**Postnatal differential expression of chemoreceptors of free fatty acids along the gastrointestinal tract of supplemental feeding versus grazing kid goats**

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**Animal Supplementary Material S1**

Protein isolation and Western Blot analysis

Mucosal samples (0.2 g each) were frozen in liquid nitrogen, crushed into powder, and dissolved in 300 μL RIPA lysate (Applygen Technologies, Beijing, China) with 1% protease inhibitor cocktail (Roche Diagnostics GmbH, Mannheim, Germany), followed by 30 min of cleavage on ice. Then, samples were centrifuged at 12,000 × g for 15 min at 4°C, and the supernatant was taken. Protein concentrations were measured utilizing a BCA Protein Assay kit (Hin biotech, Beijing, China). Exact amounts of protein needed for each sample were calculated and mixed with 5 × loading buffer, incubated at 95°C for 5 min and stored at -20°C.

Equal amounts of protein of each sample and pre-stained standards (Bio-Rad Laboratories) were separated by electrophoresis in 10% SDS-polyacrylamide gels. Separated proteins were transferred onto polyvinylidene difluoride (PVDF) membranes (Bio-Rad, USA) under constant electric current of 200 mA for 70 min. The PVDF membranes were then incubated with 5% skim-milk in TBST buffer [10 mL 1 mol/L Tris·HCl (pH = 7.5) and 8.8 g NaCl dissolved in 1000 ml distilled water, containing 0.2% Tween20] for 1 h to suppress non-specific binding of immunoglobulins. The pre-blocked membranes were incubated with the first antibody (diluted 1:200 in 1 × TBST) at 4°C overnight, washed in 1 × TBST for 3 times (15 min each), and then incubated with horseradish-peroxidase (HRP)-labeled secondary antibody (1:3,000 diluted in 1 × TBST) (Proteintech Treatment, Inc., Chicago, USA) for 1 h at room temperature, washed in 1 × TBST for 3 times (10 min each). Bands of target proteins were detected by WesternBright ECL Western Blotting HRP Substrate (APGBio, Shanghai, China), and the images were taken and analyzed by AlphaImager 2200 digital imaging system (Digital Imaging System, Kirchheim, Germany).