Applied Research into Amino Acid Nutrition

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TAKE HOME MESSAGE

- Rumen-protected methionine and rumen-protected lysine added to the diet of Holstein cows improve:
  - Reproductive performance.
  - Oxidative stress.
  - Health and milk production.

INTRODUCTION

Studies over the last 2 decades clearly established the link between nutrition and fertility in ruminants (Robinson et al., 2006; Wiltbank et al., 2006; Grummer et al., 2010; Santos et al., 2010; Cardoso et al., 2013; Drackley and Cardoso, 2014). Dietary changes can cause an immediate and rapid alteration in a range of humoral factors that can alter endocrine and metabolic signaling pathways crucial for reproductive function (Boland et al., 2001; Diskin et al., 2003). Moreover, periconceptional nutritional environment in humans and other animals is critical for the long-term setting of postnatal phenotype (Fleming et al., 2015). Restricting the supply of B-vitamins and methionine during the periconceptional period in sheep, e.g., resulted in adverse cardiometabolic health in postnatal offspring (Sinclair et al., 2007). Feeding female mice a low-protein diet during the preimplantation period of pregnancy resulted in a reduction in amino acid (AA) concentration in uterine fluid and serum and attendant changes in the AA profile of the blastocyst (Eckert et al., 2012).

Strategies have been used to improve the reproductive performance of dairy cows through alteration of nutritional status (Santos et al., 2001, 2008a). In other species, dietary supplementation with specific AA (e.g., arginine, glutamine, leucine, glycine, and methionine) had beneficial effects on embryonic and fetal survival and growth through regulation of key signaling and metabolic pathways (Del Curto et al., 2013; Wang et al., 2012). Methionine is the most limiting AA in lactating cows (NRC, 2001), but supplementation of diets with crystalline methionine has been excluded because free methionine is quickly and almost totally degraded by the microorganisms in the rumen (NRC, 2001). In contrast, supplementing rumen-protected methionine (RPM) has a positive effect on milk protein synthesis in dairy cows (Pisulewski et al., 1996; Ordway, 2009; Osorio et al., 2013). Although the role of methionine in bovine embryonic development is unknown, there is evidence that methionine availability alters the transcriptome of bovine preimplantation embryos in vivo (Penagaricano et al., 2013) and its contents (Acosta et al., 2016).

The DNA methylation in promoters is an important mechanism for regulation of gene expression and gene silencing. However, DNA methylation in other regions may have a more complex role in regulation of transcription (Bird and Wolfe, 1999; Van de Veyver, 2002; Suzuki and Bird, 2008). Methylation of the DNA depends on the availability of methyl donors supplied by AA such as methionine and by compounds of one-carbon metabolic pathways such as choline (Van de Veyver, 2002). Increased methionine bioavailability is likely to increase the entry of methionine into the one-carbon metabolism cycle where it is initially converted into S-adenosylmethionine, the major biological methyl donor (Martinov et al., 2010). Nonruminants fed diets deficient in methyl donors (e.g., choline and methionine) have hypomethylated DNA (Locker et al., 1986; Tsujiuchi et al., 1999). These changes occur not only in global methylation (Wilson et al., 1984) but also in the methylation of specific genes (Bhave et al., 1988). However, effects of methionine in preimplantation embryos are still controversial. Bonilla et al. (2010) suggested that extracellular methionine is not required for DNA methylation in the cultured blastocyst. Nevertheless, gene expression changes caused by alteration of DNA methylation (i.e., absence of the methylase genes) can result in embryo death or developmental defects in preimplantation embryos (Reik et al., 2001).

REPRODUCTION AND NUTRITION

Nutrient demands for milk synthesis are increased in early lactation, and if no compensatory intake of nutrients is achieved to cope with milk production requirements, reproductive functions (i.e., synthesis and secretion of hormones, follicle ovulation, and embryo development) may be depressed. The incidence of diseases and disorders can be high during the periparturient period and have a negative impact on reproductive performance. The risk of pregnancy was reduced if cows lost more than one body condition score (BCS) unit (Butler, 2003, 2005; Santos et al., 2008b). Milk production increases faster than energy intake in the first 4 to 6 weeks after calving. High yielding cows will experience negative energy balance (NEB) and blood concentrations of non-esterified fatty acids (NEFA) increase, and concentrations of insulin-like growth factor-I (IGF-I), glucose, and insulin are low. If extreme, these changes in blood metabolites and hormones may compromise ovarian function and fertility (Butler, 2005).

Different nutritional strategies have been proposed to improve reproduction of the dairy cow with no detrimental effect on lactation performance. Feeding high quality forages, controlled-energy diets, or adding supplemental fat to diets are some of the most common ways to improve energy intake in cows (Cardoso et al., 2013; Drackley and Cardoso, 2014; Mann et al., 2015). Reproduction of dairy cattle may be benefited by maximizing DMI during the transition period, minimizing the incidence of periparturient problems (Cardoso et al., 2013; Drackley and Cardoso, 2014).

THE IMPORTANCE OF AMINO ACIDS

Some AA are limiting for optimal milk production as evidenced by an increase in milk yield, percentage of milk protein, and milk protein yield after supplementation with specific, rumen-protected AA. The first 3 limiting AA for milk production are considered to be methionine, lysine (NRC, 2001), and histidine (Hutannen, 2002). In addition, many AA can have positive effects on physiological processes that are independent of their effects on synthesis of proteins (Wu, 2013). Fertilization and the first few days of embryo development occur in the oviduct. By about 5 days after estrus, the embryo arrives in the uterine horn. The embryo reaches the blastocyst stage by 6 to 7 days after estrus. The embryo hatches from the zona pellucida by about day 9 after estrus and then elongates on days 14 to 19. The elongating embryo secretes the protein interferon-tau that is essential for rescue of the corpus luteum and continuation of the pregnancy. By day 25 to 28 the embryo attaches to the caruncles of the uterus and begins to establish a vascular relationship with the dam through the placenta. During all the time prior to embryo attachment, the embryo is free-floating and is dependent upon uterine secretions for energy and the building blocks for development, including AA. Thus, it is critical to understand the changes in AA concentrations in the uterus that accompany these different stages of embryo development.

The lipid profile of oocytes and early embryos can be influenced by the environment of the cow. Our group ran a trial with the objective to determine the effect of supplementing rumen-protected methionine on DNA methylation and lipid accumulation in preimplantation embryos of dairy cows (Acosta et al., 2016). Lactating Holsteins entering their second or greater lactation were randomly assigned to 2 treatments from 30 ± 2 DIM to 72 ± 2 DIM; control (CON; n = 5, fed a basal diet with a 3.4:1 Lys:Met) and methionine (MET; n = 5, fed the basal diet plus Smartamine M to a 2.9:1 Lys:Met). Embryos were flushed 6.5 days after artificial insemination. Embryos with stage of development 4 or greater were used for analysis. For lipids, fluorescence intensity of Nile Red staining was compared against a negative control embryo (subtraction of background). A total of 37 embryos were harvested from cows (MET = 16; CON = 21). Cows receiving MET had greater lipid accumulation (7.3 arbitrary units) when compared with cows receiving CON (3.7 arbitrary units). There were no treatment effects on number of cells or stage of development. In conclusion, cows supplemented with methionine produced embryos with higher lipid concentration when compared to CON, which could potentially serve as an important source of energy for the early developing embryo.

Hugentobler et al. (2010) summarized the concentrations of AA in plasma (average of days 0, 2, 3, 4 and 6 of estrous cycle), in the oviduct of crossbred beef heifers, and in the uterus (average days 6, 8, and 14 of estrous cycle). There was no effect of day of the cycle on oviductal concentrations of AA. Nine of the 20 AA were present at significantly greater concentrations in the oviduct than plasma, indicating that mechanisms are present in the cells of the oviduct that allow concentration of AA. The uterus also had greater concentrations of many AA than found in plasma from cows on the same days of the estrous cycle. The AA that were most elevated in the uterus, Asp, Asn, Glu, were mostly similar to the oviduct.

In addition to the mechanisms that concentrate AA in the uterus in non-pregnant ruminants, there are additional mechanisms that result in further increases in concentrations of AA in the uterine lumen in pregnant ruminants near the time of embryo elongation (day 14 to 18). Three studies have provided AA concentrations near the time of embryo elongation; 2 in sheep (Gao et al., 2009) and 1 in cattle (Groebner et al., 2011). Although there seems to be very little change in AA concentrations between day 10 and 16 in non-pregnant sheep, there are large increases from 3 to 23-fold in specific AA in the uterine lumen of pregnant sheep (Gao et al., 2009). In order to provide some idea of changes in uterine amino acids during early pregnancy, Wiltbank et al. (2014) combined the results from these 3 studies into a fold increase in AA during the time of embryo elongation. There is an increase in almost all AA at the time of embryo elongation. Of particular interest for dairy cattle, the 3 AA that are considered limiting for milk production, Met, His, and Lys, are the AA with the greatest increase in concentrations in the uterine lumen during embryo elongation (> 10-fold increase on average from these 3 studies). Disturbances in the temporal relationship between uterine blood flow, induction of uterine AA transport, uterine AA concentrations, embryonic growth, embryonic interferon-tau production, and rescue/regression of the corpus luteum may reduce fertility and increase pregnancy losses.

EFFECT OF METHIONINE AND LYSINE ON EMBRYO DEVELOPMENT

One particularly interesting study (Coelho et al., 1989) used serum from lactating dairy cows in the media to grow head-fold stage rat embryos (day 9.5 after breeding). Complete development of these embryos requires serum and development is normal in rat serum. When embryos are grown in serum from dairy cows, embryonic development is abnormal when measured as total embryo protein, somite pairs, or percentage of the embryos that are abnormal (no neural tube closure, abnormal shape, no development of eyes and branchial arches). Supplementation of bovine serum with AA and vitamins produced normal development. Amino acid supplementation alone but not vitamin supplementation produced normal development. Use of serum from cows that were supplemented with rumen-protected methionine also produced normal embryo development. Thus, bovine serum has such low methionine concentrations that normal development of rat embryos is retarded.

The requirements for complete development of bovine embryos have not yet been determined. Current culture conditions allow development of bovine embryos to the blastocyst stage (day 7 to 8) and even allow hatching of a percentage of embryos (day 9); however, conditions have not been developed in vitro that allow elongation of embryos. The methionine requirements for cultured pre-implantation bovine embryos (day 7to 8) was determined in studies from University of Florida (Bonilla et al., 2010). There was a surprisingly low methionine requirement (7 µM) for development of embryos to the blastocyst stage by day 7; however, development to the advanced blastocyst stage by day 7 appeared to be optimized at around 21 µM (Bonilla et al., 2010). Thus, the results of these studies indicated that development of morphologically normal bovine embryos did not require elevated methionine concentrations (> 21 µM), at least during the first week after fertilization.

Ikeda et al. (2012) evaluated whether methionine metabolism was required for normal development of bovine embryos. The researchers added ethionine or additional methionine to cultures of bovine embryos. Ethionine blocks metabolism of methionine into the one-carbon pathway (termed antimetabolite of methionine). Ethionine did not block development to the morula stage but blocked development to the blastocyst stage (Control = 38.5%; Ethionine = 1.5%). Development to the blastocyst stage in the presence of ethionine was partially restored by adding S-adenosylmethionine (SAM), which would restore the methylation pathway but not restore protein synthesis. Thus, methionine has an essential role in the development of the bovine embryo from morula to blastocyst that is probably partially mediated by hypomethylation in the absence of sufficient methionine.

Souza et al. (2012a,b) evaluated the effect of supplementation with rumen-protected methionine on early embryo development in super-ovulated cows. Super ovulation increased the number of embryos available and thus the statistical power to test the in vivo effects of methionine supplementation on early embryo development in lactating dairy cows. In this experiment, animals were blocked by parity and calving date and randomly assigned to 2 treatments differing in level of dietary methionine supplementation: 1) methionine (MET); diet composed of (% DM) corn silage (39.7), alfalfa silage (21.8), HMSC (17.2), roasted soybeans (8.6), grass hay (4.6), canola meal (4.0), mineral-vitamin mix (2.7) and ProVAAL Ultra (w/Smartamine®, 1.4), formulated to deliver 2,875 g MP with 6.8 Lys % MP and 2.43 Met % MP; 2) control (CON); cows fed the same basal diet but replacing ProVAAl Ultra by ProVAAL Advantage (no added Smartamine®), formulated to deliver 2,875 g MP with 6.8% Lys (% MP) and 1.89% Met (% MP). There was an increase in both kg of milk protein produced and percentage of protein in the milk (Souza et al., 2012b). Thus, from a milk protein synthesis standpoint, methionine was concluded to be the first limiting AA. A large significant effect of feeding the rumen-protected methionine on circulating methionine concentrations (control = 16.8 μM vs. Met-supplemented = 22.9 μM) was observed.

Even though methionine supplementation during the later stages of follicle development and early embryo development may not have produced morphological changes in the early embryo, it is well known that methionine during this time can have effects on the epigenome of the embryo (Sinclair et al., 2007). This means that the genes can be changed in such a way that they are not expressed in the same way due to addition of groups, generally methyl groups, to the DNA of the cells. To test this hypothesis, Penagaricano et al. (2013) evaluated whether embryos that were recovered from cows that had been supplemented or not supplemented with methionine had differences in gene expression. The objective was to evaluate the effect of maternal methionine supplementation on the transcriptome of bovine pre-implantation embryos. Only high quality embryos from individual cows were pooled and then analyzed by a powerful technique that allows evaluation of all genes that are expressed in these embryos, called RNA sequencing. Remarkably, the small difference in circulating methionine produced a substantial difference in expression of genes in the embryo. Methionine supplementation seemed to change gene expression in a way that may lead to improved pregnancy outcomes and improved physiology of the offspring.

Researchers from the same laboratory at the University of Wisconsin conducted a trial with a total of 309 cows (138 primiparous and 171 multiparous) that were blocked by parity and randomly assigned to 2 treatments; 1) CON: Cows fed a ration formulated to deliver 2,500 g of MP with 6.9% Lys (% MP) and 1.9% Met (% MP) and 2) RPM: Cows fed a ration formulated to deliver 2,500 g of MP with 6.9% Lys (% MP) and 2.3% Met (% MP). Cows were randomly assigned to 3 pens with headlocks and fed a single basal TMR twice daily. From 28 to 128 DIM, after the AM milking, cows were head-locked for 30 minutes, and the TMR of CON and RPM cows were individually top dressed with 50 g of DDG or 50 g of a mix of DDG (29 g) and Smartamine M (21 g) respectively. Following a double ovsynch protocol, cows were inseminated and pregnancy checked at 28 days (plasma pregnancy specific protein-B concentration), and at 32, 47, and 61 days (ultrasound). Individual milk samples were taken once a month and analyzed for composition. There were no statistical differences in milk production, but RPM cows had a higher milk protein concentration. Cows fed the methionine-enriched diet had a lower pregnancy loss from 21 to 61 days after AI (16.7% RPM cows vs. 10.0% from CON cows). Pregnancy losses between days 28 and 61 were not different in the primiparous cows (12.8% CON and 14.6% RPM); however, pregnancy losses between treatments were significant for the multiparous cows (19.6% CON vs. 6.1% RPM; Toledo et al., 2015).

CONCLUSIONS

The elevated concentration of Met, His, and Lys in the uterine fluid of pregnant cows near the time of embryo elongation suggests that elevated amounts of these AA may be critical for this important stage of embryo development. Supplementation of cows with methionine during the final stages of follicular development and early embryo development, until day 7 after breeding, led to lipid accumulation changes in the embryos and resulted in differences in gene expression in the embryo. Methionine supplementation seems to impact the preimplantation embryo in a way that enhances its capacity for survival, because there is strong evidence that endogenous lipid reserves serve as an energy substrate. Lower pregnancy losses from cows fed a methionine-enriched diet suggest that methionine favors embryo survival, at least in multiparous cows. Further studies are needed to corroborate whether supplementation with methionine would have a beneficial impact on embryo survival and if these changes in the early embryo translate into changes in pregnancy outcomes or physiology of the resulting calf.

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