The 6th ISTAP International Seminar on Tropical Animal Production

“Integrated Approach in Developing Sustainable Tropical Animal Production”

PROCEEDINGS

PART I

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LIST OF CONTENTS

PREFACE ....................................................................................................................................iii
REPORT FROM ORGANIZING COMMITTEE........................................................................ iv
WELCOME ADDRESS............................................................................................................v
OPENING REMARKS .............................................................................................................vi
LIST OF CONTENTS ..............................................................................................................vii

PLENARY SESSION

1. Strategies to Increase the Domestic Production of Raw Milk in Indonesia and Other South East Asian Countries
   John Moran and Phillip Morey ..........................................................................................1-11

2. Nutritional Challenges of Lactating Dairy Cattle in a Tropical Climate
   J. K. Bernard ....................................................................................................................12-17

3. Feed, Land, and Landscape for Sustainable Animal Production
   Shaukat A. Abdulrazak a and Isaac M. Osugab .................................................................18-18

4. Food Safety Regulation and Halal Food Issues in Indonesia
   Roy Sparringa ...................................................................................................................19-19

5. Extension System for Livestock Development in Developing Countries: Knowledge Management Application
   Budi Guntoro ...................................................................................................................20-27

6. Structural Development of Livestock Farms in a Global Perspective
   Henning Otte Hansen ........................................................................................................28-50

7. Whole Farm Problems with Heat Stress – It’s Not Just for Lactating Dairy Cows
   Allen Young ....................................................................................................................51-57

LEAD PAPER

1. Antimicrobial Peptides Expression for Defense System in Chicken Gastrointestinal and Reproductive Organs
   Yukinori Yoshimura, Bambang Ariyadi, and Naoki Isobe .................................................58-60

2. Improving Technology Adoption and Sustainability of Programs to Increase Bali Cattle Productivity in West Nusa Tenggara Province, Indonesia
   Yusuf A. Sutaryono, T. Panjaitan, and Dahlanuddin .........................................................61-66

3. The Role of Family Poultry Systems in Tropical Countries
   Yusuf L. Henuk, Monchai Duangjinda, and Chris A. Bailey .............................................67-71
SUPPORTING PAPERS

Part I

Animal Feed and Nutrition

1. NM-03-P The Marl and Kaolin in Broiler Diet: Effects on the Bone Weight and the Cutting Yield
   D. Ouachem, A. Meredef, and N. Kaboul.................................72-75

2. NM-04-P The Effect of Liquid Nanocapsule Level on Broiler Fat Quality
   Andri Kusmayadi, Zuprizal, Supadmo, Nanung Danar Dono, Tri Yuwanta, Ari Kusuma Wati, Ronny Martien, Sundari................76-79

3. NM-05-O Production and Egg Quality of Quail Layer Given Diets Containing Different Levels of Crab (Portunus pelagicus) by-Product Meal
   K.G. Wiriyawan, Syamsuhaidi, D.K. Purnamasari, and T.S. Binetra.................................................................80-84

4. NM-08-P A Preliminary Study on the Use of Enzyme and Organic Acids in Rice Bran-containing Diet at Two Levels of Dietary Protein for Rabbit
   Tuti Haryati and Yono C. Raharjo........................................85-89

5. NM-09-O Efficacy of Toxin Binder in Reducing Induced Aflatoxin B and Ochratoxin A in Broiler Feed
   Anjum Khalique, Muhammad Umer Zahid, Jibran Hussain, Zahid Rasool.................................................................90-93

6. NM-10-O Evaluation of Local Feed in Broiler Diets in Small Scale Farm in Palu Central Sulawesi

7. NM-11-O Digestibility and Nutritional Value of Gedi (Abelmoschus manihot (L.) Medik) Leaves Meal in the Diet of Broilers
   Jet Saartje Mandey, Hendrawan Soetanto, Osfar Sjofjan, Bernat Tulung.........................................................100-104

8. NM-12-O Utilization of Skipjack Tuna (Katsuwonus pelamis L.) Gill in Diet as a Source of Protein on Carcass Quality of Broiler Chickens
   Jein Rinny Leke, Jet S. Mandey, Meity Sompie, Fenny R. Wolayan..........................................................105-109

9. NM-13-O The Dynamics of Indigenous Probiotics Lactic Acid Bacteria on Growth Performance, Total Adherence Bacteria, and Short-Chain Fatty Acids Production in the Ileum of Male Quail
   Sri Harimurti, Sri Sudaryati and Bambang Ariyadi...............110-110
10. NM-14-O  Selection of Human-origin Lactobacillus Strains as Probiotics with Capability in Synthesizing Conjugated Linoleic Acid and Alleviating Hyperglycemia in Rats (Rattus norvegicus) in Vivo
   **Widodo, Pradipta Ayu Harsita, Samuel Aditya, Nosa Septiana Anindita, Tutik Dwi Wahyuningsih and Arief Nurrochmad**.................................................................111-116

   **Lilik Retna Kartikasari, Adi Magna Patriadi Nuhriawangsa, Winny Swastike and Bayu Setya Hertanto**..........................117-117

12. NM-16-O  Performance of Japanese Quails Fed Different Protein Levels and Supplemented with Betaine
   **Adi Ratriyanto, Rysca Indreswari, Adi Magna Patriadi Nuhriawangsa, Apriliana Endah Haryanti**..........................118-122

13. NM-17-O  The Influence of Vitamin D3 Levels on Diets with Phytase on Production Performance of Layer Quail (Coturnix coturnix japonica)
   **Adi Magna Patriadi Nuhriawangsa, Adi Ratriyanto, Winny Swastike, Rysca Indreswari, Ahmad Pramono and Try Haryanto**.........123-126

14. NM-20-O  Phytobiotics Habbatus Sauda and Garlic Meal: Are Still Efficacious During the Spread of Marek's Disease Outbreak?
   **N.D. Dono, E. Indarto, Kustantinah, Zuprizal**........................................127-131

15. NM-22-O  The Effect of Dietary Calcium and Phosphorus Level on Serum Mineral Contents of the Bantul Local Duck within a Day
   **H. Sasongko, T. Yuwanta, Zuprizal, Supadmo, and I. Widiyono**.................................................................132-132

16. NR-01-P  Supplementation Local Feed Urea Gula Air Multinutrient Block and Different Levels of Sulphur for Increase Lactation Productivity Doe Also Decrease Kid Mortality Bligon Goat Grazed at Timor Savannah
   **Arnold E. Manu, Yusuf L. Henuk, H.L.L.Belli, M.M. Kleden**......133-137

17. NR-02-P  Methane Production and Rumen Fermentation Characteristics of Buffalo Ration Containing Sorghum Silage with Rumen Simulation Technique (RUSITEC) Methods
   **Teguh Wahyono, Dewi Apri Astuti, Komang G. Wiryawan, Irawan Sugoro, Suharyono**..........................................................138-142

18. NR-04-O  Body Weight Gain Response of Sumba Ongole Cattle to the Improvement of Feed Quality in East Sumba District, East Nusa Tenggara, Indonesia
   **Debora Kana Hau and Jacob Nulik**..................................................143-146
19. NR-05-O Daily Body Weight Gain of Bali Cattle Fed with Leucaena Leucocephala as the Main Ration in West Timor, East Nusa Tenggara, Indonesia
Jacob Nulik and Debora Kana Hau.................................................................147-150

20. NR-06-O Tannin Anthelmintic Doses, Metabolizable Energy and Undegraded Protein Contents of Rubber Leaves (Hevea brasiliensis) as Herbal Nutrition for Goats
Sri Wigati, Maksudi Maksudi, Abdul Latief and Eko Wiyanto .151-155

21. NR-07-P Consumption and Digestibility of Nutrients in Bali Cattle at the Last Period of Pregnancy Kept under Semi Intensive System Supplemented with Nutritive Rich Feed Contained Lemuru Oil and Zinc
Erna Hartati, E.D. Sulistijo, A. Saleh...............................................................156-160

22. NR-08-P Preliminary Screening for Anthelmintic Potential of Sesbania grandiflora Leaves for Parasitic Infected Goats in Short-Term Trial
Mohd Azrul Lokman, Kanokporn Phetdee, Sathaporn Jittapalapong and Somkiert Prasanpanich.................................................................161-165

23. NR-09-O The Effect of Urea Treated Straws and Urea-Molasses Feed Blocks (UMB) on Reproductive Performance of Libyan Barbary Sheep


25. NR-11-O Chemical Composition, Antioxidant Compounds and Antioxidant Capacity of Ensiled Coffee Pulp

26. NR-12-O Influence of Starch Type as Substrate Material in Dry Lactic Acid Bacteria Inoculant Preparation on Fermentation Quality and Nutrient Digestibility of King Grass Silage
B. Santoso, B. Tj. Hariadi and Jeni.................................................................182-186

27. NR-13-O Responses of Growing-Female Crossbred Etawa Goats Fed Concentrates Containing by product of Traditional Fried Snack Industry with Different Levels of Urea
A R. S. Asih, K G. Wiryawan, I. N. Sadia, and Kertanegara........187-190
28. NR-14-O  Restriction Feed and Refeeding Evaluation for Consumption, Feed Cost, Income Over Feed Cost, Percentage of Carcass and Meat Quality Kacang Goat
Bambang Suwignyo, Miftahush Shirothul Haq, Setiyono, and Edi Suryanto ...............................................................191-197

29. NR-15-O  Characteristics of polyunsaturated fatty acids and nutrient digestibility feed cattle of the fermented rumen fluid by one and two stage in vitro

30. NR-16-P  Performance and Economic Efficiency of young Anglo-Nubian Goat Fed Different Protein and Energy
I-G.M.Budiarsana, Supriyati and L. Praharani .................................................203-207

31. NR-17-P  Effect of Choline Chloride Supplementations on Productive Performance of Etawa Crossbred Goats
Supriyati Kompiang, I Gusti Made Budiarsana, Rantan Krisnan, Lisa Praharani.................................208-212

32. NR-18-O  Body Weight Gain of Donggala Bull Given Supplement Feed on Basis of Cocoa Pod Husks Fermentation
F.F. Munier, Mardiana Dewi, and Soeharsono...........................................213-217

33. NR-19-O  Influence of Cellulolytic Bacteria from Rumen Fluid on In Vitro Gas Production of Robusta Coffee Pulp (Coffea canephora Sp.) Fermented
Chusnul Hanim, Lies Mira Yusiai, and Fahriza Anjaya Jazim.........218-222

34. NR-20-P  Growth and Productivity of Brachiaria brizantha cv MG 5 under the effect of different dose of NPK fertilization
Nafiatul Umami, Meita Puspa Dewi, Bambang Suhartanto, Cuk Tri Noviandi, Nilo Suseno, Genki Ishigaki, Ryo Akashi.........................................................223-227

35. NR-21-O  Indigofera Sp as a Source of Protein in Forages for Kacang Goat in Lactation and Weaning Period
A. Nurhayu and Andi Baso Lompengeng Ishak..................................228-232

36. NR-22-O  Supplementing Energy and Protein at Different Degradability to Basal Diet on Total Protozoa and Microbial Biomass Protein Content of Ongole Grades Cattle
Dicky Pamungkas, R. Utomo, dan M. Winugroho..........................233-237

37. NR-24-O  Nutritive Evaluation of Pineapple Peel Fermented by Cellulolytic Microbe and Lactic AcidBacteria by In Vitro Gas Production Technique
Lies Mira Yusiai, Chusnul Hanim and Caecilia Siska Setyawati......................................................238-242
38. NR-25-O The Supplementation of ZnSO₄ and Zn-Cu Isoleusinate in the Local Feed Based at Last Gestation Period on Dry Matter Consumption and Digestibility and Calf Birth Weight of Bali Cattle
   FMS Telupere, E Hartati, and A. Saleh........................................243-247

39. NR-26-O Local Micro Organisms (LOM) as an Activator to Enhance the Quality of Various Plant Waste as Feed
   Andi Ella, A. Nurhayu and A. B. Lompengeng Ishak.........................248-251

40. NR-27-O Organic Acid and Inhibition of Complete Silage Ration on the Growth of Salmonella enteritidis
   Allaily, Nahrowi, M. Ridla, M. Aman Yaman.................................252-256

41. NR-28-O The utilization of some feed supplement by using or without molasses on local male sheep on fermentation results in rumen liquid, daily live weight gain, production, C/N ratio and water content of feces
   Suharyono, Teguh Wahyono, C. Ellen, K and Asih Kurniawati.................................................................257-260

42. NR-29-O Evaluation of Albaizia chinensis as Tannins Source for in Vitro Methane Production Inhibitor Agents Sheep Rumen Liquor
   Anas, M. A., Yusiati, L. M., Kurniawati, A., Hanim, C.................261-265

43. NR-30-O Growth and Productivity of Sorghum Bicolor (L.) Moench in Merapi Eruption Soil with Organic Fertilizer Addition
   Suwignyo, B, B. Suhartanto, G. Pawening, B.W. Pratomo...............266-270

44. NR-31-P Quality and Storability of Pelleted Cassava (Manihot utilisima) Leaves var. Bitter
   Ristianto Utomo, Subur Priyono Sasmito Budhi, Cuk Tri Noviandi,
   Ali Agus, and Fidrais Hanafi ..................................................271-274

45. NR-32-O Biomass Production of Pueraria javanica Using Rhizobium Inoculant and Urine Bali Cattle in East Borneo
   Ida Ketut Muhita, Nafiut Umami, Subur Priyono Sasmito Budhi and
   Endang Biliarti ........................................................................275-280

46. NR-33-P The Effect of Using Different Sources of Carbohydrates to Feed Efficiency on Indigenous Thin Tailed Male Lamb
   Muktiani, A, A. Purnomoadi, E. Prayitno........................................281-285

47. NR-35-O Substitution of Concentrate by Protein Source Forage for Growing Heifer of Friesian Holstein (FH)
   Y. Widiawati and M. Winugroho...............................................286-290

48. NR-38-O The Use of Tricodherma sp. as a Starter of Fermentation Dry Teak Leaves (Tectona grandis) as Animal Feed
   Yunianta and Hartatik.........................................................291-295
49. NR-39-P Nutritive Values of Rice Straw Fermentation Used Carbon Sources on Different Level With Various of Inoculant Levels *Aspergillus niger* and *Lactobacillus plantarum*  
*R. Agus Tri Widodo Saputro, Nono Ngadiyono, Lies Mira Yusiati, I Gede Suparta Budisatria* ...............................................................296-300

50. NR-40-O The Fat Protective Effect of Fish Oil, Sunflower Seed Oil and Corn Oil on Fluid Rumen Fermentation Parameters  
*Agustinah Setyaningrum, Soeparno, Lies Mira Yusiati and Kustantinah* .................................................................301-305

51. NR-41-O The Effect of Supplementation of Gliricidia or Rice Bran on Liveweight Gain, Feed Intake and Digestibility of Kacang Goat Fed Mulato Grass  
*Marsetyo, Damry and Mustaring* ........................................................................306-310

52. NR-42-P In Sacco Feeding Value of Multi-Stage Ammoniated Palm Press Fiber  
*Armina Fariani, Arfan Abrar and Gatot Muslim* ...............................................311-311

53. NR-43-O Alternative Rations to Maintain High Growth Rate of Bali Bulls Fattened with *Leucaena* Based Diet in Sumbawa, Eastern Indonesia  
*T. S. Panjaitan* ................................................................................................312-315

54. NR-44-O The Use of Ramie By-Product (*Boehmeria nivea*) Materials as Complete Feed on the Growth and Hematology of Weaning Etawah Cross Breed Goat  
*Emmy Susanti, Ali Agus, Y. Y. Suranindyah, and F. M. Suhartati* .................................................................316-320

55. NR-45-O Study on Complete Feed Fermentation of Agricultural By-Product on Performance Etawah Goat  
*Yusdar Zakaria, Yurliasmi, Cut Intan Novita* ................................................321-325

56. NR-46-P Carcass Production and Component of Lamb Provided Metanogenic Inhibitor Feed  

**Small Ruminant, Beef Cattle, Animal Draught and Companion Animal**

57. PPO-01-O Correlation between the Slaughter Weight, Carcass Weight, with Body Measurements of Cattle in Kebumen, Central Java  
*Setiyono, Suharjono Triatmojo, Trisakti Haryadi, Dino Eka Putra* .................................................................331-334

58. PPO-02-O Production of Stingless Bees (*Trigona sp.*) Propolis in Various Bee Hives Design  
*Agus salim, Nafiatul Umami, Erwan* .................................................................335-338
<table>
<thead>
<tr>
<th>No.</th>
<th>Code</th>
<th>Title</th>
<th>Authors</th>
<th>Pages</th>
</tr>
</thead>
<tbody>
<tr>
<td>59.</td>
<td>PPO-03-P</td>
<td>Morphological Characteristics and Performance Boerawa Goat in Tanggamus District Lampung Province</td>
<td><em>Kusuma Adhianto and M. Dima Iqbal Hamdani</em></td>
<td>339-342</td>
</tr>
<tr>
<td>60.</td>
<td>PPO-04-P</td>
<td>Growth, Carcass Production and Meat Quality of Ongole Grade Cattle, Simmental Ongole Crossbred Cattle and Brahman Cross</td>
<td><em>N. Ngadiyono, Soeparno, Panjono, Setiyono and I. Akhmadi</em></td>
<td>343-347</td>
</tr>
<tr>
<td>61.</td>
<td>PPO-06-O</td>
<td>Growth and Rumen Environment of Pre-weaning Bali Calves Offered Different Forage Based Calf Supplements</td>
<td><em>IGN Jelantik, ML Mullik, TT Nikolaus, T Dami Dato, IG Mahardika, NP Suwiti, C Leo Penu, J. Jeremias, A. Tabun</em></td>
<td>348-352</td>
</tr>
<tr>
<td>63.</td>
<td>PPO-08-O</td>
<td>Effect of Different Lands on Heat Tolerance Coefficient and Body Weight Gain of Ram Fat Tailed Sheep</td>
<td><em>Rachmawati, A., H. Nugroho and E. Y. Wanto</em></td>
<td>359-359</td>
</tr>
<tr>
<td>64.</td>
<td>PPO-09-O</td>
<td>The Effects of Hair Colors Differences on the Performance of Etawah Grade Doe</td>
<td><em>I Gede Suparta Budisatria, Panjono, Dyah Maharani</em></td>
<td>360-364</td>
</tr>
<tr>
<td>65.</td>
<td>PPO-10-P</td>
<td>Age and Body Weight at Puberty and Service per Conception of Ongole Crossbred Heifer on Smallholder Farming System</td>
<td><em>Endang Baliarti, Bayu Andri Atmoko, Febri Aryanti, Nono Ngadiyono, I Gede Suparta Budisatria, Panjono, Tri Satya Mastuti Widi, M. Danang Eko Yulianto, Sigit Bintara</em></td>
<td>365-369</td>
</tr>
<tr>
<td>66.</td>
<td>PPO-11-O</td>
<td>Performance of Three Breeds of Sudanese Cattle</td>
<td><em>Hassan Ishag Hassan Haren and Hatim Idris</em></td>
<td>370-373</td>
</tr>
</tbody>
</table>

**Poultry Science**

<table>
<thead>
<tr>
<th>No.</th>
<th>Code</th>
<th>Title</th>
<th>Authors</th>
<th>Pages</th>
</tr>
</thead>
<tbody>
<tr>
<td>67.</td>
<td>PU-01-P</td>
<td>Biosecurity Measurements in Poultry Farming System in Kuwait</td>
<td><em>A.A. Alsaffar</em></td>
<td>374-376</td>
</tr>
<tr>
<td>68.</td>
<td>PU-03-O</td>
<td>Effect of Mating and Polymorphism Insulin Like Growth Factor Binding Protein 2 Gene on Body Weight and Heritability of Kampung Chicken</td>
<td><em>Sri Sudaryati, J.H.P. Sidadolog, Wihandoyo, W.T. Artama</em></td>
<td>377-381</td>
</tr>
<tr>
<td>69.</td>
<td>PU-05-O</td>
<td>The Residue Profile of Ciprofloxacin in Broiler Muscle and Liver</td>
<td><em>Agustina Dwi Wijayanti, Ambarwati, Wa Ode Sitti Falah Ramli</em></td>
<td>382-386</td>
</tr>
<tr>
<td>Paper ID</td>
<td>Title</td>
<td>Authors</td>
<td>Pages</td>
<td></td>
</tr>
<tr>
<td>---------</td>
<td>-----------------------------------------------------------------------------------------</td>
<td>---------------------------------------------------------------------------------------------</td>
<td>-------</td>
<td></td>
</tr>
<tr>
<td>PU-06-O</td>
<td>Selection for 10 Weeks Old Body-Weight on Sentul Chicken</td>
<td>Sofjan Iskandar and Tike Sartika</td>
<td>387-390</td>
<td></td>
</tr>
<tr>
<td>PU-07-O</td>
<td>Analysis of Reproductive Potential and Hatchability of Naked Neck and Normal Hens</td>
<td>Jafendi H.P.Sidadolog, Tri Yuwanta, Wihandoyo, Sri Harimurti, Sri Sudaryati, Heru Sasongko and Bambang Ariyadi</td>
<td>391-396</td>
<td></td>
</tr>
<tr>
<td>PU-08-O</td>
<td>Localization and Molecular Size of Mucin2 Glycoproteins Forming the Gut Mucosal Barrier in the Indonesian Indigenous Naked Neck and Normal Feathered Chickens</td>
<td>B. Ariyadi, J.H.P. Sidadolog, S. Harimurti, S. Sudaryati, and Wihandoyo</td>
<td>397-400</td>
<td></td>
</tr>
</tbody>
</table>

**Dairy Science and Industry**

<table>
<thead>
<tr>
<th>Paper ID</th>
<th>Title</th>
<th>Authors</th>
<th>Pages</th>
</tr>
</thead>
<tbody>
<tr>
<td>PPP-01-P</td>
<td>Milk Quality Of Anglo Nubian X Etawah Grade Goats And Saanen X Etawah Grade Goats At First Kidding Period</td>
<td>Lisa Praharani, Supryati, and Rantan Krisnan</td>
<td>401-405</td>
</tr>
<tr>
<td>PPP-02-O</td>
<td>Performance of Dairy Cattle with Supplementation of Rumensin, Garlic Husk (Allium sativum) and Organic Minerals in Ration</td>
<td>Caribu Hadi Prayitno, Suwarno, and Aiffah Noor Hidayah</td>
<td>406-409</td>
</tr>
<tr>
<td>PPP-06-P</td>
<td>Diacylglycerol Acyltransferase1 (DGAT1) Gene Polymorphism in New Zealand Holstein Friesian Cattle under Dairy Breeding Station and Its Correlation with Milk Quality</td>
<td>SA. Asmarasari, C. Sumantri, IW Mathius, A. Anggraeni</td>
<td>418-422</td>
</tr>
<tr>
<td>PPP-07-O</td>
<td>Reaction of Cathelicidin-2 secreted from goats milk leukocytes to lipopolysaccharide</td>
<td>Moemi Nishikawa, Yukinori Yoshimura, and Naoki Isobe</td>
<td>423-425</td>
</tr>
</tbody>
</table>
PART II

Animal Breeding and Reproduction

79. PPE-01-P Identification of Pure Breed Bali Cattle by Using Molecular Approach
    Endang Tri Margawati, Indriawati, Slamet Diah Volkandari and Muhammad Ridwan...
    426-431

80. PPE-02-P Milk Transmitting Ability of Saanen Bucks under Intensive Management
    Anneke Anggraeni.......................................................... 432-436

81. PPE-03-O Genetic Markers of Twinning Births of Local Beef Cattle and Its
    Crossbreds in Indonesian
    A. Anggraeni, S. A. Asmarasari, H. Hasinah, C. Talib and
    B. Tiesnamurti................................................................. 437-441

82. PPE-04-P Association of Prolactin Gene with Egg Production in PMp Ducks
    T. Susanti and I. P. Sari................................................ 442-446

83. PPE-05-P Microsatellite analysis of genetic diversity in Pekin, Alabio, and their
    crossbred duck populations
    L. Hardi Prasetyo, T. Susanti, T. Purwadaria........................ 447-447

84. PPE-08-P Genotypic Profile of Ettawa Grade Goat with Different Head and Neck
    Color Based on MC1R Gene
    Dyah Maharani, I Gede Suparta Budisatria, Panjono, Tety Hartatik
    and Slamet Diah Volkandari.................................................. 448-451

85. PPE-09-O Polymorphism of Promoter Prolactine Gene and Its Association with Egg
    Production of Selected Indonesian Kampung Chicken (KUB)
    Tike Sartika................................................................. 452-452

86. PPE-10-O Qualitative And Quantitative Traits of Kokok Balenggek Chicken, the
    Rare Indigenous Chicken in West Sumatera
    Firda Arlina, Hafil Abbas, Sarbaini Anwar, Jamsari............. 453-457

87. PPE-11-O Phenotype Measurements of Bali Cattle Combined with Interviews
    of Farmers from Multiple Locations in Indonesia as a Resource for
    Development of Breeding Programs
    Ann Eriksson, Endang Tri Margawati, Indriawati, Ronny Rachman
    Noor, Goran Andersson, Emma M Svensson.............................. 458-462

88. PPE-12-O Investigating the genetic status of Bali cattle in Indonesia using large scale
    genotyping
    Emma Svensson, Ann Eriksson, Ida Clemensson Lindell, Endang Tri
    Margawati, Rere Indriawati, Ronny Rachman Noor and
    Göran Andersson.............................................................. 463-463
89. **PPE-14-P** Genetic Variation and Phylogenetic Tree of Indonesian domestic Goat  
*Tety Hartatik, Kustantinah, Ristianto Utomo and Lies Mira Yusiati* .......................................................... 464-469

90. **PRP-01-O** Identification of GH/Alu-I Gene Polymorphisms in Indonesian Simeulue Buffalo  

91. **PRP-02-O** Reproduction Performance of Bali Cow at Three Areas of Bali Province  
*Andoyo Supriyantono* .......................................................... 475-479

92. **PRP-03-O** Blood Lipid Profile of Hypercholesterolemia Rattus norvegicus L. Fed with Sausages Containing Omega 3 and Omega 6 Fatty Acids  
*Rio Olympias Sujarwanta, Edi Suryanto, Setiyono, Supadmo, Rusman, Jamhari, Yuny Erwanto* .......................................................... 480-484

93. **PRP-04-O** The Effect of Kayu Akway (*Drymis sp*) Extract on The Number of Leukocyte of The Male Mice (*Mus musculus L*)  
*Purwaningsih, Angelina N. Tethool* .......................................................... 485-488

94. **PRP-05-O** In Vitro Maturation Rate of Bligon Goat Oocytes Supplemented with Gonadotrophin  
*Diah Tri Widayati and Mulyoto Pangestu* .......................................................... 489-493

95. **PRP-06-P** A Preliminary Study of the Use of Hormones on the Reproductive Performance of some Breeds of Rabbits  
*Bayu D. P. Soewandi and Yono C. Raharjo* .......................................................... 494-497

96. **PRP-08-P** The use of vaginal smear method based on the morphology of the vaginal mucosa epithelial cells for the dairy cows cycle estrus detection  
*Riyanto, J., Sunarto, S. D. Widyawati and Lutojo* .......................................................... 498-501

97. **PRP-09-P** Optimization of Bovine Sperm Sexing: Modification of Column Length and Separation Time  
*Riasari Gail Sianturi and D.A. Kusumaningrum* .......................................................... 502-506

98. **PRP-10-O** The Detailed Motility and Velocity Characteristics of Rams Spermatozoa as Assessed by Computer-Aided Semen Analysis.  
*Ismaya* .......................................................... 507-511

99. **PRP-11-O** The Effect of Trehalose Level In Tris-based Medium On Sperm Membrane Integrity of Boer Goat Semen After Cooling  
*Nurul Isnaini, Trinil Susilawati and Luqman Hakim* .......................................................... 512-514
100. PRP-12-O  Reproductive Efficiency Of Filial Ongole (Po), Limousin And Simmental Crossbred Cattle At Situbondo District
   Kuswati, Doni sonta, Sri Wahyuningsih, Trinil Susilawati and Aulia Puspita Anugra Yekti..................................................515-520

101. PRP-13-O  Reproductive Performances of Ongole Crossbred Cattle Using Artificial Insemination  Sexed Semen with Diferrent Methods
   Trinil Susilawati, Lieyo Wahyudi, Nurul Isnaini and Aulia.....521-525

102. PRP-14-P  Physiology and Reproduction Responses of Crossing Beef Cows
   Aryogi and Y. Adinata..........................................................526-531

103. PRP-16-O  Supplementation of Cysteine on Plasma Membrane Integrity of Buck Spermatozoa
   Sri Wahjuningsih, Nuryadi and Achadiah Rachmawati........532-535

104. PRP-17-P  Estrous Behavior in the Thoroughbred-Indonesian Local Crossbred Mares
   Muhammad Danang Eko Yulianto, Bambang Purwantara,
   Amrozi................................................................................536-540

105. PRP-19-O  Preservation of Bull Cement Technology Applications without Freezing Proceed and Utilization of Epididymis as A Slaughterhouse as A Waste Product to Optimizational Bali Cattle Artificial Insemination in Remote Areas
   Agung B, Mirandy S. Hermilinda P, T. Considus, Gustari S.....541-545

106. PRP-21-P  Sperm Quality of Gembrong Goat In Bali, East Java and North Sumatera After Extended With Citrate-egg Yolk, Tris-egg Yolk and Andromed®
   Sigit Bintara, Dyah Maharani, I Gede Suparta, Jafendi H, Sumadi,
   Simon Eleuser, Aron Batubara..............................................546-549

107. PRP-22-P  The Response of Gonadotropin Hormone at Different Dosage on Time of Oestrus, The Profile of Progesterone, Estrogen and Corpus Luteum of Ongole Crossed Cows
   Lukman Affandhy, D.M. Dikman, Y. Widyaningrum..............550-553

108. PRP-23-O  Reproductive performance of Ekor Tipis and Garut ewes raised in the same condition
   Panjono, E. Baliarti, N. Ngadiyono, I. G. S. Budisatria, T. S. M. Widi,
   M. D. E. Yulianto and Sigit Bintara........................................554-556

109. PRP-24-P  Effect of Doe Blood Serum Supplementation to Buck Semen on the Head to Head Agglutination Test
   Hassan Ishag Haren, Mohamed Abd Elmoneim Salih, Abdel Aziz
   Makkawi and Hatim Idris.....................................................557-561
Agribusiness and Livestock Socioeconomics

110. SA-01-P Determinant of Intangible Benefit and Cost in Integrated Biosystem Cattle In Yogyakarta
    T.A Kusumastuti, S. Nurtini, R. Widiati, S.P. Syahlani, and M.A.U. Muzayyanah
    ........................................................................................................562-565

111. SA-02-P The Sustainability of Community Development in Area Pig Farming with Serasah System Based on Spiritual and Cultural Aspect
    Suci Paramitasari Syahlani, F. Trisakti Haryadi, and Yans Pangerungan
    ........................................................................................................566-570

112. SA-03-O Exploration of Potential Regional Resources for Beef Cattle Farming Development in Java, Indonesia
    Rini Widiati, Tri Anggraeni Kusumastuti, Mujtahidah Anggriani Ummul Muzayanah
    ........................................................................................................571-576

113. SA-04-O Technical, Economic and Social Feasibilities of Beef Cattle Development in Bintuni Papua Barat Indonesia
    T.W. Widayati, B. Santoso, J. Woran, I.U. Warsono and J.A. Palulungan
    ........................................................................................................577-581

114. SA-05-P Economic Analysis and the Impact of Technology IB Livestock Buffalo of Income Farmer
    Rusdiana S. and L. Praharani
    ........................................................................................................582-585

115. SA-06-P Economic Analysis of the Effects of Conservation Land to Provide Feed in Dry Land Farming on the Island East
    Helena Dasilva and Sophia Ratnawaty
    ........................................................................................................586-595

116. SA-08-O Analysis of Champion of Milk Cluster Industry in The Province of Central Java-Indonesia
    Tridjoko W. Murti, Adiarto, Soedjatmogo, B. Purbaya and R. Kawuri
    ........................................................................................................596-600

117. SA-10-O Small Scale Livestock Farmers’ Disincentives for Animal Disease Prevention and How Incentives Can Be Improved: A Case of Uganda
    Juliet Biira
    ........................................................................................................601-605

118. SA-11-O Production Cost Evaluation and Effect of Lactic Acid Bacteria (Lactobacillus Plantarum) as Starter with Different Molasses Addition
    Zaenal Bachruddin, Mujtahidah Anggriani and Afif Fakhruddin
    ........................................................................................................606-609

119. SA-12-P Livestock Commodities Income Contribution of Farming in the Village of Catur, Kintamani, Bangli
    Ida Ayu Putu Parvati and Nyoman Suyasa
    ........................................................................................................610-614
120. SA-13-O Assessment of the Calorie-Protein Consumption Pattern among Rural and Low-Income Urban Households in Indonesia
Mujtahidah Anggriani Ummul Muzayyanah, Sudi Nurtini, Suci Paramitasari Syahlaniphrase 615-618

121. SA-14-O Constraints of Value Chain in Dairy Industry in Central Java
Budi Guntoro, Rochijan, Budi Prasetyo Widyobroto, Indratiningsih, Nafiatul Umami, Sudi Nurtini, and Ambar Pertiwiningrum 619-623

122. SK-02-O The Agricultural Technology Transfer Agencies Role on Transferring the Biogas Technology to Farmers: A Study Case of Dairy Farmer’s Network Analysis in Umbulharjo Village, Yogyakarta Province, Indonesia
R. Ahmad Romadhoni Surya Putra 624-628

123. SK-03-O Combined Effect of Message Framing and Endorser Credibility to Buying Interest of Yoghurt Product
Tian Jihadhan, Suci Paramitasari Syahlan, F. Trisakti H 629-633

124. SK-04-O The Alternative Livestock and Sustainability of Farmers in Mexico
Ricardo E. Caicedo Rivas, A. Moreno Oceguera, A. de M. Parra Gallegos and M. Paz Calderón Nieto 634-637

125. SK-05-P Farmers’ Perception of Etawah Grade Goat Productivity Based on the Hair Color Differences
I Gede Suparta Budisatria, Panjono, Dyah Maharani 638-642

126. SK-06-O Regional Development for Beef Cattle Farming based on Agricultural by Product in Serdang Bedagai District, North Sumatra Province, Indonesia
Tri Hesti Wahyuni, Sya’ad Afifuddin, Ma’ruf Tafsin and Rahmanta Ginting 643-650

127. SK-07-O Farmers Motivation in Exerting Dairy Goats at the Slope Area of Merapi Volcano
Trisakti Haryadi F., Kustantinah, Tommy Andjar C.K 651-654

128. SK-08-O Enhancing Farmer’s Creativity in Dairy Goat Farming (A Case Study in Banyumas District)
Moch. Sugiarto 655-658

129. SK-10-O Utilization of Communication Media in the Process of Extension to Develop Farm Business at Minahasa District North Sulawesi Province
Anneke K. Rintjap, Jolanda K.J. Kalangi, Maasye T. Massie 659-663

130. SK-11-O The Influence of Dairy Farming Motivation on Dairy Cows Productivity in Different Disaster Prone Areas of Merapi Volcano
S. Andarwati, F. Trisakti Haryadi, B. Guntoro, E. Sulastri 664-667
131. SK-12-P Potential and Opportunities of Livestock Development in 24 Locations
PSDSK Assistance of BPTP Support for Food Security
Titim Rahmawati and Yoshi Tri Sulistyaningsih ......................... 668-672

132. SK-13-O Cattle Farmer’s Characteristics In West Timor (Case Study on Nekmese Farmers Group, Usapinonot, North Central Timor, Nusa Tenggara Timur)
Paulus Klau Tahuk., Endang Baliarti., Subur Priyono Sasmito Budhi and Panjono ................................................................. 673-677

133. SK-15-P Estimation of the Peranakan Ongole Cattle Output in Klirong, Kebumen, Central of Java
Sumadi, N. Ngadiyono, C. T. Noviandi, D. T. Widayati ............. 678-682

Animal Products Technology

134. TD-01-O Effects of Hibiscus sabdariffa and Schleichera oleosa Liquid Smoke on Lipid Content, Lipid Oxidation and Residual Nitrite in Se’i (Rotenese Smoked Beef)
Gemini E.M. Malelak, I.G.N. Jelantik, Maria R. Denoratu, Geertruida M Sipahelut, I.G.N. Jelantik ................................. 683-687

135. TD-02-O Chemical Composition and Antioxidative Potential of Chicken Sausage with Substitution of Tempe Jamhari, Yuny Erwanto, Listia Kusumasari Nurhanifah ............. 688-692

136. TD-04-O In Vitro Antioxidant Activity of Beef Lung Protein Hydrolysates Khothibul Umam Al Awwaly, Suharjono Triatmojo, Wayan T. Artama, Yuny Erwanto ....................................................... 693-693

137. TD-05-O Carcass Production and Chevon Quality of Kacang Buck Reared Traditionally in Grobogan, Central Java, Indonesia
Retno Adiwinarti, I Gede Suparta Budisatria, Kustantinah, Rusman ................................................................. 694-698

138. TD-06-O Fraud Identification in Meatballs Product Using Porcine Detection KIT and Multiplex Polymerase Chain Reaction Methods Tridjoko Murti, Christina Admantin, Umar Santoso, Dyah Widiasih, Aris Haryanto ................................................................. 699-703

139. TD-07-O Identification of Dog Meat Species by Polymerase Chain Reaction (PCR) Dyah Ayu Widiasih, Cynthia Debbi Ratnasari, Yatri Drastini, Tridjoko Wisnu Murti ................................................................. 704-708

140. TD-08-O Study on the Physico-Chemical Characteristics and Microstructure of Meat from Goat Given Ration Papaya Leaves (Carica papaya L.) Muh. Ichsan Haris, Soeparno, Umar Santoso, Rusman ........... 709-713
<table>
<thead>
<tr>
<th>No.</th>
<th>Code</th>
<th>Title</th>
<th>Authors</th>
<th>Pages</th>
</tr>
</thead>
<tbody>
<tr>
<td>141</td>
<td>TD-09-O</td>
<td>The Effect of Acetic Acid Concentration and Curing Time on the Characteristics of Native Chicken Legs Skin Gelatin</td>
<td>Meity Sompie, S. E. Siswosubroto and J. H. W Pontoh</td>
<td>714-718</td>
</tr>
<tr>
<td>142</td>
<td>TST-02-O</td>
<td>Antibacterial Activity of Fermented Milk Cultured with Yeast-LAB and Added Sweet Corn Against Pathogenic Bacteria</td>
<td>Yurliasni, Yusdar Zakaria, Zuraida Hanum and Sitti Wajizah</td>
<td>719-723</td>
</tr>
<tr>
<td>143</td>
<td>TST-03-P</td>
<td>Effect of Storage Period Eggs on Egg Quality Characteristics Naked Neck Chicken</td>
<td>Tatan Kostaman and Soni Sopiyan</td>
<td>724-728</td>
</tr>
<tr>
<td>144</td>
<td>TST-04-O</td>
<td>Study The Quality of Multi Probiotic Fermented Milk Made from Cow’s Milk and Goat’s Milk</td>
<td>Eni Robiyati, Tridjoko Wisnu Murti, Harisuddin Lutfan Jundi, Fajar Ramadhan</td>
<td>729-732</td>
</tr>
<tr>
<td>145</td>
<td>TST-05-O</td>
<td>Development of Halal Goat Cheese using Rennet Like from Vegetable Source as Replace to Those of Commercial Rennet Source</td>
<td>Widitya Tri Nugraha, Tridjoko Wisnu Murti, Irma Sri Novitasari, Tri Kartika Sari, Gangga Murcita, Gregorius Riswan Timur Wijakangka</td>
<td>733-737</td>
</tr>
<tr>
<td>146</td>
<td>TST-06-O</td>
<td>The Characteristics of Salted Chicken and Duck Egg by using Traditional Roasting</td>
<td>Nurliyani, Anggi Hartawan, Yulianto Adi Nugroho, Indratiningsih</td>
<td>738-742</td>
</tr>
<tr>
<td>147</td>
<td>TST-07-O</td>
<td>Capability of Isolates Probiotic Bacteria, Isolated From Spontaneous Fermented goat Milk as Starter In milk Fermentation</td>
<td>Afriza Yelnetty, Purwadi, Arie Mirah</td>
<td>743-743</td>
</tr>
<tr>
<td>148</td>
<td>TST-09-O</td>
<td>Changes in physico-chemical and sensory characteristics of concentrated yogurt made from goat milk during storage</td>
<td>Juni Sumarmono, Mardiati Sulistyowati, and Triana</td>
<td>744-748</td>
</tr>
</tbody>
</table>

**Waste and Environmental Issues**

<table>
<thead>
<tr>
<th>No.</th>
<th>Code</th>
<th>Title</th>
<th>Authors</th>
<th>Pages</th>
</tr>
</thead>
<tbody>
<tr>
<td>149</td>
<td>TLL-01-O</td>
<td>Development of New Biostarter Medium Using Local Raw Materials for Composting of Elephant Feces</td>
<td>Nanung Agus Fitriyanto, Suharjono Triatmojo, Tri Sunu Dane Wibawa</td>
<td>749-753</td>
</tr>
<tr>
<td>150</td>
<td>TLL-02-P</td>
<td>Implementation of Good Manufacturing Practices System in Halal Certified Chicken Slaughterhouses in Daerah Istimewa Yogyakarta</td>
<td>Edi Suryanto, Tridjoko Wisnu Murti, Yatri Drastini, Rusman, Bastoni, Umar Al Faruqi and Ismatullah Salim</td>
<td>754-760</td>
</tr>
</tbody>
</table>
151. TLL-03-O  The Influence of Tanning Material Difference on the Physical Quality of the Skin of Puffer Fish (*Arothon reticularis*)
RL.M. Satrio Ari Wibowo, Titik Anggraini, Ambar Pertiwininggrum .................................................................761-765

152. TLL-04-P  The Effect of Composting Liquid Organic Fertilizer Processing Residues on Compost Quality
Eulis Tanti Marlina, Yuli Astuti Hidayati, Tb. Benito A. Kurnani .................................................................766-769

153. TLL-05-P  Utilization of Bee Nest Waste as a Natural Disinfectant on Hatching Eggs Poultry
Ellin Harlia, Andriyanto, Eulis Tanti Marlina, Denny Suryanto ........................................................................770-773

154. TLL-06-P  Quality Vermicompost (Content N, P, K) From Beef Cattle Waste Treatment Through Integrated
Yuli Astuti Hidayati, Sudiarto, and Wowon Juanda........................................................................774-777

155. TLL-08-O  The Application of Secang Natural Dye on Sheep Leather Crust Suede Using Ikat Jumputan Method
Entin Darmawati, Suharjono Triatmojo and Diana Ross Arief ........................................................................778-784

156. TLL-09-O  New Technique to Detect Pig Hair by Immunochromatographic Rapid Test
Yatri Drastini, Sumantri, Christina Yuni Admantin, Tridjoko Wisnu Murti ..........................................................785-788

157. TLL-10-O  Isoptericola sp. A10-1, Chitinase Producing Actinobacterium Isolated from Indonesian Tropical Shrimp Pond Waste Water
Amrih Prasetyo, Lies Mira Yusiatu, Yuny Erwanto, Wihandoyo, Nanung Agus Fitriyanto, Tomoyuki Nakagawa and Takashi Hayakawa .................................................................789-792

158. TLL-11-O  Production and Application of Keratinase Enzyme of Bacillus spp. Isolate by Using Raw Feather as Substrate
Theresia Galuh Wandita, Nanung Agus Fitriyanto, Suharjono Triatmojo ..........................................................793-797

159. TLL-12-O  Different Effect on the Quality of Organic Fertilizer Fermentor of Ongole Crossbred Cattle’s Feces
Dedes Amertaningtyas, Trinil Susilawati and Lilik Eka Radiati ........................................................................798-802

160. TLL-13-P  Implementation of Good Manufacturing Practices System in Halal Certified Cattle Slaughterhouses in Daerah Istimewa Yogyakarta
Bastoni, Nasrul Hidayat, Edi Suryanto, Rusman, Tridjoko Wisnu Murti, Yatri Drastini ........................................... 803-809
Nutritive Evaluation of Pineapple Peel Fermented by Cellulolytic Microbe and Lactic Acid Bacteria by In Vitro Gas Production Technique

Lies Mira Yusiati, Chusnul Hanim and Caecilia Siska Setyawati

Faculty of Animal Science, Gadjah Mada University
Corresponding email: yusiati@yahoo.com

ABSTRACT: The research was conducted to evaluate the nutritive value of pineapple peel fermented by rumen cellulolytic microbes and followed by addition of lactic acid bacteria (LAB) at the 4th, 8th, 12th and 16th days of fermentation. Cellulolytic microbes was added as much as 5% of dry matter (DM) into 300 g of pineapple peel with three replicates for each treatment. Fermentation without addition of cellulolytic microbes and LAB was carried out as control. Fermentation was done up to 21 d and the sample of fermented pineapple peel were taken out for physical quality measurements including odor, color, the presence of fungi, as well as the lactic acid content. The sample was dried at 55°C, and then prepared for chemical composition analysis including dry matter (DM), organic matter (OM), crude fibre (CF), crude protein (CP), extract ether (EE) and nitrogen free extract (NFE). The samples were also prepared for evaluation of kinetic fermentation by in vitro gas production technique proposed by Menke and Steingass. The data obtained were analyzed by analysis of variance using one way design and continued by Duncan’s new multiple range test to examine the differences among the mean values. The result showed that pineapple peel fermented by cellulolytic microbes with addition of LAB in all treatment as well as the control had brown colour, and without fungus. The more acidic odor were found in fermented pineapple added with cellulolytic microbes and LAB, due to lactic acid content which increased significantly. Cellulolytic microbes and LAB addition didn’t affect DM and OM content, while the content of CF tended to decrease. The treatments increased NFE as well as CP content, and decreased EE content significantly. Gas production, and the values of a, b, c fractions were not affected by addition of cellulolytic microbes and LAB. It could be concluded that lactic acid bacteria which was added after 12 days of cellulolytic fermentation) gave the best nutritive quality of fermented pineapple peel.

Keywords: Pineapple peel, Cellulolytic microbes, Lactic acid bacteria, Fermentation, In vitro gas production.

INTRODUCTION

In spite of the large amounts of industrial by product and agricultural waste, the problems of inadequate nutrition and prohibitive cost of conventional feedstuff during dry season remained unsolved. It is driving some farmers to find alternative feedstuffs. One of those materials which interested to be considered was pineapple peel. The problems of pineapple peel as animal feed are high water content, and low digestibility due to high fibre content. As a compound of Total Mix Fiber which was produced from agricultural byproduct as an alternative roughage feed, pineapple peel silage contained 23.98% of crude fibre (Maneerat et al., 2013). It was expected the nutritive quality of pineapple silage could be increased by reducing crude fiber content, therefore addition of cellulolytic microbes along with lactic acid bacteria (LAB) should be considered in the pineapple peel fermentation.
MATERIALS AND METHODS

Microbes Preparation

Donor Animal. In this experiment, 2 head of rumen fistulated Ongole crossbred cattle were used as the donor animal to get the rumen fluid for a source of fibrolytic microbes needed for fermentation as well as for in vitro gas production technique.

Microbes enrichment. Rumen fluid were collected from the both donor animals early in the morning before feeding time, composited, prepared and kept anaerobically in waterbath at 39°C. A quantity of rumen fluid samples were taken out and subjected into enzymes assays (carboxymethyl cellulase/ CMC-ase) which were done in duplicate (Halliwel et al., 1985). Rumen fluid as much as 10% of medium volume was pipeted into glass fermenter which was already filled with enrichment medium based on Omelianski (1902) cit. Skinner (1971). Fermentation was done anaerobically at 39°C for 7 days.

Rumen cellulolytic microbes cultivation. The inoculum grown in the enrichment media was taken out, and it was added into the glass fermenter filled with growing medium as much as 10% from the total medium which used 4 g cellulose as substrate (Omelianski (1902) cit. by Skinner (1971). The fermenters was kept anaerobically at 39°C for 7 days. The culture was ready to be applied for pineapple fermentation immediately after enzymes assays.

Pineapple fermentation

After growing for 7 days, the cellulolytic culture as much as 5% DM was mixed with 300 g of air-dried pineapple peel, incubated anaerobically at room temperature and followed by addition of lactic acid bacteria (LAB) at the 4th, 8th, 12th and 16th days of fermentation. Fermentation without cellulolytic microbes and LAB addition was carried out as control. Fermentation was done up to 21 days.

Evaluation of fermented pineapple peel quality

At the end of this fermentation period the glass-silos were opened for sampling. The fermented pineapple peel samples were taken out and examined for their physical quality including odor, colour, texture, the presence of fungi and lactic acid content following Baker and Summerson method (Hawk, 1976). Content of dry matter was directly determined in the fresh fermented samples prior to 55°C drying for fermentation end products measurements. The dried sample was then analyzed for dry matter (DM), organic matter (OM), crude fibre (CF), crude protein (CP), extract ether (EE) and nitrogen free extract (NFE) content (AOAC Intl., 2005). The samples were also prepared for evaluation of kinetic fermentation by in vitro gas production technique proposed by Menke and Steingass (1988).

Statistical Analysis

Data obtain were analyzed by analysis of variance using one way design and the means were compared by Duncan’s Multiple Range Test (Rosner, 1990).

RESULT AND DISCUSSION

Specific activity of Carboxy methyl cellulase (CMC-ase) in the rumen fluid was 0.08 U/mg. The specific activity of CMC-ase increased as the inoculums transferred to the enrichment media and also during cultivation in the growing media (0.16 and 1.74 U/mg) respectively. Physical quality of fermented pineapple peel was shown in Table 1.
Table 1. Physical quality and lactic acid content of pineapple peel fermented by cellulolytic bacteria and different time of lactic acid bacteria addition

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Without addition of inoculum (P0)</th>
<th>The day of lactic acid bacteria addition</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>4 (P1)</td>
</tr>
<tr>
<td>Odour</td>
<td>Slightly acid</td>
<td>Acid</td>
</tr>
<tr>
<td>Texture</td>
<td>Rough</td>
<td>rough</td>
</tr>
<tr>
<td>Presence of fungi</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Lactic acid (%)</td>
<td>0.03&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.11&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>abc</sup>: Means within the same row with different superscript letters differ significantly (P<0.01)

The addition of cellulolytic microbes and LAB in the fermentation of pineapple peel significantly increased lactic acid content (P<0.01), therefore the odor became more acid compared with control. There were no fungi found in all treatments and the color of all fermented material remained the same, while the texture of fermented material became smoother as the effect of cellulolytic microbe and LAB addition. The increasing of acidity which was shown by the decreasing of pH and the change of odor to be more acid also reported by Yusiati et al. (2011) when cellulolytic microbes was added into the coffee pulp fermentation. The addition of LAB at the 4th day of fermentation gave the highest lactic acid content of fermented material. It was about 3.67 times lactic acid content of the control. Lactic acid content decreased by the addition time of LAB due to the shortage of LAB resident time in the fermentation media.

Chemical compositions of pineapple peel fermented by cellulolytic microbes and lactic acid bacteria addition were presented in Table 2.

Table 2. Chemical composition of fermented pineapple peel (%DM)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Without addition of inoculum (P0)</th>
<th>The day of lactic acid bacteria addition</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>4&lt;sup&gt;th&lt;/sup&gt; (P1)</td>
</tr>
<tr>
<td>Dry matter</td>
<td>47.64</td>
<td>46.66</td>
</tr>
<tr>
<td>Organic matter</td>
<td>92.16</td>
<td>91.72</td>
</tr>
<tr>
<td>Crude protein</td>
<td>7.16&lt;sup&gt;c&lt;/sup&gt;</td>
<td>7.78&lt;sup&gt;xy&lt;/sup&gt;</td>
</tr>
<tr>
<td>Ether extract</td>
<td>11.10&lt;sup&gt;a&lt;/sup&gt;</td>
<td>11.19&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Crude fibers</td>
<td>17.19</td>
<td>17.06</td>
</tr>
<tr>
<td>Nitrogen free</td>
<td>56.70&lt;sup&gt;b&lt;/sup&gt;</td>
<td>55.76&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>abc</sup>: Means within the same row with different superscript letters differ significantly (P<0.05)
<sup>xyz</sup>: Means within the same row with different superscript letters differ significantly (P<0.01)
<sup>ns</sup>: Not significant

Lactic acid bacteria addition into cellulolytic fermentation of pineapple peel did not give significant effect on dry matter and organic matter content. Nitrogen free extract (NFE) increased significantly when LAB was added after 12 days of cellulolytic fermentation. The increasing of NFE might be as an effect of the crude fiber content which have a tendency (P<0.07) to decrease. The increasing of cellulolytic fermentation time prior to LAB addition gave extended time to the
cellulolytic microbes to degrade the fiber content of pineapple peel and converted it to glucose which is component of NFE.

**In vitro degradation of fermented pineapple waste**

Data on gas production are given in Table 3. It indicated, there was no significant effect of cellulolytic and LAB addition on total gas production, gas produced from a and b fraction as well as the rate of gas production (c values). Hanim *et al.* (2010) reported the same finding that addition of cellulolytic inoculums did not give any effect on a, b and c values of fermented cocoa pod, although its CF content decreased significantly.

**Table 3.** Total gas production, fraction a, b and, c value of fermented pineapple peel

<table>
<thead>
<tr>
<th>Parameter</th>
<th>without addition of inoculum (P0)</th>
<th>The day of Lactic Acid Bacteria addition</th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>4th (P1)</td>
</tr>
<tr>
<td>Gas Volume ns</td>
<td>80.529</td>
<td>84.384</td>
</tr>
<tr>
<td>a fraction ns</td>
<td>-0.425</td>
<td>-1.501</td>
</tr>
<tr>
<td>b fraction ns</td>
<td>83.077</td>
<td>89.649</td>
</tr>
<tr>
<td>c value (ml/h) ns</td>
<td>0.055</td>
<td>0.052</td>
</tr>
</tbody>
</table>

\(\text{ns: non significant}\)

Total gas volume and gas produced from insoluble fraction in this present study were higher compared with the gas produced by cellulolytic fermented cacao pod. It seems to be an effect of lower CF content of fermented pineapple peel compared with CF content of fermented cacao pod (16.35-17.19% vs. 22.28%). The increasing of NFE in fermented pineapple with addition of LAB at 12th days of fermentation, followed by the increasing of a fraction value.

Yusiati *et al.* (2010) reported that fermentation using 5% fibrolytic inoculums originally from rumen fluid with CMC-ase activity 4.71 U/mg and xylanase 0.028 U/mg, increased OM and DM in vitro digestibility of palm kernel cake, although CF content was not decrease. It seem that increasing of fermented pineapple peel digestibility could be expected by increasing the level of inoculums as well as by applying mix inoculums such as cellulolytic and xylanolytic.

**CONCLUSION**

The addition of cellulolytic microbes originally from the rumen fluid as much as 5% and lactic acid bacteria 2.5% after 12 days cellulolytic fermentation is the best way to increase the nutritive quality of fermented pineapple peel.

**REFERENCES**


Hanim, C., L.M. Yusiati, and V.P. Budyastuti. 2010. In vitro gas production of fermented cocoa pod (Theobroma cacao) added with cellulolytic inoculum from cattle rumen fluid. Proceeding of The 5th International Seminar on Tropical Animal Production Community Empowerment...


