The Veterinary Medicine International Conference (VMIC)

12-14 July 2017

ISSN 2413-0877

www.KnEpublishing.com
The Veterinary Medicine International Conference (VMIC)

VMIC—The Veterinary Medicine International Conference—is one of the world's leading conference focusing on a wide array of topics including Veterinary Medicine and Biomedical Science. It offers a stimulating venue for scientists, researchers, lecturers, general practitioners, and others to broaden their social scientific network. This conference contributes to improving human and public health by improving agricultural and food systems, advancing biomedical and comparative medical research, preventing and addressing zoonotic diseases, kit diagnostic, enhancing environmental and ecosystem health, and helping manage the 21st-century public health challenges.

**Conference date:** 12–14 July 2017  
**Location:** Surabaya, East Java, Indonesia  
**Editors:** Sri Agus Sudjarwo, Fedik A. Rantam, rer. nat. Gunawan Indrayanto, Muchammad Yunus, Rimayant, Wiwik Misaco, Tita Damayanti Lestari, Mustofa Helmi Effendi, Dikky Eka Mandala Putranto, and Shafia Khairani  
**Organizer:** Faculty of Veterinary Medicine, Universitas Airlangga, Indonesia  
**Sponsors:** Ministry of Research, Technology and Higher Education of the Republic of Indonesia, Universitas Airlangga, ROMINDO PRIMA VETCOM, Gadjah Mada University, Bogor Agriculture Institute, ILRI (International Livestock Research Institute), Kagoshima University, Miyazaki University, Faculty of Veterinary Science, Chulalongkorn University, College of Veterinary Medicine, Tarlac Agricultural University, Yamaguchi University, Erasmus MC, Kasetsart University, Wyndham Veterinary Clinic, GERBU – Germany, USAID  
**Published:** 29 November 2017  
**ISSN:** 2413-0877
# Table of Contents

The Veterinary Medicine International Conference (VMIC) | pages 1-9

**Toll-Like Receptors (TLRs) Play Role in Adaptive Immunity in Rabbits Immunized by Sarcoptes scabiei Proteins**

Nunuk Dyah Retno Lastuti, Fedik Abdul Rantam, Poedji Hastutiek, Dony Chrismanoto  
The Veterinary Medicine International Conference (VMIC) | pages 10-20

**Preservation Effect of Grouper (Epinephelus sp) Fillet Against Survival of Anisakidae**

Hartanto M. Raharjo, Setiawan Koesdarto, A.T Soelih Estoeplangsetie, Kusuma Wardhani L.D  
The Veterinary Medicine International Conference (VMIC) | pages 21-27

**Acanthocephalan in Xenochrophis piscator Snake in Sidoarjo Indonesia**

Inggarastya Syah Audini, Lucia Tri Suwanti, Setiawan Koesdarto, Emmanuel Djoko Poetranto  
The Veterinary Medicine International Conference (VMIC) | pages 28-33

**The Identification Blood Parasites On Pig (Sus domesticus) In Polewali Mandar District**

Silvana Arfin, Lucia Muslimin, Adryani ris  
The Veterinary Medicine International Conference (VMIC) | pages 34-40

**Spirometra in Ptyas mucosus Snake in Sidoarjo, Indonesia**

Garindra Tiara Pranashinta, Lucia Tri Suwanti, Setiawan Koesdarto, Emmanuel Djoko Poetranto  
The Veterinary Medicine International Conference (VMIC) | pages 41-47

**Toxicity of Citrus mitis, Citrus aurantifolia, and Citrus maxima leaf extract toward mortality of Aedes aegypti larvae (Diptera: Culicidae)**

Hamidah Hamidah, Hebert Adrianto  
The Veterinary Medicine International Conference (VMIC) | pages 48-61

**An In Vitro Antibacterial Activity Test of Meniran Herbs (Phyllanthus Niruri L.) Ethanol Extract Against Mycoplasma gallisepticum causes Chronic Respiratory Disease (CRD) in Broiler Chickens**

Emy Koestanti Sabdoningrumr, Sri Hidana, Retno Sri Wahjuni, Sri Chusniati, Arimbi Arimbi  
The Veterinary Medicine International Conference (VMIC) | pages 62-68

**Bioremediation of Mercury (II) Contaminated Seawater Using the Diatom Skeletonema costatum**

Thin Soedarti, Tini S., Sucipto H., Eko P. Kuncoro  
The Veterinary Medicine International Conference (VMIC) | pages 69-76

**BMP-2 Expression of Post Tooth Extraction that Catfish Oil Application**

Theresia Indah B., Bambang Sumaryono, Ketut Suwardita, Amelia Putri R.  
The Veterinary Medicine International Conference (VMIC) | pages 77-83

**Inhibition of Apoptosis in Retinal of Newborn Mice Due to Congenital Toxoplasmosis**

Lucia Tri Suwanti, Mufasirin Mufasirin, Hani Plumeriastuti
Effect of Spirulina Platensis on The Number of Spermatogenic Cells in The Seminiferous Tubules of Rat (Rattus Norvegicus) with Excessive Physical Exercise
Rahmah Wahyu Rosidawati, Rimayanti Rimayanti, Koesnolo Supranianondo
The Veterinary Medicine International Conference (VMIC) | pages 93-104

Phytochemicals, Antioxidant and Antifungal Properties of Acorus calamus, Curcuma mangga, and Allium sativum
Bayinatal Muchitaromah, Mujahidin Ahmad, Emy Koestanti S, Yuni Ma’rifatul A, Velayati Labone A
The Veterinary Medicine International Conference (VMIC) | pages 105-110

Shark Species on Export Products from East Java and Bali by Dna Barcoding Based on Internal Transcribed Spacer-2 (Its-2) Locus in Mitochondrial
Eduardus Birno Aksono
The Veterinary Medicine International Conference (VMIC) | pages 111-124

Bioactivity of human Menopausal Gonadotrophin (hMG) and Deglycosylated hMG (hMGdG) from Urine of Post-Menopausal Women On invitro Bovine Embryonic cleavage
Herry Agoes Hermadiv
The Veterinary Medicine International Conference (VMIC) | pages 125-138

The Effect of Frequency Acoustic Stimulation Sound on Intrauterine Weakening of Pregnant Sheep
Djamil Suherman, Hermanto Tri Joewono, I Komang Wiarsa Sardjana
The Veterinary Medicine International Conference (VMIC) | pages 139-152

The Potency of AF 508-T Gen Mutant the Coding of Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) As Prototype at the Congenital Bilateral Absence of Vas Deferens (CBAVD) Disease in Indonesia
Masichah Mafuchati, Mas’ud Harijadi, Widjiati Widjati, Boerhan Hidayat
The Veterinary Medicine International Conference (VMIC) | pages 153-165

A Retrospective Study of Canine Pyometra in Segar Veterinary Hospital, Kuala Lumpur, Malaysia Year 2012-2016
Ng Xin Hui, Mas’ud Harijadi, Hardany Primarizky
The Veterinary Medicine International Conference (VMIC) | pages 166-174

Oocyte Quality and Subsequent In Vitro Maturation of Sheep Oocyte-Cumulus Complex from Ovary with Presence and Absence of Corpus Luteum
Rini Widjastuti, Mas Rizky A.A. Syamsunarno, Takdir Salli, Arief Boediono
The Veterinary Medicine International Conference (VMIC) | pages 175-188

The Effect of Insulin-Like Growth Factor-I of Pregnant Crossbred Mare Serum and Insulin-Like Growth Factor-I Recombinant Mouse on Estrous Cycles and Litter Sizes of Mice (Mus musculus)
Tjuk Imam Resiadi, Imam Mustofa, Suzaanita Utama, Sri Mulya
The Veterinary Medicine International Conference (VMIC) | pages 189-196
Adding of L-Arginin Amino Acid in Skin Milk Diluent to Maintain Quality of Buck Sperm in Cold Temperature

Tri Wahyu Suprayogy, Suberni Susilowati, Tatik Hernawati
The Veterinary Medicine International Conference (VMIC) | pages 197-204

Profile of Crude Protein Tyrosine Kinase on Plasma Membrane of Merino Sheep Spermatozoa Using the Method of SDS-Page (Sodium Dodecyl Sulphate-Polyacrylamide Gel Electrophoresis)

Yilda Carlena Wardani, Sri Pantja Madyawati, Poedji Hastutiek
The Veterinary Medicine International Conference (VMIC) | pages 205-211

Comparison of Morula and Blastula Embryo Vitrification by Using Cryoprotectant Ethylene Glycol, Propanediol, DMSO and Insulin Transferrin Selenium

Widjia Widjia, Epy Muhammad Luqman, Portia Sumarsono
The Veterinary Medicine International Conference (VMIC) | pages 212-223

Effect of L-Arginine on the Thickness Iliac Arteries Wall Post Fogarty Balloon Embolectomy Catheter in Rabbit (Oryctolagus cuniculus)

Gavrial Amadei Puspitarani, Ngakan Made Rai Widjaja, Hardany Primarizky
The Veterinary Medicine International Conference (VMIC) | pages 224-233

Ellagic Acid Activity in Healing Process of Incision Wound on Male Albino Rats (Rattus norvegicus)

Hardany Primarizky, Wiwik Misaco Yuniarti, Bambang Sektari Lukiswanto
The Veterinary Medicine International Conference (VMIC) | pages 234-240

Control and Preventive Study of Brucellosis by Using

Rahmahani J, Hardijatno D, Tyaningsih W, Suwarko Suwarko
The Veterinary Medicine International Conference (VMIC) | pages 241-251

Histopathology of Coronary artery of male rat (Ratus Norvegicus) with high fat diet after being given ethanol extract of Indian acalypha (Acalypha indica, L)

The Veterinary Medicine International Conference (VMIC) | pages 252-265

Immunohistochemical Detection of Porcine Reproductive and Respiratory Syndrome Virus Antigen in Formalin-Fixed, Paraffin-Embedded Tissues with Correlation to Clinicopathologic Data

Lavina Gracia G. Manzano
The Veterinary Medicine International Conference (VMIC) | pages 266-277

Antelmintic Activity of Ocimum sanctum Linn. Leaves Ethanolic Extract Against Fasciola gigantica in vitro

Mesia Margi Mahardika, Sri Agus Sudjarwo, Setiawan Koesiarto
The Veterinary Medicine International Conference (VMIC) | pages 278-285

Scabies in Rabbit

Miyayu Soneta Sofyan, Doni Chismanto
The Veterinary Medicine International Conference (VMIC) | pages 286-295
Detection of Newcastle Disease Virus by Immunohistochemistry on the Brains of Laying Birds with Clinical Signs Torticollis and Curled Toe Paralysis

Ocie Harum Wulan, Niken Yunita, Hastari Wuryastuty, Raden Wasito
The Veterinary Medicine International Conference (VMIC) | pages 296-307

Implementation of Meniran Extract (Phyllanthus Niruri Lina) on the Performance of Broiler Chickens Infected by Mycoplasma gallisepticum Caused Chronic Respiratory Disease

Sri Hidanah, Emy Koestanti Sabduningrum, Retno Sri Wahjuni, Arimbi Arimbi
The Veterinary Medicine International Conference (VMIC) | pages 308-315

Blood Parasite Infection Prevalence in Kampung Chicken Breeder's Group in Garut

Djoko Legowo, Syifa Husnul Khotimah, Lucia Tri Suwanti
The Veterinary Medicine International Conference (VMIC) | pages 316-326

Amino Acid Analysis of Fusion (F) Gene and Prediction of Epitope B-Cell Newcastle Disease Surabaya Isolate As Vaccine Candidate

Indah Laili Rahmaswati, Fedik Abdul Rantam, Wiwik Tyaasningsih
The Veterinary Medicine International Conference (VMIC) | pages 327-337

Mini Review: Liver Fibrosis Mechanism

Wiwik Misaco Yuniarti, Hardany Primarizky
The Veterinary Medicine International Conference (VMIC) | pages 338-343

The Nutrients Contents, Dry Matter Digestibility, Organic Matter Digestibility, Total Digestible Nutrient, and NH3 Rumen Production of Three Kinds of Cattle Feeding Models

M. Anam Al-Arif, Lucia Tri Suwanti, AT Soelih Estoeangestie, Mirni Lamid
The Veterinary Medicine International Conference (VMIC) | pages 344-355

Immunogenicity of Bone Graft Using Xenograft Freeze-Dried Cortical Bovine, Allograft Freeze-Dried Cortical New Zealand White Rabbit, Xenograft Hydroxyapatite Bovine, And Xenograft Demineralized Bone Matrix Bovine In Bone Defect Of Femoral Diaphysis White Rabbit Experimental Study In Vivo

Ferdiansyah Ferdiansyah, Dwikora Novembri Utomo, Hori Suroto
The Veterinary Medicine International Conference (VMIC) | pages 356-368

Genotyping Analysis of Mycobacterium leprae isolated in Water Environment of Leprosy Endemic Places in Lamongan, East Java

Cita Rosita Sigit Prakoeswa, Nanny Herwanto, Ratna Wahyuni, Iswahyudi Iswahyudi, Dinar Adriaty, Indropo Agusni, Shinizo Izumi
The Veterinary Medicine International Conference (VMIC) | pages 369-377

Iron Overload Reduces Cholesterol and Triglyceride Serum of Mice

Devi Agustin Setiawati, Mas Rizky A.A. Syamsunarno, Pandji Irani Fianza, Nur Atik, Neni Anggraeini, Mohammad Ghozali, Ratu Safitri, Ramdan Panigoro
The Veterinary Medicine International Conference (VMIC) | pages 378-385
Measurement of Alkaloids Achyranthes Aspera Linn Level Using Thin Layer Chromatography Method and High-Performance Liquid Chromatography

Dewa Ketut Meles, Wurliana Wurliana, Dewa Putu Anom Adnyana
The Veterinary Medicine International Conference (VMIC) | pages 386-394

Propolis Potential Toward the Amount of Lymphoblast and Spleen Diameter of Male Mice (Mus musculus)

Werstant Adhityananda Rinaldi, Eka Pramythra Hestianah, Sri Mumpuni Sosiawati, Lita Rakhma Yustinasarı
The Veterinary Medicine International Conference (VMIC) | pages 395-403

Identification the Gene Nucleotide Sequence of Outer Membrane Protein Aeromonas Hydrophilla Bacteria from East Java Local Isolates Using Polymerase Chain Reaction

M. Gandul Atik Yuillian, Didik Handijatno, Sri Pantja Madyawati
The Veterinary Medicine International Conference (VMIC) | pages 404-412

Postmortem Interval Estimation Time from Algornortis Temperature of Rats Expressed by MARS Model Approach

Dwi M. Syabani, Hana Eliyani, Suharsono Suharsono, Fedik A. Rantam, Anwar Ma’ruf
The Veterinary Medicine International Conference (VMIC) | pages 413-421

Aqueous Extract of Neem Leaves (Azadirachta Indica) Decrease Expression of Immunoglobulin E (IgE) and Interleukin 4 (IL-4) in Gingiva Tissue of BALB/c Mice Injected by Ovalbumine

I Dewa Ayu Ratna Dewanti, D Dewa Ayu Susilawati, Pujianna Endah Lestari, Roedy Budirahardjo, Erawati Wulandari, Risya Widi, Sunlip Wibisono
The Veterinary Medicine International Conference (VMIC) | pages 422-435

Experimental Models Point Mutations In Plasmodium falciparum pfatpase6 Gene Exposed to Recuring Artemisinin In Vitro

Liik Maslachah, Yoes Prijatna Daechlan, Chairul A. Nidom, Loekl Enggar Fitr
The Veterinary Medicine International Conference (VMIC) | pages 436-442

Sinensetin-Rich Fraction Solid Dispersion Inhibits Cancer Cell Cycle

Lusiana Arifianti, Sukardiman Sukardiman, Mulia Hadi Santosa
The Veterinary Medicine International Conference (VMIC) | pages 443-449

Blunted Expression of PPARα in Mice with FABP-4 and -5 Deficiency under Acute Cold Exposure

Mas Rizky A A Syamsunarno, Mirasari Putri, Tatsuya Iso, Rini Widayastuti, Ramdan Panigoro, Masahiko Kurabayashi
The Veterinary Medicine International Conference (VMIC) | pages 450-459

Increased Iron in Pediatric B-Thalassaemia Major Associates with CD3+, Not γδ T Lymphocytes

Mohammad Ghozali, Ulrike Panjaitan, Adi Imam Cahyadi, Reni Ghrahani, Lelani Reniarri, MRAA. Syamsunarno, Ramdan Panigoro
The Veterinary Medicine International Conference (VMIC) | pages 460-467

Low Serum Cholesterol in Mice Pre-treated with Imperata cylindrica L. after Acute Olive Oil Gavage
The Role Of IL-6 In TMPD-Treated Lupus Arthritis Mice
Niken Indriyanti
The Veterinary Medicine International Conference (VMIC) | pages 476-485

Immunopathological Approach for Avian Influenza Virus Detection in Brain of Laying Bird with Clinical Signs of Torticollis and Carled Toe Paralysis
Niken Yunita, Ocie Harum Wulan, Hastari Wuryastuty, Raden Wasito
The Veterinary Medicine International Conference (VMIC) | pages 486-496

The Efficacy of Permot (Passiflora Foetida Linn.) Leaves Crude Extract Ointment on the Healing of Skin of Rabbit with Scabies
Poedji Hastutiek, Hana Eliyani
The Veterinary Medicine International Conference (VMIC) | pages 497-512

The Effect of Sappan Wood Extract (Caesalpinia sappan), Wheat grass and Vitamin E Treatment on the Liver Structure of Iron overload of Rat (Rattus norvegicus)
Ratu Safitri, Lelani Reniarti, Madiah Madiah, Lila Delia, Mas Rizky A.A Syamsunarno, Ramdan Panigoro
The Veterinary Medicine International Conference (VMIC) | pages 513-519

The Effectiveness of Antibiotics and Hematopoietic Stem Cell Treatment in Periodontitis Rat Model Toward TNF α Expression
Retno Indrawati R, Indoswati Diyatri, Dwi Rahmawati
The Veterinary Medicine International Conference (VMIC) | pages 520-527

Construction Hybrid immunoglobulin All Four Dengue serotype Using Mesenchymal Stem
Rofiqul A'la, Rahaju Ernawati, Nuruk Dyah Retna L, Muafisirin Muafisirin, Anwar Ma'ruf, Fedik A. Rantam
The Veterinary Medicine International Conference (VMIC) | pages 528-535

The Potential of Black Gluten and Red Rice in Rations on the Biological Values and Ideally Body Score of Healthy “Mini Rex Rabbit”
Romziah Sidik
The Veterinary Medicine International Conference (VMIC) | pages 536-548

Antiviral Activity Effect of Silver Nanoparticles (Agnps) Solution Against the Growth of Infectious Bursal Disease Virus on Embryonated Chicken Eggs with Elisa Test
Rosa Pangestika, Rahaju Ernawati
The Veterinary Medicine International Conference (VMIC) | pages 549-556

Identification and Characterization Indigenous of Lactobacillus sp from Bovine Rumen Fluid of Slaughterhouse
Tri Nurhajati, Koesnoro Soepriamando, Widy Paramita Lokapimasari, Adriana Monica Sahidu
The Veterinary Medicine International Conference (VMIC) | pages 557-565
Protective Effect of Propolis Extract Against Lead Acetate Toxicity in Mice (Mus Musculus) Testes

Tuni Widawati, Sri Agus Sudjarwo, Herry Agoes Hermadi
The Veterinary Medicine International Conference (VMIC) | pages 566-578

Detection of Antibiotic Residues and Concentration in Raw Milk from Lembang Small Holder Dairy Farm

Virgianty Vivi
The Veterinary Medicine International Conference (VMIC) | pages 579-587

Potency of Bacillus cereus WPI 415 to Increase Crude Protein and Decrease Crude Fiber of Animal Feed Stuff

Widya Paramita Lokapinasari, Adriana Monica Sahidu, Tri Nurhayati, Koesnomo Soepranianondo, Andreas Bernay Yulianto
The Veterinary Medicine International Conference (VMIC) | pages 588-695

Acute Toxicity Tests of Alkaloid Pare (Momordica Charantia) Fruit on The Histopathology of Liver

Wulrina Wulina, Dewa Ketut Meles, Sunarni Zakaria, Imam Mustofa, Suhermi Susilowati, I Dewa Putu Anom Adnyana
The Veterinary Medicine International Conference (VMIC) | pages 696-602

Teratogenic Effect of Congenital Toxoplasmosis in Chicken Embryo

Lucia Tri Suwanti, Mufasirin Mufasirin, Hani Plumeriastuti, Erma Safitri
The Veterinary Medicine International Conference (VMIC) | pages 603-608

Case Study: Dysuria on Beef Cattle in Kunir Regency of Lumajang District, East Java, Indonesia in 2015 and 2016

Rosiana Febrianila, Widia P Lokapirasari, Tjuk I Restadi, Imam Mustofa, Herry A Hermadi, Erma Safitri
The Veterinary Medicine International Conference (VMIC) | pages 609-618

The Effectiveness of Honey in Physiological NaCl to Maintain of Viability and Motility of Spermatozoa

Elsa Agustina, Herry Agoes Hermadi, Hario Puntoedewo S, Tatik Hernawati, Indah Norman Triana, Erma Safitri
The Veterinary Medicine International Conference (VMIC) | pages 619-626

Utilization of Sumbawa Tropical Forest Honey Apis Dorsata to Improve Fertility of Indonesia Oriental Magpie Robin (Copsychus saularis) as Effort Animal Population Increasement

Abdullah Hasib, Risalid Muhamad, Talita Yuanda Reksa, Alvina Ulimaz Artha, Erma Safitri
The Veterinary Medicine International Conference (VMIC) | pages 627-632

Utilization of Honey Apis dorsata as Antiosteoporosis on Requirements of Bone Calcium Ash Density on Ovariolyosterecomized White Rat (Ratus norvegicus)

Muhammad Huda Ramadhan Ibrahim, Abdullah Hasib Hasib, Siti Nur Rohmah, Salsabilla Abani, Samsi Yordan, Ira Sari Yudaniyanty
The Veterinary Medicine International Conference (VMIC) | pages 633-641

Increased Integrity of Plasma Membrane and Acrosome Cap Spermatozoa Limousin Cattle at Post Thawing in Frozen Media by adding Seawater Extract

Nur Faidah, Tatik Hernawati, Mirni Lamid, Imsudiono Imsudiono, Tri Wahyu Suprayogi, Sri Mulyati
The Veterinary Medicine International Conference (VMIC) | pages 642-649

The Relation of Body Temperature and Vaginal Cytology Examination in Time Artificial Insemination Rate Fat-tailed Sheep (Ovis Aries) in The District Sidoarjo East Java

Rhendyka Prasetya Anggriawan, Suzanita Utama, Hana Eliyani
The Veterinary Medicine International Conference (VMIC) | pages 650-657

Effect of Laser Acupuncture Shoot on Ova Point of Male Mojosari Duck (Anas plathyrynchos) on The Number of Sertoli and Leydig Cells

Yuanara AR Adikara, Abdul Samik, Ira S Yudaniayanti, Tatang S Adikara, Eka P Hestianah, Suzanita Utama
The Veterinary Medicine International Conference (VMIC) | pages 658-667

Insulin-Like Growth Factor-I (IGF-I) from Crossbred Pregnant Mare Serum to Increase Follicle Number of Mice (Mus musculus)

Abdullah Abdullah, Tjuk IRestiadi, Nunuk DR Lastuti, Tita Damayanti, Wurlina Wurlina, Erma Safitri
The Veterinary Medicine International Conference (VMIC) | pages 668-676

Morphological Identification Nematodes Tanqua tiara Found on Gastric Varanus salvator at East Java

Alfiana Laili Dwi Agusti, Setiawan Koesarto, Bambang Sektiary Lukiswanto, Lucia Tri Suwanti, Zainal Arifin, Emmanuel Djoko Putranto
The Veterinary Medicine International Conference (VMIC) | pages 677-683

Effect of Propolis on Spermatogenic Cells Number and Diameter of Seminiferous Tubules in Male Mice (Mus musculus)

Dona Astari Nurkarimah, Eka Pramythra Hestianah, Retno Sri Wahjuni, Mas'ud Hariadi, Suryo Kuncorojakti, Herry Agoes Hermadi
The Veterinary Medicine International Conference (VMIC) | pages 684-693

The Effect of Blue Green Algae (Spirulina platensis) Extract in White Rat (RattusNorvegicus) Treated with Excessive Physical Exercise on Leydig Cell Number and Seminiferous Tubules Diameter

Dimas Yuzrifar Rhavindra Lazuardi, Rimayanti Rimayanti, Hardasy Primarizky, Sri Agus Sudjarwo, Suzanita Utama, Kadek Rachmawati
The Veterinary Medicine International Conference (VMIC) | pages 694-701

The Effect of Mahkota Dewa (Phaleria macrocarpa) Pulp Extract by Peroral Administration Toward The Percentage of Capacitation and Acrosome Reaction in Rat (Rattus norvegicus)

Dhanang Estu Bagyo, Budi Utomo, Rudy Sukamoto Setiabudi
The Veterinary Medicine International Conference (VMIC) | pages 702-711

Protection of Dayak Onion Tuber Extract (Eleutherine Palmifolia) Against Kidney Histopathological Appearance of Albino Male Rat Strain Wistar which was Induced by Alloxan

Dwi Gayatri Nurcahyawati, Hanu Plumeriastutti, Liliq Maslachah
The Veterinary Medicine International Conference (VMIC) | pages 712-717

Test Various Estrus Detection Device Against Pregnancy Rates on Dairy Cows in Cooperative Tunas Setia Barn Kabupaten Pasuruan

Silvia Rani Andriyanti, Mas'ud Hariadi, Roesno Darsono, Pudji Sriaanto
Effect of Laser puncture Shot on Reproduction Point of Male Mojosari Duck (Anas Platyrhynchos) on the Numbers of Spermatogonium Cells and Seminiferous Tubules Diameter
Melyandari Ayu Qomar, Rimayanti Rimayanti, Tri Nurhajati
The Veterinary Medicine International Conference (VMIC) | pages 727-733

Cases of Reproduction Disorder in Beef Cattle of Modo District, Lamongan in 2015
Azharuddin Anshoria, Tri Nurhajati, Budi Utomo
The Veterinary Medicine International Conference (VMIC) | pages 734-741

The Application of Equine Chorionic Gonadotropin (Ecg) and Prostaglandin F2α to Increase the Rate of Pregnancy in Bali Cattle at Baleleng, Bali
Yugenthri A/P Chandran, Herry Agoes Hernadi, Eka Pramyrrha Hestianah
The Veterinary Medicine International Conference (VMIC) | pages 742-752

Morphometry Study of Hemipenis Biawak Air Varanus Salvator on Length Measurement of Snouth Vent Length (Svl) And Body Weight
Ilham Adi Kusuma, Dicky Boro Alfiananto, Pudji Srianto, Nusdianto Triakoso, Djoko Legowo
The Veterinary Medicine International Conference (VMIC) | pages 753-762

Effect of Polygonum Minus (Knotweed) Leaves Extract on the Histopathological Changes of Kidney in Mice (Mus Musculus) Induced by Mercuric Chloride
Winni Apriani, Thomas Valentinus Widiatno, Sri Agus Sudjarwo
The Veterinary Medicine International Conference (VMIC) | pages 763-775

Skin Stem Cell Resource Potential for Peripheral Nerve Repair Due to Trauma of Post Regional Anesthesia
Sumartono Christrijoko, Fedik A Rantam, Eddy Rahardjo, Martia R Tucharina
The Veterinary Medicine International Conference (VMIC) | pages 776-781

Public Awareness in ensuring Animal Originated Food Safety: A Review on “One Health” Approach in Veterinary Medicine
A. T. Soelih Estoeopangestie
Protein and Decrease Crude Fiber of Animal Feed Stuff

Widya Paramita Lokapirnasari¹, Adriana Monica Sahidu², Tri Nurhajati¹, Koesnoto Soepranianondo¹, and Andreas Berny Yulianto³

¹Department of Animal Husbandry, Faculty of Veterinary Medicine, Airlangga University, Surabaya, Indonesia
²Department of Marine, Faculty of Fisheries and Marine, Airlangga University, Surabaya, Indonesia
³Faculty of Veterinary Medicine, Wijaya Kusuma Surabaya University, Surabaya, Indonesia

Abstract

This research aims to identify isolate as a probiotic candidate derived from liquor rumen of local beef cattle and to know the ability of isolates as biofermentor on basal feed to the changes in the nutrient value. The selected samples were obtained from a slaughterhouse in Surabaya. This study consisted of two stages. The first stage was the identification of bacteria through the test of morphology, biochemical, resistance to acidity and 16S rDNA sequencing. The second stage was a test of the ability of the isolates on the nutrient of basal feeds by fermentation for three days in an aerobic condition. Based on the findings of the first phase, it has been identified that probiotic bacterium rods, motility positive, Gram-negative, have viability at pH 2 and pH 3 for 90 minutes and 24 hours and have the ability to ferment lactose, sucrose, galactose, ribose, cellobiose and xylose. Furthermore, based on test results of 16S rDNA sequencing, the probiotic bacterium was identified as Bacillus cereus WPL 415. Based on the research results at the second stage, Bacillus cereus WPL 415 at doses of 0.25% and 0.5% could improve the nutrient content of the basal feed. The results of the proximate analysis revealed that there was an increase in crude protein content of 6.78% until 8.12% compared to the control and was able to lower the crude fiber content of 15.19% and 17.40% compared to the control. Based on these results it can be concluded that Bacillus cereus WPL 415 from local beef cattle can be used as a probiotic candidates to improve the quality of animal feed.

Keywords: Bacillus cereus, probiotic, crude protein, crude fiber.
1. Note

Please read these instructions carefully and print them. At the end of the instructions you will find a button that removes this text and prepares the document for your text. (Note that this button may not work properly if you change in any way this text.) Use the styles, fonts and point sizes as defined in this template, but do not change or redefine them in any way as this will lead to unpredictable results.

2. Introduction

Probiotics are beneficial living microorganisms, either mono culture or mixed cultures that if applied to humans and animals will provide beneficial effects for the host by improving the properties of the indigenous flora, improving the health status of man or animals [1] and have the ability to modulate the balances and activities of the gastrointestinal (GI) microbiota [2]. Probiotic should be able to stimulate growth, improve feed conversion ratio and inhibit enteropathogens, without causing any undesirable effect. In the application process, probiotics must survive the stress produced during manufacturing, storage and administration at farm conditions [3]. Several strains have been used as probiotics i.e. Lactobacillus, Pediococcus, Bacteroides, Bifidobacterium, Bacillus, Streptococcus and Escherichia coli, alone or consortiated [4].

Based on the results of characterization, it was identified that Bacillus species (Bacillus cereus, Bacillus clausii, Bacillus pumilus) had potential as a probiotic based on the ability of colonization, immunostimulant, and antimicrobial activity [5]. Lactobacillus plantarum and Bacillus spp. spores have been reported to decrease the amount of Vibronaceae in rotifers fed with these additives, and subsequently increase weight and survival of turbot larvae [6]. Although some strains of Bacillus species have been used as probiotics, but the information related to the advantages of using Bacillus have not been widely reported, thus, this study aims to carry out the exploring bacteria as a probiotic candidate sourced from isolated indigenous of local beef cattle from slaughterhouses in Surabaya.

3. Materials and Methods

The materials used are fresh gut of local cattle from abattoirs, alcohol 70%, medium selective MRSB (de Man Rogosa Sharpe Broth), MRSA (de Man Rogosa Sharpe Agar) (OXOID),
medium Luria Bertani (LB) (MERCK), Gram stain (Crystal Violet, Lugol, alcoholacetone, and safranin), physiological NaCl, 0.1 N HCl. **Sample Preparation.** The part of intestinal organs were rinsed with sterile distilled water and crushed using a mortar and weighed as much as 1 g and put into a sterile physiological NaCl solution and diluted with multilevel dilution \((10^{-1}-10^{-6})\). A total of 1 ml of \(10^{-6}\) dilution was inoculated on MRSA (Man Ragosa Sharpe Agar) medium, then incubated for 24-48 hours at 37°C. **Purification of Bacteria Probiotic Candidate.** Purification was done by selecting a colony on the surface and then inoculated on the surface of MRSA (Man Ragosa Sharpe Agar) medium with scratch method to obtain a separate colony. It was incubated at 37°C for 2x24 hours. The purification step could be done 2-3 times to obtain pure isolates. Furthermore, the isolate was inoculated on MRSA medium slant as stocks for further testing. The observation of cell morphology was done by using the Gram stain. **Test of Resistance on Acidity (pH).** The resistance test on acidity was done using MRS broth medium supplemented with 0.1 N HCl to obtain pH 23 (according to the pH of the stomach). As much as 1 ose each bacterial isolates was taken from the stock culture and was inoculated on MRSB-HCl medium. After that, it was incubated for 2x24 hours at 37°C. If there was a growth of bacteria on MRSB-HCL medium, it showed positive results, and it showed negative results if there was no growth of bacteria on the MRSB-HCL medium. **Identification of the gene encoding 16S rDNA.** DNA isolation was using the method of Ausubel [7]. **The second stage.** In the second phase of the study was carried out a test of B.cereus WPL 415 ability against basal feed through the fermentation process. The study was divided into three treatments with each of eight repetitions. The treatment consisted of: P0: feed without B.cereus WPL 415, P1: feed + 0.25% B.cereus WPL 415, and P2: feed + 0.50% B.cereus WPL 415. The fermentation of basal feed was done by adding a solution of inoculant appropriate treatment dose, then dissolved in a 3% drop and 20% non-chlorine water. After the solution was mixed homogeneously, sprayed on basal feed. Fermentation was done under anaerobic conditions using a plastic bag as a silo for 3 days. After the fermentation period ended, the plastic was opened, then proximate analysis was performed to determine changes in the nutritional content of crude protein and crude fibre. Data were analysed by analysis of variance. If the results were significantly different (\(P <0.05\)) between treatments, the analysis was proceeded by Duncan’s multiple range test [8].

### 4. Results and Discussion

Based on identification results, the isolate obtained from the small intestine of cattle, had the following characteristics: a rod-shaped cells, Gram negative and positive
motility. The results of biochemical tests isolates obtained in this study demonstrated the ability of fermentation as listed in Table 1.

The arrangement of obtained nucleotide isolates, further was identified with the program BLAST (Basic Local Alignment Search Tool) in www.ncbi.com and isolates obtained which had some similarities with the arrangement of nucleotide similarity level 92% - 88%. (Bacillus cereus ATCC 14579, identity 92%, sequence ID ref|NC_004722.1; Bacillus cereus Rock4-18, identity 92%, sequence ID ref|NZ_CM000735.1; Bacillus cereus AH621, identity 92%, sequence ID ref|NZ_CM000719.1; Bacillus megaterium DSM319, identity 89%, sequence ID ref|NC_014103.1; Bacillus licheniformis ATCC 14580, identity 88%, sequence ID ref|NC_006270.3; Bacillus hemicellulosilyticus JCM 9152, identity 88%, sequence ID ref|NZ_BAUU01000088.1; Bacillus amylyoliquefaciens DSM7, identity 88%, sequence ID ref|NC_014551.1; Bacillus subtilis subsp. subtilis str. 168, identity 88%, sequence ID ref|NC_00964.3; Bacillus subtilis subsp. spizizenii TU-B-10, identity 88%, sequence ID ref|NC_016047.1; Bacillus cellulosilyticus DSM 2522, identity 88%, sequence ID ref|NC_014829.1)

This research showed new isolate namely Bacillus cereus WPL 415. Based on the research results of Navinchandran, had been identified through sequencing of 16 S rRNA, a probiotic bacterium Bacillus cereus from the gut of wild shrimp Penaeus monodon [9]. The probiotic bacterium had antagonistic activity against pathogenic bacteria in shrimp as well as having the ability to produce extracellular enzymes. Probiotic B. cereus at a concentration of 0.4% / 100 g of feed was efficient in stimulating the growth (specific growth rate / SGR of 4.40 ± 0.179%
and a better feed conversion ratio (FCR of 1.27 ± 0.081) and immunity (total count haemocyte, lysozyme activity, plasma protein concentration and bactericidal activity) in shrimp.  

T 2: Viability of the bacteria *Bacillus cereus* WPL 415 at pH 3 and pH 4.

<table>
<thead>
<tr>
<th>Time</th>
<th>MRS Agar (control) CFU/ml</th>
<th>MRS Agar pH 3 CFU/ml</th>
<th>MRS Agar pH 4 CFU/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>90 minutes</td>
<td>1.4 × 10⁸</td>
<td>1.0 × 10⁸</td>
<td>2.1 × 10⁸</td>
</tr>
<tr>
<td>24 hours</td>
<td>2.1 × 10⁸</td>
<td>1.2 × 10⁸</td>
<td>2.0 × 10⁸</td>
</tr>
</tbody>
</table>

Among the various species of probiotics, those belong to the genus of *Bacillus* which had the advantage that, due to their capacity to sporulate, they survive at ambient temperatures as well as during desiccation by methods that involve; moderate heating, such as spray dryers, avoiding the use of lyophilization or other expensive technologies [10]. *B. cereus* strains were shown to persist in the mouse gastrointestinal tract for up to 18 days post administration, demonstrating that these organisms had some abilities to colonize. The spores of one *B. cereus* strains were extremely sensitive to simulated gastric conditions and simulated intestinal fluids [11].

*Bacillus* is able to grow on various kinds of sugar, these isolates also has a cellulolytic activity shown on its ability to degrade trinitrophenyl-carboxymethyl cellulose and growth on medium containing glucose cellobiose or produce the largest cellulolytic activity. Cellulolytic activity is not generated until the stationary phase of growth. Maximum cellulolytic activity test occurs at pH 4.8 and a temperature of 58 °C [12].

The research results in Table 2 indicated that the isolates of *Bacillus cereus* was able to sustain life in the acidic conditions of pH 3-pH 4. It was shown from the comparison with the controls, which in 90 minutes showed the viability of control about 1.4 × 10⁸ - 2.1 × 10⁸ CFU / ml. On the condition of acid pH 3 for 90 minutes, *Bacillus cereus* isolates demonstrated the viability of 1.0 × 10⁸ - 1.2 × 10⁸ CFU / ml, whereas at pH 4 for 90 minutes showed the viability of 2.0 × 10⁸ - 2.1 × 10⁸ CFU / ml. Later in the control condition for 24 hours, *Bacillus cereus* isolates demonstrated the viability of 7.5 × 10⁸ 6.6 × 10⁸ CFU / ml, whereas at pH 3 for 24 hours
demonstrated the viability of $0.4 \times 10^8 - 1.0 \times 10^8$ CFU/ml. On the condition of acid pH 4 demonstrated the viability of $3.6 \times 10^8 - 4.8 \times 10^8$ CFU/ml.

Resistance to acidity test results showed that isolates of *Bacillus cereus* was considered able to survive through the digestive tract system that had a low pH conditions, so as to reach the intestine to be able to do activities to maintain the balance of microflora. *Bacillus cereus* has the ability to survive in the intestinal tract and during manufacturing, such as interaction with enteropathogens, resistance to heat and to variation of pH.

Variations in pH of the gastrointestinal tract could affect the viability of the isolate as a probiotic candidate. Bacillus genus have strong adaptability to diverse conditions and that several species produce highly resistant spores; they have been isolated from fish [13;14]. Based on the results of statistical analysis, the use of *B. cereus* WPL 415 in the fermentation basal feed showed significant differences among treatments on the content of crude protein and crude fibre (Table 3).

The results of the proximate analysis the crude protein content was the lowest for the P0 treatment (control, without the use of *B. cereus* WPL 415), while the use of 0:25 % *B. cereus* WPL 415 and 0.5% crude protein were able to increase the content of 6.78 % until 8:12% compared with control. The highest results of the proximate analysis of crude fibre content was at P0 treatment (control, without the use of *B. cereus* WPL 415), while the use 0.25% *B. cereus* WPL 415 and 0.5% were able to lower crude fibre content of 15.19% to 17:40% compared to control patients. The results of this study indicate that WPL 415 *B. cereus* was able to increase the nutrient of the forage. The increase in crude protein content was due to the increased biomass of single cell protein *B. cereus* WPL 415. In the fermentation process, nutrients were available for the media used for the breeding biomass of *B. cereus* WPL 415, thus, it increased the number of microbe biomass, in which the increase was detected from the results of proximate analysis of crude protein.
The use of *B. cereus* WPL 415 in the fermentation basal feed could also reduce the content of crude fibre. It was supported by the test results that demonstrates the ability of isolates to ferment crude fibre, namely xylose and cellobiose. Cellulose fraction was the biggest component of a constituent of plant cell walls that were very difficult and even could not be digested by monogastric digestive enzymes, so that the cellulose must be broken down first into low molecular weight compounds such as mono, di and tri saccharides. The degradation involved a complex of cellulase enzymes produced by microbes are endo-beta-glucanase and beta-glucosidase.

Cellulase enzyme is a complex enzyme consists of a group of enzymes that work synergistically to degrade cellulose i.e. 1, 4-beta-D-glucan-4-glucanohydrolases or endoglucanase; 1, 4-beta-D-glucan glucanohydrolases or exoglucanases and beta-glucoside glucohydrolases or cellobiase. Endoglucanase enzyme randomly cut the internal amorphous on the chain of 1, 4-beta polysaccharides cellulose into cellulo-oligosaccharides. Exoglucanases enzyme take a role in glucose unit at the polar end of the reduction or nonreduction of the chain of cellulo-oligosaccharides produces Cellobiose (disaccharide). The beta-Glucosidases enzyme hydrolyse Cellobiose into glucose [15]. Supplementation of 0.20% -0.40% rumen bacterial culture cellulolytic isolates buffalo can improve the body weight gain and feed efficiency of ducks. This is due to the addition of a bacteria culture acts as probiotics can stimulate the synthetic enzyme digestion enhancing the utilization of nutrients [16].

Based on the results of the study [17], the use of cow’s rumen fluid was very potential as inoculant that contained of high nutrients and ready fermentable microbial and fibre degrading enzymes. The use of rumen fluid was capable of producing an inoculant with high nutrient and microbiology that were effective to be used as a starter. The high population of microbial inoculant and the support by the substrate degradation ability was high, and the high activity of cellulose and xylanase enzymes had been able to decrease crude fibre content of the ration so that the nutrient of the ration fermented inoculants’ quality produced increases. The improvement quality of the nutrient of the fermented feed gave a positive response to the increase in the digestibility [18]. The results of other studies indicate the existence of extracellular products produced by Bacillus sp which indicates strongly inhibited the growth of *Aeromonas hydrophila* and *Vibrio alginolyticus* isolated from diseased fish. APIZYM Enzyme assays showed that both bacteria have esterase lipase, leucine arylamidase, acid phosphatase, lipase and Naphthol-AS-BI-phosphohydrolase activities [19].
5. Conclusion

Based on these results it can be concluded that *Bacillus cereus* WPL 415 from local beef intestine can be used as a probiotic candidates to improve the nutrient value of animal feed stuff.

Acknowledgments

The author would like to thank to the Rector of the University of Airlangga, Chairman of the Institute for Research and Innovative of Airlangga University, and Ministry of Research, Technology and Higher Education who has funded this research on Commodity Research Universities (PUPT). Researchers also would like to thank to all who has helped this research.

References


