



# 动物营养学报

CHINESE JOURNAL OF ANIMAL NUTRITION



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动物营养学报 » 2014, Vol. 26 » Issue (3) : 694-700 DOI: 10.3969/j.issn.1006-267x.2014.03.019

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## 丙氨酰-谷氨酰胺二肽对仔猪小肠上皮细胞间紧密连接蛋白occludin定位与表达的影响

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### Effects of Alanyl-Glutamine Dipeptide on Localization and Expression of Tight Junction Protein Occludin in Small Intestinal Epithelial Cells from Piglets

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**摘要** 本试验以分离自仔猪空肠上皮的IPEC-1细胞为模型, 探讨丙氨酰-谷氨酰胺二肽(Ala-Gln)对仔猪小肠上皮细胞间紧密连接蛋白occludin定位与表达的影响。试验选取同代IPEC-1细胞接种至6块6孔细胞培养板上, 分别用含0(对照组)、0.25、0.50、1.00、2.00、4.00 mmol/L Ala-Gln的DMEM高糖培养基进行培养, 待细胞生长融合达70%以上时, 对occludin进行免疫荧光定位和蛋白质印迹(Western Blot)检测。结果表明: 1) 对照组细胞可见其胞质内存在团状的阳性荧光染色, 而细胞间连接处阳性荧光染色不明显; 随着Ala-Gln浓度的升高, 细胞间连接处荧光信号逐渐增强, 细胞轮廓更加清晰, 而胞质内荧光信号逐渐减弱。2) 各添加组IPEC-1细胞间occludin的相对表达量均极显著高于对照组( $P<0.01$ )。随着Ala-Gln浓度的升高, occludin的相对表达量呈先增后减的趋势, 添加量为2.00 mmol/L时达到峰值, 且极显著高于其他添加量( $P<0.01$ )。由此可知, Ala-Gln可以上调仔猪小肠上皮细胞间紧密连接蛋白occludin的表达, 促进细胞间紧密连接结构的形成, 进而增强仔猪小肠黏膜的屏障功能。

**关键词:** 仔猪 小肠上皮细胞 紧密连接 occludin 丙氨酰-谷氨酰胺二肽

**Abstract:** This experiment was conducted to evaluate the effects of alanyl-glutamine dipeptide (Ala-Gln) on localization and expression of tight junction protein occludin in small intestinal epithelial cells from piglets using IPEC-1 cells isolated from jejunal epithelium as the model. The IPEC-1 cells of the same generation were planted into 6-six-well cell culture plates and were incubated with DMEM high-glucose medium containing 0 (control group), 0.25, 0.50, 1.00, 2.00, 4.00 mmol/L Ala-Gln, respectively. When the cell fusion had reached more than 70%, the immunofluorescence localization and Western Blot of occludin were detected. The results showed as follows: 1) the positive fluorescence staining clouds in cytoplasm of cells were observed in the control group, but that was not obvious among the junction of cells. The fluorescence signals among the junction of cells appeared to be strengthen gradually and the cell outline appeared clearer with the increase of Ala-Gln concentration. Contrarily, the fluorescence signals in cytoplasm of cells were weakened gradually. 2) Compared with the control group, the relative expression level of occludin in IPEC-1 cells in supplemental groups was significantly increased ( $P<0.01$ ) and a trend from increase to decrease was observed with the increase of Ala-Gln concentration. The relative expression level of occludin in 2.00 mmol/L group was significantly higher than that in other supplemental groups ( $P<0.01$ ). Therefore, Ala-Gln can up-regulate the expression of tight junction protein occludin in small intestinal epithelial cells from piglets, and promote the formation of intercellular tight junction structure, and then strengthen the barrier function of intestinal mucosa in piglets.

**Keywords:** piglet, intestinal epithelial cell, tight junction, occludin, Ala-Gln

收稿日期: 2013-10-09;

基金资助:

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引用本文:

邓宸玺, 王自蕊, 游金明等 . 丙氨酰-谷氨酰胺二肽对仔猪小肠上皮细胞间紧密连接蛋白occludin定位与表达的影响[J]. 动物营养学报, 2014,V26(3): 694-700

DENG Chenxi, WANG Zirui, YOU Jinming etc . Effects of Alanyl-Glutamine Dipeptide on Localization and Expression of Tight Junction Protein Occludin in Small Intestinal Epithelial Cells from Piglets[J]. Chinese Journal of Animal Nutrition, 2014,V26(3): 694-700.

链接本文:

[http://118.145.16.228/Jweb\\_dwyy/CN/10.3969/j.issn.1006-267x.2014.03.019](http://118.145.16.228/Jweb_dwyy/CN/10.3969/j.issn.1006-267x.2014.03.019) 或 [http://118.145.16.228/Jweb\\_dwyy/CN/Y2014/V26/I3/694](http://118.145.16.228/Jweb_dwyy/CN/Y2014/V26/I3/694)

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