

飼糧中添加益生菌在雞隻腸道中存活之探討

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摘要

本研究主要探討於飼糧中添加含益生菌發酵乳對雞隻腸道中益生菌的存活率之影響。試驗動物為購自商業孵化場之一日齡的離雞42隻，逢機分成三個試驗組，每試驗組各14隻。試驗期間為八週。A、B兩組於基礎飼糧中分別添加3%、5%之含益生菌發酵乳，在第五週開始，A、B改餵食不含益生菌發酵乳之基礎飼糧，C組為對照組僅餵飼基礎飼糧。試驗結果顯示：飼餵含益生菌發酵乳的雞隻與對照組雞隻之生長性能無顯著差異。益生菌在雞隻各階段腸道中存活的分析上，以LAMVAB和BIM-25培養基，測得其迴腸、盲腸及結腸內容物之乳酸桿菌菌數和雙歧桿菌菌數，A、B兩組之雞隻較對照組為高 ($P < 0.05$)，而其盲腸菌數較迴腸、結腸菌數為高 ($P < 0.05$)；另外，於雞隻排泄物之乳酸桿菌菌數和雙歧桿菌菌數，分別於第5週和第4週的菌數較其他週數為高 ($P < 0.05$)。此外，益生菌經過細菌培養，萃取DNA後，利用PCR之方法檢測益生菌專一性。結果發現有餵食含益生菌發酵乳之雞隻，可於排泄物或腸內容物檢測到添加飼料的益生菌，此顯示益生菌可於雞隻腸道中存活。另以掃描式電子顯微鏡亦可於雞隻盲腸組織切片觀察到益生菌菌株之存在。根據實驗結果顯示，於飼糧中添加含益生菌發酵乳可於雞隻腸道內存活。

關鍵詞：益生菌、雞隻、腸道

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參考文獻

- 1.光岡知足。1992。腸內細菌?家畜?生產性。Feeding. 32 (7) :19-27.
- 2.林志勳、江世楷。1996。消化道微生物，腸道微生物對動物營養的重要性。台灣養豬科學研究所，竹南，台灣。第38-208頁。
- 3.邱美雲。2003。雞隻腸道來源之乳酸桿菌 *Lactobacillus reuteri* Pg 4菌株作為益生菌之探討。碩士論文。國立中興大學。台中。
- 4.周其勳。2006。發酵乳產品中乳桿菌屬的菌數測定與菌種鑑定。碩士論文。大葉大學。彰化。
- 5.洪偉盛。2005。以分子標定法鑑別市售產品之雙叉乳桿菌。碩士論文。國立台灣大學。台北。
- 6.施宗雄。1998。台灣乳酸菌類研究之介紹及在飼料添加劑上之應用。生物產業。9 (4) : 239-245。
- 7.楊媛絢。1997。乳酸菌專輯:原生保健性菌種 (probiotics) 與益菌助生質 (prebiotics) 之應用。財團法人食品工業發展研究所。新竹。
- 8.黃崇貞。2004。腸道微生物與益生菌。食品工業月刊。36 (3) : 4-15。
- 9.劉振鳳。2004。利用tuf gene和PCR技術於乳酸桿菌之檢測與乳酸菌之安全評估。碩士論文。國立中興大學。台中。
- 10.謝豪晃。2002。生物技術應用於飼料工業。家禽世界月刊。30 (4) :11-14。
- 11.Barnes, E. M., G. C. Mead, D. A. Barnum, and E. G. Harry.

1972. The intestinal flora of the chicken in the period 2-6 weeks of age, with particular reference to the anaerobic bacteria. Br. Poultry Sci. 13:311-326.
- 12.Barnes, E. M., C. S. Impey, and B. J. H. Stevens. 1979. Factors affecting the incidence and anti-salmonella activity of the anaerobic caecal flora of the young chick. J. Hygiene, Cambridge. 82:263-283.
- 13.Barnes, E. M., C. S. Impey, and D. M. Cooper. 1980. Manipulation of the crop and intestinal flora of the newly hatched chick. Am. J. Clin. Nutr. 33: 2426-2433.
- 14.Buenrostro, J. L., and F. H. Kratzer. 1983. Effects of Lactobacillus inoculation and antibiotic feeding of chickens on availability of dietary biotin. Poultry Sci. 62: 2022-2029.
- 15.Cavazzoni, V., A. Adami, and C. Castrovilli. 1993. Performance of broiler chickens supplemented with *Bacillus coagulans* as probiotic. Br. Poult. Sci. 39: 526-529.
- 16.Chiang, S. H., and W. M. Hsieh. 1995. Effect of direct-fed microorganisms on broiler growth performance and litter ammonia level. Asian-Aus. J. Anim. Sci. 8: 159-162.
- 17.Cocolin, L., M. Manzano, C. Cantoni and G. Comi. 2001. Denaturing gradient gel electrophoresis analysis of the 16S rRNA gene V1region to monitor dynamic changes in the bacterial population during fermentation of Italian Sausages. Appl. Environ. Microb. 67: 5113-5121.
- 18.Choct, M. 2000. Alternatives to in-feed antibiotics in monogastric animal industry. ASA Technical Bulletin. Vol. AN30-2001.
- 19.Dubernet S., N. Desmases and M. Gueguen. 2002. A PCR-based method for identification of Lactobacilli at the genus level. FEMS Microbiology Letters, 214:271-275.
- 20.Du Plessis, E.M., and L.M. Dick. 1995. Evaluation of random amplified polymorphic DNA (RAPD) -PCR as a method to differentiate *Lactobacillus acidophilus*, *Lactobacillus crispatus*, *Lactobacillus amylovorus*, *Lactobacillus gallinarum*, *Lactobacillus gasseri*, and *Lactoabcillus johnsonii*. Curr. Microbiol. 31: 114-118.
- 21.Edens, F. W. 2003. An alternative for antibiotic use in poultry: Probiotics. Rev.Bras. Clenc. Avic, Vol.5, no.2, p. 75-97.
- 22.Eyssen, H., E. Swaelen, Z. Kowsyk-Gindifer, and G. Parmenteer. 1965. Nucleotide requirement of *Lactobacillus acidophilus* variants isolated from the crop of chicks. Antonie van Leeuwenhoek. 31:241-248.
- 23.Francis, C., D. M. Janky, A. S. Arafa, and R. H. Harms. 1978. Interrelationship of *Lactobacillus* and zinc bacitracin in the diets of turkey poult. Poultry Sci. 57: 1687-1689.
- 24.Fuller, R. 1973. Ecological studies on the *Lactobacillus* flora associated with the crop epithelium of the fowl. J. Appl. Bacteriol. 36: 131-139.
- 25.Fuller, R. and B. E. Brooker. 1974. Lactobacilli which attach to the crop epithelium of neonatal pig. Appl. Envir. Microbiol.35: 582-591.
- 26.Fuller, R. 1978. Epithelial attachment and other factors controlling the colonization of the intestine of the gnotobiotic chicken by *Lactobacilli*. J. Appl. Bacteriol. 45: 389-395.
- 27.Fuller, R. 1989. Probiotics in man and animals. J Appl. Bacteriol. 66: 365-378.
- 28.Goodling, A. C., G. J. Cerniglia, and J. A. Hebert. 1987. Production performance of white leghorn layers fed *Lactobacillus* fermentation products. Poultry Sci. 66: 480-486.
- 29.Holzapfel, W. H. Haberer, P. Snel, J. Schillinger, U., and Huis, H. J. 1998. Overview of gut flora and probiotics. Int. J. Food Microbiol. 41: 85-101.
- 30.Huis in 't Veld, J. H. J., Havenaar, R., and Matrau, P. 1994. Establishing a specific basic for probiotic R&D. TIBTECH J. 12:6-8.
- 31.Hutanen, C. N. and J. M. Pensack. 1965. The development of the intestinal flora of the young chick. Poultry Sci. 44: 825-830.
- 32.Jin, L. Z., Y. W. Ho, N. Abdullah, and S. Jalaludin. 1996. Influence of dried *Bacillus subtilis* and *Lactobacilli* cultures on intestinal microflora and performance in broilers. Asian-Aus. J. Anim. Sci. 9: 397-404.
- 33.Jin, L. Z., Y. W. Ho, N. Abdullah, and S. Jalaludin. 1998a. Effects of adherent *Lactobacillus* cultures on growth, weight of organs and intestinal microflora and volatile fatty acids in broilers. Anim. Feed Sci. Technol. 70: 197-209.
- 34.Jin, L. Z., Y. W. Ho, N. Abdullah, and S. Jalaludin. 1998b. Growth performance, intestinal performance, intestinal *Lactobacillus* culture. Poultry Sci. 77: 1259-1265.
- 35.Jin, L. Z., Y. W. Ho, N. Abdullah, and S. Jalaludin. 1998c. Effect of adherent *Lactobacillus* spp. On in vitro adherence of *Salmonellae* to the intestinal epithelial cells of chicks. J. Appl. Bacteriol. 81: 201-206.
- 36.Jin, L. Z., Y. W. Ho, N. Abdullah, and S. Jalaludin. 2000. Digestive and bacterial enzyme activities in broilers fed diets supplemented with *Lactobacillus* cultures. Poultry Sci. 79:886-891.
- 37.Johansson, M.-L., G. Molin., B.Pettersson., M. Uhlen., and S. Ahrnee. 1995a. Characterization and species recognition of *Lactobacillus plantarum* strains by restriction fragment length polymorphism (RFLP) of the 16S rRNA gene. J. Appl. Bacteriol. 79: 536-541.
- 38.Johansson, M.-L., G. Molin., B.Pettersson., M. Uhlen., and S. Ahrnee. 1995b. Classification of *Lactobacillus plantarum* by restriction endonuclease analysis of total chromosomal DNA using conventional agarose gel electrophoresis. Int. J. Syst. Bacteriol. 45: 670-675.
- 39.Johansson, M.-L., G. Molin., B.Pettersson., M. Uhlen., and S. Ahrnee. 1995c. Randomly amplified polymorphic DNA (RAPD) for rapid typing of *Lactobacillus plantarum* strains. Lett. Appl. Microbiol. 21:115-159.
- 40.Kalbande, V. H., M. A. Gaffar, and S. V. Deshmukh. 1992. Effect of probiotic and nitrofuran on performance of growing commercial pullets. Indian J. Poultry Sci. 27:116-117.
- 41.Langhout, P. 2000. New additives for broiler chickens. World Poultry-Misset. 16:22-27.
- 42.Lilly, D. M., and R. H. Stillwell. 1965. Probiotics: Growth promoting factors produced by microorganisms. Science 147: 747-748.
- 43.Maiolino, R., A. Fioretti, L. F. Menna, and C. Meo. 1992. Research on the efficiency of probiotics in diets for broiler chickens. Nutr. Abstr. Rev. Series 62 B:482.
- 44.Mohan, B., R. Kadirvel, A. Natarajan, and M. Bhaskaran. 1996. Effect of probiotic supplementation on growing, nitrogen utilization and serum cholesterol in broilers. Br. Poultry Sci. 37: 395-401.
- 45.Morishita, Y., T. Mitsuoka, C. Kaneuchi, S. Yamamoto, and M. Ogata. 1971. Specific establishment of lactobacilli in the digestive tract of germ-free chickens. Japanese J. Microbiol. 15:531-538.
- 46.Nahashon, S. N., H. S. Nakaue, S. P. Snyder, and L. W. Mirosh. 1996. Performance of Single Comb White Leghorn layers fed a diet with a live microbial during the growth and egg laying phases. Anim. Feed Sci. and Technol. 57:25-38.
- 47.Ochi, Y., T. Mitsuoka, and T. Segi. 1964. Untersuchungen über die Dar mflora des Huhn Mitteilung: die Entwicklung der Darmflora von Kuken bis zum Huhn. Zntralbl. Bakteriol. Parasitenkd. Infektionskr. Hyg. Abt. 1 Orig. 193:80-85.
- 48.Parker, R. B. 1974. Probiotics, the other half the antibiotic story. Anim. Nutr. And Health 29: 4-8.
- 49.Rodrigues, U. M., M. Aguirre., R.R. Facklam., and M.D. Collins. 1991. Specific and intraspecific molecular typing of lactococci based on polymorphism of DNA encoding rRNA. J. Appl. Bacteriol. 71: 509-516.
- 50.Roy, D., and S. Sirois. 2001. Molecular differentiation of *Bifidobacterium* species with amplified ribosomal DNA restriction analysis and alignment of short regions of *ldh* gene. FEMS Microbiol. Lett. 191:17-24.
- 51.Saiki, R. K., D. H. Gelfand., S. Stoffel., S.J. Scharf., R. Higuchi., G. T. Horn., K.B. Mullis., and H.A.Erlich. 1988. Primer-directed enzymatic amplification of DNA with thermostable DNA polymerase. Science 239: 487-494.
- 52.Salanitro, J. P., I. G. Blake, P. A.

M. Maglio, and J. R. Goodman. 1978. Bacteria isolated from the duodenum, ileum, and cecum of young chicks. *Appl. Environ Microbiol.* 35:782-790. 53.Sarra, P. G., F. Dellaglio and V. Bottazzi. 1985. Taxonomy of Lactbacilli isolated from the alimentary tract of chickens. *Syst. Appl. Microbiol.* 6:86-89. 54.Sarrela, M., Lahteenmaki, L., Crittenden, R., Salminen, S. and Mattila-Sandholm, T. 2002. Gut bacteria and health foods-the European perspective. *Int. J. Food Microbiol.* 78:99-117. 55.Shafey, T. M., S. Al-Mufarej, M. I. Shalaby, and A. J. Jarelnabi. 2001. The effect of feeding mannan-oligosaccharides (Bio-Mos) on the performance of meat chickens under two different vaccination programs. *Asian-Aus. J. Anim. Sci.* 14: 559-563. 56.Simth, H. W. 1965. The Development of the flora of the alimentary tract in young animals. *J. Pathol. Bacteriol.* 90: 495-513. 57.Spring, P. 1997. Understanding the development of the avian gastrointestinal microflora: An essential key for developing competitive exclusion products. p. 313-324. In: Lyons, T. P., and K. A. Jacques (eds.) *Biotechnology in the Feed Industry*. Proc. Alltech ' 13th Ann. Symp. Nottingham University Press, Noughborough, UK. 58.Song, Y., N. Kato., C. Liu., Y. Matsumiya., H. Kato., and K. Watanabe. 2000. Rapid identification of 11 human intestinal Lactobacillus species by multiplex PCR assay using group- and species-specific primers derived from 16S-23S rRNA intergenic spacer region and its flanking 23S rRNA. *FEMS Microbiol. Lett.* 187: 167-173. 59.Torriani S, F. Clementi ., M. Vancanneyt., B. Hoste., F. Dellaglio., K. Kersters. 2001. Differentiation of Lactobacillus plantarum, L. pentosus and L. paraplanatum species by RAPD-PCR and AFLP. *Syst. Appl. Microbiol.* 4:554-560. 60.Tortuero, F. 1973. Influence of implantation of Lactobacillus acidophilus in chicks on the growth, feed conversion, malabsorption of fats syndrome and intestinal flora. *Poultry Sci.* 52: 197-203. 61.Tortuero, F., and E. Fernandez. 1995. Effects of inclusion of microbial cultures in barley-based dietsfed to laying hens. *Anim. Feed Sci. Technol.* 53:255-265. 62.Ventura, M., M. Elli, R. Reniero and R. Zink. 2001. Molecular microbial analysis of Bifidobacterium isolates from different environments by the species-specific amplified ribosomal DNA restriction analysis (ARDRA) . *FEMS Microb. Ecology.* 36: 113-121. 63.Visek, W. J. 1978. The mode of growth promotion by antibiotics. *J. Anim. Sci.* 45: 1447-1469. 64.Walter J., G. W. Tannock, A. Tilsala-Timisjarvi, S. Rodtong, D. M. Loach, K. Munro, and T. Alatossava. 2000. Detection and identification of gastrointestinal Lactobacillus species by using denaturing gradient gel electrophoresis and Species-Species PCR primers. *Appl. Environ Microbiol.* 66: 297-303. 65.Watkins, B. A., and F. H. Kratzer. 1983. Effect of oral dosing of Lactobacillus acidophilus against pathogenic Escherichia coli in gnotobiotic chicks. *Poultry Sci.* 61: 1298-1308. 66.Watkins, B. A., and F. H. Kratzer. 1984. Drinking water treatment with commercial preparation of a concentrated Lactobacillus culture for broiler chickens. *Poultry Sci.* 63: 1671-1673. 67.Wrong, O. M. 1981. The large intestinal: Its role in manmanlian nutrition and homeostasis. P. 133-211. In: Wrong, O. M., C. J. Edminds, and V. S. Chadwick (eds.) *Nutrogen Compounds*. 68.Yeo, J., and K. Kim. 1997. Effect of feeding diets containing an antibiotic, a probiotic or yucca extract on growth and intestinal urease activity in broiler chicks. *Poultry Sci.* 76: 381-385.