DISTRIBUTION OF NUTRIENT TRANSPORTER RELATED GENES IN DIFFERENT SEGMENTS IN SMALL INTESTINE OF RABBITS

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ABSTRACT

The objective of this study was to investigate the distribution of nutrient transporter related genes in different segments in small intestine of rabbit. Ten healthy rabbits of at 110 day, with similar weight were chosen to slaughter, intestinal segments samples were collected from duodenum, jejunum, ileum for detection intestinal tissue distribution of oligopeptide transporter PepTl, amino acid transporter CAT1, B^0AT, EAAT3, rBAT, glucose transporter SGLT1, GLUT2, GLUT5 and fatty acid transporter FATP4 mRNA abundance by Real-time PCR. The results show that PepT1 mRNA in duodenum was the highest in intestinal segments, and the jejunum was slightly lower. CAT1, rBAT, and B^0AT mRNA were highest in ileum, and the jejunum was slightly lower. EAAT3 mRNA expression was higher in jejunum and ileum. SGLT1 and GLUT5 mRNA was higher in duodenum and jejunum. GLUT2 and FATP4 mRNA were highest in jejunum, and the duodenum was slightly lower. The result indicates that the main part of the intestinal transport absorption of oligopeptide, glucose and fatty acid is the front half of the small intestine, the main part of the transport absorption of amino acid is the posterior segment of the small intestine.

Key words: Rabbit, Small intestine, Nutrient transporter, mRNA expression abundance.

INTRODUCTION

Small intestine is one of the main places for digestion and absorption of nutrients, the digestion and absorption capacity of nutrient molecules at different sections of small intestine is different. The digestion and absorption of small intestine depends on the absorption area and the individual cell transport capacity (Zhang 2014; Speier *et al*. 2012), so the normal expression of the intestinal transit vector plays an important role in the digestion and absorption of nutrients in the daily ration in the animal's body. This test studied the expression and distribution of nutrient transporter related genes in different segments of small intestine in rabbits, which helped to better understand the process of digestion and absorption of nutrients in the intestine of rabbits, providing a theoretical basis for the law of digestion and absorption of protein in the small intestine of rabbits, and for the reference of reasonable ratio for the ration of rabbits and nutrition regulation on the production of rabbits.

MATERIALS AND METHODS

Experimental animals and sample collection

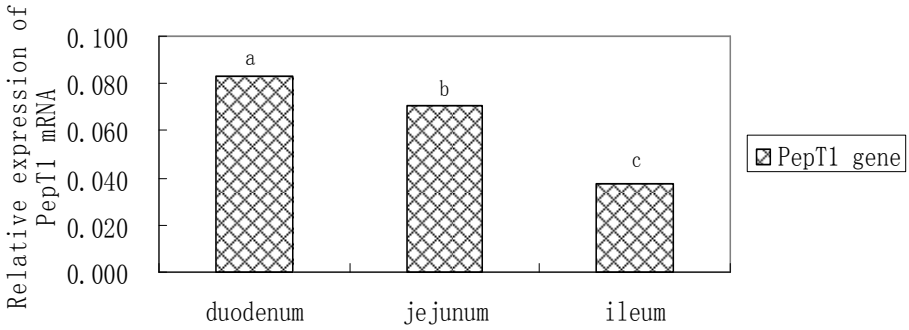
Experimental rabbits were provided by the rabbit farm used for experimental teaching in the school living collection. Feeding the complete formula granulated feed. Ten 110-day-old healthy rabbits with close weight were killed through carotid arteries bloodletting, and the intestines were separated rapidly after the abdominal cavity was dissected. All parts of the intestine were washed with 4℃ precooled saline and PBS buffer, respectively, and blotted with absorbent paper; about 2 cm of duodenum, jejunum and ileum samples were took respectively and placed into a 1.5 mL centrifuge tube, which was quickly frozen with liquid nitrogen, in cryopreservation at -70℃ for standby application.

Real Time PCR
Select β-actin as a reference gene, according to the sequence of the Oryctolagus cuniculus β-actin, Pep T1, CAT1, rBAT, B^0AT, EAAT3, SGLT1, GLUT2, GLUT5 and FATP4, ten pairs of primer were designed. The Real Time PCR reaction was carried out using the β-actin gene as an internal control. SYBR green fluorescence signal was collected at the last step of each cycle, dissolution curve analysis was carried out as the temperature was raised from 60℃ to 95℃ at the end of the cycle.

Data processing and statistical analysis
The relative gene expression was calculated by $2^{-\Delta CT}$ method, n=10; the data were expressed as mean ± standard deviation (x- ± s); the one-way variance (ANOVA) was analyzed on the mRNA expression of each gene using SPSS 13.0 statistical software, and performed multiple comparisons using Duncan.

RESULTS AND DISCUSSION

Expression of small peptide transporter related genes in different sections of rabbit intestine



The same series of data different lowercase letters that significant difference $P<0.01$, same as below.
Figure 1. Tissue specific expression of PepT1 mRNA.

As it can be seen from Figure 1, the expression level of PepT1 mRNA in different segments of small intestine in rabbits was significantly different ($P<0.01$), of which the expression level of PepT1 mRNA in proximal duodenum was the highest; the expression in jejunum was slightly lower than that in duodenum, and the ileum was the lowest. This is consistent with the Freeman et al. 1995 study results. Previous studies showed that the expression level of PepTl mRNA in duodenum and jejunum in monogastric animals such as chicken (Gilbert et al. 2007) is higher. This shows the main part of intestinal absorption of small peptides in rabbits is the front half of small intestine.

Expression of amino acid transporter related genes in different sections of rabbit intestine
As it can be seen from Figure 2, the relative expression level of CAT1 and B^0AT mRNA in different segments of small intestine in rabbits was significantly different ($P<0.01$), and the expression levels increased from the proximal to the distal part of the small intestine, the expression levels of B^0AT mRNA in different segments of small intestine were higher than those in CAT1. The expression level of rBAT mRNA in ileum of the distal end of small intestine was significantly higher than duodenum and jejunum ($P<0.01$); the expression level of EAAT3 mRNA in duodenum of the proximal of small intestine was significantly lower than jejunum and ileum ($P<0.01$). The results indicating that the main part of intestinal transport of amino acids in rabbits is the posterior segment of small intestine.

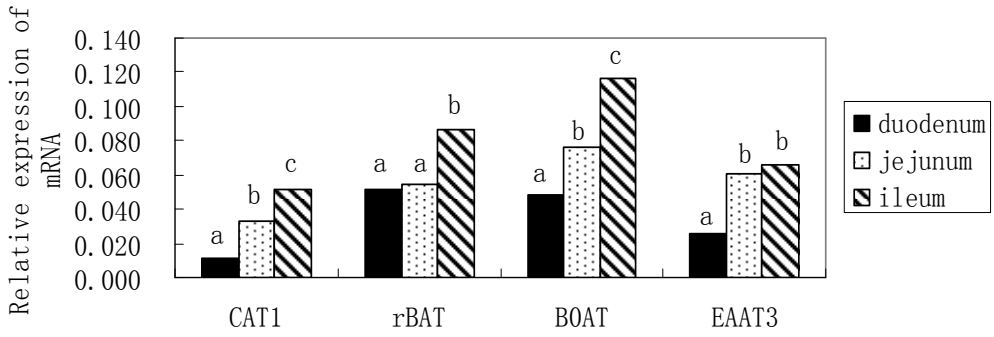


Figure 2. Tissue specific expression of CAT1, rBAT, B^0AT and EAAT3 mRNA.

Expression of glucose transporter related genes in different sections of rabbit intestine

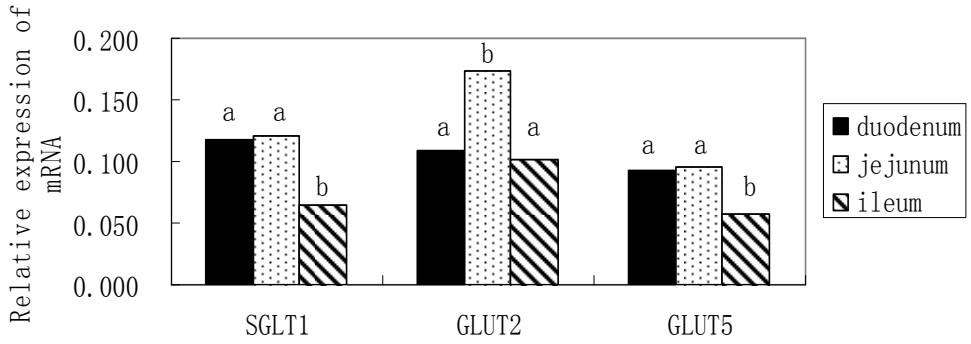


Figure 3. Tissue specific expression of SGLT1, GLUT2 and GLUT5 mRNA.

As it can be seen from Figure 3, the expression level of SGLT1 mRNA in ileum was significantly lower than duodenum and jejunum (P<0.01). The expression level of GLUT2 mRNA in jejunum was significantly higher than that in duodenum and ileum (P<0.01); the expression level of GLUT5 mRNA in ileum was significantly lower than that in duodenum and ileum (P<0.01). Previous studies showed that the expression levels of mRNAs in these two transporters were higher in the proximal part of small intestine than in the distal part (Byers *et al*. 2017; Zhang 2014). This shows that the main part of intestinal transport of glucose in rabbits is the front half of small intestine.

Expression of fatty acid transporter related genes in different sections of rabbit intestine

As it can be seen from Figure 4, the expression level of FATP4 mRNA in jejunum was the highest, the expression in duodenum was slightly lower than that in jejunum, and the ileum was the lowest(P<0.01). Zhang (2014) found that the expression abundance of FATP4 mRNA in broiler gut was the highest in jejunum, followed by duodenum and the lowest in ileum. The results indicating that the main part of intestinal absorption of fatty acids in rabbits is the front half of small intestine.

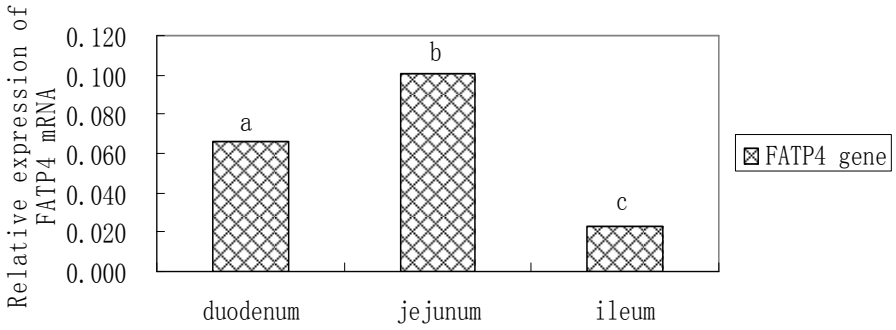


Figure 4. Tissue specific expression of FATP4 mRNA.

CONCLUSIONS

The main part of intestinal transport and absorption of small peptides, glucose and fatty acid in rabbits is the front half part of small intestine, and the main part of intestinal transport of amino acids is the latter part of small intestine.

ACKNOWLEDGMENTS

This work was supported by the Science and Technology Research Projects in Colleges from Hebei Province of P.R.China (#bj2014035); the Excellent Going Abroad Experts' Training Program in Hebei Province; the Modern Agriculture (rabbit) Industrial Science and Technology System Projects from the Ministry of Agriculture of P.R.China (#CARS-43-B-2).

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