Impact d'un supplément combiné d'acide folique et de vitamine B_{12} en période prépartum et en début de la lactation chez la vache laitière

Thèse

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Résumé

Les objectifs de cette thèse étaient d'évaluer les effets d'un supplément combiné d'acide folique et de vitamine B_{12} sur les performances zootechniques en fermes laitières (première étude) et sur la cinétique du glucose en début de lactation (deuxième étude). Les traitements suivants ont été donnés par injections intramusculaires hebdomadaires de trois semaines avant le vêlage jusqu'à huit ou neuf semaines postpartum : pour la première étude 1) saline ou 2) acide folique et vitamine B_{12} et pour la deuxième étude 1) saline; 2) acide folique; 3) vitamine B_{12}; ou 4) acide folique et vitamine B_{12}.

Pour la première étude, 805 vaches dans 15 troupeaux ont été impliquées. L'incidence de dystocie a été plus basse et la première insémination postpartum a eu lieu plus tôt pour les vaches multipares recevant le supplément que pour les vaches multipares ne le recevant pas. Le supplément n'a pas eu d'effet sur la production laitière. Toutefois, la concentration en gras du lait a diminué et celle en protéines a augmenté pour les vaches recevant le supplément. De plus, ces vaches ont perdu moins de poids corporel en début de lactation suggérant un effet du supplément sur la répartition de l'énergie. L'analyse économique a indiqué que ce supplément a été rentable pour la moitié des fermes à l'étude. Le supplément a augmenté la concentration en vitamine B_{12} du lait dans tous les troupeaux.

Pour la deuxième étude, 24 vaches multipares ont été utilisées. Les concentrations plasmatiques de folates et de vitamine B_{12} des animaux témoins étaient plus élevées que ce qui a été rapporté dans la littérature. Étonnamment, le supplément d'acide folique a diminué le taux d'apparition du glucose à la neuvième semaine postpartum. L'ingestion, la concentration en glycogène hépatique et le pourcentage de glucose synthétisé provenant du propionate étaient semblables entre les traitements.

Les résultats de ces études indiquent que, sous certaines circonstances, les apports d'acide folique et de vitamine B_{12} par la microflore du rumen semblent adéquats pour optimiser les performances des vaches. Cependant, il n'existe actuellement aucun moyen de prédire les apports en ces vitamines pour l'animal selon la ration reçue.

Abstract

The objectives of this thesis were to evaluate the effects of a combined supplement of folic acid and vitamin B_{12} on performance in commercial dairy herds (first study) and on glucose kinetic in early lactation (second study). The following treatments were given by weekly intramuscular injections from three weeks before calving until eight or nine weeks postpartum: for the first study 1) saline or 2) folic acid and vitamin B_{12} and for the second study 1) saline; 2) folic acid; 3) vitamin B_{12} or; 4) folic acid and vitamin B_{12}.

For the first study, 805 cows in 15 herds were involved. The incidence of dystocia was lower and the first breeding postpartum occurred at an earlier time for multiparous cows receiving the vitamin supplement than for multiparous cows that did not. Vitamin supplement did not affect milk production. However, milk fat concentration decreased and milk protein concentration increased for dairy cows receiving the supplement. Moreover, these cows lost less body weight in early lactation suggesting an effect of the vitamin supplement on energy partitioning. The economic analysis showed that this supplement was profitable for half of the studied herds. The vitamin supplement increased vitamin B_{12} concentration in milk within each herd.

For the second study, 24 multiparous cows were involved. Plasma concentrations of folates and vitamin B_{12} from controls were higher than previously reported in the literature. Surprisingly, the folic acid supplement decreased whole-body glucose rate of appearance at week nine postpartum. However, dry matter intake, liver glycogen concentration, and percentage of glucose synthesized from propionate were similar among treatments.

Results from these two studies highlighted that, under some circumstances, supplies of folic acid and vitamin B_{12} from ruminal microflora seem to be adequate to optimize cow performance. However, it is not possible under the actual state of knowledge to predict supplies of these vitamins for the animal according to the diet.

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Liste des abréviations

AA = amino acid

ACTB = β-actin

ADF = acid detergent fiber

ADN = acide désoxyribonucléique

AGL = acides gras libres

ATP = adenosine triphosphate

BCAA = branched-chain amino acids

BCS = body condition score

BEN = bilan énergétique négatif

BF = butterfat

BHBA = acide β-hydroxybutyrique

BHMT = betaine homocysteine methyltransferase

BW = body weight

CBS = cystathionine β-synthase

CP = crude protein

CR = conception rate

CV = coefficient of variation

CVMS = consommation volontaire de matière sèche

DCAD = dietary cation-anion difference

DHI = dairy herd improvement

DIM = days in milk

DM = dry matter

DMI = dry matter intake

EAA = essential amino acids

EB = energy balance

ECM = energy corrected milk

ÉN = énergie nette

GAPDH = glyceraldehyde-3-phosphate dehydrogenase

GC-MS = gas chromatography-mass spectrometry

GNF = glucides non fibreux

GNMT = glycine N-methyltransferase

Hcy = homocysteine

IE = isotopic enrichment

ILR = irreversible loss rate

IU = international unit

JEL = jours en lait

LH = hormone lutéinisante

MMA = methylmalonic acid

MTHFR = 5,10-methylene-tetrahydrofolate reductase

MUT = methylmalonyl-CoA mutase

MP = metabolisable protein

MS = matière sèche

MUN = milk urea nitrogen

NDF = neutral detergent fiber

NEAA = nonessential amino acids

NEB = negative energy balance

NEFA = nonesterified fatty acids

NE_L = net energy for lactation

NFC = nonfiber carbohydrates

NSC = nonstructural carbohydrates

PCR = polymerase chain reaction

PPIA = peptidylprolyl isomerase A

PV = poids vif

Ra = rate of appearance

Rd = rate of disappearance

RDP = rumen-degradable protein

RNA = ribonucleic acid

RUP = rumen-undegradable protein

SAHH = S-adenosylhomocysteine hydrolase

SD = standard deviation

SE = standard error

TAA = total amino acids

THF = tétrahydrofolate

TMR = total mixed ration

TS = total solids

UXT = ubiquitously-expressed transcript

WB = whole body

YWHAZ = tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, zeta polypeptide

Patience et longueur de temps font
plus que force ni que rage
Jean de La Fontaine

Remerciements

Je tiens tout d'abord à souligner le travail de deux personnes remarquables m'ayant épaulée tout au long de mes études supérieures : Doris Pellerin et Christiane Girard, respectivement directeur et codirectrice de thèse. Je me sens choyée d'avoir été encadrée par deux personnes telles que vous. Merci Doris pour ta confiance à mon égard et pour ton éternel optimisme même quand je n'y croyais plus. Merci Christiane pour ta patience lors des nombreuses relectures d'articles et pour ta compréhension. « Mieux vaut vous connaître que de connaître quelque chose. » ☺

Un merci spécial à Hélène Lapierre d'Agriculture et Agroalimentaire Canada de m'avoir dévoilé les rudiments des perfusions d'isotopes stables, mais surtout d'avoir cru en moi dès mes premiers pas en recherche.

À Débora Santschi de Valacta, à qui je dois mes débuts avec le logiciel SAS, merci. Comme une grande sœur, tu me traces le chemin!

J'aimerais remercier Dany Cinq-Mars de l'Université Laval pour son soutien tout au long de mes études supérieures.

Je remercie Jean Bernier et Yvan Chouinard de l'Université Laval de m'avoir dirigée vers les bonnes personnes à un moment opportun de mon parcours universitaire.

Le premier projet réalisé dans le cadre de cette thèse n'aurait pas été possible sans l'implication hors pair des producteurs laitiers de 15 fermes de la région de la ville de Québec. Je suis très reconnaissante du temps que vous avez consacré à faire avancer la science. Ces 14 mois de collecte de données passés auprès de vous ont été très enrichissants. Merci à Ferme Bellaska, Ferme Boisjoly, Ferme Clémar, Ferme Conrad Giroux, Ferme Degil, Ferme Émeraude, Ferme Jeanmiber, Ferme Laurier, Ferme LP Laflamme, Ferme Maco, Ferme Mipado, Ferme Noël Fortin, Ferme Rivière Portneuf, Ferme Vachard et Ferme Volait. Merci également à Jean-Luc Boutin, Jacques Laflamme, Gilles Lacasse, Daniel Forgues et Julie Duval, vétérinaires, pour leur précieuse collaboration lors du recrutement des fermes.

I would like to thank Tom Overton from Cornell University for welcoming me in his lab for a 6-month internship during my Ph. D. It was a real pleasure to work with all of you and to discover Ithaca and surroundings!

J'exprime ma gratitude envers le Conseil de recherches en sciences naturelles et en génie du Canada (CRSNG), les Fonds de recherche du Québec – Nature et technologies (FRQ-NT) et les Fonds Germain-Brisson de l'Université Laval pour avoir subventionné mes finances personnelles tout au long de mes études supérieures.

À tous les étudiants du Centre de recherche de Sherbrooke que j'ai eu la chance de côtoyer, j'ai grandement apprécié partager toutes ces activités avec vous, me permettant ainsi d'en apprendre un peu plus sur votre culture. Merci, thank you, obrigada, gracias, grazie!

À mes parents, Luc Duplessis et Marie-Ange Marcotte, merci de m'avoir transmis les gènes « persévérance », « goût du travail bien fait » et « passion pour l'agriculture ». Merci pour votre soutien et votre écoute lors des moments plus difficiles, mais aussi d'avoir partagé mes joies. Merci à mes sœurs Annie et Carole de faire partie de ma vie. Merci à ma bonne amie d'enfance Émilie Dionne pour son « support », son écoute et ces moments où un seul regard suffit!

Avant-propos

Ce travail de thèse porte sur les effets d'un supplément combiné d'acide folique et de vitamine B_{12} en période péripartum et en début de la lactation chez la vache laitière et a été réalisé en deux projets distincts. Cette thèse comprend une introduction (Chapitre 1), une revue des travaux antérieurs (Chapitre 2), six articles (Chapitres 3 à 8), une discussion/conclusion (Chapitre 9) et un article présenté en annexe (Chapitre 10). Dans tous les cas, je suis la première auteure de ces articles. Pour les deux projets, c'est moi qui ai récolté les données, les ai analysées et ai rédigé les articles sous la supervision de mon directeur et ma codirectrice de thèse. Deux exceptions à cela sont expliquées directement en dessous des chapitres concernés. J'ai également participé au processus de révision des articles publiés et celui accepté.

Chapitre 3 : Duplessis, M., Girard, C. L., Santschi, D. E., Laforest, J.-P., Durocher, J. et 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. Cet article est publié.

Chapitre 4 : Duplessis, M., Girard, C. L., Santschi, D. E., Lefebvre, D. M. et Pellerin, D. 2014. Milk production and composition, and body measurements of dairy cows receiving intramuscular injections of folic acid and vitamin B_{12} in commercial dairy herds. Livest. Sci.167: 186-194. Cet article est publié.

Chapitre 5 : Duplessis, M., Girard, C. L., Santschi, D. E. et Pellerin, D. 2014. An economic model evaluating the supplementation of folic acid and vitamin B_{12} given around parturition and in early lactation on dairy farms in Québec, Canada. Can. J. Anim. Sci. DOI : 10.4141/CJAS-2014-026. Cet article a été accepté pour publication le 20 juin 2014.

Chapitre 6 : Duplessis, M., Pellerin, D. et Girard, C. L. 2014. Vitamin B_{12} concentration in milk of cows receiving weekly intramuscular injections of folic acid and vitamin B_{12} in commercial dairy herds.

Chapitre 7 : Duplessis, M., Lapierre, H., Pellerin, D., Laforest, J.-P. et Girard, C. L. 2014. Effects of intramuscular injections of folic acid and vitamin B_{12}, alone or combined, on lactational performance and energy status of multiparous dairy cows.

Chapitre 8 : Duplessis, M., Lapierre, H., Ouattara, B., Bissonnette, N., Pellerin, D., Laforest, J.-P. et Girard, C. L. 2014. Whole-body metabolism of glucose and propionate of multiparous dairy cows receiving a folic acid and vitamin B_{12} supplement, alone or combined.

Exception : Pour cet article, les analyses de laboratoire concernant l'expression des gènes et l'activité de la méthylmalonyl-CoA mutase dans le foie ont été réalisées par B. Ouattara sous la supervision de N. Bissonnette.

Chapitre 10 (Annexe 1) : Duplessis, M., Mann, S., Nydam, D. V., Girard, C. L., Pellerin, D. et Overton, T. R. 2014. Short communication: Folates and vitamin B_{12} in colostrum and milk from dairy cows fed different energy levels during the dry period.

Exception : Les données de cet article ont été récoltées lors du projet de thèse de S. Mann réalisé à la *Cornell University* sous la supervision de T. R. Overton et de D. V. Nydam.

1 Introduction

La période entourant le vêlage représente un défi de taille pour la vache laitière. En effet, la période de transition, souvent considérée comme étant de trois semaines avant le vêlage jusqu'à trois semaines postpartum (Drackley, 1999), implique beaucoup de changements physiologiques, métaboliques et nutritionnels pour l'animal (Goff et Horst, 1997; NRC, 2001). En plus de donner naissance à son veau, la vache entame sa lactation, ce qui requiert une augmentation brusque des apports énergétiques, protéiques et calciques pour ne nommer que ces éléments. À titre d'exemple, vers la fin de la gestation, le développement quotidien du fœtus nécessite environ 0,82 Mcal en énergie pour la vache tandis que la production de 10 kg de colostrum suite au vêlage demande 11 Mcal (Goff et Horst, 1997). Au Québec, en 16 ans, la production laitière moyenne par vache a augmenté de 39 % (Fédération des producteurs de lait du Québec, 2010). Aux États-Unis, une augmentation de la production laitière par vache de l'ordre de 33 % entre 1990 et 2005 a été rapportée par Weiss et Ferreira (2006), alors que ces auteurs estiment à seulement 15 % l'augmentation de la consommation volontaire de la matière sèche (CVMS) durant la même période. Également, une diminution de 30 % de CVMS de deux jours avant le vêlage à deux jours après la parturition est fréquemment observée chez les vaches laitières (Goff et Horst, 1997). En début de la lactation, l'énergie disponible est prioritairement distribuée à la glande mammaire pour la sécrétion de lait. Ce phénomène, nommé homéorhésie, a été décrit pour la première fois chez la vache laitière par Bauman et Currie (1980).

Pour l'animal, tous ces éléments font en sorte que l'énergie requise pour la production laitière en début de la lactation est plus élevée que l'énergie ingérée provenant des aliments et cette condition entraine un bilan énergétique négatif (BEN) (Butler, 2003; McArt et al., 2013). Les vaches s'adaptent à cette situation en mobilisant leurs réserves adipeuses et protéiques. Il est donc normal, dans une certaine limite, de retrouver dans le plasma de ces animaux une diminution de la concentration de glucose accompagnée d'une hausse de la concentration d'acides gras libres (AGL) et de corps cétoniques tels que l'acide β-hydroxybutyrique (BHBA) (McArt et al., 2013). Les AGL circulant dans le sang peuvent être prélevés par le foie et être oxydés afin d'entrer dans le cycle de Krebs pour fournir de l'énergie à l'animal (Goff et Horst, 1997; McArt et al., 2013). Le BHBA, pour sa part, est

majoritairement formé lorsque le foie ne parvient plus à oxyder les AGL (Goff et Horst, 1997; Walsh et al., 2007) et peut lui aussi entrer dans le cycle de Krebs après transformation (Bergman, 1971; McArt et al., 2013). Un excès de ces métabolites dans le sang est un indicateur d'un BEN excessif pouvant causer divers problèmes tels qu'une baisse de la production laitière (Chapinal et al., 2012), une augmentation des risques de souffrir de maladies métaboliques ou autres maladies (Ospina et al., 2010; Esposito et al., 2014) ainsi qu'une diminution des performances reproductives (Butler, 2003; Leroy et al., 2008). En 2013, la principale cause de réforme dans les troupeaux laitiers québécois était la reproduction, comptant pour 17,6 % des réformes totales (Valacta, 2014).

Tous les problèmes soulevés ci-haut représentent des pertes monétaires importantes pour les entreprises laitières. La période de transition est l'étape de vie de la vache laitière la moins bien comprise (Drackley, 1999). Il est alors primordial que la recherche en production laitière tente de mieux comprendre cette période afin de diminuer les impacts négatifs du BEN énumérés ci-haut.

Depuis quelques années, la recherche s'intéresse aux possibles effets positifs d'un supplément combiné d'acide folique (vitamine B_9) et de vitamine B_{12} sur la vache laitière en période de transition et en début de la lactation. En première partie de cette thèse, une revue de littérature sur ce sujet sera présentée. Entre autres, les résultats sur les effets de ces suppléments vitaminiques sur les performances laitières et la reproduction des vaches seront énumérés. Par la suite, quatre chapitres présenteront les résultats d'une expérience réalisée en fermes commerciales portant sur les effets d'un supplément combiné d'acide folique et de vitamine B_{12} sur la vache laitière en période de transition et en début de la lactation. Deux autres chapitres présenteront les résultats d'une étude conduite au Centre de recherche et de développement sur le bovin laitier et le porc de Sherbrooke portant sur les effets de suppléments d'acide folique et de vitamine B_{12}, seuls ou combinés, sur la vache laitière en période de transition et en début de la lactation. Une discussion générale/conclusion clôturera cette thèse suivie d'un article en annexe réalisé à partir de données récoltées à la *Cornell University*, Ithaca, New York, États-Unis.

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2 Revue des travaux antérieurs

2.1 Présentation des vitamines B_9 et B_{12}

2.1.1 Vitamine B_9

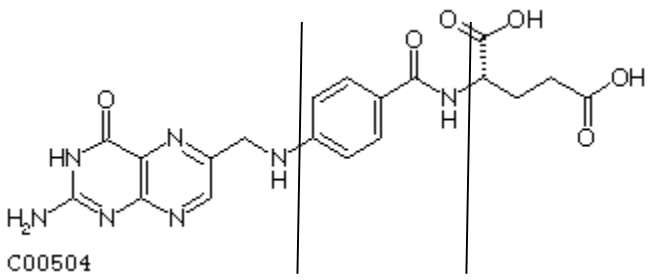
La vitamine B_9, l'acide ptéroylglutamique ou l'acide folique sont tous des synonymes pour nommer cette molécule qui a été découverte dans les années 1930. Cette substance hydrosoluble était alors connue sous plusieurs appellations selon la propriété qui y était associée à l'époque; vitamine M, vitamine Bc, facteur U et bien plus encore. Par exemple, un facteur contre l'anémie chez le singe a été trouvé dans les levures et un extrait de foie et a été nommé vitamine M (McDowell, 1989). Les chercheurs ont noté que l'absence de cette substance provoquait une anémie chez le singe rhésus, une anémie macrocytaire chez le rat, un arrêt de croissance de *Lactobacillus casei* et de *Streptococcus lactis* (Le Grusse et Watier, 1993) ainsi qu'une diminution du nombre total de globules blancs chez les agneaux (Draper et Johnson, 1952). Un peu plus tard, un facteur de croissance pour *Lactobacillus casei* et *Streptococcus lactis* a été isolé de la feuille d'épinard; le terme « acide folique » provient d'ailleurs de cette découverte (McDowell, 1989).

Le terme « folates » est également utilisé pour désigner cette vitamine. En effet, plusieurs molécules différant par leur état d'oxydation, par la longueur de la chaine d'acides glutamiques et par l'unité monocarbonée y étant attachée possèdent une activité vitaminique (Bailey, 2007).

2.1.1.1 Structure chimique et propriétés physico-chimiques de la vitamine B_9

La structure chimique de l'acide folique possède trois parties (Figure 2.1). Sur ce schéma, de gauche à droite, il y a tout d'abord le noyau ptéridine, l'acide para-aminobenzoïque et, pour terminer, l'acide glutamique. Les deux premiers éléments forment l'acide ptéroïque. Cela explique pourquoi la vitamine B_9 est parfois appelée acide ptéroylglutamique (McDowell, 1989). Comme il a été mentionné précédemment, le nombre d'acide glutamique est variable selon la molécule et se chiffre normalement entre trois et sept. Cela

est applicable pour les folates retrouvés dans la plupart des tissus. Cependant, la vitamine B_9 synthétique possède un seul acide glutamique (McDowell, 1989; Laanpere et al., 2010). Lorsque plusieurs acides glutamiques sont attachés à l'acide ptéroïque, le terme polyglutamate est utilisé.



Noyau ptéridine | Acide para-aminobenzoïque | Acide glutamique

Figure 2.1 Structure chimique de la vitamine B_9 (Adapté de Hill, 2010)

La forme de l'acide folique qui accepte les unités monocarbonées est réduite et se nomme tétrahydrofolate (THF) (Forges et al., 2008).

Cette vitamine hydrosoluble est sensible à la lumière et doit être protégée des rayons ultraviolets. Elle est reconnaissable par sa couleur jaune orangé, ainsi que son absence de goût et d'odeur (McDowell, 1989).

2.1.1.2 Besoins en vitamine B_9 chez différentes espèces animales

Les besoins en acide folique changent selon l'espèce animale et le stade de vie (Tableau 2.1). Chez la chèvre, le mouton et la vache, les microbes contenus dans leur rumen ont la capacité de synthétiser les vitamines du complexe B, qui seront par la suite absorbées par l'animal hôte. Ce phénomène sera décrit plus en détail dans la section *2.2 Synthèse des vitamines du complexe B chez les ruminants*. Les veaux, les agneaux et les chevreaux, n'ayant pas un rumen totalement développé, doivent obtenir l'acide folique par leur alimentation. Comme pour plusieurs espèces, la gestation chez la vache laitière augmente la demande des tissus en acide folique (Girard et Matte, 1995). En ce qui concerne l'être humain, les besoins augmentent avec l'âge, mais surtout avec la grossesse et la lactation (Laanpere et al., 2010). Certains animaux comme le rat ont l'habitude d'ingérer leurs fèces

riches en vitamines B. Ce comportement est connu sous l'appellation « coprophagie ». Le cæcum de ces espèces contient des microbes qui, comme chez les ruminants, synthétisent les vitamines du complexe B. Ces dernières peuvent être absorbées directement du cæcum ou rejetées dans les fèces (Barki et al., 1949).

Tableau 2.1 Besoins en vitamine B_9 selon différentes espèces animales

Espèce	Stade de vie	Besoin
Rat	-	1,0 mg/kg
Poule	Ponte	0,25 mg/kg
Poule	Reproduction	0,35 mg/kg
Poulet	Leghorn 0-6 semaines	0,55 mg/kg
Poulet	Leghorn 6-14 semaines	0,25 mg/kg
Porc	-	0,30 mg/kg
Humain	Bébé	30-45 µg/jour
Humain	Enfant	100-300 µg/jour
Humain	Adulte	400-800 µg/jour
Chèvre	Adulte	Synthèse microbienne
Mouton	Adulte	Synthèse microbienne
Vache laitière	Adulte	Synthèse microbienne

Adapté de McDowell (1989).

2.1.1.3 Fonctions de la vitamine B_9

L'acide folique est reconnu pour son rôle de transporteur (accepteur/donneur) d'unités monocarbonées. Ces dernières peuvent être du méthyle, du méthylène, du formyle, du formimino ou du hydroxyméthyle (Le Grusse et Watier, 1993). Elles s'associent avec les THF et, ensemble, contribuent à plusieurs réactions dans l'organisme. Par exemple, ils sont impliqués dans le métabolisme de certains acides aminés et dans la synthèse des purines et des pyrimidines (McDowell, 1989; Bailey, 2007). Plus spécifiquement, les purines et les pyrimidines sont des types de bases azotées contenues dans l'ADN (Griffiths et al., 2006). La synthèse de ces bases azotées est un processus complexe dans lequel l'acide folique agit comme source d'unités monocarbonées (Bailey, 2007). L'ADN est essentiel lors de la formation des cellules (McDowell, 1989). Une carence en acide folique entraine une

diminution de la division cellulaire et est surtout néfaste pour les tissus en croissance ou se régénérant rapidement comme les globules rouges.

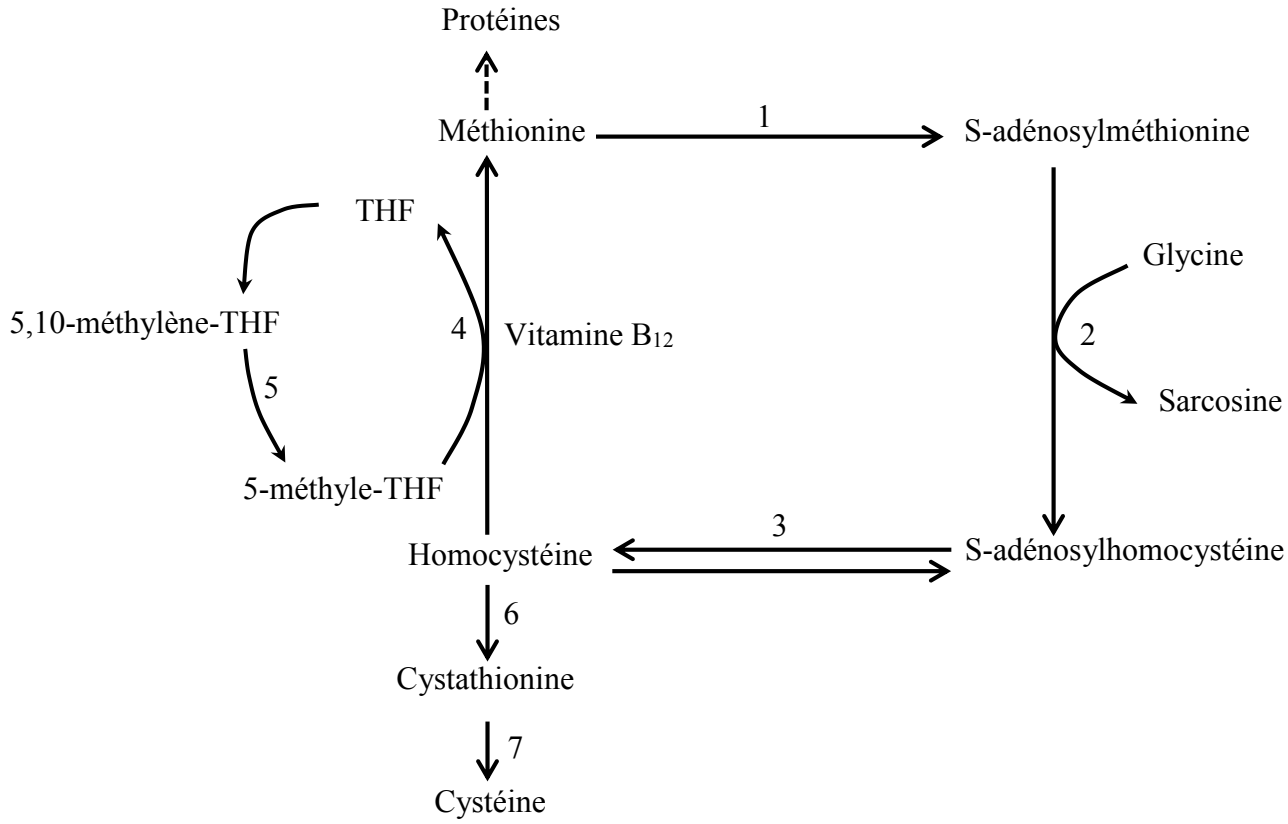


Figure 2.2 Voies métaboliques impliquant les vitamines B_9 et B_{12} (THF = tétrahydrofolate; 1 = méthionine adénosyltransférase; 2 = glycine N-méthyltransférase; 3 = S-adénosylhomocystéine hydrolase; 4 = méthionine synthase; 5 = 5,10-méthylènetétrahydrofolate réductase; 6 = cystathionine β-synthase; 7 = cystathionine β-lyase) (Adapté de Girard et Matte, 2005b; Matte et al., 2006; Preynat et al., 2010)

L'acide folique est également indirectement impliqué dans la synthèse des protéines (Figure 2.2). Avec l'aide de la vitamine B_{12} comme coenzyme et de la méthionine synthase, le 5-méthyle-THF transfère son groupement méthyle à l'homocystéine pour former la méthionine, un des premiers acides aminés essentiels limitant la production laitière avec des rations typiquement nord-américaines (Schwab et al., 1992). La méthionine, avec 19 autres acides aminés, est nécessaire pour la synthèse des protéines (Bailey, 2007). Cet acide aminé peut aussi se transformer en S-adénosylméthionine qui agit en tant que donneur majeur de groupements méthyles (Girard et Matte, 2005b; Forges et al., 2007; Berker et al.,

2009). La S-adénosylméthionine est responsable de plus de 100 réactions de méthylations enzymatiques chez les mammifères telles que la méthylation de l'ADN qui intervient dans la transcription des gènes et la synthèse de la choline, de la créatinine, de la créatine (constituants du muscle) et de la sérotonine (neurotransmetteur) (Girard et Matte, 2005b). Les besoins en méthionine et en groupes méthyles sont augmentés par la lactation (Girard et Matte, 2005b; 2006).

2.1.2 Vitamine B_{12}

La vitamine B_{12} a été nommée ainsi en 1948. Auparavant, cette vitamine était désignée sous le nom « Animal Protein Factor » (Friesecke, 1982), car la nature du composé actif était inconnue (McDowell, 1989). À cette époque, des travaux avaient permis de découvrir un facteur extrinsèque dans l'alimentation et un facteur intrinsèque provenant de la muqueuse gastrique pouvant combattre l'anémie pernicieuse. Il a fallu attendre jusqu'en 1955 pour connaître la formule chimique complexe de la vitamine B_{12}.

Seulement les microorganismes procaryotes possèdent la capacité de synthétiser la vitamine B_{12} (McDowell, 1989). Ainsi, les plantes ne contiennent pas ou très peu de cette molécule dans leurs tissus.

Tout comme l'acide folique, vitamine B_{12} est un terme générique pour représenter un groupe de molécules : la cyanocobalamine, l'hydroxocobalamine, la méthylcobalamine et l'adénosylcobalamine (McDowell, 1989). Cependant, les formes méthylcobalamine et adénosylcobalamine sont les formes actives agissant comme coenzymes (Combs, 2012).

2.1.2.1 Structure chimique et propriétés physico-chimiques de la vitamine B_{12}

La structure de la vitamine B_{12} est très complexe et comporte 4,5 % de cobalt (Figure 2.3) (McDowell, 1989). Il s'agit de la vitamine la plus lourde; elle possède une masse moléculaire de 1355 (Friesecke, 1982), comparativement à 441 pour l'acide folique (Le Grusse et Watier, 1993). La vitamine B_{12} se compose d'un noyau tétrapyrrolique renfermant un atome de cobalt relié à quatre atomes d'azote (Le Grusse et Watier, 1993). La structure reliée à l'atome de cobalt diffère et permet de nommer la molécule

vitaminique. Par exemple, la cyanocobalamine possède un groupement cyanure fixé à l'atome de cobalt. Si ce groupement est remplacé par un groupement hydroxyle, la molécule s'appellera hydroxocobalamine. En recherche, la forme cyanocobalamine est souvent utilisée, car elle est relativement stable et disponible (McDowell, 1989; Green et Miller, 2007).

La vitamine B_{12} est une poudre cristalline de couleur rouge foncé qui est sensible à la lumière et aux rayons ultraviolets (Le Grusse et Watier, 1993).

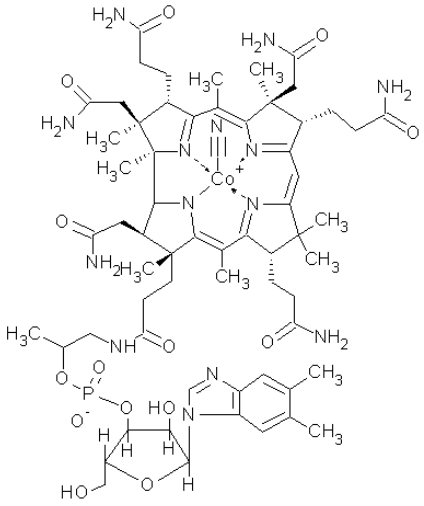


Figure 2.3 Structure chimique de la vitamine B_{12} (Adapté de Helmenstine, 2010)

2.1.2.2 Besoins en vitamine B_{12} chez différentes espèces animales

Les besoins en vitamine B_{12} diffèrent selon l'espèce et le stade de vie de l'animal (Tableau 2.2). Les besoins en cette vitamine sont minimes comparativement à ceux des autres vitamines (McDowell, 1989). Tout comme pour l'acide folique et les autres vitamines du complexe B, il a été établi que les microorganismes du rumen d'un animal en santé synthétisaient de la vitamine B_{12} lorsque le cobalt est présent en quantités suffisantes. Ce phénomène sera décrit plus en détail dans la section *2.2 Synthèse des vitamines du complexe B chez les ruminants*. En ce qui concerne les monogastriques, l'ampleur des besoins dépend également de la synthèse par les microorganismes du caecum et du comportement de coprophagie ou caecotrophie chez certaines espèces comme le lapin

(Couch et al., 1950). Par contre, un apport alimentaire est nécessaire pour éviter les symptômes de carences chez ces espèces.

Tableau 2.2 Besoins en vitamine B_{12} chez différentes espèces animales

Espèce	Stade de vie	Besoin
Rat	Croissance	50 µg/kg
Poule	Leghorn Ponte et reproduction	4 µg/kg
Poulet	Leghorn 0-6 semaines	9 µg/kg
Poulet	Leghorn 6-20 semaines	3 µg/kg
Porc	Croissance-finition	5-20 µg/kg
Porc	Reproduction-lactation	15 µg/kg
Humain	Bébé	0,5-1,5 µg/jour
Humain	Enfant	2-3 µg/jour
Humain	Adulte	3 µg/jour
Humain	Grossesse, lactation	4 µg/jour
Chèvre	Adulte	Synthèse microbienne
Mouton	Adulte	Synthèse microbienne
Vache laitière	Adulte	Synthèse microbienne
Vache laitière	Veau	0,34-0,68 µg/kg poids corporel

Adapté de McDowell (1989).

2.1.2.3 Fonctions de la vitamine B_{12}

Chez les mammifères, la vitamine B_{12} joue un rôle de coenzyme dans deux réactions enzymatiques (Friesecke, 1982; McDowell, 1989; Le Grusse et Watier, 1993).

La vitamine B_{12} est essentielle dans la formation des purines et des pyrimidines (McDowell, 1989). En effet, la vitamine B_{12} sous forme de méthylcobalamine agit comme coenzyme avec la méthionine synthase lors de la déméthylation du 5-méthyle-THF en THF (Figure 2.2). En conséquence, une carence en vitamine B_{12} entraine une accumulation du 5-méthyle-THF, une forme de l'acide folique qui ne peut pas être utilisée pour la synthèse des purines et pyrimidines (Friesecke, 1982; Scott, 1999; Girard et Matte, 2006). Cela explique pourquoi il est difficile de distinguer une carence en vitamine B_{12} ou en vitamine B_9. Tout

comme pour l'acide folique, une carence en vitamine B_{12} entraine une diminution de la division cellulaire (McDowell, 1989).

La vitamine B_{12} sous forme d'adénosylcobalamine agit comme coenzyme avec l'aide de la méthylmalonyl-CoA mutase (MUT) afin de permettre l'isomérisation du méthylmalonyl-CoA en succinyl-CoA dans la mitochondrie (Figure 2.4). Il s'agit d'une étape requise lors de la transformation de l'acide propionique pour permettre son entrée dans le cycle de Krebs générant des molécules qui produiront de l'ATP ou du glucose (néoglucogenèse) (Friesecke, 1982; McDowell, 1989). L'acide propionique provient soit de l'alimentation ou du métabolisme, en particulier chez les ruminants chez lesquels la fermentation des glucides dans le rumen en produit une quantité importante (McDowell, 1989). Dans une étude de Strobel (1992), la bactérie ruminale *Prevotella ruminicola* 23 a produit davantage d'acide propionique et a obtenu un meilleur taux de croissance en présence de vitamine B_{12} qu'en son absence. Ces résultats montrent que les bactéries du rumen synthétisant l'acide propionique à partir du glucose ont besoin de la vitamine B_{12} selon le procédé inverse de celui présenté à la Figure 2.4.

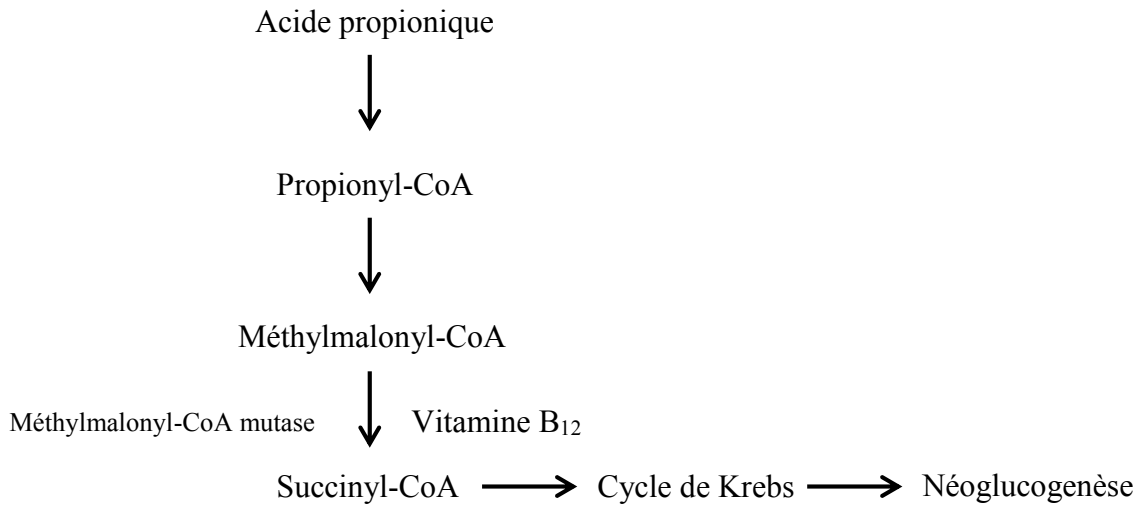


Figure 2.4 Voie métabolique de l'acide propionique avant son entrée dans le cycle de Krebs impliquant la vitamine B_{12} (Adapté de Preynat et al., 2010)

2.2 Synthèse des vitamines du complexe B chez les ruminants

Dans les années 1920, des recherches ont été réalisées afin d'étudier si les microorganismes du rumen avaient la capacité de synthétiser les vitamines du complexe B. Entre autres, Bechdel et al. (1928) sont arrivés à cette conclusion. Pour ce faire, ces chercheurs ont alimenté une génisse Holstein d'une ration pratiquement dépourvue en vitamines du complexe B. Ils ont remarqué que cet animal ne présentait pas de signe de carence et continuait à se développer normalement malgré la ration ingérée pauvre en vitamines du complexe B. Une fistule ruminale a été installée sur l'animal afin de faciliter l'accès au rumen et le prélèvement du contenu de l'organe. Par la suite, un échantillon du contenu du rumen de la génisse a été offert à des rats et la croissance de ces rats était comparée à un groupe témoin recevant une ration pauvre en vitamines du complexe B. Les cages dans lesquelles les rongeurs étaient logés possédaient un fond spécial afin que ces derniers n'aient pas accès à leurs fèces et ainsi éviter la coprophagie. Les courbes de croissance des deux groupes de rats ont différé significativement. En effet, le groupe recevant le contenu du rumen de la génisse Holstein présentait un taux de croissance plus élevé et un meilleur état de santé que le groupe témoin. Une deuxième expérience a été réalisée, cette fois-ci en offrant à un groupe de rats un supplément de bactéries ruminales séchées comparativement à une ration pauvre en vitamines du complexe B au groupe témoin. Le même résultat a été obtenu, c'est-à-dire que le taux de croissance était plus élevé chez les rats recevant le supplément de bactéries ruminales séchées que celui des rats témoins carencés en vitamines du complexe B. Ces résultats ont montré que les microorganismes du rumen synthétisent les vitamines du complexe B et expliquent pourquoi les bovins, contrairement aux rats, peuvent grandir, se reproduire et produire du lait sans montrer de signes de carence même en consommant une ration pauvre en vitamines du complexe B (Bechdel et al., 1928).

D'autres études se sont intéressées à la différence entre la quantité de vitamines du complexe B contenue dans la ration versus celle dans le contenu du rumen des ovins et des bovins. Les résultats obtenus ont montré que le contenu du rumen contenait jusqu'à 16 fois plus de vitamines du complexe B que la ration ingérée, appuyant ainsi l'hypothèse de leur synthèse dans le rumen (McElroy et Goss, 1939; Wegner et al., 1940; Lardinois et al., 1944).

Il est maintenant bien établi que les microorganismes d'un rumen en santé et bien développé synthétisent les vitamines du complexe B dont font partie l'acide folique et la vitamine B_{12} (Kon et Porter, 1954; NRC, 2001; Girard et al., 2009b). Santschi et al. (2005a) ont estimé la synthèse microbienne apparente de vitamines B_9 et B_{12} dans le rumen de vaches laitières à 21,0 et 73,0 mg/j, respectivement. Il est à noter que ces valeurs ne tiennent pas compte de la destruction et de l'utilisation de ces vitamines dans le rumen ni de leur absorption à travers la paroi ruminale. Cela dit, ces données sous-estiment probablement la synthèse microbienne réelle. Cependant, dans les études de Girard et al. (2009a) et Girard et Desrochers (2010), l'absorption d'acide folique et de vitamine B_{12} à travers la paroi du rumen n'a pas été différente de zéro. Dans une étude impliquant des vaches laitières, Schwab et al. (2006) ont estimé la synthèse microbienne de vitamine B_9 à 16,5 mg/j et à 79,8 mg/j pour la vitamine B_{12}; ce sont des données similaires à celles de Santschi et al. (2005a). Girard et al. (2009b) ont calculé que les microorganismes du rumen produisaient environ 38 % de vitamine B_{12} sous sa forme active (vitamine B_{12} vraie), le restant étant produit sous une forme sans activité biologique connue pour l'animal appelé analogues de la vitamine B_{12}.

Il a été montré que la destruction dans le rumen des suppléments alimentaires d'acide folique et de vitamine B_{12} des vaches laitières est élevée (Santschi et al., 2005a). Elle a été de l'ordre de 97,0 % pour le supplément d'acide folique et de 62,9 % pour le supplément de vitamine B_{12} (Santschi et al., 2005a). Cependant, cette destruction a été surestimée pour l'acide folique, car elle a été calculée à partir d'échantillons récoltés à l'aide d'une canule duodénale et l'absorption de cette vitamine se produit en partie dans le duodénum proximal. En effet, dans un autre essai, en moyenne 25 % de l'acide folique infusé dans l'abomasum n'a pas été retrouvé dans les échantillons de la canule duodénale (Santschi et al., 2005a). La destruction d'un supplément alimentaire de vitamine B_{12} a été de l'ordre de 80 % dans l'étude de Girard et al. (2009b).

2.3 Variation des concentrations sanguines des vitamines B_9 et B_{12} chez la vache laitière

2.3.1 Vitamine B_9

Girard et al. (1989b) se sont intéressés à la variation des folates dans le sérum de la vache laitière au cours de la lactation. Les 70 vaches multipares étaient réparties en cinq groupes, chacun d'entre eux représentait un stade physiologique différent : 1) vêlage (0 ou 1 jour suivant la parturition); 2) deux mois postpartum; 3) trois mois de gestation; 4) six mois de gestation et; 5) deux mois avant la parturition. Des échantillons de sang ont été récoltés à partir de la veine jugulaire. La concentration sérique en folates a fluctué au cours des différents stades physiologiques étudiés (Figure 2.5). Une différence dans la concentration sérique de folates de 40 % a été notée entre le moment du vêlage et deux mois postpartum. La concentration sérique de folates au vêlage (16,8 ng/ml) a été significativement plus basse qu'à deux mois postpartum (24,3 ng/ml) et à trois mois de gestation (21,9 ng/ml). Girard et al. (1995) et Girard et Matte (1999) ont obtenu des résultats semblables.

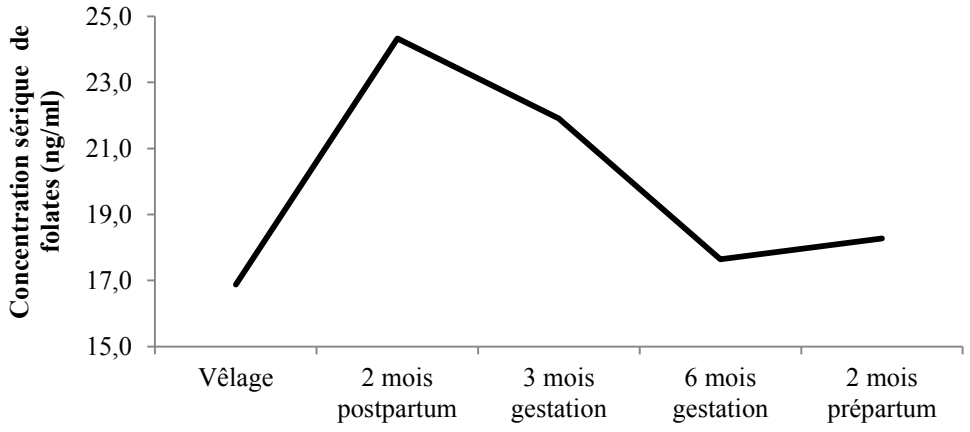


Figure 2.5 Concentration de folates dans le sérum de vaches laitières selon différents stades physiologiques (Adapté de Girard et al., 1989b)

Girard et al. (1989b) ont étudié les effets d'une injection intramusculaire d'acide folique selon quatre doses (40, 80, 160 ou 320 mg) sur la concentration sérique de cette vitamine chez 20 vaches taries et gestantes et 20 vaches en lactation mais non gestantes. Pour les vaches taries recevant l'injection de vitamine B_9, peu importe la dose, la concentration

sérique de folates a atteint un maximum une journée après l'injection puis a diminué jusqu'au jour 16 après l'injection. Cependant, la plus haute concentration en folates a été obtenue avec les doses de 160 et 320 mg. Les injections d'acide folique n'ont pas eu d'effet significatif sur la concentration sérique en folates des vaches non gestantes en début de lactation.

Girard et al. (1995) se sont intéressés aux effets d'injections intramusculaires hebdomadaires d'acide folique de 160 mg sur la concentration sérique en folates. Les chercheurs ont utilisé 24 vaches multipares et 16 vaches primipares. La moitié des animaux recevait une injection intramusculaire hebdomadaire de 4 ml d'eau saline stérile (témoin) et l'autre moitié, 4 ml d'acide folique à raison de 40 mg/ml (160 mg). L'étude débutait lorsque les vaches étaient confirmées gestantes, environ 45 jours après l'insémination, et se terminait six semaines après le vêlage. Contrairement aux résultats de Girard et al. (1989b), les injections d'acide folique ont permis d'augmenter la concentration sérique moyenne de folates avant et après la parturition. Les concentrations sériques étaient plus élevées chez les vaches multipares (environ 17,0 ± 0,8 ng/ml) que chez les vaches primipares (environ 14,4 ± 0,8 ng/ml) avant et après le vêlage (Girard et al., 1995).

Girard et Matte (1999) ont utilisé 32 vaches primipares et 31 vaches multipares et ont ajouté à la ration soit 0, 2 ou 4 mg/kg de poids vif d'un supplément alimentaire d'acide folique, et ce, d'un mois avant la parturition jusqu'à 305 jours en lait (JEL). Le supplément d'acide folique (2 ou 4 mg/kg de poids vif) a augmenté la concentration sérique en folates par rapport aux animaux témoins. L'augmentation de la concentration sérique en folates des animaux recevant le supplément a été davantage marquée lors des 16 premières semaines de la lactation, contrairement à l'étude de Girard et al. (1995) dans laquelle le supplément administré par voie intramusculaire a permis une augmentation stable de la concentration sérique d'acide folique au cours de la lactation.

Les résultats de l'étude de Girard et al. (1989b) suggèrent que la synthèse microbienne d'acide folique dans le rumen n'a pas été suffisante pour empêcher les fluctuations sériques en folates. D'autres études ont montré qu'il est possible d'augmenter la concentration sérique en folates par la supplémentation (Girard et al., 1995; Girard et Matte, 1999; Graulet et al., 2007).

2.3.2 Vitamine B_{12}

Anthony et al. (1951b) n'ont pas observé de différences dans la concentration sanguine de vitamine B_{12} chez les vaches gestantes et les vaches non gestantes. Ils ont noté une concentration sanguine en vitamine B_{12} significativement plus élevée chez les vaches Holstein que chez les Jersey. Les auteurs ne sont pas parvenus à expliquer cette différence. Elliot et al. (1965) ont étudié la concentration de vitamine B_{12} dans le sang des vaches laitières selon leur stade de lactation. Des prélèvements de sang sur 24 vaches Holstein primipares et multipares ont été réalisés débutant trois semaines avant le vêlage jusqu'à 37 semaines postpartum. Les auteurs ont estimé que les besoins en cobalt des animaux étaient comblés par la ration. Une diminution significative d'environ 12 % du taux sanguin de vitamine B_{12} a été observée en début de la lactation comparativement à la mi-lactation, vers 22 semaines postpartum. Par la suite, la concentration sanguine de vitamine B_{12} a atteint un plateau entre la mi-lactation et la fin de la lactation, à 37 semaines postpartum.

Corse et Elliot (1970) n'ont pas remarqué de différence significative dans la concentration sérique de vitamine B_{12} entre la période de tarissement, trois semaines après le vêlage et quatre mois après la parturition. Cependant, les données obtenues suggèrent une concentration sérique de vitamine B_{12} numériquement plus basse pendant le tarissement.

Girard et Matte (1999) ont noté une diminution sérique en vitamine B_{12} du vêlage jusqu'aux deux premiers mois de la lactation, surtout chez les vaches primipares. Kincaid et al. (2003) ont observé une diminution significative dans la concentration sérique de vitamine B_{12} chez les vaches primipares et multipares au cours de la lactation, la plus basse concentration a été atteinte à 120 JEL. Girard et al. (2005) ont noté que le plus bas niveau de concentration sérique de vitamine B_{12} chez les vaches multipares a été atteint à la huitième semaine de la lactation pour ensuite augmenter au fur et à mesure que la lactation a progressé. Dans cette étude, la concentration sérique en vitamine B_{12} était de 263,2 ± 12,6 pg/ml à quatre semaines avant le vêlage. À la 8^{e} semaine de la lactation, elle était de 181,3 (variant de 167,3 à 198,3) pg/ml contre 232,8 (214,9-254,7), 257,2 (235,1-278,7) et 265,1 (242,3-287,1) pg/ml à la 18^{e}, 28^{e} et 38^{e} semaine de la lactation, respectivement.

De la même façon que pour l'acide folique, la synthèse dans le rumen n'a pas été suffisante pour éviter les fluctuations sériques en vitamine B_{12}. De plus, la période critique où les niveaux sont les plus bas est autour du vêlage. Tout comme pour la vitamine B_9, il est possible d'augmenter significativement la concentration sanguine de vitamine B_{12} par la supplémentation (Graulet et al., 2007; Preynat et al., 2009a,b; Akins et al., 2013).

2.3.3 Lien entre les concentrations sanguines de vitamines B_9 et B_{12}

Girard et Matte (1999) ont étudié la concentration sérique de vitamine B_{12} selon trois différents niveaux de supplément d'acide folique ajoutés à la ration de vaches laitières, soit 0, 2 ou 4 mg/kg de poids vif. Les traitements ont débuté quatre semaines avant la date de vêlage prévue jusqu'à 305 JEL. Au vêlage, les deux groupes ayant reçu le supplément de vitamine B_9 (2 ou 4 mg/kg de poids vif) présentaient un taux sérique en vitamine B_{12} plus bas que le groupe témoin (0 mg/kg de poids vif d'acide folique). De plus, la concentration sérique en acide folique du vêlage jusqu'à huit semaines de la lactation était significativement plus haute pour les animaux ayant reçu le supplément d'acide folique. Girard et al. (2005) ont obtenu des résultats similaires. Comme il a été expliqué précédemment, la vitamine B_{12} agit comme coenzyme afin de transférer le groupement méthyle de l'acide folique à l'homocystéine pour former la méthionine (Scott, 1999). Il est donc possible de déduire que l'ajout d'un supplément d'acide folique dans la ration des animaux a augmenté la demande pour la vitamine B_{12}. Cette dernière devient alors limitante et sa concentration trop basse provoque une accumulation de l'acide folique dans le sérum sous sa forme méthylée, une forme ne pouvant plus être utilisée par les cellules de l'organisme (Girard et Matte, 2005b; 2006).

2.4 Variation des concentrations hépatiques des vitamines B_9 et B_{12} chez les ruminants

Les vitamines B_9 et B_{12} sont entreposées dans le foie (Le Grusse et Watier, 1993). Dans une étude où un supplément combiné de 2,6 g d'acide folique et 500 mg de vitamine B_{12} a été

donné par l'alimentation à quatre vaches laitières, 28 % de folates et 46 % de la vitamine B_{12} absorbés en veine porte ont été prélevés par le foie (Girard et al., 2001).

2.4.1 Vitamine B_9

Graulet et al. (2007) ont observé une augmentation de la concentration hépatique de folates de deux à huit semaines postpartum chez la vache laitière. Similairement, la concentration hépatique de folates a augmenté de deux à huit semaines de la lactation pour ensuite atteindre un plateau jusqu'à 16 semaines postpartum dans l'étude de Preynat et al. (2010). En moyenne, la concentration hépatique de folates a été de 6,7 ± 0,3 µg/g de tissu frais entre deux et 16 semaines après le vêlage (Preynat et al., 2010).

Le supplément d'acide folique a permis d'augmenter significativement la concentration hépatique de folates par rapport aux animaux ne recevant pas la vitamine (Graulet et al., 2007; Preynat et al., 2010).

2.4.2 Vitamine B_{12}

Wilson et al. (1967) et Walker et Elliot (1972) ont observé que le niveau de vitamine B_{12} dans le foie des bovins a augmenté au cours de la lactation (de 2 à 26 semaines postpartum). Cependant, Graulet et al. (2007), Preynat et al. (2010) et Grace et Knowles (2012) n'ont pas observé de différence significative dans la concentration hépatique de vitamine B_{12} au début de la lactation (de 0 à 16 semaines postpartum). La concentration hépatique moyenne a été de 0,94 ± 0,02 µg/g entre 2 et 16 semaines de la lactation (Preynat et al., 2010).

Le supplément de vitamine B_{12} a augmenté de façon significative la concentration de cette vitamine dans le foie (Graulet et al., 2007; Preynat et al., 2010; Akins et al., 2013).

2.5 Besoin des bovins laitiers pour les vitamines B_9 et B_{12}

Le rumen se développe à partir de deux mois chez le bovin, à condition que celui-ci reçoive une ration le permettant (Lagerlof, 1929 cité par Savage et McCay, 1942). Les études ont montré que les jeunes ruminants ont besoin d'un apport extérieur de vitamines du complexe B, sauf la niacine, pour croître normalement (Draper et al., 1952; Kon et Porter, 1954). Dumoulin et al. (1991) ont rapporté que le gain moyen quotidien des génisses laitières âgées entre 7 et 12 semaines pouvait être augmenté de 7,6 % par des injections intramusculaires hebdomadaires d'acide folique. Girard et al. (1989a) ont montré que les veaux âgés d'environ 18 jours possédaient une concentration sérique de folates plus basse que les génisses de quatre mois. En effet, les jeunes animaux de 18 jours avaient une concentration sérique de folates de 8,2 comparativement à 14,8 ng/ml pour les génisses de quatre mois. Ces dernières présentaient une concentration sérique de folates semblable à celle des vaches adultes, en moyenne de 18,8 ng/ml au cours de la lactation (Girard et al., 1989b). Ces résultats suggèrent que les animaux dont le rumen n'est pas totalement développé peuvent souffrir d'une carence en acide folique si leur alimentation en est dépourvue.

Eckles et Williams (1925) se sont intéressés aux effets de l'ajout de levures à la ration des vaches laitières. Les levures sont reconnues comme étant une bonne source de vitamines B dont l'acide folique (Eckles et al., 1924). Cependant, cela n'est pas valable pour la vitamine B_{12} qui est synthétisée seulement par les bactéries (McDowell, 1989). À l'époque du déroulement de l'étude, les besoins des bovins laitiers pour plusieurs vitamines n'étaient pas établis et un des objectifs du projet d'Eckles et Williams (1925) était de déterminer si ces ruminants nécessitaient un apport de vitamines B comme les animaux de laboratoire pour optimiser les performances. Deux groupes de vaches laitières ont été utilisés : le groupe 1 a reçu un supplément de levures (25 g pour chaque livre de lait produit par jour) contrairement au groupe 2 qui n'en a pas reçu. La production moyenne du groupe 1 a été de 26,4 lb/j (12,0 kg/j) contre 25,6 lb/j (11,6 kg/j) pour le groupe 2. Les auteurs ont conclu que l'ajout de levures à l'alimentation n'apportait pas de bénéfices à la vache laitière pour la production laitière, la concentration de gras du lait et l'état de santé. Cela a suggéré que les

microorganismes du rumen synthétisaient suffisamment de vitamines du complexe B pour subvenir aux besoins des vaches laitières de l'époque.

Selon le NRC (2001), les microorganismes du rumen synthétisent suffisamment de vitamines du complexe B pour satisfaire les besoins de la vache laitière. Selon Kon et Porter (1954), les ruminants en santé n'ont pas besoin d'un apport extérieur en vitamines du complexe B. Le NRC (2001) a dressé un tableau des besoins en acide folique et en vitamine B_{12} des vaches laitières selon une extrapolation des besoins chez le porc et selon les recherches de Miller et al. (1986) et de Zinn et al. (1987) (Tableau 2.3).

Tableau 2.3 Estimation des besoins journaliers des vitamines B_9 et B_{12} chez la vache laitière

	Besoins journaliers estimés (mg/j)[1]			Synthèse dans le rumen
Vitamine	Tissus	Lait	Total	(mg/j)
Vitamine B_9	33	2	35	7
Vitamine B_{12}	0,4	0,2	0,6	70

[1] Les besoins ont été estimés à l'aide d'une vache type de 650 kg produisant 35 kg de lait à 4 % de gras par jour.
Adapté du NRC (2001).

Selon le Tableau 2.3, les microorganismes produisent amplement de vitamine B_{12} pour les besoins des tissus et de la production laitière à condition que l'apport en cobalt soit suffisant (McDowell, 1989). Cependant, la situation est toute autre pour l'acide folique pour lequel la synthèse semble insuffisante pour satisfaire les besoins. Toutefois, la synthèse estimée d'acide folique dans le rumen présentée au Tableau 2.3 est de 2 à 3 fois moins élevée que celle rapportée dans les études de Santschi et al. (2005a) et de Schwab et al. (2006).

2.6 Effet de l'alimentation sur la synthèse des vitamines du complexe B chez les ruminants

La flore microbienne du rumen s'adapte à la ration servie aux animaux. En effet, une ration riche en concentrés et une ration riche en fourrages ne solliciteront pas les mêmes microorganismes lors de la dégradation des aliments (Hayes et al., 1966). Suite aux études

mettant en évidence la synthèse des vitamines du complexe B par les microorganismes du rumen, des chercheurs se sont intéressés aux effets de l'alimentation des animaux sur leur synthèse dans le rumen.

Lardinois et al. (1944) ont évalué la synthèse de différentes vitamines du complexe B à partir du contenu ruminal de bovins adultes et de veaux laitiers recevant huit différentes rations. L'objectif de ce projet était de déterminer si l'ajout d'azote non protéique sous forme d'urée et/ou de glucides modifiait la synthèse microbienne des vitamines du complexe B. En ce qui concerne l'acide folique, les auteurs ont conclu que la synthèse dans le rumen de cette vitamine ne semblait pas corrélée avec la composition de la ration. Selon cette équipe, pour maximiser la synthèse des vitamines du complexe B dans le rumen, l'animal doit recevoir suffisamment de glucides. Hunt et al. (1954) ont découvert que l'ajout d'amidon (0 vs 9 g d'amidon) dans un rumen artificiel stimulait la synthèse de vitamine B_{12}.

Hayes et al. (1966) ont nourri 48 bouvillons Angus selon six rations se distinguant par les ingrédients (par exemple maïs seulement, mélange maïs-foin) ainsi que par la forme physique des aliments servis afin d'étudier les changements sur la synthèse des vitamines du complexe B dans le rumen. La concentration d'acide folique dans le fluide ruminal était plus élevée lorsque les animaux n'ingéraient que du maïs floconné ou moulu, sans fourrage. Le fluide ruminal des bouvillons qui recevaient du maïs floconné, du maïs moulu ou du maïs moulu avec du foin long contenait significativement plus de vitamine B_{12} que celui des animaux nourris que de foin moulu. Dans cette étude, les rations à base de fourrages contenaient plus de vitamines du complexe B que les rations à base de maïs. Cependant, dans le fluide ruminal, les concentrations de la plupart des vitamines du complexe B étaient plus élevées pour les rations à base de maïs, sans fourrage.

Dans un essai impliquant des génisses Holstein, Dryden et Hartman (1971) ont conclu que le contenu du rumen de ces animaux contenait davantage de vitamine B_{12} lorsqu'une ration à base d'ensilage leur était servie comparativement à une ration à base de foin haché, de foin moulu ou de foin haché et de grain. Chez le mouton, Sutton et Elliot (1972) ont conclu que la synthèse de vitamine B_{12} dans le rumen était significativement plus basse lorsque le pourcentage de grain dans la ration augmentait.

Girard et al. (1994) ont observé les effets de quatre rations sur le contenu ruminal d'acide folique chez des bouvillons : 1) ratio fourrage : concentré de 30 : 70; 2) ratio de 30 : 70 + 2 mg/kg de poids vif par jour d'un supplément d'acide folique servi dans l'alimentation; 3) ratio de 70 : 30 et; 4) ratio de 70 : 30 + 2 mg/kg de poids vif par jour d'un supplément d'acide folique. Le contenu ruminal en vitamine B_9 des animaux ingérant une ration riche en concentrés était plus élevé que celui des bouvillons recevant une ration riche en fourrages. Les auteurs ont conclu que la synthèse d'acide folique par les microorganismes du rumen était augmentée lorsque l'animal ingérait des aliments riches en glucides rapidement fermentescibles. En ce qui concerne les rations avec le supplément d'acide folique, elles ont permis d'augmenter significativement les concentrations ruminale et sérique en cette vitamine, et ce, indépendamment de la nature de la ration.

Les études présentées ci-haut ont utilisé des ratios fourrages : concentrés différents de ceux couramment adoptés en production laitière. Santschi et al. (2005b) ont mesuré l'effet de ratios fourrages : concentrés fréquemment utilisés en production laitière sur les concentrations d'acide folique et de vitamine B_{12} dans le contenu ruminal chez des vaches primipares et multipares. Deux ratios différents ont été utilisés : 1) haut en fourrages (fourrage : concentré; 58 : 42) et 2) bas en fourrage (37 : 63). Les auteurs n'ont pas remarqué de différences significatives sur la concentration d'acide folique et de vitamine B_{12} dans le fluide ruminal selon les ratios et la parité. Cependant, il a été noté qu'une ration avec un ratio fourrage : concentré bas (40 : 60) diminuait la synthèse de la vitamine B_{12} vraie dans les fractions solide et liquide associées aux bactéries du rumen.

Schwab et al. (2006) se sont intéressés à la synthèse d'acide folique et de vitamine B_{12} dans le rumen selon deux proportions de fourrages (35 et 60 %) et deux proportions de glucides non fibreux (GNF; 30 et 40 %) dans la ration selon un dispositif factoriel 2 × 2. La quantité d'acide folique synthétisée (mg/j) a été plus haute pour les rations contenant 40 % de GNF et 35 % de fourrages. Cela suggère que la synthèse d'acide folique par les microorganismes du rumen est plus élevée lorsque les rations ingérées ont une vitesse de dégradation dans le rumen élevée. La synthèse de vitamine B_{12} dans le rumen était plus basse lorsque les pourcentages de fourrages et de GNF étaient à 60 et à 40 %, respectivement. La synthèse microbienne de vitamine B_{12} dans le rumen a été la plus haute pour les animaux nourris

avec la ration à 35 % de fourrages, pour laquelle la quantité de sucres ingérés était la plus élevée.

Dans le mémoire de maîtrise de Seck (2012), 14 vaches multipares ont reçu soit une alimentation haute en fourrages (ratio fourrage : concentré de 61 : 39) ou basse en fourrages (45 : 55). La synthèse apparente de vitamine B_{12} dans le rumen a été augmentée par la ration haute en fourrages, passant de 0,46 à 0,50 mg de vitamine B_{12}/kg de matière sèche ingérée pour les rations basse et haute en fourrages, respectivement. Cela a suggéré qu'en plus de la quantité de sucres contenue dans la ration comme conclu par Schwab et al. (2006), la synthèse de la vitamine B_{12} dans le rumen est également influencée par le ratio fourrage : concentré comme il a été rapporté par Santschi et al. (2005b).

Dans une étude visant à quantifier l'impact de l'espèce fourragère (légumineuse versus graminée) dans la ration sur la synthèse de vitamine B_{12} dans le rumen, aucune différence significative n'a été notée sur la synthèse pour des vaches ayant reçu de la luzerne (*Medicago sativa L.*) ou du dactyle (*Dactylis glomerata L.*) (Seck, 2012).

La plupart des résultats présentés, malgré la variabilité entre les études, suggèrent que la flore microbienne du rumen s'adapte à la ration reçue et que cela a un impact sur la synthèse apparente des vitamines B_9 et B_{12} dans le rumen et sur la quantité disponible pour la vache.

2.7 Impact des vitamines B_9 et B_{12} sur la performance laitière des vaches

2.7.1 Quantité de lait produit

Girard et al. (1995) n'ont pas observé de différence significative sur la production laitière de vaches primipares et multipares ayant reçu hebdomadairement une injection de 160 mg d'acide folique commençant à 45 jours de gestation et durant jusqu'à six semaines après la parturition comparativement au groupe témoin. En effet, les chercheurs ont noté une production laitière moyenne de 24,6 ± 1,4 kg/j pour les vaches ayant reçu le supplément et

de 24,8 ± 1,4 kg/j pour les animaux témoins. Cependant, de 45 jours de gestation jusqu'au tarissement, la production laitière des vaches recevant les injections hebdomadaires d'acide folique a numériquement été augmentée de 14 %.

Girard et Matte (1998) ont étudié les effets de l'ajout d'un supplément d'acide folique à raison de 0, 2 ou 4 mg/kg de poids vif par jour incorporé dans l'ensilage de légumineuses sur les performances laitières (Tableau 2.4). Pour ce faire, 32 vaches primipares et 31 vaches multipares ont été utilisées et ont reçu le supplément d'un mois avant la date prévue du vêlage jusqu'à 305 JEL. Le supplément d'acide folique offert à raison de 4 mg/kg de poids vif par jour a permis d'augmenter la production laitière de 6 % (+ 2,2 kg/j) et de 10 % (+ 3,0 kg/j) chez les multipares pour les 100 premiers JEL et entre 100 et 200 JEL, respectivement, par rapport aux animaux témoins (Tableau 2.4). En ce qui concerne les vaches primipares, pour les 100 premiers JEL, la production laitière a été plus basse pour celles ayant reçu le supplément que pour les animaux témoins, mais il n'y a pas eu d'effet entre 100 et 200 JEL. Indépendamment de la parité, le supplément n'a pas eu d'effet sur la production laitière en fin de lactation (entre 200 et 300 jours). Pour expliquer les réponses du supplément de vitamine B_9 chez les vaches primipares, les auteurs ont relevé le fait que ces dernières avaient une concentration sérique en vitamine B_{12} plus basse que les vaches multipares, surtout pendant les 100 premiers JEL. Comme il a déjà été mentionné, la vitamine B_{12} agit comme coenzyme afin de transférer le groupe méthyle de l'acide folique à l'homocystéine et ainsi permettre l'entrée de la vitamine B_9 dans les cellules pour, entre autres, contribuer à la formation des purines et pyrimidines (Scott, 1999). La basse concentration sérique de vitamine B_{12} chez les vaches primipares a pu freiner cette réaction et a possiblement créé une carence en acide folique à l'échelle cellulaire (Girard et Matte, 1998).

La production laitière 305 jours de 54 vaches multipares n'a pas été affectée (moyenne de 10 584 ± 137 kg) par un supplément d'acide folique distribué dans l'alimentation à raison de 0, 3 ou 6 mg/kg de poids vif. Les traitements étaient offerts d'un mois avant la date prévue du vêlage jusqu'à 305 JEL (Girard et al., 2005). Dans une autre étude utilisant les mêmes doses d'acide folique et commençant à 56 JEL, le supplément alimentaire de

vitamine B_9 n'a pas permis non plus d'augmenter la production laitière (Girard et al., 2009a).

Tableau 2.4 Production laitière moyenne selon la parité et les traitements (0, 2 ou 4 mg/kg PV[1] d'un supplément d'acide folique par jour)

	Primipares			Multipares			
JEL[2]	0 mg/kg	2 mg/kg	4 mg/kg	0 mg/kg	2 mg/kg	4 mg/kg	SE[3]
3 à 305	27,0	25,1	25,2	27,4	29,0	29,6	0,96
3 à 100	28,8	27,0	26,6	34,8	35,3	37,0	0,89
100 à 200	28,6	26,6	26,9	29,0	31,4	32,0	1,09
200 à 300	24,3	22,4	23,0	19,4	19,1	20,8	1,29

[1] PV= Poids vif.
[2] JEL = Jours en lait.
[3] SE = Erreur type de la moyenne.
Adapté de Girard et Matte (1998).

Les résultats de Girard et Matte (1998) concernant les vaches primipares ont donné suite à une étude impliquant 14 vaches primipares recevant un supplément hebdomadaire de vitamine B_{12} (0 ou 10 mg) sous forme d'injections intramusculaires tout en leur fournissant un supplément d'acide folique à raison de 4 mg/kg de poids vif par l'alimentation, et ce, peu importe la dose du supplément de vitamine B_{12} reçue (Girard et Matte, 2005a). Le projet a commencé à 4 semaines et a duré jusqu'à 18 semaines de lactation. Ce traitement n'a pas permis d'augmenter significativement la production laitière. En effet, elle était de 31,1 et de 28,5 ± 1,6 kg/j pour les vaches recevant le supplément de vitamine B_{12} et pour les animaux témoins, respectivement. Cependant, le lait corrigé pour l'énergie a augmenté significativement de 3,2 kg/j, passant de 25,8 à 29,0 ± 1,6 kg/j pour les vaches témoins et les vaches recevant le supplément de vitamine B_{12}, respectivement. Les résultats de l'étude de Girard et Matte (1998) combinés à ceux-ci suggèrent une réponse optimale du supplément d'acide folique lorsque la vitamine B_{12} n'est pas limitante, c'est-à-dire lorsque la concentration sérique de vitamine B_{12} est plus élevée que 200 pg/ml (Girard et Matte, 2005a).

Graulet et al. (2007) ont assigné 24 vaches multipares à l'un des quatre traitements alimentaires suivants : 1) pas de vitamines (témoin); 2) 2,6 g/j d'un supplément

d'acide folique; 3) 0,5 g/j d'un supplément de vitamine B_{12} et; 4) les deux vitamines ensemble. Les suppléments de vitamines étaient donnés directement dans la ration. Pour les huit premières semaines de la lactation, le supplément d'acide folique, avec ou sans le supplément de vitamine B_{12}, a permis d'augmenter la production laitière de 3,4 kg/j.

Sacadura et al. (2008) ont noté une augmentation de la quantité de lait produit par jour chez des vaches en début de lactation (93 JEL en moyenne) ayant reçu un supplément alimentaire protégé contre la dégradation dans le rumen contenant quatre vitamines du complexe B dont l'acide folique. Les animaux recevant les vitamines ont produit 40,5 ± 0,4 kg/j tandis que le groupe témoin a produit 39,6 ± 0,4 kg/j. Les mêmes traitements ont été étudiés chez des vaches ayant en moyenne 219 JEL et le supplément n'a pas permis d'augmenter significativement la production laitière. Les auteurs ont affirmé que le supplément de vitamines du complexe B semble plus efficace chez les animaux en début de la lactation, période pendant laquelle la CVMS est plus basse qu'en milieu de lactation et ne permet pas de fournir toute l'énergie nécessaire pour la production laitière.

Preynat et al. (2009b) ont noté une augmentation de la production laitière chez les vaches recevant un supplément par injections intramusculaires hebdomadaires de vitamines B_9 et B_{12} (40,3 ± 0,9 kg/j) dans les quatre premières semaines de la lactation comparativement aux animaux témoins (37,5 ± 0,9 kg/j) et ceux ne recevant que de la vitamine B_9 (37,7 ± 0,9 kg/j).

Dans une sous-étude impliquant 24 vaches multipares provenant de l'étude de Preynat et al. (2009b), le supplément de 160 mg d'acide folique et de 10 mg de vitamine B_{12} donné hebdomadairement par voie intramusculaire a permis d'augmenter la production laitière de 12 % à la semaine 12 de la lactation, passant de 34,8 à 38,9 ± 1,0 kg/j, par rapport au groupe témoin (Preynat et al., 2009a).

Ghaemialehashemi (2013) a noté une augmentation de 3,6 kg/j de la production laitière pour les vaches multipares recevant hebdomadairement 320 mg d'acide folique et 10 mg de vitamine B_{12} de trois semaines avant la date prévue du vêlage jusqu'à la huitième semaine postpartum par voie intramusculaire.

Akins et al. (2013) ont injecté hebdomadairement 0 ou 10 mg de vitamine B_{12} à des vaches primipares et multipares de 60 jours avant le vêlage jusqu'à 150 jours après la parturition. Les auteurs n'ont pas noté d'amélioration de la production laitière suite à ce traitement. Ils ont conclu que le niveau plasmatique en folates des vaches impliquées était bas et que cela a pu freiner la réponse.

Le Tableau 2.5 présente une synthèse des résultats concernant la production laitière des vaches de cinq études dans lesquelles des suppléments de vitamines B_9 et/ou B_{12} étaient donnés aux vaches laitières.

Tableau 2.5 Production laitière de vaches ayant reçu des suppléments d'acide folique et/ou de vitamine B_{12} selon différentes études

	Production laitière (kg/j)				
	Traitements				
Études	B_9-B_{12}-	B_9+B_{12}-	B_9-B_{12}+	B_9+B_{12}+	SE[1]
Girard et Matte, 2005[2]	-	28,5	-	31,1	1,6
Graulet et al., 2007[3]	39,5	41,8	36,5	40,9	1,6
Preynat et al., 2009a[4]	35,4	-	-	38,6	1,5
Preynat et al., 2009b[5]	37,7	36,8	-	38,9	1,1
Ghaemialehashemi, 2013[6]	40,1	-	-	43,7	1,3

[1] SE = Erreur type de la moyenne.
[2] B_9+B_{12}- : 4 mg d'acide folique/kg poids vif/j; B_9+B_{12}+ : 4 mg d'acide folique/kg poids vif/j et 10 mg de vitamine B_{12}/semaine.
[3] B_9-B_{12}- : pas de vitamines; B_9+B_{12}- : 2,6 g d'acide folique/j; B_9-B_{12}+ : 0,5 g de vitamine B_{12}/j; B_9+B_{12}+ : 2,6 g d'acide folique/j et 0,5 g de vitamine B_{12}/j.
[4] B_9-B_{12}- : pas de vitamines; B_9+B_{12}+ : 160 mg d'acide folique/semaine et 10 mg de vitamine B_{12}/semaine. Il s'agit des résultats présentés sans l'ajout de méthionine.
[5] B_9-B_{12}- : pas de vitamines; B_9+B_{12}- : 160 mg d'acide folique/semaine; B_9+B_{12}+ : 160 mg d'acide folique/semaine et 10 mg de vitamine B_{12}/semaine. Il s'agit des résultats présentés sans l'ajout de méthionine.
[6] B_9-B_{12}- : pas de vitamines; B_9+B_{12}+ : 320 mg d'acide folique/semaine et 10 mg de vitamine B_{12}/semaine.

2.7.2 Constituants du lait

Les mêmes études que dans la section *2.7.1 Quantité de lait produit* seront reprises afin de présenter, cette fois-ci, les résultats qui tiennent compte des effets d'un supplément d'acide folique et/ou de vitamine B_{12} sur la composition du lait.

2.7.2.1 Protéines

Girard et al. (1995) ont noté une augmentation du pourcentage de protéines du lait chez les vaches multipares recevant hebdomadairement un supplément de 160 mg d'acide folique par injections intramusculaires pendant les six premières semaines de la lactation. Ce résultat ne s'applique pas aux vaches primipares chez qui le supplément n'a pas eu d'effet sur le pourcentage en protéines du lait.

Le supplément d'acide folique a permis d'augmenter la quantité de caséine du lait pour les vaches multipares pendant 305 JEL (Girard et Matte, 1998). La caséine est la principale protéine contenue dans le lait de vache, représentant en moyenne 87,5 % des protéines totales (Cayot et Lorient, 1998).

Un supplément d'acide folique ajouté dans l'alimentation des bovins laitiers (3 ou 6 mg/kg de poids vif) a augmenté la concentration en protéine brute et en caséine du lait chez les vaches multipares (Girard et al., 2005) et la concentration et la quantité journalière de protéine brute dans le lait (Girard et al., 2009a) par rapport aux animaux témoins (0 mg d'acide folique/kg de poids vif).

Un supplément de 4 mg/kg de poids vif d'acide folique combiné à une injection de vitamine B_{12} n'a pas eu d'effet significatif sur la quantité de protéine brute journalière dans le lait chez les vaches primipares comparativement aux animaux ne recevant que le supplément d'acide folique (Girard et Matte, 2005a).

La quantité de protéine brute quotidienne dans le lait a augmenté de l'ordre de 75 g/j dans l'étude de Graulet et al. (2007) lors de l'ajout d'un supplément d'acide folique dans l'alimentation des vaches. Les résultats sont similaires pour l'équipe de Sacadura et al. (2008) et de Preynat et al. (2009a) tandis qu'aucune augmentation n'a été notée par Preynat et al. (2009b). Une augmentation de la quantité de protéine produite par jour a été observée chez les vaches multipares recevant un supplément combiné d'acide folique et de vitamine B_{12} en début de la lactation (Ghaemialehashemi, 2013).

Un supplément de vitamine B_{12} n'a pas eu d'impact sur le pourcentage ou la quantité de protéines dans le lait (Akins et al., 2013).

Le Tableau 2.6 résume les résultats des quantités de protéines sécrétée quotidiennement selon cinq études où des suppléments de vitamines B_9 et/ou B_{12} étaient donnés aux vaches laitières.

Tableau 2.6 Quantité de protéines sécrétées dans le lait de vache ayant reçu des suppléments d'acide folique et/ou de vitamine B_{12} selon différentes études

	Quantité de protéines (kg/j)				
	Traitements				
Études	B_9-B_{12}-	B_9+B_{12}-	B_9-B_{12}+	B_9+B_{12}+	SE[1]
Girard et Matte, 2005[2]	-	0,95	-	1,03	0,04
Graulet et al., 2007[3]	1,20	1,24	1,14	1,25	0,04
Preynat et al., 2009a[4]	1,03	-	-	1,07	0,05
Preynat et al., 2009b[5]	1,11	1,08	-	1,13	0,03
Ghaemialehashemi, 2013[6]	1,23	-	-	1,39	0,04

[1] SE = Erreur type de la moyenne.
[2] B_9+B_{12}- : 4 mg d'acide folique/kg poids vif/j; B_9+B_{12}+ : 4 mg d'acide folique/kg poids vif/j et 10 mg de vitamine B_{12}/semaine.
[3] B_9-B_{12}- : pas de vitamines; B_9+B_{12}- : 2,6 g d'acide folique/j; B_9-B_{12}+ : 0,5 g de vitamine B_{12}/j; B_9+B_{12}+ : 2,6 g d'acide folique/j et 0,5 g de vitamine B_{12}/j.
[4] B_9-B_{12}- : pas de vitamines; B_9+B_{12}+ : 160 mg d'acide folique/semaine et 10 mg de vitamine B_{12}/semaine. Il s'agit des résultats présentés sans l'ajout de méthionine.
[5] B_9-B_{12}- : pas de vitamines; B_9+B_{12}- : 160 mg d'acide folique/semaine; B_9+B_{12}+ : 160 mg d'acide folique/semaine et 10 mg de vitamine B_{12}/semaine. Il s'agit des résultats présentés sans l'ajout de méthionine.
[6] B_9-B_{12}- : pas de vitamines; B_9+B_{12}+ : 320 mg d'acide folique/semaine et 10 mg de vitamine B_{12}/semaine.

2.7.2.2 Gras

Frobish et Davis (1977) ont administré quotidiennement pendant sept jours 6 ou 18 mg d'un supplément de vitamine B_{12} à des vaches en début et en milieu de lactation. Les deux traitements n'ont pas eu d'impact sur le pourcentage de gras dans le lait. Le même résultat a été obtenu lors de l'administration d'un supplément combiné d'acide folique et de vitamine B_{12}, fournissant 6 ou 18 mg de chaque vitamine, pendant onze et sept jours, respectivement.

Elliot et al. (1979) ont injecté 0 ou 10 mg de vitamine B_{12} dans le muscle de vaches laitières deux fois par semaine en commençant quatre semaines avant le vêlage jusqu'à huit semaines après. La ration que les animaux ingéraient avait été formulée pour provoquer le syndrome du lait faible en gras. En effet, elle était constituée de 80 % de concentrés et de 20 % d'ensilage. Le pourcentage moyen de gras du lait n'a pas été significativement

différent entre les deux traitements. Avec une injection intramusculaire de 150 mg de vitamine B_{12}, James Croom et al. (1981) ont obtenu les mêmes résultats qu'Elliot et al. (1979).

Pour les 100 premiers JEL, la quantité de gras sécrété dans le lait des vaches multipares recevant un supplément d'acide folique par l'alimentation (0, 2 ou 4 mg/kg de poids vif) a augmenté linéairement selon la dose du supplément, passant de 138,1 à 150,4 ± 4,9 kg pour les 100 premiers JEL (Girard et Matte, 1998). Toutefois, la quantité de gras produit a été diminuée par le supplément chez les vaches primipares. Le pourcentage de gras du lait n'a pas différé significativement entre les traitements et les parités.

Il n'y a pas eu de différence significative dans la concentration en gras du lait de vache recevant un supplément d'acide folique seul (Girard et al., 2005) ou un supplément combiné d'acide folique et de vitamine B_{12} (Girard et Matte, 2005a) par rapport aux animaux témoins. Par contre, en ce qui concerne la quantité de gras sécrété dans le lait, elle a été de 0,87 et de 1,01 ± 0,06 kg/j pour les vaches primipares ayant reçu 0 et 10 mg d'un supplément de vitamine B_{12}, respectivement, avec 4 mg/kg de poids vif d'un supplément d'acide folique (Girard et Matte, 2005a).

Le pourcentage de gras du lait a été augmenté par le supplément de vitamine B_{12} seul, mais n'a pas différé lorsque combiné avec un supplément d'acide folique (Graulet et al., 2007).

Sacadura et al. (2008) ont observé une augmentation de la quantité de gras sécrété par jour dans le lait des vaches ayant reçu un supplément de quatre vitamines du complexe B, dont l'acide folique, donné dans la ration par rapport aux animaux témoins.

La quantité de gras sécrété par jour dans le lait a eu tendance à augmenter avec l'administration d'un supplément de vitamines B_9 et B_{12}, passant de 1,20 à 1,31 ± 0,05 kg/j pour les animaux témoins et ceux recevant le supplément, respectivement. Cependant, il n'y a pas eu d'effet de traitement sur la concentration en matière grasse du lait (Preynat et al., 2009a). Pour leur part, Preynat et al. (2009b) et Ghaemialehashemi (2013) n'ont pas noté de différence dans la concentration ni dans la quantité de matière grasse sécrétée par jour suite aux injections intramusculaires hebdomadaires des vitamines B_9 et B_{12}.

La concentration ainsi que la quantité de matière grasse sécrétée par jour ont augmenté linéairement avec l'augmentation de la dose du supplément d'acide folique (0, 3 ou 6 mg/kg de poids vif par jour) donné aux vaches multipares de l'étude de Girard et al. (2009a).

Le Tableau 2.7 présente un résumé des résultats des quantités de gras sécrété quotidiennement selon cinq études où des suppléments de vitamines B_9 et/ou B_{12} étaient donnés aux vaches laitières.

Tableau 2.7 Quantité de gras sécrété dans le lait de vache ayant reçu des suppléments d'acide folique et/ou de vitamine B_{12} selon différentes études

	Quantité de gras (kg/j)				
	Traitements				
Études	B_9-B_{12}-	B_9+B_{12}-	B_9-B_{12}+	B_9+B_{12}+	SE[1]
Girard et Matte, 2005[2]	-	0,87	-	1,01	0,06
Graulet et al., 2007[3]	1,42	1,65	1,49	1,50	0,06
Preynat et al., 2009a[4]	1,22	-	-	1,27	0,06
Preynat et al., 2009b[5]	1,38	1,33	-	1,38	0,04
Ghaemialehashemi, 2013[6]	1,70	-	-	1,91	0,14

[1] SE = Erreur type de la moyenne.
[2] B_9+B_{12}- : 4 mg d'acide folique/kg poids vif/j; B_9+B_{12}+ : 4 mg d'acide folique/kg poids vif/j et 10 mg de vitamine B_{12}/semaine.
[3] B_9-B_{12}- : pas de vitamines; B_9+B_{12}- : 2,6 g d'acide folique/j; B_9-B_{12}+ : 0,5 g de vitamine B_{12}/j; B_9+B_{12}+ : 2,6 g d'acide folique/j et 0,5 g de vitamine B_{12}/j.
[4] B_9-B_{12}- : pas de vitamines; B_9+B_{12}+ : 160 mg d'acide folique/semaine et 10 mg de vitamine B_{12}/semaine. Il s'agit des résultats présentés sans l'ajout de méthionine.
[5] B_9-B_{12}- : pas de vitamines; B_9+B_{12}- : 160 mg d'acide folique/semaine; B_9+B_{12}+ : 160 mg d'acide folique/semaine et 10 mg de vitamine B_{12}/semaine. Il s'agit des résultats présentés sans l'ajout de méthionine.
[6] B_9-B_{12}- : pas de vitamines; B_9+B_{12}+ : 320 mg d'acide folique/semaine et 10 mg de vitamine B_{12}/semaine.

Il n'y a pas eu de changement significatif dans la concentration de matière grasse ainsi que la quantité de gras sécrété quotidiennement suite à des injections intramusculaires hebdomadaires de 0 ou 10 mg vitamine B_{12} (Akins et al., 2013).

2.7.2.3 Lactose

Girard et Matte (1998) ont observé une augmentation du lactose produit pour les 100 premiers JEL pour les vaches multipares recevant un supplément d'acide folique par

l'alimentation, et ce, linéairement avec l'augmentation de la concentration vitaminique (0, 2 ou 4 mg/kg de poids vif par jour).

Contrairement aux études de Girard et al. (2005; 2009a) et de Graulet et al. (2007) où la quantité de lactose produite par jour chez les vaches multipares recevant un supplément d'acide folique, avec ou sans supplément de vitamine B_{12}, n'a pas augmenté significativement, les résultats des études de Girard et Matte (2005a), de Preynat et al. (2009a,b) et de Ghaemialehashemi (2013) ont indiqué une augmentation significative du lactose produit par jour ou de la concentration de lactose dans le lait de vache ayant reçu un supplément combiné d'acide folique et de vitamine B_{12} comparativement aux vaches témoins. Cependant, Preynat et al. (2009b) ont observé une augmentation moins marquée à mesure que la lactation progressait.

Les résultats concernant la performance laitière suite à l'ajout d'un supplément d'acide folique et/ou de vitamine B_{12} sont variables. Néanmoins, selon la littérature, les réponses obtenues sont généralement meilleures lorsqu'un supplément combiné d'acide folique et de vitamine B_{12} est offert à la vache en période péripartum et en début de la lactation.

2.7.3 Vitamines B_9 et B_{12} contenues dans le lait

2.7.3.1 Vitamine B_9

Le colostrum ainsi que le lait des premiers jours après le vêlage des bovins laitiers sont riches en folates (Collins et al., 1951; Karlin, 1966; Girard et al., 1995). Ensuite, la concentration diminue pour atteindre un plateau vers deux ou trois mois de la lactation.

Girard et al. (1995) n'ont pas noté d'effet significatif sur la concentration de folates du lait de vache laitière recevant hebdomadairement un supplément d'acide folique ou non par voie intramusculaire durant les six premières semaines de la lactation. Pour leur part, Girard et Matte (1998; 1999) ont observé une augmentation de la concentration de folates dans le lait lorsque les vaches recevaient un supplément alimentaire d'acide folique selon une dose de 0, 2 ou 4 mg/kg de poids vif par jour durant la lactation entière. Il n'y a pas eu de différence dans la concentration de folates du lait lorsque les animaux recevaient un

supplément d'acide folique de 2 ou 4 mg/kg de poids vif par jour. Des résultats similaires ont été observés par Girard et al. (2005), étude dans laquelle le supplément d'acide folique était donné aux animaux selon des concentrations différentes, soit 0, 3 ou 6 mg/kg de poids vif par jour. Cependant, l'augmentation de la concentration de folates du lait a été moins marquée chez les vaches recevant un supplément d'acide folique de 6 mg/kg de poids vif par jour que chez ceux en recevant quotidiennement 3 mg/kg de poids vif (Girard et al., 2005). Ces résultats suggèrent que la glande mammaire des bovins possède un mécanisme régulant la concentration de cette vitamine dans le lait. Les folates doivent être liés à une protéine pour être sécrétés dans le lait. Cette protéine devient le facteur limitant. Ainsi, il ne peut y avoir davantage de folates dans le lait s'il n'y a pas également augmentation de la disponibilité de cette protéine (Girard et al., 2005).

La concentration ainsi que la quantité sécrétée dans le lait (mg/j) de folates ont augmenté significativement dans l'étude de Graulet et al. (2007) suite à l'ajout de 2,6 g/j d'un supplément d'acide folique dans la ration des vaches laitières. Des observations semblables ont été rapportées sur la concentration en folates dans le lait de vaches multipares par Preynat et al. (2009a,b), étude dans laquelle le supplément a été injecté hebdomadairement par voie intramusculaire.

2.7.3.2 Vitamine B_{12}

Collins et al. (1951) n'ont pas rapporté de différence dans la concentration en vitamine B_{12} du lait chez trois races de bovins (Holstein, Jersey et Guernsey). Cependant, une grande variabilité dans la concentration de cette vitamine dans le lait a été notée entre les animaux. Gregory et al. (1958) ont observé des résultats similaires; la concentration de vitamine B_{12} du lait entre les races Shorthorn et Friesian n'était pas différente et une grande variation dans la concentration de vitamine B_{12} entre animaux a été rapportée.

Dans une étude d'Anthony et al. (1951a), le colostrum ainsi que le lait des vaches de race Holstein avaient une concentration en vitamine B_{12} plus élevée comparativement aux vaches de race Jersey. En effet, la concentration moyenne de vitamine B_{12} du colostrum et du lait de vache Holstein était de 49 et 22 ng/ml contrairement à 21 et 10 ng/ml pour les vaches Jersey, respectivement. Miller et al. (1966) ont obtenu des résultats semblables

concernant la concentration en vitamine B_{12} du lait de vache Holstein et Jersey. Tout comme dans l'étude de Gregory et al. (1958), le colostrum des vaches était plus concentré en vitamine B_{12} que le lait (Anthony et al., 1951a).

Une injection hebdomadaire de 10 mg de vitamine B_{12} entre les semaines 4 et 18 de la lactation a permis d'augmenter significativement la concentration ainsi que la quantité de cette vitamine dans le lait. Cela représente une augmentation de plus de 300 % par rapport au lait des vaches témoins (Girard et Matte, 2005a).

Une augmentation de la concentration et de la quantité sécrétée de vitamine B_{12} dans le lait a été observée par Graulet et al. (2007) et par Preynat et al. (2009a,b) lorsque les vaches recevaient un supplément de vitamine B_{12}, avec ou sans supplément d'acide folique. Similairement, la concentration de vitamine B_{12} du colostrum et du lait a augmenté significativement chez les vaches primipares et multipares recevant un supplément de 10 mg de vitamine B_{12} par semaine comparativement aux animaux qui n'en recevaient pas (Akins et al., 2013).

Le Tableau 2.8 résume les résultats obtenus concernant la concentration de vitamine B_{12} dans le lait de cinq études impliquant des vaches ayant reçu un supplément d'acide folique et/ou de vitamine B_{12}.

Il est intéressant de noter au Tableau 2.8 que le supplément de vitamine B_{12}, soit donné par l'alimentation ou par voie intramusculaire, a augmenté significativement la concentration de cette vitamine dans le lait augmentant ainsi sa valeur nutritive, et ce, dans les cinq études présentées au Tableau 2.8. De plus, il faut mentionner la variabilité des concentrations obtenues; Girard et Matte (2005a) ont noté une concentration de 3214 pg/ml alors que Preynat et al. (2009a) ont rapporté 7287 pg/ml pour la même dose de vitamine B_{12} administrée. Cependant, cette variabilité peut être partiellement expliquée par les méthodes de dosage de la vitamine B_{12} différentes entre les études.

Tableau 2.8 Concentration en vitamine B_{12} dans le lait provenant de vaches ayant reçu un supplément d'acide folique et/ou de vitamine B_{12} lors de différentes études

	Vitamine B_{12} dans le lait (pg/ml)				
	Traitements				
Études	B_9-B_{12}-	B_9+B_{12}-	B_9-B_{12}+	B_9+B_{12}+	SE[1]
Girard et Matte, 2005[2]	-	736	-	3214	137
Graulet et al., 2007[3]	1731	1834	5172	4406	391
Preynat et al., 2009a[4]	4666	-	-	7287	828
Preynat et al., 2009b[5]	4781	4976	-	6816	270
Akins et al., 2013[6]	1575	-	4431	-	184

[1] SE = Erreur type de la moyenne.
[2] B_9+B_{12}- : 4 mg d'acide folique/kg poids vif/j; B_9+B_{12}+ : 4 mg d'acide folique/kg poids vif/j et 10 mg de vitamine B_{12}/semaine.
[3] B_9-B_{12}- : pas de vitamines; B_9+B_{12}- : 2,6 g d'acide folique/j; B_9-B_{12}+ : 0,5 g de vitamine B_{12}/j; B_9+B_{12}+ : 2,6 g d'acide folique/j et 0,5 g de vitamine B_{12}/j.
[4] B_9-B_{12}- : pas de vitamines; B_9+B_{12}+ : 160 mg d'acide folique/semaine et 10 mg de vitamine B_{12}/semaine. Il s'agit des résultats présentés sans l'ajout de méthionine.
[5] B_9-B_{12}- : pas de vitamines; B_9+B_{12}- : 160 mg d'acide folique/semaine; B_9+B_{12}+ : 160 mg d'acide folique/semaine et 10 mg de vitamine B_{12}/semaine. Il s'agit des résultats présentés sans l'ajout de méthionine.
[6] B_9-B_{12}- : pas de vitamines; B_9-B_{12}+ : 10 mg de vitamine B_{12}/semaine.

Chassaing et al. (2011) ont étudié la concentration de vitamine B_{12} dans le lait de vache selon différentes méthodes d'alimentation : 1) ration à base d'herbe dans les montagnes; 2) ration à base d'herbe à basse altitude; 3) ration à base d'ensilage de maïs dans les montagnes et; 4) ration à base d'ensilage de maïs à basse altitude. Des échantillons de lait provenant des réservoirs réfrigérés de 100 fermes participantes ont été prélevés à différents temps dans l'année pour refléter les impacts sur la régie de l'alimentation (pâturage l'été versus fourrage entreposé). La concentration la plus élevée de vitamine B_{12} dans le lait a été notée lorsque les animaux étaient nourris d'ensilage de maïs, peu importe le temps de l'année. Ainsi, l'alimentation a des impacts sur la concentration de vitamine B_{12} dans le lait et peut expliquer la variabilité observée dans les études citées plus haut (Tableau 2.8).

Dans une étude néerlandaise, la concentration moyenne de vitamine B_{12} dans le lait provenant de 544 vaches Holstein de première lactation a été de 4400 pg/ml, variant entre 1000 et 12 900 pg/ml (Rutten et al., 2013). De plus, les auteurs ont estimé que l'héritabilité

de la concentration de vitamine B_{12} dans le lait était de 0,37, suggérant que le contenu en vitamine B_{12} du lait peut être modifié par la sélection génétique (Rutten et al., 2013).

2.7.3.3 Fortification des aliments en vitamine B_9

Depuis 1998, il est obligatoire de fortifier certains aliments en acide folique au Canada et aux États-Unis (MacFarlane et al., 2011). Cette mesure a pour but d'augmenter la consommation de cette vitamine chez les femmes en âge de procréer pour ainsi réduire les malformations du tube neural chez le nouveau-né (Tamura et Picciano, 2006). La farine blanche et autres grains figurent parmi les aliments touchés par cette exigence (Colapinto et al., 2011). Il a été montré que la carence en acide folique chez la population canadienne est pratiquement inexistante et que 40 % de la population générale en présente une haute concentration dans le sang (Colapinto et al., 2011). Cependant, la fortification en acide folique est matière à débat puisqu'elle est possiblement néfaste pour les personnes présentant une carence en vitamine B_{12} (Selhub et Paul, 2011). Ces deux vitamines étant hautement interreliées dans le métabolisme, un excès d'acide folique peut masquer la carence en vitamine B_{12} et ainsi aggraver les symptômes d'anémie et de troubles cognitifs, particulièrement chez les personnes âgées (Morris et al., 2007; Selhub et Paul, 2011). Au Canada, il a été estimé qu'environ 5 % de la population est carencée en vitamine B_{12} (MacFarlane et al., 2011). Selon Selhub et Paul (2011), tout aliment fortifié en acide folique devrait également l'être en vitamine B_{12}.

Seulement les bactéries peuvent synthétiser la vitamine B_{12}, les animaux, les végétaux et les champignons en étant incapables (McDowell, 1989; Martens et al., 2002). Les êtres humains doivent compter sur les produits animaux (Ferlay et al., 2013), en particulier ceux des ruminants, ou sur les suppléments alimentaires pour combler leur besoin journalier en cette vitamine. Une étude de Matte et al. (2012) a montré que la vitamine B_{12} présente dans le lait de bovin était davantage assimilable par l'intestin que la forme synthétique des suppléments. Cette équipe a utilisé le porc comme modèle animal, car il possède un mécanisme d'absorption intestinale de la vitamine B_{12} semblable à celui de l'être humain. À 28 jours d'âge, dix porcelets femelles ont été utilisés dans un dispositif en tiroirs, dans lequel le premier facteur correspondait à la concentration en vitamine B_{12} dans le lait (lait enrichi en vitamine B_{12} provenant de vaches ayant reçu un supplément de vitamine B_{12} ou

non). Trois traitements consistaient à une ration à base de céréales supplémentée de lait de vache selon trois formes différentes : 1) lait cru; 2) microfiltré et; 3) pasteurisé. Les deux autres traitements consistaient en des rations supplémentées de cyanocobalamine (forme synthétique) ou non. La quantité de vitamine B_{12} sous la forme synthétique fournie aux porcelets a été adaptée selon le groupe appartenant au lait enrichi de vitamine B_{12} (80 µg) ou non (50 µg). En conclusion, l'absorption intestinale de la vitamine B_{12} a été améliorée de 8 à 10 % lorsque cette vitamine provenait du lait, enrichi ou non et peu importe la forme (cru, microfiltré ou pasteurisé), en comparaison avec la forme synthétique. Conséquemment, le lait est une bonne source de vitamine B_{12} permettant de prévenir les carences chez les êtres humains (Matte et al., 2012).

2.8 Impact des vitamines B_9 et B_{12} sur le bilan énergétique de la vache laitière

2.8.1 Concept du bilan énergétique négatif

Tout d'abord, il est bien de définir le concept du bilan énergétique chez la vache laitière. Dans la littérature, cela se définit comme étant la différence entre l'énergie que l'animal ingère par son alimentation et l'énergie requise pour son entretien et sa production laitière, plus sa croissance et sa gestation, s'il y a lieu (Remppis et al., 2011). Cela dit, lorsque le bilan énergétique est négatif, cela signifie que l'énergie ingérée par l'animal n'est pas suffisante pour combler ses besoins et celui-ci doit donc aller puiser dans ses réserves corporelles pour obtenir l'énergie nécessaire pour son métabolisme et sa production (Butler et Smith, 1989; Goff et Horst, 1997). Chez la vache laitière, le BEN se produit particulièrement en début de la lactation (McArt et al., 2013). La Figure 2.6 illustre le concept du bilan énergétique au courant de la lactation. Il est possible de voir qu'à partir de 80 JEL environ, l'animal n'est généralement plus en BEN (Villa-Godoy et al., 1988; Allen et al., 2005).

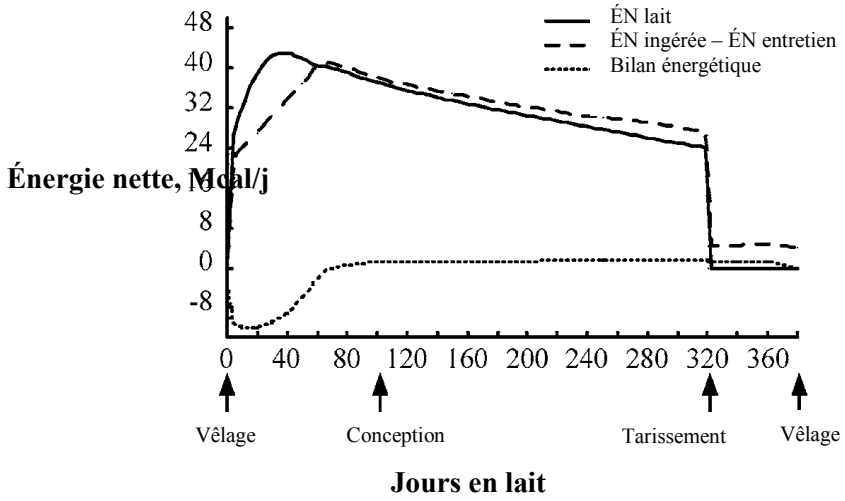


Figure 2.6 Bilan énergétique théorique d'une vache lors d'une lactation complète (ÉN = énergie nette) (Adapté d'Allen et al., 2005)

2.8.2 Mobilisation des réserves corporelles et ses conséquences

La mobilisation des réserves corporelles lors d'un BEN cause une augmentation des AGL dans la circulation sanguine de l'animal (McArt et al., 2013). Les acides gras ainsi libérés peuvent être utilisés directement par différents tissus comme source d'énergie, par la glande mammaire pour la synthèse du gras du lait ou par le foie. Ce dernier est responsable d'oxyder les AGL afin de leur permettre d'entrer dans le cycle de Krebs et de contribuer à la néoglucogenèse. Les AGL peuvent également être partiellement oxydés dans le foie afin de former des corps cétoniques tels que l'acétone, l'acide acétoacétique et le BHBA ou convertis en triglycérides (Figure 2.7). La Figure 2.7 illustre également que l'acide propionique, l'acide lactique, certains acides aminés et, de moindre importance chez le ruminant, le glucose peuvent entrer dans le cycle de Krebs afin de fournir de l'énergie à l'animal.

Il est normal pour la vache en début de la lactation de présenter des concentrations sanguines d'AGL et de corps cétoniques plus élevées qu'en fin de la lactation. Ils représentent une source d'énergie supplémentaire pour l'animal. Toutefois, un excès d'AGL et de corps cétoniques retrouvés dans la circulation sanguine est néfaste pour la productivité et la santé de la vache et suggère un bilan énergétique trop négatif (McArt et

al., 2013). Lorsqu'en trop grande quantité, le foie ne parvient pas à oxyder la totalité des AGL et ceux-ci sont alors re-estérifiés à nouveau en triglycérides (Goff et Horst, 1997). Cela mène à leur accumulation dans le foie et altère le fonctionnement des hépatocytes. Cette pathologie se nomme foie gras ou lipidose hépatique (Bobe et al., 2004). Dans cette condition, l'acétyl-CoA n'est plus incorporé dans le cycle de Krebs, mais il est transformé en acétoacétyl-CoA puis en corps cétoniques (Figure 2.7). L'acétonémie se caractérise par une accumulation importante de corps cétoniques dans les fluides corporels tels que le lait, le sang et l'urine (Bergman, 1971). Le BEN augmente les risques d'acétonémie, de déplacement de caillette et de rétention placentaire en début de la lactation (LeBlanc, 2010).

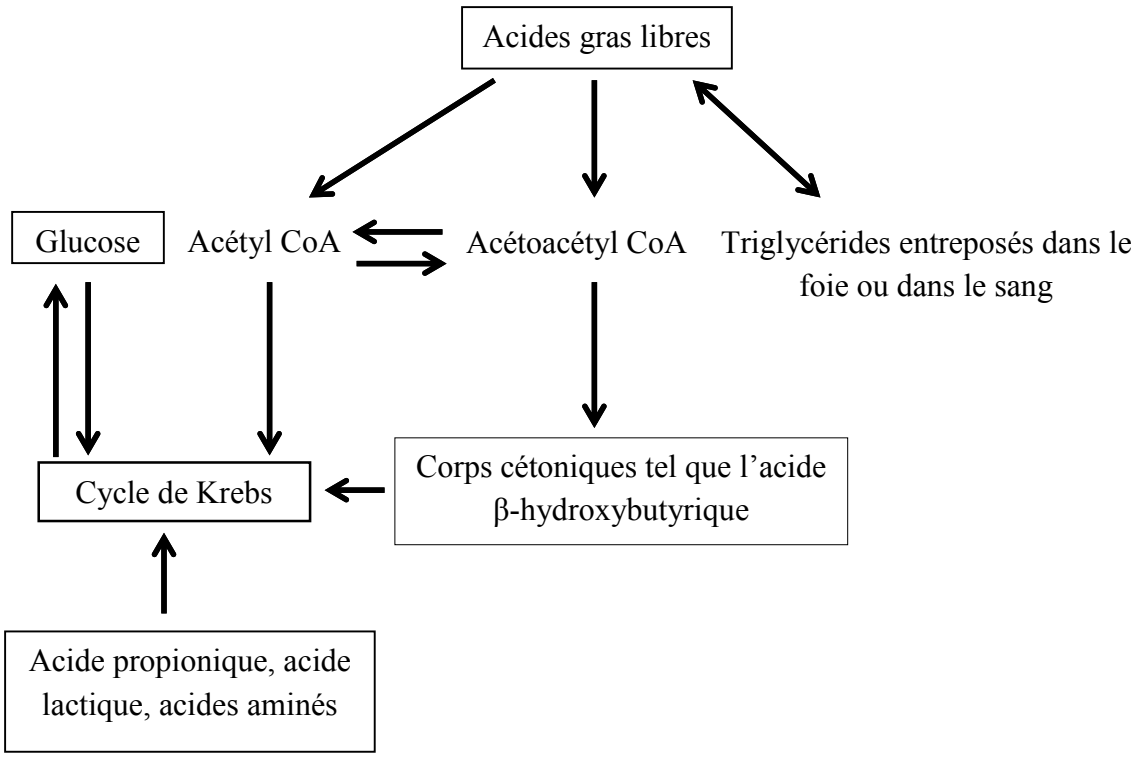


Figure 2.7 Résumé du métabolisme des acides gras libres, des corps cétoniques et de la néoglucogenèse dans le foie (Adapté de Bergman, 1971)

2.8.3 Impact des vitamines B_9 et B_{12} sur l'acide méthylmalonique et la méthylmalonyl-CoA mutase

Comme il a été mentionné plus haut, la vitamine B_{12} agit comme coenzyme dans la réaction métabolique permettant de transformer le méthylmalonyl-CoA en succinyl-CoA sous l'action de l'enzyme MUT (Le Grusse et Watier, 1993). En d'autres termes, il s'agit d'une des étapes permettant à l'acide propionique d'entrer dans le cycle de Krebs afin de fournir de l'énergie à la vache. L'acide propionique contribue pour environ 50 à 60 % de la synthèse de glucose chez le ruminant (Amaral et al., 1990; Danfær et al., 1995). Ainsi, lors d'une carence en vitamine B_{12}, l'activité de la MUT est réduite et le méthylmalonyl-CoA se dégrade en acide méthylmalonique (Scott, 1999; Selhub et al., 2007).

Girard et Matte (2005a) ont noté une diminution de la concentration sérique d'acide méthylmalonique de l'ordre de 0,103 µmol/L en début de la lactation chez des vaches primipares ayant reçu un supplément d'acide folique et des injections intramusculaires hebdomadaires de vitamine B_{12} comparativement aux vaches ayant reçu un supplément d'acide folique seul. Cela indique que les animaux ne présentaient probablement pas un niveau optimal en vitamine B_{12} en début de la lactation. De plus, ce résultat suppose que le méthylmalonyl-CoA n'a pas été gaspillé et a été utilisé. Les auteurs ont conclu qu'un niveau sous-optimal de vitamine B_{12} ralentit l'activité de la MUT et, par le fait même, l'utilisation de l'acide propionique affectant ainsi le métabolisme énergétique chez la vache laitière.

Contrairement à ce qui avait été rapporté par Graulet et al. (2007), l'expression du gène codant pour l'enzyme MUT a été augmentée de 15 % pour les vaches ayant reçu le supplément combiné d'acide folique et de vitamine B_{12} par rapport aux animaux témoins (Preynat et al., 2010).

2.8.4 Impact des vitamines B_9 et B_{12} sur les acides gras libres et les corps cétoniques

Selon l'étude de Graulet et al. (2007) dans laquelle des suppléments d'acide folique et de vitamine B_{12} étaient donnés aux vaches, soit seuls ou combinés, la concentration

plasmatique d'AGL n'a pas différé significativement entre les traitements. Cependant, la plus basse concentration d'AGL a été obtenue chez les vaches ayant reçu le supplément des deux vitamines combinées; 481 ± 56, 442 ± 60, 420 ± 56 et 344 ± 56 μ*M* pour le groupe témoin, le groupe supplémenté d'acide folique, le groupe supplémenté de vitamine B_{12} et les deux vitamines ensemble, respectivement. La concentration de lipides totaux dans le foie après deux semaines de la lactation a été de 14,0 ± 1,4, 29,7 ± 1,5, 15,8 ± 1,4 et 14,1 ± 1,5 g/g d'ADN pour le groupe témoin, le groupe supplémenté d'acide folique, le groupe supplémenté de vitamine B_{12} et les deux vitamines ensemble, respectivement. Les lipides hépatiques totaux ont été plus élevés pour les animaux supplémentés d'acide folique seul, mais ont diminué plus rapidement aux semaines deux à huit de la lactation que pour les autres traitements. Les concentrations en triglycérides hépatiques ont montré un patron similaire. Selon les résultats de cette étude, lorsque les deux vitamines ont été données ensemble aux animaux, leur utilisation chez les vaches semble avoir été accrue et cela a été suivi d'une amélioration de l'efficacité métabolique par rapport aux vaches supplémentées avec l'acide folique seul (Graulet et al., 2007). En effet, avec une production laitière et une CVMS similaires pour les animaux recevant le supplément d'acide folique seul et ceux recevant les deux vitamines, il y a eu une diminution de la concentration hépatique des lipides totaux et des triglycérides lorsque les vaches recevaient les deux vitamines ensemble. Il est à noter que les productions laitières des groupes témoin et vitamine B_{12} seule étaient plus basses que celles des groupes acide folique seul et les deux vitamines combinées. Cela renforce l'hypothèse que le métabolisme énergétique en début de la lactation est amélioré lorsque les vaches reçoivent un supplément combiné d'acide folique et de vitamine B_{12}. Cependant, Preynat et al. (2010) n'ont pas observé d'effet sur les concentrations hépatiques de lipides et de triglycérides lorsque les animaux recevaient un supplément d'acide folique seul ou combiné avec un supplément de vitamine B_{12}.

Dans l'étude de Preynat et al. (2009b), aucune différence significative n'a été notée dans les concentrations plasmatiques de BHBA et d'AGL pour les vaches recevant un supplément combiné d'acide folique et de vitamine B_{12} ou non autour de la parturition et en début de la lactation, et ce, malgré l'augmentation de la production laitière observée pour le groupe recevant le supplément combiné d'acide folique et de vitamine B_{12}.

Une diminution de la concentration plasmatique de BHBA a été notée dans les études de Gagnon (2012) et de Ghaemialehashemi (2013) pour les vaches multipares ayant reçu le supplément combiné d'acide folique et de vitamine B_{12} en période de transition et en début de la lactation. Tandis qu'il n'y a pas eu d'effet de traitement sur les AGL en début de la lactation dans l'étude de Gagnon (2012), les vaches ayant reçu le supplément combiné de vitamines ont présenté une concentration plasmatique d'AGL plus basse que les vaches témoins dans l'étude de Ghaemialehashemi (2013).

2.8.5 Impact des vitamines B_9 et B_{12} sur le glucose

Graulet et al. (2007) ont noté une augmentation de la concentration plasmatique de glucose lorsque les vaches ont reçu un supplément d'acide folique accompagné d'un supplément de vitamine B_{12} (Tableau 2.9).

Dans les études de Preynat et al. (2009b), de Gagnon (2012) et de Ghaemialehashemi (2013), contrairement à celle de Graulet et al. (2007), le supplément combiné de vitamines B_9 et B_{12} n'a pas permis d'augmenter la concentration de glucose plasmatique (Tableau 2.9). Cependant, grâce à la perfusion intraveineuse de glucose marqué avec des isotopes stables (D-[U^{13}-C]glucose), Preynat et al. (2009a) ont noté que le taux de perte irréversible (ILR pour *irreversible loss rate*) du glucose a eu tendance à augmenter de 160 g/j avec l'administration du supplément combiné d'acide folique et de vitamine B_{12} par rapport aux animaux témoins. À l'équilibre, l'ILR équivaut au taux d'apparition qui représente la somme du glucose disponible provenant soit de l'absorption portale, de la glycogénolyse ou de la néoglucogenèse (Preynat et al., 2009a). Les deux premiers éléments ont été écartés pour être la cause de cette augmentation. Ainsi, l'augmentation de l'ILR du glucose dans cette étude peut probablement s'expliquer par une augmentation de la néoglucogenèse. L'acide propionique étant le principal précurseur glucogénique chez les ruminants (Reynolds, 2006) et la vitamine B_{12}, un coenzyme lors de la transformation de l'acide propionique pour son entrée dans le cycle de Krebs, le supplément combiné d'acide folique et de vitamine B_{12} semble avoir amélioré l'efficacité d'utilisation de ce précurseur glucogénique en vue de la néoglucogenèse.

Tableau 2.9 Glucose plasmatique provenant de vaches ayant reçu un supplément d'acide folique et/ou de vitamine B_{12} lors de différentes études

	Glucose plasmatique (μM)				
	Traitements				
Études	B_9-B_{12}-	B_9+B_{12}-	B_9-B_{12}+	B_9+B_{12}+	SE[1]
Graulet et al., 2007[2]	3,37	3,15	3,35	3,49	0,07
Preynat et al., 2009a[3]	3,62	-	-	3,47	1,07
Preynat et al., 2009b[4]	3,66	3,63	-	3,60	0,05
Gagnon, 2012[5]	2,98	-	-	3,00	0,04
Ghaemialehashemi, 2013[5]	3,22	-	-	3,13	0,12

[1] SE = Erreur type de la moyenne.
[2] B_9-B_{12}- : pas de vitamines; B_9+B_{12}- : 2,6 g d'acide folique/j; B_9-B_{12}+ : 0,5 g de vitamine B_{12}/j; B_9+B_{12}+ : 2,6 g d'acide folique/j et 0,5 g de vitamine B_{12}/j.
[3] B_9-B_{12}- : pas de vitamines; B_9+B_{12}+ : 160 mg d'acide folique/semaine et 10 mg de vitamine B_{12}/semaine. Il s'agit des résultats présentés sans l'ajout de méthionine.
[4] B_9-B_{12}- : pas de vitamines; B_9+B_{12}- : 160 mg d'acide folique/semaine; B_9+B_{12}+ : 160 mg d'acide folique/semaine et 10 mg de vitamine B_{12}/semaine. Il s'agit des résultats présentés sans l'ajout de méthionine.
[5] B_9-B_{12}- : pas de vitamines; B_9+B_{12}+ : 320 mg d'acide folique/semaine et 10 mg de vitamine B_{12}/semaine.

2.8.6 Conclusion des études sur l'impact d'un supplément de vitamines B_9 et B_{12} sur le métabolisme énergétique

Selon Girard et Matte (2005a), Graulet et al. (2007), Preynat et al. (2009a,b) et Ghaemialehashemi (2013), un supplément combiné de vitamines B_9 et B_{12} semble améliorer le bilan énergétique en début de la lactation. En effet, ce supplément a permis d'augmenter significativement certaines composantes du lait sans augmenter la CVMS des vaches. De plus, il n'y a pas eu de différences significatives sur le poids corporel (Graulet et al., 2007; Preynat et al., 2009b), la concentration plasmatique d'AGL et de BHBA (Preynat et al., 2009b) et la condition d'état de chair des vaches suite aux traitements (Graulet et al., 2007). En plus de l'augmentation de la production laitière et de certains composants chez les vaches multipares ayant reçu le supplément combiné de vitamines dans l'étude de Ghaemialehashemi (2013), elles ont également perdu moins de poids et les concentrations plasmatiques d'AGL et de BHBA étaient plus basses que les vaches témoins. Cela suggère que le supplément a permis de diminuer la mobilisation des réserves corporelles en début de la lactation.

Selon Sacadura et al. (2008), l'augmentation de la production laitière observée chez les vaches laitières ayant reçu le supplément oral protégé de la dégradation ruminale de quatre vitamines du complexe B, incluant l'acide folique, sans augmentation de la CVMS reflète une amélioration de l'efficacité du métabolisme énergétique et protéique causée par le supplément.

Akins et al. (2013) n'ont pas observé une amélioration du métabolisme énergétique chez des vaches laitières ayant reçu hebdomadairement 0 ou 10 mg de vitamine B_{12} de 60 jours avant la date prévue du vêlage jusqu'à 150 JEL. En effet, la production laitière ainsi que les concentrations plasmatiques de glucose, d'AGL et de BHBA n'ont pas été différentes selon les traitements. Ces auteurs ont rapporté que la concentration plasmatique de folates était basse lors de cette étude et que cela a pu influencer les résultats (Akins et al., 2013).

2.9 Impact des vitamines B_9 et B_{12} sur la reproduction

2.9.1 Impact chez la vache laitière

Peu de recherches étudiant les performances reproductives des vaches laitières recevant un supplément combiné d'acide folique et de vitamine B_{12} ont été conduites. Comme il a été mentionné précédemment, les recherches précédentes semblent montrer que ce supplément, donné en période péripartum et au début de la lactation, améliore le bilan énergétique chez la vache laitière (Girard et Matte, 2005a; Graulet et al., 2007; Preynat et al., 2009a,b; Ghaemialehashemi, 2013). L'intensité et la durée du BEN ont un impact sur la reproduction en diminuant les fréquences de pulsation de la neurohormone gonadolibérine (mieux connue sous l'acronyme GnRH) et, par le fait même, de l'hormone lutéinisante (LH) qui est nécessaire à la croissance du follicule ovarien et à l'ovulation (Walsh et al., 2007). Effectivement, la LH est une glycoprotéine qui stimule la production d'œstrogènes et de progestérone chez les femelles et provoque l'ovulation (Campbell, 1995). Juchem et al. (2012) ont noté une augmentation du taux de conception de 4,8 % à 42 jours après la première saillie et cela s'est maintenu jusqu'à 150 JEL chez des vaches laitières multipares ayant reçu un supplément oral de vitamines du complexe B protégées contre la dégradation dans le rumen incluant l'acide folique et la vitamine B_{12}. Gagnon (2012) a rapporté une

augmentation de 25 % de la taille des follicules préovulatoires à 57 JEL chez des vaches multipares recevant un supplément combiné d'acide folique et de vitamine B_{12} contrairement aux vaches témoins et a conclu que cela pourrait se traduire par une ovulation plus hâtive. Une amélioration du bilan énergétique chez les vaches recevant le supplément explique possiblement cette observation (Gagnon, 2012). Le diamètre du follicule dominant chez les vaches multipares recevant le supplément combiné d'acide folique et de vitamine B_{12} était significativement plus large que chez les animaux témoins, et ce, de un à trois jours avant l'ovulation (Ghaemialehashemi, 2013). De plus, le supplément combiné a augmenté la concentration plasmatique d'œstradiol (Ghaemialehashemi, 2013).

Bien que les objectifs concernant la reproduction ne soient pas les mêmes dans les productions laitière, ovine et porcine, des études concernant l'impact des suppléments de vitamine B_9 et de vitamine B_{12} sur la reproduction chez la brebis et la truie seront présentées. En effet, pour une santé économique optimale de l'entreprise porcine ou ovine, chaque femelle doit produire le nombre maximum possible de petits viables par portée. En production laitière, cette condition n'est aucunement désirée; une vache ayant des jumeaux court plus de risques de souffrir de maladies métaboliques, voit sa production laitière et ses performances reproductives diminuées (Nielen et al., 1989) et peut donner naissance à une femelle stérile (free-martin) lorsque son jumeau est un mâle (Padula, 2005).

2.9.2 Impact chez la brebis

Dans l'étude de Méthot et al. (2008), le supplément de 210 mg d'acide folique/j donné aux brebis de trois semaines avant l'arrivée du bélier dans l'enclos jusqu'à 30 ou 32 jours de gestation n'a pas permis d'améliorer la fertilité, le nombre d'agneaux nés par portée, le taux de mortalité embryonnaire ni le taux d'ovulation des brebis. Ces résultats regroupent les données obtenues sur trois bergeries commerciales indépendamment de la saison où le supplément a été offert (saison ou contre-saison sexuelle) et du génotype des brebis (prolifique ou non prolifique). Lorsque les taux d'ovulation de la race non prolifique et prolifique ont été comparés en saison sexuelle incluant les résultats d'une bergerie seulement selon les traitements, il a été conclu que ceux-ci ont été plus élevés chez les

brebis de race prolifique recevant le supplément d'acide folique (1,8, 1,6, 1,9 et 2,3 ± 0,2 pour les brebis non-prolifiques recevant 0 ou 210 mg/j d'un supplément d'acide folique et pour les brebis prolifiques recevant 0 ou 210 mg/j d'un supplément d'acide folique, respectivement).

2.9.3 Impact chez la truie

Plusieurs projets ont été conduits afin d'étudier l'effet d'un supplément d'acide folique et, dans une moins grande mesure, l'effet d'un supplément de vitamine B_{12} sur les performances reproductives des truies.

Dans une étude où un supplément de vitamine B_{12} (0 ou 400 µg/j) a été donné dans l'alimentation des truies, le nombre de porcelets vivants par portée a été augmenté pour les animaux ayant reçu le supplément de vitamine (Frederick et Brisson, 1961). En effet, en incluant toutes les portées (8 dans chaque traitement), 101 et 81 porcelets sont nés des animaux recevant le supplément de vitamine B_{12} et des animaux témoins, respectivement.

Dans une étude de Matte et al. (1984), 209 truies ont été impliquées et réparties dans quatre traitements selon un dispositif factoriel 2 × 2; animaux témoins (sans vitamine) ou recevant un supplément de 15 mg d'acide folique nourris selon une ration répondant à leurs besoins et animaux témoins ou recevant un supplément de 15 mg d'acide folique nourris selon une ration servie à volonté (*flush*) du sevrage de la portée précédente jusqu'aux premiers signes de chaleur. Le meilleur résultat concernant le nombre de porcelets nés et vivants a été obtenu pour le groupe de truies ayant reçu le supplément d'acide folique nourri avec la ration *flush*. En effet, ce traitement a permis d'augmenter le nombre de porcelets nés vivants de 1,5 par portée comparativement aux animaux témoins recevant la ration répondant à leurs besoins. Le supplément d'acide folique a possiblement joué un rôle sur la taille de la portée en fournissant un milieu utérin approprié afin de diminuer la mortalité embryonnaire (Matte et al., 1984; 1996).

Lindemann et Kornegay (1989) ont alimenté des truies avec ou sans supplément d'acide folique (0 ou 1 ppm) pendant trois gestations consécutives. Le supplément a permis d'augmenter le nombre total de porcelets nés et vivants. De plus, cette performance a

augmenté numériquement au cours des parités. En effet, pour les animaux recevant le supplément, le nombre de porcelets nés par portée a augmenté de 0,19 et de 1,96 pour les truies primipares et lors de la troisième mise-bas, respectivement, par rapport aux animaux témoins.

Tremblay et al. (1989) ont assigné 162 truies multipares selon un dispositif factoriel 2 × 3; soit deux niveaux d'un supplément d'acide folique servi dans l'alimentation (0 ou 5 mg/kg de ration) et trois traitements pour stimuler l'ovulation (une ration témoin, une ration *flush* ou une injection de gonadotrophine provenant du sérum d'une jument gestante). Le supplément d'acide folique a augmenté le taux de survie des fœtus de 7,1 % et diminué le nombre de fœtus morts de 3,2 par portée à 30 jours de gestation.

Lindemann (1993) a effectué une revue de littérature portant sur l'effet d'un supplément d'acide folique sur les performances reproductives des truies. Il a présenté plusieurs études dans lesquelles un supplément d'acide folique a permis d'augmenter, pas toujours significativement par contre, la taille de la portée chez la truie lorsque celui-ci était offert en début de gestation. L'auteur affirme que les truies qui ont un taux d'ovulation plus élevé, qui sont plus âgées et qui reçoivent un « *flushing* » alimentaire trois semaines avant l'ovulation bénéficient davantage de ce type de supplément. En d'autres termes, il s'agit des animaux qui risquent d'être plus productifs au sein du troupeau. Matte et al. (1993) ont également conclu qu'un supplément d'acide folique est davantage bénéfique aux truies multipares, car aucun effet de traitement n'a été observé suite à une supplémentation de vitamine B_9 chez les cochettes.

Harper et al. (1994; 1996) ont obtenu des résultats contradictoires à ceux de Matte et al. (1984) et aux résultats présentés dans la revue de littérature de Lindemann (1993). Ils n'ont rapporté aucune augmentation du nombre de porcelets nés par portée suite à une supplémentation d'acide folique donnée aux truies multipares 21 jours avant la mise à l'accouplement ainsi que tout au long des trois ou quatre gestations suivantes. Néanmoins, le pourcentage de fœtus vivants à 42 jours de la gestation était numériquement plus élevé lors de l'ajout du supplément par rapport aux animaux témoins (Harper et al., 1996).

Dans une étude où 32 truies multipares ont été utilisées, le supplément d'acide folique (0 ou 15 mg/kg) a triplé la quantité utérine de prostaglandine E_2 comparativement aux animaux témoins (Matte et al., 1996). Le rôle de cette hormone sur le développement embryonnaire est mal connu, mais pourrait agir sur la survie embryonnaire en réduisant la réponse immunitaire chez la truie lors de la conception.

Selon Matte et al. (2006), un supplément avec un ratio optimal vitamines B_9 : B_{12} tenant compte du nombre de parturitions des truies serait bénéfique afin de profiter pleinement des impacts positifs de l'acide folique sur les performances reproductives des truies. Connaissant l'étroite relation entre l'acide folique et la vitamine B_{12} chez les mammifères, les auteurs ont émis l'hypothèse qu'une carence en vitamine B_{12} puisse être à l'origine de la variabilité entre les études sur l'effet d'un supplément d'acide folique sur les performances reproductives. Cette condition peut mener à une augmentation de la quantité d'homocystéine dans l'endomètre et causer, entre autres, un développement anormal des embryons.

2.9.4 Impact chez les autres espèces

Chez le rat, une injection de 1 mg d'acide folique par jour durant la gestation a permis d'augmenter significativement le poids, le contenu en protéines, en ADN et en ARN des fœtus et des placentas comparativement aux témoins (Morgan et Winick, 1978).

Une étude a été conduite par Habibzadeh et al. (1986) auprès de femelles cochons d'Inde dans le but de découvrir l'effet d'un supplément d'acide folique donné dans l'alimentation sur les performances reproductives. Le supplément de vitamine B_9 a permis de diminuer la mortalité fœtale et ainsi d'augmenter le nombre de fœtus vivants à 37 jours de gestation.

Chez les femmes enceintes, un bas taux d'acide folique sérique augmente les chances de donner naissance avant terme (moins de 37 semaines de grossesse) et d'accoucher d'un enfant ayant un petit poids à la naissance (moins de 2500 g) (Scholl et al., 1996). Une revue de littérature par Laanpere et al. (2010) mentionne qu'une carence en acide folique chez la femme peut interférer avec la maturation de l'ovocyte et du follicule et de la croissance et du développement embryonnaire. De plus, il a été montré qu'un supplément d'acide folique

prévient les anomalies de fermeture du tube neural pendant la période entourant la conception (Forges et al., 2008; Laanpere et al., 2010).

2.9.5 Homocystéine

Une carence en acide folique ou en vitamine B_{12} augmente la concentration plasmatique de l'homocystéine en diminuant sa reméthylation en méthionine (Selhub et al., 2007). Guay et al. (2002) ont observé une diminution non significative de 10 % d'homocystéine dans le plasma de truies nullipares Yorkshire-Landrace et de truies multipares Landrace suite à une alimentation supplémentée de 15 mg d'acide folique. Le contenu utérin présentait également une diminution de l'homocystéine lors de l'ajout du supplément vitaminique dans la ration. Une observation semblable, significative cette fois, a été faite chez des femmes recevant un supplément d'acide folique (Szymanski et Kazdepka-Zieminska, 2003 cités par Forges et al., 2007). L'homocystéine est reconnue pour avoir des effets négatifs sur la fertilité des femmes (Tamura et Picciano, 2006; Forges et al., 2008). Par exemple, une forte concentration d'homocystéine dans le sang augmente les risques d'avortement au début de la grossesse (Forges et al., 2007; Laanpere et al., 2010). De plus, une forte concentration d'homocystéine dans le fluide folliculaire a des impacts négatifs sur la qualité des ovocytes, sur le taux de fertilisation ainsi que sur la qualité des embryons (Berker et al., 2009).

Dans l'étude de Graulet et al. (2007), un supplément de vitamine B_{12} donné en période péripartum a augmenté la concentration plasmatique d'homocystéine tandis qu'un supplément combiné d'acide folique et de vitamine B_{12} a diminué la concentration plasmatique d'homocystéine chez des vaches multipares (Preynat et al., 2009b).

2.10 Isotopes stables

Dans le cadre du deuxième projet réalisé pour la présente thèse de recherche, des isotopes stables ont été utilisés afin d'étudier le métabolisme du glucose chez la vache laitière. La prochaine section présente quelques principes de base derrière l'utilisation des isotopes stables pour l'étude du métabolisme.

2.10.1 Qu'est-ce qu'un isotope stable?

Un atome possède un noyau comprenant des protons et des neutrons. Pour les éléments plus légers, dont l'abondance naturelle est élevée, comme le carbone et l'hydrogène, le noyau contient environ autant de protons que de neutrons, mais c'est le nombre de protons qui définit l'élément. La somme de la masse des protons et des neutrons est appelée la masse atomique. Un isotope stable est un atome qui se différencie par le nombre de neutrons dans son noyau et possède ainsi une masse atomique différente de l'élément dit de base (Wolfe et Chinkes, 2005). Cependant, cela n'altère pas ses fonctions chimiques. Par exemple, la masse atomique du ^{12}C est 12 (6 protons et 6 neutrons) et celle du ^{13}C est 13 (6 protons et 7 neutrons). Le ^{13}C est un isotope stable. Le terme stable est utilisé, car cet isotope ne se désintègre pas en émettant un rayonnement, comme le ^{14}C, par exemple. L'utilisation des isotopes stables pour étudier le métabolisme des êtres vivants a augmenté de façon considérable au cours des dernières années, car cette méthode est précise et sécuritaire en permettant de marquer des nutriments sans utiliser la radioactivité (Coggan, 1999).

2.10.2 Terminologie utilisée pour désigner les isotopes stables

Dans le cadre des travaux de recherche qui seront présentés dans cette thèse, deux molécules marquées avec des isotopes stables seront utilisées : il s'agit du [6,6-$^{2}H_2$]-glucose et du [1-$^{13}C_1$]-sodium propionate. Les structures chimiques de ces molécules marquées sont illustrées à la Figure 2.8.

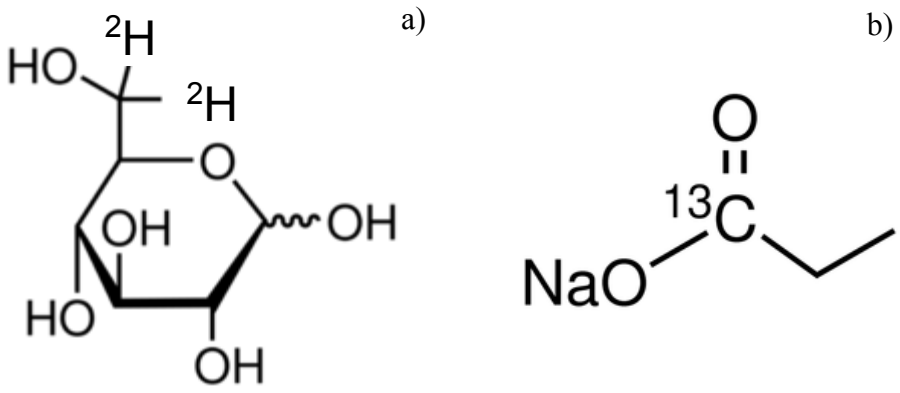


Figure 2.8 Structures chimiques du D-[6,6-$^{2}H_{2}$]-glucose (a) et du [1-$^{13}C_{1}$]-sodium propionate (b) (Adapté de Sigma-Aldrich, 2013)

Par exemple, en ce qui concerne la molécule D-[6,6-$^{2}H_{2}$]-glucose, les positions des atomes marqués dans la molécule sont indiquées en considérant les numéros de carbone. Pour la molécule de glucose en exemple, les deux atomes marqués sont positionnés au 6^{e} carbone, d'où le 6,6 entre les crochets. Ensuite, le 2 en exposant à gauche du H indique la masse atomique de l'atome d'hydrogène et le 2 en indice à droite du H signifie que deux atomes d'hydrogène sont marqués.

2.10.3 Hypothèses de départ pour utiliser des isotopes stables

Selon Wolfe et Chinkes (2005), quelques hypothèses doivent être respectées lors de l'étude du métabolisme du glucose à l'aide d'isotopes stables :

1. Le glucose nouvellement formé est relâché directement dans le plasma.

2. Il n'y a pas de discrimination par l'organisme entre la molécule marquée perfusée et la molécule naturellement présente. La molécule marquée est utilisée de la même manière, dans les mêmes réactions.

3. La perfusion de la molécule marquée n'a pas d'impact sur le métabolisme endogène du glucose.

4. Les molécules marquées ne se recyclent pas pendant la période de perfusion/prélèvement. Par exemple, les molécules marquées ne doivent pas être

prises par un tissu, être métabolisées et relâchées dans le sang sous leur forme marquée initiale.

2.10.4 Principes des perfusions d'isotopes stables

La Figure 2.9 représente un pool dans lequel il y a perfusion d'une molécule marquée d'isotopes stables à l'état d'équilibre, c'est-à-dire que la molécule marquée disparait du pool au même taux qu'elle apparait dans ledit pool (i.e. le taux de perfusion) (Wolfe et Chinkes, 2005). Au début de la perfusion, la molécule marquée quitte le pool moins vite que le taux de perfusion ce qui entraine une hausse de la proportion de la molécule marquée dans le pool jusqu'à l'atteinte de l'équilibre : cette proportion se nomme enrichissement isotopique. C'est pour cette raison qu'il faut respecter une période d'attente pour l'atteinte de l'équilibre entre le début de la perfusion et le prélèvement d'échantillons sanguins.

Suite à l'atteinte de l'équilibre dans le pool perfusé et suivant l'hypothèse que l'organisme ne fait pas de distinction entre une molécule marquée ou non, il est possible de calculer l'ILR à l'aide de la formule suivante : ILR = (taux de perfusion/enrichissement isotopique) – taux de perfusion (Wolfe et Chinkes, 2005; Preynat et al., 2009a).

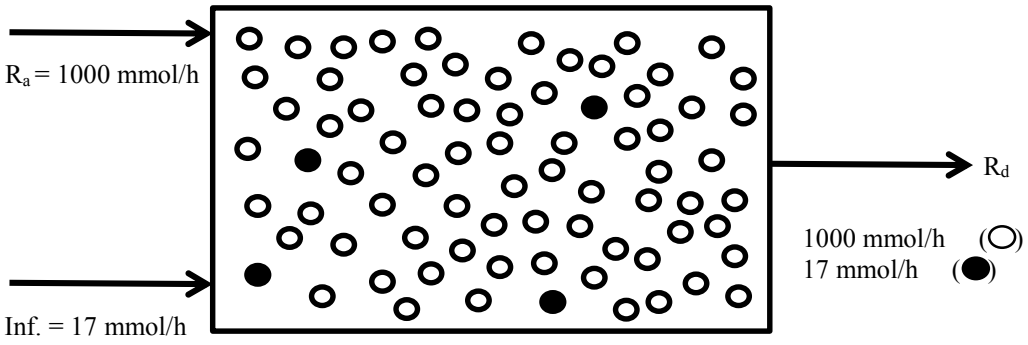


Figure 2.9 Représentation schématique d'un état d'équilibre d'un pool lors de perfusion d'isotopes stables (R_a = taux d'apparition; R_d = taux de disparition; Inf. = taux de perfusion) (Adapté de Wolfe et Chinkes, 2005)

2.11 Hypothèses et objectifs

Cette revue de littérature a mis en évidence que les performances zootechniques des vaches laitières en début de la lactation pouvaient être optimisées par un supplément combiné d'acide folique et de vitamine B_{12}. De plus, les études précédentes suggèrent que ce supplément améliore le métabolisme énergétique des animaux, probablement en permettant à davantage d'acide propionique d'entrer dans le cycle de Krebs pour contribuer à la néoglucogenèse. Afin d'étudier si l'amélioration du métabolisme énergétique suite à cette supplémentation a un impact sur la reproduction et les maladies métaboliques, un grand nombre d'animaux est nécessaire pour augmenter la puissance statistique.

Deux projets distincts ont été effectués pour mener à bien cette thèse. Un premier projet a été fait sur des fermes commerciales situées près de la ville de Québec et le deuxième a été réalisé à la ferme expérimentale du Centre de recherche et de développement sur le bovin laitier et le porc de Sherbrooke. Le premier projet réalisé dans le cadre de cette thèse permettait d'inclure un grand nombre de vaches et d'étudier si l'adoption de ce supplément sur les fermes laitières québécoises est bénéfique. Le deuxième projet permettait de conclure si l'amélioration du métabolisme énergétique observée dans les autres études suite au supplément combiné était causée par une augmentation de la synthèse de glucose à partir de l'acide propionique. Les hypothèses et objectifs des deux projets sont présentés séparément.

2.11.1 Premier projet

2.11.1.1 Hypothèses

Un supplément d'acide folique et de vitamine B_{12} a un impact positif sur le métabolisme énergétique et la reprise de l'activité ovarienne après le vêlage et améliore ainsi les performances reproductives, augmente la production laitière et ses composantes et réduit l'incidence des maladies dans un troupeau laitier. Ainsi, ce type de régie améliore la rentabilité des entreprises agricoles québécoises. De plus, ce supplément améliore la valeur nutritive du lait en augmentant sa concentration en vitamine B_{12}.

2.11.1.2 Objectifs

Les objectifs de ce projet étaient de mesurer les effets d'injections d'un supplément combiné d'acide folique et de vitamine B_{12} données de trois semaines avant la date prévue du vêlage jusqu'à huit semaines postpartum sur la reproduction, la production laitière, l'incidence des maladies ainsi que sur la concentration du lait en vitamine B_{12} dans les troupeaux laitiers québécois. Ce projet a aussi permis d'évaluer la rentabilité de l'utilisation de ce supplément autour du vêlage et en début de la lactation dans les troupeaux laitiers québécois.

2.11.2 Deuxième projet

2.11.2.1 Hypothèses

Un supplément d'acide folique et de vitamine B_{12} permet d'améliorer le métabolisme du glucose en début de lactation en augmentant l'efficacité de l'utilisation de l'acide propionique pour la synthèse du glucose. Cette étude permet de déterminer si un supplément d'acide folique est nécessaire pour l'expression de l'effet du supplément de vitamine B_{12} sur cette voie métabolique.

2.11.2.2 Objectif

L'objectif de ce projet était de mesurer les effets d'injections de suppléments d'acide folique et de vitamine B_{12}, seuls ou combinés, données de trois semaines avant la date prévue du vêlage jusqu'à neuf semaines postpartum sur l'efficacité du métabolisme du glucose en début de la lactation.

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3 Article sur la reproduction et les maladies – Premier projet

Effets d'une supplémentation en acide folique et en vitamine B_{12} sur le taux de réforme, les maladies et la reproduction dans les troupeaux laitiers

Effects of folic acid and vitamin B_{12} supplementation on culling rate, diseases, and reproduction in commercial dairy herds

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Cet article est publié dans le *Journal of Dairy Science*.

Référence : Duplessis, M., Girard, C. L., Santschi, D. E., Laforest, J.-P., Durocher, J. et 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.

3.1 Résumé

Cette étude a été réalisée pour déterminer l'impact d'un supplément combiné d'acide folique et de vitamine B_{12} sur le taux de réforme, les maladies métaboliques et autres maladies et la reproduction en début de la lactation sur 805 vaches dans 15 troupeaux. Les vaches étaient assignées à des injections intramusculaires hebdomadaires de soit 5 ml de 1) saline 0,9 % NaCl (témoin) ou 2) 320 mg d'acide folique et 10 mg de vitamine B_{12} (vitamines) de 3 semaines avant la date prévue du vêlage jusqu'à 8 semaines suivant la parturition. Le taux de réforme n'a pas été influencé par le traitement et a été de 27,5 %. Le supplément n'a pas diminué les incidences d'acétonémie, de rétention placentaire, de déplacement de caillette, de fièvre du lait, de métrite et de mammite. Cependant, l'incidence de dystocie a diminué de 50 % chez les vaches multipares recevant le supplément, mais aucun effet n'a été noté pour les vaches primipares. La première saillie suivant la parturition a eu lieu 3,8 jours plus tôt chez les vaches multipares recevant le supplément comparativement aux vaches multipares témoins; aucun effet n'a été observé chez les vaches primipares. Les jours ouverts, les taux de conception à la première et à la deuxième saillie, le nombre de saillies/conception ainsi que le pourcentage d'animaux gestants à 150 JEL n'ont pas été affectés par le traitement. La réduction de l'incidence des dystocies combinée à la réduction des jours en lait à la première saillie chez les vaches multipares recevant le supplément combiné d'acide folique et de vitamine B_{12} ont suggéré que le supplément a eu des effets positifs sur les vaches plus âgées.

Mots clés : vache laitière, acide folique, vitamine B_{12}, troupeaux commerciaux

3.2 Abstract

This study was undertaken to determine the impact of a combined folic acid and vitamin B_{12} 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 months from February to December 2010 and within each herd, cows were assigned according to parity, previous 305-day milk production, and calving interval to either 5 mL of 1) saline 0.9% NaCl (Control) or 2) 320 mg of folic acid + 10 mg of vitamin B_{12} (Vitamins). Treatments were administered weekly by intramuscular injections starting 3 weeks before the expected calving date until 8 weeks 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 primiparous cows (18.8%). Within the first 60 days 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 at or above 100 μmol/L of β-hydroxybutyrate in milk was 38.3 ± 2.9% for the vitamin group and 41.8 ± 3.0% for the control group, and was not affected by treatment. The combined supplement of folic acid and vitamin B_{12} did not decrease incidence of retained placenta, displaced abomasum, milk fever, metritis, and 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 days earlier with the vitamin supplement as compared to controls whereas no treatment effect was seen for primiparous cows. Days open, first and second-breeding conception rates, number of breedings per conception as well as 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 B_{12} suggested that the vitamin supplement had a positive effect on older cows.

Key words: dairy cow, folic acid, vitamin B_{12}, commercial herds

3.3 Introduction

It is well known that ruminal bacteria can synthetize B vitamins including folic acid and vitamin B_{12} (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 B_{12} as being 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 B_{12} 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 B_{12} increased milk production by approximately 12% when administered to multiparous cows 3 weeks before the expected calving date until 16 weeks of lactation (Preynat et al., 2009a).

Methylmalonyl-CoA mutase is a vitamin B_{12} dependent enzyme which 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 B_{12} 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 B_{12} as compared to folic acid alone. Furthermore, a combined supplement of folic acid and vitamin B_{12} given to dairy cows during the transition period increased the mRNA abundance of methylmalonyl-CoA mutase in liver (Preynat et al., 2010). These results suggested that a combined supplement of folic acid and vitamin B_{12} 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 of plasma concentration of glucose and whole-body irreversible loss rate of glucose, respectively, in response to the supplement of folic acid and vitamin B_{12} to dairy cows. An increased blood concentration of glucose leaded to a reduced blood BHBA concentration and reduced the risk of ketosis in dairy cows (Nielsen and Ingvartsen, 2004). Ketotic cows had lower blood glucose concentration than non-ketotic cows (Tehrani-Sharif et al., 2012).

Furthermore, cows with subclinical ketosis are thereafter more likely to develop metritis, clinical ketosis, and displaced abomasum (Suthar et al., 2013) and more at risk to be culled (Roberts et al., 2012). Previous findings showed that, 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 impact of a supplementation in folic acid and vitamin B_{12} on reproduction in commercial dairy herds. However, Juchem et al. (2012) reported a significant improvement of conception rate 42 days 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 previously reported that folic acid supplements increased 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 B_{12} on energy balance and reproduction previously reported in other studies described above, it was hypothesized that a combined folic acid and vitamin B_{12} supplement would reduce culling rate and incidence of metabolic disorders and other diseases. Hence, it would be translated into better reproductive performance. The purpose of this study was therefore to determine the effects of a supplement of folic acid and vitamin B_{12} given from 3 weeks prepartum until 8 weeks of lactation on culling rate, incidence of metabolic disorders and diseases, and reproduction performance of cows in 15 commercial dairy herds.

3.4 Materials and Methods

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

3.4.1 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 Québec City, QC, Canada were enrolled. Herd size ranged from 25 to 120 cows. All lactating cows were kept in tie-stall barns and were milked twice daily. Average 305-day milk yield for the lactation preceding the study for multiparous cows was 9,662 ± 114 kg and average calving interval was 393 ± 3 days, with no difference between treatments (P = 0.97 and 0.40, respectively). In 2010, on average, a dairy herd in Québec had 57 cows producing 8,800 kg of milk during a 305-day lactation (Valacta, 2011). Therefore, dairy herds participating in this experiment corresponded to average dairy herds in Québec except that cows produced slightly more milk. All cows from each herd were included in the project, except those with an estimated calving interval greater than 500 days. 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 trans-rectal palpation or ultrasonography, and evaluation and treatment of any sick cows. Timed artificial insemination was not used routinely.

Herds were visited from February 2010 to April 2011 every other week on the same schedule. Every 2 months and within each herd, cows were randomly assigned to treatments, based on parity (primiparous vs. multiparous), predicted 305-day milk yield, and calving interval. Treatments consisted of weekly intramuscular injections of either 5 mL of 1) saline 0.9% NaCl (Control) or 2) 320 mg of folic acid + 10 mg of vitamin B_{12} (Vitamins; pteroylmonoglutamic acid, MP Biomedicals, Solon, OH and cyanocobalamin, 5,000 µg/mL, Vétoquinol, Lavaltrie, QC, Canada) and were given from 3 weeks before the expected calving date until 8 weeks after parturition. On the weeks between visits, dairy producers were asked to inject treatments. Disposable syringes containing the studied solutions were prepared every 2 weeks and were kept refrigerated in a box to protect them from light until use.

About 3 weeks before calving, body weight (BW) was 667.0 and 673.7 ± 4.3 kg, and body condition score (BCS) was 3.45 and 3.47 ± 0.04 for dairy cows receiving the vitamin

supplement and control cows, respectively ($P > 0.27$). During the first 60 days in milk (DIM), milk yield was on average 35.0 ± 0.3 kg/d and no treatment effect was observed ($P = 0.68$).

3.4.2 Data Collection

Reasons for culling with corresponding DIM when cows left the herd were obtained from producers and Valacta (Dairy Production Center of Expertise, Québec and Atlantic Provinces, Ste-Anne-de-Bellevue, QC, 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 removed from the herd because they did not meet the dairy producer standards anymore, 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 non-favourable 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 non-favourable 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, a drop of milk from one quarter was placed on the strip for 3 seconds and reading was done after a waiting period of 60 seconds. If BHBA concentration was below 100 µmol/L, cows were declared non-ketotic; if BHBA concentration was at or over 100 and less than 200 µmol/L, cows were considered mild ketotic; and BHBA concentration at

or greater than 200 μmol/L indicated severe ketosis. If a cow was tested on 2 consecutive visits, the highest result was kept for analysis.

Definition of metabolic disorders and other diseases was standardized within herds as previously described by Santschi et al. (2011). Briefly, occurrence of retained placenta, metritis and endometritis, mastitis, milk fever, and displaced abomasum was recorded during the visit following each calving. A retained placenta was described as a failure to expel foetal membranes partly or completely within 24 hours 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) or mild (feeble cow whose symptoms were improved after calcium or other mineral doses) and severe (cow cannot get up). In the present 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 considered as number of days open, and number of breedings per conception were obtained from Valacta. 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 non-culled cows. In some herds, to increase chance of pregnancy per oestrus, cows were bred 2 times within 3 days. 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.

3.4.3 Statistical Analysis

Culling rate was analysed with the GLIMMIX procedure of SAS (version 9.2, SAS Institute, 2008, Cary, NC) using treatment, parity, block, herd, as well as treatment × parity interaction as fixed effects. Parity refers to primiparous and multiparous cows after calving

and block, to 2-month assignations. Logit transformation after conversion to binomial distribution was performed on these data. Proc FREQ was used in order to compute proportions of cows among culling reasons. A chi-square test was performed for detecting any difference between treatments.

Incidence of metabolic disorders and other diseases, dystocia incidence, twin birth, and calf size at birth were analysed with the GLIMMIX procedure of SAS using the same model as described above. Incidence of ketosis was transformed into a binomial distribution to facilitate the analysis. In a first analysis, cows were considered non-ketotic when BHBA level was below 100 µmol/L and ketotic when BHBA concentration was at or above 100 µmol/L. In a second analysis, severe ketotic cows had a BHBA level at or above 200 µmol/L compared to all others with a BHBA level below 200 µmol/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 as well as breedings/conception were compared with the MIXED procedure of SAS. Treatment, parity, block, herd, and the interaction treatment × 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 were computed using the LIFETEST procedure of SAS.

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 $0.05 < P \leq 0.10$.

3.5 Results and Discussion

3.5.1 Culling Rate

Culling rate was on average 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 3.1 and no treatment effect was observed on proportion of cows among culling reasons ($P = 0.48$). The primary culling reason was reproduction (17.0%), followed by mastitis/high somatic cell count (16.6%). As expected, significantly more multiparous cows were culled than primiparous cows ($P < 0.0001$). Culling rates were 18.8% and 32.2% for primiparous and multiparous cows, respectively. During the first 60 DIM, 21.3% of total culling occurred (47 culled cows) and was not affected by treatment ($P = 0.55$). Primary culling reasons for 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 3.1).

Table 3.1 Proportion and number of culled cows during the entire studied lactation and within the first 60 days in milk

	Proportion, % (n)	
Item	Entire lactation[1]	First 60 days in milk[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/high SCC[3]	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/unknown	13.6 (30)	12.8 (6)

[1] Proportion based on 221 culled cows. Culling data are from calving to the beginning of the next dry period.
[2] Proportion based on 47 culled cows.
[3] SCC = somatic cell count.

In 2010, the average culling rate from dairy herds subscribing to dairy herd improvement (DHI) in Québec was 36.1% (percentile 10 = 50.8% and percentile 90 = 22.5%) according

to Valacta (2011). The lower culling rate obtained in this trial could be explained by cows with previous calving interval greater than 500 days not being enrolled. It could be hypothesized 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 culling reason pattern was similar between this study and Valacta data for 2010 (Valacta, 2011).

3.5.2 Metabolic Disorders and Other Diseases

Based on the threshold at or above 100 μmol/L BHBA from Keto-Test results, ketosis incidence was 38.3 ± 2.9% and 41.8 ± 3.0% for vitamin and control cows, respectively (P = 0.37; Table 3.2). Incidence of severe ketosis defined by Keto-Test results at or above 200 μmol/L BHBA was about 12.8 ± 1.9% and did not differ between treatments either (P = 0.91). Ketosis incidences in the current study were slightly higher than previously reported in commercial dairy herds in Québec using the same method and the same cut-off points as in the current study (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 forage quality harvested during the summer 2009 due to 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 B_{12} supplement around parturition as compared to control cows (Preynat et al., 2009a,b). Furthermore, plasma BHBA concentrations of dairy cows receiving weekly injections of vitamin B_{12} alone did not differ from control cows (Akins et al., 2013). However, a vitamin B_{12} supplement increased plasma glucose concentration of dairy cows fed folic acid during the transition period but had no effect in cows not fed folic acid supplement (Graulet et al., 2007). Moreover, a combined supplement of folic acid and vitamin B_{12} increased glucose irreversible loss rate by 160 g/d suggesting an 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 B_{12} would reduce ketosis. Nonetheless, results from the current study do not support this hypothesis.

Surprisingly, primiparous cows had a higher incidence of severe ketosis than multiparous cows with 17.4 ± 3.0% versus 10.3 ± 1.8%, respectively ($P = 0.005$; Table 3.2). This is not in accordance with van der Drift et al. (2012) and McArt et al. (2013) reporting that cows in first and second lactations had a lower prevalence of hyperketonemia determined by a plasma BHBA concentration ≥ 1,200 μmol/L.

There was 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 3.2). Experiments studying effects of a supplement of folic acid and vitamin B_{12} on metabolic and other diseases are lacking. However, a study in which vitamin B_{12} injections were given weekly to dairy cows reported that metabolic and uterine disorders recorded were unlikely related to the treatment (Akins et al., 2013). Previous studies concluded that a supplement of folic acid and vitamin B_{12} 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 risks of developing metabolic disorders in early lactating cows (LeBlanc, 2010b; Ingvartsen and Moyes, 2013). In the current study, 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.

Table 3.2 Incidences of diseases and dystocia according to treatments and parity (adjusted means ± standard error)

	Primiparous		Multiparous		*P*-value		
	Treatments[1] (Trt)						
Item	Control	Vitamins	Control	Vitamins	Trt	Parity	Trt × parity
Number 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

[1] 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}.
[2] Non-ketotic cow if BHBA concentration less than 100 µmol/L and mild or severe ketotic cow if over 100 µmol/L.
[3] Severe ketosis if BHBA concentration greater than 200 µmol/L.
[4] Incidence of milk fever for primiparous was less than 0.01% (data not shown) and was not affected by treatment ($P = 1.0$).
[5] No treatment effect for primiparous cows ($P = 0.16$) but the vitamin supplement reduced dystocia in multiparous cows as compared to controls ($P = 0.02$).

3.5.3 Calf Size and Dystocia Incidence

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 multiparous cows, 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 ± 1.8% and 11.8 ± 1.9% for vitamin and control groups, respectively, and was not affected by treatment ($P = 0.53$). However, a significant treatment × parity interaction was observed ($P = 0.008$; Table 3.2). For multiparous cows, supplementation in folic acid and vitamin B_{12} decreased incidence of dystocia by 50% ($P = 0.02$), from 10.8 ± 2.0% to 5.3 ± 1.4% but it had no effect on primiparous cows ($P = 0.16$). The lower incidence of difficult calving with the vitamin supplement in multiparous cows remains difficult to explain. It is unlikely related to genetic or non-genetic (such as nutrition, sex of calf) factors known to have an impact on dystocia (Meijering, 1984) because cows were equally distributed within treatments and herds. Moreover, no significant difference was observed on 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 to 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 ± 2.5% and 7.6 ± 1.3%, respectively. Dystocia incidence for multiparous cows is higher than previously reported by Santschi et al. (2011) even if the same definition of dystocia was used.

Table 3.3 Reproductive parameters according to treatments and parity (adjusted means ± standard error)

	Primiparous		Multiparous		*P*-value		
	Treatments[1] (Trt)						
Item	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/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

[1] 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}.
[2] No treatment effect for primiparous cows ($P = 0.44$) but the vitamin supplement decreased DIM at first breeding for multiparous cows as compared to controls ($P = 0.05$).
[3] CR = conception rate.
[4] Percentage of cows confirmed pregnant after the second service excluding cows confirmed pregnant after the first service.

3.5.4 Reproduction

Days in milk at first breeding in response to treatments tended to differ according to parity (interaction treatment × parity, $P = 0.07$; Table 3.3). 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 days, for multiparous cows receiving the supplementation in folic acid and vitamin B_{12} ($P = 0.05$). Two hypotheses could be drawn from this observation. Firstly, in a study by Eaglen et al. (2011), first breeding was significantly delayed in primiparous cows that had a difficult calving compared to primiparous cows that had an easy calving. According to these 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 × calving difficulty, $P = 0.77$). Secondly, vitamin B_{12} 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 dry matter intake (DMI), nonesterified fatty acids (NEFA) or BHBA. Furthermore, cows receiving a combined folic acid and vitamin B_{12} supplement had reduced BW and BCS losses after calving combined with lower milk fat and higher milk protein contents, suggesting an 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 possibly be explained by the supplement lessening NEB in early lactation.

Despite the effects of a combined supplement of folic acid and vitamin B_{12} on energy metabolism previously reported (Girard and Matte, 2005; Graulet et al., 2007; 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/conception, and percentage of cows pregnant at 150 DIM (Table 3.3). A higher percentage of primiparous cows were pregnant at 150 DIM as compared to multiparous cows ($P = 0.03$); 76.9 ± 3.2% of primiparous cows were pregnant at 150 DIM compared to 67.7 ± 2.8% for multiparous cows. Reproductive data for multiparous cows obtained in this study are 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 pre-conception and gestational periods could 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 as compared to prolific control ewes and 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 days of gestation (Tremblay et al., 1989). Research pertaining to the effects of folic acid and vitamin B_{12} supplement in dairy cows on reproduction is scarce. Nevertheless, in a study in which multiparous dairy cows were fed a B-vitamin supplement including folic acid and vitamin B_{12} and protected from ruminal degradation in early lactation, first service CR at 42 days after artificial insemination and at 150 DIM were significantly higher for the vitamin group as compared to the control group (Juchem et al., 2012). Results from a study in which a supplement of folic acid and vitamin B_{12} was administered 3 weeks before the expected calving date until 9 weeks after parturition suggested that this combined supplement increased the expression of genes related to ovulation allowing dairy cows to have a faster follicular growth which can lead to an earlier ovulation (Gagnon, 2012).

The percentage of pregnant cows according to parity on a given DIM is represented by survival curves (Figure 3.1a,b). According to LeBlanc (2010a), this is the most accurate

method to analyse pregnancy data as culled cows are also included. In the current study, no treatment effect was observed on the percentage of pregnant cows with regard to parity ($P \geq 0.35$).

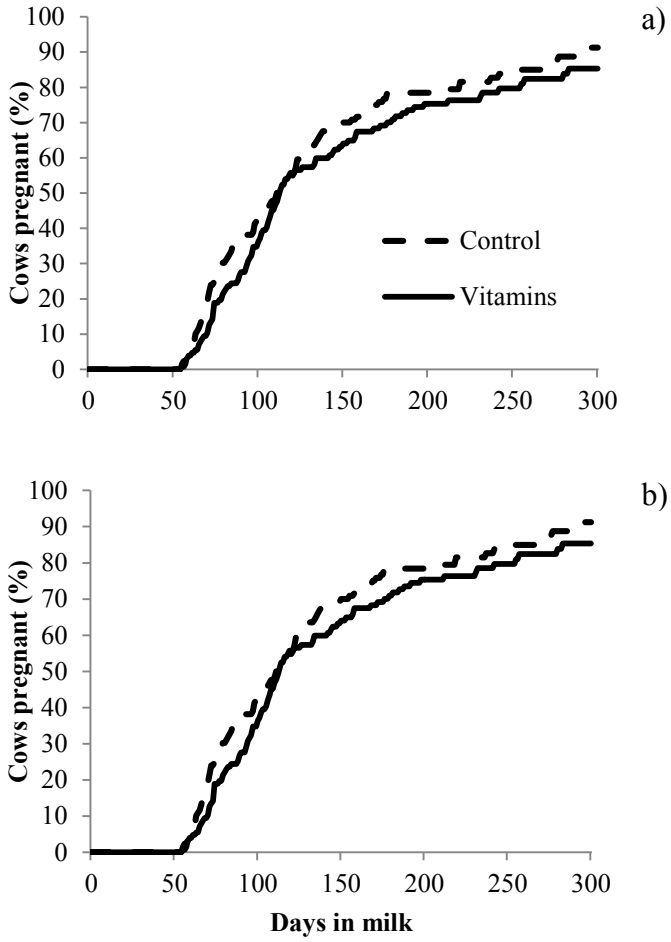

Figure 3.1 Survival curves for days to pregnancy for primiparous cows (a; *P* = 0.35) and multiparous cows (b; *P* = 0.61) according to treatments. Control = 5 mL of saline 0.9%; Vitamins = 3 mL of 320 mg of folic acid and 2 mL of 10 mg of vitamin B_{12}

3.6 Conclusion

In summary, a combined supplement of folic acid and vitamin B_{12} did not decrease culling rate and 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 multiparous control cows. Although no treatment

effect was observed for the majority of reproductive parameters such as first-breeding CR, breedings/conception, the first breeding postpartum for multiparous cows occurred 3.8 days earlier with the vitamin supplement and was not related to difficulty at calving.

3.7 Acknowledgements

The authors are grateful to dairy producers, veterinarians, and nutritionists who actively participated to this study. We acknowledge the help of 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}. We also thank Steve Méthot (Agriculture et Agroalimentaire Canada, Sherbrooke, QC, Canada) for his statistical advices. 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.

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4 Article sur la performance laitière – Premier projet

Production et composition du lait et mesures corporelles de vaches laitières recevant des injections intramusculaires d'acide folique et de vitamine B_{12} dans des troupeaux laitiers commerciaux

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

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Cet article est publié dans le *Livestock Science*.

Référence : Duplessis, M., Girard, C. L., Santschi, D. E., Lefebvre, D. M. et Pellerin, D. 2014. Milk production and composition, and body measurements of dairy cows receiving intramuscular injections of folic acid and vitamin B_{12} in commercial dairy herds. Livest. Sci. 167: 186-194.

4.1 Résumé

L'objectif de cette étude était de mesurer les effets d'une supplémentation en acide folique et en vitamine B_{12} donnée avant le vêlage et en début de la lactation sur la production laitière et ses composantes pendant les 60 premiers jours en lait (JEL) et la production 305 jours et sur les indicateurs de bilan énergétique pour 805 vaches réparties dans 15 troupeaux. Les vaches ont été assignées à des injections intramusculaires hebdomadaires (5 ml) de soit 1) saline 0,9 % NaCl (témoin) ou 2) 320 mg d'acide folique et 10 mg de vitamine B_{12} (vitamines) de 21 jours avant la date prévue du vêlage jusqu'à 60 JEL. Pour les 60 premiers JEL, la production laitière moyenne a été de 35,0 kg/j et n'a pas été affectée par les traitements. En moyenne, la concentration en gras du lait a été diminuée de 42,1 à 40,3 g/kg en début de la lactation pour les vaches recevant le supplément vitaminique comparativement aux animaux témoins tandis que la concentration en protéines du lait a été augmentée de 30,9 à 31,5 g/kg par le supplément vitaminique. Le supplément de vitamines a réduit la production de gras pendant 305 jours pour les vaches primipares par rapport aux témoins tandis qu'aucun effet de traitement n'a été observé pour les vaches multipares. Comme indicateurs du bilan énergétique, le ratio gras : protéine du lait a diminué de 0,06 pour les vaches recevant le supplément de vitamines comparativement aux animaux témoins. Les vaches ayant reçu le supplément de vitamines ont perdu moins de poids corporel pendant les 60 premiers JEL que les vaches témoins. La réduction observée de la perte de poids corporel combinée à la réduction du ratio gras : protéine sans effet sur la production laitière suggèrent que le supplément d'acide folique et de vitamine B_{12} peut avoir un effet sur la répartition de l'énergie au début de la lactation.

Mots clés : vache laitière, troupeau commercial, acide folique, vitamine B_{12}, répartition de l'énergie

4.2 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-day yield, and on indicators of energy balance for dairy cows in commercial 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 months and within each herd, cows were assigned, according to parity, predicted 305-day 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 (SD 8) days 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 controls, 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-day milk and protein yields. The vitamin supplement reduced 305-day 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 controls. 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.

Key words: dairy cow, commercial herd, folic acid, vitamin B_{12}, energy partitioning

4.3 Introduction

It is well known that rumen microorganisms can synthetize B vitamins including folic acid and vitamin B_{12} (Bechdel et al., 1928; NRC, 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; NRC, 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, a reduction of dry matter (DM) intake, and an acute raise in milk production (Goff and Horst, 1997). Nadir of serum folate concentration in dairy cows was observed at calving (Girard et al., 1989) and at 8 weeks after parturition regarding serum vitamin B_{12} concentration (Girard and Matte, 1999). Those results demonstrated that supply of these B vitamins from rumen bacteria synthesis 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 weeks 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 controls (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-day 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-day 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 days before the expected calving date until 60 DIM to primiparous and multiparous cows on milk and component yields during the first 60 DIM and the 305-day 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.

4.4 Materials and Methods

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

4.4.1 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 8,800 kg of milk for a 305-day 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-day milk, fat, and protein yields were 9,662 ± 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 days for both treatment groups ($P = 0.40$).

4.4.2 Treatments

The study lasted 14 months 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 months from February to December 2010 and within each herd, cows were assigned, based on parity (primiparous vs. multiparous), predicted 305-day 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, 5,000 µg/mL, Vétoquinol, 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 days 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 weeks and were kept refrigerated in a box to protect them from light.

4.4.3 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-day 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 (Wildman et al., 1982; Ferguson et al., 1994) starting 21 ± 8 days 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 days before calving, at the first visit after calving (7 ± 4 DIM) and at 55 ± 13 DIM. Because weight 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 days before parturition to calving) and during early lactation (from calving to 60-100 DIM) are described in Table 4.1. All dairy herds used a pre-calving and an early-lactation diets. However, data regarding pre-calving diet from one herd were lacking and were not included (Table 4.1). Among herds, rations were computed to meet or exceed cobalt requirements (0.11 mg/kg of DM) according to the NRC (2001).

Table 4.1 Average composition (range) of pre-calving and early lactation diets in the studied herds[1]

Composition	Pre-calving diet	Early-lactation diet
Herds, n	14	15
CP[2] (% DM)	14.5 (12.8-16.2)	17.2 (16.3-18.5)
RDP[2,3] (% DM)	9.1 (7.5-11.1)	10.7 (9.5-11.4)
RUP[2,3] (% CP)	30.1 (26.3-34.0)	37.5 (32.0-42.6)
NE_L[2,3] (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[2] (% DM)	26.9 (21.3-32.1)	20.4 (17.1-23.3)
NDF[2] (% 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[2,3] (% 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[4] (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)

[1] Diet compositions were provided by each herd nutritionist, based on individual forage analyses done in the Valacta laboratory. Data from one herd regarding pre-calving diet were lacking.
[2] 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.
[3] Calculated according to NRC (2001).
[4] Based on the equation: dietary cation-anion difference (DCAD; mEq/kg) = [(% Na × 435) + (% K × 256)] – [(% Cl × 282) + (% S × 624)] (Ender et al., 1971).

4.4.4 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 NRC (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 (Duffield et al., 1997; Buttchereit et al., 2010).

4.4.5 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 (Grieve et al., 1986; de Vries and Veerkamp, 2000; Buttchereit et al., 2010) 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).

4.4.6 Statistical Analysis

Lactation curves for the whole lactation (0 to 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 $e^{-0.05 \times DIM}$) and the MIXED procedure of SAS (version 9.2, SAS Institute, 2008, Cary, NC, USA). 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-month 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 2 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 analyses for the current 305-day 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 days 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 to 14 days before calving; 2) 13 to 0 days before calving; 3) 1 to 14 DIM; 4) 15 to 28 DIM; 5) 29 to 42 DIM; 6) 43 to 56 DIM; 7) 57 to 70 DIM; 8) 71 to 84 DIM; and 9) 85 to 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 days before calving until 7 days after calving and 7 days after calving until 55 DIM were also analysed

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 $0.05 < P \leq 0.10$.

4.5 Results

4.5.1 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 4.2); 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 averaged 34.0 ± 0.3 and 34.9 ± 0.3 kg/d for vitamin and control groups, respectively. The vitamin supplement decreased milk fat concentration from 42.1 to 40.3 ± 0.4 g/kg ($P = 0.004$; Figure 4.1a) but increased milk protein concentration from 30.9 to 31.5 ± 0.2 g/kg ($P = 0.04$; Figure 4.1b). No treatment effect on milk lactose concentration was observed ($P = 0.22$; Table 4.2). Daily yields of protein and lactose secreted in milk did not differ between treatments ($P \geq 0.42$; Table 4.2) and were greater for multiparous cows than primiparous cows ($P < 0.0001$). 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 4.2). 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 4.2).

Table 4.2 Milk production and components according to treatments and parity for the first 60 DIM (adjusted means ± SE)

	Primiparous		Multiparous		*P*-value		
	Treatment[1] (Trt)						
Item	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	<.0001	0.68
ECM[2] (kg/d)	30.0 ± 0.5	28.7 ± 0.5	39.7 ± 0.4	39.3 ± 0.4	0.06	<.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[3] (kg/d)	1.26 ± 0.02	1.18 ± 0.03	1.66 ± 0.02	1.63 ± 0.02	0.42	<.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	<.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	<.0001	0.62
Yield (kg/d)	1.40 ± 0.02	1.38 ± 0.03	1.84 ± 0.02	1.84 ± 0.02	0.77	<.0001	0.72
Fat: protein ratio[4]	1.41 ± 0.02	1.34 ± 0.02	1.36 ± 0.01	1.32 ± 0.01	0.001	0.18	0.07
MUN[5] (mg/dL)	8.49 ± 0.25	8.63 ± 0.26	8.39 ± 0.19	8.11 ± 0.19	0.79	0.20	0.15

[1] 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} administered weekly.
[2] Energy-corrected milk (ECM; kg/d) = 12.55 × fat (kg/d) + 7.39 × protein (kg/d) + 5.34 × lactose yield (kg/d). Adapted from NRC (2001) and Tyrrell and Reid (1965).
[3] 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).
[4] Vitamin supplement decreased the fat: protein ratio at a higher extent in primiparous cows (P = 0.0005) than in multiparous cows (P = 0.02).
[5] MUN = milk urea nitrogen.

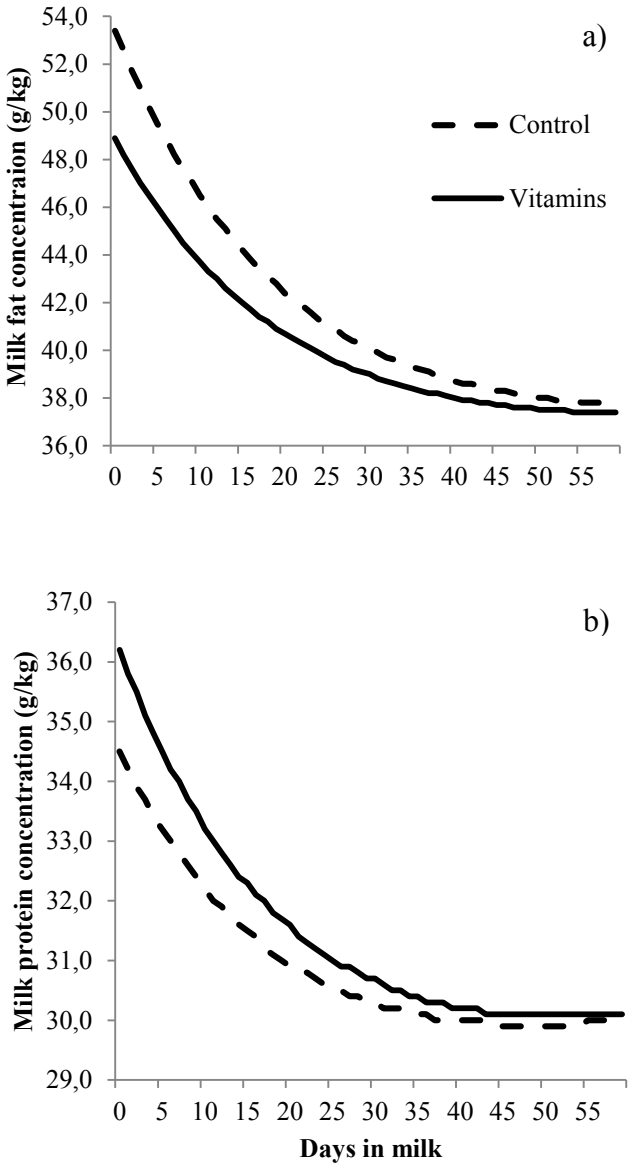

Figure 4.1 Effects of a weekly supplementation in folic acid and vitamin B_{12} during the first 60 days in milk on milk fat (a; $P = 0.004$) and milk protein (b; $P = 0.04$) concentrations. Control = 5 mL of saline 0.9% NaCl; Vitamins = 3 mL of 320 mg of folic acid and 2 mL of 10 mg of vitamin B_{12}. The average standard errors were 0.4 and 0.2 g/kg for milk fat concentration and milk protein concentration curves, respectively

Table 4.3 Lactation length, 305-day milk and component yields according to treatments and parity (adjusted means ± SE)

	Primiparous		Multiparous		*P*-value		
	Treatment[1] (Trt)						
Item	Control	Vitamins	Control	Vitamins	Trt	Parity	Trt × Parity
Number of cows[2]	118	113	202	218			
Lactation length[3] (days)	351 ± 6	356 ± 5	361 ± 5	355 ± 5	0.99	0.40	0.33
Milk yield (305 days; kg)[3]	8,782 ± 124	8,655 ± 126	10,396 ± 94	10,306 ± 91	0.31	<.0001	0.86
Fat yield (305 days; kg)[3,4]	357 ± 5	341 ± 5	405 ± 4	405 ± 4	0.07	<.0001	0.08
Protein yield (305 days; kg)[3]	286 ± 4	281 ± 4	335 ± 3	333 ± 3	0.35	<.0001	0.61

[1] 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} administered weekly.
[2] Cows with lactation length less than 250 days were not included.
[3] Herd effect, $P < 0.0001$.
[4] The vitamin supplement reduced 305-day milk fat yield in primiparous cows as compared with control ($P = 0.03$) whereas no treatment effect was observed for multiparous cows ($P = 0.94$).

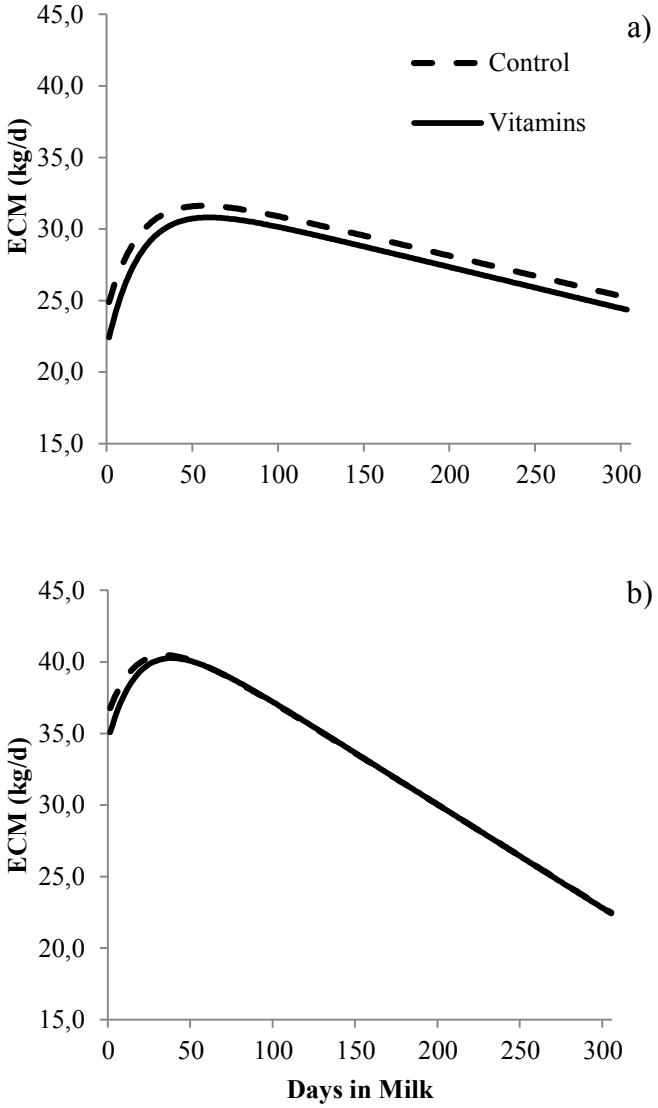

Figure 4.2 Energy corrected milk (ECM) 305-day lactation curves for primiparous (a) and multiparous (b) cows according to treatments (treatment × parity interaction, *P* = 0.20). Control = 5 mL of saline 0.9% NaCl; Vitamins = 3 mL of 320 mg of folic acid and 2 mL of 10 mg of vitamin B_{12}. ECM (kg/d) = 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

Lactation length was not different between treatments and parity (treatment × parity interaction, $P = 0.33$). The current 305-day milk yield was not affected by treatment nor was 305-day protein yield ($P \geq 0.31$; Table 4.3). Pertaining to 305-day milk fat yield, a tendency treatment × parity interaction was observed ($P = 0.08$; Table 4.3). The 305-day 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-day yield for primiparous and multiparous cows are shown in Figure 4.2a,b (treatment × parity interaction, P = 0.20). Primiparous cows had a lower ECM peak but a better persistency than multiparous cows.

4.5.2 Body Condition Score and Estimated Body Weight

At 21 ± 8 days 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 4.4). 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 days 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 days before calving to 7 days postpartum did not differ between treatments (P = 0.29; Table 4.4) 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 averaged 22.8 and 30.3 ± 2.9 kg for vitamin and control groups, respectively.

Table 4.4 Body condition score (BCS) and estimated body weight (BW) after calving and estimated BW losses before and after calving (adjusted means ± SE)

	Primiparous		Multiparous		*P*-value		
	Treatment[1] (Trt)						
Item	Control	Vitamins	Control	Vitamins	Trt	Parity	Trt × Parity
Number of cows	136	135	263	271			
BCS at 7 DIM[2,3,4]	3.41 ± 0.05	3.43 ± 0.05	2.97 ± 0.05	3.03 ± 0.05	0.12	<.0001	0.55
Estimated BW at 7 DIM[3,4,5] (kg)	599.7 ± 5.6	592.1 ± 5.6	664.7 ± 4.0	664.2 ± 4.0	0.56	<.0001	0.73
Estimated BW loss[5] (kg)							
Before calving until 7 DIM[4,6]	33.9 ± 3.5	30.6 ± 3.6	23.4 ± 2.9	21.4 ± 2.9	0.29	0.0001	0.81
After calving[4,7]	32.5 ± 3.8	24.7 ± 3.8	28.1 ± 3.1	21.0 ± 3.1	0.007	0.14	0.89

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

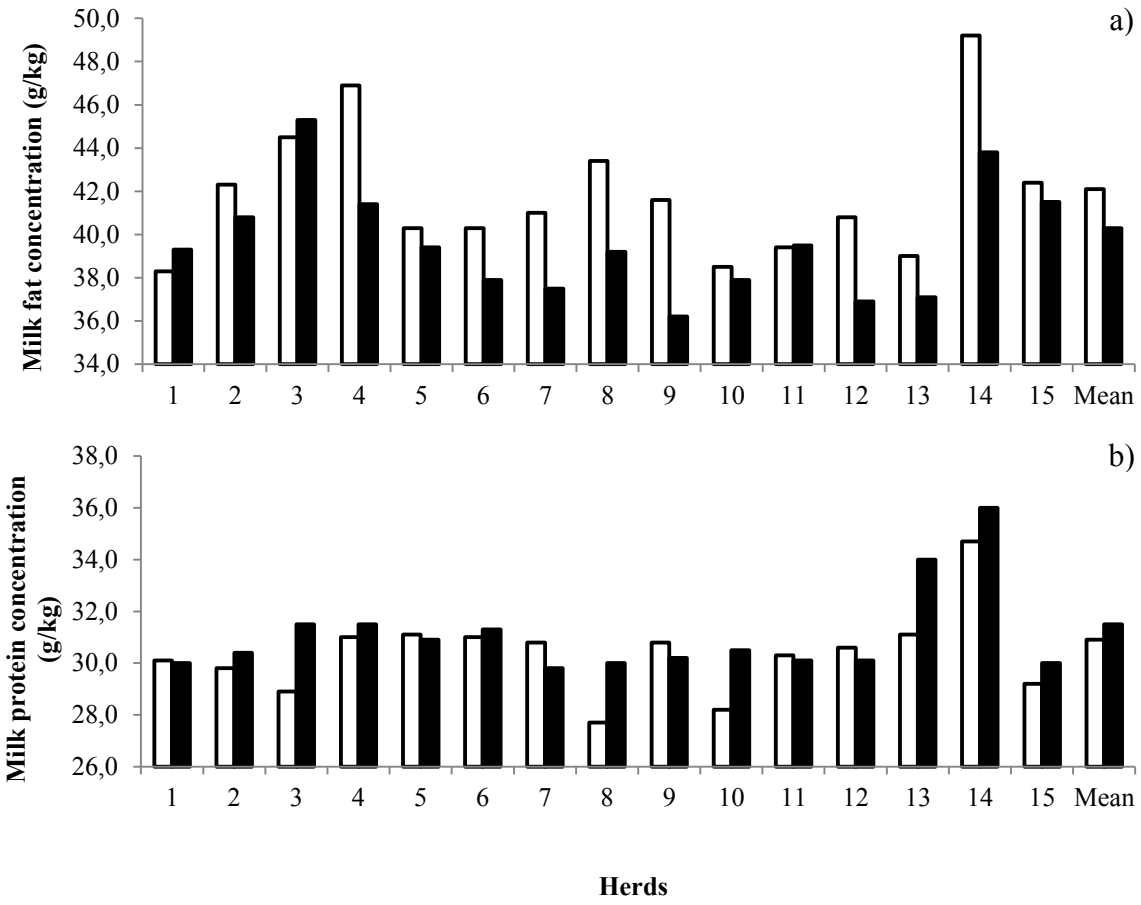

Figure 4.3 Milk fat (a) and milk protein (b) concentrations during the first 60 days in milk according to treatments (open bars = control (5 mL of saline 0.9% NaCl) and closed bars = vitamins (3 mL of 320 mg of folic acid and 2 mL of 10 mg of vitamin B_{12})) among the 15 herds involved and the mean (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

4.5.3 Variation Among Herds

Responses to treatments among the 15 herds involved in this experiment are illustrated in Figures 4.3a,b and 4.4. 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 (Figures 4.3a,b and 4.4). 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.

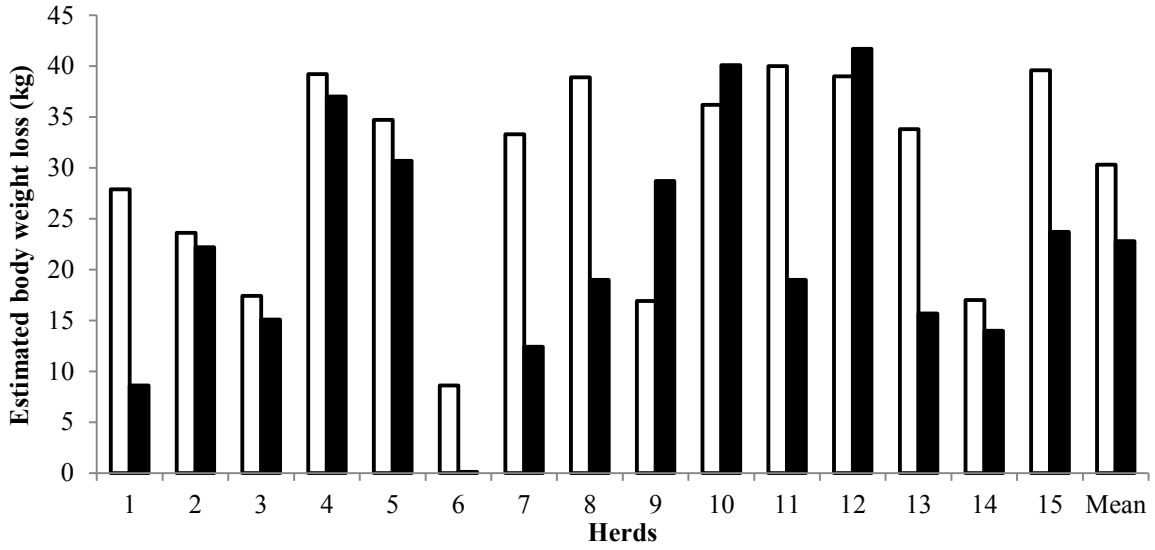

Figure 4.4 Estimated body weight loss from 7 to 55 days in milk according to treatments (open bars = control (5 mL of saline 0.9% NaCl) and closed bars = vitamins (3 mL of 320 mg of folic acid and 2 mL of 10 mg of vitamin B_{12})) among the 15 herds involved and the mean (herd effect; *P* = 0.002). Number 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

4.6 Discussion

The folic acid and vitamin B_{12} supplement administered by intramuscular injections during the first 60 DIM did not increase milk yield during the injection period and over the 305-day 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 weeks 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 weeks of lactation in multiparous cows. In these 2 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 analyse folate and vitamin B_{12} status of cows. Responses among herds 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 the 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. (2009a; 2010) 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 (Grieve et al., 1986; de Vries and Veerkamp, 2000; 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 released from body reserves can be taken up by the mammary gland and secreted into 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 (Heuer et al., 1999; Buttchereit et al., 2010). 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 weight 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 2 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 weeks 1 to 8 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,b).

4.7 Conclusion

Supplementation of folic acid and vitamin B_{12} given 21 days before the expected calving date until 60 DIM did not increase milk yield of dairy cows in early lactation and during the 305-day 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.

4.8 Acknowledgements

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.

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5 Article sur la rentabilité – Premier projet

Un modèle économique évaluant une supplémentation d'acide folique et de vitamine B_{12} donnée autour de la parturition et en début de la lactation sur les fermes laitières québécoises

An economic model evaluating the supplementation of folic acid and vitamin B_{12} given around parturition and in early lactation on dairy farms in Québec, Canada

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Cet article a été accepté pour publication dans le *Canadian Journal of Animal Science*.

Référence : Duplessis, M., Girard, C. L., Santschi, D. E. et Pellerin, D. 2014. An economic model evaluating the supplementation of folic acid and vitamin B_{12} given around parturition and in early lactation on dairy farms in Québec, Canada. Can. J. Anim. Sci. DOI : 10.4141/CJAS-2014-026.

5.1 Résumé

L'objectif de cette étude était d'estimer la rentabilité potentielle d'un supplément combiné d'acide folique et de vitamine B_{12} dans les troupeaux laitiers québécois. Un total de 791 vaches réparties dans 14 troupeaux laitiers ont participé au projet. Les vaches étaient assignées à une injection intramusculaire hebdomadaire de saline ou 320 mg d'acide folique et 10 mg de vitamine B_{12}. Les traitements ont débuté 3 semaines avant la date de vêlage prévue et ont duré jusqu'à 8 semaines de la lactation. Pour tous les troupeaux, des données sur la production laitière, la reproduction et l'incidence des maladies ont été prises. Deux scénarios ont été étudiés compte tenu du système de gestion de l'offre propre à l'industrie laitière canadienne : 1) quota gardé constant et 2) nombre de vaches gardé constant. Pour les scénarios 1 et 2, 8 et 7 troupeaux sur 14, respectivement, ont obtenu une marge annuelle nette positive par vache suite à l'utilisation du supplément. Des marges nettes moyennes de 31,18 $ et -4,86 $ CAN par vache par année ont été obtenues pour les scénarios 1 et 2, respectivement. Ces réponses variables soulignent que les apports de ces vitamines par la flore du rumen étaient probablement différents entre les troupeaux et que les connaissances actuelles ne permettent pas de prédire les apports selon les rations servies.

Mots clés : vache laitière, acide folique, vitamine B_{12}, rentabilité

5.2 Abstract

The aim of this study was to estimate the potential profitability of a combined supplement of folic acid and vitamin B_{12} given around parturition and in early lactation in commercial dairy herds in Québec. A total of 791 dairy cows from 14 herds were enrolled. Cows were assigned to weekly intramuscular injections of saline or 320 mg of folic acid and 10 mg of vitamin B_{12}. Treatments began 3 weeks before the expected calving date and lasted until 8 weeks of lactation. Within each herd, data on production, reproduction, and incidence of metabolic disorders and other diseases were recorded. With regard to the Canadian dairy industry which operates under a supply management system, 2 scenarios were studied: 1) quota kept constant and 2) number of cows kept constant. For scenarios 1 and 2, 8 and 7 herds out of 14, respectively, obtained a positive annual net margin per cow following the vitamin supplement. The average net margins were \$31.18 and -\$4.86 CAN cow^{-1} yr^{-1} for scenarios 1 and 2, respectively. The variability of the response highlights that supplies of these vitamins by ruminal synthesis were probably different among herds and actual knowledge does not allow predicting supplies according to the diet.

Key words: dairy cow, folic acid, vitamin B_{12}, profitability

5.3 Introduction

In dairy production, use of folic acid and vitamin B_{12} supplement, either oral or parenteral, is not a common practice. According to the NRC (2001), ruminal microorganisms from healthy ruminants produce sufficient amounts of B vitamins to avoid deficiency. Previous studies showed that ruminal synthesis ranged between 16.5 and 21.0 mg d^{-1} for folic acid, and between 73.0 and 79.8 mg d^{-1} for vitamin B_{12} (Santschi et al., 2005; Schwab et al., 2006). Nonetheless, milk yield was increased by 3.4 kg d^{-1} and protein yield by 75 g d^{-1} in multiparous cows fed a folic acid supplement alone or combined with a vitamin B_{12} supplement around parturition and in early lactation (Graulet et al., 2007). A combined supplementation in folic acid and vitamin B_{12} given to multiparous cows 3 weeks before the expected calving date until 16 weeks of lactation increased milk, fat and protein yields, particularly during the first 4 weeks of lactation as compared with cows fed no vitamin supplementation or folic acid alone (Preynat et al., 2009b). Moreover, previous studies concluded that a folic acid and vitamin B_{12} supplement given in early lactation seems to improve energy metabolism efficiency of dairy cows (Girard and Matte, 2005; Graulet et al., 2007; Preynat et al., 2009a; 2010). Indeed, cows receiving the vitamin supplement had better lactational performance without increasing dry matter (DM) intake as compared with control ones.

In Canada, the dairy industry is regulated by a supply management system (Canadian Dairy Commission, 2013). The objective of this system is to avoid milk surpluses, to stabilize milk prices at the farm level and to ensure that milk production is equal to the Canadian demand. To achieve this, Canada limits milk importation and controls milk production at the farm level. Therefore, each dairy producer should access a part of the total production quota which is based on kg of butterfat (BF) d^{-1}. In other words, the quantity of fat produced every day on a farm must be within a range according to the quota allocated to this farm. Under- and over-quota productions represent monetary losses for dairy producers. Selling or buying cows or quota should therefore be considered for any significant changes in daily BF production on a dairy farm. In addition, milk payment is

based on the quantity of each milk component sold (i.e. fat, protein, and lactose and other solids).

To our knowledge, no field study has been conducted to calculate the economic impact of a combined supplement of folic acid and vitamin B_{12} given during the periparturient period and in early lactation. Based on the effects of a combined supplement of folic acid and vitamin B_{12} on milk production and on efficiency of energy metabolism in early lactation reported in previous studies, it was hypothesized that this supplement would be economically beneficial for dairy herds in Québec. Consequently, the aim of this study was to evaluate the profitability within a 365 day period of using this supplementation around parturition and in early lactation in commercial dairy herds in Québec. In addition, because several herds were involved, this experiment allowed computing differences among herds.

5.4 Materials and Methods

All procedures of this study were approved by the Animal care committee from Université Laval, QC following the guidelines of the Canadian Council on Animal Care (2009). In the current paper, all economic values are reported in Canadian dollars (Can$); in 2010, 1.00 Can$ averaged 0.97 US$ according to the Bank of Canada. It is noteworthy that all costs and values were based on dataset of the province of Québec in 2010.

5.4.1 Experimental Procedures

The description of herds and cows involved in this study has been reported previously (Duplessis et al., 2014a). Briefly, a total of 805 dairy cows located in 15 herds around Québec city, QC participated in this experiment. However, because some data needed for an accurate economic analysis were missing from one herd, this herd was discarded from the dataset. The current economic analysis was therefore performed on data for 791 cows from 14 herds (269 primiparous and 522 multiparous cows; 780 Holstein and 11 Jersey cows). All cows from each herd were included in the project, except those with a previous calving interval greater than 500 days. The size of herds ranged from 38 to 120 dairy cows (mean = 64 cows). All cows were milked twice daily and housed in tie-stall barns. Seven

herds used a total mixed ration whereas the 7 others used an individual concentrate feeding system.

To join the study, dairy herds had to record milk production, fat and protein contents on a monthly basis through the Dairy Herd Improvement agency (DHI; Valacta, Ste-Anne-de-Bellevue, QC), to be visited at least once a month by a veterinarian, and to do not use routinely timed artificial insemination. Veterinarians working close to Québec city, QC were asked to contact dairy producers that met the above conditions and participation was on a voluntary basis.

From February to December 2010, every 2 months and within each herd, dairy cows were blocked by parity (primiparous or multiparous), previous 305-day milk production, and calving interval and randomly assigned to either 5 mL of 1) saline 0.9% NaCl (Control) or 2) 320 mg of folic acid and 10 mg of vitamin B_{12} (Vitamins; pteroylmonoglutamic acid, MP Biomedicals, Solon, OH, USA and cyanocobalamin, 5000 μg mL^{-1}, Vétoquinol, Lavaltrie, QC). Folic acid solutions were prepared using ultrapure water and treatments were administered in one 5 mL syringe. Treatments consisted of weekly intramuscular injections and began 3 weeks before the expected calving date and lasted until 8 weeks after parturition. Intramuscular injections were chosen to overcome the extensive destruction by the rumen microflora of dietary supplements of folic acid and vitamin B_{12} (Santschi et al., 2005). Throughout the study, herds were visited every other week from February 2010 to April 2011. Dairy producers had to inject treatments on weeks between visits. To protect vitamin supplement from light, syringes were stored in opaque boxes and syringes for both treatments were kept refrigerated until use.

Effects of a supplement of folic acid and vitamin B_{12} on milk production, culling rate, metabolic disorders and other diseases, and reproductive performance were reported elsewhere (Duplessis et al., 2014a,b). Data on milk yield and components per cow for the whole lactation, culling and reproduction data were obtained from Valacta. Incidences of metabolic disorders and other diseases were obtained from dairy producer and veterinarian records. Calving ease was evaluated on a 5-scale score as follows: 1) no assistance; 2) light assistance; 3) difficult calving; 4) surgery; and 5) non-favourable calf presentation (Jamrozik et al., 2005). Dystocia was considered for a score at or greater than 3 (Mee,

2008). For the calculation of animals sold, it was considered that 50% of calves born were male, that the mortality rate was 5% and that female calves not required for replacement were sold.

5.4.2 Economic Values and Calculations

The approach previously described by Santschi et al. (2011) was used to compute the economic effect of a supplementation in folic acid and vitamin B_{12}. However, some changes described below have been made to better suit this project.

A spreadsheet developed in Excel software (2010, Microsoft Corp., Redmond, WA, USA) was used in order to compute the effect of a folic acid and vitamin B_{12} supplement on farm profitability. Dairy cows were divided in 4 groups: control primiparous cows, control multiparous cows, vitamin primiparous cows, and vitamin multiparous cows. For each group and within each herd, the following parameter means were entered in the spreadsheet for the studied lactation: calving interval, number of breedings to get pregnant, milk yield and milk fat, protein, and lactose and other solids yields for the whole lactation, incidences of retained placenta, metritis, milk fever, severe ketosis, displaced abomasum and clinical mastitis, and replacement rate. Incidences were calculated as number of new cases of a given disease divided by the number of cows at risk during the whole experimental period. Data were averaged for the 14 herds, although these means were not always different between treatments, and were entered in the spreadsheet for each parameter within each group in order to represent the actual economic impact for each dairy producer (Santschi et al., 2011). Calving intervals were computed by adding days in milk at last breeding obtained from Valacta to 282 days as an average gestation length and were obtained from cows that completed the lactation; data from culled cows were discarded (115 cows for vitamin group and 120 for control group). For the purposes of the current analysis, the FREQ procedure of SAS (version 9.2, SAS Institute, 2008, Cary, NC, USA) was used to compute incidences of diseases and replacement rate by treatment and parity within each herd. Definitions of each metabolic disorder and other disease have been previously described (Duplessis et al., 2014a). Ketosis incidence was assessed using Keto-test (Elanco Animal Health, Guelph, ON). A mild ketotic cow had a Keto-test result between 100 and

200 μmol L^{-1} whereas a severe ketotic cow had a Keto-test result over 200 μmol L^{-1}. In the current paper, only incidence of severe ketosis was used in the dataset as mild ketosis is generally not treated and, therefore, considered without veterinarian fees. Replacement rate was considered to be equal to the culling rate as one cow that leaves the herd has to be replaced.

Economic values of parameters used in this study are shown in Table 5.1. The cost of the supplement of folic acid and vitamin B_{12} was set to $16.50 for the entire administration period or $1.50 wk^{-1} for 11 weeks. Because this combined supplement is not commercially available, this price has been estimated from the separate costs of 320 mg of folic acid ($0.56) and 10 mg of vitamin B_{12} ($0.94). The right to produce 1 kg of BF d^{-1} in Québec in 2010 cost $25 000 according to the Fédération des producteurs de lait du Québec (FPLQ, 2010b). When necessary, the purchase of quota was financed over 10 years at an interest rate of 6%. On the other hand, if quota needed to be sold, the 6% interest from the quota sold decreased herd costs in the partial budget. Annual interest payment was calculated using the PMT function in Excel software. Based on constant interest rate and constant payments, this function allows calculating the payment of a loan. The average milk component prices detailed in Table 5.1 were obtained from FPLQ (2010a). Total breeding cost included average semen dose cost plus the visit of the artificial insemination technician (Table 5.1). Other variable costs were first obtained on a hL basis and multiplied by milk production (hL cow^{-1} yr^{-1}), and included DHI services ($0.77 hL^{-1}), bedding ($1.11 hL^{-1}), manure management ($0.68 hL^{-1}), dairy equipment for milking ($1.07 hL^{-1}), other costs ($0.82 hL^{-1}), and short-term interest ($0.13 hL^{-1}) (Agritel Web, Longueuil, QC). The average cost for these other variables in the 14 studied herds was $434.29 cow^{-1} yr^{-1}. Replacement cost represents variable costs associated to heifer raising (Agritel Web, Longueuil, QC). The average values of a culled cow and a male calf were estimated from the average prices of $0.95 kg^{-1} and $2.20 kg^{-1} in 2010 for a culled cow and a male calf, respectively (Fédération des producteurs de bovins du Québec, 2010). The value of a female calf was obtained from the study of Santschi et al. (2011). As stated by OMAFRA (2009), injection site lesions could reduce carcass value. In the current study, no monetary loss caused by injection site lesions was taken into account in the average price of a culled

cow as time of culling was difficult to predict and a total volume of 5 mL per injection was given which is low (Léonard, 2001).

Table 5.1 Economic parameters used in this study to compute the economic effect of a combined supplement of folic acid and vitamin B_{12}

Item	Economic parameters[1]
Supplement cost ($ 11 wk^{-1} cow^{-1})[2]	16.50
Quota price ($ kg^{-1} of BF d^{-1})[3]	25 000.00
Milk price[4]	
Fat ($ kg^{-1})	9.62
Protein ($ kg^{-1})	8.49
Lactose + other solids ($ kg^{-1})	1.69
Transportation costs ($ hL^{-1})	2.56
Milk marketing costs ($ kg^{-1} of solids)	0.14
Breeding costs ($ $breeding^{-1}$)[5]	44.88
Other variable costs ($ hL^{-1})[6]	4.58
Replacement cost ($ $heifer^{-1}$)[6]	1 960.00
Value of animal sold	
Culled cow ($ cow^{-1})[7]	600.00
Male calf ($ $calf^{-1}$)[7]	100.00
Female calf ($ $calf^{-1}$)[8]	250.00

[1] All prices are in Canadian dollars (Can$).
[2] Cost for the entire period of administration of a combined supplement of 320 mg of folic acid and 10 mg of vitamin B_{12} i.e. 3 weeks before the expected calving date until 8 weeks after the parturition. One injection was evaluated to cost $1.50.
[3] Price to pay in 2010 for the right to produce 1 kg of butterfat (BF) d^{-1} in the province of Québec according to the Fédération des producteurs de lait du Québec (FPLQ, 2010b).
[4] Average prices for the year 2010 according to the FPLQ (2010a).
[5] Including the semen cost (averaging $29.38 in 2010) and the visit of the artificial insemination technician ($15.50) (M. Roy, CIAQ, St-Hyacinthe, QC, personal communication).
[6] Calculated from Agritel Web database. 2010. (http://agritel.fgcaq.com/Agritel), Fédération des groupes conseils agricoles du Québec (Longueuil, QC).
[7] In 2010, a culled cow (635 kg) was sold in average $0.95 kg^{-1} and a male calf (45 kg), $2.2 kg^{-1} (Fédération des producteurs de bovins du Québec, 2010).
[8] According to Santschi et al. (2011).

Veterinary costs associated with metabolic disorders and other diseases taken into account in this study were obtained from the experiment of Santschi et al. (2011) and were

estimated by 3 veterinarians from 2 clinics located in Québec (Table 5.2). However, because the calculation of these costs was done 2 years before the beginning of the current study, a total inflation rate of 5% was applied. These costs include prices of the medication and veterinarian fees if required. In the current study, incidence of mild milk fever (feeble cow whose symptoms were improved after calcium or other mineral doses) and severe milk fever (cow cannot get up) were combined. Because severe cases were rare (n=8), only cost of mild milk fever from the study of Santschi et al. (2011) was considered in the present analysis. Time required for treating a sick cow according to diseases was estimated by the same 3 veterinarians (D. E. Santschi, unpublished data; Table 5.2).

Table 5.2 Veterinary costs associated with diseases studied in this experiment[1]

Disease	Cost[2] ($ case^{-1})	Time required[3] (min cow^{-1} case^{-1})
Retained placenta	4.95	20
Metritis	52.27	20
Milk fever (mild)	18.73	25
Ketosis (severe)	19.23	40
Displaced abomasum	168.28	60
Mastitis	23.33	60

[1] All values are in Canadian dollars (Can$).
[2] According to Santschi et al. (2011) with an augmentation of 5% to compensate for the inflation rate. The cost includes the medication and the veterinarian visit fees, if needed.
[3] Time required to treat the animal (D. E. Santschi, unpublished data).

Rations used (early, mid and late lactation, far-off, and pre-calving) were obtained from herd nutritionists throughout the study. If available, ration cost was taken directly from the nutritionist software. When this information was not available, the daily cost per cow was calculated using the cost of each ingredient multiplied by the quantity given on a DM basis.

It was estimated that the time required to inject the vitamin supplement throughout the supplementation period was 10 min cow^{-1}. According to a study conducted by Valacta, 14.9 minutes are needed to attend to one lactating cow per day regardless of the lactation stage whereas 9.6 min d^{-1} are needed for one dry or pre-calving cow. These data were obtained from herds averaging 50-100 cows housed in tie-stall barns (Roy et al., 2011). Average

employee wage rate to quantify farm labor was \$12.80 h^{-1} (ranging from \$10.00 to \$16.00 h^{-1}) in 2010 as estimated by the 14 participating producers.

The break-even point for the supplementation cost per cow was calculated as follows: (variation in net income + supplement cost)/number of cows required to fulfill the quota.

5.4.3 Calculations Allowing Comparisons on a Yearly Basis per Herd

Economic parameter results were brought together considering the respective proportions of primiparous and multiparous cows within each herd and treatment group (i.e. control and vitamins). The average proportion of primiparous cows in each herd for 2010 was obtained from DHI records and ranged from 25.4 to 38.2% (Table 5.3).

All economic parameters were divided by the calving interval and multiplied by 365 days in order to analyze data on a yearly basis and to allow comparison among herds. More precisely, for the calculation of treatment and veterinary services expressed in \$ cow^{-1} yr^{-1}, incidence of each disease was multiplied by its cost per case, adjusted for the calving interval and multiplied by 365 days for annual cost. Similarly, labor required for disease treatment per cow per year was computed as follows: time required (hour) for a given disease multiplied by its incidence, and then corrected for calving interval and multiplied by 365 days to obtain annual cost. In order to calculate the yearly feeding cost per cow in early lactation, daily cost per cow was multiplied by the average number of days during which the early-lactation ration was offered and then a correction for the calving interval length was done. A similar calculation for mid- and late-lactation rations was made.

Table 5.3 Additional information about dairy herds involved in this experiment

	Herds													
Item	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Herd size[1]	45	55	103	47	122	53	76	45	42	57	38	47	91	81
Milk yield (kg)[2]	8 341	8 043	9 786	8 599	11 034	9 611	8 987	10 937	9 733	10 997	10 065	10 602	9 022	10 210
Fat content (%)	3.88	3.66	4.38	4.12	3.70	3.86	3.78	3.89	3.81	3.82	3.82	3.98	3.93	4.23
Protein content (%)	3.26	3.16	3.36	3.28	3.11	3.24	3.12	3.12	3.23	3.27	3.17	3.24	3.31	3.15
Primiparous cows (%)	28.8	25.5	27.2	36.0	33.3	36.6	38.2	26.2	35.3	25.4	27.7	27.5	34.3	37.3

[1] According to DHI records from each herd. Including lactating and dry cows.
[2] Average 305-day milk yield for 2010 according to DHI records.

5.4.4 Partial Budget

Two situations with regard to the Canadian dairy industry were studied within each herd involved in this study: 1) quota kept constant and number of cows adjusted to fulfill the quota and 2) number of cows kept constant and quota sold or bought depending on herd milk fat production. For both scenarios, parameters to compute variation in income and costs were first calculated on a herd basis as follows: (average result per cow per year from the vitamin group × number of cows to fulfill the quota if the vitamin supplement is used) - (average result per cow per year from the control group × number of cows to fulfill the quota if the vitamin supplement is not used). A positive result therefore represents a gain or increase, whereas a negative result indicates a loss or decrease attributed to the vitamin supplement.

Within each herd, the final result of the partial budget was divided by the respective number of cows required if the vitamin supplement is adopted. This allows proper comparison among herds regardless of herd size. Production, reproduction and incidence of disease results of the 14 herds were averaged and divided by the number of cows required to fulfill the average quota according to scenarios 1 and 2 to generate a partial budget for an average herd.

5.4.5 Sensitivity and Break-Even Point Analyses

Sensitivity analyses were done in order to evaluate the effects of price or cost changes on partial budget input values according to each scenario. Animal sold price, replacement cost, reproduction cost, treatments and veterinary service cost, feeding cost, labor cost and other variable costs were multiplied by 0.8 and 1.2 to study the impact of 20% changes in these input values as previously performed by De Vries (2006). An increment of 5% of milk price was considered being more realistic and all inputs were varied one by one.

Two analyses were carried out to study the decrease of calving interval length and replacement rate required with the use of the vitamin supplement at the break-even point.

Calving interval and replacement rate were chosen as these parameters are of economic importance for dairy herds (Pellerin and Gilbert, 2008; Włodarek et al., 2011). Moreover, it has been reported that the vitamin supplement significantly decreased days at first breeding by 3.8 days for multiparous cows (Duplessis et al., 2014a) suggesting that the vitamin supplement could have an indirect impact on calving interval length. Differences between treatment groups in the response to the supplement were entered in the model only when parameter results reached significance or tendency ($P \leq 0.10$). As milk fat concentration was significantly decreased by the vitamin supplement within the first 60 days in milk (DIM) (Duplessis et al., 2014b) and a tendency for a lower milk fat concentration during the whole lactation was noted for cows receiving the vitamin supplement as compared with control cows (M. Duplessis, unpublished data), milk fat concentration of each herd was decreased by 0.05 percentage point which represents the average decreased of milk fat concentration for the whole lactation. Other parameters remained unchanged for control and vitamin groups for the purpose of this analysis as they were not significantly different for the whole lactation.

5.5 Results and Discussion

The 14 dairy herds involved in this study corresponded to the average dairy herds in Québec in 2010 although milk production was higher. Indeed, in 2010, the average herd size was 57 cows mainly housed in tie-stall barns and producing on average 8800 kg of milk on a 305-day basis (Valacta, 2011). Milk, fat, and protein yields and size in 2010 for herds participating in this study are shown in Table 5.3.

For the present analysis, it was assumed that the use of a supplement of folic acid and vitamin B_{12} did not have an impact on housing costs and DM intake as showed by previous studies (Girard and Matte, 2005; Graulet et al., 2007; Preynat et al., 2009a).

The 305-day milk production for multiparous cows during the previous lactation was 9732 ± 113 kg and the calving interval was 394 ± 3 days and did not differ between treatment groups ($P = 0.94$ and 0.38, respectively).

5.5.1 Economic Variables

Means of economic parameters per cow on a yearly basis according to treatments from the 14 dairy herds presented in Table 5.4 slightly differ from data reported on a lactation basis by Duplessis et al. (2014a,b).

On a yearly basis, no increase of milk yield was noted following the use of the vitamin supplement (Table 5.4) nor for the first 8 weeks of lactation (Duplessis et al., 2014b). This is not in accordance with previous results in which the combined vitamin supplement increased milk yield in early lactation (Graulet et al., 2007; Preynat et al., 2009b). Within the first 60 DIM, cows receiving the vitamin supplement from the current study significantly had a lower milk fat content than control cows (Duplessis et al., 2014b). This is in accordance with results in Table 5.4. Although not significant, on average, the vitamin supplement decreased calving interval by 5.9 days and replacement rate by 2.7% (Table 5.4). The shorter calving interval following the vitamin supplementation is in accordance with multiparous cows receiving the vitamin supplement being statistically bred 3.8 days before multiparous control cows at the first service (Duplessis et al., 2014a). As previously reported, incidence of diseases, such as ketosis and displaced abomasum, was not affected by treatments (Duplessis et al., 2014a) which explains that veterinary costs were similar for both treatments (Table 5.4).

The average incidence of dystocia was decreased by 50% in multiparous dairy cows receiving the vitamin supplement, from 10.8 ± 2.0% to 5.3 ± 1.4% (Duplessis et al., 2014a). Using a similar dystocia scoring scale to the one used in the current experiment, Dematawena and Berger (1997) concluded that a difficult calving was associated with monetary losses. However, the present experiment was not designed to get accurate data on the cost of dystocia and this cost was not taken into account in the partial budget.

When reported on a yearly basis, the combined supplement of folic acid and vitamin B_{12} cost \$14.55 cow^{-1} and 0.2 h cow^{-1} was required to prepare and inject the vitamin supplement.

Table 5.4 Economic parameter results according to treatments on a yearly basis

Item	Means (Ranges)[1]	
	Control[2]	Vitamins[3]
Supplement cost ($ cow^{-1} yr^{-1})[4]	0.00	14.55 (12.97-16.05)
Production (kg cow^{-1} yr^{-1})		
Milk	9 767 (8 182-11 186)	9 765 (7 800-11 031)
Fat	392.0 (327.1-447.5)	385.9 (303.4-435.9)
Protein	322.0 (272.8-364.3)	323.1 (253.7-366.7)
Lactose + other solids	540.4 (454.2-626.7)	541.4 (428.9-622.4)
Calving interval (days)	419.9 (366.7-458.7)	414.0 (375.2-471.0)
Breeding (cow^{-1} yr^{-1})	2.2 (1.6-2.8)	2.1 (1.4-2.8)
Veterinary costs ($ cow^{-1} yr^{-1})[5]	21.43 (4.18-41.44)	21.64 (5.08-38.28)
Replacement rate (%)	29.2 (13.1-48.0)	26.5 (13.1-42.1)
Labor (h cow^{-1} yr^{-1})[6]		
Supplement	0.0	0.2 (0.1-0.2)
Far-off	5.9 (2.8-9.3)	5.9 (2.9-8.3)
Pre-calving	2.9 (2.7-3.4)	3.0 (2.6-3.3)
Early lactation	21.4 (12.6-30.0)	21.9 (11.7-32.4)
Mid lactation	21.6 (20.0-24.7)	21.9 (19.5-24.2)
Late lactation	33.9 (26.1-44.9)	33.3 (21.6-48.2)
Diseases' treatment	0.3 (0.2-0.5)	0.4 (0.1-0.5)
Feeding costs ($ cow^{-1} yr^{-1})		
Far-off	81.91 (44.15-131.86)	83.50 (40.22-110.94)
Pre-calving	55.45 (22.76-78.23)	56.24 (20.68-77.78)
Early lactation	512.16 (276.27-803.36)	519.38 (256.67-868.53)
Mid lactation	447.09 (284.17-704.88)	453.40 (278.13-688.85)
Late lactation	660.44 (412.15-995.51)	647.90 (400.93-967.83)

[1] None of the parameters reported are significantly different between the vitamin and control groups on a yearly basis.
[2] Control = 5 mL of saline 0.9% NaCl.
[3] Vitamins = 3 mL of 320 mg of folic acid and 2 mL of 10 mg of vitamin B_{12}.
[4] All amounts are in Canadian dollars (Can$).
[5] Including treatment and veterinary service costs.
[6] Calculated using 14.9 min d^{-1} to attend to one lactating cow and 9.6 min d^{-1} to one dry cow according to dairy herds averaging 50-100 cows housed in tie-stall barns (Roy et al., 2011).

5.5.2 Partial Budget

Data presented in Table 5.4 were used to calculate partial budgets according to scenario 1, quota kept constant or scenario 2, number of cows kept constant. Partial budgets were generated for a herd of 64 cows having 69.66 kg of BF d^{-1} which represents the averaged quota allocated to the 14 herds involved in this study. Number of cows or quota was adjusted according to the scenario studied (Tables 5.5 and 5.6).

5.5.2.1 Scenario 1: Quota Kept Constant

Table 5.5 summarizes variations in income and costs following a supplementation in folic acid and vitamin B_{12} when quota is kept constant. Cows receiving the vitamin supplement produced slightly less fat per year and because quota is expressed in kg of BF d^{-1}, one cow should be bought to fulfill the quota. The additional cow required to fulfill a quota of 69.66 kg of BF d^{-1} explained the increase of income of milk sold (Table 5.5). The lower replacement rate following a supplementation in folic acid and vitamin B_{12} decreased the income from animals sold as fewer cows were culled (-$908) even if more calves were sold ($550). Yearly income increased by $3712 following the adoption of a folic acid and vitamin B_{12} supplement. Replacement cost (-$2965) decreased in accordance with the decrease of replacement rate observed with the vitamin supplement. Reproduction cost (-$33) decreased as vitamin supplemented cows required, on average, 0.1 less breeding yr^{-1} to get pregnant than control cows. On the other hand, treatments and veterinary services ($36), feeding ($1980), labor ($1260), and other variable costs ($448) increased, mainly as a consequence of the decreased calving interval with the vitamin supplement. Indeed, within a year, more cows calve and require more treatments and veterinary services as calving is the critical period. Moreover, this scenario required an additional cow to fulfill the quota. The vitamin supplement cost $959 yr^{-1} which led to an overall increase of costs of $1685. Therefore, for an average dairy herd in Québec having 69.66 kg of BF d^{-1} of quota, a combined folic acid and vitamin B_{12} supplement given 3 weeks before the expected calving data until 8 weeks after the parturition increased the annual net income by $2027 (Table 5.5) which represents $31.18 cow^{-1} yr^{-1}.

Table 5.5 Partial budget following a supplementation in folic acid and vitamin B_{12} when the quota is kept constant (scenario 1)[1]

Input	$ yr^{-1} [2]
Variation in income	
Milk sold[3]	4070
Animals sold[4]	-358
Total	3712
Variation in costs	
Replacement	-2965
Reproduction[5]	-33
Treatments and veterinary services	36
Vitamin supplement	959
Feeding[6]	1980
Labor[6,7]	1260
Other variable costs	448
Total	1685
Variation in net income	2027
Per cow	31.18

[1] Treatment consisted of a supplement of 320 mg of folic acid and 10 mg of vitamin B_{12} given 3 weeks before the expected calving date until 8 weeks after parturition. The partial budget is based on a herd of 65 cows having a quota of 69.66 kg of butterfat d^{-1}. All calculations were made as follows: results from vitamin cows – results from control cows. Herd average values were used for the partial budget calculations.
[2] All values are in Canadian dollars (Can$).
[3] Canadian dairy producers are paid according to the amount of milk components sold.
[4] Including culled cows (value of $600), male calves sold after 7 days of age ($100), and female calves not needed for replacement ($250).
[5] Represents the difference in cost from artificial insemination of the vitamin group minus artificial insemination cost of the control group.
[6] Includes far-off, pre-calving, early, mid-, and late-lactation stages.
[7] Includes labor required for administration of supplement and disease treatments. Calculated using 14.9 min d^{-1} to attend to one lactating cow and 9.6 min d^{-1} to one dry cow according to dairy herds averaging 50-100 cows housed in tie-stall barns (Roy et al., 2011).

5.5.2.2 Scenario 2: Number of Cows Kept Constant

Table 5.6 presents the results of the partial budget for scenario 2. As described above, dairy cows receiving the vitamin supplement around calving had a lower yearly milk fat yield than control cows. To keep the number of cows constant within the herd following the adoption of the vitamin supplement, about 1.1 kg of BF d^{-1} of quota cannot be fulfilled. For

the calculation purposes, it was assumed that this amount of quota would be sold. The decreased income for milk sold (-$3111) could be explained by the vitamin supplement lessening the annual amount of fat produced. As for scenario 1, the number of culled cows (-$1071) decreased following the vitamin supplementation and the number of calves sold ($446) increased as a consequence for the lower replacement rate. The income decreased by $3736 with the vitamin supplement. The reduction of replacement cost (-$3500) is due to the decrease of the replacement rate with the vitamin supplement. The same explanation for the diminution of reproduction cost (-$131) used for the scenario 1 applies to the scenario 2, as the number of breedings decreased with the vitamin supplement. The slight increase of treatment and veterinary service ($14), feeding ($123), and labor ($103) costs was due to the decreased calving interval when using the vitamin supplement as for scenario 1. However, these supplementary costs are low. The combined supplement of folic acid and vitamin B_{12} cost $944 for this scenario. No depreciation was considered for quota as it keeps its value over time. Total costs were diminished by the vitamin supplement by $3425. Nonetheless, annual net income was decreased by $311 for an average herd of 64 cows by switching to the vitamin supplement management around parturition which represents a loss of $4.86 cow^{-1} yr^{-1}.

5.5.2.3 Comparison Between the 2 Scenarios

According to the results presented in Tables 5.5 and 5.6, it is clear that the scenario in which the quota is kept constant is more profitable for the dairy industry in Québec than the scenario in which the number of cows is kept constant. Indeed, the annual net income increased in scenario 1 unlike scenario 2 in which the annual net income decreased. To keep the quota constant with the vitamin supplementation given around calving and in early lactation, one cow might be bought (or increment of 1.5% of herd size). Thus, this scenario is possible only if there are stalls left in the barn.

The cost of supplementation at the break-even point for scenario 1 was $45.31 cow^{-1} yr^{-1} and for the scenario 2, $9.77 cow^{-1} yr^{-1}. In other words, if the vitamin supplement costs $45.31 or less in the case of the scenario 1, it is profitable for a dairy herd to give these vitamins around parturition and in early lactation, otherwise, it is not. For the entire period of administration, the cost of the combined supplement of folic acid and vitamin B_{12} was

estimated to \$16.50 regardless of the calving interval or \$14.55 cow^{-1} yr^{-1}. The vitamin supplement seems too expensive for the scenario 2 in which the number of cows is kept constant.

Table 5.6 Partial budget following a supplementation in folic acid and vitamin B_{12} in which the number of cows is kept constant (scenario 2)[1]

Input	\$ yr^{-1} [2]
Variation in income	
Milk sold[3]	-3111
Animals sold[4]	-625
Total	-3736
Variation in costs	
Replacement	-3500
Reproduction[5]	-131
Treatments and veterinary services	14
Vitamin supplement	944
Feeding[6]	123
Labor[6,7]	103
Interest on quota[8]	-978
Total	-3425
Variation in net income	-311
Per cow	-4.86

[1] Treatment consisted of 320 mg of folic acid and 10 mg of vitamin B_{12} given 3 weeks before the expected calving date until 8 weeks after parturition. To keep the number of cows at 64, about 1.1 kg of butterfat d^{-1} should be sold to avoid under-production. All calculations were made as follows: results from vitamin cows – results from control cows. Herd average values were used for the partial budget calculations.
[2] All amounts are in Canadian dollars (Can\$).
[3] Canadian dairy producers are paid according to the amount of milk components sold.
[4] Including culled cows (value of \$600), male calves sold after 7 days of age (\$100), and female calves not needed for replacement (\$250).
[5] Represents the difference in cost from artificial insemination of the vitamin group minus artificial insemination cost of the control group.
[6] Includes far-off, pre-calving, early, mid-, and late-lactation stages.
[7] Includes labor required for administration of supplement and disease treatments. Calculated using 14.9 min d^{-1} to attend to one lactating cow and 9.6 min d^{-1} to one dry cow according to dairy herds averaging 50-100 cows housed in tie-stall barns (Roy et al., 2011).
[8] The interest rate on quota was set to 6% which is financed over 10 years.

5.5.3 Sensitivity and Break-Even Point Analyses

5.5.3.1 Sensitivity Analyses

With the exception of replacement cost, changes in the input prices or costs by multiplying them by 0.8 or 1.2 did not have major impacts on variations in net income per cow nor on the break-even points of the supplement cost (Table 5.7). However, by increasing replacement cost by 20%, which represents the cost of raising heifers, variation in net income per cow per year for scenario 2 (number of cows kept constant) reached a positive value ($6.02 cow^{-1} yr^{-1}) and the break-even point for the cost of the supplement ($20.56 cow^{-1} yr^{-1}) was higher than the current cost of the supplement per cow per year ($14.55 cow^{-1} yr^{-1}). For scenario 1 in which quota was kept constant, the highest net income per cow per year was also obtained when replacement cost was increased by 20%. These results could be explained by the vitamin supplement decreasing replacement rate by 2.7%.

5.5.3.2 Break-Even Point Analyses

When milk fat concentration was decreased by 0.05 percentage point within each herd and other parameters remained unchanged between treatments, calving interval length would need to be decreased on average by 2.4 and 3.5 days by the use of the vitamin supplement for scenarios 1 and 2, respectively, to reach the break-even point. Regarding replacement rate, the vitamin supplement would need to decrease it by 1.7 and 3.1 percentage points for scenario 1 and 2, respectively, to reach the break-even point.

Table 5.7 Effects of changing the input costs or prices on variation in net income and break-even point following the use of a supplement of folic acid and vitamin B_{12} according to scenario 1 (quota kept constant) and scenario 2 (number of cows kept constant)

	Variation in net income		Supplement cost break-even point	
	$ cow^{-1} yr^{-1}			
Input[1]	Scenario 1	Scenario 2	Scenario 1	Scenario 2
Field study	31.18	-4.86	45.31	9.77
Milk price (+5%)	34.05	-7.24	48.18	7.39
Animal sold price				
+20%	30.09	-5.20	44.22	7.84
-20%	32.56	-2.93	46.39	11.70
Replacement cost				
+20%	40.18	6.02	54.30	20.56
-20%	22.18	-15.65	36.31	-1.10
Reproduction cost				
+20%	31.28	-4.45	45.40	10.17
-20%	31.08	-5.26	45.21	9.37
Treatment and veterinary service cost				
+20%	31.07	-4.90	45.20	9.73
-20%	31.29	-4.82	45.41	9.81
Feeding cost				
+20%	25.15	-5.25	39.28	9.38
-20%	37.20	-4.47	51.33	10.16
Labor cost				
+20%	27.35	-5.17	41.48	9.46
-20%	35.00	-4.54	49.13	10.08
Other variable costs				
+20%	29.82	-	43.94	-
-20%	32.54	-	46.67	-

[1] Prices and costs were multiplied by 0.8 (-20%) and 1.2 (+20%) in order to study the impact of 20% changes in these input values. Milk prices were multiplied by 1.05 (+5%) because it was considered as a more realistic increase.

5.5.4 Variation Among Herds

The study design allowed comparison of the economic response among herds following the use of a combined supplement of folic acid and vitamin B_{12} (Figure 5.1). Results are quite variable among herds. Indeed, for the scenario 1 in which the quota is kept constant, 8 herds out of 14 had a positive annual net margin per cow; varying from $3.75 to $304.23 cow^{-1} yr^{-1} for herds with positive net margins and from -$18.04 to -$445.60 cow^{-1} yr^{-1} for herds with the negative annual net margins. Similarly, for scenario 2 in which the number of cows is kept constant, the annual net margin per cow was positive for half of the herds involved in this experiment. The annual net margin per cow for herds with positive results ranged from $40.65 to $422.56 whereas it varied from -$67.24 to -$633.80 for herds with a negative annual net margin per cow.

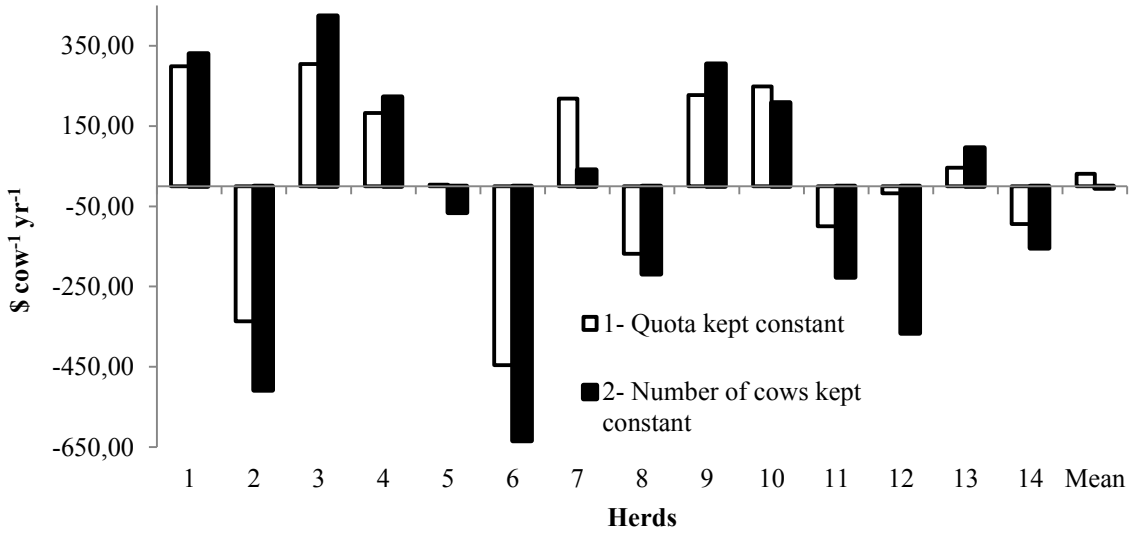

Figure 5.1 Results from partial budgets of the 14 herds studied and mean according to the 2 scenarios investigated: 1) quota kept constant and number of cows adjusted and 2) number of cows kept constant and quota sold or bought to avoid under- or over-production. Variations in net income are in Canadian dollars and are calculated from results of folic acid and vitamin B_{12} supplemented cows minus results from control cows

The reason why the response following the vitamin supplement was so variable among herds remains unclear. No link with milk production can be found while milk yield ranged from 8 341 to 11 034 kg on a 305-day basis for herds with a positive annual net margin per

cow. The variation could not be explained by diet distribution either as the half of herds with a positive annual net margin per cow used a total mixed ration and the other half, an individual concentrate feeding system. The current state of knowledge does not allow predicting the vitamin supply from ruminal synthesis under different dietary managements or diet composition. Non-dietary factors could not be discarded as being a source of variation among dairy herds (Bach et al., 2008).

In scenario 1, a positive annual net margin per cow was mainly due to a decrease in costs (7 herds out of 8) such as replacement rate and reproduction whereas costs were increased for all herds with a negative annual net margin per cow following the supplementation. In scenario 2, income was increased in 5 herds out of 7 with a positive annual net margin per cow while no herd having a negative annual net margin increased their income.

It could be hypothesized that, in some herds, ruminal synthesis of folic acid and vitamin B_{12} was not sufficient to meet requirements of dairy cows and to optimize performance, then, those cows benefit from an exogenous supply of these vitamins. The huge variation in results among herds suggests that each dairy herd should evaluate the profitability of a combined supplement of folic acid and vitamin B_{12} before giving it around parturition and in early lactation.

5.6 Conclusion

In summary, results from partial budgets studying the potential profitability of a combined supplement of folic acid and vitamin B_{12} given 3 weeks before the expected calving date until 8 weeks after parturition in dairy herds in Québec were variable. Within the 14 herds involved in this trial, the vitamin supplement was profitable for 8 and 7 herds for the scenario in which the quota is kept constant and for the scenario in which the number of cows is kept constant, respectively. Considering that solely milk fat concentration was decreased by the vitamin supplement within each herd, calving interval would need to be shortened by 2.4 and 3.5 days for scenarios 1 and 2, respectively, to reach the break-even point. Regarding replacement rate, it would need to be decreased by 1.7 and 3.1 percentage points by the use of the vitamin supplement for scenarios 1 and 2, respectively, to achieve

the break-even point. One of the pitfalls of evaluating the profitability of a combined supplement of folic acid and vitamin B_{12} in a dairy herd is that the current knowledge does not allow predicting the response on an individual herd basis mostly caused by the difficulty to predict the amount of vitamin synthesized by ruminal microflora. Moreover, to facilitate its adoption by dairy producers, the combined supplement of folic acid and vitamin B_{12} should become commercially available.

5.7 Acknowledgements

The authors acknowledge the invaluable implication of dairy producers, veterinarians, and nutritionists in this study. We are grateful to Véronique Roy (Agriculture et Agroalimentaire Canada, Sherbrooke, QC) for preparing folic acid solutions, and Vétoquinol (Lavaltrie, QC) for generously providing vitamin B_{12}. This project was financed by the Action Concertée Novalait-FQRNT-MAPAQ-AAC and Conseil pour le développement de l'agriculture du Québec (CDAQ). For the latter, funding was provided through Agriculture and Agri-Food Canada's Advancing Canadian Agriculture and Agri-Food (ACAAF) program.

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6 Article sur la vitamine B_{12} dans le lait – Premier projet

Concentration en vitamine B_{12} du lait de vaches recevant des injections intramusculaires hebdomadaires d'acide folique et de vitamine B_{12} dans les troupeaux laitiers commerciaux

Vitamin B_{12} concentration in milk of cows receiving weekly intramuscular injections of folic acid and vitamin B_{12} in commercial dairy herds

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Cet article est une première version du manuscrit qui sera soumis au *Journal of Dairy Science*.

6.1 Résumé

Seulement les bactéries et les archaebactéries peuvent synthétiser la vitamine B_{12} si l'apport en cobalt est suffisant. En conséquence, la source naturelle de vitamine B_{12} dans le régime alimentaire des êtres humains provient des produits animaux, particulièrement ceux des ruminants. Cette étude a été réalisée afin d'évaluer l'effet d'un supplément combiné d'acide folique et de vitamine B_{12} donné durant la période de transition et en début de la lactation sur la concentration en vitamine B_{12} du lait dans les troupeaux laitiers et de décrire la variabilité concernant la concentration en vitamine B_{12} du lait entre les troupeaux et les vaches d'un même troupeau selon les traitements. Un total de 805 vaches (271 primipares et 534 multipares) dans 15 troupeaux ont été impliquées. Les vaches ont été assignées à des injections intramusculaires hebdomadaires de soit 5 ml de 1) saline 0,9 % NaCl (témoin) ou 2) 320 mg d'acide folique et 10 mg de vitamine B_{12} (vitamines). Les traitements ont débuté 3 semaines avant la date prévue du vêlage et ont duré jusqu'à 8 semaines après la parturition. Les échantillons de lait ont été prélevés à 27,9 ± 4,1 et 55,8 ± 4,3 jours en lait. La concentration de vitamine B_{12} dans le lait a été significativement augmentée par le supplément de vitamines comparativement aux vaches témoins, de 3140 à 5420 pg/ml. La concentration en vitamine B_{12} du lait n'a pas été différente entre les vaches témoins primipares et multipares tandis que celle des vaches primipares ayant reçu le supplément a été plus élevée que celle des vaches multipares dans le groupe vitamines. Les jours en lait n'ont pas affecté la concentration en vitamine B_{12} dans le lait. Pour les deux traitements, la concentration en vitamine B_{12} du lait a été hautement variable entre les troupeaux, mais également à l'intérieur de chaque troupeau. En résumé, un supplément combiné d'acide folique et de vitamine B_{12} a amélioré la valeur nutritive du lait en augmentant sa concentration en vitamine B_{12} pour les 15 troupeaux laitiers, mais selon des ampleurs différentes.

Mots clés : vache laitière, lait, vitamine B_{12}, acide folique

6.2 Abstract

Only bacteria and archaebacteria can synthetize vitamin B_{12} if cobalt supply is adequate. Consequently, the natural source of vitamin B_{12} in human diets comes from animal products, especially those from ruminants. This study was undertaken to evaluate the effect of a combined supplement of folic acid and vitamin B_{12} given during the transition period and in early lactation on milk concentration of vitamin B_{12} in commercial dairy herds as well as to describe the variability regarding vitamin B_{12} concentration in milk among and within herds according to treatments. A total of 805 dairy cows (271 primiparous and 534 multiparous) in 15 commercial herds were involved. Every 2 months and within each herd, from February to December 2010, cows were assigned, based on parity, predicted 305-day milk yield, and calving interval, to weekly intramuscular injections of either 5 mL of 1) saline 0.9% NaCl (Control) or 2) 320 mg of folic acid and 10 mg of vitamin B_{12} (Vitamins). Treatments began 3 weeks before the expected calving date and lasted until 8 weeks after parturition. Milk samples were taken at 27.9 ± 4.1 and 55.8 ± 4.3 days in milk. Vitamin B_{12} concentration in milk was significantly increased by the vitamin supplement as compared with milk of control cows, from 3,140 to 5,420 pg/mL. Milk concentration of vitamin B_{12} was not different between primiparous and multiparous control cows whereas primiparous cows receiving the vitamin supplement had greater vitamin B_{12} concentration in milk than multiparous cows in the vitamin group. Vitamin B_{12} concentration in milk was unaffected by days in milk. In both treatments, vitamin B_{12} concentration in milk was highly variable among and within dairy herds. Among herds, vitamin B_{12} concentration in milk ranged from 2,246 to 3,851 pg/mL for control group and from 4,344 to 6,581 pg/mL for cows receiving the vitamin supplement. In summary, a combined supplement of folic acid and vitamin B_{12} enhanced the nutritive value of milk by increasing its vitamin B_{12} concentration for the 15 dairy herds involved in the present experiment, but according to a variable extent among herds.

Key words: dairy cow, milk, vitamin B_{12}, folic acid

6.3 Introduction

Only prokaryotes as bacteria and archaebacteria are able to synthesize vitamin B_{12} if cobalt supply is sufficient; plants and fungi being unable to (McDowell, 1989; Martens et al., 2002). However, animal products contain fair amounts of this vitamin, because of the close link between animals and prokaryotes dwelling in their digestive systems. Consequently, the natural source of vitamin B_{12} in human diets comes from animal products, especially those from ruminants. Studies showed that vitamin B_{12} in cow milk is absorbed more efficiently than its synthetic form used in supplements (Matte et al., 2012) and in fish and meat products (Vogiatzoglou et al., 2009).

Since 1998, in Canada and in USA, it is mandatory to fortify white flour and other grains with folic acid (Colapinto et al., 2011) in order to decrease the risk of birth neural tube defects. A Canadian survey showed that folate deficiency was virtually nonexistent in the Canadian population and 40% of the people surveyed had a high red blood cell folate concentration (Colapinto et al., 2011) whereas vitamin B_{12} deficiency was detected in 4.6% of them (MacFarlane et al., 2011). In human, high folate status could be detrimental to those having vitamin B_{12} deficiency (Selhub and Paul, 2011). Indeed, low vitamin B_{12} status was associated with both anemia and cognitive impairment when serum folate concentration was high (Selhub et al., 2007). Optimizing vitamin B_{12} concentration in milk of dairy cows could be a natural way to partially overcome this issue.

In the experiment of Girard and Matte (2005), vitamin B_{12} concentration in milk of dairy cows receiving a weekly injection of 10 mg of vitamin B_{12} and a dietary supplement of 4 mg of folic acid/kg of body weight per day increased by more than 300% as compared with milk from cows receiving a supplement of folic acid alone. Subsequent studies also showed a significant increase of vitamin B_{12} concentration in milk from cows receiving a combined supplement of folic acid and vitamin B_{12} (Graulet et al., 2007; Preynat et al., 2009a,b) or a supplement of vitamin B_{12} alone (Akins et al., 2013).

Chassaing et al. (2011) observed that concentration of vitamin B_{12} in milk of cows fed a corn silage-based diet was higher than milk from cows fed a grass-based ration. Similarly,

Hunt et al. (1954) noted that vitamin B_{12} synthesis by ruminal microorganisms was stimulated *in vitro* with starch as a source of energy as compared with no starch added in the artificial rumen. Previous studies concluded that apparent ruminal synthesis of vitamin B_{12} was increased by a high forage to concentrate ratio as compared with a low forage to concentrate ratio (Santschi et al., 2005; Seck, 2012). Schwab et al. (2006) concluded that apparent ruminal synthesis of vitamin B_{12} was positively correlated with dietary sugars and NDF and negatively correlated with dietary NFC, NSC, and starch content.

Given previous results, it was therefore hypothesized that vitamin B_{12} concentration in milk would be increased by the use of the combined supplement of folic acid and vitamin B_{12}, enhancing its nutritive value. Moreover, as ruminal synthesis of vitamin B_{12} is affected by diet composition and this synthesized vitamin B_{12} is then partially secreted in milk (Ferlay et al., 2013), it was hypothesized that vitamin B_{12} concentration in milk would be variable among dairy herds as diet compositions and managements were different. The purpose of this work was to assess the effect of a combined supplement of folic acid and vitamin B_{12} given during peripartum period and in early lactation on vitamin B_{12} concentration in milk of dairy cows in commercial herds. Moreover, it was aimed to describe the variability regarding vitamin B_{12} concentration among herds and individuals.

6.4 Materials and Methods

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

6.4.1 Herds and Cows

Herds and cows were previously described by Duplessis et al. (2014a). Briefly, 15 commercial dairy herds located around Québec City, QC, Canada and 805 dairy cows (271 primiparous and 534 multiparous cows; 780 Holstein and 25 Jersey cows; 14 Holstein and 1 Jersey herds) were involved. Size of dairy herds ranged from 25 to 120 cows. Cows were housed in tie-stall barns and milked twice daily. Diet managements differed among herds; 7

herds used a total mixed ration and 8 herds had individual concentrate feeding systems. Rations among herds were mainly based on legume-grass silage, corn silage, and concentrate as ground corn. Among herds, rations were computed to meet or exceed cobalt requirements (0.11 mg/kg of dry matter (DM)) according to the NRC (2001) and averaged 0.56 mg/kg of DM (ranging from 0.26 to 0.94 mg/kg of DM) for rations in early lactation.

Data on culling rate, diseases, reproduction, lactational performance, and the economic impact following a combined supplement of folic acid and vitamin B_{12} in commercial dairy herds were presented elsewhere (Duplessis et al., 2014a,b,c).

6.4.2 Treatments

The study lasted 14 months from February 2010 to April 2011. During this period, each herd was visited every 2 weeks on the same schedule. Every 2 months and within each herd, cows were assigned, based on parity (primiparous vs. multiparous), previous 305-day milk yield, and calving interval, to weekly intramuscular injections of 5 mL of either 1) saline 0.9% NaCl (Control) or 2) 320 mg of folic acid and 10 mg of vitamin B_{12} (Vitamins; pteroylmonoglutamic acid, MP Biomedicals, Solon, OH and cyanocobalamin, 5,000 µg/mL, Vétoquinol, Lavaltrie, QC, Canada). Treatments began 3 weeks before the expected calving date and lasted until 8 weeks after parturition. Producers were asked to inject the treatment to dairy cows weeks among visits. Disposable syringes containing the studied solutions were prepared every 2 weeks and were kept refrigerated in a box to protect them from light.

6.4.3 Measurements and Analyses

Milk samples for determination of vitamin B_{12} concentration were taken at 27.9 ± 4.1 and 55.8 ± 4.3 days in milk (DIM). After collection, milk samples were delicately shaken to obtain a homogeneous blend, poured in 15 mL cone-shaped tubes, and put on ice for transportation. Upon arrival at the laboratory, they were frozen at 20°C and stored until analysis. The technique based on folate analysis was adapted for vitamin B_{12} according to DeVries et al. (2005), Hyun and Tamura (2005), and Chen and Eitenmiller (2007). Milk

samples were defrosted in a water bath at 37°C for 30 minutes. Sample preparation was done as follows: 2.5 mL of milk, 2.5 mL of 0.1 M of Na_2HPO4, and 25 µL of 1 M NaCN were poured in a 15 mL cone-shaped tube and shaken. Then, 100 µL of protease (protease Type XIV: bacterial, from *Streptomyces griseus*; EC number = 232-909-5; Sigma-Aldrich, Oakville, ON, Canada) was added, incubated for 90 minutes and shaken frequently. The tubes were autoclaved for 5 minutes at 100 °C to stop enzyme activity and then placed in cold water for 5 minutes. Tubes were centrifuged at 5,000 × *g* for 10 minutes at 4°C. A volume of 50 µL of supernatant and 150 µL of ultra-pure water were placed in 2.0 mL Eppendorf tubes and kept frozen at -20°C or used immediately for analysis by radioassay with a commercial kit (SimulTRAC® B_{12}/FOLATE-S, MP Biomedicals, Solon, OH). The inter-assay CV was 2.2%.

6.4.4 Statistical Analysis

Vitamin B_{12} concentration in milk was analyzed with the MIXED procedure of SAS (version 9.2, SAS Institute, 2008, Cary, NC) with repeated measures. Treatment, parity, block, herd and, time as well as treatment × parity, treatment × herd, treatment × time, parity × time, and treatment × parity × time interactions were fixed effects. Blocks meant 2 months assignation intervals and parity referred to primiparous and multiparous cows after calving. Seven different covariance structures were tested (CS, CSH, AR(1), ARH(1), TOEP, TOEPH, and UN), and UN was chosen because fit statistics were the smallest.

When an interaction 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 $0.05 < P \leq 0.10$.

6.5 Results and Discussion

Vitamin B_{12} concentration in milk increased by 73% for cows receiving the supplement of folic acid and vitamin B_{12} as compared with control cows, from 3,140 to 5,420 ± 75 pg/mL ($P < 0.0001$). A glass (250 mL) of milk from cows receiving the vitamin supplement provides 56% of the vitamin B_{12} recommended daily allowance (2.4 µg) for adults and

children over 13 years of age (Health Canada, 2006). This result is in accordance with previous studies in which intramuscular injections of vitamin B_{12}, alone or combined with a supplement of folic acid, increased significantly the concentration of vitamin B_{12} in milk (Girard and Matte, 2005; Preynat et al., 2009b; Akins et al., 2013). An interaction treatment × parity was observed ($P = 0.002$). Indeed, there was no significant difference in vitamin B_{12} concentration in milk between primiparous and multiparous control cows ($P = 0.47$) whereas, in cows receiving the vitamin supplement, milk concentration of vitamin B_{12} was greater for primiparous cows (5,660 ± 114 pg/mL) than for multiparous cows (5,179 ± 82 pg/mL; $P = 0.0003$). Even though no parity effect was noted in control cows, a dilution effect could not be ruled out to explain the previous result as milk yield the day of sampling was not recorded and, within the first 60 DIM, multiparous cows produced a greater amount of milk per day than did primiparous cows (Duplessis et al., 2014b). Akins et al. (2013) did not report any effect of parity on vitamin B_{12} concentration in milk of dairy cows receiving intramuscular injections of vitamin B_{12} or not. In the current study, vitamin B_{12} concentration in milk of cows was not affected by DIM ($P = 0.11$) as previously reported within the first 8 weeks of lactation (Graulet et al., 2007).

Concentration of vitamin B_{12} in milk was variable among herds for both treatments (treatment × herd interaction, $P = 0.0009$; Figure 6.1) although the combined supplement of folic acid and vitamin B_{12} significantly increased vitamin B_{12} concentration in milk within each herd ($P \leq 0.0007$). Among herds, vitamin B_{12} concentration in milk ranged from 2,246 to 3,851 pg/mL for control cows ($P = 0.03$; Table 6.1) and from 4,344 to 6,581 pg/mL for cows receiving the vitamin supplement ($P < 0.0001$; Table 6.2).

According to the literature, vitamin B_{12} concentration in milk of cows not receiving a vitamin B_{12} supplement is highly variable in early lactation; vitamin B_{12} concentration in milk between 1,575 to 4,781 pg/mL has been reported (Preynat et al., 2009b; Akins et al., 2013). This variation could partially be explained by different type and quality of ingredients fed, and different management among experiments and herds involved in the current trial. Indeed, different classes of microorganisms in the rumen are involved in the degradation of forage and concentrate (Goff and Horst, 1997; Petri et al., 2012) and Dryden

et al. (1962) showed that some strains of ruminal bacteria could synthesize a greater amount of vitamin B_{12} than others.

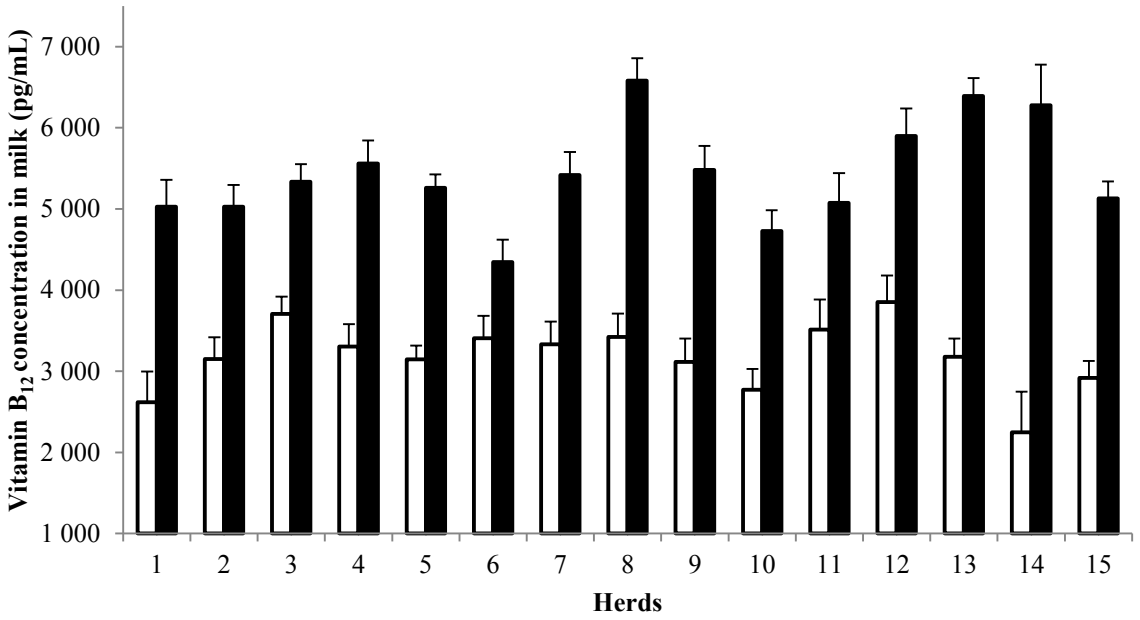

Figure 6.1 Vitamin B_{12} concentration in milk of cows receiving 5 mL of saline 0.9% NaCl (open bars) or 3 mL of 320 mg of folic acid and 2 mL of 10 mg of vitamin B_{12} (closed bars) among herds involved in this study (treatment × herd interaction, *P* = 0.0009). Number of cows per herd involved in 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

The lowest milk concentration of vitamin B_{12} for control cows (herd 14 on Figure 6.1) was observed in the Jersey herd. Previous studies also reported that concentration of vitamin B_{12} in milk from Jersey cows was lower than milk from Holstein cows (Anthony et al., 1951; Miller et al., 1966).

A variation in vitamin B_{12} concentration in milk from cows receiving a supplement of vitamin B_{12}, alone or combined with a supplement of folic acid, has also been reported, between 4,431 to 6,816 pg/mL (Preynat et al., 2009b; Akins et al., 2013). In the current study, extent of the response to the vitamin supplement on vitamin B_{12} concentration was variable as well. Indeed, the increase of vitamin B_{12} concentration in milk varied from 936 to 4,031 pg/mL among herds (Figure 6.1).

Table 6.1 Vitamin B_{12} concentration in milk of control cows[1] in early lactation according to dairy herds

Herds	Cows (n)	Vitamin B_{12} concentration in milk (pg/mL)			
		Average[2]	SD	Minimum	Maximum
1	13	2,617	531	1,795	3,333
2	25	3,150	910	1,857	5,017
3	39	3,706	1,057	1,671	6,121
4	23	3,305	848	1,878	5,040
5	61	3,148	581	1,710	4,589
6	22	3,407	914	2,057	5,012
7	22	3,333	678	1,972	5,116
8	21	3,422	908	1,874	5,236
9	21	3,113	1,163	1,213	6,702
10	27	2,772	711	1,754	5,454
11	13	3,515	751	2,256	4,730
12	16	3,851	1,067	2,509	6,755
13	35	3,179	850	1,741	5,787
14	7	2,246	607	1,626	3,292
15	40	2,919	910	1,553	5,668

[1] Control = 5 mL of saline 0.9% NaCl.

[2] Vitamin B_{12} concentrations in milk were variable among herds ($P = 0.03$).

Milk concentration of vitamin B_{12} was also highly variable among individual control cows within each herd (Table 6.1) as similarly reported by Collins et al. (1951), Gregory et al. (1958), and Rutten et al. (2013). Rutten et al. (2013) showed that genotype of the cow affects vitamin B_{12} concentration in milk and concluded that genetic selection could increase vitamin B_{12} concentration in milk. Variability regarding vitamin B_{12} concentration in milk among individual cows receiving the vitamin supplement was observed as well (Table 6.2). As blood samples were not taken on these cows, it was not possible to study correlations between plasma and milk concentrations of vitamin B_{12}.

Table 6.2 Vitamin B_{12} concentration in milk of cows receiving the vitamin supplement[1] in early lactation according to dairy herds

		Vitamin B_{12} concentration in milk (pg/mL)			
Herds	Cows (n)	Average[2]	SD	Minimum	Maximum
1	17	5,028	1,396	3,205	8,259
2	25	5,029	1,086	3,433	7,767
3	38	5,336	1,434	2,273	8,461
4	22	5,559	1,413	3,199	7,950
5	63	5,259	1,337	2,739	9,524
6	23	4,344	983	2,512	7,160
7	21	5,417	2,333	2,975	10,565
8	23	6,581	1,805	3,481	11,054
9	21	5,482	1,522	2,781	10,124
10	27	4,729	1,523	2,694	9,584
11	13	5,075	1,084	3,493	7,075
12	15	5,898	1,765	3,632	10,761
13	35	6,392	1,857	2,984	11,572
14	7	6,277	2,213	3,762	9,809
15	40	5,130	1,157	2,902	8,268

[1] Vitamins = 3 mL of 320 mg of folic acid and 2 mL of 10 mg of vitamin B_{12}.
[2] Vitamin B_{12} concentrations in milk were variable among herds ($P < 0.0001$).

6.6 Conclusion

Milk concentration of vitamin B_{12} was increased by 73% by the combined supplement of folic acid and vitamin B_{12}, from 3,140 to 5,420 ± 75 pg/mL. For both treatments, vitamin B_{12} concentration in milk was highly variable among dairy herds and cows. The lowest vitamin B_{12} concentration in milk of control cows was observed in the Jersey herd. Among herds, one glass of 250 mL from control cows provided between 23 and 40% of the vitamin B_{12} recommended daily allowance.

6.7 Acknowledgements

The authors are grateful to the dairy producers, nutritionists, and veterinarians who actively participated in this experiment. We thank Véronique Roy and Valérie Beaudet (Agriculture et Agroalimentaire Canada, Sherbrooke, QC, Canada) for technical assistance. We acknowledge Vétoquinol (Lavaltrie, QC, Canada) for generously providing vitamin B_{12}. This project has been made possible through the support from Entente de collaboration pour l'innovation en production et transformation laitière Novalait-AAC-FQRNT-MAPAQ and from Conseil pour le développement de l'agriculture du Québec (CDAQ). For the latter, funding was provided through Agriculture and Agri-Food Canada's Advancing Canadian Agriculture and Agri-Food (ACAAF) program.

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7 Article sur la production laitière et le statut énergétique – Deuxième projet

Effets d'injections intramusculaires d'acide folique et de vitamine B_{12}, seules ou combinées, sur les performances laitières et le statut énergétique de vaches laitières multipares

Effects of intramuscular injections of folic acid and vitamin B_{12}, alone or combined, on lactational performance and energy status of multiparous dairy cows

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Cet article est une première version du manuscrit qui sera soumis au *Journal of Dairy Science*.

7.1 Résumé

L'objectif de cette étude était d'évaluer les effets d'un supplément d'acide folique et de vitamine B_{12}, donné seul ou combiné, par injections intramusculaires, de 3 semaines avant la date de vêlage prévue jusqu'à 7 semaines postpartum sur les performances laitières et le statut énergétique des vaches laitières. Vingt-quatre vaches multipares ont été assignées à soit 0 ou 320 mg d'acide folique et 0 ou 10 mg de vitamine B_{12} selon un dispositif factoriel 2 × 2. La production laitière des vaches ayant reçu le supplément d'acide folique a atteint un plateau plus tôt que celles ne l'ayant pas reçu et a diminué de 4,9 et 5,1 kg/j aux semaines 6 et 7 de la lactation, respectivement. La quantité de lactose produit a également diminué aux semaines 6 et 7 avec le supplément d'acide folique. Cependant, les concentrations et les quantités produites de gras, protéines et solides totaux dans le lait n'ont pas été affectées par les traitements. La cote d'état de chair postpartum était plus élevée pour les vaches recevant le supplément d'acide folique comparativement aux vaches ne l'ayant pas reçu. La concentration plasmatique de glucose a été augmentée par le supplément d'acide folique. La concentration plasmatique d'acides gras libres postpartum a été plus basse pour les vaches qui ont reçu le supplément d'acide folique comparativement à celles ne l'ayant pas reçu. La concentration plasmatique d'acide méthylmalonique était basse et n'a pas été affectée par les traitements suggérant que l'apport de vitamine B_{12} était adéquat, même pour les animaux témoins. En conclusion, le supplément d'acide folique, seul ou combiné avec le supplément de vitamine B_{12}, a été bénéfique pendant les 5 premières semaines de la lactation tel que suggéré par la production laitière similaire entre les traitements, la plus basse concentration plasmatique d'acides gras libres et la plus haute concentration plasmatique de glucose et cote d'état de chair pour les vaches recevant le supplément d'acide folique comparativement aux vaches ne l'ayant pas reçu. Néanmoins, les effets bénéfiques du supplément d'acide folique se sont estompés à mesure que la lactation a progressé.

Mots clés : vache laitière, acide folique, vitamine B_{12}, état énergétique

7.2 Abstract

The purpose of this experiment was to evaluate the effects of a folic acid and vitamin B_{12} supplement, given alone or combined, by intramuscular injections, from 3 weeks before the expected calving date until 7 weeks postpartum on lactational performance and energy status of dairy cows. Twenty-four multiparous cows were assigned to 6 blocks of 4 cows each according to previous 305-day lactation yield to either 0 or 320 mg of folic acid and 0 or 10 mg of vitamin B_{12} in a 2 × 2 factorial arrangement. Milk yield of cows receiving the folic acid supplement reached a plateau earlier than cows that did not. Moreover, the folic acid supplement decreased milk production by 4.9 and 5.1 kg/d at weeks 6 and 7 of lactation, respectively. Milk yield of lactose was also diminished at weeks 6 and 7 by the folic acid supplement. However, fat, protein, as well as total solid concentrations and yields were unaffected by treatments. Postpartum body condition score was higher for cows receiving the folic acid supplement as compared with cows that did not. Plasma concentration of folates was increased by the folic acid supplement when combined with the vitamin B_{12} supplement. The vitamin B_{12} supplement increased concentration of vitamin B_{12} in plasma. Plasma concentration of glucose was increased by the folic acid supplement and was 3.40 and 3.20 ± 0.05 μM with the use of folic acid supplement or not, respectively. Postpartum plasma concentration of nonesterified fatty acids was lower for cows that received weekly folic acid supplement as compared with cows that did not. Plasma concentration of methylmalonic acid was low and unaffected by treatments suggesting that vitamin B_{12} supply was adequate, even for unsupplemented cows. Plasma concentrations of Cys, His, and Phe were increased whereas plasma concentrations of Gly and Ile were decreased by the folic acid supplement. Folic acid and vitamin B_{12} supplements, alone or combined, increased plasma Hcy and Tyr concentrations throughout the experimental period. It could be concluded that the folic acid supplement, alone or combined with vitamin B_{12}, was beneficial within the first 5 weeks of lactation as suggested by similar milk yield among treatments, lower plasma nonesterified fatty acid concentration and higher plasma glucose concentration and body condition score for cows receiving the folic acid supplement than cows that did not. However, the effects of the folic acid supplement faded out as lactation progressed.

Key words: dairy cow, folic acid, vitamin B_{12}, energy status

7.3 Introduction

In mammals, vitamin B_{12} is involved in 2 metabolic pathways as a coenzyme. First, this vitamin is essential to transfer a methyl group from 5-methyl-tetrahydrofolate, the methylated form of folic acid, to homocysteine (Hcy) to form Met (Le Grusse and Watier, 1993), often a first limiting amino acid (AA) for milk production with typical North American diets (Schwab et al., 1992). Furthermore, Met forms S-adenosylmethionine which is the major methyl group donor in mammals and milk production increases requirements for methyl groups (Girard and Matte, 2005b). Second, before entering into the Krebs cycle for providing energy, propionate must be transformed to methylmalonyl-CoA and then to succinyl-CoA involving vitamin B_{12} as a coenzyme (Scott, 1999). In ruminants, between 50 to 60% of glucose synthesis originates from propionate (Danfær et al., 1995; Reynolds, 2006). A lack of vitamin B_{12} causes an accumulation of methylmalonyl-CoA in blood which is converted into methylmalonic acid (MMA). It has been showed that a supplement of vitamin B_{12} given in early lactation to primiparous cows also receiving dietary folic acid decreased the serum concentration of MMA by 17.5% as compared with control cows receiving a supplement of folic acid alone (Girard and Matte, 2005a). This result suggested to the authors that vitamin B_{12} status of cows not receiving the vitamin B_{12} supplement was not sufficient and had probably consequences for bioenergetics.

In some trials, a dietary supplement of folic acid given to the dairy cows increased milk production or milk components in early lactation (Girard and Matte, 1998; Graulet et al., 2007; Girard et al., 2009) whereas sometimes it did not (Girard et al., 2005). The authors concluded that these different responses in milk production were accounted for availability of vitamin B_{12} estimated from plasma concentration. Indeed, cows with plasma concentration of vitamin B_{12} higher than 200 pg/mL had a higher milk response to folic acid supplementation than cows with concentrations lower than 200 pg/mL. In accordance with this statement, milk production increased by 12% in early lactation for multiparous cows intramuscularly injected with both folic acid and vitamin B_{12} as compared with control cows or cows receiving a supplement of folic acid alone (Preynat et al., 2009a).

Previous studies cited above concluded that a combined supplement of folic acid and vitamin B_{12} seems to improve energy metabolism in early lactation (Girard and Matte, 2005a; Graulet et al., 2007; Preynat et al., 2009a) by enhancing lactational performance without increasing dry matter intake (DMI). In 15 commercial dairy herds, fat to protein ratio was decreased in early lactation by weekly injections of folic acid and vitamin B_{12} suggesting that cows receiving the vitamin supplement had a better energy status than control cows (Duplessis et al., 2014).

Given previous results and respective roles of folic acid and vitamin B_{12} described above, it was hypothesized that a combined supplement of folic acid and vitamin B_{12} given by weekly intramuscular injections around parturition and in early lactation would increase lactational performance and enhance energy metabolism. This study was then undertaken to measure lactational performance and energy status of multiparous dairy cows receiving supplements of folic acid and vitamin B_{12}, alone or combined, from 3 weeks before the expected calving date until 7 weeks of lactation. It is noteworthy that results presented in the current paper were obtained from a study in which it was aimed to evaluate if better energy metabolism following a combined folic acid and vitamin B_{12} supplementation observed in previous studies was caused by more propionate entering into the Krebs cycle to provide energy (Chapter 8, companion paper). It was chosen to give folic acid and vitamin B_{12} supplements alone or combined to study if the supplement of folic acid is required for the expression of the supplement of vitamin B_{12} on the propionate metabolic pathway described above.

7.4 Materials and Methods

7.4.1 Cows and Treatments

Twenty-four multiparous Holstein cows from the dairy herd at the Agriculture and Agri-Food Canada Research Centre (Sherbrooke, QC, Canada) were involved in this study. The experimental protocol was approved by the Institutional Committee for Animal Care of the Sherbrooke Research Centre. Care of cows followed the recommended code of practice of the National Farm Animal Care Council (2009) and the guidelines of the Canadian Council

on Animal Care (2009). Animals were kept in a tie-stall barn under 1730 h of light per day (0530 to 2300 h) and milked twice daily (0730 and 1930 h). The experimental period began 3 weeks before the expected calving date and lasted until 9 weeks of lactation. However, only data collected until 7 weeks are presented in the current paper because the cows were submitted to a feed restriction at week 8 before a surgery to insert a catheter into one ruminal vein to infuse tracers labelled with stable isotope the following week (Chapter 8, companion paper). Before parturition, cows were fed a close-up diet and a lactation diet after calving (Table 7.1). Long hay was given at 0730 h and total mixed ration was served once daily at 0830 h for the entire experimental period. Feed was pushed toward cows throughout the day. Orts were removed at 0700 h. Daily refusals were weighed, DMI was calculated and feed offered was adjusted if needed allowing 10% refusals. Cows had free access to water.

Cows were assigned to 6 blocks of 4 animals each according to previous 305-day milk production to one of the following treatments: 1) saline 0.9% NaCl (B_9-B_{12}-); 2) 320 mg of folic acid (B_9+B_{12}-; pteroylmonoglutamic acid, MP Biomedicals, Solon, OH); 3) 10 mg of vitamin B_{12} (B_9-B_{12}+; cyanocobalamine, 5,000 µg/mL, Vétoquinol, Lavaltrie, QC, Canada) or; 4) 320 mg of folic acid and 10 mg of vitamin B_{12} (B_9+B_{12}+). Intramuscular injections of 5 mL were given weekly during the experimental period. The injected amount of vitamins was chosen according to the experiments of Girard et al. (1989) and Preynat et al. (2009a). The parenteral route was chosen instead of dietary supplementation to bypass the utilization and destruction of vitamins by the rumen microorganisms (Santschi et al., 2005) and therefore to assess only the metabolic role of these vitamins.

The previous 305-day milk yield did not differ among treatments and averaged 9,227 ± 592 kg ($P = 0.94$). Before the beginning of the experiment, 4 weeks before the expected calving date, body weight (BW) averaged 703, 722, 736, and 735 ± 22 kg, and body condition score (BCS) averaged 3.00, 3.13, 3.25, and 2.88 ± 0.18 for B_9-B_{12}-, B_9+B_{12}-, B_9-B_{12}+, and B_9+B_{12}+, respectively ($P \geq 0.18$).

Table 7.1 Ingredients and nutrient composition of diets fed to dairy cows

	Diet	
Item	Close-up	Lactation
Ingredients (% DM)		
Grass hay	39.4[1]	4.8[2]
Legume-grass silage	-	28.6[3]
Corn silage	33.0[4]	28.5[5]
Cracked corn	-	19.4
Soybean meal	13.9	9.4
Distiller grain (corn)	-	1.2
Corn gluten meal	-	1.2
Canola meal	-	0.8
Micronized soybean	-	0.8
Beet pulp	12.4	3.2
Mineral and vitamin premix	1.0[6]	1.5[7]
Calcium carbonate	0.4	0.5
Nutrient composition		
DM (%)	47.7	46.3
CP (%)	13.6	16.5
RUP[8] (%)	6.1	7.4
MP[9] (g/d)	1,322	2,284
Met[9] (% of MP)	1.78	1.83
ADF (%)	18.9	20.5
NDF (%)	30.4	31.2
NE_L[9] (Mcal/d)	22.1	32.5
K (%)	1.26	1.66
Ca (%)	0.65	0.83
P (%)	0.38	0.42
Mg (%)	0.32	0.24
S (%)	0.15	0.17
Cl (%)	0.27	0.44
Co (mg/kg)	1.45	1.04

[1] 10.9 ± 1.7% CP; 2.2 ± 0.5% soluble protein; 35.2 ± 3.5% ADF; 55.2 ± 2.1% NDF (n = 4).
[2] 11.3 ± 0.9% CP; 2.7 ± 0.6% soluble protein; 33.5 ± 4.0% ADF; 54.2 ± 3.8% NDF (n = 10).
[3] 20.7 ± 0.8% CP; 12.7 ± 1.2% soluble protein; 27.5 ± 1.2% ADF; 36.5 ± 2.9% NDF (n = 10).
[4] 8.5 ± 0.2% CP; 2.7 ± 0.7% soluble protein; 19.3 ± 1.1% ADF; 35.5 ± 3.9% NDF (n = 7).
[5] 7.9 ± 0.4% CP; 3.0 ± 0.7% soluble protein; 16.0 ± 0.7% ADF; 30.2 ± 2.0% NDF (n = 10).
[6] Contains per kg: 63 g Ca, 63 g P, 211 g Mg, 132 g NaCl, -756 mEq DCAD, 6,905 mg Mn, 7,726 mg Zn, 1,653 mg Cu, 115 mg of Co, 53 mg Se, 736,842 IU vitamin A, 200,000 IU vitamin D, and 13,221 IU vitamin E.
[7] Contains per kg: 109 g Ca, 60 g P, 55 g Mg, 161 g Na, 27 g K, 24 g S, 2,385 mg Fe, 2,095 mg Mn, 3,111 mg Zn, 514 mg Cu, 65 mg Co, 22 mg Se (32% organic selenium), 507,864 IU vitamin A, 65,169 IU vitamin D, and 3,025 IU vitamin E.
[8] According to the NRC (2001).
[9] Calculated from average DMI of control cows (B_9-B_{12}-) during the close-up (13.9 kg/d) and lactation (20.8 kg/d) periods using NRC (2001) model.

7.4.2 Measurements

7.4.2.1 Feed

All ingredients from the diet were sampled once a week during the entire studied period. Forage samples were divided in two subsamples; one subsample was immediately analysed by near infrared reflectance spectrometry (Agri-Analyse Agricultural Laboratory, Sherbrooke, QC, Canada) in order to change, if needed, the quantity of energy and protein supplements to keep similar amounts of energy and degradable and undegradable proteins in diet throughout the trial and the second one was frozen at -20°C until analysis. Samples were dried using an air-forced oven at 55°C for 48 h, ground and analyzed by wet chemistry for DM, CP, ADF, NDF, and minerals (Agri-Food Laboratories, Guelph, ON, Canada). Rumen undegradable protein (RUP) and NE_L were calculated according to the NRC (2001).

7.4.2.2 Milk Yield and Samples

Milk yield was recorded at each milking. Thereafter, daily yield averages were calculated. Milk samples were taken weekly from 2 consecutive milkings and milk composition (fat, protein, and lactose) was analyzed by Valacta (Dairy Production Center of Expertise, Québec and Atlantic Provinces, Ste-Anne-de-Bellevue, QC, Canada) by mid infrared reflectance spectrometry. Energy-corrected milk (ECM) was calculated as follows: ECM (kg/d) = 12.55 × fat yield (kg/d) + 7.39 × protein yield (kg/d) + 5.34 × lactose yield (kg/d), based on NRC (2001) and energy value of milk of 0.74 Mcal/kg (Tyrrell and Reid, 1965).

7.4.2.3 Blood

Blood samples were taken once a week at 1315 h by caudal venipuncture using a Vacutainer system (Becton, Dickinson and Co., Franklin Lakes, NJ) before calving and between weeks 4 to 7 of lactation. For the first 3 weeks of lactation, blood samples were taken twice a week. Tubes with EDTA were used to analyze plasma folates, vitamin B_{12}, nonesterified fatty acids (NEFA), and β-hydroxybutyrate (BHBA) whereas heparinized tubes were used to analyze plasma glucose, MMA, urea, and AA concentrations. Blood samples were centrifuged within 1 hour after collection for 15 minutes at 3,000 × *g* and

4°C. For AA analysis, on a weight basis, 1,000 µL of plasma was mixed with 200 µL of an internal standard of AA labelled with stable isotopes (Cdn Isotopes Inc., Montréal, QC, Canada and Cambridge Isotope Laboratories Inc., Andover, MA) according to concentration as described by Doepel and Lapierre (2010). Samples were frozen at -20°C until analysis, exept AA which were frozen at -80°C.

7.4.2.4 Body Weight

Cows were weighed after the morning milking for 2 consecutive days at 13 ± 5 days before calving, and at 6, 20, 34, and 48 ± 2 days in milk (DIM). Average BW for those 2 consecutive days was used for statistical analysis.

7.4.2.5 Body Condition Score

Body condition score was recorded by the same individual once a week from 3 weeks before the expected calving date until 7 weeks postpartum according to a 1 to 5 scale with quarter points (Wildman et al., 1982; Ferguson et al., 1994).

7.4.3 Blood Plasma Analyses

7.4.3.1 Folates and Vitamin B_{12}

Folates and vitamin B_{12} were analyzed in duplicate by radioassay using a commercial kit (SimulTRAC® B_{12}/FOLATE-S, MP Biomedicals, Solon, OH). The inter-assay coefficients of variation were 3.6 and 2.5% for folate and vitamin B_{12} analyses, respectively.

7.4.3.2 Glucose, BHBA, NEFA, and Urea

Commercial kits were used to determine concentrations of plasma glucose (Glucose (Trinder) assay, Genzyme Diagnostics P.E.I. Inc., Charlottetown, PEI, Canada), BHBA (β-hydroxybutyrate reagent set, Pointe Scientific Inc., Canton, MI), NEFA (HR Series NEFA-HR(2), Wako Chemicals USA, Inc., Richmond, VA), and urea (BUN Urea Nitrogen reagent set, Pointe Scientific Inc., Canton, MI).

7.4.3.3 Methylmalonic Acid

Plasma concentration of MMA was determined by isotopic dilution as described by Girard and Matte (2005a) and according to the modified method of McMurray et al. (1986) using GC-MS (model CG6890-MS5973, Hewlett Packard Co., Wilmington, DE) in electron ionization mode. A total of 500 µL of plasma was taken and vortexed with 10 µL of internal standard (30 µ*M* $^2H_3$-methylmalonic acid, 98%, Cambridge Isotope Laboratories Inc., Andover, MA). Measurements were done for m/z ions, 119.0 and 122.0.

7.4.3.4 AA

Analyses of AA were performed according to Maxin et al. (2013). Briefly, plasma were deproteinized with 0.25 mL of sulfosalicylic acid (38%), and then centrifuged at 16,200 × *g* for 10 minutes at 4°C. Samples were derivatized with 0.05 mL of N-tert-butyldimethylsilyl-N-methyltrifluoroacetamide and dimethylformamide (Sigma-Aldrich, Oakville, ON, Canada) according to a 1:1 ratio after supernatants being eluted by means of poly-prep chromatography columns (Bio-Rad, Hercules, CA). Measurements of AA were made using GC-MS using electron ionization (model CG6890-MS5973, Hewlett Packard Co., Wilmington, DE).

7.4.4 Statistical Analyses

Two levels of folic acid (0 or 320 mg/wk) and 2 levels of vitamin B_{12} (0 or 10 mg/wk) were used in a 2 × 2 factorial arrangement in a six randomized complete block design. Variables were analyzed using the MIXED procedure of SAS (version 9.2, SAS Institute, 2008, Cary, NC) with repeated measures in time according to the following model:

$$Y_{ijk} = \mu + B_i + V_j + BV_{ij} + T_k + VT_{jk} + \varepsilon_{ijk}$$

In which Y_{ijk} is the studied variable, μ is the overall mean, B_i is the block effect, V_j is the treatment effect, BV_{ij} is the error a, T_k is the time effect, and ε_{ijk} is the residual error. According to the factorial arrangement used, the treatment effect was decomposed in folic acid, vitamin B_{12} and the interaction folic acid × vitamin B_{12} effects and their interactions with time (VT_{jk}).

Milk yield and DMI were analysed using weekly means. Separate analyses were performed for BW, DMI and BCS data before and after calving. For milk yield and components, DMI, BCS, and after calving BW data, seven covariance structures were tried out as measures were taken according to equal time intervals (CS, CSH, AR(1), ARH(1), TOEP, TOEPH, and UN) and the one with smallest fit statistics was chosen for each analysis. Body weight data before calving (13 ± 5 d before calving) were analyzed with the MIXED procedure of SAS according to the same model described above except repeated measures were not used. Blood plasma metabolites were analyzed using repeated measures as described above except that different covariance structures were compared as data were collected according to unequal time intervals (SP(POW), SP(GAU), SP(EXP), SP(LIN), SP(LINL), SP(SPH), ANTE(1), and UN); the structure with the smallest fit statistics was chosen for each analysis. When an interaction was significant, the SLICE option in the LSMEANS statement of SAS was used to help interpretation of results. As BW and BCS were numerically different among treatments at 4 weeks before the expected calving date, these data were entered as covariable ($P < 0.0001$) for BW and BCS analyses. Results were considered significant when $P \leq 0.05$ and as a tendency at $0.05 < P \leq 0.10$.

7.5 Results

7.5.1 Prepartum Data

During the pre-calving period, DMI of dairy cows that received the supplement of folic acid, alone or combined with the supplement of vitamin B_{12}, was lower than for cows that did not, with this difference being larger at week 2 prepartum (folic acid × time interaction, $P = 0.02$). At weeks 3, 2, and 1 prepartum, DMI was respectively 13.7, 12.8, and 12.3 ± 0.36 kg/d for cows receiving the folic acid supplement, alone or combined with the supplement of vitamin B_{12}, and 14.1, 14.4, and 13.0 ± 0.36 kg/d for cows that did not. From 3 weeks before parturition to calving, BCS tended to be higher for cows receiving the folic acid supplement than for cows that did not ($P = 0.08$; Table 7.2). At 13 ± 5 days before calving, BW was unaffected by treatments ($P \geq 0.11$; Table 7.2).

Table 7.2 Effects of intramuscular injections of folic acid and vitamin B_{12}, alone or combined, given from 3 weeks before the expected calving date until 7 weeks of lactation on DMI, BW, BCS, and milk yield and components (least square means)

	Treatment[1]					*P*-value		
Item	B_9-B_{12}-	B_9+B_{12}-	B_9-B_{12}+	B_9+B_{12}+	SEM	B_9	B_{12}	$B_9 \times B_{12}$
Cows (n)	6	6	6	6				
DMI (kg/d)								
Pre-calving[2,3]	13.9	12.9	13.7	13.0	0.4	0.05	0.90	0.69
Postcalving[2]	20.8	21.1	20.2	20.6	0.8	0.67	0.53	0.98
BW (kg)								
Pre-calving	747	758	747	750	4	0.11	0.44	0.40
Postcalving[2]	649	661	645	641	12	0.75	0.35	0.55
BCS[4]								
Pre-calving	3.04	3.12	3.03	3.09	0.03	0.08	0.49	0.77
Postcalving[2,3]	2.50	2.65	2.60	2.73	0.09	0.11	0.32	0.87
Milk production[2,3] (kg/d)	41.5	37.8	40.6	39.8	3.0	0.47	0.85	0.64
ECM[2,5] (kg/d)	39.2	34.8	38.4	37.6	2.3	0.28	0.69	0.45
Milk composition								
Fat[2] (g/kg)	37.9	37.0	38.8	37.4	2.0	0.59	0.76	0.91
Protein[2] (g/kg)	30.7	31.6	31.5	33.0	0.8	0.18	0.21	0.73
Lactose[2] (g/kg)	46.7	46.0	46.1	45.8	0.5	0.31	0.41	0.66
TS[2] (g/kg)	116.3	115.5	117.3	116.6	2.5	0.76	0.67	0.96
Milk yields								
Fat (kg/d)	1.54	1.33	1.53	1.44	0.10	0.16	0.60	0.56
Protein[2] (kg/d)	1.29	1.18	1.27	1.30	0.09	0.64	0.56	0.41
Lactose[2,3] (kg/d)	1.93	1.71	1.84	1.82	0.13	0.39	0.98	0.46
TS[2] (kg/d)	4.77	4.24	4.64	4.57	0.30	0.34	0.74	0.45
TS/DMI[2]	0.23	0.21	0.25	0.22	0.01	0.11	0.36	0.92

[1] B_9-B_{12}- = No vitamin supplement; B_9+B_{12}- = 320 mg of folic acid; B_9-B_{12}+ = 10 mg of vitamin B_{12}; B_9+B_{12}+ = 320 mg of folic acid and 10 mg of vitamin B_{12}.
[2] Time effect, $P \leq 0.01$.
[3] Folic acid × time interaction, $P \leq 0.10$.
[4] According to a 1 to 5 scale with quarter points (Wildman et al., 1982; Ferguson et al., 1994).
[5] ECM (kg/d) = 12.55 × fat yield (kg/d) + 7.39 × protein yield (kg/d) + 5.34 × lactose yield (kg/d), adapted from NRC (2001) and Tyrrell and Reid (1965).

7.5.2 Postpartum Data

7.5.2.1 DMI, BW, and BCS

Postcalving DMI was on average 20.8 ± 0.8 kg/d and did not differ among treatments ($P \geq 0.53$; Table 7.2). As expected, DMI increased by 9.6 kg/d from the week following parturition (14.5 ± 0.5 kg/d) to week 7 after calving (24.1 ± 0.4 kg/d; time effect, $P < 0.0001$). After calving, BW was not affected by treatments ($P \geq 0.35$; Table 7.2), but it decreased from 658 ± 7 kg at 6 ± 2 DIM to 646 ± 7 kg at 48 ± 2 DIM (time effect, $P = 0.0009$). Postpartum BCS was greater for cows receiving the folic acid supplement from weeks 5 to 7 (folic acid × time interaction, $P = 0.10$). Figure 7.1 shows prepartum and postpartum BCS for cows that received folic acid supplement as compared with cows that did not.

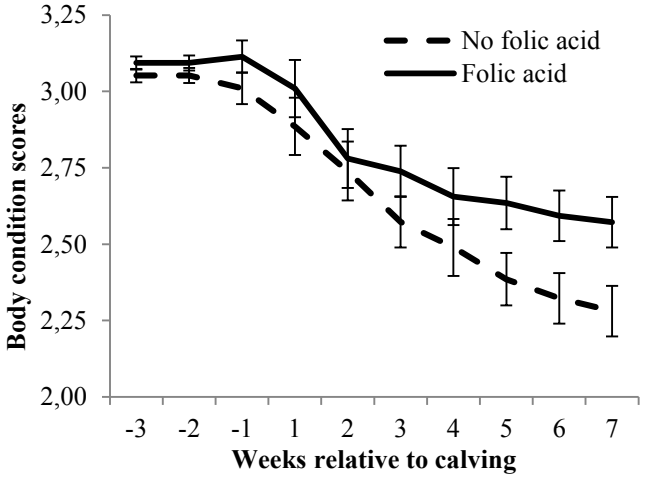

Figure 7.1 Effects of weekly intramuscular injections of folic acid (0 or 320 mg) on BCS given from 3 weeks before the expected calving date to 7 weeks postpartum (folic acid × time interaction, $P = 0.10$) using BCS at week 4 prepartum as a covariable ($P < 0.0001$). According to a 1 to 5 scale with quarter points

7.5.2.2 Milk Yield and Components

Milk yield of cows receiving the folic acid supplement, alone or combined with the supplement of vitamin B_{12}, reached a plateau at week 2 following by a small decrease at

weeks 6 and 7 than cows that did not receive a folic acid supplement (folic acid × time interaction, $P = 0.01$; Figure 7.2). However, ECM was unaffected by treatments neither were total solids (TS)/DMI, milk fat, protein, and TS concentrations and yields nor milk lactose concentration ($P \geq 0.11$; Table 7.2). Milk lactose yield during the first 7 weeks of lactation followed the same pattern as milk yield (folic acid × time interaction, $P = 0.02$).

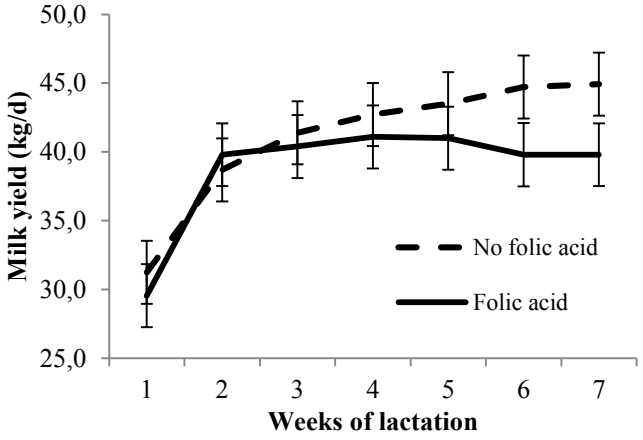

Figure 7.2 Milk yield within the first 7 weeks of lactation according to folic acid supplementation (320 mg) or not (0 mg) given by weekly intramuscular injections from 3 weeks before the expected calving date until 7 weeks of lactation (folic acid × time interaction, *P* = 0.01)

7.5.3 Plasma Variables

7.5.3.1 B Vitamins

Before the beginning of the study, 4 weeks before the expected calving date, folate and vitamin B_{12} plasma concentrations averaged 13.8 ± 0.6 ng/mL and 256 ± 21 pg/mL and were not different among treatments ($P \geq 0.2$). Over the whole experimental period, the folic acid supplement increased plasma folate concentration ($P = 0.0005$) when the vitamin B_{12} supplement was given whereas it was not affected when vitamin B_{12} supplement was not given ($P = 0.38$; Table 7.3). Folate plasma concentration was increased by the folic acid supplement, alone or combined with vitamin B_{12}, but from week 4 until week 7 of lactation, this increase was no longer significant (folic acid × time interaction, $P = 0.05$; Figure 7.3a). Plasma vitamin B_{12} concentration was significantly higher for dairy cows receiving the vitamin B_{12} supplement, alone or combined with the supplement of folic acid ($P = 0.0004$;

Table 7.3), throughout the experimental period (vitamin B_{12} × time interaction, $P = 0.28$; Figure 7.3b). Regardless of treatments, vitamin B_{12} concentration in plasma decreased from week 1 of lactation (415 ± 29 pg/mL) to week 7 (241 ± 11 pg/mL; time effect, $P < 0.0001$).

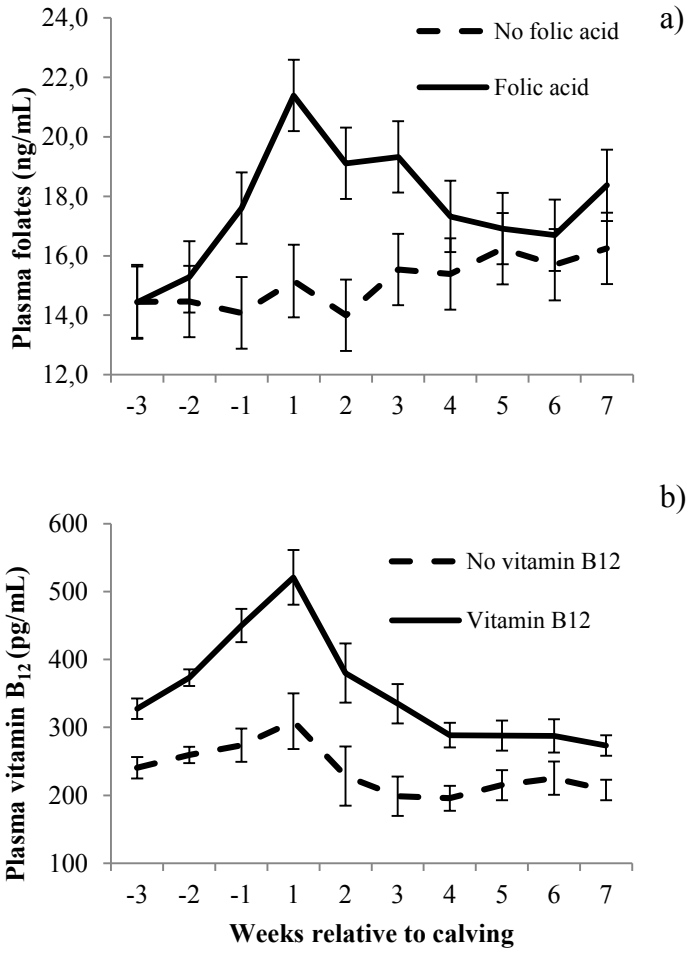

Figure 7.3 Effects of weekly intramuscular injections of folic acid (0 or 320 mg) given from 3 weeks before the expected calving date until 7 weeks after parturition on plasma folic acid concentration (a; folic acid × time interaction, $P = 0.05$) and plasma vitamin B_{12} concentration (b; vitamin B_{12} × time interaction, $P = 0.28$)

Table 7.3 Effects of weekly intramuscular injections of folic acid and vitamin B_{12}, alone or combined, given from 3 weeks before the expected calving date until 7 weeks of lactation on plasma B vitamins, glucose, BHBA, NEFA, methylmalonic acid (MMA), urea and AA (least square means)

	Treatment[1]					*P*-value		
Item	B_9-B_{12}-	B_9+B_{12}-	B_9-B_{12}+	B_9+B_{12}+	SEM	B_9	B_{12}	$B_9 \times B_{12}$
Cows (n)	6	6	6	6				
Folates[2,3] (ng/mL)	16.2	17.2	14.0	19.1	0.8	0.002	0.88	0.02
Vitamin B_{12}[2] (pg/mL)	217	252	339	381	28	0.19	0.0004	0.90
Glucose[2] (μ*M*)	3.14	3.42	3.25	3.39	0.06	0.006	0.50	0.27
BHBA[2,4] (m*M*)	0.76	0.71	0.78	0.69	0.03	0.06	0.86	0.61
NEFA[2,3] (μ*M*)	221	171	264	179	17	0.002	0.16	0.34
MMA[2,3] (μ*M*)	0.50	0.60	0.52	0.50	0.04	0.42	0.33	0.21
Urea[2] (m*M*)	5.52	5.43	5.07	5.20	0.21	0.93	0.11	0.60
AA (μ*M*)								
Ala[2]	229.3	216.5	209.6	225.0	6.6	0.85	0.41	0.05
Asn[2]	45.6	43.5	44.1	45.2	1.4	0.72	0.94	0.26
Asp	26.6	25.9	27.4	25.4	2.2	0.54	0.93	0.76
Cys[2]	99.5	105.9	97.9	107.5	2.0	0.001	0.99	0.43
Glu[2,3]	44.0	44.2	44.8	46.9	1.3	0.42	0.20	0.48
Gln[2]	295.0	299.5	315.8	309.1	5.9	0.86	0.02	0.36
Gly[2,5]	347.0	312.5	357.2	322.8	9.9	0.003	0.32	0.99
His[2]	55.4	60.8	54.5	60.6	1.9	0.008	0.79	0.84
Hcy[2,3]	5.18	5.58	5.54	6.13	0.25	0.07	0.09	0.71
Ile	114.3	106.3	122.9	108.1	6.3	0.09	0.42	0.59
Leu[2]	146.2	142.8	153.4	140.2	6.8	0.24	0.74	0.48
Lys[2]	68.6	70.6	66.9	66.6	3.2	0.79	0.39	0.73
Met[3,4]	28.2	27.7	27.9	27.7	0.8	0.67	0.89	0.86

Table 7.3 Continued

Item	Treatment[1]				SEM	*P*-value		
	B_9-B_{12}-	B_9+B_{12}-	B_9-B_{12}+	B_9+B_{12}+		B_9	B_{12}	$B_9 \times B_{12}$
Phe	48.0	51.9	48.8	50.4	0.9	0.01	0.73	0.24
Pro[2]	76.8	79.2	74.0	77.9	2.5	0.23	0.44	0.76
Ser[2]	87.9	85.2	89.1	84.5	2.8	0.21	0.94	0.74
Thr[2]	83.8	87.7	92.6	87.6	3.7	0.89	0.26	0.25
Trp[2]	42.1	41.1	40.6	39.6	1.4	0.51	0.30	0.99
Tyr[2]	45.2	47.9	47.4	51.5	1.3	0.02	0.05	0.62
Val[2]	195.6	190.1	201.6	189.2	9.2	0.34	0.79	0.72
BCAA[2,6]	456.1	439.2	477.9	437.4	22.0	0.21	0.65	0.60
EAA[2,7]	782.6	778.0	809.3	770.0	25.0	0.39	0.71	0.49
NEAA[2,8]	1,297.2	1,259.5	1,307.3	1,295.0	19.9	0.23	0.27	0.53
TAA[2,9]	2,086	2,044	2,123	2,072	31	0.15	0.32	0.89

[1] B_9-B_{12}- = No vitamin supplement; B_9+B_{12}- = 320 mg of folic acid; B_9-B_{12}+ = 10 mg of vitamin B_{12}; B_9+B_{12}+ = 320 mg of folic acid and 10 mg of vitamin B_{12}.
[2] Time effect, $P \leq 0.08$.
[3] Folic acid × time interaction, $P \leq 0.09$.
[4] Vitamin B_{12} × time interaction, $P \leq 0.06$.
[5] Folic acid × vitamin B_{12} × time interaction, $P = 0.03$.
[6] BCAA = Branched-chain amino acids = Ile + Leu + Val.
[7] EAA = Essential amino acids = His + Ile + Leu + Lys + Met + Phe + Thr + Trp + Val.
[8] NEAA = Nonessential amino acids = Ala + Asn + Asp + Cys + Gln + Glu + Gly + Pro + Ser + Tyr.
[9] TAA = Total amino acids.

7.5.3.2 Glucose, BHBA, NEFA, MMA, and Urea

Plasma concentration of glucose averaged 3.40 and 3.20 ± 0.04 μ*M* for cows receiving the folic acid supplement or not, respectively ($P = 0.006$; Table 7.3), and was higher for cows receiving the folic acid supplement than the cows that did not. Plasma concentration of glucose was on average 3.67 ± 0.07 μ*M* from 3 weeks before calving to parturition; it decreased at the onset of lactation and then it increased with weeks of lactation from 3.16 ± 0.07 μ*M* at week 1 to 3.36 ± 0.07 μ*M* at week 7 (time effect, $P < 0.0001$). Plasma concentration of BHBA tended to be lower for cows receiving the folic acid supplement (0.70 ± 0.02 m*M*), alone or combined with the supplement of vitamin B_{12}, than for cows that did not (0.77 ± 0.02 m*M*; $P = 0.06$; Table 7.3). Plasma BHBA concentration tended to be higher for cows receiving the vitamin B_{12} supplement, alone or combined with the supplement of folic acid, one week postpartum (vitamin B_{12} × time interaction, $P = 0.06$) but no difference was observed thereafter. Indeed, at week 1 of lactation, plasma BHBA concentrations were 0.94 and 0.70 ± 0.11 m*M* for the vitamin B_{12} supplement or not, respectively. From weeks 2 to 7 of lactation, plasma concentration of NEFA was lower for cows receiving the folic acid supplement than for those that did not (folic acid × time interaction, $P = 0.009$; Figure 7.4). Plasma concentration of MMA was unaffected by treatments ($P \geq 0.21$; Table 7.3). However, a time effect was noted ($P < 0.0001$); plasma concentration of MMA averaged 0.94 ± 0.12 μ*M* before parturition and 0.41 ± 0.02 μ*M* post-partum. Plasma concentration of urea was unaffected by treatments ($P \geq 0.11$; Table 7.3). The lowest plasma concentration of urea was observed at week 1 after calving (4.61 ± 0.22 m*M*) and then it increased until week 7 of lactation (6.09 ± 0.22 m*M*; time effect, $P < 0.0001$).

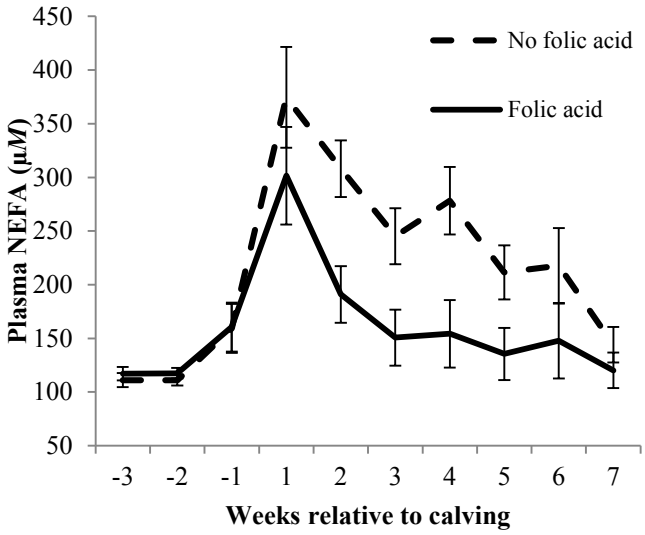

Figure 7.4 Effects of weekly intramuscular injections of folic acid (0 or 320 mg) given from 3 weeks before the expected calving date until 7 weeks after parturition on plasma NEFA concentration (folic acid × time interaction, $P = 0.009$)

7.5.3.3 AA

Plasma concentrations of Cys, His, and Phe increased ($P \leq 0.01$; Table 7.3) whereas plasma concentrations of Gly and Ile decreased with the folic acid supplement, alone or combined with the supplement of vitamin B_{12} ($P \leq 0.09$). Both folic acid and vitamin B_{12} supplements, alone or combined, increased plasma Hcy and Tyr concentrations ($P \leq 0.09$; Table 7.3). Supplementary folic acid had no marked effect on plasma concentration of Hcy from 3 weeks before calving until 2 weeks postpartum but from weeks 3 to 7 postpartum, plasma Hcy concentration was increased by the folic acid supplement as compared with no folic acid supplementation (folic acid × time interaction, $P = 0.04$).

During the prepartum experimental period, plasma concentrations of Cys and Hcy were 119.5 ± 2.4 and 8.11 ± 0.33 μ*M*, respectively, and, after calving, plasma concentrations of Cys increased from week 1 (89.2 ± 2.4 μ*M*) to week 7 (106.3 ± 2.4 μ*M*) of lactation whereas plasma concentration of Hcy decreased from week 1 (5.29 ± 0.28 μ*M*) to week 4 (4.67 ± 0.28 μ*M*) of lactation and increased thereafter until week 7 (5.25 ± 0.18 μ*M*; time effect, $P < 0.0001$). Overall, plasma concentrations of Ala, Leu, Lys, Pro, Thr, Trp, Tyr, Val, branched-chain AA, total essential AA, and total AA decreased from 3 weeks before calving until parturition and then increased from parturition until week 7 of lactation (time

effect, $P \leq 0.08$) while plasma concentrations of Gly, Ser, and total nonessential AA followed the opposite trend (time effect, $P < 0.0001$). Plasma concentration of Asn increased and plasma concentrations of Glu, Gln, and His decreased throughout the experimental period (time effect, $P \leq 0.0006$).

7.6 Discussion

At the beginning of the experiment, plasma folate and vitamin B_{12} concentrations were 86 and 11% higher than in the study of Preynat et al. (2009b), respectively. Plasma concentration of vitamin B_{12} throughout the experimental period for cows not receiving the vitamin B_{12} supplement was higher than in the study of Girard and Matte (2005a) and Graulet et al. (2007) but followed a similar pattern than reported by Girard et Matte (1999) for multiparous dairy cows. Plasma vitamin B_{12} concentration was increased by the vitamin B_{12} injections as previously reported (Girard and Matte, 2005a; Preynat et al., 2009b; Akins et al., 2013). As in the current study, Preynat et al. (2009b), using intramuscular injections of folic acid and vitamin B_{12}, observed a decrease of plasma folate concentration as lactation progressed for cows receiving the folic acid supplement.

Grace and Knowles (2012) concluded that a serum concentration of vitamin B_{12} higher than 173 pg/mL was adequate for grazing cows. It has been reported in the literature that supplemental folates improved lactational performance in multiparous dairy cows having a serum concentration of vitamin B_{12} higher than 200 pg/mL (Girard and Matte, 2005a). Moreover, in a previous experiment in which plasma concentration of vitamin B_{12} was below 200 pg/mL for early lactating cows, a combined supplement of folic acid and vitamin B_{12} enhanced metabolic efficiency of dairy cows as compared with folic acid supplement alone (Graulet et al., 2007). In the present study, plasma concentration of vitamin B_{12} for dairy cows not receiving the vitamin B_{12} supplementation was greater than 200 pg/mL throughout the experimental period which could explain the similar response on plasma glucose, BHBA, and NEFA concentrations to folic acid supplement, alone or combined with vitamin B_{12}. In the current study, plasma concentration of MMA after parturition in control cows was lower by 31% than control cows in the study of Girard and Matte (2005a) in which plasma concentration of vitamin B_{12} was below 200 pg/mL. Plasma

MMA is a good indicator of vitamin B_{12} deficiency (Selhub et al., 2009). Indeed, a deficiency in vitamin B_{12} leads to an accumulation of methylmalonyl-CoA and it is then transformed into MMA instead of succinyl-CoA. Moreover, as no treatment effect was observed on plasma MMA concentration, it suggests that vitamin B_{12} was not limiting.

As in the current study, Girard and Matte (2005a), Graulet et al. (2007), and Preynat et al. (2009b) did not report any effect of folic acid and vitamin B_{12} supplement, alone or combined, on DMI after parturition. However, no other study reported a decrease of DMI with the use of a folic acid supplement before parturition as observed in the present experiment.

In early lactation, Graulet et al. (2007) reported an increase of 3.4 kg/d of milk production for multiparous dairy cows fed dietary folic acid, alone or combined with a supplement of vitamin B_{12}. A combined supplement of folic acid and vitamin B_{12} given by intramuscular injections increased milk production, especially during the first 4 weeks of lactation as compared with folic acid supplement alone and control cows (Preynat et al., 2009b). In contrast, in the current study, folic acid supplement, alone or combined with the supplement of vitamin B_{12}, did not increase milk yield within the first 5 weeks of lactation; milk production was even lower for cows receiving the folic acid supplement during the weeks 6 and 7 of lactation. Milk fat concentration and yield were unaffected by the folic acid supplement, alone or combined with the supplement of vitamin B_{12}, as in previous studies (Girard et al., 2005; Graulet et al., 2007; Preynat et al., 2009b). However, Girard and Matte (2005a) reported that a combined supplement of folic acid and vitamin B_{12} increased milk fat yield as compared with folic acid supplement alone. Graulet et al. (2007) observed an augmentation of milk protein yield by 75 g/d for cows receiving the dietary folic acid supplement. Likewise, an increase of milk protein concentration or yield was observed by Girard et al. (1995) and Preynat et al. (2009b) with folic acid supplement given by intramuscular injections. This is not in agreement with results from the current study in which milk protein concentration and yield were remained unchanged by the folic acid supplement. Results from the trial of Akins et al. (2013) are in accordance with those from the present experiment as injection of vitamin B_{12} alone did not increase milk yield, milk fat and protein yields and concentrations.

Although milk production at weeks 6 and 7 decreased for multiparous cows receiving the folic acid supplement, no significant treatment effect was noted on ECM and TS yield, and plasma concentration of glucose and postpartum BCS were higher, and starting at week 2 after parturition, plasma NEFA and BHBA concentrations were lower for cows that received the folic acid supplement as compared with cows that did not. In early lactation, when the energy demand for milk production is greater than energy provided by DMI, negative energy balance (NEB) occurs (Bauman and Currie, 1980). A normal adaptation to NEB and to the decrease of available glucose in early lactation consists in cows mobilizing body fat reserves releasing NEFA in blood that could be partially oxidized in liver to generate BHBA (McArt et al., 2013). These metabolites could enter into the Krebs cycle to provide energy after being oxidized in the liver or NEFA could be taken up by the mammary gland and secreted in milk fat (Bauman and Griinari, 2003; McArt et al., 2013). Higher plasma concentration of glucose and postpartum BCS and lower plasma concentrations of NEFA and BHBA combined with similar DMI, milk production, and milk TS secretion between weeks 2 to 5 of lactation suggest that cows receiving the folic acid supplement, alone or combined with the supplement of vitamin B_{12}, had a better energy status than cows that did not. Moreover, even if prepartum DMI was lower for cows receiving the folic acid supplement, prepartum plasma NEFA and BHBA concentrations were not affected by treatments. Graulet et al. (2007) and Preynat et al. (2009a,b) reported that a combined supplement of folic acid and vitamin B_{12} improved metabolic efficiency of dairy cows in early lactation by increasing plasma glucose or glucose irreversible loss rate, improving lactational performance without increasing DMI and plasma NEFA and BHBA concentrations. Vitamin B_{12} is a coenzyme for methylmalonyl-CoA mutase which transform methylmalonyl-CoA to succinyl-CoA, an intermediate step in the transformation of propionate for its entry into the Krebs cycle (Le Grusse and Watier, 1993; Scott, 1999). Even if folic acid has no known role in this last pathway, Selhub et al. (2007) concluded that folate supply seemed to affect both pathways in which vitamin B_{12} is involved as a coenzyme. Similarly, Graulet et al. (2007) reported a greater affinity of the methylmalonyl-CoA mutase for vitamin B_{12} when dairy cows received the combined supplement of folic acid and vitamin B_{12} in comparison with no supplementation or these 2 vitamins given separately. As dairy cows had a high plasma vitamin B_{12} status throughout the present trial,

it could explain why the folic acid supplement given alone improved metabolic efficiency. In the current study, plasma NEFA concentrations of control cows were half plasma NEFA values reported from 3 weeks before calving until 8 weeks of lactation for control cows by Graulet et al. (2007) coupled with similar milk production. It suggests that, overall, dairy cows in the present study mobilized less body fat and had probably a better energy status than in the study of Graulet et al. (2007).

At weeks 6 and 7 postpartum, milk and lactose yields were decreased by the folic acid supplement. As lactose is the major osmo-regulator in the mammary gland for uptake of water for milk secretion (Linzell, 1972; Mepham, 1993), these results go in the same direction. Nonetheless, these results are difficult to explain. In the literature, a supplement of folic acid given in early lactation, combined or not with vitamin B_{12} supplement, had either no effect on lactose yield or increased it (Girard and Matte, 2005a; Graulet et al., 2007; Preynat et al., 2009b).

In contrast with the current experiment, Graulet et al. (2007) did not report effect of dietary folic acid supplement, alone or combined with vitamin B_{12} supplement, on Gln, His, Phe and, Tyr. The increase of plasma Gln concentration for cows receiving the vitamin B_{12} supplement could be explained by more ammonia being incorporated into Glu to form Gln (Salway, 2004). This is supported by a numerical decrease of plasma urea concentration on these cows.

Regardless of treatments, average plasma concentration of Met was higher than what reported in the literature even if rumen-protected Met (Girard et al., 2005; Girard and Matte, 2005a; Preynat et al., 2009b) or a combined supplement of folic acid and vitamin B_{12} (Graulet et al., 2007) were given. Folic acid and vitamin B_{12} work closely in the Met salvage pathway; using vitamin B_{12} as a coenzyme for Met synthase, the 5-methyl-tetrahydrofolate gives its methyl group to Hcy to form Met (Scott, 1999). Methionine could be used for protein synthesis or, requiring ATP, transformed in S-adenosylmethionine, the major donor of methyl group (Finkelstein, 1990). After giving its methyl group, S-adenosylmethionine is transformed into S-adenosylhomocysteine and then into Hcy (Finkelstein and Martin, 2000). Homocysteine could then be remethylated again or catabolized in Cys through the transsulfuration pathway according to a regulatory

mechanism (Finkelstein, 1990). In rats, an increased availability of Met led to an augmentation of the percentage of Hcy converted into cystathionine, a precursor for Cys formation, followed by a more rapid cycling involving a decreased half-life of the methyl group of Met (Finkelstein, 1990). As Met status was high regardless of treatments and vitamin B_{12} was not lacking in the current study, by supplying folic acid as a donor of methyl group to regenerate Met from Hcy, the availability of Met was probably increased and, therefore, a more rapid cycling occurred in cows receiving a folic acid supplement, alone or combined with vitamin B_{12}. This regulatory mechanism could explain why dairy cows receiving the folic acid supplement, alone or combined with the supplement of vitamin B_{12}, had higher plasma levels of Hcy and Cys with a similar plasma concentration of Met among treatments. In accordance, this could have led to the lower plasma concentration of Gly observed for dairy cows receiving the folic acid supplement as this AA accepts the methyl group of S-adenosylmethionine to form sarcosine and S-adenosylhomocysteine to alleviate Met excess (Finkelstein, 1990; Luka et al., 2009). Graulet et al. (2007) also observed an increase of plasma Hcy concentration with the use of dietary vitamin B_{12} whereas, in contrast, Hcy tended to decrease with folic acid injections alone or combined with vitamin B_{12} supplement in the study of Preynat et al. (2009b).

As discussed above and as in the current study, previous studies concluded that a combined supplement of folic acid and vitamin B_{12} enhanced metabolic efficiency in early lactation (Girard and Matte, 2005a; Graulet et al., 2007; Preynat et al., 2009a,b; 2010). A study conducted in commercial dairy herds in Québec, Canada concluded that the combined vitamin supplement given by intramuscular injections seems to change energy partitioning in early lactating dairy cows (Duplessis et al., 2014). In the experiment of Graulet et al. (2007), folic acid and vitamin B_{12} supplements were given in the diet whereas only folic acid supplement was given in the ration in the trial of Girard and Matte (2005a). Even if it has been showed that dietary folic acid did not impact rumen metabolism (Girard et al., 2009), the mode of distribution of the folic acid supplement among the present study (intramuscular injections) and these previous experiments (dietary) could not be discarded as a factor affecting treatment responses. Nevertheless, Preynat et al. (2009b), using weekly intramuscular injections, reported an increase of milk production for cows receiving the combined supplement without an increase of plasma BHBA and NEFA concentrations. The

discrepancy between results from the study of Preynat et al. (2009b) and the present experiment could be possibly explained by higher plasma folate, vitamin B_{12}, and Met concentrations throughout the experimental period in the current trial. Despite the folic acid supplement, alone or combined with the supplement of vitamin B_{12}, improved metabolic efficiency before calving and from weeks 2 to 5 postpartum, milk production was lower for cows receiving the folic acid supplement, especially at weeks 6 and 7. It suggests that, in the present experiment, the folic acid supplement alone might be given only during the prepartum period and within the first 5 weeks of lactation to be beneficial. Moreover, supply of folic acid for cows receiving the folic acid supplement was probably in excess at weeks 6 and 7 postpartum. The current state of knowledge does not allow predicting total supply of folic acid and vitamin B_{12} from microbial synthesis to dairy cows fed different diets and, thus, it is not possible to evaluate ahead of vitamin supplementation which cows could benefit from this.

7.7 Conclusion

Throughout the experimental period, plasma folate, vitamin B_{12}, and Met concentrations of dairy cows were higher than previously reported in the literature. Plasma concentration of MMA indicated that vitamin B_{12} was not deficient. Cows receiving the folic acid supplement, alone or combined with vitamin B_{12} supplement, had a better energy status than cows that did not receive the folic acid supplement within the first 5 weeks of lactation. This conclusion is supported by lower plasma BHBA and NEFA concentrations and higher plasma glucose concentration and BCS for cows receiving the folic acid supplement combined with similar milk yield and components among treatments. However, at weeks 6 and 7 postpartum, the folic acid supplement decreased milk and lactose yields suggesting that supplies of folic acid and vitamin B_{12} were sufficient, or even in excess, to optimize performance. However, foreseeing which cow would benefit from a folic acid and vitamin B_{12} supplement, alone or combined, is currently not possible.

7.8 Acknowledgements

Authors are grateful to Véronique Roy, Valérie Beaudet, Chrystiane Plante, and Jocelyne Renaud (Agriculture et Agroalimentaire Canada, Sherbrooke, QC, Canada) for technical assistance. We acknowledge the invaluable help of Marie-Ève Bouchard, Étienne Viens and the barn staff (Agriculture et Agroalimentaire Canada, Sherbrooke, QC, Canada) for special care of dairy cows throughout the experiment. We also thank Vétoquinol (Lavaltrie, QC, Canada) for generously providing vitamin B_{12}. This project was financed by the Entente de collaboration pour l'innovation en production et transformation laitière Novalait-AAC-FQRNT-MAPAQ.

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8 Article sur le métabolisme du glucose – Deuxième projet

Métabolisme corporel du glucose et de l'acide propionique de vaches laitières multipares recevant des suppléments d'acide folique et de vitamine B_{12}, seuls ou combinés

Whole-body metabolism of glucose and propionate of multiparous dairy cows receiving supplements of folic acid and vitamin B_{12}, alone or combined

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Cet article est une première version du manuscrit qui sera soumis au *Journal of Dairy Science*.

8.1 Résumé

Cette étude a été réalisée pour évaluer l'effet de suppléments d'acide folique et de vitamine B_{12}, seuls ou combinés, sur la cinétique du glucose à la semaine 9 de la lactation chez la vache laitière. Les concentrations de métabolites étroitement reliés aux voies métaboliques du cycle de méthylation et de la néoglucogenèse à partir de l'acide propionique ont également été mesurées. Vingt-quatre vaches multipares ont été assignées selon une étude factorielle 2 × 2 à soit l'un des traitements suivant : 1) saline 0,9 % NaCl; 2) 320 mg d'acide folique; 3) 10 mg de vitamine B_{12} ou; 4) 320 mg d'acide folique et 10 mg de vitamine B_{12}. Les injections intramusculaires hebdomadaires ont été données à partir de 3 semaines avant la date de vêlage prévu jusqu'à 9 semaines postpartum. À 63 jours en lait (JEL), des perfusions simultanées de D-[6,6-$^{2}H_2$]-glucose (16,5 mmol/h) dans une veine jugulaire et [1-^{13}C]-sodium propionate (13,9 mmol/h) dans une veine ruminale ont été réalisées. Des biopsies hépatiques ont été faites à 64 JEL. Les suppléments d'acide folique et de vitamine B_{12} ont augmenté les concentrations de folates et de vitamine B_{12} dans le lait, respectivement. Une réponse similaire a été observée dans le foie. À la semaine 9 postpartum, la consommation volontaire de matière sèche (CVMS) n'a pas été affectée par les traitements. La production laitière ainsi que le lactose sécrété ont eu tendance à être plus bas de 5,0 kg et de 0,25 kg/j, respectivement, pour les vaches recevant le supplément d'acide folique que pour les vaches ne l'ayant pas reçu. La concentration hépatique de glycogène n'a pas été différente entre les traitements. Le taux d'apparition corporel de glucose a diminué de 229 g/j pour les animaux recevant le supplément d'acide folique. Comme la CVMS et la concentration hépatique de glycogène n'ont pas différé entre les traitements, elles ne peuvent pas être considérées comme étant la source de cette baisse. La proportion de glucose synthétisé à partir de l'acide propionique n'a pas été différente entre les traitements et a été de 59,7 %. Ces résultats suggèrent que les apports d'acide folique et de vitamine B_{12} provenant de la synthèse du rumen étaient adéquats pour optimiser les performances des vaches laitières sous les conditions expérimentales de cette expérience.

Mots clés : vache laitière, acide folique, vitamine B_{12}, acide propionique, néoglucogenèse

8.2 Abstract

This study was undertaken to evaluate the effect of supplements of folic acid and vitamin B_{12}, alone or combined, on glucose kinetics at week 9 of lactation in dairy cows. Concentrations of metabolites and hepatic expression of genes closely related to the pathways of the methylation cycle and gluconeogenesis from propionate were also measured. Twenty-four multiparous cows were assigned according to a complete block design in a 2 × 2 factorial arrangement to either one of the following treatments: 1) saline 0.9% NaCl; 2) 320 mg of folic acid; 3) 10 mg of vitamin B_{12} or; 4) 320 mg of folic acid and 10 mg of vitamin B_{12}. Intramuscular injections were given weekly from 3 weeks before the expected calving date until 9 weeks postpartum. At 63 days in milk, simultaneous infusions of D-[6,6-$^{2}H_2$]-glucose (16.5 mmol/h) in one jugular vein and [1-^{13}C]-sodium propionate (13.9 mmol/h) in one ruminal vein were performed. Liver biopsies were carried out the following day. Supplements of folic acid and vitamin B_{12} increased folate and vitamin B_{12} concentrations in milk, respectively. A similar response was observed in liver. At week 9 postpartum, dry matter intake was unaffected by treatments. Milk and lactose yields tended to be lower by 5.0 and by 0.25 kg/d, respectively, for cows receiving the folic acid supplement than for cows that did not. Plasma β-hydroxybutyrate concentration tended to decrease with the folic acid supplement. Liver concentration of glycogen was not affected by treatments and averaged 1.93 ± 0.32% of wet weight. Gene expressions of methylmalonyl-CoA mutase and S-adenosylhomocysteine hydrolase in liver were higher for cows receiving the combined folic acid and vitamin B_{12} supplement as compared with cows receiving a supplement of folic acid alone whereas no treatment effect was noted for cows not receiving a folic acid supplement. Whole-body glucose rate of appearance decreased by 229 g/d for animals receiving the folic acid supplement. As dry matter intake and liver concentration of glycogen were not different among treatments, they cannot be considered as a source of this decrease. The proportion of glucose synthesized from propionate did not differ among treatments and averaged 59.7 ± 8.5%. These data suggest that the supply of folic acid and vitamin B_{12} from ruminal synthesis was sufficient to optimize performance of dairy cows under the present experimental conditions.

Key words: dairy cow, folic acid, vitamin B_{12}, propionate, gluconeogenesis

8.3 Introduction

The transition from late gestation to early lactation represents a challenge for high yielding dairy cows. Major metabolic, physiological, and nutritional changes occur at parturition and the onset of lactation (Goff and Horst, 1997). Detrimental health effects may be induced by excessive negative energy balance in dairy cows unable to adapt to this challenging period (McArt et al., 2013). Previous studies concluded that a combined supplement of folic acid and vitamin B_{12} seems to improve energy metabolism in early lactation (Girard and Matte, 2005; Graulet et al., 2007; Preynat et al., 2009b) as suggested by the enhancement of lactational performance without an increase of dry matter intake (DMI) for cows receiving the combined vitamin supplement.

In ruminants, approximately 20% of glucose required is provided by glucose absorbed from starch digestion into the hepatic portal vein whereas the remaining is supplied from gluconeogenesis (Galindo et al., 2011). In fed cows, propionate is the major precursor of glucose (Reynolds, 2006; Larsen and Kristensen, 2013) and contributes up to 60% of glucose hepatic release (Amaral et al., 1990; Danfær et al., 1995). Before entering into the Krebs cycle, propionate needs to be transformed into propionyl-CoA, then into methylmalonyl-CoA, and, finally, into succinyl-CoA (Scott, 1999; Preynat et al., 2010). The latter transformation is vitamin B_{12}-dependent; indeed, this vitamin plays a role of coenzyme for methylmalonyl-CoA mutase (MUT) (Scott, 1999). In vitamin B_{12} deficiency, methylmalonyl-CoA accumulates and is then converted into methylmalonic acid (MMA). In a previous study, the combined dietary supplement of folic acid and vitamin B_{12} increased plasma glucose of multiparous cows in early lactation but no effect was noted for cows receiving vitamin B_{12} supplement alone (Graulet et al., 2007). Moreover, by infusing D-[U^{13}-C]glucose at 87 days in milk (DIM) to multiparous cows, Preynat et al. (2009a) observed an increase of irreversible loss rate (ILR) of glucose by 160 g/d for cows receiving weekly intramuscular injections of a combined supplement of folic acid and vitamin B_{12} as compared with control cows. At equilibrium, ILR equals the rate of appearance which represents the sum of glucose entry rates from portal absorption, glycogenolysis, and gluconeogenesis. These authors hypothesized that this increase was

likely due to an enhanced gluconeogenesis caused by the combined vitamin supplement. Variation of glucose absorption and glycogenolysis were discarded as being the cause of the increased glucose ILR as DMI was similar between treatments and no study reports an effect of a combined supplement of folic acid and vitamin B_{12} on glycogen kinetic (Preynat et al., 2009a).

Vitamin B_{12} also acts as a coenzyme in the methylation cycle (Figure 8.1) and is closely interrelated with folate metabolism. Indeed, 5-methyl-tetrahydrofolate, the methylated form of folic acid, can give its methyl group to homocysteine (Hcy) to form Met using vitamin B_{12} as a coenzyme of Met synthase. Methionine could be used to form protein or could be transformed in S-adenosylmethionine which is the major methyl donor in mammals (Scott, 1999). After its demethylation, tetrahydrofolate could be involved in purine and pyrimidine syntheses, both constituents of DNA. Consequently, a lack of folic acid and vitamin B_{12} impedes cell division (Scott, 1999).

It was hypothesized that increasing folic acid and vitamin B_{12} supplies would improve gluconeogenesis by allowing more propionate entering into the Krebs cycle. This study was undertaken to evaluate whole-body (WB) kinetics of glucose and propionate and the proportion of glucose synthetized from propionate at week 9 of lactation following a supplementation of folic acid and vitamin B_{12}, alone or combined, given by weekly intramuscular injections from 3 weeks pre-partum to 9 weeks post-partum. By giving the vitamin B_{12} supplement alone or combined with the supplement of folic acid, this experiment allowed studying if folic acid supplement is required for the expression of the effect of a supplement of vitamin B_{12} on the propionate metabolic pathway described above. Moreover, concentrations of metabolites and hepatic expression of genes closely related to the methylation cycle and propionate pathway were measured in order to better characterize the effects of these vitamin supplements on dairy cows during the week of labeled glucose and propionate infusions.

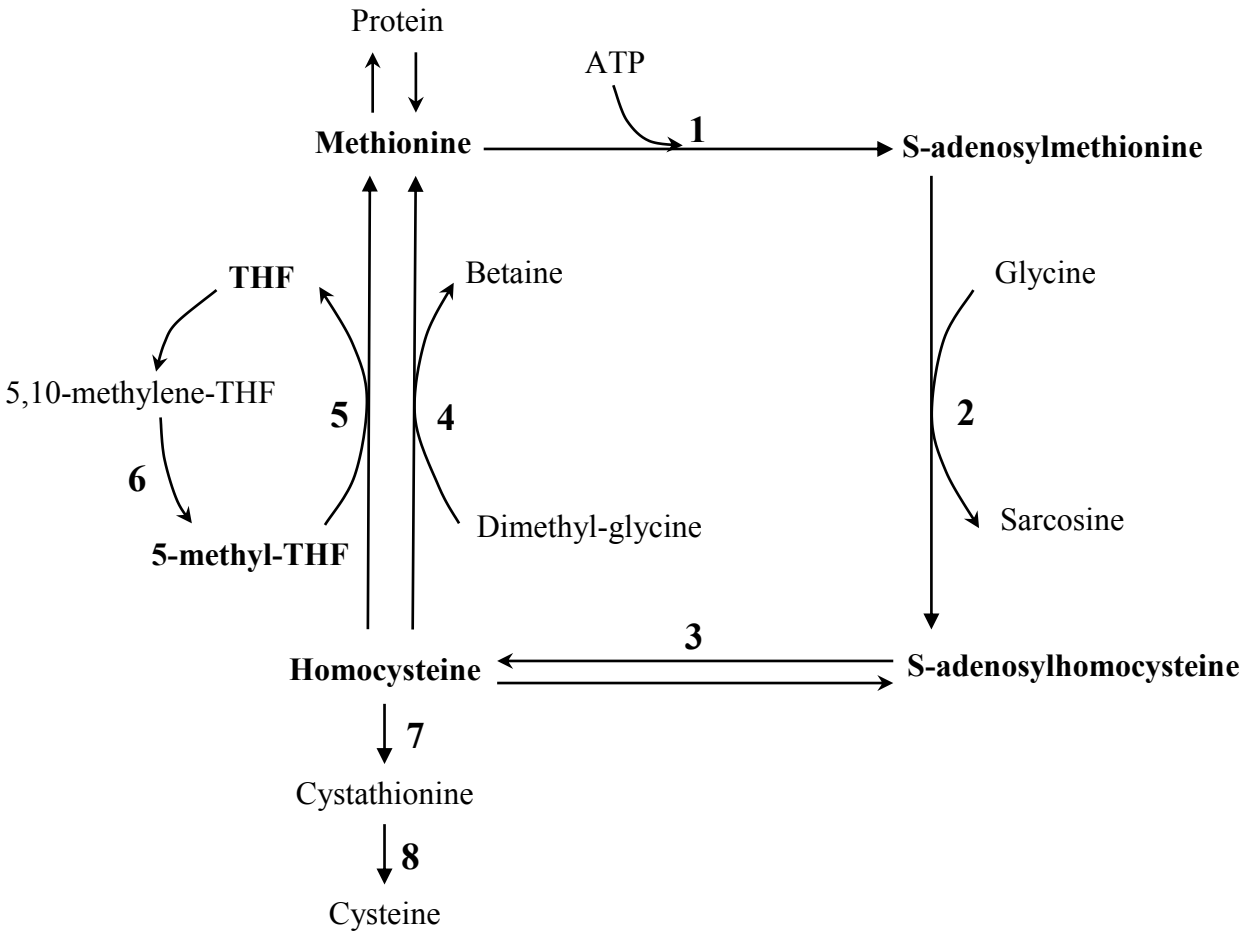

Figure 8.1 Simplified metabolic pathways involving folic acid and vitamin B_{12}. THF = tetrahydrofolate; Enzymes: 1 = methionine adenosyltransferase; 2 = glycine N-methyltransferase; 3 = S-adenosylhomocysteine hydrolase; 4 = betaine homocysteine methyltransferase; 5 = methionine synthase and vitamin B_{12} as a coenzyme; 6 = 5,10-methylenetetrahydrofolate reductase; 7 = cystathionine β-synthase; 8 = cystathionine β-lyase (Adapted from Preynat et al., 2010)

8.4 Materials and Methods

8.4.1 Cows and Treatments

Twenty-four multiparous Holstein cows from the dairy herd at the Agriculture and Agri-Food Canada Research Centre (Sherbrooke, QC, Canada) were enrolled in this experiment as previously described in Chapter 7 (companion paper). Briefly, animals were kept in a tie-stall barn and milked twice daily at 12-hour intervals under 1730 h of light per day. Cows were assigned to 6 blocks of 4 animals each according to their previous 305-day milk production to one of the following treatments: 1) saline 0.9% NaCl (B_9-B_{12}-); 2) 320 mg of folic acid (B_9+B_{12}-; pteroylmonoglutamic acid, MP Biomedicals, Solon, OH); 3) 10 mg of vitamin B_{12} (B_9-B_{12}+; cyanocobalamin, 5,000 µg/mL, Vétoquinol, Lavaltrie, QC, Canada) or; 4) 320 mg of folic acid and 10 mg of vitamin B_{12} (B_9+B_{12}+). Intramuscular injections of 5 mL were administered weekly from 3 weeks before the expected calving date until 9 weeks of lactation. The parenteral route was chosen instead of dietary supplementation as it has been shown that the utilization and destruction of vitamins by the rumen microorganisms are large (Santschi et al., 2005).

Before parturition, a pre-calving diet was fed to dairy cows and a lactation diet was offered postpartum (Chapter 7; companion paper). Daily intake and orts of each cow were weighed and feed offered was adjusted if needed allowing 10% refusals. Cows had free access to water. Six days before the infusions of labelled glucose and propionate, beginning approximately at 8 weeks of lactation, 12 equal meals per day served as a total mixed ration were given every 2 hours to dairy cows using automated feeders (Ankom, Fairport, NY) to minimize post-prandial variations during the measurements of WB glucose and propionate rate of appearance (Ra). Care of cows followed the guidelines of the National Farm Animal Care Council (2009). All procedures were approved by the Institutional Committee on Animal Care of the research center according to the guidelines of the Canadian Council on Animal Care (2009).

8.4.2 Surgery

A surgery to insert a catheter (Tygon Microbore Tubing, Formula S-54-HL, Saint-Gobain Performance Plastics, Courbevoie, France) into a ruminal vein for performing infusion of labeled propionate was carried out at 54 ± 2 DIM. The day before the surgery, a catheter was inserted into each jugular vein: one for infusion of labeled glucose and the other one for blood sampling. Two and one days before the surgery, 2/3 and 1/3, respectively, of the TMR were offered to the animals to prepare them for the surgery. Water access was cut off 12 hours before the surgery. However, throughout the night, a solution of ultra-pure water (19.0 L), NaCl (90.0 g) and dextrose 50% (1.0 L) was infused in one jugular vein. At least 30 minutes before the surgery, 40 mL of penicillin G procaine (300,000 IU of penicillin G/mL; Zoetis Canada Inc., Kirkland, QC, Canada) and 20 mL of ketoprofen (100 mg of ketoprofen/mL; Merial Canada Inc., Baie-d'Urfé, QC, Canada) were injected intramuscularly.

The surgery was done under paravertebral anesthesia on the left side of the standing cow. A skin incision of 20-30 cm was performed on the ventral part of the paralumbar fossa. After incising muscles and peritoneum, dorsal sack of the rumen was exteriorized in order to localize one ruminal vein. In average, a catheter length of 40 cm was inserted into one left ruminal vein. Catheter was then exteriorized between left transverse processes of lumbar vertebras 3-4 or 4-5. Immediately after the surgery, cows had free access to water and hay was offered. A few hours after the surgery, half of the TMR was offered. A solution of ultra-pure water (19.0 L), NaCl (110.0 g), KCl (30.0 g), dextrose 50% (0.5 L), and calcium borogluconate (0.5 L) was infused in one jugular vein to help the cows recovering from the surgery. During the 2 days following the surgery, cows received intramuscular injections of 40 mL of procainic penicillin G twice a day and 20 mL of ketoprofen once a day.

8.4.3 Sampling and Measurements

8.4.3.1 Blood

Blood samples were taken at 61 ± 3 DIM at 1315 h by venipuncture of the coccygeal vein using a vacutainer system (Becton, Dickinson and Co., Franklin Lakes, NJ) and were treated as described in the Chapter 7 (companion paper).

8.4.3.2 Body Weight and Body Condition Score

Cows were weighed after the morning milking for 2 consecutive days at 70 and 71 ± 3 DIM. Average body weight (BW) for those 2 consecutive days was used for statistical analysis. Body condition scores (BCS) were done by the same individual at 61 ± 3 DIM according to a 1 (very thin) to 5 (very fat) scale using quarter points (Wildman et al., 1982; Ferguson et al., 1994).

8.4.3.3 Milk Yield and Samples

Milk yields were recorded at each milking the week before the infusion, i.e. between 56 and 63 DIM and daily yields were averaged for the whole week. Milk samples were taken from the 2 consecutive milkings before performing tracer infusions at 63 ± 3 DIM and milk composition (fat, protein, and lactose) was analyzed by Valacta (Dairy Production Center of Expertise, Québec and Atlantic Provinces, Ste-Anne-de-Bellevue, QC, Canada) by mid infrared reflectance spectrometry. Milk fat, protein, and lactose yields were computed from average milk yield the week before the tracer infusion multiplied by respective milk concentrations.

8.4.4 Blood and Milk Analyses

8.4.4.1 Blood Samples

Plasma concentrations of folates, vitamin B_{12}, glucose, β-hydroxybutyrate (BHBA), nonesterified fatty acids (NEFA), and urea were analyzed using commercial kits as previously described in Chapter 7 (companion paper). Plasma concentrations of MMA and

amino acids (AA) were analyzed using GC-MS (model CG6890-MS5973, Hewlett Packard Co., Wilmington, DE) as described in Chapter 7 (companion paper).

8.4.4.2 Milk Samples

Folates and vitamin B_{12} in milk were analyzed in duplicate by radioassay using a commercial kit (SimulTRAC® B_{12}/FOLATE-S, MP Biomedicals, Solon, OH).

Regarding milk folates, milk samples were first thawed in a water bath at 37°C for 30 minutes and analyzed according to the AOAC procedure (AOAC, 2000). A total of 2.0 g of milk were weighed in a 50 mL cone-shape tube, 10 mL of 0.1 M of Na_2HPO_4, and 100 µL of protease (protease Type XIV: bacterial, from *Streptomyces griseus*; EC number = 232-909-5; Sigma-Aldrich, Oakville, ON, Canada) was added and vortexed. Tubes were incubated in a water bath for 90 minutes at 37°C and frequently shaken. After the incubation, tubes were put in the autoclave for 3 minutes at 121°C to stop enzyme activity and then placed in cold water for 5 minutes. A total of 400 µL of conjugase (from lyophilized chicken pancreas powder; Pel-Freez® Biologicals, Rogers, AR) was put in tubes and incubated at 37°C for 180 minutes. Then, tubes were autoclaved as described above and were centrifuged at 5,000 × *g* for 10 minutes at 4°C. A volume of 50 µL of supernatant and 150 µL of ultra-pure water were placed in 2.0 mL Eppendorf tubes and kept frozen at -20°C until analysis by radioassay. The inter-assay CV was 2.3%.

For milk concentration of vitamin B_{12}, the technique based on folates analysis was adapted by DeVries et al. (2005), Hyun and Tamura (2005), and Chen and Eitenmiller (2007) as previously described in the Chapter 6. The inter-assay CV was 1.7%.

8.4.5 Tracer Infusions

At 63 ± 3 DIM, D-[6,6-$^2H_2$]-glucose (99 mole percent excess; Cambridge Isotope Laboratories Inc., Andover, MA) at a rate of 16.5 ± 0.4 mmol/h and [1-^{13}C]-sodium propionate (99 mole percent excess; Cambridge Isotope Laboratories Inc., Andover, MA) at a rate of 13.9 ± 0.4 mmol/h were simultaneously infused in one jugular vein and in one

ruminal vein, respectively, using a syringe pump. Tracers were previously dissolved in sterile saline. Infusions began on average at 0930 h and lasted 4 hours.

8.4.5.1 Blood Sampling

The day of tracer infusions, one blood sample was collected on average 1 hour before the initiation of infusions to determine isotopic natural abundance. Two hours following the beginning of the labeled glucose and propionate infusions, blood samples were taken every 15 minutes from the contralateral jugular catheter for the next 2 hours (n = 9) and placed in heparinized vacutainer tubes (Becton, Dickinson and Co., Franklin Lakes, NJ). Tubes were kept on ice until centrifugation and were centrifuged within 60 minutes after collection at 3,000 × *g* and 4°C for 15 minutes. Aliquots of plasma were transferred into 2-mL tubes and stored at -80°C until analysis.

8.4.5.2 Enrichment Analyses

The isotopic enrichment (IE) of glucose was performed with GC-MS (model CG6890-MS5973N, Hewlett Packard Co., Wilmington, DE) as described by Preynat et al. (2009a) in the positive chemical ionization mode. The IE of glucose isotopomers were assessed by monitoring ions at m/z 331, 332, and 333 to quantify IE of m+1 and m+2.

Propionate IE was measured using a GC-MS (model CG6890-MS5973N, Hewlett Packard Co., Wilmington, DE) with mass selective detector operating in the positive chemical ionization mode. Plasma (500 µL) was first deproteinized with 0.2 g of 5-sulfosalisilic acid hydrate, vortexed for 1 minute and then centrifuged at 16,200 × *g* for 5 minutes at 4°C. Supernatants were collected, 1.5 mL of ethyl ether was added into 2 mL eppendorf tubes and the mixture was vortexed 5 times before a centrifugation at 16,200 × *g* for 2 minutes at 4°C. Supernatants were transferred into 2 mL eppendorf tubes with drierite in the bottom (calcium sulfate; W. A. Hammond Drierite Co. LTD, Xenia, OH), kept at room temperature for 2 hours and frequently shaken. Two hours later, tubes were centrifuged at 16,200 × *g* for 2 minutes at 4°C. A total of 60 µL of acetonitrile mixed with sodium sulphate was pipetted into 2-mL glass vials (reacti-Vial) and a pen mark was done at the level of the acetonitrile sodium sulphate blend on the vial. Supernatants from the last centrifugation were transferred into the glass vials which were capped and then vortexed. Samples were

then evaporated under N using a multineedle manifold (Reacti-Therm III, Heating module, Pierce, Rockford, IL) and the volume was decreased until the pen mark on the glass vials. A total of 10 µL of N-tert-butyldimethylsilyl-N-methyltrifluoroacetamide (Sigma-Aldrich, Oakville, ON, Canada) was added and samples were heated at 60°C for 10 minutes and analyzed for IE by monitoring ions at m/z 131 and 132.

8.4.5.3 Calculations

Glucose and propionate IE are expressed as mole percent excess. Whole-body glucose and propionate Ra were computed as follows: Ra (mmol/h) = (infusion rate/IE) - infusion rate where infusion rate is expressed in mmol/h. For WB glucose Ra, the calculation was made using IE from m+2 glucose isotopomer. The proportion of glucose synthesized from propionate was obtained as following: percentage (%) of glucose synthesized from propionate = (m+1 glucose IE/propionate IE) × 2. This factor 2 needed to be used to take into account that half of the labeled C from [1-^{13}C]-sodium propionate would be lost as CO_2 out of the mitochondria during the transformation of malate into oxaloacetate, then into phosphoenolpyruvate to form glucose (Mayes, 1981). Glucose flux from propionate (mmol/h) was calculated by multiplying the % of glucose synthesized from propionate by WB glucose Ra.

8.4.6 Liver Biopsies and Analyses

Liver biopsies were performed at 64 ± 3 DIM according to the procedure previously described by Graulet et al. (2007). Briefly, liver biopsies were done at the level of the 10th intercostal space under local anesthesia by a veterinarian using an ultrasound scanner to view liver tissues and then to avoid liver hemorrhage. On average, 1,658 ± 338 mg of liver tissue was taken off. Excess of fat or blood was removed from liver samples and then they were frozen in liquid N and stored at -80°C until analysis.

8.4.6.1 Folates and Vitamin B_{12}

Methods for folate and vitamin B_{12} analyses in liver were previously described by Graulet et al. (2007). About 40 mg and 30 mg of frozen liver samples were used for folate and

vitamin B_{12} quantifications, respectively. Commercial kits designed for human plasma were used to determine concentrations of folates and vitamin B_{12} in liver by radioassay (SimulTRAC® B_{12}/FOLATE-S, MP Biomedicals, Solon, OH).

8.4.6.2 Glycogen

Glycogen concentration of liver was determined according to the protocol of Hawk and Bergeim (1926) with modifications as described by Bernal-Santos et al. (2003). About 100 mg of liver tissue was used for the purpose of the analysis. After conversion of liver glycogen to glucose by amyloglucosidase (from *Aspergillus niger*, Sigma A-1602; EC number = 3.2.1.3; Sigma-Aldrich, Oakville, ON, Canada), glucose was analyzed using a commercial kit (Glucose (Trinder) assay, Genzyme Diagnostics P.E.I. Inc., Charlottetown, PEI, Canada).

8.4.6.3 Total RNA Isolation and Purification

Total RNA was extracted from hepatic tissue by using a QIAzol Lysis Reagent (QIAGEN, Toronto, ON, Canada) following the original manufacturer protocol with slight modifications. Briefly, frozen samples (100 mg of liver tissue) were homogenized in 2 mL of QIAzol Lysis Reagent on ice using a tissue-tearor. A volume of 600 µL QIAzol Lysis Reagent was added to 400 µL of homogenate; the mixture was vigorously vortexed and kept at room temperature for 5 minutes to promote dissociation of nucleoprotein complexes. A volume of 200 µL of chloroform was added; the mixture was shaken and left at room temperature for 3 minutes followed by a centrifugation at 12,000 × *g* for 15 minutes at 4°C to remove lipids. After centrifugation, the aqueous fraction (upper layer) which contained RNA was carefully pipetted, and RNA was precipitated by adding an equal volume of 70% ethanol and mixed thoroughly by vortexing. RNA was then purified using RNeasy Mini Kit (QIAGEN, Toronto, ON, Canada), including on-column DNase digestion.

8.4.6.4 Quantitative Real Time-PCR

Quantifications by real-time PCR following reverse transcription were performed as previously described by Lévesque-Sergerie et al. (2007) with minor modifications

(Dudemaine et al., 2014). Table 8.1 provides primer sequences and optimal conditions for PCR of studied genes. The real time-PCR reactions (10 μL, final volume) were performed on 96-well plates using Fast SYBR Green PCR Master Mix (Life Technologies, Burlington, ON, Canada) in a 7,500 Fast real time-PCR System (Life Technologies, Burlington, ON, Canada). The expression of 5 reference genes, namely β-actin (ACTB), peptidylprolyl isomerase A (PPIA), glyceraldehyde-3-phosphate dehydrogenase (GAPDH), ubiquitously-expressed transcript (UXT), tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, zeta polypeptide (YWHAZ) was determined for all samples. Genes ACTB and PPIA were taken as housekeeping genes for normalization as their stability value was the highest (0.029) among the others (≥ 0.060). Their geometrical means were used for normalization of longitudinal gene expression profiling as described previously (Bionaz and Loor, 2007).

8.4.6.5 Methylmalonyl-CoA Mutase Activity

Determination of hepatic total activity of MUT and holomutase were performed as described by Ouattara et al. (2013).

Table 8.1 Primer sequences for PCR analysis of studied genes of dairy cows

Primer names[1]		Primer sequences (5'-3')	Primer position (nt)	GenBank accession no.	Length (bp)
ACTB	Forward	TGGCACCCAGCACAATGA	1051-1068	NM_173979	123
	Reverse	CCTGCTTGCTGATCCACATCT	1053-1173		
BHMT	Forward	GTGACCCCTGGCGAGTGT	640-657	NM_001011679	67
	Reverse	GGCAGTTTACCCCCACGAT	688-706		
CBS	Forward	GGGCAAGCTCTCGCACAT	1593-1610	NM_001102000	114
	Reverse	ACGACCCCGAACACCATCT	1688-1706		
GAPDH	Forward	GCCTCCTGCACCACCAACT	513-531	NM_001034034	112
	Reverse	TCTTCTGGGTGGCAGTGATG	605-625		
GNMT	Forward	TGAACAACAAGGCCCACATG	647-666	NM_001206116	117
	Reverse	CCAGGCAGTGAGGGTAGTAGGA	742-763		
MTHFR	Forward	CCCAACCAGAATGGCTACAAA	1515-1535	NM_001011685	150
	Reverse	CGGCTTCCCGTTGATGTT	1647-1664		
MUT	Forward	CCCTTGGACGGCCAGATATT	2257-2276	NM_173939	84
	Reverse	AAACACCAACTTCAAACAGAAATTCA	2315-2340		
PPIA	Forward	ATGCTGGCCCCAACACAA	317-334	NM_178320	101
	Reverse	CCCTCTTTCACCTTGCCAAA	398-417		
SAHH	Forward	GTGGATCGCTACTTGTTGAAGAAC	1064-1087	NM_001034315	114
	Reverse	GAAGGAGTTGCTCATCACAAAGC	1155-1177		
UXT	Forward	TGGCAGAAGCTCTCAAGTTCATT	339-361	NM_001037471	105
	Reverse	CATGTGGATATGGGCCTTGAT	423-443		
YWHAZ	Forward	AATGCAACCAACACATCCTATCAG	530-553	NM_174814	131
	Reverse	GTTCAGCAATGGCTTCATCAAAT	638-660		

[1] ACTB = β-actin; BHMT = betaine homocysteine methyltransferase; CBS = cystathionine β-synthase; GAPDH = glyceraldehyde-3-phosphate dehydrogenase; GNMT = glycine N-methyltransferase; MTHFR = 5,10-methylene-tetrahydrofolate reductase; MUT = methylmalonyl-CoA mutase; PPIA = peptidylprolyl isomerase A; SAHH = S-adenosylhomocysteine hydrolase; UXT = ubiquitously-expressed transcript; YWHAZ = tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, zeta polypeptide.

8.4.7 Statistical Analyses

Two levels of folic acid (0 or 320 mg/wk) and 2 levels of vitamin B_{12} (0 or 10 mg/wk) were used in a 2 × 2 factorial arrangement in a 6 randomized block design. Milk yield and DMI the week before tracer infusions were averaged. All variables were analyzed using the MIXED procedure of SAS (version 9.2, SAS Institute, 2008, Cary, NC). Fixed effects were folic acid, vitamin B_{12}, blocks as well as folic acid × vitamin B_{12} interaction. Results were considered significant when $P \leq 0.05$ and as a tendency at $0.05 < P \leq 0.10$. When the interaction folic acid × vitamin B_{12} was significant or a tendency, the SLICE option in the LSMEANS statement of SAS was used to help interpretation. The insertion of the catheter into the ruminal vein was not possible for one cow due to a problem with the ruminal vein conformation and the ruminal catheter was not working for one cow the day of infusion. For these 2 cows (B_9+B_{12}+), only data from labeled glucose infusion was used in the analysis. One cow (B_9+B_{12}-) was not included in the statistical analyses for WB kinetics because her DMI the day of tracer infusions was 5.8 kg which was on average lower by 15.0 kg than other cows involved in the study. Liver sample from one control cow (B_9-B_{12}-) was not sufficient to perform folate, vitamin B_{12}, and glycogen analyses.

8.5 Results and Discussion

8.5.1 Production Data

Dry matter intake, BW, and BCS were not different among treatments at week 9 postpartum (Table 8.2) as previously observed (Girard and Matte, 2005; Graulet et al., 2007; Preynat et al., 2009a,b).

At week 9 of lactation, milk yield tended to be lower by 5.0 kg/d for cows receiving the folic acid supplement, alone or combined with the supplement of vitamin B_{12} as compared with cows that did not receive the folic acid supplement ($P = 0.10$; Table 8.2). This is in agreement with results reported in the companion paper (Chapter 7) as milk production for cows receiving a folic acid supplement was lower at weeks 6 and 7 of lactation as

compared with cows that did not receive the folic acid supplement. In contrast, Preynat et al. (2009a) reported that, at week 12 postpartum, a supplementation of folic acid plus vitamin B_{12} increased milk yield by 12%. Moreover, supplementary folic acid increased milk production by 3.4 kg/d during the first 8 weeks of lactation in a previous experiment (Graulet et al., 2007). No treatment effect was noted on milk concentrations of fat, protein, lactose, and total solids (TS) and milk yields of fat, protein, and TS ($P \geq 0.11$; Table 8.2). The tendency for a decrease of milk yield of lactose by 0.25 kg/d for cows receiving the folic acid supplement ($P = 0.07$; Table 8.2) could explain the decrease in milk production at week 9 as lactose is the primary osmo-regulator for uptake of water in the mammary gland for milk production (Linzell, 1972; Mepham, 1993).

The supplement of folic acid increased milk concentration of folates ($P = 0.01$; Table 8.2) but did not increase milk yield of folates ($P = 0.36$; Table 8.2). Graulet et al. (2007) reported an increase of both milk concentration and yield of folates whereas Preynat et al. (2009b) only observed an augmentation of milk yield of folates following supplementary folic acid. Milk concentration and yield of vitamin B_{12} were increased by the vitamin B_{12} supplement ($P < 0.0001$; Table 8.2) as frequently reported in the literature (Girard and Matte, 2005; Graulet et al., 2007; Akins et al., 2013). In the current study, vitamin B_{12} concentration of milk was greater by 1,814 pg/mL for cows receiving the folic acid supplement as compared with cows that did not ($P = 0.05$; Table 8.2).

8.5.2 Plasma Variables

An interaction folic acid × vitamin B_{12} was observed on plasma concentration of folates ($P = 0.05$; Table 8.3). However, results from the SLICE option of SAS indicated no significant effect of folic acid within vitamin B_{12} supplementation ($P \geq 0.11$). At week 9 postpartum, plasma concentration of folates was unaffected by folic acid supplement ($P = 0.92$; Table 8.3) and averaged 18.4 ± 1.6 ng/mL, in line with observations made in the same cows from weeks 4 to 7 of lactation (Chapter 7; companion paper). Preynat et al. (2009a) also reported a similar observation at week 12 of lactation for cows receiving a combined supplement of folic acid and vitamin B_{12} or not. Plasma concentration of vitamin B_{12} was significantly higher for dairy cows receiving the vitamin B_{12} supplement (416.6 ± 30.4 pg/mL), alone or

combined with folic acid, than cows that did not (319.9 ± 30.4 pg/mL; $P = 0.04$; Table 8.3) as similarly reported by Graulet et al. (2007), Preynat et al. (2009b), and Akins et al. (2013). However, plasma concentration of vitamin B_{12} of control cows was between 22 and 78% higher at week 9 postpartum in the current study than in other studies conducted in early lactation (Graulet et al., 2007; Preynat et al., 2009a; Akins et al., 2013). Plasma concentration of folates was higher by between 14 and 68% for control cows in the current trial than in the studies by Graulet et al. (2007), Preynat et al. (2009a), and Akins et al. (2013). These comparisons indicate that, regardless of vitamin supplementation, control cows in the current study already had a higher plasma folate and vitamin B_{12} status than in other studies cited above.

Plasma concentrations of glucose, NEFA, MMA, urea, Ala, Glu, Gly, Ile, Leu, Lys, Met, Phe, Pro, Ser, Trp, Tyr, Val, branched-chain AA, essential and nonessential AA, and total AA were unaffected by treatments at week 9 postpartum ($P \geq 0.11$; Table 8.3). Plasma concentrations of MMA was lower, even in control cows, than results reported by Girard and Matte (2005) suggesting that vitamin B_{12} was not lacking in the current experiment. Plasma concentration of BHBA tended to decrease from 0.76 to 0.61 ± 0.06 m*M* with the folic acid supplement, alone or combined with vitamin B_{12} ($P = 0.09$; Table 8.3) as reported for previous weeks in the Chapter 7 (companion paper). The supplement of vitamin B_{12} tended to increase plasma concentrations of Asn and Gln ($P \leq 0.08$; Table 8.3). Plasma concentration of Asp decreased ($P = 0.006$) whereas plasma concentrations of His and Hcy increased with the folic acid supplement ($P = 0.04$; Table 8.3). The highest plasma concentrations of Cys and Thr were observed for cows receiving the combined supplement of folic acid and vitamin B_{12} ($P \leq 0.10$). Plasma concentration of Met was higher than in the trials of Graulet et al. (2007) and Preynat et al. (2009a). Graulet et al. (2007) observed that a supplement of vitamin B_{12} increased plasma concentration of Hcy and Cys whereas in the study of Preynat et al. (2009a,b), plasma concentration of Hcy was decreased by the combined vitamin supplement and plasma concentration of Cys was unaffected by treatments. It has been reported that Hcy is a biomarker for Met excess in rats (Toue et al., 2006) and that an increased availability of Met leads to a decreased half-life of the methyl group of Met and increases cystathionine synthesis, a precursor for Cys formation (Finkelstein, 1990). These results suggest that, regardless of treatments, Met supply was

probably sufficient with the diet fed to these cows. By supplying a combined supplement of folic acid and vitamin B_{12}, a more rapid transmethylation cycle probably occurred followed by increased syntheses of Hcy and Cys to alleviate increased Met availability.

8.5.3 Liver Variables

8.5.3.1 B Vitamins

Liver concentration of folates was increased by 13.9 μg/g of fresh tissue by the folic acid supplement ($P < 0.0001$; Table 8.4). Graulet et al. (2007) and Preynat et al. (2010) also reported that a folic acid supplement, alone or combined with vitamin B_{12}, increased hepatic concentration of folates. As compared with the study of Preynat et al. (2010), liver concentrations of folates in the current experiment were greater by 202 and 161% for cows receiving the folic acid supplement and for cows that did not, respectively. However, it is noteworthy that, in the present study, the folic acid supplement was twice (320 mg) the amount used in the experiment of Preynat et al. (2010) (160 mg).

Intramuscular injections of vitamin B_{12} increased liver concentration of vitamin B_{12} ($P = 0.007$; Table 8.4) as previously reported (Preynat et al., 2010; Akins et al., 2013). Hepatic concentration of vitamin B_{12} in the present study was slightly lower than in the trial of Preynat et al. (2010).

8.5.3.2 Glycogen

Liver glycogen concentration did not differ among treatments and was on average 1.93 ± 0.32% of wet weight at 64 ± 3 DIM ($P \geq 0.32$; Table 8.4). Values reported here are higher than observed by Bernal-Santos et al. (2003) at 10 and 21 DIM but liver glycogen was reported to increase from 14 to 49 DIM (Weber et al., 2013).

Table 8.2 Effects of intramuscular injections of folic acid and vitamin B_{12}, alone or combined, on DMI, BW, BCS, and milk yield and components at week 9 of lactation (least square means)

	Treatment[1]					*P*-value		
Item	B_9-B_{12}-	B_9+B_{12}-	B_9-B_{12}+	B_9+B_{12}+	SEM	B_9	B_{12}	$B_9 \times B_{12}$
Cows (n)	6	6	6	6				
DMI (kg/d)	18.1	19.0	18.5	19.4	1.9	0.65	0.85	0.99
BW[2] (kg)	606	640	616	621	22	0.39	0.85	0.53
BCS[3]	2.21	2.42	2.38	2.42	0.12	0.30	0.49	0.49
Milk yield (kg/d)	36.4	30.0	38.1	34.4	2.9	0.10	0.31	0.64
Milk composition[4]								
Fat (g/kg)	33.7	38.8	36.0	35.4	2.0	0.30	0.79	0.18
Protein (g/kg)	28.6	30.7	29.3	32.2	1.4	0.11	0.44	0.77
Lactose (g/kg)	47.2	46.1	46.9	47.2	6.0	0.51	0.57	0.27
TS (g/kg)	109.6	115.6	112.3	114.8	2.7	0.14	0.73	0.53
Folates (ng/mL)	76.5	102.0	78.8	103.3	8.5	0.01	0.84	0.96
Vitamin B_{12} (pg/mL)	2,271	3,711	6,720	8,908	843	0.05	<.0001	0.66
Milk yields								
Fat (kg/d)	1.25	1.17	1.38	1.21	0.13	0.34	0.53	0.74
Protein (kg/d)	1.04	0.92	1.12	1.09	0.08	0.39	0.14	0.56
Lactose (kg/d)	1.72	1.38	1.79	1.62	0.13	0.07	0.26	0.51
TS (kg/d)	4.01	3.46	4.28	3.92	0.33	0.19	0.28	0.78
Folates (mg/d)	2.97	3.24	3.05	3.58	0.43	0.36	0.63	0.76
Vitamin B_{12} (µg/d)	91.5	114.2	258.3	300.3	29.5	0.29	<.0001	0.75

[1] B_9-B_{12}- = No vitamin supplement; B_9+B_{12}- = 320 mg of folic acid; B_9-B_{12}+ = 10 mg of vitamin B_{12}; B_9+B_{12}+ = 320 mg of folic acid and 10 mg of vitamin B_{12} given weekly from 3 weeks before the expected calving date until 9 weeks postpartum.

[2] Average BW at 70 and 71 ± 3 DIM.

[3] Data on BCS were measured at 61 ± 3 DIM according to a 1 to 5 scale with quarter points (Wildman et al., 1982; Ferguson et al., 1994).

[4] Data measured from 2 consecutive milkings before tracer infusions.

Table 8.3 Effects of intramuscular injections of folic acid and vitamin B_{12}, alone or combined, on plasma concentrations of B vitamins, glucose, BHBA, NEFA, methylmalonic acid, urea and, AA at 61 ± 3 days in milk (least square means)

	Treatment[1]					*P*-value		
Item	B_9-B_{12}-	B_9+B_{12}-	B_9-B_{12}+	B_9+B_{12}+	SEM	B_9	B_{12}	$B_9 \times B_{12}$
Cows (n)	6	6	6	6				
Folates (ng/mL)	20.5	16.7	16.5	19.9	1.6	0.92	0.81	0.05
Vitamin B_{12} (pg/mL)	298.9	340.8	385.4	447.9	42.9	0.24	0.04	0.81
Glucose (μ*M*)	3.45	3.70	3.61	3.55	0.13	0.48	0.99	0.25
BHBA (m*M*)	0.68	0.62	0.84	0.61	0.08	0.09	0.39	0.31
NEFA (μ*M*)	189.9	145.5	203.6	157.1	39.6	0.27	0.75	0.98
Methylmalonic acid (μ*M*)	0.37	0.31	0.29	0.38	0.04	0.69	0.94	0.11
Urea (m*M*)	5.46	5.65	5.04	5.51	0.45	0.47	0.54	0.76
AA (μ*M*)								
Ala	241.8	237.7	234.0	231.1	16.0	0.83	0.66	0.97
Asn	48.5	49.4	53.9	58.2	3.4	0.46	0.06	0.64
Asp	45.7	35.1	49.4	29.1	4.6	0.006	0.80	0.32
Cys	111.0	114.8	113.2	126.5	4.0	0.05	0.10	0.25
Glu	45.1	45.9	48.4	44.6	3.4	0.67	0.76	0.51
Gln	264.4	271.7	285.3	304.9	14.3	0.36	0.08	0.68
Gly	253.1	262.8	272.1	261.5	14.1	0.97	0.54	0.48
His	53.8	59.2	55.6	64.3	3.1	0.04	0.28	0.61
Hcy	4.86	5.08	4.93	5.75	0.25	0.04	0.13	0.21
Ile	135.0	130.7	170.2	143.0	19.9	0.44	0.25	0.57
Leu	181.2	180.7	210.2	183.7	20.7	0.52	0.45	0.54
Lys	89.7	85.7	78.0	85.6	7.7	0.82	0.45	0.46
Met	28.2	28.3	28.6	29.0	1.6	0.88	0.75	0.91

Table 8.3 Continued

Item	Treatment[1]				SEM	*P*-value		
	B_9-B_{12}-	B_9+B_{12}-	B_9-B_{12}+	B_9+B_{12}+		B_9	B_{12}	$B_9 \times B_{12}$
Phe	58.4	61.0	58.6	60.8	2.9	0.41	1.0	0.93
Pro	83.7	97.7	87.4	92.5	8.2	0.22	0.92	0.57
Ser	75.9	81.6	80.4	79.3	5.5	0.68	0.85	0.54
Thr	92.9	104.7	106.0	114.5	5.3	0.08	0.05	0.76
Trp	49.8	48.6	48.1	50.9	2.1	0.72	0.88	0.36
Tyr	62.5	60.8	64.8	70.3	4.2	0.66	0.18	0.40
Val	238.8	243.3	267.4	241.5	22.2	0.64	0.55	0.50
BCAA[2]	555.0	554.6	647.7	568.2	61.8	0.53	0.40	0.53
EAA[3]	927.8	942.1	1,022.6	979.2	73.8	0.85	0.40	0.71
NEAA[4]	1,231.6	1,257.4	1,288.9	1,331.4	47.5	0.50	0.20	0.87
TAA[5]	2,164	2,205	2,316	2,238	102	0.86	0.38	0.57

[1] B_9-B_{12}- = No vitamin supplement; B_9+B_{12}- = 320 mg of folic acid; B_9-B_{12}+ = 10 mg of vitamin B_{12}; B_9+B_{12}+ = 320 mg of folic acid and 10 mg of vitamin B_{12} given weekly from 3 weeks before the expected calving date until 9 weeks postpartum.
[2] BCAA = branched-chain amino acids = Ile + Leu + Val.
[3] EAA = essential amino acids = His + Ile + Leu + Lys + Met + Phe + Thr + Trp + Val.
[4] NEAA = nonessential amino acids = Ala + Asn + Asp + Cys + Gln + Glu + Gly + Pro + Ser + Tyr.
[5] TAA = total amino acids.

Table 8.4 Effects of a folic acid and vitamin B_{12} supplement, alone or combined, on liver concentrations of folates, vitamin B_{12} and glycogen, relative mRNA abundance of selected genes, and MUT activity at 64 ± 3 days in milk (least square means ± standard error)

	Treatment[1]				*P*-values		
Item	B_9-B_{12}-	B_9+B_{12}-	B_9-B_{12}+	B_9+B_{12}+	B_9	B_{12}	$B_9 \times B_{12}$
Cows (n)	6[2]	6	6	6			
Vitamins (μg/g of fresh tissue)							
Folates	17.3 ± 2.1	30.4 ± 1.9	17.6 ± 1.9	32.4 ± 1.9	<.0001	0.57	0.68
Vitamin B_{12}	0.78 ± 0.08	0.63 ± 0.07	0.89 ± 0.07	0.97 ± 0.07	0.65	0.007	0.11
Glycogen (% of wet weight)	1.78 ± 0.36	2.17 ± 0.32	2.02 ± 0.32	1.74 ± 0.32	0.87	0.79	0.32
Relative mRNA abundance[3]							
BHMT	1.09 ± 0.22	0.72 ± 0.22	0.92 ± 0.22	0.90 ± 0.22	0.35	0.97	0.41
CBS	1.38 ± 0.24	1.25 ± 0.24	1.24 ± 0.24	1.29 ± 0.24	0.85	0.82	0.64
GNMT	0.86 ± 0.27	0.94 ± 0.27	0.72 ± 0.27	0.55 ± 0.27	0.84	0.24	0.57
MTHFR	0.98 ± 0.15	0.73 ± 0.15	1.06 ± 0.15	0.94 ± 0.15	0.23	0.32	0.67
MUT	1.18 ± 0.20	0.80 ± 0.20	1.10 ± 0.20	1.37 ± 0.20	0.77	0.21	0.09
SAHH	1.64 ± 0.16	1.22 ± 0.16	1.41 ± 0.16	2.03 ± 0.16	0.51	0.07	0.004
MUT activity (nmol/mg protein/min)							
Total	13.84 ± 0.71	14.11 ± 0.71	14.97 ± 0.71	14.65 ± 0.71	0.97	0.25	0.68
Holomutase	0.51 ± 0.06	0.58 ± 0.06	0.62 ± 0.06	0.72 ± 0.06	0.17	0.05	0.81
Holomutase/total (%)	3.73 ± 0.39	4.13 ± 0.39	4.15 ± 0.39	4.87 ± 0.39	0.18	0.16	0.65

[1] B_9-B_{12}- = No vitamin supplement; B_9+B_{12}- = 320 mg of folic acid; B_9-B_{12}+ = 10 mg of vitamin B_{12}; B_9+B_{12}+ = 320 mg of folic acid and 10 mg of vitamin B_{12} given weekly from 3 weeks before the expected calving date until 9 weeks postpartum.

[2] Regarding vitamin and glycogen contents, liver sample from one cow was not sufficient to perform these analyses.

[3] BHMT = betaine homocysteine methyltransferase; CBS = cystathionine β-synthase; GNMT = glycine N-methyltransferase; MTHFR = 5,10-methylene-tetrahydrofolate reductase; MUT = methylmalonyl-CoA mutase; SAHH = S-adenosylhomocysteine hydrolase.

8.5.3.3 Gene Expressions

No treatment effect was observed on mRNA abundance of betaine homocysteine methyltransferase, cystathionine β-synthase, glycine N-methyltransferase, and 5,10-methylenetetrahydrofolate reductase ($P \geq 0.23$; Table 8.4). A tendency for a folic acid × vitamin B_{12} interaction on relative mRNA abundance of MUT was noted ($P = 0.09$; Table 8.4). No treatment effect was observed on relative mRNA abundance of MUT when cows did not receive the folic acid supplement ($P = 0.75$) whereas relative mRNA abundance of MUT was affected by the level of vitamin B_{12} supplement when cows received the folic acid supplement ($P = 0.04$). Indeed, the highest MUT mRNA relative abundance was obtained with the combined supplement of folic acid and vitamin B_{12} as in the study of Preynat et al. (2010). However, Graulet et al. (2007) did not report any treatment effect in early lactation on gene expression of MUT for cows receiving dietary folic acid and vitamin B_{12} supplement. The lowest hepatic concentration of vitamin B_{12} and gene expression of MUT were observed in cows receiving the folic acid supplement alone. In rat liver, Nakao et al. (2009) noted that mRNA level of MUT was decreased by a vitamin B_{12} deficient diet as compared with diet providing sufficient amount of vitamin B_{12}. In the current study, even though vitamin B_{12} was probably not deficient for dairy cows receiving the folic acid supplement alone, it could explain why gene expression of MUT was lower for these cows as vitamin B_{12} concentration in liver was also lower than in other treatment groups. A folic acid × vitamin B_{12} interaction was observed on gene expression of S-adenosylhomocysteine hydrolase (SAHH; $P = 0.004$; Table 8.4); the highest gene expression of SAHH was obtained for cows receiving the combined supplement of folic acid and vitamin B_{12} as compared with folic acid or vitamin B_{12} supplementation alone ($P \leq 0.009$). In the methylation cycle (Figure 8.1), SAHH is required to transform S-adenosylhomocysteine to Hcy (Finkelstein, 1990). The greatest gene expression of SAHH for cows receiving the combined vitamin supplement is in accordance with the highest plasma concentration of Hcy and Cys observed in these cows at week 9 of lactation but also within the first 7 weeks postpartum (Chapter 7; companion paper).

8.5.3.4 Methylmalonyl-CoA Mutase Activity

As reported by Graulet et al. (2007), total MUT specific activity was unaffected by a supplementation of folic acid and vitamin B_{12}, alone or combined, and averaged 14.39 ± 0.71 nmol/mg protein per minute ($P \geq 0.25$; Table 8.4). Holomutase activity was increased by 0.13 nmol/mg protein per minute by the vitamin B_{12} supplement ($P = 0.05$; Table 8.4). This result could be explained by vitamin B_{12} being a coenzyme of MUT (Le Grusse and Watier, 1993). Nevertheless, the percentage of holomutase activity from total MUT specific activity was not different among treatments ($P \geq 0.16$; Table 8.4) and was less than 5% as reported by Nakao et al. (2009) in rat liver.

8.5.4 Whole-Body Kinetics

Plasma concentration of glucose during the tracer infusions was unaffected by treatments and averaged 3.51 ± 0.13 μ*M* ($P \geq 0.66$; Table 8.5). Whole-body glucose Ra and the percentage of milk lactose from WB glucose Ra tended to decrease with the folic acid supplement by 229 g/d and 5.0%, respectively ($P \leq 0.10$; Table 8.5). Whole-body glucose Ra in the current study was greater than previously reported in some studies infusing D-[6,6-$^2H_2$]-glucose to dairy cows at later stages of lactation whereas the percentage of milk lactose from WB glucose Ra was similar (Lemosquet et al., 2009a; Galindo et al., 2011; Maxin et al., 2013). Net portal fluxes of propionate of early lactating cows reported by Reynolds et al. (2003) and Raun and Kristensen (2011) were in the same range as the WB propionate Ra in the current experiment.

Table 8.5 Effects of intramuscular injections of folic acid and vitamin B_{12}, alone or combined, on whole-body glucose and propionate kinetics at 63 ± 3 days in milk (least square means ± standard error)

	Treatment[1]				*P*-value		
Item	B_9-B_{12}-	B_9+B_{12}-	B_9-B_{12}+	B_9+B_{12}+	B_9	B_{12}	$B_9 \times B_{12}$
Cows (n)	6	5	6	6[2]			
Glucose							
Plasma glucose (μ*M*)	3.52 ± 0.11	3.48 ± 0.11	3.48 ± 0.11	3.55 ± 0.11	0.92	0.93	0.66
WB glucose Ra[3] (mmol/h)	848 ± 29	772 ± 32	810 ± 29	780 ± 29	0.10	0.64	0.45
Milk lactose/WB glucose Ra[4] (%)	50.8 ± 2.7	44.7 ± 3.1	53.7 ± 2.7	49.6 ± 2.7	0.09	0.19	0.73
Propionate							
WB propionate Ra[3] (mmol/h)	1,132 ± 138	964 ± 157	930 ± 138	994 ± 180	0.75	0.58	0.46
Glucose from propionate (%)	64.6 ± 6.6	61.3 ± 7.5	58.3 ± 6.6	54.7 ± 8.5	0.65	0.39	0.98
Glucose flux from propionate (mmol/h)	545 ± 60	467 ± 68	473 ± 60	437 ± 78	0.42	0.45	0.75

[1] B_9-B_{12}- = No vitamin supplement; B_9+B_{12}- = 320 mg of folic acid; B_9-B_{12}+ = 10 mg of vitamin B_{12}; B_9+B_{12}+ = 320 mg of folic acid and 10 mg of vitamin B_{12} given weekly from 3 weeks before the expected calving date until 9 weeks postpartum.

[2] Regarding propionate results, 2 cows were discarded in the treatment B_9+B_{12}+ due to problem with ruminal catheterization.

[3] Whole-body glucose and propionate rate of appearance (WB Ra) measured using D-[6,6-$^2H_2$]-glucose and [1-^{13}C]-sodium propionate continuous and simultaneous infusions.

[4] Ratio calculated from milk lactose and WB Ra of glucose expressed in g/d.

The lower WB glucose Ra observed for cows receiving the folic acid supplement, alone or combined with the supplement of vitamin B_{12}, indicates a reduction in glucose availability, in line with the lower milk yield and lactose synthesis observed in these cows. As WB glucose Ra represents the sum of glucose synthesis via gluconeogenesis, glucose absorption from the hepatic portal-drained viscera, and glycogenolysis (Lemosquet et al., 2009b; Galindo et al., 2011) and DMI was not significantly different among treatment groups, it is then unlikely that the decrease of WB glucose Ra observed with supplementary folic acid was due to change in portal glucose absorption, and neither was the WB Ra of propionate affected by treatments. In addition, the concentration of liver glycogen was not statistically different among treatments suggesting that glycogenolysis was unaffected by the supplement of folic acid and vitamin B_{12}, alone or combined. Whole-body glucose Ra in the study of Preynat et al. (2009a) tended to increase by 160 g/d with the combined supplement of folic acid and vitamin B_{12} at week 12 of lactation, likely due to an enhancement of propionate usage for gluconeogenesis as concluded by these authors. However, in the current study, the proportion of glucose from propionate was not affected by treatments and averaged 59.7 ± 8.5% ($P \geq 0.46$; Table 8.5). This proportion is in accordance with the literature in which the contribution of propionate to glucose synthesis was reported to vary between 50 to 58% for cows in early lactation (Amaral et al., 1990; Danfær et al., 1995; Larsen and Kristensen, 2013). Propionate is the major glucogenic precursor in fed ruminants although lactate and glucogenic AA can also contribute to gluconeogenesis but to a lesser extent (Reynolds, 2006; Larsen and Kristensen, 2013). To enter into the Krebs cycle, propionate has to be transformed into propionyl-CoA, then into methylmalonyl-CoA, and then into succinyl-CoA (Scott, 1999). The latter reaction involves MUT and vitamin B_{12} as a coenzyme. A lack of vitamin B_{12} impedes this reaction (Scott, 1999). In lactating ewes, an improvement of gluconeogenesis from propionate has been observed when using intramuscular injections of vitamin B_{12} (Peters and Elliot, 1983). In the current experiment, an increase of WB glucose Ra caused by an enhancement of using propionate for gluconeogenesis was expected following a combined supplement of folic acid and vitamin B_{12}. As net hepatic lactate and AA removal was not measured, it could not be answered if the decrease of WB glucose Ra following the folic acid supplement was caused by affecting lactate and AA metabolism as a precursor of glucose. However, no data

in the literature suggest that a supplement of folic acid could impair this pathway. Moreover, plasma concentrations of Ala and Gly, known as major glucogenic nonessential AA (Larsen and Kristensen, 2009), were not affected by treatments at week 9 of lactation (Table 8.3).

In the study of Galindo et al. (2011), it has been showed that the mammary gland was the primary user of glucose; total utilisation accounting for 73% of WB glucose Ra. Moreover, these authors also reported that milk lactose yield to mammary net glucose flux ratio was 73% (Galindo et al., 2011) supporting that milk lactose secretion is the major enquirer of glucose to the mammary gland. It could be hypothesized that the lower WB glucose Ra observed in cows receiving the folic acid supplement could have led to lower milk yield of lactose and to glucose partitioning as supported by the lower percentage of milk lactose from WB glucose Ra. Indeed, glucose utilization in tissues other than mammary gland or glucose utilization in the mammary gland for other purposes than lactose synthesis could have been favored with the use of the folic acid supplement. Unfortunately, as mammary uptake of nutrients was not measured in the present study, this hypothesis cannot be confirmed. Altogether these results indicate that the decreased lactose yield and associated milk yield with folic acid supplementation could have originated from systemic effect, through a reduced WB Ra of glucose, or through a mammary control, with a reduced lactose yield to WB Ra ratio. There is no clear explanation of this negative impact of folic acid on glucose kinetics in the current study.

The major difference between the present study and Preynat et al. (2009a) was the plasma status of folic acid, vitamin B_{12}, and Met of control cows being higher in the current study, as reported above. It seems that, in contrast with control cows in the study of Preynat et al. (2009a), the amounts of folic acid and vitamin B_{12} synthesized in rumen were sufficient to meet requirements and to optimize transmethylation pathway and performance of control cows in the present study. By supplying the folic acid supplement to the dairy cows, it could be hypothesized that this vitamin was then probably in excess as suggested by negative effects of the folic acid supplement on milk and lactose yields and WB glucose Ra. These results highlight the necessity to develop a tool to predict supplies of folic acid

and vitamin B_{12} from ruminal synthesis according to diet management which is currently impossible under the state of knowledge.

8.6 Conclusion

Milk and lactose yields tended to decrease with the folic acid supplement as did the WB glucose Ra. As DMI and liver concentration of glycogen were not different among treatments, changes in glucose portal absorption and glycogenolysis cannot be considered responsible for this decrease. As well, the WB propionate Ra, the major glucogenic precursor in ruminants, and the proportion of glucose synthesized from propionate, averaging 60%, were not different among treatments. Plasma concentrations of folic acid, vitamin B_{12}, and Met of control cows in the present experiment were higher than previously reported which suggest that the supply of folic acid and vitamin B_{12} from ruminal synthesis was sufficient to optimize transmethylation pathway and performance of dairy cows under conditions of the current study.

8.7 Acknowledgements

Authors are grateful to Véronique Roy, Valérie Beaudet, Jocelyne Renaud, Chrystiane Plante, Mario Léonard, and Catherine Thibault (Agriculture et Agroalimentaire Canada, Sherbrooke, QC, Canada) for technical assistance, Maris McCarthy (Cornell University, Ithaca, NY) for the glycogen protocol, and Steve Méthot (Agriculture et Agroalimentaire Canada, Sherbrooke, QC, Canada) for his statistical advices. We acknowledge the invaluable help of Marie-Ève Bouchard, Étienne Viens and the barn staff (Agriculture et Agroalimentaire Canada, Sherbrooke, QC, Canada) for special care of dairy cows throughout the experiment. We appreciate the help of Antoine Bourgeois, DMV (Clinique Vétérinaire de Sherbrooke, QC, Canada) and Éric Martineau, DMV (Clinique Vétérinaire de Coaticook, QC, Canada) who performed surgeries and liver biopsies, respectively. We also thank Vétoquinol (Lavaltrie, QC, Canada) for generously providing vitamin B_{12}. This project was financed by the Entente de collaboration pour l'innovation en production et transformation laitière Novalait-AAC-FQRNT-MAPAQ.

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9 Discussion générale et conclusion

Les résultats présentés dans cette thèse ont été obtenus en effectuant deux projets distincts. Comme il a déjà été mentionné, le premier projet a eu lieu sur 15 fermes commerciales et le deuxième projet a été réalisé en centre de recherche. Pour cette raison, ce chapitre sera divisé en trois parties : la première partie portera sur le premier projet; la deuxième partie sur le deuxième projet et; une conclusion portant sur les deux projets clôturera cette thèse.

9.1 Premier projet

L'objectif premier de ce projet était de mesurer l'impact d'un supplément combiné d'acide folique et de vitamine B_{12} sur la reproduction et les maladies en fermes commerciales étant donné les résultats des études précédentes suggérant que ce supplément améliorait le métabolisme énergétique. Pour ce faire, un grand nombre d'animaux devait être impliqué dans l'étude, car les variabilités des données de reproduction et de santé sont grandes. Plus de 1000 vaches par traitement auraient été requises pour détecter un possible résultat significatif pour certains paramètres de reproduction et de santé. Cependant, il a été décidé d'inclure un total de 800 vaches dans l'étude provenant de fermes commerciales; 400 animaux par traitement. Les études précédentes évaluant les effets d'un supplément combiné d'acide folique et de vitamine B_{12} avaient été réalisées sur un nombre restreint d'animaux en centre de recherche. La réalisation de l'étude en fermes permettait également d'évaluer l'effet sur la production laitière et l'impact économique d'un tel supplément sur les fermes laitières québécoises.

En 2010, année durant laquelle la majorité des données de l'étude ont été recueillies, la ferme laitière québécoise comptait en moyenne 57 vaches (Valacta, 2011). Afin de compléter l'expérience dans un délai raisonnable, 15 fermes ont été recrutées. L'avantage que confère le recrutement de plusieurs troupeaux par rapport à un seul est la possibilité d'étudier la variabilité de la réponse au supplément de vitamines comme il a été réalisé aux chapitres 4 à 6. Cette étude a d'ailleurs révélé une grande variabilité de la réponse au supplément entre les fermes. Cependant, à partir des résultats, il demeure tout de même difficile d'expliquer complètement cette variabilité. À titre d'exemple, les troupeaux 3 et 9

ont tous deux obtenu une marge par vache positive ($/vache/année) suite à l'adoption du supplément vitaminique (Chapitre 5). Cependant, le pourcentage de gras du troupeau 3 a légèrement augmenté pour les vaches ayant reçu le supplément tandis que celui du troupeau 9 a diminué de façon importante suite à la supplémentation (Chapitre 4). De plus, les animaux ayant reçu le supplément ont perdu légèrement moins de poids que les animaux témoins pour le troupeau 3 tandis qu'il s'agit de la situation inverse pour le troupeau 9 (Chapitre 4).

Une hypothèse peut toutefois être formulée afin de tenter d'expliquer la variabilité de la réponse au supplément entre les troupeaux. Cette dernière peut probablement provenir des différentes régies d'alimentation employées sur les fermes utilisant plusieurs types d'aliments de qualité diverse. En effet, comme l'acide folique et la vitamine B_{12} sont synthétisés par les microorganismes du rumen (Santschi et al., 2005) et que la microflore s'adapte à la ration reçue (Goff et Horst, 1997), il est possible que l'apport au duodénum de ces vitamines pour les vaches était variable entre les fermes. Aucun échantillon de sang n'a été prélevé sur les animaux participant à l'étude. Il n'a donc pas été possible de déterminer la concentration sanguine de folates et de vitamine B_{12} selon les troupeaux. Cependant, la Figure 6.1 montre que la concentration en vitamine B_{12} du lait pour les animaux témoins a été variable entre les troupeaux suggérant un apport en vitamine B_{12} également différent (Chapitre 6). Toutefois, à partir de ces résultats, il est impossible de déterminer si les apports en vitamine B_{12} provenant de la microflore du rumen pour les animaux témoins dans les troupeaux ayant la concentration en vitamine B_{12} la plus élevée (par exemple les troupeaux 3 et 12) étaient suffisants pour optimiser les performances laitières et reproductives.

Un inconvénient de la grande variabilité de la réponse au supplément entre les troupeaux est que cela réduit les chances d'obtenir des résultats significatifs lorsque les moyennes des traitements des 15 troupeaux étudiés sont utilisées. D'un autre côté, une analyse statistique indépendante pour chaque troupeau s'avère être un exercice plutôt futile considérant le nombre d'animaux par traitement par troupeau trop bas (entre 7 et 66 vaches/traitement) pour détecter une différence significative à l'intérieur du troupeau, particulièrement en ce qui concerne les paramètres de reproduction et de santé. Pour cette raison, dans le cadre du

Chapitre 5 portant sur l'impact économique potentiel d'un supplément combiné d'acide folique et de vitamine B_{12}, les données brutes pour effectuer les calculs économiques ont été utilisées dans l'objectif de souligner la variabilité entre les troupeaux. De plus, cette méthode permet d'évaluer l'impact économique réel pour un producteur laitier participant à cette étude.

Les visites à la ferme pendant le projet s'effectuaient aux deux semaines, mais les injections intramusculaires de vitamines devaient s'effectuer chaque semaine. Les producteurs avaient la charge d'effectuer les injections sur les animaux les semaines entre les visites. Il fallait donc qu'ils soient bien informés de l'importance de respecter l'horaire établi et de signaler tout changement à celui-ci. Au cours des 14 mois durant lesquels les visites ont eu lieu, deux producteurs ont oublié à deux reprises d'effectuer les injections entre les visites. Ces oublis n'ont donc pas été fréquents.

Un aspect particulier de ce projet est que la consommation volontaire de matière sèche (CVMS) des animaux traités n'a pas pu être évaluée, le design de l'étude rendant cette mesure impossible : visites aux deux semaines sur 15 troupeaux pendant 14 mois. L'information la plus fiable qui a pu être récoltée afin de décrire l'alimentation des animaux provient des rations alimentaires formulées par les conseillers en nutrition de la ferme. Toutefois, il subsiste une marge d'erreur entre les rations calculées et ce qui est réellement servi et consommé par les animaux à la ferme.

Lors de la dernière visite, les producteurs (14 sur 15) ont répondu à un questionnaire permettant de réaliser l'étude de l'impact technico-économique de ce supplément sur les fermes laitières. Une des questions consistait à connaitre la préférence des producteurs quant à la forme que devrait prendre le supplément combiné d'acide folique et de vitamine B_{12} advenant la commercialisation. Neuf producteurs sur 14 (64 %) ont répondu que le supplément devrait être commercialisé sous forme de supplément alimentaire, deux (14 %) sous forme injectable tandis que trois producteurs (21 %) n'avaient pas de préférence particulière. De ce sondage, il en ressort que pour faciliter son adoption, le supplément vitaminique devrait être disponible préférentiellement sous forme alimentaire. Une autre question portait sur l'éventualité de la commercialisation d'un lait enrichi en vitamine B_{12}, les producteurs devaient se prononcer sur leur intérêt ou non de respecter un cahier des

charges dans cette optique. Neuf producteurs sur 14 ont montré leur intérêt pour la production de ce lait à valeur ajoutée tandis que cinq ont dit que cela ne les intéressait pas. Parmi ces derniers producteurs, trois ont précisé qu'ils considèrent avoir assez de cahiers de charges à respecter pour le moment.

9.2 Deuxième projet

Le principal objectif de ce projet était d'évaluer si la synthèse du glucose à partir de l'acide propionique était affectée suite à des suppléments d'acide folique et de vitamine B_{12}, seuls ou combinés. Cette étude découle de résultats précédents où un supplément combiné d'acide folique et de vitamine B_{12} a augmenté le taux de perte irréversible du glucose en début de la lactation.

Pour réaliser ce projet, une chirurgie visant à insérer un cathéter dans une veine du rumen a été réalisée vers 53 jours en lait afin de permettre la perfusion de l'acide propionique marqué avec un isotope stable. Au début de l'étude, certaines vaches avaient de la difficulté à se remettre de la chirurgie faisant en sorte que les perfusions simultanées de glucose et d'acide propionique devaient être retardées d'une semaine pour ces vaches. Il est certain qu'une chirurgie au moment du pic de la lactation est un événement stressant pour ces vaches. Malgré le jeûne total la veille de la chirurgie, la production laitière des vaches ne diminuait pas en conséquence. Les animaux se retrouvaient, entre autres, en hypocalcémie entrainant l'atonie du système digestif qui favorisait le déplacement de la caillette. Les soins pré et postopératoires ont été revus pour tenter de corriger la situation. Ainsi, la veille de la chirurgie, le jeûne complet a été remplacé par un jeûne partiel où un tiers de la ration habituelle était offert. Les vaches recevaient également une fluidothérapie postopératoire consistant en une infusion intraveineuse d'un soluté contenant du NaCl, du KCl et du borogluconate de calcium. Ces solutions ont amélioré le rétablissement post chirurgical des animaux.

Les résultats obtenus concernant les perfusions d'isotopes stables ne sont pas ceux qui étaient attendus lors de l'élaboration initiale de l'expérience. Comme il a été mentionné précédemment, des études antérieures ont rapporté qu'un supplément combiné d'acide

folique et de vitamine B_{12} améliorait le métabolisme énergétique en début de la lactation en augmentant le glucose plasmatique et le taux de perte irréversible du glucose (Graulet et al., 2007; Preynat et al., 2009). Au même titre, dans la présente étude, il était attendu qu'un tel supplément augmente le taux d'apparition du glucose causé par une augmentation du glucose synthétisé à partir de l'acide propionique. Les suppléments d'acide folique et de vitamine B_{12} ont été donnés seuls ou combinés afin d'étudier si un supplément d'acide folique était nécessaire pour l'expression du supplément de vitamine B_{12}. Dans le Chapitre 8, il a été rapporté que le supplément d'acide folique, seul ou combiné avec le supplément de vitamine B_{12}, a diminué le taux d'apparition du glucose et qu'aucun effet de traitement n'a été observé sur le pourcentage du glucose synthétisé à partir de l'acide propionique. Une explication plausible de ces résultats est que les concentrations plasmatiques de folates et de vitamine B_{12} des animaux, même ceux ne recevant pas de suppléments, étaient plus élevées tout au long de l'étude que ce qui a été précédemment rapporté dans la littérature, suggérant des apports déjà suffisants en ces vitamines.

Les deux projets réalisés dans le cadre de cette thèse faisaient partie d'un projet plus large comportant deux autres objectifs qui ont fait l'objet de mémoires de maitrise déposés à l'Université Laval (Gagnon, 2012; Ghaemialehashemi, 2013). Ces projets visaient à étudier les effets d'injections intramusculaires hebdomadaires d'acide folique et de vitamine B_{12} données de trois semaines avant la date prévue du vêlage jusqu'à huit ou neuf semaines postpartum sur la reprise de l'activité ovarienne et sur la qualité des follicules dominants après la parturition. Le Tableau 9.1 est un résumé de quelques résultats obtenus après le vêlage dans le cadre de ces deux projets de maîtrise et du projet présenté au Chapitre 7. Il est à noter que seulement les résultats des animaux témoins et ceux recevant le supplément combiné d'acide folique et de vitamine B_{12} sont présentés. Ce tableau offre un intéressant point de comparaison sur les effets de ce supplément en lien avec les concentrations plasmatiques de folates et de vitamine B_{12} postpartum. En ce qui concerne les animaux témoins (B_9-B_{12}-), les concentrations plasmatiques de folates et de vitamine B_{12} après la parturition étaient 56 et 65 % plus élevées, respectivement, dans la présente étude que dans celle de Ghaemialehashemi (2013). La concentration plasmatique de folates pour les animaux témoins était 48 % plus élevée dans l'expérience présentée dans cette thèse que lors du projet de Gagnon (2012) tandis que les concentrations plasmatiques de vitamine B_{12}

étaient semblables. La production laitière a augmenté entre 1,2 et 3,6 kg/j avec le supplément de vitamines combinée à une CVMS semblable entre les traitements dans les essais de Gagnon (2012) et de Ghaemialehashemi (2013) contrairement à la présente étude où la production laitière a diminué chez les vaches recevant le supplément. Dans tous les cas, le supplément vitaminique a permis de diminuer les concentrations plasmatiques d'AGL et de BHBA. Cependant, les concentrations plasmatiques d'AGL et de BHBA des animaux témoins étaient entre 33 et 42 % plus basses dans l'expérience présentée dans cette thèse que pour les deux autres études. Il ressort de ce tableau que le statut sanguin en folates et en vitamine B_{12} est un facteur influençant la réponse au supplément de vitamines. Selon le Tableau 9.1, la plus grande réponse au supplément a été obtenue dans le projet où les concentrations plasmatiques en folates et en vitamine B_{12} des animaux témoins étaient les plus basses (Ghaemialehashemi, 2013). Il semblerait qu'à un certain niveau sanguin de folates et de vitamine B_{12}, les effets du supplément sur la production laitière soient négatifs comme il a été le cas dans l'étude présentée dans cette thèse.

Tableau 9.1 Comparaison de certains résultats obtenus en début de la lactation dans trois études où un supplément combiné d'acide folique et de vitamine B_{12} était donné à des vaches laitières

	Chapitre 7		Ghaemialehashemi (2013)		Gagnon (2012)	
	Traitements[1]					
Item	B_9-B_{12}-	B_9+B_{12}+	B_9-B_{12}-	B_9+B_{12}+	B_9-B_{12}-	B_9+B_{12}+
CVMS[2] (kg/j)	21,0	20,6	21,4	23,0	22,5	23,9
Lait (kg/j)	41,5	39,8	40,1	43,7	39,5	40,7
AGL[3] (μ*M*)	251	192	434	368	422	427
BHBA[3] (m*M*)	0,74	0,69	1,23	0,82	1,10	0,81
Folates[3] (ng/ml)	16,4	20,0	10,5	14,0	11,1	14,4
Vitamine B_{12}[3] (pg/ml)	216	373	131	266	223	342

[1] B_9-B_{12}- = 0,9 % saline NaCl (témoin); B_9+B_{12}+ = 320 mg d'acide folique et 10 mg de vitamine B_{12}.
[2] CVMS = consommation volontaire de matière sèche.
[3] Concentrations plasmatiques; AGL = acides gras libres; BHBA = acide β-hydroxybutyrique.

9.3 Conclusion

Les deux projets réalisés dans le cadre de cette thèse font ressortir le fait que, sous certaines circonstances, les vaches laitières en période de transition et en début de la lactation ont bénéficié d'un supplément combiné d'acide folique et de vitamine B_{12} tandis que dans d'autres cas, le statut des animaux en ces vitamines était probablement adéquat pour optimiser les performances. Toutefois, il n'existe actuellement aucun moyen de prédire l'apport en acide folique et en vitamine B_{12} provenant de la synthèse microbienne du rumen selon le type d'alimentation servi aux animaux. Il n'existe pas non plus de seuil de concentrations plasmatiques d'acide folique et de vitamine B_{12} en dessous duquel une supplémentation est nécessaire pour les animaux ni au-dessus duquel une supplémentation pourrait diminuer les performances laitières. Le développement d'un modèle de prédiction pouvant estimer les apports en acide folique et en vitamine B_{12} selon la ration ingérée permettrait d'établir le type d'animaux qui pourrait bénéficier ou non de ce supplément vitaminique.

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10 Annexe – Travaux réalisés en collaboration avec la *Cornell University,* Ithaca, NY, USA

Courte communication : Folates et vitamine B_{12} dans le colostrum et le lait de vaches laitières nourries selon différents niveaux d'énergie pendant la période du tarissement

Short communication: Folates and vitamin B_{12} in colostrum and milk from dairy cows fed different energy levels during the dry period

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Cet article est une première version du manuscrit qui sera soumis au *Journal of Dairy Science*.

10.1 Résumé

Cette étude a été entreprise pour évaluer les concentrations et les quantités journalières sécrétées de folates et de vitamine B_{12} dans le colostrum et dans le lait au début de la lactation chez des vaches laitières nourries selon différents niveaux d'énergie pendant la période de tarissement. Un total de 84 vaches Holstein multipares ont été assignées 60 jours avant la date prévue du vêlage à un des traitements alimentaires suivants donnés en ration totale mélangée : 1) Ration haute en énergie pendant la période de tarissement (HE; 1,35 Mcal NE_m/kg de matière sèche (MS)); 2) Ration à énergie contrôlée pendant la période de tarissement (EC; 1,14 Mcal NE_m/kg MS)) ou; 3) Ration intermédiaire (EI; ration à énergie contrôlée du tarissement à 30 jours avant la date du vêlage prévue, suivie d'une ration représentant un mélange de 50 : 50 des rations contrôlée et haute en énergie jusqu'à la parturition; 1,24 Mcal NE_m/kg MS). Après le vêlage, toutes les vaches ont reçu la même ration totale mélangée jusqu'à 42 jours en lait (JEL). Les échantillons de colostrum ont été pris à la première traite suivant le vêlage et les échantillons de lait ont été prélevés lors de la traite du matin à 11 et 39 ± 2 JEL. Les concentrations et les quantités sécrétées de folates dans le colostrum et dans le lait n'ont pas différé entre les traitements. La concentration de vitamine B_{12} dans le colostrum a été plus haute pour les vaches ayant reçu la ration EC (31.7 ± 1.4 ng/mL) que les vaches EI (23.5 ± 1.4 ng/mL) tandis qu'aucun effet de traitement n'a été noté pour la concentration en vitamine B_{12} dans le lait. Les quantités de vitamine B_{12} sécrétées dans le colostrum et le lait ont été en moyenne plus élevées pour le groupe HE (176 ± 8 µg/j) que pour le groupe EI (151 ± 8 µg/j). En conclusion, ces résultats suggèrent que les niveaux d'énergie dans les rations pendant la période de tarissement peuvent changer la concentration et la quantité sécrétée de vitamine B_{12} dans le colostrum et dans le lait, mais n'ont pas eu d'effet sur les folates.

Mots clés: acide folique, vitamin B_{12}, colostrum, lait, vache laitière

10.2 Abstract

This study was undertaken to evaluate folate and vitamin B_{12} concentrations and daily yields of colostrum and milk in early lactation of dairy cows fed different levels of energy during the dry period. A total of 84 multiparous Holstein cows were assigned to one of the following dietary treatments fed as a total mixed ration 60 days before the expected calving date: 1) High energy one-group dry cow diet (HE; 1.35 Mcal NE_m/kg dry matter (DM); 56% corn silage, 12% wheat straw, and 32% concentrate mix on a daily DM basis); 2) Controlled energy one-group dry cow diet (CE; 1.14 Mcal NE_m/kg DM; 28% corn silage, 36% wheat straw, and 36% concentrate mix on a daily DM basis) or; 3) Intermediate step-up diet (IE; controlled energy diet from dry off until 30 days before the expected calving and then switching to a diet representing a 50: 50 blend of the controlled and high energy diets until parturition; 1.24 Mcal NE_m/kg DM). After calving, all cows were fed the same diet served as a total mixed ration (44% corn silage, 13% grass silage, and 43% concentrate mix on a daily DM basis) until 42 days in milk (DIM). Colostrum samples were taken at the first milking after the parturition and milk samples were taken during the morning milking at 11 and 39 ± 2 DIM. Colostrum from the first milking and milk were weighed the day of sampling. Colostrum yield from the first milking postpartum and milk yields at 11 and 39 DIM were unaffected by treatments. Colostrum yield averaged 6.8 ± 0.7 kg whereas milk yields at 11 and 39 DIM were on average 40.3 ± 1.5 kg and 48.9 ± 1.3 kg, respectively. Folate concentrations and yields in colostrum and milk were not different among treatments. Folate concentration of colostrum (440.3 ± 18.8 ng/mL) was higher than folate concentration in milk at 11 DIM (93.7 ± 3.0 ng/mL) and at 39 DIM (78.4 ± 2.6 ng/mL). Vitamin B_{12} concentration in colostrum was higher for CE cows (31.7 ± 1.4 ng/mL) than IE cows (23.5 ± 1.4 ng/mL) while in contrast no treatment effect was noted for vitamin B_{12} concentration in milk. At 11 and 39 DIM, milk concentrations of vitamin B_{12} averaged 3.8 and 3.2 ± 1.4 ng/mL, respectively. Yields of vitamin B_{12} in colostrum and milk samples were on average greater for HE cows (176 ± 8 µg/d) than IE cows (151 ± 8 µg/d). In summary, results suggest that energy levels in diets during the dry period could change vitamin B_{12} concentration and yield in colostrum and milk but had no effect on milk concentration and yield of folates.

Key words: folate, vitamin B_{12}, colostrum, milk, dairy cow

10.3 Introduction

In contrast to folic acid, vitamin B_{12} is neither synthesized nor used by plants and fungi; only bacteria and archaebacteria are known to synthesize vitamin B_{12} when cobalt supply is adequate (Martens et al., 2002). It has been shown that apparent ruminal synthesis of folic acid and vitamin B_{12} in dairy cows were between 16.5 and 21.0 mg/d, and 73.0 and 79.8 mg/d, respectively (Santschi et al., 2005; Schwab et al., 2006) with a proportion of these synthesized vitamins secreted into milk (Ferlay et al., 2013). At birth, the rumen of the calf is immature, no B-vitamin synthesis by ruminal microorganisms occurs and digestion in the abomasum is similar to the nonruminant (NRC, 2001). Bovine colostrum and milk are an excellent source of vitamin B_{12} (Foley and Otterby, 1978; Matte et al., 2012) and, to a lesser extent, folates for nonruminant calves and humans, respectively, who rely on exogenous sources to meet their B-vitamin requirements (NRC, 2001; Graulet, 2014).

It has been reported that, in humans, a glass of 250 mL of milk from cows not receiving a B-vitamin supplement could contribute up to 56 and 4% of required daily intake of vitamin B_{12} and folates, respectively (Graulet, 2014). Moreover, vitamin B_{12} in bovine milk is more efficiently absorbed in humans than its synthetic form used in supplement (Matte et al., 2012). Since 1998, in Canada and in USA, folic acid fortification of white flour is mandatory in an attempt to decrease risk of neural tube defects at birth (MacFarlane et al., 2011). Although the fortification with folic acid has been reported as being effective to decrease the risk of neural tube defect, it raises new concerns (Colapinto et al., 2011). Indeed, high folate status could hide vitamin B_{12} deficiency by masking anemia but not neurological symptoms (Matte et al., 2012). In that case, it could lead to irreversible neurological damage if diagnostic is delayed. Optimizing vitamin B_{12} concentration of milk by diet management of dairy cows could be a natural way to partially overcome this issue.

A recent study conducted in 15 commercial dairy herds showed that vitamin B_{12} concentration in milk was highly variable among farms, varying from 2.2 to 3.9 ng/mL (Chapter 6). Current knowledge on factors affecting folic acid and vitamin B_{12} secretion in colostrum and milk is limited. Nevertheless, some studies concluded that diet management had an impact on folate and vitamin B_{12} concentrations of bovine milk (Miller et al., 1966;

Chassaing et al., 2011). The aim of the experiment was to evaluate folate and vitamin B_{12} concentrations and yields of colostrum and milk in early lactating dairy cows fed diets with different levels of energy during the dry period. Moreover, as recent data on folate and vitamin B_{12} concentrations and yields of colostrum are scarce in the literature, this study allows updating these data. To our knowledge, no other research has assessed folate and vitamin B_{12} concentrations and yields of colostrum and milk according to different levels of energy of dry cow diets.

10.4 Data Collection and Analyses

All procedures of this experiment were approved by the Cornell University Institutional Animal Care and Use Committee. A total of 84 Holstein cows entering their second or greater lactation were enrolled at the Cornell University Dairy Teaching and Research facility to one of the following dietary treatments consisting of total mixed ration (TMR) formulated to supply different levels of energy 60 days before the expected calving date: 1) High energy one-group dry cow diet (HE; 1.35 Mcal NE_m/kg dry matter (DM)); 2) Controlled energy one-group dry cow diet (CE; 1.14 Mcal NE_m/kg DM) or; 3) Intermediate step-up diet (IE; controlled energy diet from dry off until 30 days before the expected calving and then switching to a diet representing a 50: 50 blend of the low and high energy diets until parturition; 1.24 Mcal NE_m/kg DM). After calving, all cows were fed a common fresh diet using a TMR until 42 DIM. Cows were fed once daily for ad libitum intake and diets were formulated using the Cornell Net Carbohydrate and Protein System (Fox et al., 1992). Ingredients and nutrient compositions of dry and fresh cow diets are showed in Table 10.1. Dry cow diets were formulated to exceed Co requirements (0.11 mg/kg of DM; NRC, 2001). Cows were housed in tie stalls and milked twice daily. Colostrum samples were taken at the first milking after the parturition and milk samples were taken during the morning milking at 11 and 39 ± 2 DIM. Colostrum from the first milking and milk were weighed the day of sampling.

Folates and vitamin B_{12} in colostrum and milk were analyzed in duplicate by radioassay using a commercial kit (SimulTRAC® B_{12}/FOLATE-S, MP Biomedicals, Solon, OH) as previously described in Chapters 6 and 8 except that some modifications were done for

colostrum analyses. Regarding folate analysis, a total of 0.5 g of colostrum was weighed instead of 2.0 g for milk and 1.5 g of ultra-pure water was added to get a total weight of approximately 2.0 g. Then, the colostrum analysis of folates was performed as for milk. For vitamin B_{12} analysis, 0.5 g of colostrum was weighed instead of 2.5 g for milk followed by an addition of about 2.0 g of ultra-pure water. The analysis of vitamin B_{12} in colostrum was then carried out as for milk but a high-speed centrifugation performed at 46,500 × *g* for 22 minutes at 4°C was required to separate the supernatant from the pellet. The inter-assay coefficients of variation were 3.3 and 2.0% for folates and vitamin B_{12}, respectively.

Table 10.1 Ingredients and chemical analysis of diets fed to dairy cows

Item	Dry diet[1]			Fresh diet
	HE	CE	IE	
Ingredients, % of DM				
Corn silage	55.9	28.5	42.2	44.2
Wheat straw	12.4	35.6	24.0	-
Grass hay	-	-	-	13.5
Concentrate mix	27.2	31.2	29.2	38.9
Vitamin and mineral mix	4.5	4.7	4.6	3.4
Chemical analysis, % of DM				
CP	13.0	16.2	14.6	18.8
NDF	41.9	51.0	46.5	36.0
NFC	35.5	24.2	29.9	34.3
Ether extract	3.4	3.1	3.3	5.4
DCAD (meq/kg of DM)	-139	-94	-115	86
NE_m[2] (Mcal/kg of DM)	1.35	1.14	1.24	-
Co (mg/kg of DM)	1.10	0.98	1.05	-

[1] HE = high energy one-group dry cow diet; CE = controlled energy one-group dry cow diet; and IE = intermediate step-up dry cow diet.
[2] Calculated from CNCPS (v. 6.1).

Data were analyzed using Proc MIXED of SAS (version 9.2, SAS Institute, 2008, Cary, NC) with repeated measures. Fixed effects were treatment, time as well as treatment × time

interaction. Eight covariance structures were tried out as data were collected according to unequal time intervals (SP(POW), SP(GAU), SP(EXP), SP(LIN), SP(LINL), SP(SPH), ANTE(1), and UN) and the one with smallest fit statistics was chosen for each analysis. A Tukey's HSD test was performed when results reached significance or a tendency ($P \leq 0.10$). When the treatment × time interaction was significant or a tendency, the SLICE option in the LSMEANS statement of SAS was used to help interpretation.

10.5 Colostrum and Milk Yields

Neither treatment effect nor treatment × time interaction were noted for colostrum and milk yields at 11 and 39 DIM ($P \geq 0.60$; Table 10.2). As expected, the time effect was significant ($P < 0.0001$; Table 10.2). Indeed, colostrum yield was lower than milk yield at 11 and 39 DIM ($P < 0.0001$) and milk yield was greater at 39 DIM than at 11 DIM ($P < 0.0001$). Colostrum yield of the first milking averaged 6.8 ± 0.7 kg whereas milk yields at 11 and 39 DIM were on average 40.3 ± 1.5 kg and 48.9 ± 1.3 kg, respectively.

Table 10.2 Colostrum and milk yields the day of sampling according to different levels of energy during the dry period (least square means)

	Treatment[1]			
Yield[2] (kg)	HE	CE	IE	SEM[3]
Colostrum (first milking)	7.3	6.2	7.0	0.7
11 days in milk	41.2	40.5	39.1	1.5
39 days in milk	49.4	48.8	47.9	1.3

[1] HE = high energy one-group dry cow diet; CE = controlled energy one-group dry cow diet; and IE = intermediate step-up dry cow diet.
[2] Treatment effect ($P = 0.60$); time effect ($P < 0.0001$); and treatment × time interaction ($P = 0.84$).
[3] SEM = standard error of the mean.

10.6 Folates

Folate concentrations and yields in colostrum and milk were not affected by level of energy in prepartum diets ($P \geq 0.31$; Table 10.3). As lactation progressed, folate concentrations decreased (time effect, $P < 0.0001$; Table 10.3); colostrum had the highest concentration of

folates (440.3 ± 18.8 ng/mL) followed by milk at 11 DIM (93.7 ± 3.0 ng/mL) and at 39 DIM (78.4 ± 2.6 ng/mL). Collins et al. (1951) also reported higher folate concentrations in colostrum than milk of dairy cows. Involving primiparous and multiparous Holstein cows, the study of Girard et al. (1995) reported that folate concentration of colostrum at first milking postpartum was on average 287.0 ± 12.6 ng/mL for control cows and 312.3 ± 19.3 ng/mL for cows receiving weekly injections of folic acid. Parity effect could not be discarded as being an explanation for higher colostrum concentration of folates in the current study. Girard and Matte (2005), Graulet et al. (2007), and Preynat et al. (2009) observed lower milk folate concentration and yield for cows not receiving a folic acid supplement than in the current experiment, probably because data in these studies were collected until later stages of lactation.

10.7 Vitamin B_{12}

Vitamin B_{12} concentration in colostrum was higher than in milk samples (time effect, $P < 0.0001$) as previously reported (Anthony et al., 1951; Collins et al., 1951; Gregory et al., 1958). Vitamin B_{12} concentration of colostrum in the present study was lower than results reported in the review of Foley and Otterby (1978) but higher than those from Akins et al. (2013). Foley and Otterby (1978) concluded that composition of colostrum could be influenced by many factors including prepartum diet. A significant treatment × time interaction was observed ($P = 0.02$; Table 10.3). Vitamin B_{12} concentration in colostrum differed among treatments ($P = 0.0002$) whereas no diet effect was observed on milk samples ($P > 0.97$). Colostrum from CE cows had a vitamin B_{12} concentration higher than IE cows ($P = 0.001$; Table 10.3). Microflora in the rumen adapts to the diets offered to dairy cows (Goff and Horst, 1997). As vitamin B_{12} is synthesized by ruminal bacteria, it could be hypothesized that the lower vitamin B_{12} concentration in colostrum for IE cows was due to the shift of bacterial population in the rumen that occurred for those cows 30 days before the expected calving date. At 11 DIM, milk concentration of vitamin B_{12} was on average 3.8 ± 1,4 ng/mL and averaged 3.2 ± 1.4 ng/mL at 39 DIM. Yields of vitamin B_{12} in colostrum and milk samples were on average greater for HE cows (176 ± 8 µg/d) than IE cows (151 ± 8 µg/d, $P = 0.08$; Table 10.3).

Table 10.3 Folate and vitamin B_{12} concentrations and yields in colostrum and milk of dairy cows fed according to different levels of energy during the dry period (least square means ± SEM[1])

	Treatment[2]		
Item	HE	CE	IE
Folate concentration[3] (ng/mL)			
Colostrum	429.7 ± 18.2	463.6 ± 18.5	427.5 ± 18.8
11 days in milk	93.8 ± 2.9	93.2 ± 3.0	94.2 ± 2.9
39 days in milk	76.0 ± 2.6	80.7 ± 2.6	78.6 ± 2.6
Folate yield[4] (mg/d)			
Colostrum	3.2 ± 0.3	2.7 ± 0.3	3.0 ± 0.4
11 days in milk	3.8 ± 0.2	3.8 ± 0.2	3.6 ± 0.2
39 days in milk	3.7 ± 0.1	3.9 ± 0.1	3.7 ± 0.1
Vitamin B_{12} concentration[5] (ng/mL)			
Colostrum	27.8^{ab} ± 1.4	31.7^{b} ± 1.4	23.5^{a} ± 1.4
11 days in milk	3.9^{a} ± 1.4	3.7^{a} ± 1.4	3.8^{a} ± 1.4
39 days in milk	3.3^{a} ± 1.4	3.1^{a} ± 1.4	3.0^{a} ± 1.4
Vitamin B_{12} yield[6] (µg/d)			
Colostrum	201 ± 14	183 ± 14	167 ± 15
11 days in milk	161 ± 14	149 ± 15	145 ± 15
39 days in milk	165 ± 14	150 ± 15	140 ± 15

[1] SEM = standard error of the mean.
[2] HE = high energy one-group dry cow diet; CE = controlled energy one-group dry cow diet; and IE = intermediate step-up dry cow diet.
[3] Treatment effect ($P = 0.31$); time effect ($P < 0.0001$); and treatment × time interaction ($P = 0.39$).
[4] Treatment effect ($P = 0.87$); time effect ($P = 0.0009$); and treatment × time interaction ($P = 0.61$).
[5] Treatment effect ($P = 0.05$); time effect ($P < 0.0001$); and treatment × time interaction ($P = 0.02$).
[6] Treatment effect ($P = 0.10$; tendency for HE cows of having higher vitamin B_{12} yield than IE cows ($P = 0.08$)); time effect ($P = 0.008$); and treatment × time interaction ($P = 0.98$).
[ab] Means in the same row with different superscripts differ; $P \leq 0.05$.

As in the present trial, Collins et al. (1951), Miller et al. (1966), and Rutten et al. (2013) observed a large variability on vitamin B_{12} concentration of bovine milk among individuals, from less than 1.0 to 12.9 ng/mL. Milk concentrations of vitamin B_{12} at 11 DIM ranged from 1.0 to 7.1 ng/mL and from 1.6 to 5.6 ng/mL at 39 DIM. Rutten et al. (2013) showed

that this variability among dairy cows could be partially explained by genotype of the animal.

In summary, concentrations of folates and vitamin B_{12} were much higher in colostrum than in milk. Folate concentrations and yields of colostrum and milk were not influenced by levels of energy in diet during the dry period. Results suggest that energy levels in diets during the dry period could change vitamin B_{12} concentration in colostrum and vitamin B_{12} yields in colostrum and milk but had no effect later on milk concentration of vitamin B_{12}.

10.8 Acknowledgements

The authors are grateful to Charlene M. Ryan and Francisco Leal-Yepes (Cornell University, Ithaca, NY) and Chrystiane Plante (Agriculture et Agroalimentaire Canada, Sherbrooke, QC, Canada) for technical assistance.

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