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

Xin Shu, Zhiyuan Fang, Yuan Guan, Xiying Chen, Juan J. Loor, Hongdou Jia, Jihong Dong, Yazhe Wang, Rankun Zuo, Guowen Liu, Xiaobing Li and Xinwei Li

SUPPLEMENTARY FILE

Supplementary Table S1. General features of cows used in the study

	Clinical ketosis (n = 15)		Control (n = 15)		*P*
	Median	IQR	Median	IQR	
Body weight (kg)	624	601, 645	631	615, 652	0.7314
Milk production (kg of milk/cow per day)	24.6	22.8, 27.7	30.8	29.2, 33.6	0.0414
Milk fat (kg /per day)	1.28	1.13, 1.46	0.86	0.71, 1.01	0.0413
Milk protein (kg /per day)	0.604	0.533, 0.742	1.08	0.92, 1.22	0.0183
Milk lactose (kg /per day)	1.15	1.03, 1.28	1.20	1.12, 1.30	0.4821
Serum glucose (mM)	1.96	1.83, 2.14	4.04	3.91, 4.15	0.0048
Serum fatty acids (mM)	1.23	1.10, 1.34	0.28	0.20, 0.34	0.0089
Serum BHB (mM)	3.88	3.63, 4.22	0.43	0.37, 0.50	0.0072

The average value of data over the sampling period; IQR: interquartile range; DM, dry matter; DMI, dry matter intake; BHB, β-hydroxybutyrate.

Supplementary Table S2. Ingredient and nutrient composition of the diets.

Ingredient (%)	
Corn silage	40.00
Corn	35.00
Wheat bran	8.00
Soybean meal	5.00
Sunflower	8.00
NaCl	1.00
Premix*	1.80
$NaHCO_3$	1.20
Total	100.00
Nutrient composition (% of DM)	
NE_L (MJ/Kg)	6.70 ± 0.64
CP	15.20 ± 1.38
NDF	33.45 ± 3.22
ADF	17.20 ± 1.52
NFC	40.40 ± 3.78
Ca	0.70 ± 0.62
P	0.50 ± 0.45

*One kg of premix contained the following: Vitamin A 200,000 IU, Vitamin D 70,000 IU, Vitamin E 1,000 IU, Fe 2,000 mg, Cu 600 mg, Zn 2,400 mg, Mn 1,300 mg, I 6 mg, Co 7 mg.

DM, Dry Matter; NEL, Net energy for lactation; CP, Crude protein; NDF, Neutral detergent fibre;

ADF, Acid detergent fibre; NFC, Non-fibre carbohydrate. The data presented are the mean ± SEM.

Supplementary Table S3. The primer sequences of the genes.

Gene	Primer sequences（5’-3’）	Length（bp）
β-casein	F AATCTTCATTTGCCTCCTCTCTTG	209
	R ACAGGACCGAGTACAGGCT	
S6K1	F GGACATGGCAGGGGTGTTT	283
	R GGTATTTGCTCCTGTTACTTTTCG	
4EBP1	F GAACTCACCTGTGACCAAGA	157
	R CTCAAACTGTGACTCTTCACC	
STAT5	F AAGACCCAGACCAAGTTCGC	422
	R AGCACCGTGGCAGTAGCAT	
GAPDH	F TCTTCACTACCATGGAGAAGG	96
	R TCATGGATGACCTTGGCCAG	
Ubiquitin	F GATCCAGCATAAGGAAGGCAT	97
	R GCTCCACCTCCAGGGTGAT	
β-actin	F GCCCTGAGGCTCTCTTCCA R GCGGATGTCGACGTCACA	101

Supplementary Table S4. Antibodies used for Western Blotting and Immunofluorescence assay.

Name	Dilution	Item No.	Company	City/Country
β-casein	1:100	bs-10032R	Bioss	Beijing, China
mTOR	1:1,000	ab2833	Abcam	Cambridge, UK
p-mTOR	1:1,000	ab84400	Abcam	Cambridge, UK
S6K1	1:250	ab9366	Abcam	Cambridge, UK
p-S6K1	1:500	ab131436	Abcam	Cambridge, UK
JAK2	1:1,000	4040	CST	Danvers, USA
p-JAK2	1:1,000	3771	CST	Danvers, USA
STAT5	1:1,000	ab68465	Abcam	Cambridge, UK
p-STAT5	1:500	4322T	CST	Danvers, USA
β-actin	1:1,000	sc-47778	Santa Cruz	USA
CK-18	1:250	bs-2043R	Bioss	Beijing, China