Evaluation of the expression of H^+/K^+ ATPase gene by real time RT-PCR in fundic gastric mucosa of weaned pigs

S. De Filippi, M. Mazzoni, P. Trevisi, L. Casini, P. Bosi

Dipartimento di Protezione e Valorizzazione Agroalimentare, Università di Bologna, Italy

Corresponding author: Sara De Filippi. Dipartimento di Protezione e Valorizzazione Agroalimentare, Sezione Allevamenti Zootecnici. Via F.lli Rosselli 107, 42100 Reggio Emilia, Italy – Tel: +39 052 2209522 – Fax: +39 052 2209523 – E mail: saradefilippi@yahoo.it

ABSTRACT

Gastric acid secretion contributes to the gut barrier against pathogens. However, during suckling, pigs show a reduced hydrochloric acid secretion, in proportion to fermentative activity of milk lactose in the stomach. When the passage of maternal milk in the stomach ceases at the moment of weaning, the acid secretion in the stomach is still limited. Then, the progressive intake of solid feed will gradually stimulate this secretion. H+/K+-ATPase is responsible for acid secretion into the stomach and catalyses electro neutral exchange of cytoplasmic H+ and external K+ coupled with ATP hydrolysis. The aim of the research was to set up in fundic gastric mucosa a technique to quantify the expression levels of gene involved in H+/K+-ATPase production during weaning by Real Time RT-PCR. To quantify the copy number of the H+/K+-ATPase gene, the absolute quantification method, by using an external standard curve (MSE 0.125, r=-1, slope=-3.576; intercept=39.23), was performed on stomach samples. The nucleic acid sequence of pig gastric H+/K+-ATPase was found in GenBank (accession number: M22724) and the external and internal primers were designed by OLIGO Primer Analysis Software version 5.0, respecting the parameters suggested by Roche. Each final value was obtained from at least 2 replicates per amplification in at least 2 different days. Data obtained from 60 weaned pigs showed a great variability with a minimum of 10 copies /µl to a maximum of 471,398 copies/µl, with a standard deviation of 72,726 and an average value of 21,847. The large individual variability could be explained by the fast regulation on parietal cell, which occurs after various stimuli. The evaluation of the expression H+/K+-ATPase gene was useful to evidence the effect of different diets on gastric functionality. We also observed a positive correlation ($r=0.385$, $P<0.01$) of this parameter with the number of parietal cells. The Quantitative Real Time RT-PCR technique permitted to cover the lack of literature concerning the gene expression in pig gastric mucosa with a sensitive method, detecting even low amount on mRNA molecules. It so offers important physiological insights on mRNA expression level.