Amino Acid Balancing and Its Role on Metabolism, Inflammation, and Oxidative Stress: Future Molecular Implications

Johan S. Osorio[1]
Department of Dairy and Food Sciences, South Dakota State University

Introduction

The modern dairy cow has been selected over generations for high milk production, with many successful dairy operations averaging over 13,500 kg of milk per lactation cycle per cow. This amount of nutrient output in the form of milk components puts a tremendous amount of pressure on the metabolism of the dairy cow, especially during early lactation. In fact, it is common to all mammals, including the dairy cow, to undergo marked physiologic and metabolic changes during the transition from pregnancy to lactation. For instance, it has been estimated that in dairy cows the energy and protein requirements can dramatically increase up to 5 times from late pregnancy to lactation (i.e., transition period). Therefore, during the past 3 decades, a substantial amount of research has been conducted to understand how these biological adaptations can predispose dairy cows to negative effects on health and consequently on the lactation performance of transition dairy cows.

Metabolizable protein (**MP**) is comprised primarily of ruminally synthesized microbial CP (**MCP**) and rumen undegradable protein (**RUP**) and is the true protein digested postruminally and absorbed by the intestine in the form of amino acids (**AA**) and peptides (Schwab and Broderick, 2017). Dairy cows in early lactation commonly experience a negative MP balance condition, where the dietary MP supplied does not meet the requirements for maintenance, growth, and milk synthesis (Bell et al., 2000). Among AA, the availability of methionine (**Met**) in MP across a wide range of diets for dairy cows is low (NRC, 2001), hence, limiting its use for mammary and liver metabolism and also for the synthesis of the methylated compound S-adenosylmethionine (**SAM**) (Martinov et al., 2010). Therefore, supplementation of rumen-protected Met to transition dairy cows has consistently improved milk yield and DMI (Osorio et al., 2013; Zhou et al., 2016b; Batistel et al., 2017), milk protein yield (Ordway et al., 2009; Osorio et al., 2013; Zhou et al., 2016b; Batistel et al., 2017), and milk fat yield (Osorio et al., 2013; Zhou et al., 2016b). The latter effects have been associated at the metabolic level with considerable improvements in liver function and antioxidant precursor synthesis (Osorio et al., 2014b; Zhou et al., 2016a; Batistel et al., 2018). At the molecular level the effects of Met as a precursor of SAM has been previously investigated (Osorio et al., 2016a). Due to the multiple biological processes that require SAM, including transsulfuration, polyamine biosynthesis, DNA methylation (Lu and Mato, 2012), and histone methylation (Shima et al., 2017), the requirements for

[1]Contact: 1111 College Avenue, Alfred Dairy Science Hall, Brookings, SD 57007; 605-688-5490; Email: Johan.Osorio@sdstate.edu

methyl donors, such as choline and Met, increases at the onset of lactation (Preynat et al., 2009). Histone methylation is one of the mechanisms by which the genetic information contained in the DNA is made available or unavailable (i.e., chromatin status) for transcription and translation into proteins. Within the context of the dairy cow, Bionaz and collaborators (2012) observed marked alterations associated with the chromatin status (i.e., euchromatin or available DNA or heterochromatin or unavailable DNA) in bovine mammary tissue from late pregnancy to lactation indicating that the mammary gland undergoes substantial changes in the available genetic information during the transition period. Taken together, is it conceivable that Met availability can affect the chromatin status at the molecular level through histone methylation, since Met is the primary source for SAM, and, in turn, SAM is the main methyl donor for histone methylation. Thus, the objective of this article is to present and discuss the effects of Met on metabolism, inflammation, and gene regulation.

Methionine and the Transition Dairy Cow

It is well-known that the most challenging stage in the lactation cycle of a dairy cow is the transition from late pregnancy to lactation, where most metabolic and infectious diseases occur. Primarily, this is due to several conditions including immunosuppression, changes in endocrine status, and decrease in DMI that collide during this relatively short period of time (Grummer, 1995; Drackley, 1999). In late pregnancy and early lactation, the nutrient demand increases quite considerably. In late pregnancy nutrient demand increases as a result of fetal development, then, at the onset of lactation, the nutrient demand further increases dramatically for milk synthesis (Ingvartsen, 2006). This demand for nutrients triggers a coordinated response in different tissues such as liver, adipose, and mammary gland resulting in endocrine and metabolic alterations to ensure high milk yield concurrently with maintenance of physiological homeostasis (Ingvartsen, 2006; Loor, 2010). Such endocrine and metabolic alterations include decreased insulin while increasing glucocorticoids, growth hormone, and NEFA. At the same time tissue sensitivity to glucocorticoids increases while insulin sensitivity decreases (Bell, 1995; Ingvartsen and Andersen, 2000). Contrasting to the energy demands, transition dairy cows commonly experience a reduction in DMI around calving time, therefore limiting the supply of dietary nutrients to sustain milk production in early lactation. This scenario renders transition dairy cows in a negative balance of nutrients not only from an energy standpoint but also from a negative protein balance, specifically negative MP balance (Bell et al., 2000). Thus, it is within this time frame that improving not only MP balance, but also the AA profile comprising the MP supplied, where the greatest beneficial effects can be obtained by supplementing key AA such as Met and lysine (**Lys**).

Methionine is commonly characterized as one of the most-limiting AA in dairy cow rations, therefore it is not surprising the amount of attention this nutrient has received over the years (**Figure 1**; PubMed search using keywords “methionine dairy cows” on January 14, 2018). And more recently, the primary focus has been in transition cows where the evident and consistent beneficial effects of Met supplementation indicate that it is during this period when dairy cows have the most benefit from this nutrient (Schwab and Broderick, 2017). From a biological standpoint,

the importance of Met resides on the plethora of biological processes that it is involved in beyond the synthesis of milk proteins, and such importance has been exposed during fragile metabolic and physiologic conditions as the transition period of dairy cows. At the metabolic level, some of the main biological areas affected by Met supplementation are the lipid metabolism, inflammation, and oxidative stress.

The Lipotropic Effect of Methionine

In transition dairy cows, the common lipolytic state of the adipose tissue is partially driven by the decreased insulin levels along with decreased insulin sensitivity, which eventually leads to elevated blood NEFA concentration. This excess NEFA will then be transported through the bloodstream to peripheral tissues for use as an energy source. The liver is the most important site for removal of NEFA from circulation (Bell, 1979). Extreme rates of NEFA or lipid mobilization lead to increased uptake of NEFA, hence, increasing the susceptibility to hepatic lipidosis (Bobe et al., 2004). The hepatic assembly/export of very-low density lipoproteins (**VLDL**) is one of the potential mechanisms utilized by this organ to limit the lipid accumulation or hepatic lipidosis (Drackley, 1999). However, the rate of hepatic VLDL synthesis has been demonstrated to be lower in ruminants than monogastrics (Pullen et al., 1990). Interestingly, McCarthy et al. (1968) hypothesized that Met deficiency in ruminants might limit hepatic VLDL synthesis and be a causative factor of ketosis. Similarly, Grummer (1993) proposed that limiting AA such as Met can have a potential impact on VLDL assembly and secretion in ruminants. Several studies have assessed the role of Met as a potentially limiting AA in the regulation of hepatic VLDL synthesis in dairy calves (Auboiron et al., 1994; Auboiron et al., 1995) and dairy cows (Durand et al., 1992). More recently, the Met effect on hepatic VLDL assembly/export has also been reported in transition dairy cows (Osorio et al., 2013), where a mild increase was observed in blood ApoB-100, a key protein for the assembly/secretion of VLDL. Similar results have been observed by Sun et al. (2016), where transition dairy cows supplemented with rumen-protected Met had an overall increased blood concentration of ApoB-100 and VLDL.

Among the potential mechanisms for the effect of Met on VLDL synthesis, is the improvement in liver function. In fact, it is commonly understood that liver functionality is depressed during the transition period of dairy cows (Trevisi et al., 2013). Albumin is primarily synthesized in the liver and is one of the main blood biomarkers associated with liver function. Albumin is commonly observed to decrease in blood during the transition period (Bertoni et al., 2008). Then, the limiting effect of Met as an AA for protein synthesis is evident when a consistent increase in blood albumin has been observed when supplementing Met to transition dairy cows (Osorio et al., 2014b; Zhou et al., 2016a; Batistel et al., 2018). An increased liver function level during the transition period will likely ensure that the synthesis of key proteins such as albumin and ApoB-100 will not be impaired or at least maintained. Therefore, it is conceivable that Met as a limiting factor for protein synthesis in the liver will improve liver function and, in turn, this will improve the VLDL assembly and secretion through ApoB-100.

Methionine Alterations on Inflammation and Oxidative Stress

Contrary to liver function, inflammation and oxidative stress commonly increase during the peripartal period in dairy cows (Bionaz et al., 2007; Trevisi et al., 2012). The

peripartal inflammatory response is characterized by an increase in the production of positive acute-phase proteins (**posAPP**) such as haptoglobin and serum amyloid A (SAA), and a concomitant decrease in the production of negative APP (**negAPP**) such as albumin (Bertoni et al., 2008). The well-established triggers of the acute-phase response are the cytokines interleukin-6 (**IL**-6), IL-1, and tumor necrosis factor-alpha (**TNF-α**) (Kindt et al., 2007). They also mediate the inflammatory response by activating leukocytes and endothelial cells (Bannerman et al., 2009). Oxidative stress is driven by the imbalance between the production of reactive oxygen metabolites (**ROM**) and the neutralizing capacity of antioxidant mechanisms in tissues and blood. Such antioxidant mechanisms include glutathione, taurine, superoxide dismutase, and vitamins A and E (Bernabucci et al., 2005). During the transition period of dairy cows, there is a common degree of oxidative stress associated with the onset of the lactation (Grohn et al., 1989). But, if there is excessive lipid mobilization in the form of NEFA reaching the liver this will likely overwhelm the cellular antioxidant capacity (Bernabucci et al., 2005); then excessive ROM can induce an inflammatory response which is controlled at the molecular level by gene expression regulators or transcription factors (**TF**) (e.g., STAT3 and NFKB) (Huang et al., 2016).

At the crossroads between inflammation and oxidative stress, Met can have profound alterations on these biological processes through improved liver function and glutathione metabolism. The latter is a major antioxidant, and structurally it is a tripeptide mainly synthesized in the liver. Glutathione is the most abundant endogenous antioxidant due to its marked ability to scavenge ROM and free radicals and consequently is commonly used as a biomarker in oxidative stress-related diseases (Romeu et al., 2010; Vetrani et al., 2013). In transition dairy cows, Met supplementation has consistently increased the concentration of glutathione in the liver (Osorio et al., 2014b; Zhou et al., 2016a; Batistel et al., 2018), which has been associated with Met being incorporated upstream in the *de novo* synthesis pathway for glutathione (Halsted, 2013). Glutathione can not only serve as an important hepatic antioxidant but also it can be exported into the bloodstream, where it can aid in the control of systemic oxidative stress response. In the liver, glutathione is commonly depleted during the transition period, primarily after calving (Osorio et al., 2014b; Zhou et al., 2016a; Batistel et al., 2018). The liver glutathione has been described as a reservoir for supplying AA such as Cys to the γ-glutamyl cycle (Lu, 2009). Then, the postpartal depletion of liver glutathione indicates that the metabolism of the transition dairy cow relies on this reservoir of AA (i.e., liver glutathione) for vital functions such as oxidative stress. In summary, the decrease of important proteins synthesized in the liver such as glutathione and albumin suggests that liver protein synthesis is compromised during the transition period and the supply of limiting AA such as Met can potentially reverse these conditions and prepare dairy cows for a smooth transition from pregnancy to lactation.

Methionine and Gene Regulation in Transition Dairy Cows

The DNA contains the genetic information to synthesize all the proteins in the body, but this information must be transcribed into mRNA (i.e., transcriptome) prior to being utilized as the template for protein synthesis. The ability to obtain transcriptomic information has facilitated the characterization of the behavior of molecular networks at multiple points during the onset of diseases or stress periods such the transition period

of dairy cows. However, major gaps in knowledge of the molecular adaptations during this crucial life state of the dairy cow remains. As mentioned above, the liver plays an essential role in the physiological adaptations of the transition dairy cow, therefore transcriptomic information from this organ has been a major focus in transition dairy cow research (Loor, 2010).

Preynat and collaborators (2010) published one of the first experiments evaluating transcriptomic changes in the liver of transition dairy cows supplemented with rumen-protected Met. This study showed that cows supplemented with Met had an upregulated transcription of genes associated with Met and the methylation cycle including phosphatidylethanolamine transferase (***PEMT***), responsible for the synthesis phosphatidylcholine (**Figure 2**), the latter being an important structural component in the assembly of VLDL in the liver. More recently, studies conducted at the University of Illinois in transition dairy cows supplemented with Met revealed a common upregulation of genes related to the Met cycle (Figure 2) such as *PEMT*, S-adenosylhomocysteine hydrolase (***SAHH***), and Met adenosyltransferase 1A (***MAT1A***) (Osorio et al., 2014a; Zhou et al., 2017). The SAHH is a substrate-dependent enzyme and might play an important role in the availability of both SAM and homocysteine (**Hcy**). In fact, the inhibition of SAHH causes the accumulation of SAH and, subsequently, suppresses SAM-dependent transmethylation via feedback inhibition (Lee et al., 2011). The *MAT1A* gene encodes both MATI and MATIII isoenzymes in mammals, which are responsible for the first step in the hepatic synthesis of SAM from Met (Martinov et al., 2010). The importance of Met in the synthesis of SAM is confirmed by the upregulation of *MAT1A* and *SAHH* genes related to the Met cycle.

The essential role of SAM within the context of the transition cow relies on multiple biological processes that require this methyl donor, including transsulfuration, polyamine biosynthesis, DNA methylation (Lu and Mato, 2012), and histone methylation (Shima et al., 2017). Among these, the epigenetic modifications caused by DNA and histone methylation are particularly important in order to understand the potential transcriptomic alterations due to Met supplementation. DNA methylation occurs through specialized enzymes called DNA methyltransferases, which utilize the methyl group provided by SAM to methylate cytosines within a Cyt-phosphate-Gua (**CpG**) region (“island”) in the DNA and eventually creating methylated CpG patterns in the mammalian genome (Kass et al., 1997). The final epigenetic effect of DNA methylation is to override the predetermined genetic information in the DNA, and then the phenotype in mammals, the methylation of the DNA can induce significant modifications to the transcriptome. Previously, we observed a prepartal upregulation of *DNMT3A*, a gene that encodes for a DNA methyltransferase in charge of the *de novo* methylation of the DNA (Osorio et al., 2014a). And, more recently the significance of these findings was confirmed by observing significant alterations due to Met supplementation in the liver of transition dairy cows in terms of global DNA methylation and specific region methylation of an important TF, the peroxisome proliferator-activated receptor alpha (**PPARα**; Osorio et al., 2016a). The uniqueness of this gene regulator or **TF** within the context of the transition dairy cow was initially presented by Drackley (1999), and since then this nuclear receptor has become an interesting area of research in dairy cattle nutrigenomics (i.e., nutrient-gene interaction) (Bionaz et al., 2013). Therefore, the

connection between Met and PPARα upregulation through DNA methylation during the transition period is another suitable mechanism to explain the consistent improvements in performance (e.g., milk yield and DMI) observed in transition dairy cows supplemented with Met.

Since the initial application of high-throughput transcriptomic analysis such as a microarray platform in the dynamic adaptations of the liver during the transition period of dairy cows (Herath et al., 2004), a number of studies have followed utilizing even more advanced techniques such as RNA sequencing (**RNA-seq**) encompassing the whole transcriptome (Loor et al., 2013). The use of these techniques has revealed the biological signatures in the liver involving complex networks through numerous regulatory mechanisms with the aim to respond accurately to metabolic and physiologic cues during the transition period (Loor et al., 2006; Bionaz and Loor, 2012). From the initial experiment conducted at the University of Illinois on Met supplementation to peripartal dairy cows (Osorio et al., 2013), a microarray analysis of the liver transcriptome was performed (Osorio et al., 2012). This study revealed transcriptomic alterations in 2,663 genes [differentially expressed genes (**DEG**)] in the liver of cows supplemented with Met during the peripartal period (**Figure 3**). The functional analysis of these DEG showed not only an expected overall impact on the metabolic pathways of Cys, Met, and glutathione but also on less known cyanoamino acid and taurine metabolisms. Additionally, the high impact of gene networks associated with AA metabolism in control cows in this experiment underlined the key role of AA metabolism in the adaptations that occur during the transition period. From a nutrient-gene interaction standpoint, the results from this microarray analysis underpin the fact that dietary nutrients such as Met can have profound effects on the molecular makeup of dairy cows through gene expression alterations and subsequently promoting a better outcome in their performance during the transition period.

The importance of nutrigenomics in dairy cows has been previously reviewed (Bionaz et al., 2015). This relatively new area of research focuses on how dietary nutrients and compounds can affect gene expression directly or indirectly via interactions with TFs. Although, a specific TF that responds directly to AA or even specifically for Met still unknown, the potential interactions between AA and TFs have been discussed (Osorio et al., 2016b). The fact that Met can produce changes in the transcriptome add another layer of complexity to the metabolic, inflammatory, and antioxidant effects discussed above. Although the gene expression alterations by Met supplementation in dairy cows are consistent and evident, the actual molecular mechanisms by which this nutrient cause such alterations remain unclear.

A Model for Gene Regulation of Methionie in Dairy Cows

A proposed model for transcriptional alterations by Met supplementation is presented in **Figure 4**. This model rests on the well-established fact that Met is a precursor for SAM, that, in turn, can cause alterations in DNA and histone methylation. However, the effects of Met on gene expression through a specific TF via intermediate metabolites or cell membrane transporters are less understood.

- Intermediate metabolites of Met, for instance, cysteine downstream in the Met cycle could potentially interact with unknown TFs (e.g., zinc finger proteins) or be

essential for the final conformational structure (e.g., protein folding) of a TF through disulfide bonds, and increasing the available functional form of such TF; then this effect could cause a transcriptomic alteration.

- The PPAR belong to a family of TF that can bind and be activated by nutrients and compounds in the diet (i.e., ligand-dependent TF) (Bionaz et al., 2015). In the case of PPAR, it is well-known that this TF responds to fatty acids by increasing the transcription of genes related to metabolism and inflammation (Bionaz et al., 2013). Similar to PPAR, it is plausible that other TF in this family could potentially respond to Met, and then generate a change in gene expression, but such TF remains unknown.
- In recent years, advances in cell physiology have broadened our understanding of cell membrane AA transporters, making evident that these transporters may have dual receptor-transporter functions and act as “transceptors” to sense AA availability. As part of this sensor activity, transceptors can potentially initiate a cascade of cell signaling to result in a transcriptomic alteration through a TF.
- The use of Met as a precursor of SAM is widely accepted, and this methyl donor has been observed to cause significant alterations in DNA methylation in transition dairy cows supplemented with Met (Osorio et al., 2016a). Until now the effects of Met on histone methylation and subsequently, on gene expression have not been evaluated in the context of dairy cows.

Histone Methylation

In the cellular nuclei, the DNA is normally packed in condensed structures called chromatins, consisting primarily of histone proteins, which serve as spools where the DNA winds around. Then, the genetic information contained in the DNA exists in two states: unavailable or wind around histone proteins, and available or unwound. Chromatin remodeling is the main mechanism by which DNA is wind or unwound from histones and these dynamic modifications occur by enzymatic modifications including acetylation, phosphorylation, ubiquitination, and methylation (Singh et al., 2010). The latter is a potential mechanism through which Met can alter gene expression in dairy cows (**Figure 3**). Currently, the limited amount of data on histone methylation in dairy cows has been conducted using immune cells (He et al., 2012) primarily related to subclinical mastitis (He et al., 2016). This work has provided nuances on the interactions between mastitis-related pathogens and histone methylation, however dietary effects on histone methylation have not been investigated.

The use of fluorescent proteins to track biological events at the cellular level has been vastly exploited and impacted the fields of biochemistry, biotechnology, and cell biology. Within the context of nutrigenomics in dairy cattle, the use of fluorescent proteins was initially proposed and reviewed by Bionaz et al. (2015). One of the first papers utilizing this technique from a nutrigenomic approach in bovine mammary epithelial cells (i.e., **MacT** cells) was published (Osorio and Bionaz, 2017). Among the advantages to using fluorescent proteins is the ability to collect “true” real-time data on a specific cellular process without harvesting or extracting cells for each time point.

Recently, we have utilized a dual-fluorescent proteins system developed at the Massachusetts Institute of Technology (Lin et al., 2004) to track histone methylation with high spatial and temporal resolution in bovine cells. This fluorescent protein reporter allows for the analysis of specific methylation sites such as K9 and K27 in histones (i.e., regions of high methylation activity). For this analysis, we used the well-established bovine mammary epithelial cells, MacT cells, and treated them with 4 levels of Met in the media (0, 125, 250, and 500 µM) for 24 hours. The final parameter utilized for this type of experiment was relative fluorescent intensities analyzed through the CellProfiler (Kamentsky et al., 2011), and these data are presented in **Figure 5**. The results on K9 show an evident increase in histone methylation since 12 h post-treatment, and by 24 h the cells treated with 125 and 500 µM of Met expressed a greater ($P < 0.01$) histone methylation than control (Figure 5A). In contrast to K9, histone methylation at K27 site seems less receptive to methylation; in fact, cells treated with 500 µM of Met had a lower ($P < 0.01$) methylation status than control (Figure 5B). The viability results indicate a consistent improvement in K9 (Figure 5C) and K27 (Figure 5D) when cells were treated with 250 µM of Met. These preliminary data confirm the potential of Met to create histone modifications through methylation and consequently altering the transcriptome of dairy cows. The fact that histone methylation is not affected in a dose-dependent manner suggests that other unknown factors governing this biological process might override the effect of Met. Further ongoing analyses on global DNA methylation, region-specific DNA methylation, and gene expression profiling on the Met cycle and cell membrane transporters will help to get a better picture of the implications of the manipulation of this biological process through Met.

The importance of the method outlined above should not be restricted to an in-lab highly-controlled environment, but rather expanded by utilizing this as a complementary analysis for on-farm research experiments, or hybrid experiments (**Figure 6**). For instance, blood serum can be isolated from dairy cows supplemented with Met, with the aim to use it as a medium for bovine cells (e.g., liver, mammary, etc.). Prior to the incubation, the genetic information of the histone methylation reporters can be introduced into the bovine cells so these specialized proteins can be present at the incubation time. Through fluorescent microscopy methods the histone methylation data can not only be tracked in real-time, but also, these qualitative data can be transformed into quantitative data via imaging analysis software (i.e., CellProfiler). The ability to combine on-farm and in-lab data can be a new approach to broaden our understanding of dietary effects at the molecular level while providing actual data from an on-farm setting where importance sources of variation such as animal and environment can be considered.

Summary

Data collected in recent years on the effects of Met supplementation during the transition period of dairy cows have delineated a clearer picture of the multiple effects exerted by this nutrient at the level of performance, metabolism, and transcriptional. At the performance and metabolic level, the supplementation of Met to peripartal dairy cows consistently improved DMI, milk yield and components, and energy balance by enhancing liver function and antioxidant capacity while ameliorating the inflammatory

response. In contrast, the effects of Met at the transcriptional level are less understood. However, the substantial number of genes altered in the liver of transition dairy cows supplemented with Met is indicative of a fundamental change at the molecular level that, in turn, can be associated with a favorable metabolic status and performance. Future research related to AA balancing in dairy cows should focus on the nutrigenomic aspect of these nutrients and how they can impact and manipulate the genetic makeup of dairy cows and consequently associate this with performance and health status.

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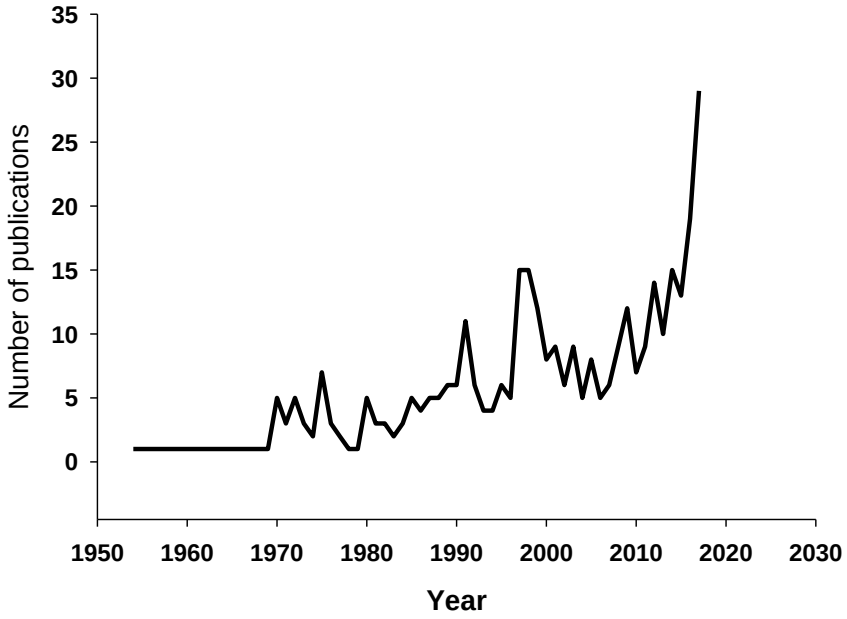

Figure 1. Number of scientific publications found in PubMed per year. The search was performed using the keywords “(methionine) AND (dairy) AND (cows)”. The search was performed on January 10, 2018.

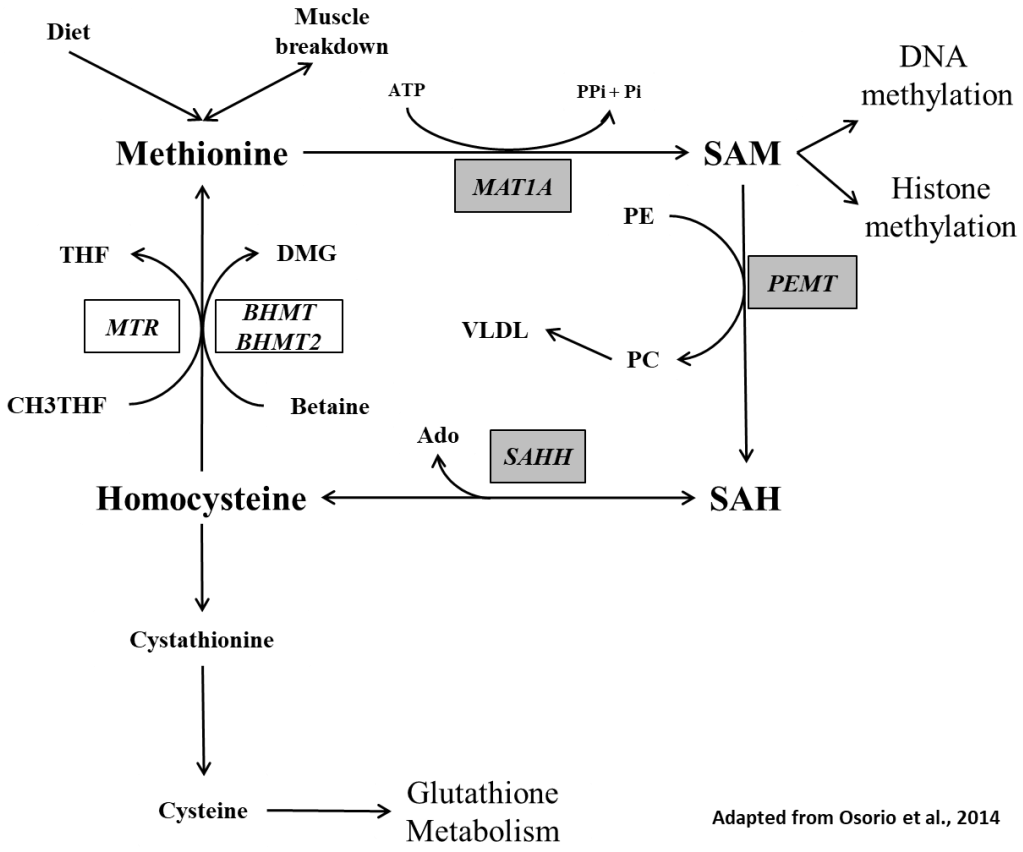

Figure 2. Key genes (squares) encoding for enzymes related to the Met cycle: Met adenosyltransferase 1A (*MAT1A*), phosphatidylethanolamine methyltransferase (*PEMT*), *S*-adenosylhomocysteine hydrolase (*SAHH*), betaine homocysteine methyltransferase (*BHMT* and *BHMT2*), and 5-methyltetrahydrofolatehomocysteine methyltransferase (*MTR*). Genes upregulated by Met supplementation in the liver of transition dairy cows are denoted by gray squares. PPi = pyrophosphate; Pi = inorganic P; SAM = *S*-adenosylmethionine; PE = phosphatidylethanolamine; PC = phosphatidylcholine; SAH = *S*-adenosylhomocysteine; Ado =adenosyl; THF = tetrahydrofolate; CH3THF = 5-methyltetrahydrofolate; DMG = dimethylglycine.

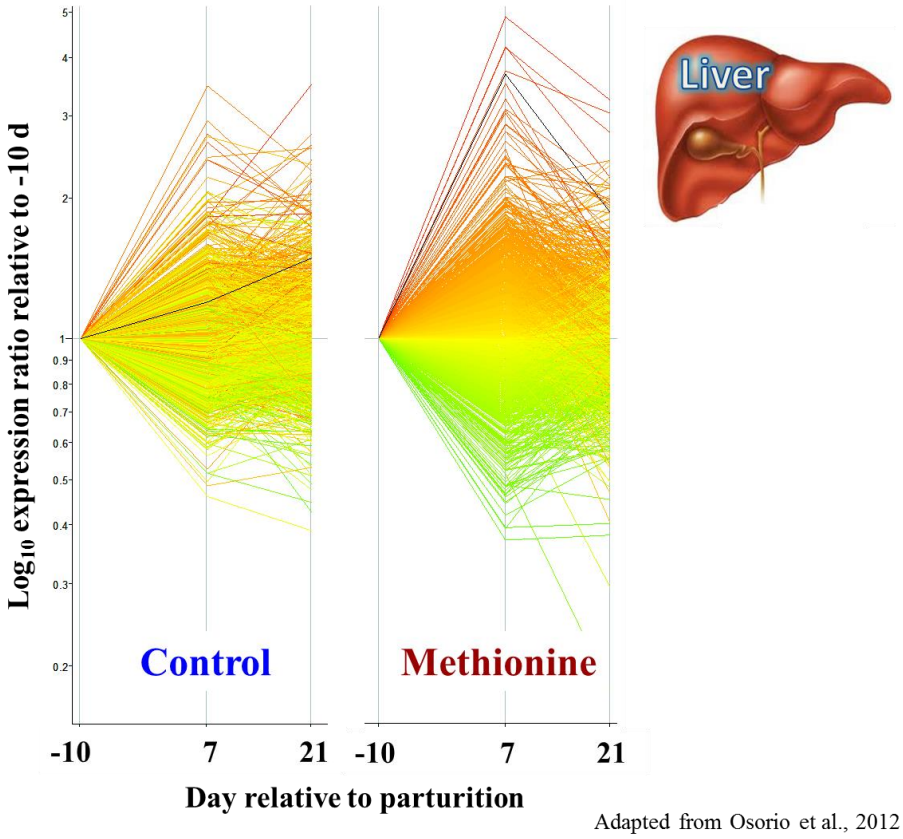

Figure 3. Overall transcriptomics adaptations in the liver of peripartal dairy cows fed a baseline diet (Control) or a baseline diet plus Met (Methionine). Shown in the image generated by Genespring GX7 (Agilent) of the 2,663 genes deemed to be differentially expressed with Time × Treatment with false discovery rate < 0.05. Green and red lines denote genes with an expression ratio lower or higher at 1 relative to -10 d, respectively.

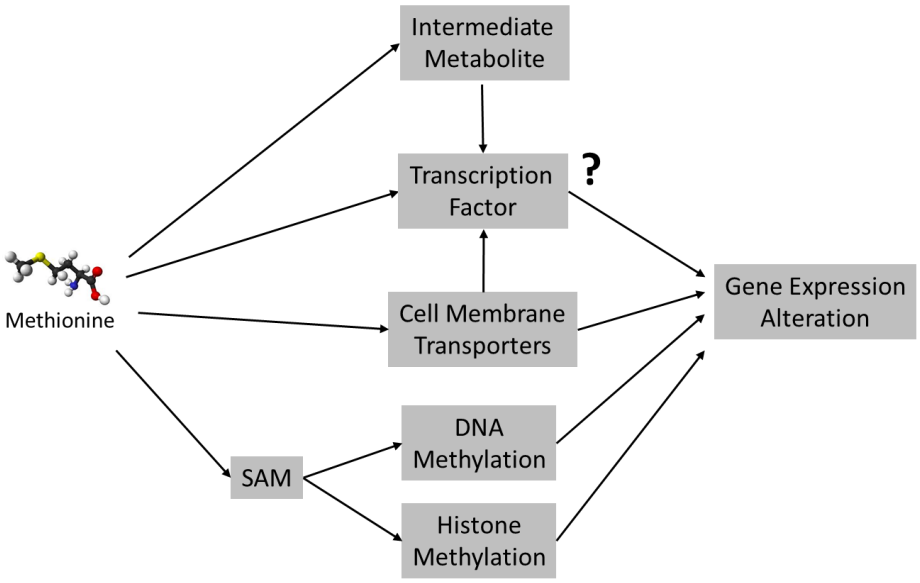

Figure 4. Proposed model for transcriptional alterations by Met supplementation in dairy cows.

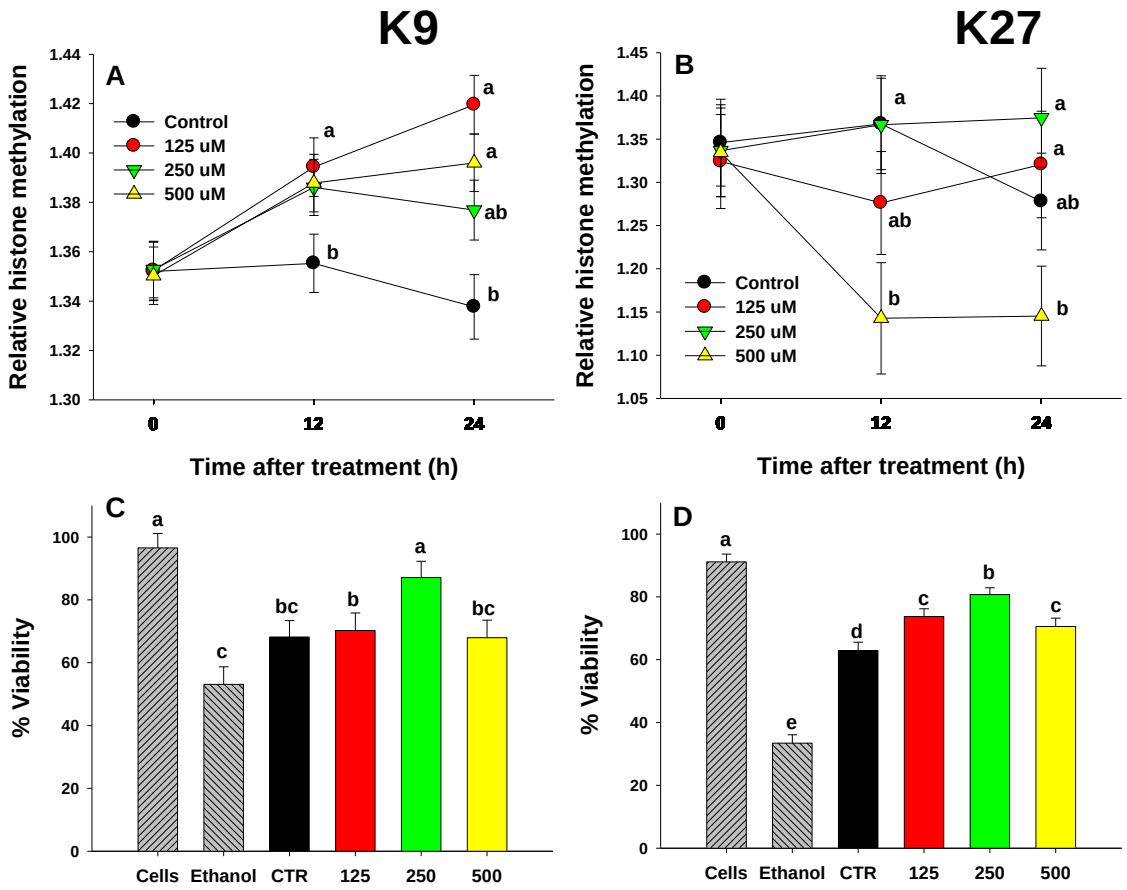

Figure 5. Relative histone methylation and cell viability in MacT cells treated with 0, 150, 250, and 500 uM of Met. MacT cells were seeded at 30,000 cells/well in a 96-well plate 24 h prior to transfection. Cells were transfected with either a K9 (**A**; Cat#22866, Addgene) or K27 (**B**; Cat# 22865, Addgene) plasmids to track histone methylation using a dual-fluorescent protein system (i.e., Fluorescence resonance energy transfer (FRET)]. Plasmids were transfected with 0.3 µL/well of Lipofectamine® 3000 (Cat# L30000001; Life Technologies) and 50 ng/well of DNA plasmid. The relative histone methylation was measured with an inverted fluorescent microscope for live imagining (Life Technologies) equipped with a motorized scanning stage. Viability of cells for K9 (**C**) and K27 (**D**) were measured at 24 h post-treatment using a staining for live cells (NucBlue®; Life Technologies) and a far-red nuclear staining (NucRed®; Life Technologies) for dead cells. The open-source software CellProfiler (Kamentsky et al., 2011) was used to analyzed each picture to quantify cell number and fluorescent intensities.

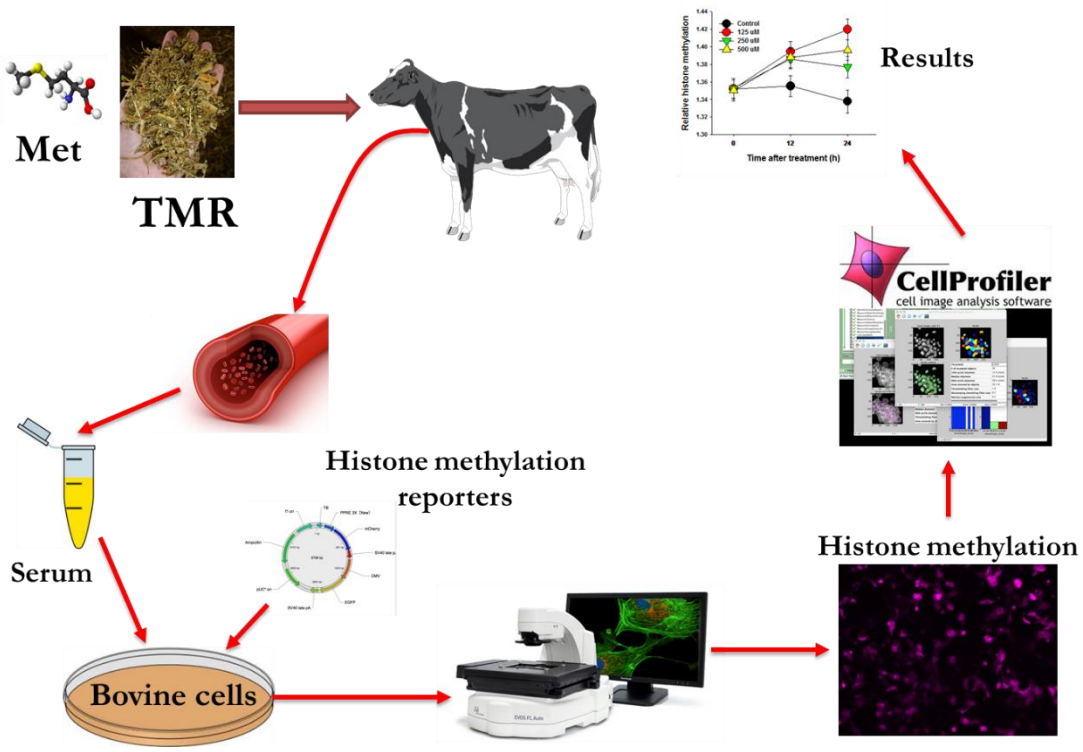

Figure 6. Schematics of a hybrid experiment for the study of histone methylation. This proposed method combines in-lab analysis of specific molecular events such as histone methylation and the tangible aspect of an on-farm experiment. Blood serum isolated from cows supplemented with Met can be utilized to incubate bovine cells. Prior to the incubation the genetic information encoding for a histone methylation reporter can be inserted (i.e., transfection) into the bovine cells. Then, the response to Met supplementation can be tracked through high-resolution imaging by multiple pictures taken by a fluorescent microscope in real-time. The qualitative data from each image taken during the incubation can be transform into quantitative data (i.e., relative intensity) through an open-source cell imaging software (CellProfiler). Finally, the relative intensity data can be statistically analyzed.

SESSION NOTES