

Proceeding
The First International Conference
Technology on Biosciences and Social

“Industry based on Knowledges”

17th-19th November 2016, Convention Hall, Andalas University

Organized by :
Animal Science Faculty of Andalas University
Co-organized by :
Alumni Center of Universiti Putra Malaysia

Penerbit Lembaga Literasi Dayak (LLD)
berkerja sama dengan Universitas Andalas, Padang

“Industry based on Knowledges”

17th-19th November 2016, Convention Hall, Andalas University

Proceeding of The First International Conference
Technology on Biosciences and Social Sciences

**Proceeding of The First International
Conference Technology on Biosciences
and Social Sciences**



ISBN 978-602-6381-22-4

“Industry based on Knowledges”

17th-19th November 2016, Convention Hall, Andalas University



**The Proceeding Of
The 1st International Conference Technology on
Biosciences and Social Science 2016**

“Industry Based On Knowledges”

**17th – 19th November 2016, Convention Hall, Andalas
University, Padang, West Sumatera, Indonesia**

Organized by:

**Animal Science Faculty of Andalas University
and
Alumbi Center of Universiti Putra Malaysia**

Organizing Committee

Steering Committee:

Rector of Andalas University
Deputy Rector II Andalas University
Dean of Animal Science Faculty
Deputy Dean I of Animal Science Faculty
Deputy Dean II of Animal Science Faculty
Prof. Dr. Ir. Salam N. Aritonang, MS
Prof. Dr. Ir. H.M. Hafil Abbas, MS
Prof. Dr. Ir. Zaituni Udin, M.Sc

Chairman :

Prof. drh. Hj. Endang Purwanti, MS., Ph.D

Co-Chairman:

Prof. Dr. Ir. Hj. Husmaini, MP

Secretary:

Dr. drh. Hj. Yulia Yellita, MP
Afriani Sandra, S.Pt., M.Sc

Secretariat:

Hendri Purwanto, S.Pt., M.Si
Yunizardi, S.Pt.
Arif Trisman, S.Pt.
Rahmat Mulyadi, SE

Treasurer :

Dr. Ir. Elly Roza, MS,

Financial

Dr. Ir. Tinda Afriani, MP.
Dr. Ir. Sabrina, MP

Editors:

drh. H. Yuherman, MS., Ph.D; Dr. Ir. Rusmana Wijaya Setia Ningrat, M. Rur.Sc.; Dr. Ir. Masrizal, MS.; Dr. Ir. Firda Arlina, MP.; Indri Juliyarsi, SP., MP.; Deni Novia, S.TP., MP.; Sri Melia, S.TP., MP.; Aronal Arief Putra, S.Pt., M.Sc; Ferawati, S.Pt, MP.; Yulianti Fitri Kurnia, S.Pt, M.Si

Meet and Greet of UPM Alummy

Prof. Dr. Marlina, Apt., MS.; Dr. Ir. Adrinal, MS.; Dr. P.K. Dewi Hayati, MS.

Contents

	Page
Organizing Committee.....	ii
Content	iii
Preface	iv
List Paper of Oral Presentation	v
List Paper of Poster Presentation	xii
Keynote Lecturer	1
Papers of Oral Presentation	25
Animal Science.....	26
Agricultures.....	198
Medicenes, Public Health, Technics and Natural Sciences	344
Economy and Social Sciences	425
Papers of Poster Presentation	491

Preface

List Paper of Oral Presentation

No.	Author's	Title	Page
ANIMAL SCIENCES			
1.	Jumatritikah Hadrawi, Asep Gunawan, Niken Ulupi, Sri Darwati and Cece Sumantri	Association Analysis of NRAMP1 Gene Related to Resistance Against <i>Salmonella pullorum</i> Infection in Kampung Chicken	27
2.	Ahmad Saleh Harahap, Cece Sumantri, Niken Ulupi, Sri Darwati, and Tike Sartika	Polymorphism Calpain-3 (CAPN3) Gene and Association with Carcass Traits and Meat Quality in Kampung Chicken	32
3.	Wahyuni, Niken Ulupi and Nahrowi	Physical Quality of Broiler Meat Fed Diets Containing Mealworm Protein Concentrate	40
4.	Mega Sofia, Cece Sumantri, Niken Ulupi and Asep Gunawan	Identification Polymorphisms of Inos Gene and Association with Body Resistance Trait in Kampung Chicken	46
5.	Risky Naully Panjaitan, Niken Ulupi and Nahrowi	Investigation of Cadmium Contamination in Mealworm, Ration and Broilers's Feces	51
6.	Woki Bilyaro, Asep Gunawan, Tuti Suryati, Cece Sumantri, and Sri Darwati	Malonaldehyde and Fat Contents of Kampong-meat Type Crossbreed Chicken	55
7.	Devi Kumala Sari, Henny Nuraini and Tuti Suryati	Quality of Gelatin Processed from Chicken Legs (<i>Tarsometa tarsus</i>) Skin with Different Method	59
8.	Linda Suhartati, Asep Gunawan, Rukmiasih, Sri Darwati, Cece Sumantri, and Tuti Suryati	Physical and Chemical Characteristic of Chicken Meat from Kampung x Meat Type Crossbred Chicken	64
9.	Teguh Rafian, Jakaria, Niken Ulupi, Yosi Fenita, and Muhammad Andriansyah	Evaluated the Effect of Fermented Palm Sludge on Burgo Chicken Performance	69
10.	Donald John Calvien Hutabarat, Fransisca Rungkat Zakaria, Endang Yuli Purwani, and Maggy Thenawidjaja Suhartono	SCFA Profile of Rice RS Fermentation by Colonic Microbiota, <i>Clostridium butyricum</i> BCC B2571, or <i>Eubacterium rectale</i> DSM 17629	73

11.	Asep Gunawan, Ahmad Furqon, Kasita Listyarini, Jakaria, and Cece Sumantri	Growth and Carcass Characteristic in Kampong x Broiler Crossbred Divergently Selected for Unsaturated Fatty Acid	84
12.	Niken Ulupi, Cece Sumatri and Sri Darwati	Resistance against <i>Salmonella pullorum</i> in IPB-D1 Crossbreed, Kampong and Commercial Broiler Chicken	88
13.	Angelia Utari Harahap	Effects of Wheat Leaf Noni (<i>Morinda citrifolia</i>) on Carcass and Production Quail Eggs (<i>Coturnix Coturnix Javonica</i>) in the Different Level Concentrate	92
14.	Armein Lusi Zeswita, Vivi Fitriani and Nursyahra	Microbial Analysis on Freshwater Shell (<i>Corbicula sumatrana</i>) in Singkarak Lake Solok District West Sumatra	96
15.	Syaiful F. L, E. Purwati, Suardi, and T.Afriani	Analysis of Estradiol and Progesterone Hormone Levels Against Various Cell Culture in TCM- 199 Medium for Cattle <i>In vitro</i>	100
16.	Harissatria, Jaswandi, and Hendri	Acceleration Time Equilibration Cauda Epididymis Spermatozoa Buffalo with Addition of Antioxidant Glutathione	109
17.	Jhon Hendri and Harris Satria	Buffalo Embryo Maturation Optimization in Vitro with Addition Glutathione	113
18.	Khalil, Reswati, Y.F. kurnia, Indahwati and Yuherman	Blood Mineral Profiles of Simmental Breed Cattle with Different Feeding Systems and Reproduction Statues in Payakumbuh Region West Sumatra, Indonesia	118
19.	Lendrawati, A. Rahmat and J. M. Nur	Performance of Broiler Chicken Fed Turmeric and Zinc Mineral under Heat	122
20.	Muslim	Utilization of Plant <i>Tithonia</i> Flowers (<i>Tithonia diversifolia</i>) in The Ration on The Performans of Broiler	126
21.	Resolinda Harly, Almasdi and Sri Mulyani	Analysis of Factors Influence Palm Oil Farmers Personal Income Trough Buffalo's Breeding	132
22.	Retno Wilyani and Moch Hisyam Hermawan	Nutritional Value of Persimmon Yoghurt (<i>Diospyros kaki</i>) as Healthy Soft Drink to Make Healthy and Fitness: An Analysis	136

23.	Zulfa Elymaizar, Arnin, Salam N Aritonang, Mardiaty Zein, and Elly Roza	In-Vitro Rumen Digestibility of Goat Feed by Patikan Kerbau (<i>Euphorbia hirta</i> L.) Herbal Supplemented	145
24.	Salam N. Aritonang, Elly Roza and Lailya Rahma	The Adding of <i>Saccharomyces cerevisiae</i> on Moisture, Acidity and Lactic Acid Bacteria Colony Count of Yogurt from Goat's Milk	150
25.	Yuherman, Nur Asmaq and Endang Purwati	Characteristics and Antimicrobial Activity of Lactic Acid Bacteria Isolated from Dadih of Agam Regency	156
26.	Yunizardi Ade Rakhmadi, and Endang Purwati	Effect of Addition White Oyster Mushroom (<i>Pleurotus ostreatus</i>) and Carrot (<i>Daucus carota</i> L) In Probiotic Duck Nugget On Protein, Calcium and Organoleptic Value	161
27.	Yulianti Fitri Kurnia and Endang Purwati	The Potential Of Dadiah From 50 Kota District, West Sumatra as a Probiotic Food Based On Total of Lactic Acid Bacteria	170
28.	Tertia Delia Nova, Sabrina and trianawati	The Effect of level Flour turmeric (<i>Curcuma domestica</i> Val) ration toward carcass local duck	174
29.	T. Astuti, G. Yelni, Nurhaita, and Y. Amir	Effect of the Form Complete Feed With Basis Fermented Palm Oil Fronds on the Content of Moisture, Crude Lipid, and Crude Protein for Ruminants	185
AGRICULTURES			
30.	Azwar Rasyidn, Gusmini, Ade Fitriadi and Yulmira Yanti	Soil Microbes Diversity Between Hilly and Volcanic Physiography And Their Effect To Soil Fertility	190
31.	Dafni Mawar Tarigan, Bambang SAS, and Hasanul Arifin Marmen	Application of Green Manure and Rabbits Urine Affect Morphological Characters of Sweet Corn Plant (<i>Zea mays saccharata</i> Sturt) in Lowland of Deli Serdang District	200
32.	Dewi Rezki, Siska Efendi, and Herviyanti	Humic Substance Characterization of Lignite as a Source of Organic Material	205
33.	Jamilah, Sri Mulyani, and Juniarti	Nutritional Composition of Ruminant Forage Derived from Rice Crops (<i>Oryza Sativa</i> L.) that Appllicated by <i>C.odorata</i> Compost	208
34.	Mega Andini, Riska, and Kuswandi	Effectiveness of Liquid Smoke to Control Mealybug on Papaya	216

35.	M.Said Siregar, Arif Kurniawan, and Syakir Naim Siregar	Study on the Manufacture of Nuggets from Natural Rubber Seed (<i>Hevea Brasiliensis</i> Mull. Arg)	220
36.	Muhammad Thamrin, Desi Novita, Fitria Darma	Factors Affecting Farmers Decision to Convert Wetland	227
37.	Riry Prihatini, Yulia Irawati, Yosi Zendra Joni, and Sri Hadiati	The Occurrence of Somaclonal Variation on The Pineapple <i>In vitro</i> Culture as Detected by Molecular Markers	238
38.	Riska and Jumjunidang	Competitiveness of <i>Fusarium oxysporum</i> . sp <i>cubeense</i> VCGs 01213/16 (Tropical race 4) Among Several VCGs in Race 4 on Ambon Hijau Cultivar	244
39.	Fridarti and Sri Mulyani	Changes nutrients by microbial fermentation chocolate waste indigenous result of the additional mineral phosphor and sulphur in-vitro	252
40.	Sri Hadiati and Fitriana Nasution	Clustering and genetic distance some salak species (<i>Salacca</i> spp) based on morphological characters	256
41.	Asep Dedy Sutrisno, YusmanTaufik, and Jaka Rukmana	Optimalization Flour Composite Nutritiose as Basic Materials Processing for Food Products	264
42.	Sri Utami, Suryawati and Ermeli	KNO ₃ Concentration and Soaking Time Effect on Breaking Seed Dormancy and Seed Growth of Sour-Sop (<i>Annona muricata</i> L.)	272
43.	Susilawati, Dewi Sartika, and Mochamad Karel Saputra	Effect of Kepok Banana (<i>musa paradisiaca linn</i>) Peel Flour Addition as a Stabilizer on Chemical and Organoleptic Properties of Ice Cream	278
44.	Ubad Badrudin, Syakiroh Jazilah, and Budi Prakoso	The effect of soil submersion duration and ameliorant types on growth and yield of shallot at Brebes Regency	287
45.	Yulfi Desi, Trimurti Habazar, Ujang Khairul, and Agustian	Disease progress of Stewart's Wilt (<i>Pantoea stewartii</i> subsp. <i>stewartii</i>) on sweet corn	293
46.	Yusnaweti	On growth response and results of upland rice due to the allotment of some a dose of compost bamboo leaves	300
47.	Fadriani Widya, Darmawan, and Adrinal	Rice husk biochar application in traditional paddy soil and its effect of nutrients vertical distribution	306

48.	Ragapadmi Purnamaningsih, Ika Roostika, and Sri Hutami	Embryogenic Callus Induction and Globular Embryo Formation of Kopyor Coconut (<i>Cocos nucifera</i> L.)	313
49.	A. Sparta, L. Octriana, Nofiarli, N. Marta, Kuswandi, M. Andini, and Y. Irawati	The Role of Cow Manure to Reduce The Need of Nutrient N Inorganic In Banana Plant Vegetative Growth	320
50.	Desi Ardilla, Herla Rusmarilin, and Adi Purnama	Study The Physical And Chemical Properties Of Bioethanol From Pineapple Skin (<i>Ananas comusus</i> L.Merr)	325
51	Masyhura MD, Budi Suarti, and Evan Ardyanto AS	Increase Moringa Leaf Powder and Long Roasting on Protein Content in the Making of Cookies from Mocaf (<i>Modified Cassava Flour</i>)	331
MEDICINES, PUBLIC HEALTH, ENGINEERING, AND NATURAL SCIENCES			
52.	Ayulia Fardila Sari ZA, Putri Nilam Sari, and Muthia Sari	Implementation of Hospital Information System in RSUP Dr. M. Djamil Padang 2016	336
53.	Dien GA Nursal, Rizanda Machmud, Eryati Darwin, Nana Mulyana	Implementation Patient Safety Standards in Basic Emergency Obstetric Care Community Health Center (BEOC_CHC) Padang	344
54.	Dewi Sartika, Susilawati, and Mumpuni Uji Kawedar	Survey of Salmonella Contaminated Vannamei Shrimps in Lampung	351
55.	Ferra Yanuar	Determinants of Birth Weight at Various Quantiles in West Sumatra	358
56.	Hardany Primarizky, Ira Sari Yudaniayanti, and Djoko Galijono	Detection Of Osteoporosis in Ovariohysterectomized Cats (<i>Felis Domesticus</i>) based on Serum Osteocalcin Levels	363
57.	Nefilinda	Influence of Education and Local Wisdom on Environment Villages in Minangkabau	368
58.	Masri, E., Asmira,S and Verawati	Local Food Development from Combination <i>Siarang</i> Variety Of Black Rice (<i>Oryza Sativa L.Indica</i>) And Yellow Pumpkin (<i>Cucurbita Moschata</i>) To Prevent Anemia For Pregnant Women	375
59.	Suryani, Zulmardi, Abdi Dharma, Yunazar Manjang, and Febria Elvy Susanti	Development of Antimicrobial Analysis of Lactic Acid Bacteria Isolated from VCO (Virgin Coconut Oil) Fermentation Process Against Bacteria in The Secretion of CSOM	380

60.	Suci Rahayu, Darmawan Saptadi, and Febi Reza Fitriani	The Influence of Dicamba in Combination with BAP on Callus Induction and Proliferation of <i>Centella (Centella asiatica L.)</i>	387
61.	Christina J. R. E. Lumbantobing, Endang Purwati, Sumaryati Syukur, and Eti Yerizel	Triglyceride lowering effect of <i>Garcinia atroviridis</i> leaf tea from Sijunjung - West Sumatra on obese subjects in Medan, North Sumatra	395
62.	Netty Suharti	Preparation and Characterization of Ethanol Extract of Mychorryzae Induced Ginger as Raw Matherial for Anti Breast cancer Nano suspension Formulation	404
63.	Misril Fuadi, Mahmud T.M. Mohamed, Mohd. Fauzi Ramlan, Yahya Awang	Effect of Benzyladenine (BA) and Duration of Shading onGrowth and Quality of <i>Dracaena sanderiana and Codiaeum variegatum</i>	408
ECONOMY AND SOCIAL SCIENCES			
64.	Andri, Ida Indrayani and Rahmi Wati	Technical Efficiency Analysis of Poultry in District of 50 Kota (Stochastic Frontier Production Function Approach)	417
65.	Arif Fadhillah	Teaching Accounting in Business School: A Personal Reflection	422
66.	Wijaya Edo Rantou	Analysis Influence of Technical Competence on Company's Performance In Electrical Engineering Company In Bandung	427
67.	Ike Revita, R. Trioclarise, Inesti Printa Elisya	Reflections Of Social Reality In The Activities Of Women Trafficking In West Sumatera	435
68.	Ira Apriyanti, Desi Novita, and Pandhu Ahmad Pangestu	Efficiency of Marketing Distribution of Palm Oil in Sub District of Selesai Regency of Langkat	440
69.	Yeyep Natrio, Afdhal Rinsik, Gusmaizal Syandri	The Occurance Of Transitivity And Suicidal Motives On Famous Public Figure`S Suicide Letters	446
70.	Yusmarni	An analysis of Marketing Efficiency of Sapodilla in Nagari Sumpur sub district of Tanah Datar, West Sumatera	457

71.	Jusuf Wahyudi, Hesti Nur'aini and Lina Widawati	Information Systems of Eradication Pests and Diseases Crops for Agriculture Extension Instructor	464
72.	Desi Novita and Ira Apriyanti	The Regional Investment Competitiveness In Binjai City	469
73.	Khairunnisa Rangkuti, Desi Novita, and Bima Mahdi	The Impact of Rising Soybean Prices to Tofu Industry Small Scale in Medan	474

List of Poster

No.	Author's	Title	Page
ANIMAL SCIENCES			
1.	Wahidin Teguh Sasongko, Teguh Wahyono, Shintia Nugrahini Wahyu Hardani, and Firsoni	Total Gas Production, Methane and Rumen Fermentation Characteristics of Rejected Soybean Meal Protected by Jackfruit Leaves	484
2.	Nita Yessirita, Tinda Afriani, and Sunadi	The Supplementation of Amino Acid Methionine-Lysine on the Protein Quality of <i>Leucaena</i> Leaf Meal Fermented with <i>Bacillus laterosporus</i>	492
AGRICULTURES			
3.	Willy Pranata Widjaja, Sumartini	Optimization Of Koji Concentration And Fermentation Time To Characteristics Of Modified Sorghum (<i>Sorghum Bicolor</i> L Monench) Flour	499
4.	Kuswandi, Makful, Sahlan, and Mega Andini	Evaluation Performance Of Some Hybrid Of Watermelon From Indonesian Tropical Fruit Research Institute	508
5.	A. Sparta, R, Triatminingsih, Y.Z. Joni, and Nofiarli	The Using of Thidiazuron to Induce the Mangoesteen Shoot (<i>Garcinia mangostana</i> L.) by Direct Organogenesis	513
6.	Ira Sari Yudaniayanti, Bambang Sektiari L., and Hardany Primarizky	Healing Quality Of Femoral Fractures In Ovariectomized Rats With Therapy Of <i>Cissus Quadrangularis</i> Extract Shown by The Expression Of Type I Collagen	517
7.	Sri Hadiati and Tri Budiyantri	Parameters Genetic of Fruit Component Characters on Snake Fruit (<i>Salacca</i> sp.)	525
8.	Riry Prihatini, Tri Budiyantri, and Noflindawati	Genetic Variability of Indonesian Papaya (<i>carica</i> spp.) as Revealed by RAPD (Rapid Amplified Polymorphic DNA)	530
9.	Regina Andayani and Fivi Yunianti	The Effects of Oxidation And Thermolysis Reaction on a-Mangostin Content in the Ethyl Acetate Extract of Mangosteen Rind (<i>Garcinia mangostana</i> L.) by High Performance Liquid Chromatography	538
10.	Nini Marta, Kuswandi, Liza Octriana, and Nofiarli	The effectiveness test of herbicides 2,4 D, glyphosate, paraquat on low dose as growth regulator on papaya seedling	545

ORAL PRESENTATION

ANIMAL SCIENCE

Association Analysis of NRAMP1 Gene Related to Resistance Against *Salmonella pullorum* Infection in Kampung Chicken

Jumatriatikah Hadrawi^{a*}, Asep Gunawan^b, Niken Ulupi^b, Sri Darwati^b
Cece Sumantri^b

^aPostgraduate School, Department of Animal Production And Technology,
Faculty of Animal Science, Bogor Agricultural University

^bDepartment of Animal Production And Technology,
Faculty of Animal Science, Bogor Agricultural University

*Corresponding author: atikahjumatri@gmail.com

Abstract

Natural Protein Resistance-Associated Makrofag 1 (NRAMP-1) gene plays a role in controlling disease resistance. Kampung Chicken is one of the local Indonesian chickens which have high diversity in term of productivity. Breeding programs to improve disease resistance through molecular selection is one of the efforts that have evolved to increase the productivity of chicken. The present research was to study the association of apolymorphism of NRAMP-1 with resistance disease in Kampung chicken. The PCR-RFLP method was applied to analyze the association between the polymorphism of NRAMP1 with resistant disease against *Salmonella Pullorum*. NRAMP-1 gene was genotyped in Kampung chickens using the PCR-RFLP method. The result showed three genotypes were identified of NRAMP-1 gene in Kampung chicken, namely TT, TC, and CC. The NRAMP-1 gene was polymorphic in all native chickens. The chi-square value of the Kampung chicken showed deviate in Hardy-Weinberg *equilibrium*. The TT and TC genotype revealed higher ($P < 0.05$) mortality bacterium compare to CC genotype. In conclusion, polymorphism in chicken NRAMP-1 gene could be used as a candidate gene to increase resistance to disease in Kampung chicken.

Keyword: Nramp-1 gene, Kampung Chicken, resistance trait, and *Salmonella pullorum*

1. Introduction

Indonesia is one of the genetic diversity center of local chickens in the world [1]. The population of local chicken from 2012 to 2015 according to Dirjen PKH [2] very small increase in the value just of 1%. Kampung chicken is a kind of Indonesian local chicken that does not have special characteristic and spread out in various regions of Indonesia. There is two big problems which become a stumbling block in developing kampung chicken. The first problem is the difficulty to get day old chick of local chicken. This problem can be solved by integrating breeder institutions belong to the government with

a research institution and with local chicken producer association.

Most of the native chicken were raised extensively with marginal feed, with environmental hygiene and low implementation of biosecurity. even though kampung chicken is able to thrive despite an increase in low population. Kampung chicken is resistant to several deadly diseases such as a *pullorum*. The disease is caused by the bacterium *Salmonella pullorum*, *Salmonella pullorum* is the cause of *pullorum* disease attacking young ages under a month with a mortality rate of 20% and 80% and adult chickens act as carriers. ([3], [4]).

Resistance immune has affected the environment and feeds also controlled by genes. One of the genes controlled is a *Natural Resistance-Associated Protein-1* (NRAMP-1) genes. In poultry, a homologue of *NRAMP1* gene has been mapped on chromosome 7 which consists of a promoter region, 15 exons, 14 introns, and flanking regions 5760 bp in length [5], *NRAMP1* gene restricts microbial access to essential micronutrients, such as Fe^{2+} , Mn^{2+} , Co^{2+} , and Zn^{2+} , within professional phagosomes. NRAMP1 gene belongs to a large gene family encoding divalent cation transporters that are localized to late endosomes/ lysosomes and are proposed to affect intraphagosomal microbial replication by modulating divalent cation content in this organelle. The many cellular functions that depend on metal ions as cofactors may explain the pleiotropic effects of NRAMP1 and its complex role in infectious diseases ([6],[7], ([8],[9]).

The results study NRAMP-1 gene is polymorphic in broiler chickens [10], the native chicken Malaysia [11], and native chickens China ([12],[13]). Until now, variations in NRAMP1 gene and their effect on disease have not been well investigated in Indonesian native chickens. Therefore, the objective of the present study was to identify the association of NRAMP1 gene polymorphisms with immune traits

2. Material and Methods

2.1. The time and place of study

The study was conducted in Juli 2015 until November 2017 at the Laboratory of Animal Breeding and Genetics IPB and Laboratory of Medical Microbiology Faculty of Veterinary Science IPB.

2.2. Blood Samples

Blood samples were 44 population kampung chicken. DNA was extracted from blood samples at the Laboratory of Animal Breeding and Genetics, Faculty of Animal

Science, Bogor Agricultural University (Indonesia).

2.3. Identification of the polymorphism NRAMP-1 gene

The first Blood samples were 44 population kampung chicken. A blood sample was taken from the brachial vein in the wing area. Identification of the polymorphism NRAMP-1 gene against kampung chicken consists of 3 phases: DNA extraction, PCR amplification and RFLP (Restriction Fragment Length Polymorphism). Genomic DNA extraction used phenol-chloroform method [14] and the DNA was dissolved in the elution buffer. The quality of the total genomic extraction was assessed by 1% agarose gel electrophoresis. Polymerase chain reaction (PCR) was carried out using primers specific for a part of exon 11 (421 bp) of NRAMP1 gene (GenBank Accession No. AY072001): forward 5'-caatgagacggtgtctgtgg-3'; reverse 5'-cccagaagaatctccctgc-3'. Amplification was carried out with a GeneAmp® PCR 9700 System (Applied Biosystems, USA). Thermal cycling conditions consisted of pre-denaturation at 95°C for 5 min, followed by 35 cycles of denaturation at 95°C for 10 s, annealing at 60°C for 20 s, and extension at 72°C for 30 s; the final extension step was at 72°C for 5 min. RFLP method was used to determine the genotype NRAMP-1 gene. PCR result of the NRAMP-1 gene fragments was cut by *SacI* restriction enzymes. DNA amplification products and a standard DNA ladder were separated on 1.5% agarose gels in 0.5X TBE buffer.

2.4. Clearance Test

Immune traits were detected in blood samples using the *clearance test* [15]. This method was used to look at normal bacterial (*S. pullorum*) population growth compared that of populations given specific treatment. The treatment impact on bacterial growth was

measured after incubating for 24-48 h at $35\pm 1^{\circ}\text{C}$. Preparation of bacteria culture begins with the rejuvenation of culture in nutrient medium at a temperature of $36\pm 1^{\circ}\text{C}$ for 18-24 h and a sub-culture on Brain Heart Broth medium at a temperature of $36\pm 1^{\circ}\text{C}$ for 18-24 h.

2.5. Data analysis

Data were analyzed with ANOVA using completely randomized design. NRAMP-1 gene genotype was as treatment and biological assays data were as a response. A statistical model was used $Y_{ij} = \mu + P_i + \varepsilon_{ij}$, where Y_{ij} is the observation on immune traits, μ is the overall mean, P_i is the effect of the single nucleotide polymorphism genotypes, and ε_{ij} is the random residual effect [16].

3. Result and Discussion

NRAMP-1 gene in chicken located on chromosome 7. The data obtained from GenBank (GenBank accession number: AY072007). The size of NRAMP1 gene was 5760pb. The structure of this gene was begun by a promoter region, exons (15), introns (14) and the end was the flanking region.

NRAMP-1 gene *Genotyping* on exon 11, and with 421 bp PCR product. NRAMP1 gene cutting by *SacI* enzyme restriction. The result showed two alleles (C and T). The genotype were identified of NRAMP-1 gene in Kampung chicken, namely TT, TC and CC where restriction fragments included a single, uncut fragment of 421 bp (TT genotype), two fragments of 258 and 163 bp (CC genotype), and three fragments of 421, 258, and 163 bp (TC genotype) (Figure.1).

Genotypes and allele frequency of NRAMP1 gene in kampung chickens

Allele and genotype frequency values of NRAMP1 genes against kampung chicken presented in Table 1.

The results showed of genotyping frequency of CC genotype dominated, could be interpreted that the gene NRAMP loci SACI-1 polymorphic trait. So for [17] which states that genetic polymorphic in an individual can be seen when there are two alleles for the like gene but different DNA configuration which occupied the same locus on a chromosome. According to Nei and Kumar [18], polymorphism can be indicated by the presence of two or more alleles in a population and the allele frequency is equal to or below 0.99.

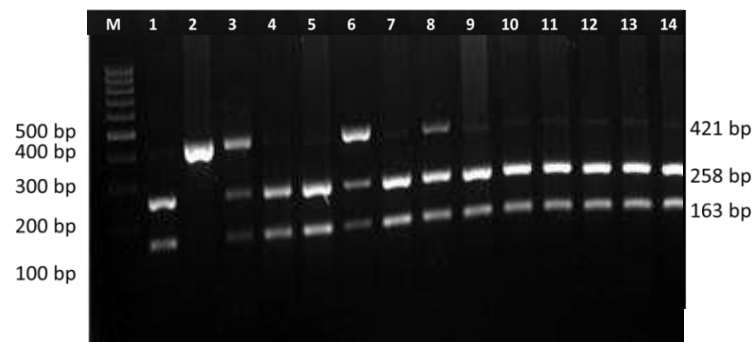


Fig. 1. PCR-RFLP amplification product of NRAMP1 gene at exon 11 that was cut by the *SacI*.

Table 1. Genotypes and allele frequencies of polymerase values of NRAMP1 genes against kampung chicken

Chicken	N	Allele frequency		genotype frequency		
		T	C	TT	TC	CC
Kampung	44	0.24	0.76	0.14 (6)	0.21 (9)	0.66(29)

Heterozygosity and Hardy-Weinberg equilibrium Genotype Gen NRAMP1

The results of heterozygosity values and Hardy-Weinberg equilibrium of NRAMP gene in locus *SacI* presented of Table 2. Genotype and allele frequencies The showed that the deviate in Hardy-Weinberg equilibrium.

Hardy-Weinberg equilibrium showed that the value of chi-squared higher than a Chi-squared table at the 1% level of confidence, that the results were significantly different. In other, the ratio deviates from expectations. The higher the degree of heterozygosity in a population, the survival of the population will be higher. The degree of heterozygosity is the average percentage of loci heterozygosity of each individual or the average percentage of heterozygous individuals in the population [18].

Resistance of Kampung Chickens

The resistance of kampung chickens in this study demonstrated the ability of chicken in ingesting and killing of bacteria. Ingesting

killing ability has shown by clearance test with infection *S. pullorum*. The association of NRAMP1 gene genotype with immune traits in kampung chickens are presented in Table 3.

The TT and TC genotype revealed higher ($P < 0.05$) mortality bacterium compare to CC genotype. The data showed polymorphism NRAMP-1 gene in kampung chicken associated with immune traits of infection *S. Pullorum*. As a candidate disease resistance gene, NRAMP1 gene has been studied by a number of researchers throughout the world.

This study conducted by Liu et al [10], demonstrated the association of an SNP polymorphism in a highly conserved region of NRAMP1 with *Salmonella enteritidis* vaccine and pathogen challenge response in young chicks, indicating that either NRAMP1 or a linked gene controls these *S. enteritidis* response traits. Hu et al [19] report about the association of a polymorphism of NRAMP1 with same immune functions in chicken takes effect.

Table 2. heterozygosity observations Value, expectations and Hardy-Weinberg equilibrium of NRAMP 1 gene

Chicken	N	Ho	He	χ^2
Kampung	44	0.36	0.21	8.389 ⁿ

n : significantly different, $\chi^2 (0.05, 1) = 3.84$

Ho: heterozygosity observation

He: heterozygosity expectations

χ^2 : Hardy-Weinberg equilibrium

Table 3. Association of NRAMP1 gene genotype in kampung chickens resistant to *Salmonella pullorum*

Genotype	Early concentration (CFU/ml)	Final concentration (CFU/ml)	Death rate of bacteria (%)
TT	6.8×10^{10}	4.17×10^8	99,39 ^{ab}
TC	6.8×10^{10}	$1,73 \times 10^8$	99,74 ^a
CC	6.8×10^{10}	$7,07 \times 10^8$	98,96 ^b

Conclusion

The TT and TC genotype revealed higher ($P < 0.05$) mortality bacterium compare to CC genotype. In chicken, NRAMP-1 gene could be used as a candidate gene to increase resistance to disease in Kampung chicken.

References

- [1] Sulandari, S, M.S.A. Zein, S. Priyanti, T. Sartika, M. Astuti, T. Widjastuti, E. Sujana, S. Darana, I. Setiawan, G. Garnida. *Sumber daya genetik ayam lokal Indonesia*. Bogor. vol. 45-104. 2007.
- [2] Directorate General of Livestock and Animal Health. 2015. Directorate General of Livestock and Animal Health Resources Statistics 2015. Directorate General of Livestock and Animal Health. Jakarta
- [3] Shivaprasad, HL. *Pullorum Disease and Fowl Typhoid*. in: Disease of Poultry. SAIF, Y.M. (Ed). 11th Edition. Ames, Iowa: Iowa State Press, 2003.
- [4] McMullin, P. *A Pocket Guide to Poultry Health and Disease*. 5M Enterprises Limited. Sheffield. 2004
- [5] Hu J, Bumstead N, Burke D, FA PdL, Skamene E, Gros P, Malo D. Genetic and physical mapping of the natural resistance-associated macrophage protein 1 (NRAMP1) in chicken. *Mamm Genome*. 6:809-815. 1995.
- [6] Boyer E, Bergevin I, Malo D, Gros P. Acquisition of Mn (II) in addition to Fe (II) is required for full virulence of *Salmonella enterica* serovar Typhimurium. *Infect. Immun.* 70: 6032-6042. 2002.
- [7] Jabado N, Cuellar-Mata P, Grinstein S and Gros P. Iron chelators modulate the fusogenic properties of Salmonella containing phagosomes. *Proc. Natl. Acad. Sci.* 100: 6127-6132. 2003.
- [8] Peracino B, Wagner C, Balest A, Balbo A. Function and mechanism of action of Dictyostelium NRAMP1 (Slc11a1) in bacterial infection. *Traffic*. 7: 22-38. 2006.
- [9] Cellier MF, Courville P and Campion C. NRAMP1 phagocyte intracellular metal with drawal defense. *Microbes. Infect.* 9: 1662-1670. 2007.
- [10] Liu W, Kaiser MG, Lamont SJ. Natural resistance-associated macrophage protein 1 gene polymorphisms and response to vaccine against or challenge with Salmonella enteritidis in young chicks. *Poult. Sci.* 82: 259-266. 2003.
- [11] Tohidi R, Idris I, Panandam JM and Bejo MH. Analysis of genetic variation of inducible nitric oxide synthase and natural resistance-associated macrophage protein 1 loci in Malaysian native chickens. *African Journal of Biotechnology* Vol. 10(8), pp. 1285-1289. 2011.
- [12] Hu JX N, Bumstead, P, Barrow, G, Sebastiani, L, Olie, K, Morgan, D, Malo. *Genome Research* 7: 693-704. 1997.
- [13] He XM, Fang MX, Zhang ZT, Hu YS, Jia XZ, He DL, Liang SD, Nie QH, Zhang XQ. Characterization of chicken natural resistance-associated macrophage protein encoding genes (NRAMP1 and NRAMP2) and association with salmonellosis resistance. *Genet. Mol. Res.* 12 (1): 618-630. 2013
- [14] George J. Jackson. *Bacteriological Analytical Manual*. Published and Distributed by AOAC International. 1998.
- [15] Sambrook J, Fritsch Ef, Maniatis T. *Molecular Cloning, A Laboratory Manual*. 2nd ed. New York: Cold Spring Harbour Lab. Press. 1989.
- [16] Mattjik AA, Sumertajaya M. *Perancangan Percobaan dengan Aplikasi SAS dan Minitab*. Ed ke-2. Bogor : IPB Press. 2002
- [17] Sofro, A.S.M. Keanekaragaman Genetik. Yogyakarta : Andi offset 1994
- [18] Nei, Kumar. *Molecular Evolution and Phylogenetics*. New York: Oxford University Press. 2000.
- [19] Hu GS, Chang GB, Zhang Y, Hong J, Liu Y, Chen GH. Association Analysis Between Polymorphisms of Nram-1 Gene and Immune Traits in Chicken. *Journal of Animal and Veterinary Advances* 10: 1133-1136. 2011.