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Assessment of the Effects of Supplemental Rumen Protected B Vitamins and Choline for Periparturient Cows: A Meta-Analysis of 28 Feeding Studies

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Abstract

There are currently no prescribed requirements for B vitamins or choline for dairy cows during the transition period, but many recent studies have shown a variety of benefits from supplying these nutrients to periparturient cows. The purpose of this research effort was to determine the potential benefit of including a rumen protected blend composed of B vitamins (riboflavin, folic acid, vitamin B12) and choline (RPBlend, Jefo Nutrition Inc., St. Hyacinthe, QC, Canada) for dairy cows during the transition period, based on results from 28 on-farm feeding studies (USA-12, Mexico-7, Canada-4, Chile-3, Australia-1, Brazil-1) conducted between 2011 and 2018. All farms participated in monthly herd management record keeping systems and were selected to participate in studies due to their excellent management. Meta-analyses in which risk differences were determined were used to assess the effects of the supplemental RPBlend on health parameters and reproduction. The effect size was used as the determinant of the possible contribution of RPblend on the yields of milk, energy corrected milk (ECM), fat and protein yields during the first four weeks of lactation. Results showed that the inclusion of RPblend reduced ($P < 0.05$) involuntary culling and mastitis by cows during the first 30 days after calving. There was a tendency ($P < 0.10$) for reduced retained placenta and metritis. The meta-analyses revealed that the risk of the displaced abomasum and milk fever did not diminish ($P > 0.10$) with the inclusion of the BPBlend. The incidence rate of subclinical ketosis, determined as blood beta hydroxy butyric acid greater than 1.2 mM was lower ($P < 0.05$) for cows offered the BPBlend (than those not supplemented. The proportion of cows confirmed pregnant by 100 days in milk was greater for cows given the BPBlend (P < 0.05). Milk yield and ECM were greater for cows receiving the RPBlend (1.13 and 0.93 kg/cow respectively, P < 0.05). There was no change in fat yield (P > 0.10) while the yield of milk protein was greater (P < 0.05) when the cows received the blend. These results suggest that the inclusion of rumen protected B vitamins and choline can assist cow health, reproduction and production at the start of lactation.

Keywords

Lactating Dairy Cows, Transition Period, Rumen Protection, B Vitamins, Choline

1. Introduction

During the transition period (3 weeks before to 3 weeks after calving) dairy cows undergo numerous changes in their metabolic status, moving from a pregnant and generally energy positive condition to a lactating and energy negative state. These changes are accompanied by stress factors associated with calving and insults to the uterus, new mammary tissue deposition as well as the onset of lactation. The success of the cow's lactation hinges on her ability to rapidly adapt and deal with these changes [1] [2] [3].

Maintenance of health during the transition period is a major obstacle to the dairy industry with 75% of the overall herd disease events occurring during that brief period of time [1]. Horst *et al.* [1] further acknowledged that health issues can result in premature removal of cows from the herd, a reduced ability to reproduce and lowered milk production, all of which are hurdles to profitability.

Improvements in feed formulation can be of great benefit during this time. Cardoso *et al.* [4] demonstrated that energy adequacy may not be possible, but a positive nutrient state remains a crucial factor for nutrients such as minerals and amino acids and that nutrient dense diets are needed to support an uneventful lactation. Even so, some nutrients may continue to be overlooked. McFadden *et al.* [5] pointed out the importance of one-carbon metabolism to support fatty acid, nucleotide, and protein synthesis, and to also support numerous methylation reactions. Required for one-carbon metabolism are choline, folic acid and vitamin B12. Recently, Duplessis *et al.* [6] [7] found that weekly injections of folic acid and vitamin B12 beginning prepartum resulted in increased energy corrected milk and numerically lower blood beta hydroxy butyric acid (BHBA) in early lactation. The inclusion of biotin in the diet, on the other hand, did not improve milk production or alter BHBA in these studies. Arshad *et al.* [8] performed a meta-analysis of 21 experiments to assess the importance of rumen protected choline in early lactation. The researchers determined that choline, provided during the transition period; increased energy corrected milk (ECM) and feed efficiency with no effect on blood BHBA. Oral B vitamin Supplementation has been shown to improve dry matter intake [9].

Gut health can be impaired in early lactation due to the increase in high energy feedstuffs needed to support lactation [10]. While data from production animal nutrition are not available, there are indications that riboflavin may benefit gut health. This vitamin has been demonstrated to increase the gut production of butyrate [11], a nutrient required by enterocytes. Riboflavin insufficiency resulted in altered gut microbial composition, and greater susceptibility to intestinal permeability leading to infectious disease [12]. Riboflavin has been shown to reduce systemic oxidative stress in humans [11] [13] as well as improve immunity and tight junction proteins in fish [14].

Despite these findings, there remains the general opinion that B vitamins are not limiting nutrients [15] and information continues to be needed to further validate the need for these nutrients. The rumen protected blend (RPBlend) evaluated in this study was a mixture of riboflavin, folic acid, vitamin B12 and choline, and has been evaluated by nutritional professionals around the world. Results have been varied, both regarding the data available to be captured as well as the extent of the response to the product. Therefore, this meta-analysis was needed to determine more accurately the likelihood of metabolic and lactational response to the supplementation of these nutrients during the transition period in dairy cows.

2. Materials and Methods

2.1. Background and Description of Trials

Results from 28 cohort field studies conducted between 2011 and 2018 were used in the development of this meta-analysis to evaluate the effects of BPBlend on periparturient health parameters, reproduction and milk yield in the first month of lactation. All herds that participated in the trials were Holstein herds, located in the USA (12), Mexico (7), Canada (4), Chile (3), Australia (1) and Brazil (1). Where monitored, data were collected on the following conditions:

- Involuntary culling: involuntary removal of cows from the herd within the first month of lactation and not related to physical injury.
- Retained placenta: placental retention as defined by each farm.
- Metritis: uterine infection post-calving requiring treatment as normally assessed on each farm.
- Abomasal displacement: damage to this organ requiring surgery or culling.
- Mastitis within the first month of lactation: all reported treatment events.
- Milk fever: reported cases of this disease requiring treatment.
- Subclinical ketosis: recorded cases of blood BHBA greater than 1.2 measured in milk within the first two to 10 days of lactation based on results obtained using the provided Precision Xtra (Abbott Laboratories, Chicago, USA) meters.
- Reproduction: cows confirmed pregnant before 100 days in milk (DIM).

In addition, information on milk yield and composition for the first 4 weeks of lactation was captured from herd records. Data for any particular parameters were not collected unless they were already part of the individual farm's routine management practice to ensure data capture accurately by not imposing changes to the normal routine. The exception was the gathering of data on subclinical ketosis, and this analysis was conducted by personnel hired for that purpose where permitted by farm management.

The BPBlend contained riboflavin, folic acid, vitamin B12 and choline imbedded in a lipid matrix. The particle size and specific gravity of the particles were designed to permit optimal passage from the rumen. Treatment cows were included in the analysis if they had received the BPBlend for a minimum of 14 days prior to calving. The blend was typically added to the total mixed ration through a service pack prepared in the feed mill.

2.2. Statistical Procedures

Within each trial, health and reproduction incidence rates were compared using Chi-Square analyses (Minitab 16, Minitab, State College, PA). Treatment effects on milk yield, fat yield, protein yield and ECM were determined using one-way analysis of variance.

Meta-analysis results were evaluated, and forest plots were generated employing Meta-Essentials Software (Creative Commons, Mountain View, CA, USA) as outlined by Suurmond *et al.* [16]. Risk differences were analyzed for health and reproduction as binary data comparing treatment outcomes (cases of event for control, cases of no recorded event for control, cases of event for test, and cases of no recorded event for test). The inverse variance method was used to provide data weighting by trial [16]. Effect sizes were calculated for yields of milk, milk fat, milk protein and ECM using raw treatment differences and standard errors (SE) from the individual. trials. Forest plots developed from the results show information on the risk difference or the effect size by trial and the 95% confidence interval (mean ± two standard deviations). Results were declared significant if the probability (P value) was less than 0.05 (*i.e.*, the probability that the differences occurred by chance is under 5%), and a tendency when the P value was greater than 0.05 but less than 0.10.

3. Results and Discussion

3.1. Data Interpretation

There are at least three methods of comparing incidence rate results from cohort studies: odds ratio, risk ratio and risk difference. Odds ratios are typically used to compare the odds of an event when exposed to a test variable, relative to non-exposure [17]. Odd ratios are computed as the portion exhibiting a particular trait in the treatment group divided by the portion exhibiting the trait in the control group. This is then often wrongly interpreted as probability [18]. Holmberg and Anderson [19] advised that risk ratios and risk differences are more clinically intuitive. Risk ratios are calculated as ratios of the outcome probability between the two cohort groups. Risk differences compare the difference in risk rates between the two groups. This method was chosen to describe the data as it was possible to have no cases of a particular event by either the test or control treatment group, requiring division by zero for either odds or risk ratios.

3.2. Analysis Findings

Involuntary culling. The first month of lactation. **Figure 1** provides the forest plot for involuntary culling. The overall average involuntary culling rate based on the 16 herds providing data was 10.3%. The risk difference for this parameter was determined to be −0.05, or 5% lower for the test group of cows (*i.e.*, 12.8% for the control and 7.8% for the test animals).

Generally speaking, the primary reason for involuntary culling of cows is poor fertility [20]. However, because these data were from the first month of lactation only, data for fertility would not be available and therefore this value would be related to metabolic events only. Rumen protected B vitamins have been demonstrated to reduce involuntary culling when supplied in early lactation in another study [21] and this suggests a general contribution towards overall health.

Retained placenta. The incidences of retained placenta were recorded in 13 feeding trials (**Figure 2**). There were 569 reported cases based on 6608 total observations. The determined risk difference was −0.01, and there was a tendency for the difference to be significant (P = 0.085). A tendency is declared when the probability of obtaining the same results is between 90% and 95%.

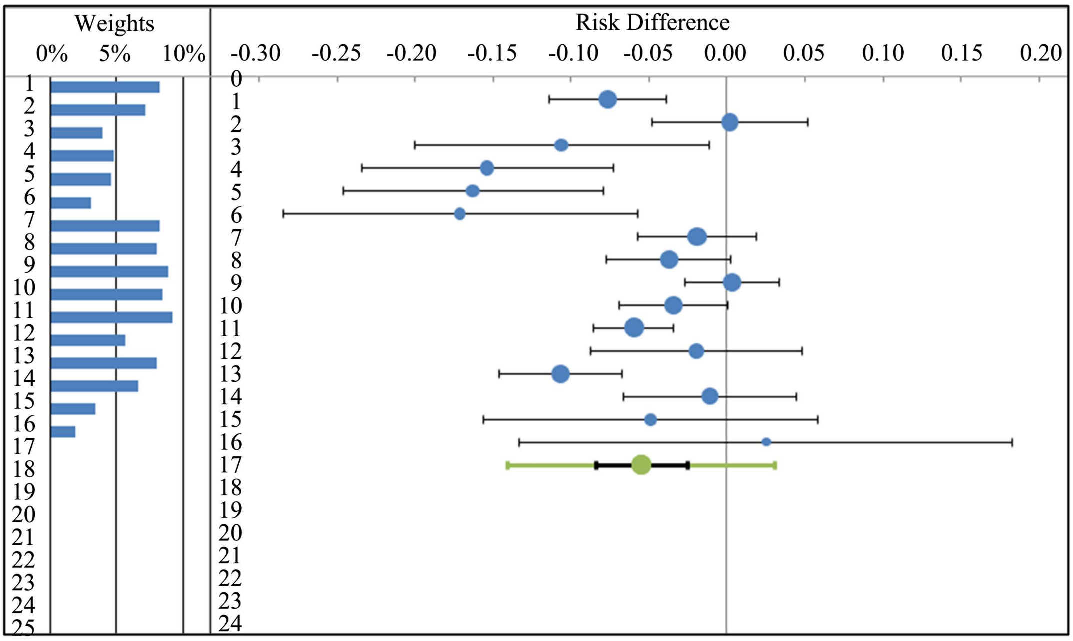

Figure 1. Forest plot of risk difference (Test-Control) for involuntary culling from 7101 subjects from 16 studies. Mean risk difference = −0.05, P = 0.004. Trial weighting is determined by inverse variance. Plots provide individual trial effect size and confidence interval. The lowermost bar shows the overall effect size, confidence interval (inner bar) and prediction interval (outer bar).

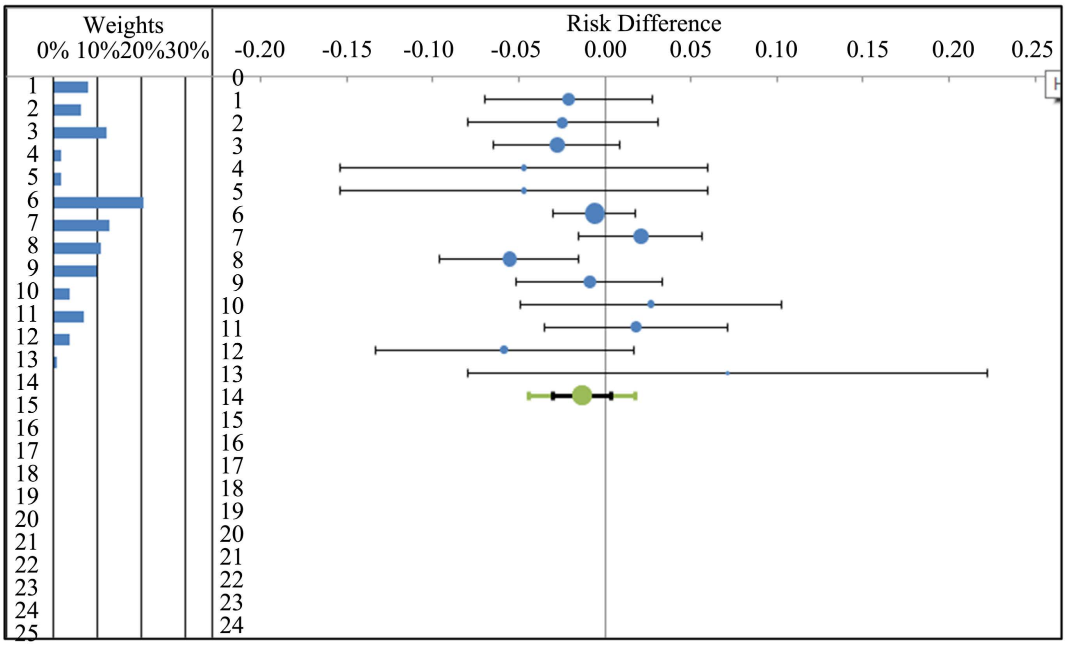

Figure 2. Forest plot of risk difference (test-Control) for retained placenta from 6608 subjects from 13 studies Mean difference = −0.01, P = 0.085. Trial weighting is determined by inverse variance. Plots provide individual trial effect size and confidence interval. The lowermost bar shows the overall effect size, confidence interval (inner bar) and prediction interval (outer bar).

Retained placenta can be precipitated by a lack of nutrients, such as energy, vitamins or minerals in late gestation [22] which may result in placental hypertrophy [23]. In a recent survey, Mahnani *et al.* [24] found an average retained placenta incidence rate of 12.3%. This value is higher than determined in the current study. The researchers likewise revealed that each case of retained placenta represented an economic loss of $US311 to $US456 due to loss in milk yield and reduced fertility. Thus, management decisions that reduce retained placenta would be of financial importance.

Metritis. Results for metritis were only available from 8 of the feeding trials. The forest plot for these results is provided in **Figure 3**. The overall rate of metritis as reported in these feeding trials was 0.235 (23.5%). The incidence rates were as normally measured by each farm. This value is similar to the 21% determined by Benzaquen *et al.* [25]. In spite of the small number of experiments, the risk difference was determined to be −0.08, with a tendency to be significant (P = 0.079).

Abomasal Displacements. No differences in the rates of occurrence of abomasal displacements were found for the 10 trials in which data were recorded (P = 0.868, **Figure 4**). The overall rates were low at 0.0185 (1.9%) for the test animals and 0.0226 (2.3%) for the controls.

Mastitis within the first month of lactation. Mastitis may occur throughout the lactation period and may persist for extended periods. However, the data shown in **Figure 5** refer to mastitis that occurred within the first month after calving. The cases of mastitis were reduced (P < 0.05) for the cows receiving the

BPBlend, based on the analysis of the 10 studies in which data were supplied. The mean difference in risk was found to be −0.04. The average overall infection rate from the studies herds was 0.084 (8.4%), indicating a substantial difference between the two groups.

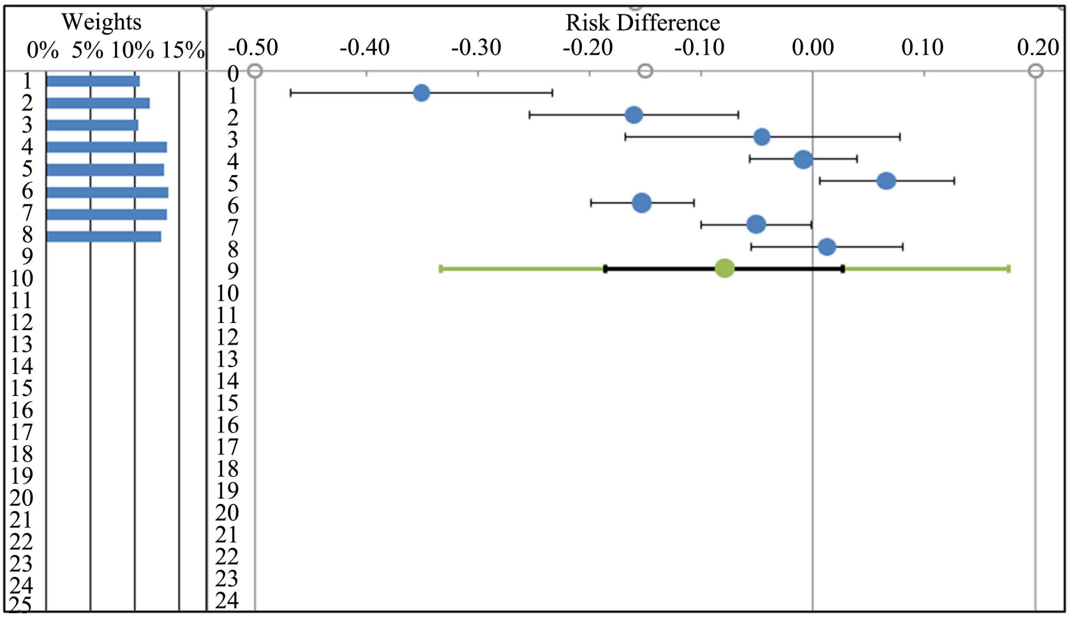

Figure 3. Forest plot of risk difference (Test-Control) for metritis from 4805 subjects from 8 studies (Test-Control). Mean difference = −0.08, P = 0.079. Trial weighting is determined by inverse variance. Plots provide individual trial effect size and confidence interval. The lowermost bar shows the overall effect size, confidence interval (inner bar) and prediction interval (outer bar).

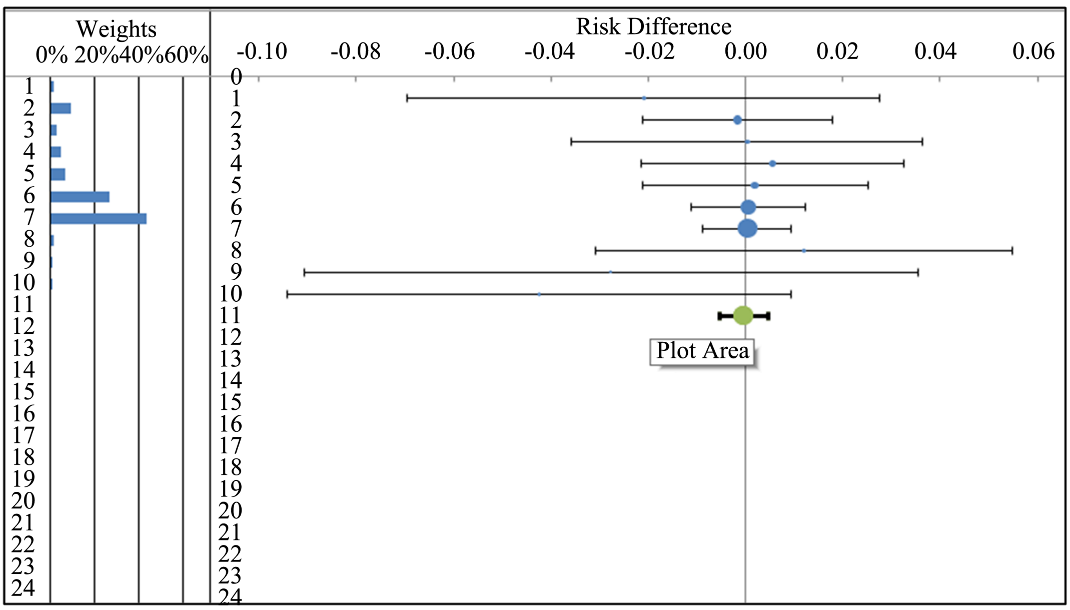

Figure 4. Forest plot of risk difference (Test-Control) for abomasal displacement from 4999 subjects from 10 studies. Mean difference = 0.00, P = 0.868 Trial weighting is determined by the inverse variance. Plots provide individual trial effect size and confidence interval. The lowermost bar shows the overall effect size, confidence interval (inner bar) and prediction interval (outer bar).

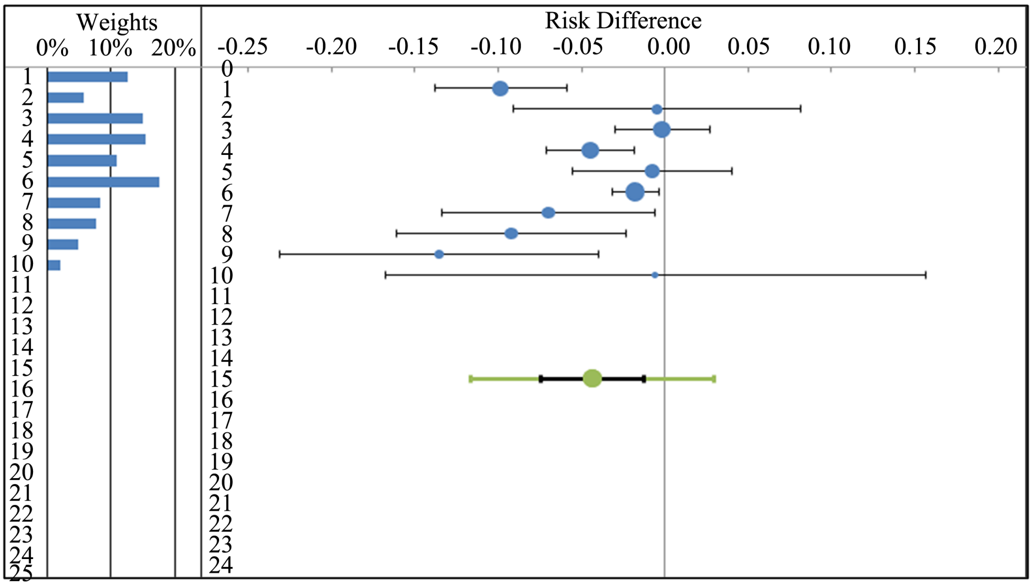

Figure 5. Forest plot of risk difference (Test-Control) for mastitis in early lactation from 4938 subjects from 10 studies. Mean difference = −0.04, P = 0.001. Trial weighting is determined by inverse variance. Plots provide individual trial effect size and confidence interval. The lowermost bar shows the overall effect size, confidence interval (inner bar) and prediction interval (outer bar).

Recent research revealed that at least one of the vitamins supplied could be beneficial in improving resistance to mastitis. Khan *et al.* [26] noted that folic acid enhanced immunity and antioxidant status, particularly during the transition period when folic acid insufficiency may exist. In an earlier review, Khan *et al.* [27] recommended the administration of folic acid to periparturient cows to assist in mastitis prevention during that period. Riboflavin as well may support antioxidant status [14].

Milk Fever. Results from 6 comparisons indicated that there were no differences in milk fever events between the test and control groups of animals (P = 0.261). The determined risk difference was −0.01. The mean incidence rate was found to be 0.019 for the test cows and 0.0.030 for the control cows. Results are provided in **Figure 6**.

Blood BHBA > 1.2 mM. BHBA levels were determined in 14 of the test herds. The forest plot of the results is shown in **Figure 7**. The determined rates of BHBA were 0.15 for the test and 0.23 for the control. Results confirmed that these results were significant (P < 0.05) with a risk difference of −0.08 for this parameter.

Brunner *et al.* [28] determined that the global average prevalence of subclinical ketosis was 24.1%, a value that is quite close to the 23% found with the control cows in this study. Clinical and subclinical ketosis in early lactation is associated with decreased milk production, and reduced fertility [29]. Deniz *et al.* [30] calculated that the average loss in milk yield was 300 kg/cow/lactation by cows with subclinical ketosis. High blood BHBA has been associated with immune suppression and increased risk of infectious diseases such as metritis and mastitis [31] [32].

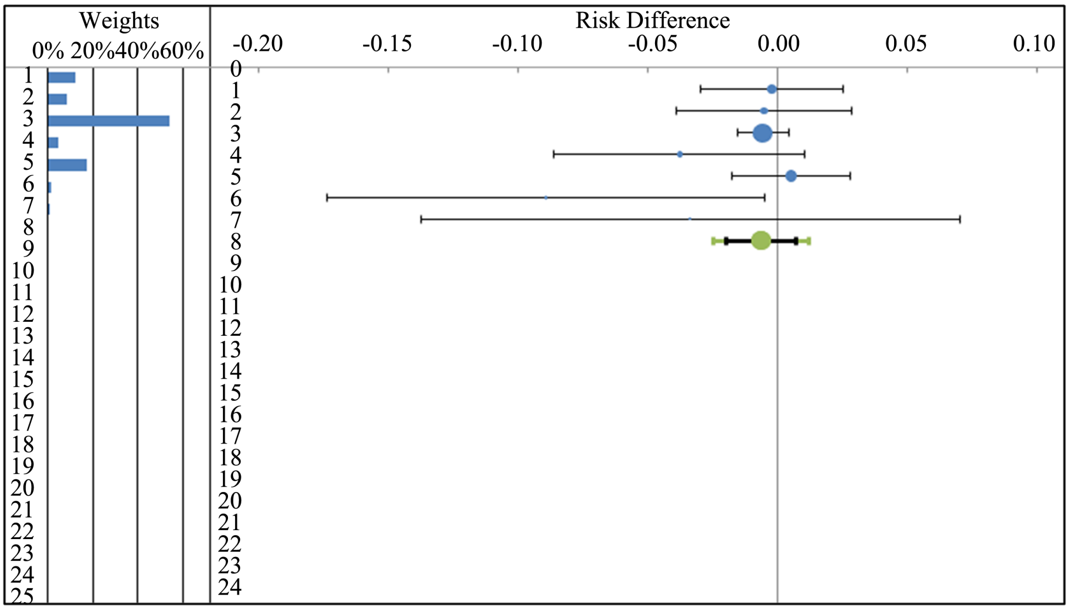

Figure 6. Forest plot of risk difference (Test-Control) for milk fever from 3045 subjects from 7 studies. Mean difference = −0.01, P = 0.261. Trial weighting is determined by inverse variance. Plots provide individual trial effect size and confidence interval. The lowermost bar shows the overall effect size, confidence interval (inner bar) and prediction interval (outer bar).

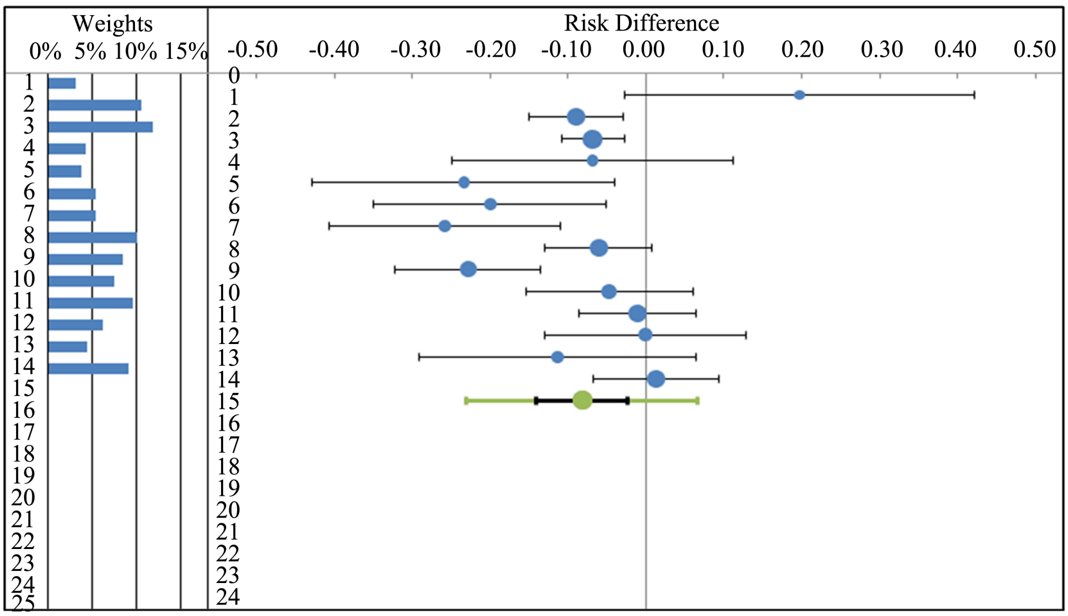

Figure 7. Forest plot of risk difference (Test-Control). Forest plot of risk difference (Test-Control) for BHBA > 1.2 from 4532 subjects from 14 studies. Mean difference = −0.08, P = 0.002. Trial weighting is determined by inverse variance. Plots provide individual trial effect size and confidence interval. The lowermost bar shows the overall effect size, confidence interval (inner bar) and prediction interval (outer bar).

Reproduction. Each farm maintained different metrics related to reproduction. The most consistent variable available or that could be readily calculated was cows confirmed pregnant by 100 DIM. Measurements were available from 12 feeding trials. Overall, the average rate of pregnancy was determined to be

0.332. The risk difference at +0.10 was determined to be significant (P < 0.05) and showed that a 10% greater number of cows were confirmed pregnant by 100 DIM when receiving the BPBlend. The forest plot of the data (Figure 8) shows that results were positive for 11 of the 12 feeding trials.

Milk Yield. Data on milk yield for the first 4 weeks of lactation were available from 16 trials. Milk yield values were not considered to be of primary importance, and values were therefore not consistently requested when the trials were initiated. The meta-analysis for these values compared effect size (Treatment-Control) weighted by the standard error of the mean for each trial. The effect size was determined to be 1.13 kg (P < 0.05, Figure 9).

Milk Fat Yield. Data for milk fat yield were computed from milk yield and milk fat percentage on a cow-by-cow basis with results available from 10 of the trials. The effect size of 0.04 kg/cow/day did not attain statistical significance (P = 0.268). As the standard error values in Figure 10 show, there was a high degree of variability with respect to fat yield.

Milk Protein Yield. There were 9 feeding trials from which data for milk protein percentages were provided again, within trials, yield of milk protein was determined on a cow-by-cow basis. Despite the small number of experiments, milk protein yield was found to increase, with an effect size of 0.05 kg/cow/day (P < 0.001) (Figure 11).

Energy Corrected Milk Yield-Figure 12. Values for energy corrected milk could be computed for 9 of the feeding trials. As with milk yield, there was a significant (P ≤ 0.05) improvement in energy corrected milk (ECM) as calculated by the formula: ECM = 0.327 * milk + 12.95 * Fat yield + 0.765 * protein yield.

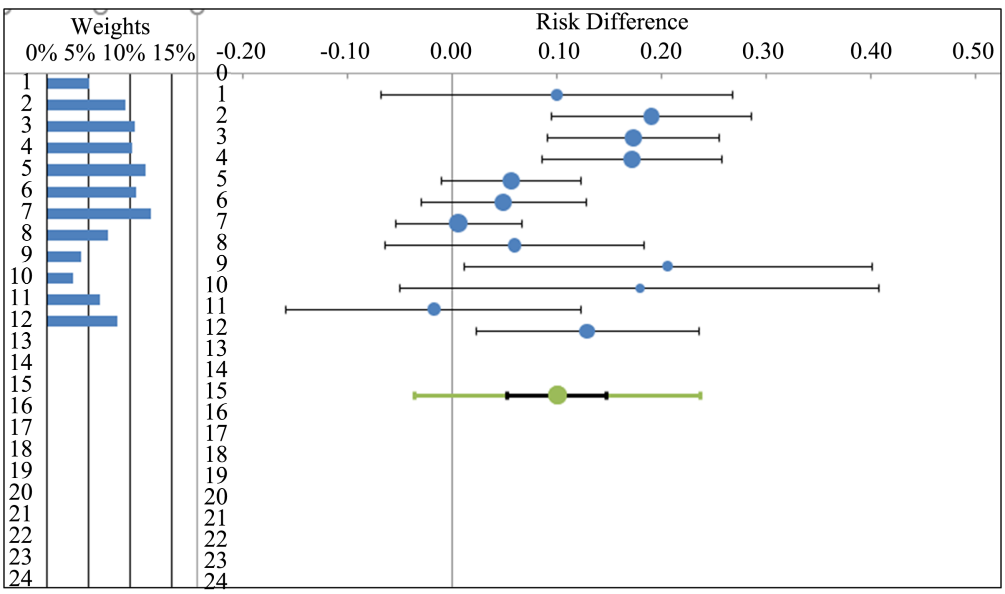

Figure 8. Forest plot of risk difference (Test-Control) for cows confirmed pregnant by 100 DIM from 4593 subjects from 12 studies (Test-Control). Mean difference = +0.10, P < 0.001. Trial weighting is determined by inverse variance. Plots provide individual trial effect size and confidence interval. The lowermost bar shows the overall effect size, confidence interval (inner bar) and prediction interval (outer bar).

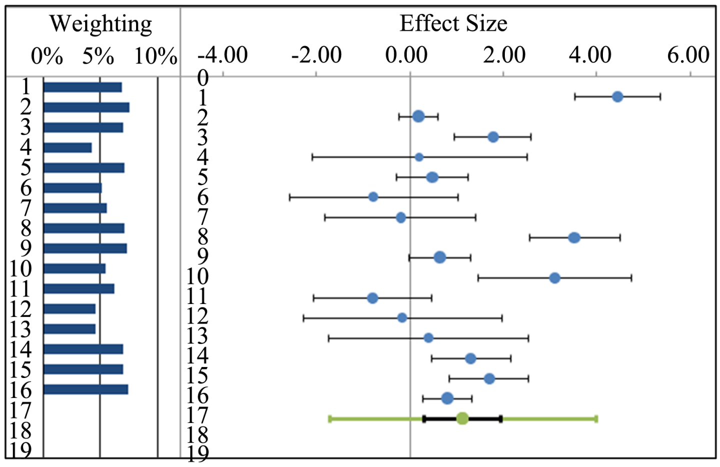

Figure 9. Forest plot of effect size for milk from 16 studies (Test-Control). Mean = 1.13, P < 0.003. Trial weighting is determined by the standard error. Plots provide individual trial effect size and confidence interval. The lowermost bar shows the overall effect size, confidence interval (inner bar) and prediction interval (outer bar).

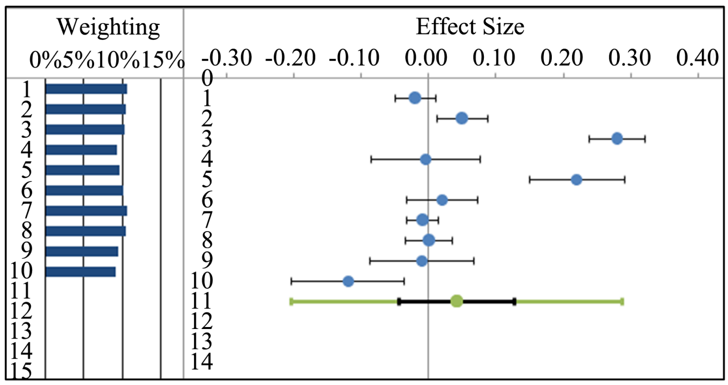

Figure 10. Forest plot of effect size for milk fat yield from 10 studies (Test-Control) Mean: 0.04, P = 0.268. Trial weighting is determined by the standard error. Plots provide individual trial effect size and confidence interval. The lowermost bar shows the overall effect size, confidence interval (inner bar) and prediction interval (outer bar).

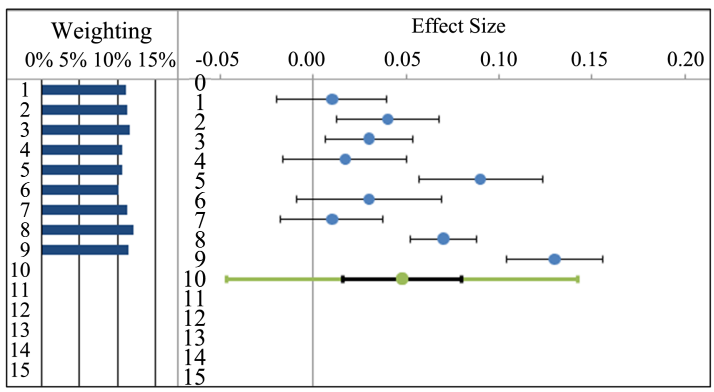

Figure 11. Forest plot of effect size for milk protein yield from 9 studies (Test-Control) Mean: 0.05 P value < 0.001. Trial weighting is determined by the standard error. Plots provide individual trial effect size and confidence interval. The lowermost bar shows the overall effect size, confidence interval (inner bar) and prediction interval (outer bar).

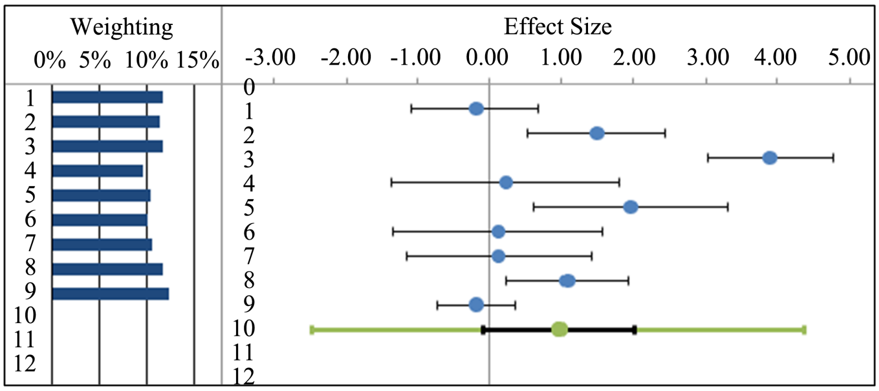

Figure 12. Forest plot of effect size for energy corrected milk from 9 studies (Test-Control) Mean: 0.93 P value = 0.036. Trial weighting is determined by the standard error. Plots provide individual trial effect size and confidence interval. The lowermost bar shows the overall effect size, confidence interval (inner bar) and prediction interval (outer bar).

4. Conclusion

The results obtained through these meta-analyses demonstrated that dairy cows benefit from the inclusion of rumen protected B vitamins and choline during the transition period. The results obtained were varied and included improvements in health parameters, reproduction and milk yield, and likely reflect the greatest intervention in the issues of the most problematic nature on the individual farms. However, taken along with previous meta-analyses regarding the use of rumen protected vitamins for lactation [33] the results strongly suggest that there are in fact needs for these nutrients by ruminant animals. More research is needed to determine dose response and nutrient synergies between B vitamins and choline for dairy cows during the transition period.

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Conflicts of Interest

There are no conflicts of interest with respect to this study.

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