

牛初乳水解物之普拉斯丁產物在體外之免疫調節及細胞活性

顧洽任、張基郁

E-mail: 364879@mail.dyu.edu.tw

摘要

本研究用母牛分娩後第2天分泌之初乳，經Flavourzyme水解後，所得之初乳水解物，在pH 5和9條件下，加入proline以Flavourzyme進行普拉斯丁反應4、8及12小時後之普拉斯丁產物，與人類單核球細胞培養1及3天後之條件培養液(MNC-CM)，再進行U937細胞生長之抑制及免疫調節之活性試驗。結果顯示，在pH 9反應8小時之普拉斯丁產物(Pp-9-8)，在濃度800 μg/mL下，其對U937細胞生長之抑制效果及細胞激素之分泌量皆具有顯著之效果。另外以3種胺基酸(Tyrosine, Leucine, Glycine)在pH 9和反應8小時之條件下進行普拉斯丁反應，所得之產物(Tp-9-8, Lp-9-8, Gp-9-8)與Pp-9-8進行相同試驗之比較。結果顯示，Pp-9-8與Lp-9-8對U937細胞生長之抑制皆具有顯著之效果，且可顯著刺激細胞激素之分泌。本研究亦對Pp-9-8及Lp-9-8進行模擬腸胃道消化試驗，探討經由腸胃道酵素作用後，對U937細胞生長之抑制及免疫調節之功效。結果顯示，經模擬腸胃道之普拉斯丁產物，對U937細胞生長之抑制及細胞激素之分泌量均有降低之趨勢。

關鍵詞：牛初乳、普拉斯丁、白血病細胞株U937、細胞激素

目錄

目錄 封面內頁 簽名頁 中文摘要iii 英文摘要iv 誌謝v 目錄vii 圖目錄xi 表目錄xiii 符號說明xiv 1. 緒論1 2. 文獻回顧2 2.1 牛初乳2 2.1.1 牛乳蛋白之水解物應用4 2.2 牛初乳中的生物活性成分4 2.2.1 乳鐵蛋白4 2.2.2 生長因子5 2.2.3 細胞激素8 2.3 酵素水解11 2.3.1 蛋白質水解物之應用11 2.3.2 酵素基質比11 2.3.3 溫度與pH12 2.3.4 普拉斯丁反應及特性12 2.4 人類白血病細胞株U93713 3. 材料與方法15 3.1 實驗材料15 3.1.1 材料15 3.1.2 藥品16 3.1.3 重要儀器16 3.2 實驗方法與分析18 3.2.1 實驗流程18 3.2.2 基本組成成分分析19 3.2.3 脫脂牛初乳之水解與普拉斯丁反應21 3.2.4 普拉斯丁經由間接模式對U937細胞抑制用實驗22 3.2.5 統計分析27 4. 實驗結果28 4.1 牛初乳之一般組成分28 4.2 加入Proline之普拉斯丁產物之免疫調節及細胞活性30 4.2.1 加入Proline普拉斯丁產物經由間接抑制U937細胞生長試驗30 4.2.2 加入Proline之普拉斯丁之MNC條件培養液中IL-1之表現量33 4.2.3 加入Proline之普拉斯丁之MNC條件培養液中IFN- β 之表現量36 4.2.4 加入Proline之普拉斯丁之MNC條件培養液中TNF- α 之表現量39 4.2.5 加入Proline之普拉斯丁產物之MNC條件培養液中一氧化氮(NO)之含量42 4.2.6 加入Proline之普拉斯丁產物刺激人類單核球細胞之細胞生長率44 4.3 加入不同胺基酸之普拉斯丁產物之免疫調節及細胞活性47 4.3.1 加入不同胺基酸之普拉斯丁產物對U937細胞生長之間接抑制試驗47 4.3.2 加入不同胺基酸之普拉斯丁產物之條件培養液對IL-1之表現量之影響49 4.3.3 加入不同胺基酸之普拉斯丁產物之條件培養液對IFN- β 之表現量之影響51 4.3.4 加入不同胺基酸之普拉斯丁產物之條件培養液對TNF- α 之表現量之影響53 4.3.5 加入不同胺基酸之普拉斯丁產物之條件培養液中一氧化氮(NO)之含量55 4.3.6 加入不同胺基酸之普拉斯丁產物之條件培養液刺激人類單核球細胞1及3天後之細胞生長57 4.4 加入Proline及Leucine之普拉斯丁產物經模擬腸胃道後之免疫調節及細胞活性60 4.4.1 經模擬腸胃道之普拉斯丁產物對U937細胞生長之間接抑制試驗60 4.4.2 經模擬腸胃道試驗後之普拉斯丁產物對單核球細胞生長率及細胞激素與一氧化氮表現量之影響62 5. 討論69 5.1 普拉斯丁產物對U937細胞生長之間接抑制69 5.2 普拉斯丁產物對人類單核球細胞生長之效果與細胞激素及NO之表現量70 5.3 經模擬腸胃道試驗之普拉斯丁產物對U937細胞生長之抑制及免疫調節之活性72 6. 結論74 參考文獻76 圖目錄 圖3.1 本研究實驗流程圖18 圖4.1 普拉斯丁產物刺激人類單核細胞條件培養液1天對U937細胞抑制率之比較31 圖4.2 普拉斯丁產物刺激人類單核細胞條件培養液3天對U937細胞抑制率之比較32 圖4.3 普拉斯丁產物刺激人類單核細胞條件培養液1天所產生IL-1之表現量之比較34 圖4.4 普拉斯丁產物刺激人類單核細胞條件培養液3天所產生IL-1之表現量之比較35 圖4.5 普拉斯丁產物刺激人類單核細胞條件培養液1天產生IFN- β 之表現量之比較37 圖4.6 普拉斯丁產物刺激人類單核細胞條件培養液3天產生IFN- β 之表現量之比較38 圖4.7 普拉斯丁產物刺激人類單核細胞條件培養液1天所產生TNF- α 含量之比較40 圖4.8 普拉斯丁產物刺激人類單核細胞條件培養液3天所產生TNF- α 含量之比較41 圖4.9 普拉斯丁產物(Pp-9-8, Tp-9-8, Lp-9-8, Gp-9-8; 800 μg/mL)刺激人類單核細胞條件培養液1及3天後對U937細胞之生長抑制率48 圖4.10 普拉斯丁產物(Pp-9-8, Tp-9-8, Lp-9-8, Gp-9-8; 800 μg/mL)刺激人類單核細胞條件培養液1及3天後之IL-1 β 含量之比較50 圖4.11 普拉斯丁產物(Pp-9-8, Tp-9-8, Lp-9-8, Gp-9-8; 800 μg/mL)刺激人類單核細胞條件培養液1及3天後之IFN- β 含量之比較52 圖4.12 普拉斯丁產物(Pp-9-8, Tp-9-8, Lp-9-8, Gp-9-8; 800 μg/mL)刺激人類單核細胞條件培養液1及3天後之TNF- α 含量之比較54 圖4.13 普拉斯丁產物經模擬腸胃道後(Lp-9-8, Pp-9-8-GS)刺激人類單核細胞條件培養液1及3天對U937細胞生長之抑制率61 圖4.14 普拉斯丁產物經模擬腸胃道試驗後(Lp-9-8-GS與Pp-9-8-GS)刺激人類單核細胞培養液1及3天產生IL-1 β 含量之比較64 圖4.15 普拉斯丁產物經模擬腸胃道試驗後(Lp-9-8-GS與Pp-9-8-GS)刺激人類單核細胞培養液1及3天產生IFN- β 含量之比較65 圖4.16 普拉斯丁產物經模擬腸胃道試驗後(Lp-9-8-GS與Pp-9-8-GS)刺激人類單核細胞培養液1及3天產生TNF- α 含量之比較66

表目錄 表2.1牛初乳與常乳組成成分比較3 表2.2牛初乳於產後不同時間之TGF- 2、IGF-1 及GH之濃度7 表4.1母牛分娩後第2天之初乳之一般組成份29 表4.2加入proline之普拉斯丁產物刺激MNC條件培養液1及3天之一氧化氮(NO)含量43 表4.3加入proline普拉斯丁產物刺激人類單核球細胞1及3天之細胞生長率45 表4.4普拉斯丁產物(Pp-9-8, Tp-9-8, Lp-9-8, Gp-9-8 ; 800 μ g/mL)刺激MNC條件培養液1及3天之一氧化氮(NO)含量56 表4.5普拉斯丁產物(Pp-9-8, Tp-9-8, Lp-9-8, Gp-9-8 ; 800 μ g/mL)刺激人類單核球細胞1及3天之細胞生長率58 表4.6普拉斯丁產物經模擬腸胃道試驗後(Lp-9-8-GS與Pp-9-8-GS)刺激MNC條件培養液1及3天之一氧化氮(NO)含量67 表4.7普拉斯丁產物經模擬腸胃道試驗後刺激人類單核球細胞1及3天之細胞生長率68

參考文獻

- 參考文獻 1.宮華婷。2006。初乳之免疫調節作用及對人類白血病細胞(U937)之抑制效果。碩士論文，國立台灣大學食品科技研究所。 2.陳志璋。2006。微膠囊化牛初乳蛋白質水解物之抗氧化性。碩士論文，大葉大學生物產業科技研究所。 3.江淑華。2005。牛初乳及其酵素水解物之抗氧化性與其蛋白質組成之相關性研究。博士論文，大葉大學生物產業科技系。 4.宋晏仁，周純芬，謝文欽。2006。醫用免疫學。合記圖書出版社，台北。 5.戴達夫。2001。酵素生化科技。華榮圖書有限公司，台北。 6.藍以政。1998。血液疾病簡介。內科新知。 7.Albina, J. E., Cui, S., Mateo R. B., and Reichner, J. S. 1993. Nitric oxide-mediated apoptosis in murine peritoneal macrophages. *Journal of Immunology*, 150, 5080-5085. 8.Alem?鴨, A., P?臘ez-Sant?潘, E., Bordenave-Juchereau, S., Arnaudin, I., G?曠ez-Guill?聲, M. C., and Montero, P. 2011. Squid gelatin hydrolysates with antihypertensive, anticancer and antioxidant activity. *Journal of Food Research International*, 44, 1044 – 1051. 9.Alluwaimi. A. M. 2004. The cytokines of bovine mammary gland:prospects for diagnosis and therapy. *Research in Veterinary Science*. 77:211-222. 10.Amarowicz, R. and Shahidi, F. 1997. Antioxidant activity of peptide fractions of capelin protein hydrolysates. *Food Chemistry*, 58, 355 – 359. 11.Anderson, J. W., Johnstone, B. M., and Cook-Newell, M. E. 1995. Meta-analysis of the effects of soy protein intake on serum lipids. *New England Journal of Medicine*, 333, 276 – 282. 12.AOAC. 1995. Official method of analysis 14th ed. Association of Official Analytical Chemists, Washington, D.C., U.S.A. 13.Aoyama, T., Fukui, K., Takamatsu, K., Hashimoto, Y., and Yamamoto, T. 2000. Soy protein isolate and its hydrolysate reduce body fat of dietary obese rats and genetically obese mice (yellow KK). *Nutrition*, 16, 349 – 354. 14.Armstrong, W. B., Kennedy, A. R., Wan, X. S., Atiba, J., McLaren, C. E., and Meyskens, F. L. 2000. Single-dose administration of Bowman-Birk inhibitor concentrate in patients with oral leukoplakia. *Cancer Epidemiology, Biomarkers and Prevention*, 9, 43 – 47. 15.Aso, K., Yamashita, M., Arai, S., and Fujimaki, M. 1974. Hydrophobic Force as a Main Factor Contributing to Plastein Chain Assembly. *Journal of Biochemistry*, 76, 341-347. 16.Barbara, J. A., Ostade, X., and Lopez, A. 1996. Tumour necrosis factor-alpha (TNF-alpha): the good, the bad and potentially very effective. *Immunology Cell Biology*, 74, 434 – 43. 17.Belitz, H. D., Gorsch, W., and Schieberle, P. 2009. *Food chemistry*, 4th edn, 83-86. Heidelberg, Germany: Springer-Verlag Berlin Heidelberg. 18.Beutler, B., and Cerami, A. 1989. The biology of cachectin/TNF-a primary mediator of the host response. *Annual Review Immunology*, 7, 625 – 55. 19.Blum, J. W., and Hammon, H. 2000. Colostrum effects on the gastrointestinal tract, and on nutritional, endocrine and metabolic parameters in neonatal calves. *Livestock Production Science*, 66, 151 – 159. 20.Carswell, E. A., Old, L. J., Kassel, R. L., Green, S., Fiore, N., and Williamson, B. 1975. An endotoxin-induced serum factor that causes necrosis of tumors, Proc. Proceedings of the National Academy of Science of USA, 72, 3666 – 3670. 21.Chen, Y. Y., and Chang, H. M. 2004. Antiproliferative and differentiating effects of polysaccharide fraction from fu-ling (*Poria cocos*) on human leukemic U937 and HL-60 cells. *Food and Chemical Toxicology* : 759 – 769. 22.Chen, C. W., Chiang, S. H., Wang, S. Y., Lin, Y. T., and Chang, C. Y. 2011. Growth inhibition and differentiating effects of protein hydrolysates frombovine colostrums on human leukemic u937 cells. *Journal of Food Biochemistry ISSN* 1745-4514. 23.Clemente A. 2000. Enzymatic protein hydrolysates in human nutrition. *Trends Food Science and Technology*, 11, 254 – 62. 24.Damiens, E., Mazurier, J., Yazidi, I., Masson, M., Duthille, I., Spik, G., and Boilly-Marer, Y. 1998. Effects of human lactoferrin on NK cell cytotoxicity against haematopoietic and epithelial tumour cells. *Acta Biochimica et Biophysica*, 1402, 277-287. 25.Das, U. N. 2004. Anti-inflammatory nature of exercise. *Nutrition*, 20 (3), 323-326. 26.Einspanier, R. and Schams, D. 1991. Changes in concentrations of insulin-like growth factors 1 and2 and their binding proteins in human milk: Effect of heat treatment on IGF andIFG binding protein stability. *Journal of Dairy Research*, 58, 171 – 178. 27.Elfstrand, L., Lindmark-Mansson, L., Paulsson, M., Nyberg, L., and Akesson, B. 2002. Immunoglobulins, growth factors andgrowth hormone in bovine colostrums and the effects of processing. *International Dairy Journal*, 12, 879 – 887. 28.Estrada, C., Gbmez, C., Martin, C., Moncada S., and Gonzlez, C. 1992. Nitric oxide mediates tumor necrosis factor-alpha cytotoxicity in endothelial cells. *Biochemical and Biophysical Research Communications*, 186, 475-482. 29.Francis, G. L., Upton, F. M., Ballard, F. J., McNell, K. A., and Wallace, J. C. 1988. Insulin-like growth factors 1 and 2 in bovine colostrum. Sequences andbiological activities comparedwith those of a potent truncatedform. *Biochemical Journal*, 251, 95 – 103. 30.Fujimaki, M., Arai, S., and Yamashita, M. 1977. Enzymic protein degradation and resynthesis for protein improvement in food proteins. *American Chemical Society*, 156-187 31.Gamble J. R., Harlan J. M., Klebanoff S. J., and Vadas M. A. 1985. Stimulation of the adherence of neutrophils to umbilical vein endothelium by human recombinant TNF. *Proceedings of the National Academy of Science of USA*, USA, 82, 8667 – 71. 32.Gauthier, S. F., Pouliot, Y., and Saint-Sauveur, D. 2006. Immunomodulatory peptides obtained by the enzymatic hydrolysis of whey proteins. *International Dairy Journal*, 16, 1315 – 1323. 33.Gruen, J. R., and Weissman S. M. 1997. Evolving views of the major histocompatibility complex. *Blood*, 90, 4252 – 65. 34.Hettiarachchy, N. S., and Kalapathy, U. 1997. Soybean protein products. In K. Liu (Ed.). *Soybeans: Chemistry, technology, and utilization*, 379 – 411. 35.Hibbs, J. B., Vavrin, Z., and Taintor, R.R. 1987. L-arginine is required for expression of the activated

macrophage effector mechanism causing selective metabolic inhibition in target cells. *Journal of Immunology*, 13X, 550-565. 36.Hsu, K. C., Cheng, M. L., and Hwang, J. S. 2007. Hydrolysates from tuna cooking juice as an anti-hypertensive agent. *Journal of Food and Drug Analysis*, 15, 169 – 173. 37.Iigo, M., Kuhara T., Ushida, Y., Sekine, K., Moore, M. A., and Tsuda H. 1999. Inhibitory effects of bovine lactoferrin on colon carcinoma 26 lung metastasis in mice. *Clinical Experimental Metastasis*, 17, 35-40. 38.Jin, Y., Cox, D. A., Knecht, R., Raschdorf, F., and Cerletti, N. 1991. Separation, purification, and sequence identification of TGF-b1 and TGF-b2 from bovine milk. *Journal of Protein Chemistry*, 10, 3411 – 3416. 39.Jolles P, and Migliore-Samour D. 1986. Preparation of immunological agents by treating lipid-free bovine casein with proteolytic enzyme and fractionating the product. Patent Assignee: Rhone-Poulenc Sante. United States Patent 4, 851, 509, European Patent 170, 550. 40.Jolles P, Migliore-Samour D, and Parker F. 1988. Immuno stimulant substances derived from bovine casein and compositions containing the same. Patent Assignee: Rhone-Poulenc Sante. United States Patent 4, 777, 243. 41.Kakuta, I. 1998. Reduction of stress response in carp, *Cyprinus carpio* L, held under deteriorating environmental conditions, by oral administration of bovine lac-toferrin. *Journal of Fish Diseases*, 21, 161-167. 42.Kapel, N., Benhamou, Y., Buraud, M., Magne, D., Opolon, P., and Gobert, J. G. 1996. Kinetics of mucosal ileal gamma- interferon response during cryptosporidiosis in immuno- competent neonatal mice. *Parasitology Research*, 82, 664 – 667. 43.Kelly, G. S. 2003. Bovine colostrums: a review of clinical uses. *Alternative Medicine Review*, 8, 378 – 394. 44.Kim, S. E., Kim, H. H., Kim, J. Y., Kang, Y. I., Woo, H. J., and Lee, H. J. 2000. Anticancer activity of hydrophobic peptides from soy proteins. *BioFactors*, 12, 151 – 155. 45.Kito, M., Moriyama, T., Kimura, Y., and Kambara, H. 1993. Changes in plasma lipid levels in young healthy volunteers by adding an extruder cooked soy protein to conventional meals. *Bioscience. Biotechnology and Biochemistry*, 57, 354 – 355. 46.Kulkarni, P. R., and Pimpale, N. V. 1989. Colostrum – a review. *Indian Journal of Dairy Science*, 42, 216. 47.Kuprash, D. V., Tumanov, A. V., Liepinsh, D. J., Koroleva, E. P., Drutskaya, M. S., and Kruglov, A. A. 2005. Novel tumor necrosis factor-knockout mice that lack Peyer ' s patches. *European Journal of Immunology*, 35, 1592 – 1600. 48.Liao, H. F., Chou, C. J., Wu, S. H., Khoo, K. H., Chen, C. F., and Wang, S. Y. 2001. Isolation and characterization of an active compound from black soybean and its effect on proliferation and differentiation of human leukemic U937 cells. *Anticancer Drugs*, 841 – 846. 49.Liu, K. 2000. Expanding soybean food utilization. *Food Technology*, 54, 46 – 58. 50.Lotem, J., and Sachs, L. 1987. Regulation of cell-surface receptors for hematopoietic differentiation-inducing protein MGI-2 on normal and leukemic myeloid cells. *International Journal of cancer prevent*. 51.Ma, C. Y., Kung, H. T., Chen, J. N., Cheng, C. Y., Chang, H. M., and Wu, S. B. 2009. Effect of bovine colostrum on the growth inhibition and differentiation of human leukemic U937 cells. *Journal of Food Agricultural*, 89, 965-969. 52.Marino, M.W., Dunn, A., Grail, D., Inglese, M., Noguchi, Y., Richards, E., Jungbluth, A., Wada, H., Moore, M., Williamson, B., Basu, S., Old, L. J. 1997. Characterization of TNF-deficient mice. *Proceedings of National Academy of Science*, 94, 8093 – 8. 53.Manev, V., Maneva, A., and Sirakov, L. 1998. Effect of lactoferrin on the phagocytic activity of polymorphonuclear leucocytes isolated from blood of patients with autoimmune diseases and *Staphylococcus aureus* allergy. *Advances in experimental medicine and biology*, 443, 321-330. 54.Makhadze, N. J. 1998. TNF locus: genetic organisation, and biological implications. *Human Immunology*, 59, 571 – 9. 55.Marcotty, C., Franken, F., van Beeumen, J., Maghuijn-Rogister, G., and Hennen, G. 1991. Insulin-like growth factor 1 (IGF-1) from cow colostrum: Purification and characterization. *Growth Regulation*, 1, 56 – 61. 56.Mead, J. R., and You, X. 1988. Susceptibility differences to *Cryptosporidium parvum* infection in two strains of gamma interferon knockout mice. *J. Parasitol*. 84, 1045-1048. 57.Mendis, E., Rajapakse, N., and Kim, S. K. 2005. Antioxidant properties of a radical scavenging peptide purified from enzymatically prepared fish skin gelatin hydrolysate. *Journal of Agricultural and Food Chemistry*, 53(3), 581?587. 58.Mestecky, J., and McGhee, J. R. 1987. Immunoglobulin A molecular, and cellular interactions involved in IgA biosynthesis, and immune response. *Advances in Immunology*, 40, 135 – 245. 59.Migliore-Samour D, and Jolles P. 1988. Casein, a prohormone with an immunomodulating role for the newborn. *Experientia*, 44(3), 188-193. 60.Miyauchi, H., Hashimoto, S., Nakajima, M., Shinoda, I., Fukuwatari, Y., and Hayasawa, H. 1998. Bovine lactoferrin stimulates the phagocytic activity of human neutrophils: identification of its active domain, *Cellular Immunology*, 187, 34-37. 61.Moldoveanu, A. I., Shephard, R. J., and Shek, P. N. 2001. The cytokine response to physical activity and training. *Sports Medicine*, 31(2), 115-144. 62.Mosmann, T. R., Cherwinski, H., Bond, M.W., Giedlin, M. A., and Coffman, R. L. 1986. Two types of murine helper T cell clone: 1. Definition according to profiles of lymphokine activities and secreted proteins. *Journal of Immunology*, 136, 2348 – 2357. 63.Mosmann, T. R., and Coffman, R. L., 1989. TH1 and TH2 cells: different patterns of lymphokine secretion lead to different functional properties. *Annual Review of Immunology*, 7, 145 – 173. 64.Munker, R., and Koeffler, H. P. 1987. Tumor necrosis factor: recent advances. *Klin Wochenschr*, 65(8), 345-52. 65.Onozaki, K., Urawa, H., Tamatani, T., Iwamura, Y., Hashimoto, T., Baba, T., Suzuki H., Yamada, M., Yamamoto, S. and Oppenheim, J. J. 1988. Synergistic interactions of interleukin 1, interferon-beta, and tumor necrosis factor in terminally differentiating a mouse myeloid leukemia cell line(M1). Evidence that interferon-beta is an autocrine differentiating factor. *Journal of Immunology*. 66.Pakkanen, R., and Aalto, J. 1997. Growth factors and antimicrobial factors of bovine colostrum. *International Dairy Journal*, 7, 285 – 297. 67.Palmar, R. M. J., Bridge, L., Foxwell N. A., and Moncada, S. 1992. The role of nitric oxide in endothelial cell damage and its inhibition by glucocorticoids. *British Journal of Pharmacology*, 105, 11-12. 68.Pasparakis, M., Alexopoulou, L., Episkopou, V., and Kollias, G. 1996. Immune and inflammatory responses in TNF alpha-deficient mice: a critical requirement for TNF alpha in the formation of primary B cell follicles, follicular dendritic cell networks and germinal centers, and in the maturation of the humoral immune response, *Journal of Experimental Medicine*, 184, 1397 – 1411. 69.Payne, C. M., Bernstein, C., Bernstein, C., Bernstein, H., Gerner, E. W. and Garewal, H. 1999. Reactive nitrogen species in colon carcinogenesis. *Antioxids Redox Signal*, 1, 499-67. 70.Petersen, A. M., and Pedersen, B. K. 2005. The anti-inflammatory effect of exercise. *Journal of Applied Physiology*, 98(4), 1154-1162. 71.Pikarsky, E., Porat, R. M., Stein, I., Abramovitch, R., Amit, S., and Kasem, S. 2004. NF-kappaB functions as a tumour promoter in

inflammation-associated cancer. *Nature*, 431 : 461 – 466. 72.Platanias, L. C., and Vogelzang, N. J. 1990. Interleukin-1:biology, pathophysiology, and clinical prospects. *American Journal of Medicine*, 89, 621-9. 73.Playford, R. J. 2001. Peptide therapy and the gastroenterologist: colostrums and milk-derived growth factors. *Clinical Nutrition*, 20, 101 – 106. 74.Puddu, P., Carollo, M.G., Belardelli, F., Valenti, P., and Gessani, S., 2007. Role of endogenous interferon and LPS in the immunomodulatory effects of bovine lactoferrin in murine peritoneal macrophages, *Journal of Leukocyte Biology*, 82, 347-353. 75.Reiner, S. L., and Seder, R. A., 1995. T helper cell differentiation in immune response. *Current Opinion in Immunology*, 7, 360 – 366. 76.Rudolf, A., Alkasrawi, M., Zacchi, G., and Lid?聳, G. 2005. A comparison between batch and fed-batch simultaneous saccharification and fermentation of steam pretreated spruce. *Enzyme and Microbial Technology*, 37(2), 195-204. 77.Rutkowski, P., Kaminska, J., Kowaksa, M., and Steffen, J. 2003. Cytokine and cytokine receptor serum levels in adult bone sarcoma patients: correlation with local tumor extent and prognoses. *Journal of Surgical Oncology*, 84, 151-159. 78.Scammell, A. W. 2001. Production and uses of colostrum. *Australian Journal of Dairy Technology*, 56, 74 – 82. 79.Sekine, K., Watanabe, E., Nakamura, J., Takasuka, N., Joong, D. K., Asamoto M., Krutovskikh V., Baba-Toriyama H., Ota T., Moore M. A., Masuda M., Sugimoto H., Nishino H., Kakizoe T. and Tsuda H. 1997. Inhibition of azoxymethane-initiated colon tumor by bovine lactoferrin administration in F344 rats. *Japan Journal of Cancer Research*, 88, 523-526. 80.Shamay, A., Cohen, N., Niwa, M. and Gertler, A. 1988. Effects of insulin-like growth factor I on dcoxyribonucleic acid synthesis and galactopocis in bovine undifferentiated and lactating mammary tissue. *Endocrinology*, 126, 804-809. 81.Shau, H., Kim, A., and Golub, S. H. 1992. Modulation of natural killer and lymphokine- activated killer cell cytotoxicity by lactoferrin, *Journal of Leukocyte Biology*, 51, 343-349. 82.Siemensma, A. D., Weijer, W. J., and Bak, H. J. 1993. The importance of peptide lengths in hypoallergenic infant formulae. *Trends in Food Science and Technology*, 4, 16-21. 83.Smith, L. M., Bonafonte, M. T., and Mead, J. R. 2000. Cytokine expression and specific lymphocyte proliferation in two strain of Cryptosporidium parvum-infected gamma-interferon knockout mice. *Journal of Parasitology*, 86, 300 – 307. 84.Sukan, G., and Andrews, A. T. 1982. Application of the plastein reaction to caseins and to skim milk powder II. Chemical and phy-sical properties of the plasteins. *Journal of Dairy Research*, 49, 279-293 85.Sun, Y., and Cheng, J. Y. 2002. Hydrolysis of lignocellulosic materials for ethanol production: a review. *Bioresource Technology*, 83, 1-11. 86.Sutas Y., Soppi E., Korhonen H. 1996. Suppression of lymphocyte proliferation in vitro by bovine caseins hydrolyzed with *Lactobacillus casei* GG-derived enzymes. *Journal of Clinical Immunology*, 98(1), 216-224. 87.Takakura, N., Wakabayashi, H., Yamauchi, K., and Takase, M., 2006. Influences of orally administered lactoferrin on IFN-gamma and IL-10 production by intestinal intraepithelial lymphocytes and mesenteric lymph-node cells, *Biochemistry Cell Biology*, 84, 363-368. 88.Tamatani, T., Kimura, S., Hashimoto, T., and Onozaki. 1989. Purification of guinea pig tumor necrosis factor (TNF) : comparsion of its antiproliferative and differentiative activities for myeloid leukemic cell lines with those of recombinant human TNF. *Journal of Biochemistry*, 105(1), 55-60. 89.Tokuyama, Y., and Tokuyama, H. 1993. Purification and identification of TGF-b2-relatedgrowth factor from bovine colostrum. *Journal of Dairy Research*, 60, 99 – 109. 90.Wang, W., and Mejia, E. G. 2005. A new frontier in soy bioactive peptides that may prevent age-related chronic diseases. *Comprehensive Reviews in Food Science and Food Safety*, 4, 63 – 78. 91.Wang, W. P., Iigo, M., Sato, J., Sekine, K., Adachi, I., and Tsuda, H. 2000. Activation of intestinal mucosal immunity in tumor-bearing mice by lactoferrin. *Janpan, Journal of Cancer Reserch*, 91, 1022-1027. 92.Wergedahl, H., Liaset, B., Gudbrandsen, O. A., Lied, E., Espe, M., and Muna, Z. 2004. Fish protein hydrolysate reduces plasma total cholesterol, increases the proportion of HDL cholesterol and lowers acyl-CoA: cholesterol acyltransferase activity in liver of Zucker rats. *Journal of Nutrition*, 134, 1320 – 1327. 93.Wu, H. C., Chen, H. M., and Shiao, C. Y. 2003. Free amino acids and peptides as related to antioxidant properties in protein hydrolysates of mackerel (*Scomber austriasicus*). *Food Research International*, 36, 949 – 957. 94.Wyatt, C. R., Brackett, E. J., and Savidge, J. 2001. Evidence for the emergence of a type-1-like immune response in intestinal mucosa of calves recovering from cryptosporidiosis. *Journal of Parasitol*, 87, 90 – 95. 95.Xu, R. J., Mellor, D. J., Birtles, M. J., Breier, B. H., and Gluckman, P. D. 1994. Effects of oral IGF-I or IGF-II on digestive organ growth in newborn piglets. *Biology of the Neonate*, 66, 280-287. 96.Yamanaka, H., Hagiwara, k., Kirisawa, R., and Iwai, H. 2003. Proinflammatory cytokines in bovine colostrum potentiate the mitogenic response of peripheral blood mononuclear cells from newborn calves through IL-2 and CD25 expression. *Microbiology of Immunology*, 47(6), 461 – 468. 97.Zablocka, A., Janusz, M., Macala, J., and Lisowski, J. 2007. A proline-rich polypeptide complex (PRP) isolated from ovine colostrum. Modulation of H₂O₂ and cytokine induction in human leukocytes. *International Immunology*, 7,981-988. 98.Zhao, X. H., and Li, Y. Y. 2009. An approach to improve ACE inhibitory activity of casein hydrolysates with plastein reaction catalyzed by Alcalase. *European Food Research and Technology*, 229, 795 – 805. 99.Zhao, X. H., Wu, D., and Li, T. J. 2010. Preparation and the radical scavenging activity of papain catalyzed casein plasteins. *Dairy Science and Technology*, 90, 521 – 535. 100.Ziegler, F., Ollivier, J. M., Cynober, L., Masinin, J. P., Coudray-Lucas, C., Levis, E., and Giboudeau, J. 1990. Efficiency of enteral nitrogen support in surgical patients: small peptides v non-degraded proteins. *Gut*, 31, 1277-1283