LAPORAN AKHIR

Penelitian Unggulan Perguruan Tinggi (U)

PANGAN NUTRASETIKA ALAMI BERBASIS BIOAKTIF PEPTIDA SUSU KAMBING UNTUK TERAPI SEHAT RHEUMATOID ARTHRITIS: KAJIAN NUTRIGENOMIK

Tahun ke-2 dari rencana 3 tahun

Ketua	**:**	**Prof. Fatchiyah, M.Kes., PhD.**	**0027116307**
Anggota	**:**	**1. Dr. Suharjono, MS.**	**0023026307**
		2. Dr. dr. Zairin N.H, Sp.OT(K), MM.FIC S	**0020116111**
		3. Bambang S., S.Ked., M.Biomed	**0009037902**

Dibiayai oleh :
Direktorat Jenderal Pendidikan Tinggi,
Kementerian Pendidikan dan Kebudayaan, Melalui DIPA Universitas Brawijaya
Nomor : DIPA-023.04.2.414989/2013, Tanggal 5 Desember 2012, dan berdasarkan
SK Rektor Universitas Brawijaya Nomor : 153/SK/2013 tanggal 28 Maret 2013

UNIVERSITAS BRAWIJAYA
DESEMBER 2013

HALAMAN PENGESAHAN

Judul	: Pangan Nutrasetika Alami Berbasis Bioaktif Peptida Susu Kambing untuk Terapi Sehat Rheumatoid Arthritis: Kajian Nutrigenomik
Peneliti/Pelaksana	
Nama Lengkap	: Prof. Fatchiyah, M.Kes., Ph.D.
NIDN	: 0027116307
Jabatan Fungsional	: Guru Besar
Program Studi	: Biologi
Nomor HP	: 082142583774
Alamat surel (e-mail)	: fatchiya@ub.ac.id
Anggota (1)	
Nama Lengkap	: Dr. Suharjono, MS.
NIDN	: 0023026307
Perguruan Tinggi	: Universitas Brawijaya
Anggota (2)	
Nama Lengkap	: Dr.dr.Zairin N.H, Sp.OT(K), MM.FIC S
NIDN	: 0020116111
Perguruan Tinggi	: Universitas Lambung Mangkurat
Anggota (3)	
Nama Lengkap	: Bambang S., S.Ked., M.Biomed.
NIDN	: 0009037902
Perguruan Tinggi	: Universitas Lambung Mangkurat
Tahun Pelaksanaan	: Tahun ke-2 dari rencana 3 tahun
Biaya Tahun Berjalan	: Rp. 300.000.000,-

Malang, 4 Desember 2013

Mengetahui,
Dekan/Ketua

(Prof. Dr. Marjono, M.Phil)
NIP. 19621116 198803 1 004

Ketua Peneliti,

(Prof. Dra. Fatchiyah, M.Kes., Ph.D)
NIP. 19631127 198903 2 001

Menyetujui,
Pjs.Ketua LPPM UB

(Prof.Dr.Ir. Siti Chuzaemi, MS.)
NIP.19530514 198002 2 001

Biaya Keseluruhan : Rp. 1.450.000.000,-

ABSTRAK

Hasil penelitian tahapan pertama telah ditetapkan bahwa kandungan lemak tertinggi pada yogurt fermentasi dengan starter komersial, jauh di atas susu segar. Kandungan karbohidrat pada yogurt fermentasi dengan starter komersial lebih rendah sedikit dibanding susu segar. Bila pada kadar lemak dan karbohidrat meningkat, maka pada kadar protein menurun pada setiap proses fermentasi dan terendah pada pada yogurt fermentasi dengan starter komersial. Pita protein dengan BM 36kDa terdapat pada susu segar dan yogurt Kambing PE dan tidak dijumpai pada susu sapi segar. Pita ini telah diidentifikasi sebagai protein α-S2 Casein pada susu segar dan yogurt keculai pada feremnatasi ganda di BM yang sama terindetifikasi sebagai protein α-S1 casein. Peptida yang teridentifikasi pada protein α-S2 Casein susu segar kambing PE yang sepsifik adalah 205AMKPWTQPK-213, dan 214-TNAIPYVRYL -223. Peptida yang teridentifikasi pada protein α-S2 Casein yogurt kambing PE yang spesifik adalah 169-LTEEEKNR-176, dan 214-TNAIPYVR-221. Hasil preleminary studi, dugaan kami pada daerah bioaktif 169-LTEEEKNR-176 adalah bioaktif peptida yang memiliki fungsi antimikrobial, dan bioaktif peptida 205 – 223 memiliki fungsi immunomodulator pada *rhemathoid arthitis* (RA). Tujuan penelitian pada tahap kedua meliputi pengujian ekspresi gen mekanismenya secara *in vitro* dan *in vivo* (bioassay) kajian nutrigenomik dan pengujian immunomodulator serta immunoalergen bioaktif peptida spesifik susu kambing. Pada tahun kedua ini, in vitro dengan menggunakan sel osteoblast (Cell line), dan in vivo atau bioassay menggunakan hewan model Tikus (*Rattus norvegicus*, galur wistar) umur 10-12 minggu dengan berat badan 150 g dan diberi perlakuan rheumatoid arthritis *Complete Freud Adjuvant* dan tikus normal sebagai kontrol. Selanjutnya dilakukan terapi dengan bioaktif peptida spesifik dan fungsi immunomodulator diuji melalui pemeriksaan: radikal bebas MDA (malonaldialdehid) dan SOD (superoksid dismutase), anatomi hewan coba dan histopatologis dengan HE. Hasil penelitian ini menunjukkan bahwa Bioaktif peptide protein alpha-S2casein susu kambing dan produknya mempunyai fungsi perbaikan persendian diduga sebagai fungsi immunomodulator. Selain itu, Bioaktif peptide protein alpha-S2casein susu kambing dan produknya mempunyai struktur secara kimia atau fisik mempunyai fungsi sebagai modulator ion chanel sel, berikatan dengan reseptor dalam inti sel atau dapat menghambat beberapa aktivitas enzim. Dan Bioaktif peptide protein alpha-S2casein susu kambing dan produknya berfungsi dapat meningkatkan proliferasi sel-sel preosteoblast dapat mengontrol stress oksidatif, ekspresi ROS dan MDA.

Keywords: nutrasetika, bioaktif peptida, gen-gen spesifik, susu kambing, rheumatoid arthritis

ABSTRACT

The results of the first phase of the study has been determined that the highest fat content in yogurt fermented with commercial starter , well above the fresh milk . Carbohydrate content in yogurt fermented with commercial starter slightly lower than fresh milk . When the levels of fat and carbohydrates increased , then the protein content decreased in every process of fermentation and the lowest in yogurt fermented with commercial starter . 36kDa molecular weight protein bands present in fresh milk and yogurt Goat PE and are not found in cow's milk . This tape has been identified as α - S2 Casein protein in fresh milk and yogurt except the double feremnatasi terindetifikasi BM same as α - S1 casein protein . Peptides identified in the protein α - S2 Casein fresh goat 's milk which is sepsifik AMKPWTQPK - 205 - 213 , and -223 214-TNAIPYVRYL . Peptides identified in the protein α - S2 Casein specific goat yogurt is LTEEEKNR - 169 - 176 , and 214 - 221 - TNAIPYVR . The results of the study preleminary , we conjecture the bioactive region LTEEEKNR - 169 - 176 is a bioactive peptide that has antimicrobial function , and bioactive peptide 205-223 has the function of immunomodulators in rhemathoid arthitis (RA) . The purpose of the study in the second stage involved testing the mechanism of gene expression in vitro and in vivo (bioassays) study of nutrigenomics and testing of bioactive peptide immunomodulator and immunoalergen specific goat's milk . In the second year , in vitro using osteoblast cells (Cell line) , and in vivo animal models or bioassay using rats (Rattus norvegicus , Wistar strain) aged 10-12 weeks with a weight of 150 g and rheumatoid arthritis treated with Complete Freud's Adjuvant and normal mice as a control . Further therapy with specific bioactive peptide immunomodulator and function tested through examination : free radicals MDA (malonaldialdehid) and SOD (superoxide dismutase) , animal anatomy and histopathologic with HE . The results of this study indicate that the bioactive peptide- protein alpha - S2casein goat's milk and its products have improved joint function is suspected as a function of immunomodulator . In addition, bioactive protein peptide alpha - S2casein goat's milk and its products have the chemical or physical structure has a function as a modulator of ion channel cell , binds to receptors in the cell nucleus or can inhibit some enzyme activity . Bioactive peptides and proteins alpha - S2casein goat's milk and its products serve to increase the proliferation of cells pre - osteoblasts can control oxidative stress , the expression of ROS and MDA .

Keywords : nutrasetic , bioactive peptides , specific genes , goat 's milk , rheumatoid arthritis

RINGKASAN

Roadmap penelitian ini merupakan salah satu bagian *roadmap* yang dikembangkan di beberapa fakultas atau program studi kajian ilmu hayati terkait dengan pemanfaatan potensi sumber daya lokal untuk pengembangan terapi sehat penyakit dengan bahan alami nabati dan hewani. Salah satu kajian bahan hewani adalah pengembangan susu kambing sebagai bahan pangan nutrasetika dan digunakan sebagai immunomodulator, immunoalergen dan nutrigenomik terapi penyakit rheumatoid arthritis. Beberapa hasil penelitian melaporkan bahwa susu kambing memilki keunggulan dibandingkan susu sapi terutama ditinjau dari aspek kesehatan. Susu kambing mengandung 35% lemak rantai sedang (medium chain fatty acids) sehingga aman dikonsumsi terutama bagi penderita hiperkolesterol. Selain itu protein susu kambing memiliki komposisi asam amino yang berbeda dengan susu dari mamalia lainnya dan tidak menyebabkan reaksi alergi. Protein susu kambing juga dapat meningkatkan immunitas karena mengandung protein yang bersifat immunomodulator. Hasil penelitian tahapan pertama telah ditetapkan bahwa kandungan lemak tertinggi pada yogurt fermentasi dengan starter komersial, jauh di atas susu segar. Kandungan karbohidrat pada yogurt fermentasi dengan starter komersial lebih rendah sedikit dibanding susu segar. Bila pada kadar lemak dan karbohidrat meningkat, maka pada kadar protein menurun pada setiap proses fermentasi dan terendah pada pada yogurt fermentasi dengan starter komersial. Kesimpulan sementara hasil riset tengah tahun kedua adalah sebagai berikut : Bioaktif peptide protein alpha-S2casein susu kambing dan produknya mempunyai fungsi perbaikan persendian diduga sebagai fungsi immunomodulator. Bioaktif peptide protein alpha-S2casein susu kambing dan produknya mempunyai struktur secara kimia atau fisik mempunyai fungsi sebagai modulator ion chanel sel, berikatan dengan reseptor dalam inti sel atau dapat menghambat beberapa aktivitas enzim. Bioaktif peptide protein alpha-S2casein susu kambing dan produknya berfungsi dapat meningkatkan proliferasi sel-sel pre-osteoblast dapat mengontrol stress oksidatif.

SUMMARY

Roadmap of this study is one part of the roadmap developed in several faculties or courses related to life science studies the potential use of local resources for the healthy development of disease therapies with natural vegetable and animal materials . One animal study materials is the development of goat 's milk as food nutrasetika and used as an immunomodulator , and nutrigenomics immunoalergen therapy of rheumatoid arthritis . Several studies reported that goat milk than cow milk has an advantage , especially in terms of health aspects . Goat's milk contains 35 % medium chain fatty (medium chain fatty acids) that are safe for consumption , especially for patients with hypercholesterolemia. Moreover goat milk proteins have different amino acid composition of the milk of other mammals and do not cause allergic reactions. Goat milk proteins can also improve immunity because it contains proteins that are immunomodulators . The results of the first phase of the study has been determined that the highest fat content in yogurt fermented with commercial starter , well above the fresh milk . Carbohydrate content in yogurt fermented with commercial starter slightly lower than fresh milk . When the levels of fat and carbohydrates increased , then the protein content decreased in every process of fermentation and the lowest in yogurt fermented with commercial starter. Conclusion The results of the research while the second half is as follows : Bioactive peptide protein alpha - S2casein goat's milk and its products have improved joint function is suspected as a function of immunomodulator . Bioactive protein peptide alpha - S2casein goat's milk and its products have the chemical or physical structure has a function as a modulator of ion channel cell , binds to receptors in the cell nucleus or can inhibit some enzyme activity . Bioactive protein peptide alpha - S2casein goat's milk and its products serve to increase the proliferation of cells pre - osteoblasts can control oxidative stress.

DAFTAR PUSTAKA

Alferez M.J.M., Aliaga I.L., Barrionuevo M. and Campos M.S. 2003. Effect of Dietary Inclusión of Goat Milk on the Bioavailability of Zinc and Selenium in Rats. J. Dairy Research (2003) 70 181-187.

Aliaga I.L., Alferez M.J.M., Nestares M.T., Ros P.B., Barrionuevo M., and Campos M.S. 2005. Goat Milk Feeding Causes an Increase in Biliary Secretion of Cholesterol and a Decrease in Plasma Cholesterol Levels in Rats. J. Dairy Sci. 88: 1024-1030.

Allen TC, 1994. Hematoxylin and eosin. In (Prophet EB, Mills B, Arringtom JB, Sobin LH, eds). Laboratory methods in histotechnology. Washington : Armed Forces Institute of Pathology, pp 53-58.

Allen TC, 1994. Mounting media. In (Prophet EB, Mills B, Arringtom JB, Sobin LH, eds). Laboratory methods in histotechnology. Washington : Armed Forces Institute of Pathology, pp 59-61.

AOAC. 1984. Official Methods of Analysis: Association of Official Analytical Chemists, Volume Two, Fivetenth Edition, The Association of Official Analytical Chemists, Inc. Virgiana.Aulanni'am. 2005. Protein & Analisisnya. Citra Mentari Group, Malang. ISBN 978508423-X

Ausubel FM, Brent R, Kingston, Moore DD, Seidman JG, Smith JA, Struhl K, 1995. Short protocol in molecular biology. Third edition, John Willey & Sons, Inc : 10.1-10.48; 11.5-11.7.

Budiarti Ismi Kurnia, Masdiana C. Padaga*, Fatchiyah*. 2013. Nutritional Composition and Protein Profile of Goat Yogurt PE with Double Culture between *Streptococcus thermophilus* and *Lactobacilus* species. Cukurova Med J. 38 (4): 681-686

Chen W.Y., Weng M.H., Chen S.E., Peh H.C., Liu W.B., Yu T.C., Huang M.C., Chen M.T., Nagahata H., and Chang C.J. 2007. Profile of Gelatinolytic Capacity of Raw Goat Milk and the Implications for Milk Quality. J. dairy Sci. 90:4954-4965.

Ciptadi, M. Nasich, Mudawamah, A. Priyanti dan b. Setyadi. 2001. Reproduksi manipulasi hormonal untuk perbaikan reproduksi ternak kambing yang ekonomis dan dapat diterapkan pada peternakan kambing rakyat di Jawa Timur. Laporan Penelitian Unibraw-Litbang Pertanian, Jakarta

Choy EHS and Panayi GS. 2001. Cytokine pathway and Joint Imflamation of RA. N Engl J Med, 344(12): 907-1006.

Clare D.A., and Swaisgood H.E. 2000. Bioactive Milk Peptides: a Prospectus, J Dairy Sci, 83, 1187–1195.

Cross M.L. and Gill H.S. 2000. Immunomodulatory Properties of Milk. British Journal of Nutrition, 84. Suppl. 1, S81-S89

Dinarello CA, Moldawer LL, 2000. Proinflamatory and anti-inflamatory cytokines in rheumatoid arthritis. Second edition, Thousand Oaks : Amgen, pp 111-118.

Fatchiyah, EL Arumingtyas, S. Widyarti dan S. Rahayu. 2009. Buku: Teknik Dasar Analisis Biologi Molekuler. Penerbit Faperta-LSIH UB. Malang.

Fatchiyah, Nurmasari DA, Ichsan M, Hilman M., Sanjaya P. 2008. Studi Pendahuluan: penentuan kadar porang, suweg dan iles-iles terhadap penurunan gula darah tikus diabet. Universitas Brawijaya. Malang.

Fatchiyah, Aulia Nurmasari, DA., Mustofa I, Firdausi L., Soeatmadji, DW. 2009. Peran Glucomannan Terhadap Kadar Gula Dan Ekspresi Gen Proinsulin Pada Tikus Diabetes: Kajian Nutrigenomik. Univ. Brawijaya. Malang (StragNas, 2009).

Fatchiyah. 2010. Buku: Nutrigenomik dalam Bahasa DNA. Penerbit Faperta UB. Malang

Fatchiyah, Masdiana C. Padaga, Nur Hidayat, Nur Permatasari, and Firli Rahmah Primula Dewi. 2013. Biological Function of Alpha-S2 Casein Peptides from Ethawah Goat Breed Dairy Milk Protein binds Calmodulin. J Dairy Science: (*Submitted*)

Fiat A.M., Samour D.M. and Jolles P. 1993. Biologically Active Peptides from Milk Proteins with Emphasis on two Examples Concerning Antithrombotic and Immunomodulating Activities. J. Dairy Sci 76:301-310

Fishman and S. Bar-Yehuda. 2010. Rheumatoid Arthritis: History, Molecular Mechanisms and Therapeutic Applications. Adenosine Receptors from Cell Biology to Pharmacology and Therapeutics, DOI 10.1007/978-90-481-3144-0_15. Springer Science+Business Media B.V. 2010.

FitzGerald RJ, and Meisel H. 2003. Caseinophosphopeptides (CPPs) as functional ingredients. In Functional dairy products Edited by Tiina Mattila-Sandholm and Maria Saarela. Woodhead Publishing Ltd and CRC Press LLC.

Farrell, H.M.; Malin, E.L.; Brown, E.M.; Mora-Gutierrez A. Review of The Chemistry of Alphas2-Casein And The Generation of A Homologous Molecular Model to Explain Its Properties. *J Dairy Sci*. **2009**, 92, 1338-53.

Geerlings A., Viliar I.C., Zarco F.H., Sanchez M., Vera R., Gomez A.Z. 2006. Identification and Characterization of Novel Angiotensin-Converting Enzyme Inhibitors Obtained from Goat Milk. J. Dairy Sci. 89: 3326-3335.

Gavino, V.C., James S. Miller, Samuel 0. Ikharebha, George E. Milo, David *G.* Cornwell. 1981. Effect of polyunsaturated fatty acids and antioxidants on lipidp eroxidation in tissue cultures. Journal of Lipid Research. Volume 22 :763-769

Gill H.S., Cross M.L., Rutherfurd K.J. and Gopal, P.K. 2001a. Dietary Probiotic Supplementation to Enhance Cellular Immunity in the Elderly. *Brit J Biomed Sci*, 58, 94–96.

Gill H.S., Darragh A.J. and Cross M.L. 2001b. Optimising Immunity and Gut Function in the Elderly. *J Nutr, Health Ageing*, 5, 80–91.

Gill H.S., Doull F., Rutherfurd K.J. and Cross M.L. 2000, Immunoregulatory Peptides in Milk. *Br J Nutr*, 84 (supplement 1), 111–117.

Gill H.S., Doull F., Rutherfurd K.J., and Cross M.L. 2000. Immunoregulatory Peptides in Bovine Milk. British Journal of Nutrition, 84. Suppl. 1, S81-S89

Gill H.S., Rutherfurd K.J. and Cross M.L. 2001c. Dietary Probiotic Supplementation Enhances Natural Killer Cell Activity in the Elderly: an Investigation of AgeRelated Immunological Changes. *J Clin Immunol*, 21, 264–271.

Gill H.S., Rutherfurd K.J., Cross M.L. and Gopal P.K. 2001d. Enhancement of Immunity in the Elderly by Dietary Supplementation with the Probiotic *Bifidobacterium lactis* HN019. *Am J Clin Nutr*, 74, 833–839.

Gill H.S., Rutherfurd, K.J., and Cross, M.L. 2000. Bovine milk : A Unique Source of Immunomodulatory Ingredients for Functional Foods. In Funtional Foods II – Claims and Evidence. Pp 82-90. Cabridge: Royal Society of Chemistry Press.

Goldsby RA, Kindt TJ, Osborne BA, 2000. Kuby Immunology. Fourth edition, New York : W.H. Freeman and Company, pp 1-514.

Haenlein G.F.W. 2001. The Many Medicinal Benefits of Goat Milk. Countryside and Small stock journal; Nov/Dec 2001; 85, 6.

Hall J, 1994. Embedding. In (Prophet EB, Mills B, Arringtom JB, Sobin LH, eds). Laboratory methods in histotechnology. Washington : Armed Forces Institute of Pathology, pp 39-43.

Haryono D. 2008. Mekanisme Immunoneuromodulasi Teknik "Assited Drainage" pada Reaksi Tikus Alergi yang Terpapar Lipopolisakarida Phorpyromonas gingivalis. Disertasi.

Korhonen H., Pihlanto-Leppälä A., Rantamäki P. and Tupasela T. 1998. Impact of Processing on Bioactive Proteins and Peptides. Trends Food Sci Technol, 8, 307– 319.

Khoiriyah Lulus K. & *Fatchiyah*. 2013. Characterization of Biochemistry & Protein Profile of Goat Milk Fermented by BAL. *JELS 2 (1)1-6*

Lamothe S, Robitaille G, St-Gelais D, and Britten M. 2007. Short Communication: Extraction of ß-casein from Goat Milk. J. Dairy Sci. 90:5380-5382.

Leporanta K. 2001. Developing Fermented Milks into Functional Foods. Innov Food Technol, 10, 46–47.

Marriot B.M. 2000. Functional foods : an ecological perspective. Am.J.Clin.Nutr. 71(suppl):1728s-1734s.

Maryam R, 2007. Metoda deteksi mikotoksin. J. Mikol. Ked. Indon. 7(1-2): 12-24

McCullough F.S.W. 2003. Nutritional Evaluation of Goat's Milk. British Food Journal; 2003; 105, 4/5.

Miller G.D., Jarvis J.K., and Mcbean L.D. 2000. Handbook of Dairy Foods and Nutrition. second edition, Boca Raton, London, New York, Washington DC, CRC Press.

Moeljanto, Rini Damayanti dan Wiryanta, B.T. Wahyu, Tahun 2002, Khasiat dan Manfaat; Susu Kambing, Agromedia Pustaka, Depok.

Padaga M, Savitry ME dan Murwani S., 2010. Potensi Protein Spesifik Susu kambing sebagai Immunomodulator dan Immunogen : Upaya Pengembangan Pangan Nutrasetika. Laporan Penelitian Unibraw. Malang.

Patil NS., Pashine A, Belmares MP., Liu W, Kaneshiro B, Rabinowitz J, McConnell B, and Mellins ED. 2001. Rheumatoid Arthritis (RA)-Associated HLA-DR Alleles Form Less Stable Complexes with Class II-Associated Invariant Chain Peptide Than Non-RA-Associated HLA-DR AllelesJ Immunol;167;71577168

Pins J. and Keenan J.M. 2002. The Antihypertensive Effects of a Hydrolysed Whey Protein Isolate Supplement (BioZate1®). Cardiovasc Drugs Ther, 16 (Suppl 1), 68.

Rooney, D.E., and Czepulkowski, B. H., 1992 in Czepulkowski, B. H., 2001, *Analyzing Chromosomes* : *The Basic from Background to Bench.* BIOS Scientific Publishers Limited

Saleh, Eniza. 2004. Teknologi Pengolahan Susu dan Hasil Ikutan Ternak. Program Studi Produksi Ternak, Fakultas Pertanian Univ. Sumatera Utara. Sumatera.

Saxelin M., Korpela R., and Makinen A.M., 2003. Classifying Functional Dairy Products. In Functional dairy products Edited by Tiina Mattila-Sandholm and Maria Saarela. Woodhead Publishing Ltd and CRC Press LLC

Sodiq, A. Dan Abidin, Z.,Tahun 2002, *Kambing Peranakan Etawa; Penghasil susu Berkhasiat Obat,* Agromedia Pustaka Depok

Saltiel AR. 2001. New perspective into the molecular pathogenesis and treatment of the type 2 diabetes. Cell, 104: 517-529. P.

Tabatabai L.B. 2004. Good News for Goat's Milk Drinkers. Agri Research; 52, 10.

Tidona, F.; Criscione, A.; Guastella A.M.; Zuccaro, A.; Bordonaro, S.; Marletta, D. Bioactive Peptide in Dairy Product. *Ital. J. Anim. Sci.* **2009**, 8, 315-340.

Tomotake H., Okuyama R., Katagiri M., Fuzita M., Yamato M., and Ota F. 2006. Comparison Between Holstein Cow's Milk and Japanese-Saanen Goat's milk in Fatty Acid Composition, Lipid Digestibility and Protein Profile. Biosci, Biotechnol. Biochem., 70 (11), 2771-2774.

Xi-Ming Xu, Jie-Ping yu, Xiao_fei he, Jun-Hua Li, Liang-Liang Yu, Hong-Gang Yu. 2005. Effects of Garlicin on Apoptosis in Rat Model of Colitis. World Journal of Gastroenterology ISSN 1007-932.

Young A., dan R.J. Levi. 1990. Diarrhoea of Famine and Malnutrition-Investigations Using a Rat Model. 2-Ileal hypersecretion Induced by Starvation. Pubmed Central February (21):162-169.

Zarnowsky, R., J. Eichel, T. Lewicka, H. Rozycki, dan S.J. Pietr. 2001. Protein Fingerprinting as A Complementary Tool for The Classification of Pseudomonas Bacteria. Cell. Mol. Bol. Lett. 6:913-923.

Ramunno, L.; Cosenza, G.; Pappalardo, M.; Longobardi, E.; Gallo, D.; Pastore, N.; DiGregorio, P.; Rando, A. Characterization of Two New Alleles at The Goat CSN1S2 Locus. *Anim. Genet*. **2001**, 32, 264-268.

Picariello, G.; Rignanese, D.; Chessa, S.; Ceriotti, G.; Trani, A.; Caroli, A.; Di-Luccia, A. Characterization and Genetic Study of The Ovine Alphas2-Casein (CSN1S2) Allele B. *Protein J.* **2009**, 28, 333-40

Södergren, E. 2000. Lipid Peroxidation In Vivo. Evaluation And Application Of Methods For Measurement. Comprehensive Summaries of Uppsala Dissertations. Acta Universitatis Upsaliensis Uppsala.

Stern, S. T., T. M. Potter, B. W. Neun. 2010. Hep G2 Hepatocyte Lipid Peroxidation Assay. Nanotechnology Characterization Laboratory. National Cancer InstituteFrederick.