

Short title: **Fatty acids decreased β -casein content**

High levels of fatty acids inhibit β -casein synthesis through suppression of the JAK2/STAT5 and mTOR signaling pathways in mammary epithelial cells of cows with clinical ketosis

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Abstract

Ketosis is a metabolic disease of dairy cows often characterized by high concentrations of ketone bodies and fatty acids, but low milk protein and milk production. The Janus kinase 2 (JAK2)-signal transducer and activator of transcription 5 (STAT5) and the mechanistic target of rapamycin (mTOR) signaling pathways are central for the regulation of milk protein synthesis. The effect of high levels of fatty acids on these pathways and β -casein synthesis are unknown in dairy cows with clinical ketosis. Mammary gland tissue and blood samples were collected from healthy ($n = 15$) and clinically-ketotic ($n = 15$) cows. In addition, bovine mammary epithelial cells (BMEC) were treated with fatty acids, methionine (Met) or prolactin (PRL), respectively. In vivo, the serum concentration of fatty acids was greater ($P > 0.05$) and the percentage of milk protein ($P > 0.05$) was lower in cows with clinical ketosis. The JAK2-STAT5 and mTOR signaling pathways were inhibited and the abundance of β -casein was lower in mammary tissue of cows with clinical ketosis ($P > 0.05$). In vitro, high levels of fatty acids inhibit the JAK2-STAT5 and mTOR signaling pathways ($P > 0.05$) and further decrease the β -casein synthesis ($P > 0.05$) in cow mammary epithelial cells. Methionine or PRL treatment, as positive regulators, activate the JAK2-STAT5 and mTOR signaling pathways to increase the β -casein synthesis. Importantly, the high concentration of fatty acids attenuated the positive effect of Met or PRL on mTOR, JAK2-STAT5 pathways and the abundance of β -casein ($P > 0.05$). Overall, these data indicate that the high concentrations of fatty acids that reach the mammary cells during clinical ketosis inhibit mTOR and JAK2-STAT5 signaling pathways, and further suppress β -casein synthesis.