

Supplementary Material

# Beta-Sitosterol Promotes Milk Protein and Fat Syntheses-Related Genes in Bovine Mammary Epithelial Cells

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**Table S1.** Bos taurus (cattle) primers used for qPCR.

Gene Name <sup>1</sup>	Accession Number	Primers Sequence	Product Size, bp
$\beta$ -actin	NM_173979.3	F: 5'-CCCTGGAGAAGAGCTACGAG-3' R: 5'-GTAGTTTCGTGAATGCCGCAG-3'	130
$\beta$ -casein	XM_015471671.2	F: 5'-GGCTATGGCTCCTAAGCACA-3' R: 5'-GTCAGGCTCTGCCTTTCAGT-3'	78
JAK2	XM_005209981.4	F: 5'-CAAGACCAGATGGATGCCCAG-3' R: 5'-ACTCGAACTGCTAGGTCTCTGA-3'	103
STAT5	NM_174617.4	F: 5'-GAGAACACCCGCAATGATTAC-3' R: 5'-TCACCGACTCTGCTCCACG-3'	151
ELF5	NM_001024569.1	F: 5'-CATCCGCTCACAAGGTTACTC-3' R: 5'-CTCGCACAAATTCCTCATAGAT-3'	170
PI3K	NM_001206047.1	F: 5'-GTCTGGACCTTCGGATGCTAC-3' R: 5'-TAAACTCCTCAATGGCTCGGT-3'	213
AKT1	NM_173986.2	F: 5'-GCACAAGCGAGGTGAGTACAT-3' R: 5'-GCCACGGAGAAGTTGTTGAG-3'	138
mTOR	XM_002694043.6	F: 5'-CGAAGAACCAATTATACCCGC-3' R: 5'-CATAGCAACCTCAAAGCAGTCC-3'	153
S6K1	NM_205816.1	F: 5'-AATGCTGCTTCTCGTCTTGGA-3' R: 5'-CAGTTCTTCCAGTTAATATGTCT-3'	90
4EBP1	NM_001077893	F: 5'-TCACTAGCCCTACAGGCGAT-3' R: 5'-AAACTGTGACTCTTCACCGC-3'	101
eIF4E	NM_174310.3	F: 5'-CCCGCCTACAGAAGAAGAGA-3' R: 5'-CAGTATCAAACCTTAGAGATCAATCG-3'	164
ACC	NM_174224.2	F: 5'-GGAGACAAACAGGGACCATTAC-3' R: 5'-GTGGAAGGAATGCTTGGGAG-3'	187
FASN	NM_001012669.1	F: 5'-GACCTGGGAGGAGTGTAAGC-3' R: 5'-GCGATAGCGTCCATGAAGTA-3'	198
SCD	NM_173959.4	F: 5'-CCACGTTCTTCATTGATTGC-3' R: 5'-CAGCCACTCTTGTAAGCTTTCC-3'	121
LPL	NM_001075120.1	F: 5'-TCACTTCAACCACAGCAGCA-3' R: 5'-GATGACGTTGGAGTCCGGTT-3'	127
SREBP1	NM_001113302.1	F: 5'-CGCTCTTCCATCAATGACA-3' R: 5'-TTCAGCGATTGCTTTTGTG-3'	188
PSMA5	NM_001015566.1	F: 5'-CATGAGTGGGCTAATTGCTG-3' R: 5'-AGCCTGAGTCACACTCTCCA-3'	115
GH1	NM_180996.1	F: 5'-GCAGATCCTCAAGCAGACCT-3' R: 5'-CAGGAGAGCAGACCGTAGTT-3'	87
GHR	NM_176608.1	F: 5'-AGACCACTTCTCATTGGTGA-3' R: 5'-ATGTCGCTTACCTGGGCATA-3'	110
IGF-1	NM_001077828.1	F: 5'-CCATCACATCCTCCTCGCAT-3' R: 5'-ATAAAGCCCTGTCTCCGC-3'	143
IGF-1R	NM_001244612.1	F: 5'-CACGAGTGGAGAAATCTGCG-3' R: 5'-ATGTGGAGGTAGCCCTCGAT-3'	102
IGFBP3	NM_174556.1	F: 5'-AGCGTGAGACAGAATACGGG-3' R: 5'-AGCCCTTCTTGTCGCAGTTG-3'	120
HIF-1 $\alpha$	NM_174339.3	F: 5'-TTCCATCTCCTCCCCACGTA-3' R: 5'-AGGCTGTCCGACTTCCAGTA-3'	81
EPO	NM_173909.2	F: 5'-TCTCCCTTCCAGATGCAACC-3' R: 5'-CTTCCCCCGCAGGAAATTGG-3'	104
EPOR	NM_001205601.1	F: 5'-CTCGTCCTCGTGCTCATTCT-3'	117

		R:5'-GCCTTCAAACCTCGCTCTCAG-3'	
		F: 5'-GGGAACCTCAGTCACACAGGTTGG-3'	
SOCS2	NM_177523.2	R:5'-TGTTAGTAGGTAGTCTGAATGCGAAC-3'	128
		F: 5'-AGAAGATCCCTCTGGTGTGAGC-3'	
SOCS3	NM_174466.2	R:5'-GTGACTTTCTCGTAGGAGTCCAGG-3'	109

<sup>1</sup> *JAK2* = Janus kinase 2; *STAT5* = Signal transducer activator of transcription 5; *mTOR* = Mammalian target of rapamycin; *S6K1* = Ribosomal protein S6 kinase beta-1; *SREBP1* = Sterol regulatory element-binding protein 1; *PPAR $\gamma$*  = Peroxisome proliferator-activated receptor  $\gamma$ ; *ACC* = Acetyl-CoA carboxylase; *FASN* = Fatty acid synthase; *LPL* = Lipoprotein lipase; *SCD* = Stearyl CoA desaturase; *GH* = Growth hormone; *IGF-1* = Insulin-like growth factor-I; *HIF-1 $\alpha$*  = Hypoxia-inducible factor-1 $\alpha$ ; *SOCS* = Suppressors of cytokine signaling; *PI3K* = Phosphoinositide 3-kinase; *AKT1* = RAC-alpha serine/threonine-protein kinase; *IGFBPs* = Insulin-like growth factor binding protein; *IGF-1R* = Type 1 insulin-like growth factor receptor; *ELF5* = E74-like factor 5; *4EBP1* = Eukaryotic translation initiation factor 4E binding protein 1; *eIF-4E* = Eukaryotic initiation factor 4E; *PSMA5* = Proteasome 20s subunit  $\alpha$ 5; *GHR* = Growth hormone receptor; *EPO* = Erythropoietin; *EPOR* = Erythropoietin receptor.

### Materials and Methods:

HiFi Script cDNA Synthesis Kit (CWBIO, Beijing, China) according to the manufacturer's instructions. The reaction was carried out in a volume of 20  $\mu$ L containing 4  $\mu$ L of dNTP mix, 2  $\mu$ L of primer mix, 4  $\mu$ L of 5  $\times$  RT buffer, 2  $\mu$ L of DTT, 1  $\mu$ L of HiFi Script, and 1  $\mu$ g of the RNA template, and the cDNA was synthesized by incubating the reaction mixture at 42 °C for 15 min followed by 85 °C for 5 min.

**Table S2.** Antibodies used for Western Blot.

Antibodies name	Diluted multiples	Accession Number	Reagent company
rabbit anti- $\beta$ -actin polyclonal antibody	1:2000	bs-0061R	Bioss
rabbit anti- $\beta$ -casein polyclonal antibody	1:2000	bs-10032R	Bioss
rabbit anti-phospho-STAT5- $\beta$ polyclonal antibody	1:2000	bs-5703R	Bioss
rabbit anti-mTOR polyclonal antibody	1:2000	bs-1992R	Bioss
rabbit anti-phospho-mTOR polyclonal antibody	1:2000	bs-3494R	Bioss
rabbit anti-S6K1 polyclonal antibody	1:2000	bs-6370R	Bioss
rabbit anti-phospho-S6K1 polyclonal antibody	1:2000	bs-5668R	Bioss
rabbit anti-SCD polyclonal antibody	1:2000	bs-3787R	Bioss
mouse anti-PSMA5 polyclonal antibody	1:2000	bsm-51520M	Bioss
rabbit anti-SREBP1 polyclonal antibody	1:2000	bs-1402R	Bioss
rabbit anti-PPAR $\gamma$ polyclonal antibody	1:2000	bs-0530R	Bioss
rabbit anti-HIF-1 $\alpha$ polyclonal antibody	1:2000	bs-0737R	Bioss
rabbit anti-SOCS2 polyclonal antibody	1:2000	bs-1896R	Bioss
rabbit anti-SOCS3 polyclonal antibody	1:2000	bs-24250R	Bioss
goat anti-rabbit IgG antibody	1:4000	ab6721	abcam
rabbit anti-mouse IgG antibody	1:4000	ab6728	abcam