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Q&A REVIEW

Dissecting the Latest RP-Choline Meta-Analysis

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1. Are you aware of any data that has looked at the impact of supplemental betaine on responses to supplemental choline (or methionine)? There are a few experiments with betaine that I recall and you can find them in the published literature:

• Frank Dunshea in Australia • Lon Whitlow at North Carolina State • Sha Tao at the University of Georgia

Just search those authors and you will find the papers from their work. Some focus on betaine during periods of heat stress. I do not recall if they fed betaine and concurrently manipulated methionine content of the diets with supplemental rumen-protected methionine. There might be other papers that I just cannot recall at this time. Just remember that, as for choline, betaine also has to be protected to avoid extensive degradation by microbes.

2. Do you have an ideal level of methionine when the 12.9 g of choline are fed? The current knowledge on methionine in postpartum diets is that it should probably represent 2.3 to 2.4% of the total metabolizable amino acids. The value (ratio of metabolizable methionine relative to MP) will vary with software used to calculate. The value I am suggesting is based on CNCPS biology. The total grams of metabolizable methionine will depend on the level of milk yield of your cows.

3. Many people formulate Methionine to Lysine in a specific ratio and also related to ME. Did you see any relationship to Lysine? I am assuming you are asking if we looked at interactions between choline and lysine in the meta-analysis. If that is the question, then the answer is no, we did not. Our focus was to look at interactions that we believe there was a biological basis to be explored. We could have looked at interactions with all essential AA or with Lysine as you asked, and make the case that Lysine is a substrate for carnitine and carnitine has a major role on fatty acid metabolism, so we could explore those aspects. Our choice was to focus on areas that were less speculative as this was not your typical randomized experiment. If there is a basis to be explored between choline and lysine, then that should be under a designed experiment with a specific hypothesis that is testable.

4. Why do you think the meta-analysis showed reduced liver glycogen in choline supplemented cows? Most likely because yield of ECM increased without an immediate increase in DM intake. Remember, hepatic tissue was collected in the first 10 DIM in those experiments we used in the meta-analysis and, in general, RPC increase yields of milk and milk fat already in the first days postpartum, but DM intake does not increase immediately. I think it is a matter of nutrient balance in the first days of lactation.

5. Are you doing any experiment to ask whether choline might have a direct positive effect on the function of cells in liver or mammary gland which could be associated to choline/betaine as methyl donor? We are not looking at mammary gland at this time. We just completed an experiment with choline to look at hepatic fatty acid metabolism and lipoprotein synthesis. The laboratory work is ongoing. However, that is an area that needs to be explored and I think there is merit to look at the effects of choline on mammary cell proliferation and apoptosis.

6. In the forest plot, within each study, all treatment means were compared with control (no supplement). Although you had no supplement group in all experiment that you included in current meta-analysis. In general, what if I found a study just reported two treatments 6 vs 12.9 g choline. Can I include this study for forest plot analyses in particular or need to have no supplement group to do proper analysis? It all depends on what the goal of your study is when analyzing data and representing with the Forest Plot. Our goal was to show the effect of supplementing choline compared with no supplementation at all. If your goal is to evaluate the effect of increasing choline from whatever baseline (e.g. from 0 to 12 or from 10 to 20, etc, etc), then yes you can add those comparisons. It changes the interpretation though. You need to define your hypothesis and the questions you want to answer to develop the methods to test the hypothesis and answer the questions you posed about the problem.

7. By taking this into account, if using RPC is prioritized to RPM in transition diets of multiparous cows? My first question was that, why RPC is not interacted with RPM to affect multiparous dairy cows performance? If you recall from the meta-analysis by Arshad et al. 2020 in Journal of Dairy Science that I presented in the webinar, there was a clear interaction between intake of choline ion and the content of metabolizable methionine as percentage of the metabolizable protein in the postpartum diet.

I.e., the response in yields of milk and energy-corrected milk to increasing amounts of choline ion were greater when metabolizable methionine was marginal than when metabolizable methionine was high. The response was present at every level of methionine, but the magnitude changed. Remember from the presentation that the precision of the estimates from the data available is much greater for:

• Choline ion between 0 and 20 g/d • Metabolizable methionine between 1.75 to 2.40% of the MP

Within those ranges, increasing choline ion from 0 to 18 g/d resulted in an estimated increase in ECM.

• When methionine is 1.80% of MP = 4 kg of ECM • When methionine is 2.40% of MP = 1.5 kg of ECM

The recent work by Potts et al. J. Dairy Sci. (2020) in press indeed did not show an interaction between choline and methionine. There is an important aspect to take into account when interpreting those results. The experiment had 4 treatments and data were analyzed for primiparous and multiparous separately. The experiment had 25 primiparous, and 29 multiparous cows. It is a transition cow experiment, so there is no opportunity to have a period with pre-treatment to use during statistical analyses as covariate for adjustment. Typical standard deviation for milk yield or energy-corrected milk yield in the first 2 months postpartum is 6 to 7 kg. It would be very difficult to detect statistical effects in production with 4 treatments and 25 to 29 experimental units, unfortunately. It is a difficult area to study, impacts of dietary interventions on production and health of cows when the target period is transition. There is a lot of variability among cows and experiment require a large sample size to be sensitive to detect important biological differences relative to production performance in dairy cows.

8. Has economic analysis been done or explored with meta-analysis results? Does it pay at all milk prices? We have not done any simulation to evaluate the economics of supplementing choline using the meta-analysis results. As any technology that has production and health impacts, the economic return will vary according to what response you expect to obtain and how you value those responses (price of milk and how you value changes in disease incidence). Conceptually, the economic return increases as response (energy-corrected milk and reduction in health events) increase and as the value of those responses (value of energy-corrected milk and the cost of a disease event) increase. There is probably a point in which the return will not be as attractive if it models marginal increases in energy-corrected milk yield, high feed cost, and the price of milk is low.

9. So does it make sense to supplement double doses of RP-choline when BCS is over 4? We do not know the ideal dose of choline ion and we do not know if every product in the market delivers equal amounts of choline ion to be absorbed in the intestine. Also, we have not seen a difference in response to choline according to body condition of cows. Yes, overconditioned cows are more likely to develop fatty liver. However, we have not seen a change in level of response to choline if a cow enters the prepartum group with a BCS of 3.00 versus a cow that enters the prepartum group with BCS of 3.75. If you decide to feed a larger dose than typically used in most experiments (e.g. 13 g of choline ion), then do that for all cows, not only for the overconditioned cows.

10. What would be your choice between Methionine and Choline if you have only one option? This is the same to ask if you can only feed one mineral, which one would use (Ca or P or Mg or Na)? That is probably not how you should look at these nutrients. Cows benefit from choline during the transition period likely because their plasma pool for phosphatidylcholine is inadequate in the last 2 weeks of gestation and first 2 to 3 weeks postpartum. Supplementing choline should restore that. Cows benefit from supplemental methionine because most dairy diets based on corn and soybean product (corn silage, corn grain, soybean meal) do not supply adequate amounts of methionine in an adequate balance with other essential amino acids to support tissue needs and milk protein synthesis. It is not a matter of selecting one versus the other, although you might make that restriction because of how much you are willing to invest. My opinion is that transition cows (3 weeks prepartum to 3 weeks postpartum) should receive diets supplemented with rumen-protected choline. It is also my opinion that postpartum cows should have diets balanced for essential amino acids, in particular methionine in early lactation.

11. Based on slide 20, would you recommend supplementing a combination of high choline and low methionine? See answer to question 10. I believe that is in part what you are asking.

12. Have you seen some effect of unprotected choline of rumen microbial protein synthesis? I am not familiar with that literature. I know that choline in feedstuffs and if you supplement as salts (choline chloride) will be mostly degraded in the rumen-reticulum before reaching the duodenum (>90%). Work in the 80's by Dr. Rich Erdman (University of Maryland) clearly showed that. Choline is degraded to trimethylamine (TMA) dimethylamine (DMA) by rumen and intestinal microbes and TMA can be protonated into trimethylammonium. I do not know if those compounds affect microbial growth. I would ask a microbiologist if rumen microbes benefit from those compounds. Microbes might use choline for phospholipid synthesis for cell wall synthesis during replication, but I just do not know the literature in the area.

13. Does Rumen-Protected Choline supplementation reduce fatty liver? Yes, it does. The dry cow model under negative nutrient balance consistently shows that effect.

14. In the case of a dairy farm, with close-up group, but no fresh group, would you recommend supplementation of RP-Choline to the close-up only? I would, but the benefits are not well characterized. Unfortunately, it is mostly based on my own interpretation of the very limited data than based on solid information from multiple randomized experiments (I know the world is full of feelings, and we need data!). For lack of strong data, my best interpretation of what is available is that response in terms of lactation performance would be limited and smaller than what we observed in the meta-analysis. I am aware of only one experiment that used nulliparous cows in which rumen-protected choline was supplemented or not during the prepartum period (treatments applied only prepartum, last 3 weeks). Cows were fed either 0 or 12.9 g of choline ion in a rumen-protected form starting at 255 d of gestation. The supplemented heifers produced 0.8 kg/day more milk in the first 80 d postpartum. The experiment has 5 pens/treatment and 580 heifers in a single farm and pen was the experimental unit. There was a tendency (P = 0.10) for increased milk yield. Supplemented cows had less NEFA and more choline in plasma prepartum. No differences postpartum. That is the only experiment I am aware of in which RPC was supplemented only prepartum. So, the benefit seems to be there, but it is small. It makes more sense to supplement before and after calving if you consider the needs for phospholipids, the limitations in supply of methyl donors in early lactation (e.g. methionine) and the intense lipid metabolism that takes place with the onset of lactation and the extensive lipomobilization to support milk synthesis when intake is inadequate.