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Methionine's Impact on Sustainable Performance

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Answers to Questions Asked, Courtesy of:

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Q: Why is the MP balance lower for the MET cows?

Brian: Because of the better AA balance, the cows were able to eat and produce more. In fact, the methionine treated cows "chose" to continue to put the extra ingested nutrients into milk as tissue mobilization was similar between Control and Met cows. Specifically, on MP balance, the provision of the supplementary methionine ensured all the other AAs (MP) were utilized more efficiently so the calculated more negative MP balance reflects this and is a positive outcome.

Q: It has been suggested that methionine supplementation in the pre-fresh period only has no impact on post-calving performance. Would you like to comment?

Johan: Based on the gathered data on methionine supplementation during the transition period of dairy cows, we can conclude that it is beneficial to supply methionine to pre-fresh cows. This primarily on the consistent increase in liver glutathione, a potent antioxidant, when pre-fresh cows are supplemented with methionine. The methionine cycle provides homocysteine, which is a precursor for glutathione. The onset of lactation and the common negative energy balance in fresh cows is accompanied by a surge in radical oxygen species (ROS), which cause oxidative stress in fresh cows. Then, if the cow already has a readily available supply of antioxidants such as glutathione in the liver, this will certainly help the cow to overcome this potential oxidative stress.

Q: TG in liver is expressed on what unit?

Johan: TG is expressed as % wet weight.

Q: Is there a recommendation for carnitine levels in the first weeks of lactation?

Johan: Depending on the site where we measure carnitine, but as a reference, carnitine will be lower in plasma>liver>muscle. For instance, in the liver of a typical transition dairy cow, carnitine levels will go from 51.3 to 24.7 nmol/g of tissue, from 2 to 28 d postpartum, respectively, and when transition cows are supplemented with methionine, we observed concentrations of ~90 to 60 nmol/g of tissue. This is consistent with results observed when transition cows have been fed low levels of L-carnitine. If you need further information on carnitine in the transition period, I suggest reading: Carlson, D. B., J. W. McFadden, A. D'Angelo, J. C. Woodworth, and J. K. Drackley. 2007. Dietary L-carnitine affects periparturient nutrient metabolism and lactation in multiparous cows. *J Dairy Sci. 90:3422-3441.*

Q: Did MetaSmart® or Smartamine® M behave similarly in the responses observed?

Brian: Overall the production responses in terms of intake and ECM production were very similar reflecting that each product treatment was contributing the same amount of additional metabolizable methionine.

Q: Is there a case for balancing methionine and lysine for the entire lactation period after doing so in the transition period given the influence on animal health?

Brian: In the transition period, the "More than Milk" roles of methionine on metabolic health are particularly important. Nevertheless, methionine and lysine are necessary as building blocks for protein synthesis at all stages of lactation and target formulation levels for LYS and MET need to be respected throughout lactation.

Q: What about norms for metabolizable (digestible) LYS and MET for freshening or high-production cows?

Brian: In the pre-fresh ration, methionine is the most important AA to pay attention to. A typical target intake is 28 to 30 g of metabolizable methionine. Ideally the lysine level should approach 2.7 to 1 with respect to methionine using the CNCPS model. In all lactation diets the target levels of Met should be 1.14 g MET per Mcal of ME and a LYS to MET ratio of 2.69 to 1.

Q: Do you think you get similar responses feeding RP-Met in high-energy and low-energy close-up dry cows?

Johan: The effects of high-energy vs. low-energy or controlled-energy diets during the close-up period have been extensively studied, and it will be interesting to understand the effects of controlling energy intake while providing an adequate amount of methionine in the close-up diet. We could speculate that cows under a restricted-energy intake (depending on the level of restriction) will benefit from an increased pool of essential AA, such as methionine. While it has been indicated that a restricted-energy intake prior to calving will prime prepartal cows for a better inflammatory and metabolic response postpartum, it will be interesting to see if methionine supplementation could have an additive effect by improving liver function and promoting a beneficial/normal inflammatory condition after calving.

Q: What's the reason for different responses between Smartamine M and MetaSmart?

Brian: Although Smartamine M and MetaSmart are both excellent sources of metabolizable methionine, MetaSmart has the additional advantage of releasing some analogue (HMTBa) in the rumen, which has been shown (Baldin et al. 2017 JDS 101:1-10) to mitigate milk fat depression. Therefore, the choice of MetaSmart to provide the additional metabolizable methionine needed in rations results in similar milk protein yields but higher milk fat yields vs Smartamine M.

Q: If glutathione liver content increases, would it be the same for milk glutathione content? A possible nutraceutical function for the trials with L-carnitine? It was rumen protected?

Johan: It is plausible that glutathione might increase in milk if transition cows are maintained in a diet supplemented with methionine postpartum. However, it was previously observed that glutathione might be a major carrier for cysteine to the mammary gland, and such AA

is essential for disulfide bonds for the final structure of proteins (e.g., casein). Then, it is plausible that the mammary gland will utilize glutathione for milk protein synthesis, and to a lesser extent, this antioxidant will be translocated as is in milk

Non-protected L-carnitine has been tested before in transition dairy cows. To my knowledge, no trials using rumen-protected carnitine have been performed. And, it was demonstrated that carnitine supplementation could decrease liver TG accumulation. However, greater doses of non-protected carnitine decreased DMI and milk yield, which was associated with an interaction of the carnitine and rumen fermentation. See more complete details in: Carlson, D. B., J. W. McFadden, A. D'Angelo, J. C. Woodworth, and J. K. Drackley. 2007. Dietary L-carnitine affects periparturient nutrient metabolism and lactation in multiparous cows. *J Dairy Sci. 90:3422-3441.*

Q: Considering the increase of glutathione with MET supplementation, do you think it could help during the heat stress period?

Johan: Yes, heat stress is actually an oxidative stress model. Then, peripartal dairy cows undergoing heat stress conditions will further benefit from increased liver storage of glutathione, a product of methionine supplementation.

Q: Are there any studies on the combination of methionine and organic selenium to overpass oxidative stress?

Johan: To my knowledge, there are no previous data looking at methionine in combination with selenium in dairy cows and less likely in transition dairy cows.

Q: Typical Met concentration in blood is 25 uM and in venous, probably 5-15 uM. Mammary cells see venous concentrations. Are you concerned with using a much higher concentrations of Met than observed *in vivo*?

Johan: No, the reason for using the high concentration is based on prior data where bovine mammary cells were observed to have a maximal casein synthesis at 500 uM. Therefore, we were trying to replicate the condition below this maximal casein synthesis at 125, 250, and 500 uM. Then, the greater histone methylation and protein synthesis in our study were observed at 125 uM, which tells us that perhaps below 125 uM we could find the actual maximal histone methylation and protein synthesis. We are aware of the much lower concentration of Met in blood in cows, especially in transition dairy cows. Therefore, we are working on developing an actual histone methylation assay using plasma/serum samples from transition dairy cows.

Q: Is it possible to expect better genetic potential with MetaSmart/methionine supplementation? I would like to put it this way, if a cow has low genetic potential and if we feed methionine, is it possible to expect better genetic performance?

Johan: I think this will depend on what is considered as low/high genetic potential, especially nowadays where there are indexes for specific traits such as mammary gland condition, legs,

health, etc. However, if we consider a classic high-yield cow that is likely to have appropriate genetics to produce large amounts of milk, it is likely that this cow will be able to mobilize a great amount of fat (lipolysis) during early lactation in order to sustain milk fat production. This is not surprising as most cows can handle this; however, high genetic merit or high-yield cows will put a tremendous amount of stress in the body in order to produce this amount of milk. Therefore, we can consider that such a cow will be at a higher risk of developing a disease during early lactation.

Q: Any explanation on why the total protein response was quadratic (250 and 500 was lower than 125)?

Johan: Likely due to a negative feedback mechanism that restricts protein synthesis past a certain level. We tested this concentration due to prior data indicating that bovine mammary cells may reach a peak of maximal casein synthesis at 500 uM. However, in transition dairy cows, we know that methionine concentrations in blood are much lower ~20-30 uM.

Q: Which one is the better methyl donor: methionine or choline?

Brian: It depends. Most individual metabolic reactions in the body are not specific to just grabbing a methyl group from anywhere but are specific to the molecule donating the methyl group or groups. The direct use of circulating choline for the synthesis of phosphatidyl choline for example is much more efficient than having to synthesize the phosphatidyl choline from methionine. Nevertheless, in practice the costs of providing these "methyl donors" may in fact predicate the inefficient use of methionine as the most cost effective.

Q: Is there a difference in gene expression and target gene between methionine and choline supplemented pre-fresh? Has that been studied?

Johan: Yes, there are a few studies in transition dairy cows where the individual and interaction effects of both methionine and choline have been investigated. Based on gene expression, it was concluded that Met-supplemented cows generated a greater synthesis of phosphatidylcholine and antioxidants than choline, and, in addition, choline supplemented cows were less likely to regenerate endogenous methionine.

Q: Why did you choose the treatments (methionine) levels of 125, 250, and 500 uM in Rosa's work?

Johan: Those concentrations were selected based on prior data indicating that bovine mammary cells may reach a peak of maximal casein synthesis at 500 uM. However, in transition dairy cows, we know that methionine concentrations in blood are much lower ~20-30 uM.

Q: What is the minimum dose to apply to a group of cows to attain the benefits explained here before? Also, where is the best response based on the product presentation (i.e., MetaSmart or Smartamine)? Should we consider using other

products such as ionophores or protected choline in combination with methionine sources?

Brian: Ionophores should be used on their own merits. The responses to ionophores and methionine will be largely independent of each other. Choline probably still has a role in association with methionine even when adequate methionine is fed, in situations where cows are over conditioned at dry off and, therefore, pre-disposed to metabolic disorders around parturition.

Q: Why does MetaSmart have the same effect as Smartamine on glutathione at 21DIM but not at 7DIM where MetaSmart was similar to the Control?

Johan: Those effects were not statistically significant. However, we could speculate that, if significant, those results could be related to some extent to the rate of depletion of liver glutathione soon after calving.

Q: A cow is expected to stay in the close-up group at least 3 weeks, but there are times that they spend even less than a week due to different factors (i.e., management, cow itself, etc.). Is there a way we can assure the benefit of supplementing Met by increasing the dose higher than recommendation? If yes, how much higher?

Brian: Methionine is a very potent molecule. Feeding above daily recommended levels will not give any additional benefit.

Q: How much per cow per day of MetaSmart Dry or Smartamine M do you recommend in close-up?

Brian: A typical recommendation is 5 to 6 g of additional metabolizable methionine, i.e. 8 to 9 of Smartamine M or 20 to 25g of dry MetaSmart.

Q: About methylation, the fact that methionine may increase methylation is a good thing or can it silence some genes that shouldn't be silenced?

Johan: Histone and DNA methylation remain to be clearly understood. Based on our data, we can strongly conclude that methionine does affect histone and DNA methylation. However, more extensive high-throughput analysis of the methylome (genome-wide DNA methylation) needs to be evaluated. Some years ago, methylation was associated with negative effects; however, our understanding of this biological effect is expanding, and with it the notion the methylation is a negative effect is less reasonable in light of the new data. For instance, transcriptional data from the mammary gland strongly suggest that this tissue needs to be highly methylated (at the histone level) as the cow reaches peak milk. Then, it is understandable that if methylation was, in fact, a negative effect on milk production, the opposite result would have been observed.

Q: Presenter showed a level of 17% CP in the diets for the studies at the beginning of lactation. Is this a maximum level or can it be decreased?

Brian: The level of CP in the ration should be a consequence of DM intake, the target level of AAs, and the degradable N needs for optimal rumen function, with respect to the ingredients and their composition, available for formulating the ration. There is no target CP level per se.

Q: Do we know the rumen degradation rate of unprotected carnitine?

Johan: Prior data from the University of Illinois indicate that 80% L-carnitine will be degraded in the rumen. See more details in: Carlson, D. B., J. W. McFadden, A. D'Angelo, J. C. Woodworth, and J. K. Drackley. 2007. Dietary L-carnitine affects periparturient nutrient metabolism and lactation in multiparous cows. *J Dairy Sci. 90:3422-3441.*

Q: Do you think choline and Met will work in the same way at the inflammation level?

Johan: No. Besides both being methyl donors, choline and Met have distinctive biological roles. Met can serve directly as an AA in the synthesis of beneficial proteins related to inflammation. Besides, it had been observed that Met is more efficient to drive the synthesis of phosphatidylcholine and antioxidants than choline. And both of these effects will help to improve liver functions and by association will help to promote a positive inflammation during the transition period.

Q: Do we have a good explanation of methionine's effect on DM intake pre- and postpartum?

Johan: This could be related to a lower inflammatory condition in cows supplemented with methionine. Inflammatory conditions have been negatively correlated with voluntary feed intake in other livestock and research animals.

Q: Can you comment about methionine and fetal programming. What are the effects on the newborn calves? Can the maternal supply of methionine improve calf health?

Johan: The studies available evaluating fetal programming effects via maternal supplementation of methionine indicate that there is indeed a beneficial effect in terms of the offspring's growth, immune status, and liver maturation. And, most of these effects are a product of maternal Met supplementation, which have been mostly associated with in utero effects rather than colostrum effects.

Q: What differences are there between using a protected methionine or increasing the use of corn gluten or fishmeal in the ration with respect to liver function and milk yield?

Brian: Feeding corn gluten meal unfortunately decreases lysine which counters the potential benefits of the extra methionine. Fishmeal is too expensive compared to using a proven protected methionine source.

Q: What is your opinion about the liver function index as a tool to check the inflammation in the fresh cows?

Johan: A liver function index will be a useful tool for studying inflammation and promoting a more consensus comparison and extrapolation across transition cow studies. However, we should be cautious about developing such an index, since inflammation is neither good or bad and all transition cows have to go through it, which is analogous to the common negative energy balance in transition cows. With this in mind, such an index should take into account liver function in the context of normal vs. detrimental inflammation.

Q: What are the major justifications for feeding methionine prepartum?

Johan: Liver glutathione accumulation, which is a potent antioxidant and may also serve as liver storage for AA.

Q: Can we correlate carnitine with any other biomarker in the blood to avoid biopsy?

Johan: It is possible to measure carnitine in blood, but this will not tell you the level of activity or efficacy to reduce TG or fatty acid accumulation in the liver in general. If you consider that the role of carnitine in the liver is to translocate fatty acids into the mitochondria, we could speculate that greater activity of carnitine will result in greater oxygen radicals from the mitochondrial oxidation as well as ketone bodies from partial beta-oxidation of fatty acids. However, other tissues such as muscle will also oxidize fatty acids, which diminish the possibility of measuring oxygen radicals and ketones as a proxy for carnitine activity. Perhaps, specific fatty acids that can only be oxidized via beta-oxidation in the liver mitochondria could be used as an alternative to measuring carnitine activity.

Q: Recent studies have shown a greater response of primiparous cows to greater amounts of metabolizable protein fed pre-calving. Do you believe that there is a specific response to some specific AAs in pre-calving diets for primiparous cows?

Johan: I'm not aware of these specific data, but it is very likely that specific AA will have a positive effect similar to what we have observed in Met or Lys:Met ratio multiparous cows. Also, it is well understood that it is not only about the level of metabolizable protein but rather the profile of AA in the metabolizable protein. Then, the increase in metabolizable protein could also increase the availability of limiting AA, such as Met and Lys.

Q: What about methionine in calf diet? Do you recommend its use?

Brian: Methionine needs to be added to all milk replacer diets but does not need to be protected. Recent work has shown that the addition of MetaSmart to starter diets enhances intake, growth, and feed conversion (Molano et al., *J. Dairy Sci. Vol 102 – Suppl. 1. Abstract # 143*).

The answers to these questions are provided in good faith and are the scientific opinions uniquely of Dr. Johan Osorio, South Dakota State University, and Dr. Brian Sloan, Adisseo.