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## Effect of rumen-protected B vitamins and choline supplementation on health, production, and reproduction in transition dairy cows

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### ABSTRACT

The objectives were to determine the effects of a rumen-protected blend of B vitamins and choline (RPBC) on the incidence of health disorders, milk yield, and reproduction in early lactation and the effects on gene expression and liver fat infiltration. A randomized controlled trial in 3 commercial dairy herds ( $n = 1,346$  cows with group as the experimental unit; experiment 1) and a university research herd ( $n = 50$  cows with cow as the experimental unit; experiment 2) evaluated the use of 100 g/cow per d of commercially available proprietary RPBC supplement (Transition VB, Jefo, St. Hyacinthe, Quebec, Canada), or a placebo, fed 3 wk before to 3 wk after calving. In experiment 2 liver biopsies were taken at 4 and  $14 \pm 1$  d in milk to measure triacylglycerol concentrations and expression of 28 genes selected to represent relevant aspects of liver metabolism. Treatment effects were assessed using multivariable mixed logistic regression models for binary health and reproductive outcomes; linear regression models for milk yield, dry matter intake, and liver outcomes; and survival analysis for time insemination and pregnancy. In experiment 1, treatment did not have an effect on the incidence of hyperketonemia (blood  $\beta$ -hydroxybutyrate  $\geq 1.2$  mmol/L; cumulative incidence to 3 wk postpartum of 28 to 30%), clinical health disorders, or udder edema. The prevalence of anovulation at 8 wk postpartum was 11% in the treatment group and 23% in the control but did not differ statistically given group-level randomization. Pregnancy at first insemination (33 and 35%) and median time to pregnancy to 200 d in milk (96 and 97 d) were not different between treatment and control, respectively. No difference was observed between treatment groups in milk yield or components through the first 3 Dairy Herd Improvement Association test days (44 kg/d in both groups, accounting for

parity and components). In experiment 2, there were no differences between treatment groups in feed intake. Mean blood  $\beta$ -hydroxybutyrate was lower at wk 3 in RPBC ( $0.6$  vs.  $0.9 \pm 0.12$  mmol/L) with no difference between treatments for mean blood concentrations of fatty acids (wk  $-1$  or  $1$ ) and  $\beta$ -hydroxybutyrate at wk 1 or 2. The gene for acyl-CoA oxidase 1 (*ACOX1*) had lower mRNA abundance in RPBC with no difference between treatments for the other genes, but the expression of half of the genes assessed differed with days in milk. Liver triacylglycerol was lower in primiparous cows at 4 d in milk in RPBC ( $2.0$  vs.  $4.4 \pm 1.2\%$ ) but not at 14 d in milk ( $2.2$  vs.  $3.2 \pm 0.97\%$ ) with no treatment effect in multiparous cows ( $4.6 \pm 0.8\%$ ). Accounting for parity, days in milk, fat and protein percentages, repeated test days, and a random effect of cow, no significant difference was observed between treatments in milk yield across the first 3 Dairy Herd Improvement Association tests ( $41.2 \pm 1.3$  in RPBC vs.  $38.0 \pm 1.4$  kg/d in control). Under the diet and management conditions of the field study including low prevalence of clinical health disorders, in experiment 1 we did not detect a benefit of RPBC, but in experiment 2 liver fat content decreased in primiparous cows.

**Key words:** hyperketonemia, ketosis, transition dairy cow, rumen-protected B vitamin, choline

### INTRODUCTION

High-producing dairy cows are at high risk of developing metabolic diseases over the transition period (LeBlanc, 2010; Abuajamieh et al., 2016), defined as the 3 wk before to 3 wk after calving, when energy demand is increased due to the onset of lactation and many other hormonal changes (Grummer, 1995; Drackley, 1999; Duplessis et al., 2014b). Most cows experience negative energy balance (**NEB**) during this period, and genetics, physiology, management, and nutrition combine to determine the success of adaptation to NEB (Herd, 2000; Ospina et al., 2013). Cows adapt to NEB by mobilizing fat reserves, which are oxidized to supply

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the Krebs's cycle with partially oxidized fatty acids, generating ketone bodies, which spare glucose by serving as alternative fuel for some tissues (Grummer, 2008). Ketone production is a normal adaptation to NEB, but when excess amounts of ketones are produced and accumulate, this can contribute to adverse health effects (Duffield, 2000; Grummer, 2008). Excess ketone bodies and circulation of fatty acids from adipose tissue can increase the risk of fatty liver (Bobe et al., 2004; Schulz et al., 2014). Hyperketonemia (**HYK**) is a concentration of circulating ketone bodies associated with undesirable subsequent outcomes and is a costly problem in the dairy industry (McArt et al., 2015) because there is a loss of milk production and increased risk of diseases such as left displaced abomasum and metritis attributable to untreated HYK (Duffield, 2000; LeBlanc, 2010; Ospina et al., 2013) and because the cumulative incidence of HYK in early lactation is, on average, >40% (McArt et al., 2012; Gordon et al., 2017a).

Dairy cattle are thought to be able to synthesize sufficient quantities of most B vitamins in the rumen to meet requirements for production of 35 L/d of milk (NRC, 2001). Vitamin B<sub>12</sub> is necessary for the production of enzymes needed for methionine regeneration and methylmalonyl CoA mutase, which is needed for propionate to enter the Krebs's cycle (Kennedy et al., 1990; Preynat et al., 2009a,b; Akins et al., 2013). Providing vitamin B<sub>12</sub> and folic acid together enhanced entry of propionate into Krebs's cycle (Graulet et al., 2007; Preynat et al., 2009a), which may be beneficial because propionate is a major substrate for glucose synthesis in dairy cows (Danfær et al., 1995).

Several studies have investigated metabolic and production responses to increased dietary supply of B vitamins. Responses to supplemental B vitamins may vary with the availability of other methyl donors (e.g., dietary methionine or choline). Foliates are used in the DNA cycle and the methylation cycle, and are necessary for reproduction and milk protein synthesis (Girard and Matte, 2005; Preynat et al., 2009a). Dietary supplementation of folate has led to increased milk yield, and the addition of dietary vitamin B<sub>12</sub> has been shown to increase plasma glucose concentrations (Graulet et al., 2007). Providing supplemental folic acid and vitamin B<sub>12</sub> by weekly injections increased milk production by 12% (Preynat et al., 2009a,b). However, in a field study of 805 cows in 15 herds (Duplessis et al., 2014a), weekly injections of folate and vitamin B<sub>12</sub> through the transition period generally did not affect health outcomes or milk yield, although milk protein percentage was increased (Duplessis et al., 2014b).

Choline is "quasi-vitamin" that, among other functions, is important in synthesis of phosphatidylcholine,

a key part of the very-low density lipoprotein membranes for export of triglycerides from the liver. Dietary choline supplementation in cattle must be protected against degradation by rumen microbes. Numerous studies have investigated the effects of supplementation of rumen-protected choline in dairy cattle, with varied results. Several reported no effect of rumen-protected choline on liver fat content (Piepenbrink and Overton, 2003; Zahra et al., 2006), fatty acids or BHB concentrations (Zahra et al., 2006; Zom et al., 2011), or milk yield (Zom et al., 2011), whereas others found a positive milk yield response, especially among fat cows (Zahra et al., 2006).

The objective of this study was to measure the effect of a commercially available proprietary rumen-protected blend of B vitamins and choline (**RPBC**) on the incidence of health disorders, milk yield, and reproduction in early lactation in commercial dairy herds. We hypothesized that the supplementation of B vitamins and choline would reduce the incidence of HYK and decrease liver fat infiltration, which would consequently improve health and pregnancy rate and increase milk production in early lactation. Anecdotal reports suggested that the prevalence of udder edema might be reduced by this supplement, so udder edema was measured in the study.

## MATERIALS AND METHODS

The University of Guelph Animal Care Committee reviewed and approved the study protocols that were accepted and followed by the herds enrolled.

### Experiment 1

**Study Size and Herds.** The study was conducted on 3 commercial freestall dairy farms in Ontario, Canada, between November 2015 and December 2016. Herd size varied from 150 to 450 lactating cows. The herds are described in Table 1. The primary outcome was the cumulative incidence of HYK (blood BHB  $\geq 1.2$  mmol/L). Recent studies (McArt et al., 2012; Gordon et al., 2017a) have shown a mean cumulative incidence of 43% of HYK with testing of blood BHB concentration at least twice per cow, weekly, in the first 2 wk of lactation. To detect a reduction from 43 to 35% with 95% confidence and 80% power requires 1,166 cows (Abramson, 2011). For our secondary outcomes, that sample size would allow for detection of a 1 kg/d (SD = 6) difference in milk yield and a difference of 32 vs. 40% pregnancy at first AI. Herds were purposely selected based on milking at least 150 Holstein cows, being enrolled in DHI milk recording, maintaining accurate dis-

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