**Leucine regulates α-amylase and trypsin synthesis in dairy calf pancreatic tissue *in vitro* via the mammalian target of rapamycin signalling pathway(*Animal* Journal)**

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Short title: Leucine regulates amylase and trypsin synthesis

**Supplementary Table S1** *Chemical composition of the calf starter feed (dry matter basis)*

|  |  |
| --- | --- |
| Items | Content (%) |
| Dry Matter | 87.06 |
| Crude Protein | 20.01 |
| Crude ash | 15.47 |
| Starch | 38.79 |
| Neutral detergent fiber | 12.20 |
| Acid detergent fiber | 6.20 |
| Calcium | 0.70 |
| Total Phosphorus | 0.38 |

**Supplementary Table S2** *Concentration (g/L) of amino acids in the milk fed to Holstein dairy calves*

|  |  |  |  |
| --- | --- | --- | --- |
| Items | Concentration1 | Items | Concentration1 |
| Arginine | 1.429±0.091 | Alanine | 1.356±0.102 |
| Histidine | 0.920±0.055 | Aspartate | 2.869±0.154 |
| Isoleucine | 1.744±0.230 | Cysteine | 0.358±0.040 |
| Leucine | 3.432±0.264 | Glutamate | 7.605±0.422 |
| Lysine | 3.055±0.144 | Tyrosine | 1.846±0.139 |
| Methionine | 0.950±0.142 | Glycine | 0.838±0.029 |
| Phenylalanine | 1.731±0.098 | Serine | 2.260±0.074 |
| Threonine | 1.804±0.059 | Proline | 6.458±0.307 |
| Valine | 2.372±0.149 |  |  |

1 Data present as mean ± SD.

**Supplementary Table S3** *The primer sequences of pancreatic digestive enzymes gene of the Holstein dairy calves*

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Primer | Reference Sequence | Sequence(5'→3') | Base Number | Annealing (℃) |
| Amylase-F | NM\_001035016 | GAAATGGCCGTGTGACAGAATTTA | 24 | 64.3 |
| Amylase-R | ACAAAGACAAGTGCCCTGTCAGAA | 24 |
| Trypsin-F | NM\_001113727 | TGTCTGCGGCTCACTGCTAC | 20 | 62.7 |
| Trypsin-R | GCTGGGATGGACGATACTCTTG | 22 |
| Lipase-F | NM\_001205820 | GTGGAAGCAAATGATGGACAAG | 22 | 61.8 |
| Lipase-R | TGGGTTGAGGGTGAGCAGA | 19 |
| Chymotrypsin-F | NM\_001098965 | CTGTGTGGATAGCCAAATGACC | 22 | 60.5 |
| Chymotrypsin-R | TGTAGCAGGGCGTGTTGTTAG | 21 |
| 18S rRNA-F | NR\_036642 | ACCCATTCGAACGTCTGCCCTATT | 24 | 61.2 |
| 18S rRNA-R | TCCTTGGATGTGGTAGCCGTTTCT | 24 |

**Supplementary material S1**

*Electrophoresis procedure and western-blot images description*

We used a pre-stained marker (Precision Plus ProteinTM Dual Color Standards, Catalogue No. 1610374, Bio-Rad, USA) as a marker of protein molecule weight. When the proteins transferred to nitrocellulose membrane (Pall Corp., USA), we cut the membranes according to the molecular weight of the target protein. The remaining binding sights on the blot were blocked (catalogue no. 37542, Thermo Scientific, USA) and each blot was incubated overnight with a polyclonal antibody recognizing the phosphorylated or total form of target proteins in blocking buffer (1:1 000, v:v). Blots were subsequently washed three times (5 min each) in TBS containing 1% Tween-20 (Bio-Rad, USA), incubated for 1 h with the secondary antibodies in blocking buffer (1:3 000), washed three times (5 min each), and scanned with a Bio-Rad Life Science Imaging system (Bio-Rad, USA). The band intensities were quantified using the Bio-Rad application software. The β-actin (Catalogue No. CW0096M, CWBIO, China) was used as the housekeeping protein. Western blots were developed and quantified using ImageJ software (National Institutes of Health, USA). The protein level was quantified by normalizing total or phosphorylated proteins with β-actin. For the target protein expression analysis, the normalized target protein expression level for each sample was compared with the positive control sample.