

Rumen-protected methionine supplementation during subclinical mastitis challenge benefit dairy cow inflammation and immune cell mTOR pathway.

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The objective of this study was to evaluate the effects of rumen-protected methionine (Smartamine M, Adisseo Inc., France) on inflammation and immune response of lactating cows during a subclinical mastitis challenge (SMC). Thirty-two Holstein dairy cows (145 ± 51 DIM) were enrolled in a randomized complete block design. At -21 d relative to SMC, cows were assigned to dietary treatments, and data were collected from 0 to 3 d relative to SMC. Cows were blocked according to parity, DIM, and milk yield. Cows received a basal diet (17.4% CP; 7.01% and 2.14% of MP as Lys and Met, respectively) supplemented with 100 g/d of ground corn (CON; n = 16) or Smartamine M (SM, 0.09% DM; n = 16). At 0 d, mammary gland rear right quarter was infused with 500,000 cfu of *Strep. uberis* (O140J). Blood samples were collected at 0, 6, 12, 24, 48, and 72 h relative to SMC for inflammation and liver function biomarkers, and blood Ca. Blood samples were also collected at 0, 12, and 24 h, and milk samples at 24 h relative to SMC for ratio of phosphorylated to total AKT, S6RP, and 4EBP1, in neutrophils (PMN), monocytes, and total leukocytes via flow cytometry. Data were analyzed using PROC MIXED of SAS. Significance was declared at $P \leq 0.05$ and trends at $P \leq 0.10$. Inflammation biomarkers showed lower ($P < 0.01$) ceruloplasmin levels for SM than CON cows. A trt × time interaction ($P = 0.05$) for blood Ca showed a trend ($P = 0.08$) for lower Ca in SM cows at 12 h post-SMC. An increased phosphorylation state of AKT, S6RP, and 4EBP1 proteins suggests an activation of the mTOR pathway. We observed greater ($P = 0.02$) p4EBP1:4EBP1 ratio in blood PMN of SM cows than CON. pAKT:AKT was greater ($P = 0.04$) in milk PMN from SM cows than CON, while a trend ($P = 0.08$) for greater milk total leukocytes in SM cows than CON was observed. SM cows had greater ($P < 0.05$) pS6RP:S6RP ratio in milk PMN and monocytes. Overall, results suggest that methionine supplementation during a SMC may attenuate inflammation while potentially enhancing protein synthesis through mTOR pathway. However, effects on inflammation need further confirmation.

Keywords: immune response, rumen-protected methionine, *Streptococcus uberis*.