EFFECT OF DIETARY SUPPLEMENTATION OF TWO FORMS OF A B-VITAMIN AND CHOLINE BLEND ON THE PERFORMANCE OF HOLSTEIN CALVES DURING THE TRANSITION AND EARLY POSTWEANING PERIOD

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INTRODUCTION

An optimal dairy replacement raising system is crucial for optimizing milk production and profit of dairy farms. The replacement system should be developed with the objective of providing targeted management and nutrition to dairy heifers during the entire course of development to meet specific and objective goals. The transition from liquid to solid diets during weaning is often an impediment for maintaining nutrient balance and growth (Weary et al., 2008) while starter formulation and nutrient content are important during this phase. Postweaning growth needs to be optimized to take advantage of the high efficiencies of growth and lower cost per unit of weight gain that can be attained and thus attention should be given to strategies for maximizing the performance through the transition and postweaning period (Kertz et al., 1998).

Weaning is a critical process that dictates significant anatomic and physiologic adaptations to facilitate appropriate solid feed intake, ruminal function and post-absorptive utilization of the fermentation end-products (Baldwin et al., 2004). Thus, the weaning period should be approached as an opportunity to adequately prepare dairy heifers to face the ruminant state while capitalizing on the benefits of enhanced growth. From a nutritional standpoint, weaning could be facilitated by the provision of a palatable diet with a nutrient profile that would enable proper ruminal fermentation for rapid development at low levels of intake, and supply the calf with an adequate profile of post-ruminally available nutrients to maintain expected growth rates.

Supplementing B-vitamins and choline to calves' diets is a consideration as they facilitate metabolic processes by acting as coenzyme factors or providing and transferring methyl-groups (McDowell, 2000), which are necessary for many metabolic functions related to health and growth. The NRC (2001) does not recommend supplementation of these nutrients to the dry feed for dairy calves under the assumption that rumen synthesis provides them in sufficient amounts after weaning. This assumption has not adequately been tested in calves with higher targeted growth rates. Nevertheless, even in fully developed ruminants, the supplementation of these vitamins has been demonstrated to improve health and performance (Girard and Matte, 2005, Lean and Rabiee, 2011). This suggests that demand for these nutrients during physiological stages of stress and high metabolic activity might exceed the dietary supply and ruminal synthesis. Before weaning, milk or milk replacer provide sufficient quantities of these vitamins to the young calf, but this supply is reduced over weaning (Waugh et al., 1947, Girard et al., 1989), while ruminal activity and microbial vitamin synthesis are still not fully developed (McDowell,

2000). During this period of decreased dietary supply, calves have a significant metabolic demand due to their lean growth and as part of change in metabolism to the ruminant state.

Research in this area is limited, however, there is evidence suggesting that B-vitamin supply during and after weaning could be insufficient to support optimal growth in dairy calves (Dumoulin et al., 1991, Girard and Matte, 1997). Although B-vitamins and choline could be added to starter feeds, the potential degradation of these vitamins by ruminal microbes might restrict the benefit of their supplementation (Santschi et al., 2005). Alternatively, rumen-protected forms of B-vitamins and choline have been used with considerable success in mature ruminants (Sacadura et al., 2008).

This study was conducted to evaluate the effect of combined supplementation of B-vitamins and choline, in non-protected and rumen-protected forms, during the transition and post-weaning period on performance of dairy calves fed a diet balanced for all nutrients, including all essential amino acids (EAA) and to achieve at least 1 kg/d gain. Our hypothesis was that these vitamins are limiting for optimal calf performance during the transition phase and, to be effective, they must be fed in a rumen-protected form.

METHODOLOGY

All protocols involving animals were reviewed and approved by the Cornell University Institutional Animal Care and Use Committee. Sixty-one Holstein calves (37 female and 24 male) born at the Cornell University Ruminant Center (Harford, NY) were enrolled. Within the first two hours of life, calves received two doses of colostrum extract (Immu-Prime; Sterling Technology, Brooking, SD) and were fed 4 L of colostrum replacer (240 g globulin proteins, Nursemate Plus 150; Sterling Technology, Brooking, SD). Twelve hours after birth, 2 L of pooled colostrum (≥ 22 on the Brix scale) were fed. Subsequently, calves were moved to a naturally ventilated calf barn, measured for BW (42.9 ± 0.8 kg) and height (76.5 ± 0.5 cm at withers and 80.2 ± 0.5 cm at hip), and housed in individual pens until 13 wk of age.

Calves were offered milk replacer (Excelerate; Milk Specialties Co., Eden Prairie, MN; Table 1) starting at a feeding rate of 0.85 kg DM/d and increasing progressively over the first 21 d up to 1.6 kg DM/d. At 49 d of age, the weaning process was started by withdrawing 0.1 kg DM/d until d 63 when weaning was completed. Milk replacer was reconstituted at 15% solids and offered at 39 ºC three times per day with nursing bottles containing 3.8L of replacer. A textured calf starter, specifically formulated for this study (Table 1), was offered ad libitum from wk 4 to 13. For adequate interpretation of the B-vitamin supplementation, the starter did not contain any added B-vitamins or choline and was formulated to meet all EAA requirements, based on body composition data (Van Amburgh et al., 2015) and the CNCPS v.7 (Higgs and Van Amburgh, 2016) predictions of amino acid supply, to ensure that they were not first limiting, since the metabolism of these nutrients is tightly interrelated (McDowell, 2000, Girard and Matte, 2006). The starter was pelleted at a commercial feed mill (Purina Animal Nutrition, Erwin, NY) and blended at Lutz Feeds (Oneonta, NY). Fresh water was available ad libitum.

At wk 3, calves were assigned to one of the three treatments in a randomized design. Treatments were as follows: a rumen protected B-vitamin and choline blend (**RPBV**, n = 20), a 70:30 mix of fat concentrate and non-protected B-vitamin and choline blend (**UPBV**, n = 22) and an unsupplemented group receiving a fat concentrate as a placebo (**CTRL**, n = 19; Equi-Calorie 100, Jefo Nutrition Inc., Saint-Hyacinthe, QC, Canada). The fat concentrate was included in UPBV and CTRL treatments to keep diets isocaloric.

Table 1. Ingredient of the formulated starter, and chemical composition and vitamin concentration of milk replacer and starter fed.

Ingredient composition (% of DM)	Starter
Pellet	
Treated soybean meal	19.8
Wheat middlings	19.3
Canola meal solvent	6.6
Dextrose	3.3
Dried whey	5.9
Blood meal	4.0
Methionine analog	0.7
Minerals	1.8
Fat	0.7
Vitamin ADE premix	0.3
Monensin	0.03
Flavor/odor enhancer	0.1
Pellet Binder	1.0
Flaked corn	20.1
Beet pulp shreds	13.2
Molasses	3.3

Item	Milk replacer	Starter[1]
DM, %	94.7	86.6
Chemical composition (% of DM)[2]		
Crude protein	28.5	25.5
Crude fat	15	2.96
aNDFom	-	25.1
Ash	6.82	8.18
Calcium	1	0.96
Phosphorus	0.71	0.63
Cobalt, mg/kg	1.22	1.4
Vitamin A, IU/kg[3]	16,530	7,273
Vitamin D, IU/kg[3]	5,510	2,424
Vitamin E, IU/kg[3]	110.2	29.9
ME, Mcal/kg	4.6	2.5
B-Vitamin concentration (mg/kg DM)		
Thiamin	17.3	3.9
Riboflavin	29.9	5.3
Niacin	152	75.6
Pantothenic acid	91.7	14.4
Pyridoxine	9.36	4.19
Biotin	0.85	0.57
Folates	1.04	0.89
Vitamin B_{12}	0.096	0.005
Choline, mg/kg DM	1,600	1,260

[1]Amino acid content (% Nitrogen): 7.1% Asp, 3.2% Thr, 4.6% Ser, 9.7% Glu, 3.6% Pro, 5.2% Gly, 5.3% Ala, 4.3% Val, 2.2% Cys, 2.1% Met, 2.6% Ile, 6.5% Leu, 2.6% Try, 3.4% Phe, 3.0% His, 7.1% Lys, 4.3% Trp, 12.2% Arg.
[2]As reported by the manufacturer for milk replacer, and as measured for the starter grain
[3]As reported by the manufacturer for milk replacer, and as formulated for the starter grain

Vitamin blends were formulated to contain all B-vitamins (thiamine, riboflavin, niacin, pantothenic acid, pyridoxine, biotin, folate and vitamin B_{12}) and choline considering the estimated weaned calf requirements. This estimation considered whole bovine milk vitamin concentrations (Davis and Drackley, 1998), reported requirements for swine (NRC, 2012) and ruminants (Marston, 1970, CSIRO, 2007) and studies reporting productive benefits with B-vitamin supplementation in dairy cattle (Dumoulin et al., 1991, Lévesque et al., 1993, Graulet et al., 2007). The B-vitamin and choline blends were mixed

and manufactured by Jefo Nutrition Inc. (Saint-Hyacinthe, QC, Canada). To ensure consumption and adequate dosing, treatments were weighed into gelatin capsules and administered orally once a day using a balling gun, based on the previous day starter intake. Choline chloride was mixed with the B-vitamin blend right before assembling the UPVB capsules. Vitamin treatments and placebo were fed at 0.39 ± 0.001% and 0.28 ± 0.001% of the starter intake, respectively.

Body weight and height were measured weekly. Milk replacer and stater intake were recorded daily. Blood was collected weekly from wk 3 to 13 for measurement of B-vitamin status and plasma urea nitrogen (PUN) and BHB. Samples of milk replacer, starter and supplements were sent to commercial labs for chemical analysis (Cumberland Valley Analytical Services, Maugansville, MD) and B-vitamins and choline analysis (Covance Inc., Princeton, NJ). For amino acid content determination, a starter sample was ground to 1 mm and analysed by HPLC following hydrolysis at 110ºC in a block heater for 21 and 168 h for Trp and the rest of the amino acids, correspondingly, following the procedure described by (Fessenden et al., 2017). Prior to the beginning of the study, the unavailable nitrogen (34.97 ± 0.61%) in the RPBV was determined in duplicates according to the in vitro indigestibility assay described by (Ross et al., 2013). In a separated analysis, in vitro rumen nitrogen stability of the RPBV was determined to be 82.73 ± 2.00%, after 18 h of fermentation.

The PUN and BHB concentrations were measured using enzymatic colorimetric assays based on commercial kits (No. 640; Sigma-Aldrich, St. Louis, MO; and ß-Hydroxybutyrate Liquicolor; Stambio Laboratory, Boerne, TX; respectively). Plasma urea nitrogen and BHB were measured from wk 5 to 13. Folates and vitamin B_{12} were determined for samples taken at wk 3, 7, 9 and 13 by radioasssay using a commercial kit (Simultrac B_{12}/ Folate-S; MP Biomedicals, Santa Ana, CA). Plasma measurements were adjusted to 35% hematocrit, to account for any variation in hydration status.

The data were organized weekly and by periods relative to weaning and were defined as follows: preweaning (wk 4 to 7), weaning (wk 8 and 9) and postweaning (wk 10 to 13). Variables for which the change over time were studied (blood parameters, BW and height) were analyzed as a completely randomized design with a mixed-effects model including the fixed effects of treatment, week and their interaction and a random effect of calf. Measurements taken at wk 3, were used as covariates for the analysis of growth traits. A fixed effects model including the effect of treatment only was used to analyze outcomes by period or at wk 3 only (weight and height ADG, DMI, ME intake, B-vitamin and choline intake, and feed efficiency). Analysis were performed using R (v. 3.3.2, R Core Team, 2016). Pairwise comparisons were done by week or by period, using a Tukey test to correct for multiple comparisons using the “lsmeans” in R. All reported mean values are arithmetic means and standard error as parameter of variation. Significance was declared at $P \leq 0.05$ and trends were stated at $0.05 < P \leq 0.10$.

RESULTS AND DISCUSSION

Dry Matter and Nutrient Intake

Intake of milk replacer was similar among groups at wk 3, before treatments were administered (1.12 ± 0.02 kg DM/d, P = 0.87). During the experimental period, no differences were observed among treatments for intake of milk replacer, starter, total DM or ME (P ≥ 0.35, Figure 1A). Calves consumed less milk replacer than what was offered to them, indicating that they were not feed restricted under the management conditions of the study. Other researchers have similarly observed no effects on DMI, before and during weaning, when supplementing a rumen-protected B-vitamin and choline blend in the diet (Wood et al., 2016) or folic acid parenterally (Dumoulin et al., 1991). Dumoulin et al. (1991) did not observe differences in the response to vitamin supplementation between calves fed restricted or ad libitum. However, they observed that calves under non-restricted feeding increased their intake of concentrate after weaning when receiving supplemental folic acid.

Crude protein intake followed the same pattern as total DMI (data not shown), as the protein content was not remarkably different among feeds. Preweaning intake of most B-vitamins and choline did not differ among treatments ($P > 0.73$), except for folates and vitamin B_{12}, for which supplemented groups showed higher intakes ($P < 0.01$, Table 2). Following the reduction in milk replacer intake due to weaning, estimated intake of B-vitamins and choline decreased for the CTRL group, while supplemented calves had higher intakes for most of these vitamins (P ≤ 0.03). As the starter intake of calves increased after weaning, the calculated intake of all vitamins, except vitamin B_{12}, increased for CTRL fed calves. The calves assigned to UPBV and RPBV treatments had higher intakes of all B-vitamins and choline than the unsupplemented calves after weaning ($P < 0.001$). For most B-vitamins and choline, all treatments reached similar or greater intakes pre- compared to post-weaning.

Folates and Vitamin B_{12} plasma concentrations

Unlike simple-stomached species, B-vitamins and choline consumed by ruminants in the diet and in supplements cannot be considered as the net supply because of alteration, utilization and synthesis of these nutrients by rumen microbes. Thus, to evaluate the net vitamin supply, circulating vitamin levels are more useful (Girard and Matte, 1988, 1997). Only plasma folates and vitamin B_{12} levels were measured to evaluate the effect and form of supplementation and the data in Figure 1B illustrates the plasma concentrations for both vitamins.

From the initial measurement at wk 3 (5.37 ± 0.19 ng/mL), plasma folates increased dramatically throughout the study reaching 12.20 ± 0.26 ng/mL at wk 13 regardless of treatment ($P > 0.14$). The increase observed with age corresponds to the progression of ruminal function and folate synthesis by the rumen microflora. Dumoulin et al. (1991) observed higher blood folate levels in ruminant calves fed ad libitum compared to restricted-fed calves despite similar intake of folates, indicating that the

greater DM being digested ruminally could have explained the differences in folate status. Recent work has corroborated the positive apparent ruminal synthesis of folates in mature ruminants (Santschi et al., 2005, Castagnino et al., 2016). Calves in the current study had the same DMI among treatments, but supplemented calves consumed more folates than CTRL group during the entire experiment. The efficacy of the non-protected folic acid provided to UPBV calves was probably diminished by the ruminal activity. In dairy cows, it has been estimated that folate ruminal disappearance is 97% (Santschi et al., 2005).

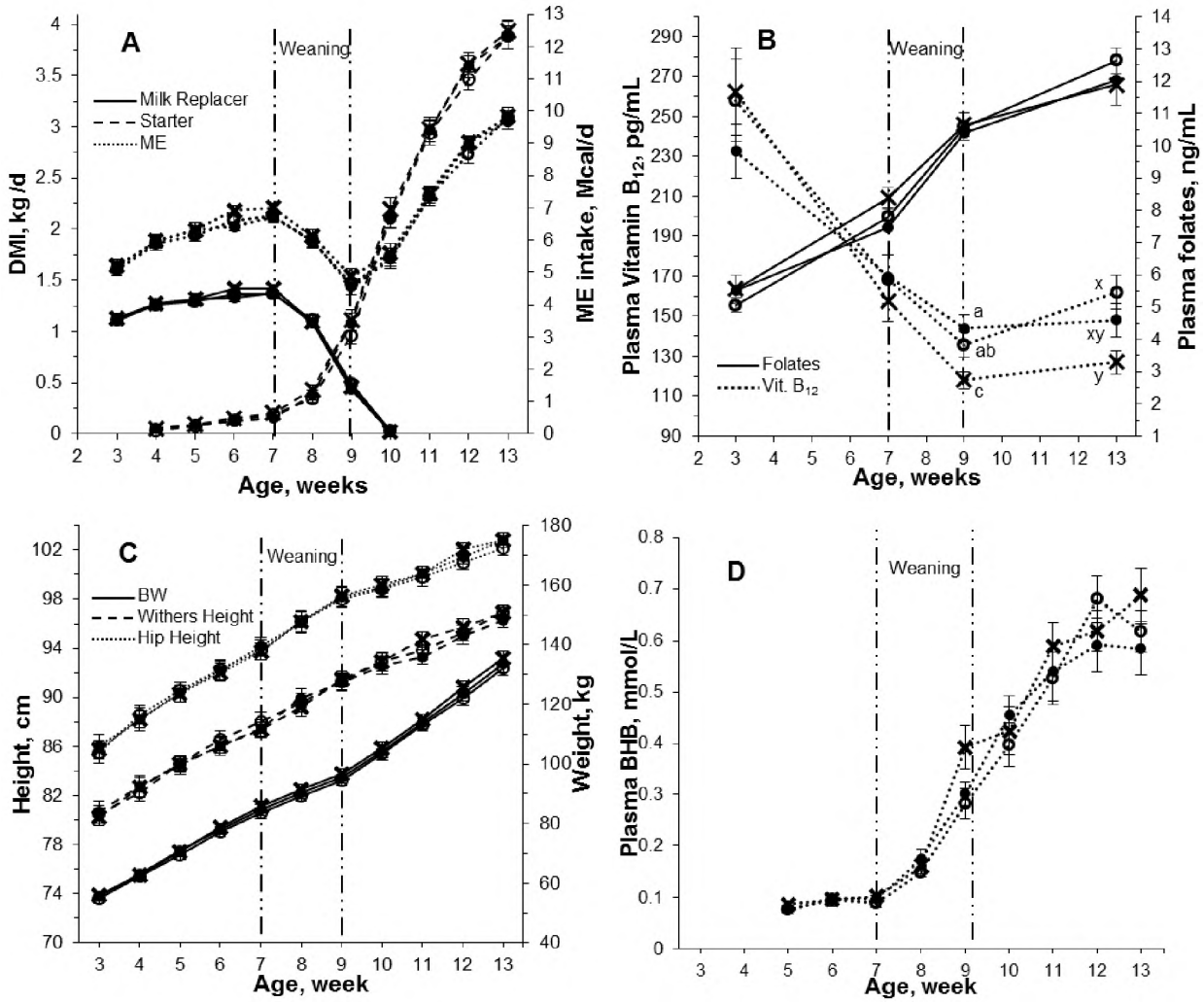

Figure 1. Mean DMI of milk replacer and starter and mean estimated total ME intake (**A**), plasma levels of folates and vitamin B_{12} (**B**), body weight, withers height and hip height (**C**) and plasma BHB (**D**) of calves supplemented with a rumen-protected B-vitamin and choline blend (●), a non-rumen protected B-vitamin and choline blend (**O**) or a placebo (**X**) from 4 to 16 wk of life. Vertical bars represent SE. a and b differ $P < 0.10$; x and y differ $P < 0.05$.

Table 2. Means of dry matter, metabolizable energy, B-vitamins and choline intake, average daily gain, and feed efficiency for calves supplemented with a placebo (CTRL), a non-rumen protected B-vitamin and choline blend (UPBV), or a rumen-protected B-vitamin and choline blend (RPBV) at periods relative to weaning.

Item[2]	Treatment[1]			SE[3]
	CTRL	UPBV	RPBV	
Preweaning				
DMI, kg/d[4]	1.48	1.43	1.43	0.02
ME intake, Mcal/d	6.55	6.34	6.35	0.11
Thiamin intake, mg/d	23.94	23.47	23.56	0.41
Riboflavin intake, mg/d	41.20	40.83	40.72	0.71
Niacin intake, mg/d	215.56	211.53	211.56	3.77
Pantothenic acid intake, mg/d	126.12	125.65	125.56	2.56
Pyridoxine intake, mg/d	13.21	13.47	13.53	0.24
Biotin intake, mg/d	1.22	1.20	1.20	0.02
Folate intake, mg/d	1.52^{b}	1.72^{a}	1.70^{a}	0.04
Vitamin B_{12} intake, ng/d	130.80^{b}	175.50^{a}	177.70^{a}	4.26
Choline intake, mg/d	2,327.43	2,301.22	2,303.68	42.09
ADG, kg/d	1.09	1.02	1.03	0.02
Feed efficiency	0.73	0.72	0.72	0.01
Weaning				
DMI, kg/d[4]	1.59	1.49	1.51	0.03
ME intake, Mcal/d	5.46	5.31	5.27	0.14
Thiamin intake, mg/d	16.42	17.37	17.27	0.62
Riboflavin intake, mg/d	27.27^{b}	31.20^{a}	29.69^{ab}	1.08
Niacin intake, mg/d	176.16	183.68	181.66	4.43
Pantothenic acid intake, mg/d	82.19^{b}	97.50^{a}	94.40^{a}	3.31
Pyridoxine intake, mg/d	10.48^{b}	13.45^{a}	13.79^{a}	0.35
Biotin intake, mg/d	1.10	1.15	1.14	0.02
Folate intake, mg/d	1.40^{b}	2.62^{a}	2.64^{a}	0.12
Vitamin B_{12} intake, ng/d	78.30^{b}	316.40^{a}	343.70^{a}	20.90
Choline intake, mg/d	2,212.48	2,356.55	2,383.41	65.29
ADG, kg/d	0.78	0.79	0.79	0.03
Feed efficiency	0.49	0.53	0.53	0.01
Postweaning				
Starter intake, kg/d	3.17	3.10	3.14	0.06
ME intake, Mcal/d	7.98	7.82	7.86	0.15
Thiamin intake, mg/d	12.51^{b}	17.10^{a}	18.66^{a}	0.63
Riboflavin intake, mg/d	17.05^{c}	35.42^{a}	31.68^{b}	1.41
Niacin intake, mg/d	241.45^{b}	299.16^{a}	297.26^{a}	10.02
Pantothenic acid intake, mg/d	46.41^{b}	118.03^{a}	112.72^{a}	5.29
Pyridoxine intake, mg/d	13.99^{b}	28.93^{a}	30.34^{a}	1.30
Biotin intake, mg/d	1.81^{b}	2.26^{a}	2.20^{a}	0.08
Folate intake, mg/d	2.46^{b}	8.66^{a}	8.40^{a}	0.12
Vitamin B_{12} intake, ng/d	16.50^{b}	1,171.60^{a}	1,246.80^{a}	78.79
Choline intake, mg/d	1,018.02^{b}	5,169.12^{a}	5,249.43^{a}	179.05
ADG, kg/d	1.40	1.33	1.36	0.02
Feed efficiency	0.44	0.43	0.43	0.01

[a,b,c]Means within a row without a common superscript differ ($P < 0.05$)

[1]CTRL, n = 19; UPBV, n = 22; and RPBV, n = 20.

[2]Preweaning = weeks 4 to 7; Weaning = Weeks 8 and 9; Postweaning = Weeks 10 to 13. Data presented as arithmetic means

[3]Overall SE is shown

[4]DMI includes milk replacer and starter intake

However, the lack of response of plasma folates to the additional folic acid provided with the RPBV treatment, suggests that, 1) the vitamin could have been rapidly cleared preventing its accumulation in plasma, 2) it was released from the matrix at a different level of the intestine than the proximal duodenum and jejunum, where its absorption takes place (Santschi et al., 2005), 3) the quantity of folate synthetized ruminally diminished the importance of the supplemental folic acid, or 4) a combination of all of these factors took place.

Plasma B_{12} decreased 34% during the preweaning period, independent of treatment ($P > 0.96$). By the end of weaning (wk 9) vitamin B_{12} continued decreasing with RPBV (143.91 ± 6.9 pg/mL) calves tending to have higher levels than CTRL (117.98 ± 4.27 pg/mL; $P = 0.09$), but no differences were detected between UPBV calves and the other group (135.8 ± 6.37 pg/L; $P > 0.23$). After weaning, plasma concentrations of vitamin B_{12} stabilized with UPBV calves having higher values (162.01 ± 8.03 pg/mL) than CTRL calves (126.94 ± 5.82 pg/mL; $P = 0.02$), while RPBV group had intermediate levels (148 ± 8.64 pg/mL; $P > 0.24$). When only evaluating the plasma concentrations of vitamin B_{12}, both forms of vitamin supplements showed the same effectiveness to supply vitamin B_{12}, thus appreciable quantities of the non-protected cyanocobalamin could potentially have reached the intestine. In previous work in adult ruminants, the ruminal disappearance of this vitamin (63%) is intermediate (Santschi et al., 2005). This suggests that if the ruminal stability and intestinal availability measured for the rumen-protected supplement are considered, RPBV calves would have received an equivalent amount of cyanocobalamin at the intestinal level as the UPBV calves. Thus, these results suggest that the ruminal use and degradation of B-vitamins described in mature ruminants occur in similar magnitude in the developing ruminant. In a similar way, they prove that, at least for cyanocobalamin, the technology of physical protection successfully prevents ruminal degradation and allows intestinal absorption.

Vitamin B_{12} plasma levels and pattern over time coincides with what has been characterized in heifers raised on milk and concentrates before weaning and fed on pasture thereafter; decreasing from 160 to 77 pg/mL from 17 to 198 days of age, and stabilizing at 130 pg/mL at 342 d (Grace et al., 2014). Plasma concentrations observed in the present study were higher during the preweaning period which could be attributed to the greater amount of liquid diet offered and the higher concentration of the vitamin in the milk replacer. The decrease in plasma vitamin B_{12} concentration with age parallels the estimated intake of the vitamin and liquid feed in the CTRL group. Serum concentrations of this vitamin in veal calves behaved differently, increasing during the first weeks of life and stabilizing at approximately 422.6 pg/mL (Girard and Matte, 1988). This observation, when linked to the vitamin B_{12} concentration in plasma while consumption of milk replacer was still elevated suggests that calves might have increased their demand for this vitamin. It is likely that the metabolic activity occurring during the ruminant state started with the intake of dry feed and augmented the demand for this vitamin even before weaning was initiated. Vitamin B_{12} serves as coenzyme of methylmanolonyl-CoA mutase, an enzyme essential for the integration of propionate into the Krebs cycle and its use as gluconeogenic substrate; consequently, the demand of this vitamin by ruminants is about 10 times the oral requirement for simple stomached species (McDowell, 2000). Thus, the decrease of this vitamin before and during weaning suggests that metabolic adaptations

to the ruminant state might occurs before calves are able to increase their ingestion of solid feed.

Ruminant animals can obtain vitamin B_{12} from ruminal synthesis. The apparent ruminal synthesis of vitamin B_{12} is positive in dairy cows (Castagnino et al., 2016) and seems to be an important source of the vitamin for those animals. To be able to carry out this synthesis, rumen microflora needs an adequate dietary supply of cobalt; the established dietary cobalt requirement for ruminants is 0.11 mg/kg of diet (NRC, 2001). The starter offered in our study should have provided sufficient cobalt to support ruminal vitamin B_{12} synthesis. Other factor that appears to affect the ruminal synthesis of this vitamin include the acidogenic capacity of the diet, being negatively associated with starch fermentability and positively correlated with fiber intake (Sutton and Elliot, 1972). Since plasma vitamin B_{12} concentrations after weaning were in parallel with the values observed in grazing heifers (Grace et al., 2014), it could be implied that ruminal conditions were favorable despite calves being fed exclusively the formulated starter. The quantity and quality of the fiber in the current starter might have enabled adequate fermentation even with the considerable levels of starch (17.8% DM) and sugars (14.1% DM). Khan et al. (2011a) found better solid feed intake, greater rumen development and higher ruminal pH by feeding forage to calves fed high volumes of milk and a calf starter. When both the grass hay and starter DMI and NDF content (18.6 and 62.4% DM, respectively) were considered, an integrated NDF content of the diet of 29.7% DM was estimated. The fiber content of the starter used in our study was not far from this value. In addition, the fibrous ingredients used in the formulation (wheat middlings and beet pulp) are characterized by containing appreciable amounts of soluble fiber and their aNDFom by having a greater potentially digestible fraction and a faster rate of digestion than most forages (Raffrenato and Van Amburgh, 2010, Zontini, 2016). These starter characteristics might have benefited DMI and ruminal pH. The estimated soluble fiber content of the starter was 6.36% DM.

Growth and Feed Efficiency

Although circulating concentrations of B-vitamins and choline might help to evaluate vitamin status and supplementation effectiveness, performance should serve as better criteria to determine adequacy of these vitamins. Body weight (55.58 ± 0.89 kg), wither height (80.52 ± 0.40 cm), hip height (85.76 ± 0.44 cm) and their respective rates of gain (0.83 ± 0.05 kg/d, 0.20 ± 0.01 cm/d and 0.19 ± 0.04 cm/d) did not differ among groups at wk 3 ($P > 0.20$). BW increased with age ($P < 0.001$) but no differences were detected among treatments during the entire period ($P = 0.64$; figure 1C). Birth weight was doubled before weaning started and tripled by the end of the experiment.

Overall ADG was 0.99 ± 0.01 kg/d over the entire experiment and differed by period. Average daily gain was 1.05 ± 0.02 kg/d before weaning, diminished to 0.78 ± 0.03 kg/d during weaning and rapidly increased to 1.36 ± 0.02 kg/d after weaning. These rates of gain were not affected by treatments ($P > 0.45$; Table 2). These ADG are consistent with growth rates obtained in other experiments providing adequate nutrients and feed availability to dairy calves (Khan et al., 2011b, Eckert et al., 2015). The slower

ADG observed during weaning is tied with the reduction in ME intake. This reduction in the rate of growth with weaning is commonly seen in the literature. However, experiments performed under a high level of nutrition have reported a more exacerbated growth slump during weaning (Terré et al., 2007) and the week after (Terré et al., 2006a, Stamey et al., 2012). This discrepancy might be attributed to differences in weaning management since in these studies calves were weaned earlier, during a shorter period and less gradually than in our study. Eckert et al. (2015) reported a similar growth reduction during the week of weaning (about 20% of preweaning ADG) of calves weaned at 8 wk of age in a step-down manner. Although in the present experiment weaning was started at the same age and performed gradually for a longer period, calves demonstrated this reduction in ADG during the two weeks of weaning. This might have been caused by the lower starter intake during the week before weaning was initiated (0.22 vs. 1.38% BW), which in turn could be related to the higher amount of milk replacer provided in our study comparted to what was offered by Eckert et al. (2015). However, the present study brings more evidence that with the provision of proper management and nutrient supply, the extent to which these postweaning ADG are reduced can be minimized. Additionally, despite the reduced gain during weaning when compared to the preweaning period, ADG in this study surpassed or equalled the rates of gain reported for calves under restricted feeding, even during the postweaning period (Kertz et al., 1979, Khan et al., 2007). Thus, the relative reduction of ADG during weaning should not discourage dairy farmers to provide more nutrients to their calves from milk or milk replacer prior to weaning.

Feed efficiency decreased as calves progressed in the feeding program, from 0.72 ± 0.01 preweaning, to 0.52 ± 0.01 during weaning, and finally 0.44 ± 0.01 postweaning. These parameters were unaffected by treatment ($P > 0.45$, Table 2). The preweaning feed efficiencies are similar other studies where calves followed an enhanced feeding program based on a high protein and low fat milk replacer (Diaz et al., 2001, Bartlett et al., 2006). Feed efficiencies obtained during the weaning and postweaning periods in this study are significantly higher than reported in other studies (Terré et al., 2006a, Khan et al., 2007, Stamey et al., 2012, Eckert et al., 2015). These differences could be due to the more gradual weaning protocol and the nutrient balance and palatability in the formulated starter. The effect of balancing for all essential amino acids in supporting the observed intake and feed efficiency is intriguing and further work is needed to explore what allowed for the greater efficiencies. Stature measurements (Figure 1C) and rates of gain (data not shown) did not differ among treatments ($P > 0.20$). Overall rates of gain were 0.23 ± 0.02 cm/d for withers and 0.25 ± 0.02 cm/d for hip height.

Hematocrit, Plasma BHB and PUN

The hematocrit, plasma BHB and PUN changed with age ($P < 0.001$) but were not affected by treatment ($P \geq 0.59$). Circulating BHB (Figure 1D) and starter intake were well correlated ($r = 0.82$; $P < 0.001$) during the treatment period, supporting the idea that starter was being fermented and consequently butyrate was being produced ruminally. This association and the fact that starter intake was similar among treatments, explains the lack of effect of vitamin supplementation on BHB concentration. However, at the same level of intake of dry feed, calves in our study seem to have higher circulating BHB than

reported in other experiments, where BHB levels range from 0 to 0.4 mmol/L (Eckert et al., 2015, Deelen et al., 2016). Differences in carbohydrate content and fermentability of the solid feed might account for this discrepancy, however, limited feed chemistry is reported in these studies. The increasing levels of BHB and folates observed in plasma confirm that functional development of the rumen started before weaning was finished.

Dumoulin et al. (1991) concluded that during the weeks preceding and following weaning, folates supplied by the diet and ruminal synthesis were not optimum for dairy heifers. They observed improvements in solid feed intake, hematocrit levels and growth post-weaning in calves supplemented with folic acid. When folic acid was added to the milk replacer of rapidly growing white veal calves, growth and hematocrit were improved while feed intake remained unchanged (Lévesque et al., 1993). Further, in dairy heifers, the supplementation of vitamin B_{12} during the rearing period did not positively impact growth performance of calves (Grace et al., 2014). Studies examining the supplementation of the other B-vitamins and choline in dairy calves are limited. The literature available and used to determine the NRC (2001) B-vitamin and choline recommendations, were concentrated on determining the lowest dietary intake level to prevent deficiency symptoms during the preweaning phase disregarding optimal performance of calves under restricted feeding conditions (Wiese et al., 1946, Johnson et al., 1947).

The B-vitamin and choline requirements depend on the levels of several nutrients in the diet (McDowell, 2000), and the discrepancy in the effect of supplemental vitamins between the previously reviewed studies and ours could be due to the availability of nutrients and the nutrient balance provided. For example, Judson et al. (1982) observed a positive response in growth to vitamin B_{12} supplementation in beef calves, but the basal diet appeared to be cobalt deficient. The dairy heifers in the study by Dumoulin et al. (1991) were fed at least half the amount of the liquid feed offered in our study and were weaned 2 wk earlier, which could have led to lower folate status and a higher susceptibility to inadequacy during weaning. Additionally, the CP content of the starter used in the present study was higher than the one used by Dumoulin et al. (1991) which, in addition to the effort to amino acid balance, might have reduced the calf's need for folate, vitamin B_{12}, pyridoxine, niacin, riboflavin and choline, related to amino acid transamination, and methionine remethylation and transsulfuration (Girard and Matte, 2005).

CONCLUSIONS

The quantities of B-vitamins and choline blends supplemented, as non-protected or rumen-protected forms, improved plasma vitamin B_{12} status postweaning, but plasma folate status was unchanged. However, the supplements did not affect growth, dry matter intake, feed efficiency or other indicators of adequacy in dairy calves fed an enhanced plane of nutrition. Supply and utilization of these vitamins by the calf seem to be influenced by the change from a liquid to a solid diet and the respective adaptations to the ruminant state. Collectively, our results suggest that, under the conditions of the study, dairy calves could obtain sufficient B-vitamins and choline from the diet and rumen

synthesis to support optimal performance and part of this response might have been due to the AA balance and profile available in the starter.

REFERENCES

Bartlett, K., F. McKeith, M. VandeHaar, G. Dahl, and J. Drackley. 2006. Growth and body composition of dairy calves fed milk replacers containing different amounts of protein at two feeding rates. J. Anim. Sci. 84:1454-1467.

Baldwin, R., K. McLeod, J. Klotz, and R. Heitmann. 2004. Rumen development, intestinal growth and hepatic metabolism in the pre-and postweaning ruminant. J. Dairy Sci. 87:E55-E65.

Castagnino, D., M. Seck, V. Beaudet, K. Kammes, J. V. Linton, M. Allen, R. Gervais, P. Chouinard, and C. Girard. 2016. Effects of forage family on apparent ruminal synthesis of B vitamins in lactating dairy cows. J. Dairy Sci. 99:1884-1894.

CSIRO. 2007. Nutrient requirements of domesticated ruminants. CSIRO publishing.

Davis, C. L. and J. K. Drackley. 1998. The development, nutrition, and management of the young calf. Iowa State University Press.

Deelen, S. M., K. E. Leslie, M. A. Steele, E. Eckert, H. E. Brown, and T. J. DeVries. 2016. Validation of a calf-side ß-hydroxybutyrate test and its utility for estimation of starter intake in dairy calves around weaning. J. Dairy Sci. 99:7624-7633.

Diaz, M., M. Van Amburgh, J. Smith, J. Kelsey, and E. Hutten. 2001. Composition of growth of Holstein calves fed milk replacer from birth to 105-kilogram body weight. J. Dairy Sci. 84:830-842.

Dumoulin, P., C. Girard, J. Matte, and G. St-Laurent. 1991. Effects of a parenteral supplement of folic acid and its interaction with level of feed intake on hepatic tissues and growth performance of young dairy heifers. J. Anim. Sci.69:1657-1666.

Eckert, E., H. E. Brown, K. E. Leslie, T. J. DeVries, and M. A. Steele. 2015. Weaning age affects growth, feed intake, gastrointestinal development, and behavior in Holstein calves fed an elevated plane of nutrition during the preweaning stage. J. Dairy Sci. 98:6315-6326.

Fessenden, S. W., T. J. Hackmann, D. A. Ross, A. Foskolos, and M. E. Van Amburgh. 2017. Ruminal bacteria and protozoa composition, digestibility, and amino acid profile determined by multiple hydrolysis times. J. Dairy Sci. 100:7211-7226.

Girard, C. and J. Matte. 1988. Blood serum concentrations of folates and vitamin B12 during growth period of white veal calves. Can. J. Anim. Sci. 68:455-460.

Girard, C. and J. Matte. 1997. Serum clearance and urinary excretion of folic acid in milk-fed and weaned calves. Can. J. Anim. Sci. 77:69-75.

Girard, C. and J. Matte. 2005. Folic acid and vitamin B 12 requirements of dairy cows: a concept to be revised. Livest Prod Sci. 98(1):123-133.

Girard, C. and J. Matte. 2006. Impact of B-vitamin supply on major metabolic pathways of lactating dairy cows. Can. J. Anim. Sci. 86(2):213-220.

Girard, C. L., J. J. Matte, and G. L. Roy. 1989. Serum folates in young dairy heifers. Br. J. Nut 61(03):595-600.

Grace, N., S. Knowles, and R. Nortjé. 2014. Vitamin B12 status and the effects of vitamin B12 supplementation during the first year of life of spring calves from pasture-fed dairy herds. NZ Vet. J. 62(5):274-278.

Graulet, B., J. Matte, A. Desrochers, L. Doepel, M.-F. Palin, and C. Girard. 2007. Effects of dietary supplements of folic acid and vitamin B 12 on metabolism of dairy cows in early lactation. J. Dairy Sci. 90(7):3442-3455.

Greenwood, R., J. Morrill, and E. Titgemeyer. 1997. Using Dry Feed Intake as a Percentage of Initial Body Weight as a Weaning Criterion1. J. Dairy Sci. 80(10):2542-2546.

Higgs, R. and M. Van Amburgh. 2016. Evolution of the CNCPS-Development of V7. Page 125 in Proc. College Nutrition Conference Proceedings, East Syracuse, NY.

Johnson, B. C., A. C. Wiese, H. Mitchell, and W. Nevens. 1947. The metabolism of nicotinic acid and its role in the nutrition of the calf. J. Biol Chem. 167:729-736.

Judson, G., J. McFarlane, M. Riley, M. Milne, and A. Horne. 1982. Vitamin B12 and copper supplementation in beef calves. Aust. Vet. J. 58:249-252.

Kertz, A., B. Barton, and L. Reutzel. 1998. Relative efficiencies of wither height and body weight increase from birth until first calving in Holstein cattle. J. Dairy Sci. 81:1479-1482.

Kertz, A., L. Prewitt, and J. Everett. 1979. An early weaning calf program: summarization and review. J. Dairy Sci. 62:1835-1843.

Khan, M., H. Lee, W. Lee, H. Kim, S. Kim, K. Ki, J. Ha, H. Lee, and Y. Choi. 2007. Pre- and postweaning performance of Holstein female calves fed milk through step-down and conventional methods. J. Dairy Sci. 90:876-885.

Khan, M., D. Weary, and M. Von Keyserlingk. 2011a. Hay intake improves performance and rumen development of calves fed higher quantities of milk. J. Dairy Sci. 94:3547-3553.

Khan, M. A., D. M. Weary, and M. A. von Keyserlingk. 2011b. Invited review: effects of milk ration on solid feed intake, weaning, and performance in dairy heifers. J Dairy Sci 94:1071-1081.

Lean, I. and A. Rabiee. 2011. Effect of feeding biotin on milk production and hoof health in lactating dairy cows: a quantitative assessment. J. Dairy Sci. 94:1465-1476.

Leclerc, H., D. Espinosa, E. Evans, R. Zambrano-Gaytan, and J. Garza-Flores. 2015. Effect of rumen protected B vitamins supplementation during the receiving period on the productive performance of beef cattle. J. Anim. Sci. 98(Suppl 2):426.

Lévesque, J., C. Girard, J. Matte, and G. Brisson. 1993. Dietary supplements of folic acid: blood and growth responses of white veal calves. Livest Prod Sci. 34:71-82.

Marston, H. 1970. The requirement of sheep for cobalt or for vitamin B 12. Bri J. Nut. 24:615-633.

McDowell, L. R. 2000. Vitamins in animal and human nutrition. John Wiley & Sons.

Meale, S., L. Leal, J. Martín-Tereso, and M. Steele. 2015. Delayed weaning of Holstein bull calves fed an elevated plane of nutrition impacts feed intake, growth and potential markers of gastrointestinal development. Anim Feed Sci Technol 209:268-273.

NRC. 2001. Nutrient requirements of dairy cattle. 7th revised ed. National Academy Press, Washington, DC.

NRC. 2012. Nutrient requirements of swine. National Academy Press, Washington, DC.

R Core Team. 2016. R: A Language and Environment for Statistical Computing. in Vienna, Austria: R foundation for statistical computing, https://www.r-project.org/.

Raffrenato, E. and M. Van Amburgh. 2010. Development of a mathematical model to predict sizes and rates of digestion of a fast and slow degrading pool and the indigestible NDF fraction. Pages 52-65 in Proc. Cornell Nutr. Conf, Syracuse, NY.

Ross, D. A., M. Gutierrez-Botero, and M. E. Van Amburgh. 2013. Development of an in-vitro intestinal digestibility assay for ruminant feeds. Pages 190-202 in Proc. Cornell Nutrition Conference, Syracuse, NY.

Sacadura, F., P. Robinson, E. Evans, and M. Lordelo. 2008. Effects of a ruminally protected B-vitamin supplement on milk yield and composition of lactating dairy cows. Anim Feed Sci Technol 144:111-124.

Santschi, D. E., R. Berthiaume, J. J. Matte, A. F. Mustafa, and C. L. Girard. 2005. Fate of Supplementary B-Vitamins in the Gastrointestinal Tract of Dairy Cows. J. Dairy Sci. 88:2043-2054.

Stamey, J. A., N. A. Janovick, A. F. Kertz, and J. K. Drackley. 2012. Influence of starter protein content on growth of dairy calves in an enhanced early nutrition program1. J. Dairy Sci. 95:3327-3336.

Sutton, A. L. and J. M. Elliot. 1972. Effect of Ratio of Roughage to Concentrate and Level of Feed Intake on Ovine Ruminal Vitamin B12 Production. J Nutr. 102:1341-1346.

Terré, M., A. Bach, and M. Devant. 2006a. Performance and behaviour of calves reared in groups or individually following an enhanced-growth feeding programme. J Dairy Res 73:480-486.

Terré, M., M. Devant, and A. Bach. 2007. Effect of level of milk replacer fed to Holstein calves on performance during the preweaning period and starter digestibility at weaning. Livest Sci 110:82-88.

Van Amburgh, M., E. Collao-Saenz, R. Higgs, D. Ross, E. Recktenwald, E. Raffrenato, L. Chase, T. Overton, J. Mills, and A. Foskolos. 2015. The Cornell Net Carbohydrate and Protein System: Updates to the model and evaluation of version 6.5. J. Dairy Sci. science 98:6361-6380.

Waugh, R., S. Hauge, and W. King. 1947. Choline Studies with Young Dairy Calves. II. Effect of Milk and Other Supplements on Blood Choline Levels1. J. Dairy Sci. 30:641-648.

Weary, D. M., J. Jasper, and M. J. Hötzel. 2008. Understanding weaning distress. Applied Animal Behaviour Science 110:24-41.

Wiese, A., B. C. Johnson, and W. Nevens. 1946. Biotin Deficiency in the Dairy Calf. Proceedings of the Society for Experimental Biology and Medicine 63:521-522.

Wood, K., E. Evans, C. Girard, H. Leclerc, L. Doepel, and G. Penner. 2016. 1466 The effects of supplementing a ruminally protected B-vitamin complex on preweaning growth and performance of Holstein heifer calves. J. Anim. Sci. 94(supplement):711-712.

Zontini, A. 2016. Characterization of fiber fractions in plant by-products and modeling fiber digestion with application in CNCPS 7.0. Page 136. Vol. PhD. Cornell University, Ithaca, NY.