Expression of the \textit{Tas1r3} and \textit{Pept1} genes in the digestive tract of wagyu cattle

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ABSTRACT: Animals have precise recognition systems for amino acids and peptides that regulate their feeding behavior as well as metabolic responses. Because of their particular gastrointestinal structure, ruminants are expected to have unique mechanisms of amino acid regulation in the digestive tract. To better understand these mechanisms in the ruminant digestive tract, the expression of \textit{Tas1r3} and \textit{Pept1} was studied along the gastrointestinal tract of Japanese Black cattle through quantitative RT-PCR and immunohistochemistry. \textit{Tas1r3} mRNA was detected ubiquitously along the gastrointestinal tract, and the most predominant expression was observed in the reticulum. In addition, the presence of \textit{Tas1r3} receptor was confirmed in the rumen through immunohistochemistry. The expression level of \textit{Pept1} mRNA was higher in the forestomach (rumen, reticulum, and omasum) and small intestine (duodenum) than that in the tongue, and predominant expression was observed in the rumen. By contrast, a negligible amount of \textit{Pept1} mRNA was detected in the abomasum and large intestine. Further studies on the roles of \textit{Tas1r3} and \textit{Pept1} in the digestive tract, in particular, in the four components of the stomach, will help us to understand the mechanisms of amino acids regulation in ruminants and provide the basis for formulating cattle diets to improve the health and productivity of cattle.

Key words: amino acids, digestive tract, peptides, Pept1, Tas1r3, wagyu cattle

INTRODUCTION

Animals need to ensure adequate absorption of amino acids from the environment. They must respond appropriately to the available sources of nutrients in their environment. Therefore, they have developed precise amino acid recognition systems that regulate their feeding behavior as well as metabolic responses.

Taste receptors of the \textit{Tas1Rs} family play a central role in their amino acid recognition (Zhao et al., 2003). The \textit{Tas1Rs} family belong to the \textit{G-protein-coupled receptor (GPCR) superfamily C} subtype and consist of three principal domains: an amino-terminal domain (ATD), a cysteine-rich domain (CRD), and a transmembrane domain (TMD) (Kunishima et al., 2000). \textit{Tas1Rs} receptors function as heterodimers; \textit{Tas1r2} and \textit{Tas1r3} bind to form sweet receptors, whereas \textit{Tas1r1} and \textit{Tas1r3} constitute a receptor for umami substances including L-amino acids (Nelson et al., 2002; Zhao et al., 2003). Recently, it has been demonstrated that \textit{Tas1Rs} receptors exist not only in oral tissues but also in various nonoral tissues (Behrens and Meyerhof, 2011).

\textit{Pept1}, also known as solute carrier family 15 member 1 (SLC15A1), utilizes a proton gradient...
(Adibi, 1997) to transport di/tri-peptides from the intestinal lumen into epithelial cells (Zhang et al., 2016). In the intestine, the Pept1 pathway constitutes a major mechanism for absorption of the products of protein digestion (Fei et al., 1994). In recent years, many researchers have reported that Pept1 is expressed in different organs and has multiple biological functions (Zhang et al., 2016; Spanier and Rohm, 2018; Viennois et al., 2018).

Although the expression of taste receptors and transporters for amino acid regulation in the digestive tract has been studied intensively in many mammalian species, little information is available for ruminants. Ruminants are characterized by their four compartments of the stomach, that is, the rumen, reticulum, omasum, and abomasum. Because of the particular gastrointestinal structure, some of the amino acids consumed by ruminants are degraded by rumen microorganisms (Diao et al., 2019). However, the mechanisms of amino acid regulation in the digestive tract of ruminants are still unclear. Because the gastrointestinal structure of ruminants is unique compared with nonruminants, the roles of taste receptors and peptide transporters must also be unique. The pattern of the expression of taste receptor and transporter genes, that is, whether they are expressed in a particular organ or not is quite different between species (e.g., Gonda et al., 2013). To date, however, there have been few reports about the expression of taste receptor and peptide transporter genes in the digestive tract of ruminants. Therefore, one receptor gene (Tas1r3) and one transporter gene (Pept1) were chosen as targets of the present study. To better understand their roles in the ruminant digestive tract, the expression of Tas1r3 and Pept1 was studied along the gastrointestinal tract of Japanese Black cattle through quantitative RT-PCR and immunohistochemistry.

### MATERIALS AND METHODS

Wagyu cattle include four types of Japanese cattle: the Black, Brown, Shorthorn, and Polled breeds (Gotoh et al., 2018). Of this, Japanese Black cattle are the most predominant breed of them because of their high-performance marbled beef and extremely high economic value (Gotoh et al., 2018). Samples of the gastrointestinal tract from Japanese Black cattle were acquired from the meat inspection center of Gifu City, Japan, following humane slaughter procedures. Tissue samples for the RT-qPCR analysis were obtained from three individuals (one female and two castrated males) reared in the same farm. Small blocks of tissues were incubated overnight at 4 °C in RNA later (Invitrogen), and stored at −20 °C until use. Total RNA was extracted from the tissues using the RNeasy Plus mini kit (QIAGEN, Germany). Elimination of genomic DNA from the RNA samples and reverse transcription of RNA into cDNA were carried out using PrimeScript RT reagent kit with gDNA Eraser (TaKaRa, Japan). The following primers for the target genes (Tas1r3 and Pept1) and an internal marker (Gapdh) were designed using Primer 3 plus software (http://www.bioinformatics.nl/cgi-bin/primer3plus/primer3plus.cgi) (Thornton and Basu, 2011): 5′-TGTGCGACAGGGTGTGCGG-3′ and 5′-AAAGGTGATAGCCACGACGC-3′ (Tas1r3), 5′-ATGGCCTTAACCGAGAGCAGA-3′ and 5′-TCACTGGCATGATGACTCCAC-3′ (Pept1), and 5′-AAAGGTCGGAGTGACCGATT-3′ and R: 5′-ATGGCCGAGCTGTCCTGTTT-3′ (Gapdh). The PCR products were checked using the standard 2% agarose gel electrophoresis. Real-time PCR was carried out with StepOne Plus (Applied Biosystems) using THUNDERBIRD SYBR qPCR Mix (Toyobo, Japan). Standard real-time PCR condition (Pre-denaturation: 95 °C, 3 min; denaturation: 95 °C, 15 s; annealing: 60 °C, 30 s; extension: 60 °C, 1 min; 40 cycles) were applied to all analyses, which were repeated three times. The relative expression of Tas1r3 and Pept1 were determined by comparative calculation with the internal marker Gapdh (Livak and Schmittgen, 2001). The ΔCt was determined by subtracting the Gapdh Ct value from the Ct value of the target gene from each organ, and the ΔΔCt was determined by subtracting the ΔCt from the baseline organ (tongue in the present study) from the ΔCt at each organ. The relative quantity of target gene mRNA was expressed as 2−ΔΔCt.

Tissues for immunohistochemistry were embedded overnight in 4% paraformaldehyde. An antigen retrieval treatment with normal horse serum was applied to unmask immunogenic epitopes for 30 min. Sections were incubated overnight at 4 °C with a primary antibody (G-2, SC-39896, CosMo Bio Co, Japan) diluted in solution (1:200 concentration). Sections were incubated at room temperature with a secondary antibody (biotinylated anti-mouse IgG horse serum; Funakoshi, Japan) and a tertiary antibody (ABC Elite; Funakoshi, Japan) at a dilution of 1:500 for 30 min. Antibody complexes were visualized with avidin–biotin-peroxidase conjugate (ABC Elite; Funakoshi, Japan) and 3,3′-diaminobenzidine (DAB; Dojindo, Japan).

### RESULTS AND DISCUSSION

When the PCR products were checked using 2% agarose gel electrophoresis, a clear single target band was observed for each gene, indicating the
high specificity of the PCR primers. Therefore, the relative expression of the *Tas1r3* and *Pept1* genes in the digestive tract was quantified using real-time PCR (Fig. 1).

**Expression of *Tas1r3* in the Gastrointestinal Tract**

The mRNA of *Tas1r3* was detected ubiquitously in both the digestive tract and tongue (Fig. 1a). The expression level in most parts of the digestive tract was higher than that in the tongue. The most predominant expression was observed in the reticulum. Following real-time PCR, the existence of the *Tas1r3* protein was validated by immunohistochemistry. The *Tas1r3* protein was clearly expressed in the reticulum (Fig. 2).

The Tas1r family members (*Tas1r1*, *Tas1r2*, and *Tas1r3*) mediate sweet and umami tastes in mammals: a heterodimer of *Tas1r2* and *Tas1r3* functions as a sweet taste receptor while a heterodimer of *Tas1r1* and *Tas1r3* functions as an umami taste receptor (Li, 2009). Disruption of the *Tas1r3* gene in knockout mice diminishes the taste responses to both sweet and umami taste stimuli (Damak et al., 2003; Zhao et al., 2003). It has been well demonstrated that the *Tas1r3* receptor is expressed in small numbers of cells in the anterior and posterior taste buds (Chaudhari et al., 2009). Recently, it has been reported that *Tas1r3* is also expressed in extra-oral tissues, including the gastrointestinal tract, brain, bladder, pancreas, male reproductive organs, immune system, adipose tissue, and bone, in many animal species including humans and mice (Laffitte et al., 2014). However, for ruminants, few reports are available except for Moran et al. (2014), which reported the existence of *Tas1r3* protein in the small intestine of sheep and cattle. The present study demonstrated that *Tas1r3* mRNA was expressed ubiquitously in the cattle digestive tract, and confirmed the existence of *Tas1r3* protein in the cattle rumen.

Although the *Tas1r3* gene was ubiquitously expressed in the cattle digestive tract, the expression level was relatively high in the reticulum, omasum, and cecum. The mRNA of *Tas1r1* and *Tas1r3* are known to be expressed in the stomach of mice and humans as well as in the small intestine and colon (Bezençon et al., 2007; Janssen and Depoortere, 2013). The existence of *Tas1r3* protein was confirmed in the mouse stomach by immunohistochemistry (Hass et al., 2010). However, studies on the expression of the Tas1rs family in the stomach

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**Figure 1.** Relative expression of the (a) *Tas1r3* and (b) *Pept1* genes in the digestive tract of wagyu cattle. The error bars indicate the standard error of the mean (SEM) from three individuals. The expression level of tongue was set at 1.

**Figure 2.** Immunohistochemistry of the *Tas1r3* receptors in the rumen of Japanese Black cattle. The scale bar represents 10 μm.
are few compared with their expression in the intestine, and little is known about their function. Intestinal Tas1r2 has been suggested to be a possible sensor of glucose or other nutritional ingredients (Cumming and Overduin, 2007; Depoortere, 2014; Calvo and Egan, 2015). The functions of intestinal Tas1rs might be related to hormonal regulation and/or defense mechanisms, as suggested for other types of taste receptors (Sternini, 2007; Breer et al., 2012; Prandi et al., 2013). Further studies, in particular, on the type of cells in the cattle stomach are important to clarify the role of Tas1rs in the stomach of ruminants.

**Expression of Pept1 in the Gastrointestinal Tract**

Although Pept1 mRNA was also detected in the gut and tongue, its expression pattern was different from that of Tas1r3 (Fig. 1b). The expression level in the forestomach (rumen, reticulum, and omasum) was more than five times higher than that in the tongue. Predominant expression was observed in the rumen. Considerable expression of Pept1 mRNA was also observed in the small intestine (duodenum). By contrast, a negligible amount of Pept1 mRNA was detected in the abomasum and as large intestine.

To the best of our knowledge, no other study except Wang et al. (2016) compared the expression of the Pept1 gene quantitatively in the gastrointestinal tract of ruminants. As a peptide transporter, it is well known that Pept1 plays a vital role in the absorption of peptides in the small intestine (Spanier and Rohm, 2018). Correspondingly, it has been reported that Pept1 mRNA was highly expressed in the small intestine compared with other organs (Spanier and Rohm, 2018). The very low level of expression in the large intestine of cattle demonstrated by the present study agrees with previous studies in humans and mice (e.g., Groneberg et al., 2001; Terada et al., 2005). By contrast, the predominant expression of Pept1 mRNA in the forestomach of cattle in the present study is a little puzzling because very weak expression in the stomach has been reported in humans and mice (Terada et al., 2005; Lu and Klaasssen, 2006). Wang et al. (2016) also reported considerable amounts of mRNA expression in the forestomach of yak (Bos grunniens) and indigenous cattle in the Qinghai-Tibetan plateau.

In ruminants, protein from the diet is generally degraded quickly and converted into microbial protein in the rumen, and then microbial protein flows into the small intestine (Gruninger et al., 2019). Therefore, it was believed that peptide absorption occurred mostly in the small intestine. However, several authors have studied amino acid net flux in the portal vein in sheep and cattle and suggested that peptide absorption occurs in the stomach (DiRienzo, 1992; Rémond et al., 2000; Tagari et al., 2004, 2008). Based on these studies, Gilbert et al. (2008) pointed out a possible important role of the rumen and omasum in the absorption of amino acids in the form of small peptides. The present study is consistent with these results suggesting significant uptake of peptides from the forestomach in ruminants. For ruminants, peptide absorption appears to be more important than was previously believed. The idea that absorption from the small intestine is the only way to ingest amino acids into the body should be reconsidered.

**Implications for the Future**

The results described above suggested that the function of the digestive tract of ruminants is not as simple as previously believed. It was believed that the absorption of peptides by animals mainly depended on Pept1, which is located on the brush border membrane of the intestine (Fei et al., 1994; Spanier and Rohm, 2018). The high level of Pept1 mRNA expression in the present study suggested that the forestomach of ruminants might play a role in the absorption of nutrients and/or regulation of homeostasis. Tas1r3 in the gastrointestinal tract is believed to detect amino acids and induce a series of downstream signals such as the release of gastrointestinal hormones (Vancleef et al., 2015). The secretion of hormones might affect the ingestion of nutrients via feeding behavior as well as the absorption of nutrients in the gastrointestinal tract. However, our knowledge about the roles of Tas1r3 and Pept1 in the digestive tract of ruminants is quite limited. Because the gastrointestinal structure of ruminants is unique compared with nonruminants, the roles of Tas1r3 and Pept1 must also be unique. Comprehensive studies on Tas1rs and Pept1 should be promising because a recent study in rats has suggested an interaction between them (Arakawa et al., 2016).

Research on Tas1r3 and Pept1 in the digestive tract will be applied to improve the efficiency of cattle production, dietary protein use, and to solve environmental issues such as reduction of the emission of greenhouse gases in the future. Ruminants are, and continue to be, the main source of protein in areas of environmentally harsh conditions and weak infrastructures because they can digest...
fibrous feeds that cannot be directly consumed by humans (Gerber et al., 2015). The global consumption of cattle meat is predicted to increase as the global population increases (Salter, 2017). However, cattle meat production is sometimes considered inefficient in terms of the conversion of natural resources into edible products (Gerber et al., 2015; Salter, 2017). Therefore, it is imminent to improve the efficiency of converting energy and protein in the cattle diet into human edible energy and protein. Further knowledge about the roles of Tas1r3 and Pept1 in the digestive tract will be indispensable to tackle this challenging task because they play central roles in the regulation of amino acids and peptides in the gut.

Japanese wagyu beef is famous for its marbling and high quality, sweet and fatty aroma, sweet rather than greasy taste, and a high quality umami flavor (Matsuishi et al., 2001). Appropriate nutritional management is indispensable to produce beef that satisfies the requirements of the Japanese beef market (Gotoh et al., 2018). However, there are many problems with the management of wagyu cattle, including dependency on imported grains for feed and high production costs accounting for more than 90% of the sale price (Gotoh et al., 2018). Further studies on Tas1r3 and Pept1 can contribute to the wagyu industry by providing the knowledge necessary for stable and efficient feeding systems and production of high quality meat.

CONCLUSION

The present study determined the mRNA expression of Tas1r3 and Pept1 quantitatively in the digestive tract of Japanese wagyu cattle. Further studies are required on the roles of Tas1r3 and Pept1 in the digestive tract, in particular, in the four components of the stomach. Such studies will help us to understand the mechanism of amino acids regulation in ruminants and provide a basis for formulating cattle diets to improve the health and productivity of cattle.

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Conflict of interest statement. None declared

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