

# Use of *Bacillus Subtilis* Var. Natto in Production of Fermented Animal Feed

林銘傑、王正仁

E-mail: 9608168@mail.dyu.edu.tw

## ABSTRACT

Soybean meal contains rich proteins and is considered as the most economically beneficial feeds for animals, but its anti-nutritional ingredients make its nutrition value and application vulnerable to limitation. On the other hand, the prohibition of antibiotics supplementation in the animal feeds by European Union countries have impacted the operation of animal farmers and feed producers. *Bacillus subtilis* var. natto (*B. natto*) bacteria can enhance the immunity of livestock and exhibits probiotic effects. It can secrete many kinds of hydrolytic enzymes with powerful fermentation abilities and is the best alternative for substituting antibiotics in the feeds. However, the price of *B. natto* probiotics on the market is substantially high and cannot be extensively used in animal feeds. However, if *B. natto* is directly used for fermentation of soybean meal, it can both increase its nutritional value by degrading its antinutritional factors and hydrolyzing other ingredients, and let fermented soybean contain a large number of *B. natto* spores with probiotic benefits and low feeding cost. The study applied solid-state fermentation method to fermentation of soybean meal by direct use of *B. natto* powder for production of fermented feeds, able to efficiently raise the number of *B. natto* spores in the feed. Direct inoculation of 0.1% commercial *B. natto* spore powder in the fermentation resulted in 109 CFU/g of spores formed in fermented feeds after 24 hours fermentation. The higher ratio of water to soybean meal led to the more growth of vegetative cells and more formation of ammonium nitrogen in fermentation of soybean meal by *B. natto*. Fermentation at higher temperature shortened the lag phase of cellular growth and therefore fastened cellular growth, but resulting in less formation of spores. Stirring of soybean meal during solid-state fermentation might create an environment for maintaining appropriate humidity and aeration and thus helped growth of *B. natto* but not formation of spores. Fermentation of sterilized soybean meal produced less ammonium nitrogen and also less spore formation. Addition of wheat bran as supplement in soybean meal during fermentation did enhance growth of cell and formation of spores but in turn increase production of ammonium nitrogen. Spiking of corn meal could increase the vegetative cell number but not significantly help formation of spores in the harvested product. Fermentation of soybean meal under room temperatures with 0.8 water ratio took only 20 hours to produce  $3 \times 10^8$  CFU/g of *B. natto* spores with a few amount of ammonia nitrogen (0.05%) in fermented soybean meal. And when a 0.6 water ratio was used, it took 28 hours to obtain  $3 \times 10^8$  CFU/g of *B. natto* spores with a few amount of ammonia nitrogen (0.02%), and even only took 32 hours to have 109 CFU/g of *B. natto* spores with a low amount of ammonium nitrogen (0.03%) in fermented soybean meal. To the operation of ordinary farmers and small entrepreneurs, fermentation of soybean meal by *B. natto* was best conducted at 37 °C with a water / meal ratio of 0.6, capable of yielding a spore number reaching 109 CFU per gram of fermented feeds with a low amount of ammonium nitrogen (0.06%) after 20 hours fermentation.

Keywords : solid-state fermentation ; soybean meal ; *Bacillus subtilis* var. natto ; spore ; ammonium nitrogen ; fermented feed

## Table of Contents

1. 緒言.....	1 2. 文獻討論.....	2 2.1 動物飼料.....	2 2.2 大豆.....
豆.....	3 2.3 大豆粕.....	4 2.3.1 大豆粕的製造過程.....	4 2.3.2 大豆粕於飼料中的應用.....
的應用.....	5 2.3.3 大豆粕中的抗營養因子.....	5 2.3.4 大豆粕的發酵.....	6 2.4 納豆菌.....
菌.....	7 2.4.1 納豆菌與微生物間的關係.....	7 2.4.2 飼料中添加納豆菌的益處.....	8 2.5 益生菌介紹.....
介紹.....	8 2.5.1 益生菌應具有的特性.....	9 2.5.2 益生菌作用機制.....	10 2.5.3 益生菌的保存.....
存.....	10 2.6 腸道菌相與動物間的關係.....	11 2.6.1 益生菌於消化系統中的好處.....	11 3. 材料與方法.....
法.....	12 3.1 實驗材料.....	12 3.1.1 大豆粕.....	12 3.1.2 玉米粕、麩皮.....
皮.....	12 3.1.3 納豆菌.....	12 3.2 儀器設備.....	12 3.2.1 儀器.....
器.....	13 3.2.2 器具與耗材.....	13 3.3 培養基.....	13 3.4 藥品.....
品.....	14 3.5 試藥配製.....	14 3.6 實驗方法.....	15 3.6.1 大豆粕發酵實驗.....
驗.....	15 3.6.2 影響大豆粕發酵的變數.....	15 3.7 分析方法.....	16 3.7.1 納豆菌之總菌數分析方法.....
分析方法.....	16 3.7.2 納豆菌之孢子數分析方法.....	17 3.7.3 氨態氮分析方法.....	17 4. 結果與討論.....
討論.....	19 4.1 以搖瓶液態發酵進行大豆粕發酵之可行性試驗..	19 4.2 納豆菌接種量對大豆粕發酵的影響.....	19 4.2.1 納豆菌接種量對氨氮產量的影響.....
影響.....	20 4.2.2 納豆菌接種量對納豆菌產孢的影響.....	21 4.3 發酵溫度.....	

與含水量對大豆粕發酵的影響.....	22	4.3.1 在室溫發酵下含水量對大豆粕發酵的影響.....	22	4.3.1.1 對氨氮產量的影響.....	22		
影響.....	23	4.3.1.2 對納豆菌產孢的影響.....	23	4.3.2 在37 發酵下含水量對大豆粕發酵的影響....	24	4.3.2.1 對 氨氮產量的影響.....	24
24 4.3.2.2 對納豆菌產孢的影響.....	25	4.3.3 在40 發酵下含水量對氨氮產量的影響.....	25				
25 4.3.3.1 對氨氮產量的影響.....	26	4.3.3.2 對納豆菌產孢的影響.....	26	4.3.4 在45 發酵下含水量對大豆粕 發酵的影響....	27		
27 4.3.4.1 對氨氮產量的影響.....	27	4.3.4.2 對納豆菌產孢的影響.....	27	4.4 大豆粕滅菌處理 對大豆粕發酵的影響.....	28		
28 4.4.1 在37 發酵下含水量對大豆粕發酵的影響....	28	4.4.1.1 對氨氮產量的影響.....	28				
29 4.4.1.2 對總菌數的影響.....	29	4.4.1.3 對納豆菌產孢的影響.....	30	4.4.2 在大豆粕滅菌處理下發酵溫度 及含水量對發酵 的影響.....	31		
31 4.4.2.1 對氨氮產量的影響.....	32	4.4.2.2 對總菌數的影 響.....	32				
32 4.4.2.3 對納豆菌產孢的影響.....	33	4.5 翻堆處理對大豆粕發酵的影響.....	33	4.5.1 對氨氮產 量的影響.....	33		
34 4.5.2 對總菌數的影響.....	34	4.5.3 對納豆菌產孢的影響.....	34	4.6 濕度控制 對大豆粕發酵的影響.....	35		
35 4.6.1 對氨氮產量的影響.....	35	4.6.2 對總菌數的影響.....	35	4.6.3 對 納豆菌產孢的影響.....	36		
36 4.7 添加麩皮及玉米粕對大豆粕發酵的影響.....	36	4.7.1 添加麩皮對大豆粕發酵的影 響.....	36	4.7.1.1 對氨氮產量的影響.....	37		
37 4.7.1.2 對納豆菌產孢的影響.....	37	4.7.1.2 對納豆菌產孢的影響.....	37	4.7.1.3 大豆粕及麩皮一 起滅菌處理對氨氮產量的影 響.....	38		
38 4.7.1.4 大豆粕及麩皮一起滅菌處理對總菌數的影響	38	4.7.1.4 大豆粕及麩皮一起滅菌處理對總菌數的影響	38	4.7.1.5 大豆粕 及麩皮一起滅菌處理對納豆菌產孢的 影響.....	38		
38 4.7.2 添加玉米粕對大豆粕發酵的影響.....	38	4.7.2.1 對 氨氮產量的影響.....	39	4.7.2.2 對納豆菌產孢的影響.....	39		
39 4.7.2.3 大豆粕及玉米粕一起滅菌處理對氨氮產 量的 影響.....	39	4.7.2.3 大豆粕及玉米粕一起滅菌處理對氨氮產 量的 影響.....	40	4.7.2.4 大豆粕及玉米粕一起滅菌處理對納豆菌產孢 的影響.....	40		
40 4.7.2.5 大豆 粕及玉米粕一起滅菌處理對納豆菌產孢 的影響.....	40	4.8 大豆粕發酵飼料放大生產試驗.....	41	4.8.1 氨 氮產量.....	41		
41 4.8.2 納豆菌產孢能力試驗.....	41	4.8.2 納豆菌產孢能力試驗.....	41	4.8.2 納豆菌產孢能力試驗.....	42		
42 參考文 獻.....	42	4.8.2 納豆菌產孢能力試驗.....	42	4.8.2 納豆菌產孢能力試驗.....	42		
	84						

## REFERENCES

1. 方維倫。1990。應用微生物（上）。復文書局，臺南市。
2. 呂向東、劉春田。1997。野生動物的營養與飼料。台灣省立鳳凰谷鳥園，台灣。
3. 陳世爵、陳潤卿。1981。黃豆油與黃豆食品手冊，台灣。
4. 黃卓志、辛志勳、張文重。1977。納豆菌之研究納豆菌培養條件之檢討。屏東農專學報。18:69-75。
5. 楊清白、吳樂民、吳春利。1986。飼料與營養。國立編譯館，台灣。
6. 詹惠雯。2005。利用*Aspergillus oryzae* 固態發酵處理大豆粕以去除寡醣暨致過敏蛋白之研究。國立中興大學食品科學系碩士論文，台中市。
7. 鄭長義。1988。飼料圖鑑與品質管制。華香園出版社，台北市。
8. 鄭長義。1991。飼料製造技術。華香園出版社。台北市。
9. 顏宏達。1988。養豬飼料學。華香園出版社。台北市。
10. Barnes, E. M., Mead, G. C. and Barnum, D. A. 1972. The intestinal flora of the chicken in the period 2 to 6 weeks of age, with particular reference to anaerobic bacteria. Br. Poult. Sci. 13: 611-626.
11. Barratt, M.E., Strachan, P.J. and Porter, P. 1979. Immunologically mediated nutritional disturbances associated with soya-protein antigens. Proc. Nutr. Soc. 38:143-150.
12. Dunsford, B.R., Knabe, D.A. and Haenly, W.E. 1989. Effect of dietary soybean meal on the microscopic anatomy of the small intestine in the early-weaned pig. J. Anim. Sci. 67:1855-1863.
13. Freitas, M., Tavan, E. Cayuela, C. Diop, L. Sapin, C. and Trugnan, G. 2003. Host-pathogens cross-talk. Indigenous bacteria and probiotics also play the game. Biol. Cell. 95:503-506.
14. Fuller, R. 1989. Probiotic in man and animals. J. Appl. Bacteriol. 66: 365-378.
15. Fuller, R. 1992. Probiotics: The Scientific Basis. Chapman and Hall, London.
16. Havenaar, R., Brink, B. T. and Huis, J. H. J. 1992. Selection of strains for probiotics use. In: R. Fuller. (Ed.). Probiotics: The Scientific Basis. P.209-222. Chapman and Hall, London.
17. Hong, K. J., Lee, C. H. and Kim, S. W. 2004. *Aspergillus oryzae* GB-107 fermentation improves nutritional quality of food soybeans and feed soybean meals. J Med Food. 4:430-435.
18. Hosoi, T., Ametani, A., Kiuchi, K. and Kaminogawa, S. 2000. Improved growth and viability of lactobacilli in the presence of *Bacillus subtilis* (natto), catalase, or subtilisin. Can. J. Microbiol. 46:892-497.
19. Hsu, A. and Allee, G. L. 1980. Limiting order of amino acids in alfalfa leaf protein concentrate. Abstracts, 72nd Annual Meeting, American Society of Animal Science. P.205.
20. Inooka, S. and Kimura, M. 1983. The Effect of *Bacillus natto* in Feed on the Sheep Red Blood Cell Antibody Response in Chickens. Avian Dis. 27:1086-89
21. Kiers, J.L., Meijer, J.C., Nout, M.J.R., Rombouts, F.M., Nabuurs, M.J.A. and van der Meulen, J. 2003. Effect of fermented soya beans on diarrhea and feed efficiency in weaned piglets. J. Appl. Microbiol. 95:545-552.
22. Komatsuzaki, T., Ohkuro, M. K. and Kuriyama, S. 1979. The difference in unspecifically immunizing effects among strains of natto bacilli. Jpn. J. Med. Sci. Biol. 99:343-345
23. Liener, I. E. 1981. Factors affecting the nutritional quality of soya products. J. Am. Oil. Chem. Soc. 58:406-415.
24. Liener, I. E. 1994. Implication of antinutritional components in soybean foods. CRC Crit Rev Food Sci Nutr. 34:31-67.
25. Lilly, D. M., and Stillwell, R. H. 1965. Probiotic: growth promoting factors produced by microorganisms. Science. 174: 747-748.
26. Liu, K. 1999. Soybeans : chemistry, technology, and utilization. Chapman and Hall, United States, P.25-411.
27. Mateos, G. G. and Lazaro, R. 2004. Whole soybeans in pigs' diets. American soybean association. P.12.
28. Murata, H., Yaguchi, H. S. and Namioka, S. 1977. Effects of administration of *Bacillus natto* upon weaning piglets. J. Jpn. Vet. Med. Assoc. 30:645-649
29. New, M. 1976. A review of shrimp and prawn nutrition. Aquaculture. 9: 277-285
30. O ' sullivan, M. G., Thornton, G., G. Osullivan, C. and Collins, J. K. 1992. Probiotic bacteria: myth or reality. Trends food technol. 3: 309-314.
31. Odunfa, S.A. 1986. Dawadawa. In:Reddy. N.R., Pierson, M.D. and Salunkhe, D.K. (Eds.), Legume-based Fermented Foods. CRC Press, Boca Raton, FL, USA, P.173-189.
32. Olguin, M. C., Hisano, N., D ' Ottavio, A. E., Zingale, M. I., Revelant, G. C. and Calderari, S. A. 2003. Nutritional and antinutritional aspects of an Argentinian soy flour

assessed on weanling rats. *Journal of Food Compo Anal.* 16:441-449. 33. Osawa, O. and Matsumoto, K. 1997. Digestion of staphylococcal enterotoxin by *Bacillus natto*. *Antonie Van Leeuwenhoek*, 71:307-311. 34. Ozawa, K., Yokota, H., Kimura, M. and Mitsuoka, T. 1981. Effect of administration of *Bacillus subtilis* strain BN on intestinal flora of weanling piglets. *Jpn. J. Vet. Sci.* 43:771-775. 35. Parker, R. B. 1974. Probiotic, the other half of the antibiotic story. *Anim. Nutr. Health.* 29:4-8. 36. Rambaud, J. C., Bouhnik, Y., Marteau, P. and Pochart, P. 1993. Manipulation of the human gut microflora. *Proc. Nutr. Sci.* 52: 357-366. 37. Sato, T., Yamada, Y., Ohtani, Y., Mitsui, N., Murasawa, H. and Araki, S. 2001. Production of menaquinone-7 (vitamin K2) by *Bacillus subtilis*. *J. Biosci. Bioeng.* 91:16-20. 38. Schwab, C. G. 1995. Protected proteins and amino acids for ruminants. In: *Biotechnology in Animal Feeds and Animal Feeding*. VCH. New York, United States, P.115. 39. Smith, H. W. 1965. The development of the flora of the alimentary tract in young animals. *J. Pathol. Bacteriol.* 90: 495-513. 40. Smith, J., Clawson, JR. A.J. and Barrick, E.R. 1976. Effect of ratio of protein from corn and soybean meal in diets of varying total protein on performance, carcass desirability and diet digestibility in swine. *J. Anim. Sci.* 26:752-758. 41. Sperti, G. S. 1971. *Probiotics*. West Point, CT: Avi Publishing Co. 42. Sumi, H. 1997. Antibacterial Activity of Bacil inhibition against *Escherichia coli* O157. *Bioindustry*. 14:47. 43. UNEP, 1999. "Agri Food Production; Facts and Figures". UNEP Industry and Environment, P.4-31. 44. USDA, 2003. "Oilseeds: World Markets and Trades". Circular series FOP 02-03 Foreign Agricultural Service. U.S. Department of Agriculture. 45. Zani, J.L., Weykamp da Cruz, F., Freitas dos Santos, A. and Gil-Turnes, C. 1998. Effect of probiotic CenBiot on the control of diarrhea and feed efficiency in pigs. *J. Appl. Microbiol.* 84: 68-71.