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Expression of genes related to sweet taste receptors and monosaccharides transporters along the gastrointestinal tracts at different development stages in goats

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ABSTRACT

In non-ruminants, sweet taste receptor and monosaccharide transporters are important components of nutrient chemosensing in the intestinal tracts. Ruminants, however, have evolved a polygastric digestive system linked to a transition in nutrient supply from readily-digestible carbohydrate (lactose) in milk during the pre-ruminant suckling phase to poorly-digestible structural carbohydrates when the rumen becomes functional. Whether these developmental and feed changes alter the mRNA expression of genes related to monosaccharide sensing (Taste receptor family 1 member 2, T1R2; Taste receptor family 1 member 3, T1R3) and transporting (monosaccharide transporters Na+/glucose co-transporter, SGLT1; glucose activated ion channel, SGLT3; solute carrier family 2 member 5, GLUT5; solute carrier family 2 member 2, GLUT2) are unknown. This study investigates the expression of sweet taste receptors (T1R2 and T1R3) and monosaccharides transporters (SGLT1, SGLT3, GLUT5 and GLUT2) along the gastrointestinal tracts (GIT) during different stages of development (suckling, weaning and grazing) in goats. The results showed that the expression of T1R2 decreased with age, and was mainly expressed in the duodenum and jejunum. Although T1R3 expression in different GIT segments fluctuated during growth, there was an increase with age, with the abomasum showing the greatest expression. Both SGLT1 and SGLT3 were mainly expressed in the jejunum at all ages, with the greatest expression in the middle jejunum during suckling, and decreased greatly ($P<0.05$) during weaning and grazing. GLUT5 was mainly expressed at the duodenum and jejunum, with differences ($P<0.05$) between suckling and the later developmental stages. No expression of GLUT5 was detected at the rumen and abomasum at any stage, but was noted in the ileum, cecum, colon and rectum during suckling, but with lower abundances during weaning and grazing. The expression of GLUT2 was detected only in the small intestine and decreased with age. The expression of T1R2 correlated ($P<0.05$) with SGLT1, SGLT3, GLUT5 and GLUT2. There were correlations ($P<0.01$) between the expressions of SGLT3 and SGLT1, as well as between SGLT3 and GLUT5. The current results indicate that: (1) the ability of the GIT of goats to absorb monosaccharides varies during development, being greatest at the suckling stage; (2) the duodenum and jejunum play a critical role in sensing and absorption of monosaccharides; (3) the mRNA expressions of sweet taste receptors and various transporters correlated well in the GIT of goats.

1. Introduction

Mammals have the ability to sense nutrient composition of ingested feeds, called 'gut nutrient chemosensing', and this plays a crucial role in controlling multiple functions during digestion, such as regulation of nutrient absorption and gut hormone secretion,

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and initiation of neural responses (Nguyen et al., 2012; Rasoamanana et al., 2012; Reimann et al., 2012). Monosaccharides sensing is of particular importance in gut nutrient chemosensing, as it provides mammals a means to identify the required energy within ingested feedstuffs. Digestible carbohydrates in ingested feedstuffs are degraded to monosaccharides (glucose, galactose and fructose) before absorption. In both non-ruminants and ruminants, monosaccharides have a sweet taste that can be detected by sweet taste receptor heterodimer T1R2/T1R3 expressed in the GIT (Nelson et al., 2001; Moran et al., 2014; Ran, 2014). After having been sensed by T1R2/T1R3, monosaccharides are transported into enterocytes by transporters (SGLT1 for glucose and galactose, GLUT5 for fructose), subsequently transporting across the basolateral membrane into the circulation via GLUT2 (Shirazi-Beechey, 1995; Kellett et al., 2008). The SGLT3, closely related to SGLT1, has been associated in the detection of monosaccharides in the intestinal mucosa (Diez-Sampedro et al., 2003; Freeman et al., 2006). During growth, ruminants face a transition in food supply from milk during the pre-ruminant suckling phase to poorly-digestible structural carbohydrates when the rumen is fully functional. Whether these developmental and feed changes alter the mRNA expression of genes related to monosaccharide sensing and transport are unknown. Therefore, the current study was designed to assay the mRNA expression of the sweet taste receptor subunits (T1R2 and T1R3) and monosaccharide transporters (SGLT1, SGLT3, GLUT5 and GLUT2) in different segments of the GIT during suckling, weaning and grazing stages in goats, with the objective of exploring their distribution and developmental changes.

2. Materials and methods

The use of animals, including welfare, husbandry and experimental procedures used for this study, was approved by the Animal Care Committee, Institute of Subtropical Agriculture, the Chinese Academy of Sciences, Changsha, China.

2.1. Experimental animal and sampling

Twenty eight newly born male and female Liuyang black goat kids (a local breed in the south of China) with similar birth weight (1.35 ± 0.12 kg) and age were used as experimental animals in this study. The total experiment lasted 70 days, separated by suckling (d 0–14), weaning (d 15–42) and grazing (d 43–70) stages. During suckling, the kids were nurtured by the ewes and had free access to nanny milk. Four kids were killed for sample collection at each of d 0, 7 and 14. During weaning, the remaining 16 kids were weaned off milk progressively and allowed to graze fresh pasture from d 20 after birth. Four kids were killed for sampling on each of d 28 and 42. During grazing, the remaining kids were only fed with fresh pasture from d 43, and 4 kids were killed for sampling on each of d 56 and 70. Mucosa samples of the rumen, abomasum, duodenum, jejunum (i.e., the proximal, middle and distal segments), ileum (i.e., the proximal, middle and distal segments), cecum, colon (i.e., the proximal and distal segments) and rectum were collected immediately after slaughter. Collected samples were quickly wrapped with sterilized tinfoil and frozen in liquid nitrogen and stored at $-80\,^{\circ}C$ until RNA extraction.

2.2. RNA extraction and cDNA preparation

Total RNA was extracted from each sample using TRIZOL (Invitrogen, California, USA) under the manufacturer's instructions. After genomic DNA was eliminated by digestion with DNase I (Thermo Scientific, Waltham, USA), the RNA quality and quantity was determined using NanoDrop 2000 (Thermo Scientific, Waltham, USA), all RNA samples showed an A260/A280 values within the range of 2.01–2.06 and A260/ A230 values above 2. And the integrity of collected RNA were analyzed with gel electrophoresis. Afterwards, 1 μg of the extracted RNA was reverse-transcribed to synthesize tissue specific cDNA using PrimeScript™ RT reagent Kit (Takara, Dalian, China) immediately. The reverse transcription procedures were conducted according to the modified steps by Ran et al. (2016). The prepared cDNA samples were further purified, quantified and diluted to the same initial concentration, and stored at $-20\,^{\circ}C$ until subsequent quantitative real-time PCR analysis.

2.3. Quantitative real-time PCR analysis

Quantitative real-time PCR was used to estimate the relative mRNA expression for interested genes. Primers used for amplification target genes (T1R2, T1R3, SGLT1, SGLT3, GLUT5 and GLUT2) were designed using the Primer-Primer 5 and online tools Primer3 (http://simgene.com/Primer3) based on the obtained sequences in our previous study (Ran, 2014), β-Actin and 18s-RNA (Fajardo et al., 2008) were selected as reference genes to normalize the expression of target genes. All primers were synthesized by Sangon Biotech (Sangon Biotech, Shanghai, China), and the primer sequences are given in Table 1. The specificity of designed primers was checked via online Primer-BLAST (NCBI) and subsequent gel electrophoresis analyzing, cloning and sequencing of PCR products of designed primers, as well as melt curve analyze during quantitative real-time PCR. The quantitative real-time PCR was performed on an ABI-7900HT qPCR system (Applied Biosystems, Foster City, CA, USA) using FG POWER SYBR GEREEN PCR MASTER MIX (Applied Biosystems, Foster City, CA, USA). Quantification of the PCR products of all interested genes was evaluated in comparison with the PCR products of β-actin and 18s-RNA. The relative changes in mRNA expression levels were calculated according to the $2^{-\Delta\Delta CT}$ method, where $-\Delta\Delta CT = -(\Delta CT_{\text{other tissue samples}} - \Delta CT_{\text{duodenal sample at d 0}})$ and $\Delta CT = CT_{\text{samples}} - (CT_{\beta\text{-actin}} + CT_{\text{18s-RNA}})/2$ (Livak and Schmittgen, 2001).

2.4. Statistical analysis

SPSS Statistical Software was used for statistical analysis. Quantitative real time PCR data were analyzed using one-way ANOVA followed by the Turkey-Kramer multiple comparison tests. Data were expressed as mean ± SEM. Regression analysis based on the Pearson coefficient was conducted to analyze correlations between any two for the expression levels of T1R2, T1R3, SGLT1, SGLT3, GLUT5 and GLUT2 in the duodenum and jejunum (proximal, middle and distal segments), taking all individuals at three stages into consideration. Statistical significance was defined as $P < 0.05$.

3. Results

3.1. T1R2 relative expression at different developing stages

Data in Table 2 showed the T1R2 expression in different GIT segments at various development stages (suckling, weaning and grazing). The T1R2 was expressed throughout the GIT during the entire experimental period, but decreased ($P \leq 0.001$) with age except for the distal colon and rectum. In detail, T1R2 was highly expressed during the suckling stage, had relatively low expression during weaning, and decreased to an extremely low level during grazing. This was especially noticeable in high T1R2 expression tissues (e.g., duodenum and jejunal middle segment).