SUSTAINABLE LIVESTOCK PRODUCTION IN THE PERSPECTIVE OF FOOD SECURITY, POLICY, GENETIC RESOURCES, AND CLIMATE CHANGE

PROCEEDINGS

FULL PAPERS

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AAAP

Asian-Australasian Association of Animal Production Societies

Scope of AAAP: AAAP is established to devote for the efficient animal production in the Asian-Australasian region through national, regional, international cooperation and academic conferences.


Organization of AAAP:
- President: Recommended by the national society hosting the next biennial AAAP Animal Science Congress and approved by Council meeting and serve 2 years.
- Two Vice Presidents: One represents the present host society and the other represents next host society of the very next AAAP Animal Science Congress.
- Secretary General: All managerial works for AAAP with 6 years term by approval by the council.
- Council Members: AAAP president, vice presidents, secretary general and each presidents or representative of each member society are members of the council. The council decides congress venue and many important agenda of AAAP

Office of AAAP: Decided by the council to have the permanent office of AAAP in Korea. Currently # 909 Korea Sci &Tech Center Seoul 135-703, Korea


Current 19 Member Societies of AAAP:
- ASAP(Australia), BABA(Bangladesh), CAASVM(China), IAAP(India), ISAS(Indonesia), IAAS(Iran), JSAS(Japan), KSAST(Korea), MSAP(Malaysia), MLSBA(Mongolia), NASA(Nepal), NZSAP(New Zealand), PAHA(Pakistan), PNGSA(Papua New Guinea), PSAS(Philippines), SLAAP(Sri Lanka), CSAS(Taiwan), AHAT(Thailand), AHAV(Vietnam).

Previous Venues of AAAP Animal Science Congress and AAAP Presidents

<table>
<thead>
<tr>
<th>I</th>
<th>1980</th>
<th>Malaysia</th>
<th>S. Jalaludin</th>
<th>II</th>
<th>1982</th>
<th>Philippines</th>
<th>V. G. Arganosa</th>
</tr>
</thead>
<tbody>
<tr>
<td>III</td>
<td>1985</td>
<td>Korea</td>
<td>In Kyu Han</td>
<td>IV</td>
<td>1987</td>
<td>New Zealand</td>
<td>A. R. Sykes</td>
</tr>
<tr>
<td>V</td>
<td>1990</td>
<td>Taiwan</td>
<td>T. P. Yeh</td>
<td>VI</td>
<td>1992</td>
<td>Thailand</td>
<td>C. Chantalakhan</td>
</tr>
<tr>
<td>VII</td>
<td>1994</td>
<td>Indonesia</td>
<td>E. Soetirto</td>
<td>VIII</td>
<td>1996</td>
<td>Japan</td>
<td>T. Morichi</td>
</tr>
<tr>
<td>IX</td>
<td>2000</td>
<td>Australia</td>
<td>J. Ternouth</td>
<td>X</td>
<td>2002</td>
<td>India</td>
<td>P. N. Bhat</td>
</tr>
<tr>
<td>XI</td>
<td>2004</td>
<td>Malaysia</td>
<td>Z. A. Jelan</td>
<td>XII</td>
<td>2006</td>
<td>Korea</td>
<td>I. K. Paik</td>
</tr>
<tr>
<td>XIII</td>
<td>2008</td>
<td>Vietnam</td>
<td>N.V. Thien</td>
<td>XIV</td>
<td>2010</td>
<td>Taiwan</td>
<td>L.C. Hsia</td>
</tr>
</tbody>
</table>

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Remark from Chairman of the 16th AAAP Congress

Dear all of the scientists, delegates, participants, ladies and gentlemen,

As the host of the 16th AAAP Animal Science Congress, we do impress, thankful, and present a high appreciation for your participation in joining the 16th AAAP Conference in Yogyakarta, Indonesia. We can see the very great enthusiasm of all the scientists to solve livestock problems as well as to share valuable information and knowledge for human prosperity all over the world.

A large numbers of representatives are participating in this conference, which indicates that the interest in the field of animal science is continuously increasing among member countries. We have invited some Plenary Speakers and Invited Papers who are qualified as scientists and bureaucrats in animal science field to share their valuable information and knowledge. Other participants can deliver their precious research through oral and poster presentations. This congress is also paralleled to symposium held by livestock organization and institution as well as some academic meetings.

The theme of the 16th AAAP Congress is “Sustainable Livestock Production in the perspective of Food security, Policy, Genetic Resources and Climate Change”. We believe that animal production in Asia and Australasia has become important and strategic sector to provide high quality food, opening up job opportunities, as well as improving farmer’s welfare. Animal science societies, therefore, have to support this growing interest by providing more appropriate and relevant technologies to improve efficiency of resources utilization to produce more animal protein food by member countries. Long term sustainable livestock production will, therefore, be significantly influenced by the national food policy, climate change issues, as well as conserved environments and genetic resources.

On behalf of 16th AAAP Committee and all associates, we wish all of the participants having a great achievement of success and fulfill the expectation as well as enjoying the interaction with all scientists participating the Congress.

High appreciation we may acknowledge to all of sectors, especially for His Majesty of Royal Palace of Yogyakarta, Sri Sultan Hamengku Buwono X, and Rector of Universitas Gadjah Mada, who have concerned to facilitate the Congress site host. Special thank to the Steering Committee, Scientific Committee, Reviewers and Editorial Boards for their great contribution to make the Congress successfully organized.

To you, your excellencies, invited guests and delegates, thank you for choosing to come to this conference and to Indonesia. We hope the arrangements we have put in place meet with your requirements. We wish you fruitful deliberations and an intellectually and socially rewarding stay in Yogyakarta.

We are looking forward to meeting you all in the future congress to continue.

Terimakasih (Thank you)

Budi Guntoro

Chairman of the 16th AAAP Congress
Selamat pagi!

Dear Ladies and Gentleman

Attendants of 16 AAAP congress:

It is my great pleasure and honor to welcome all of you at The 16th AAAP Congress on November 10 – 14, 2014 at Grha Sabha Pramana, Universitas Gadjah Mada, Yogyakarta Indonesia. This Congress is jointly organized by The Indonesian Society of Animal Science (ISAS), Indonesian Agency for Agricultural Research and Development, Indonesian Directorate General of Livestock and Animal Health Services-Ministry of Agriculture and Faculty of Animal Science Universitas Gadjah Mada. Universitas Gadjah Mada Campus is located in Yogyakarta, one of the Special Region in Indonesia where culture and tradition live in harmony with the modern nuance and educational spirit makes it a beautiful venue of this Congress.

The 16th AAAP Program consists of scientific and technical programs as well as social and cultural activities. The scientific and technical programs offer five plenary sessions, two satellite symposia, field trip, and many scientific sessions, both oral and poster presentations.

During this event distinguished scientists from all over the world will present plenary papers ranging from livestock policy, food security, local genetic resources, climate change, animal welfare, international trade, as well as global research agenda. I believe that around 1,200 scientists as well as livestock producers, companies, graduate and postgraduate students from 40 countries are attending the Congress and more than 770 research papers will be presented. The Congress also provides not only opportunities to discuss and exchange information and experience with scientists from different regions of the world, but also a good environment to build up friendship between nations is our ultimate goals for the Congress outcome. Moreover, this congress also keeps its tradition to be a forum of communication among researchers, academician, industries and related stakeholders among Asian-Australasian countries.

The social and cultural programs are specially designed to be very important for the congress participants since the promotion of friendship and future scientific cooperation are also central to this AAAP Congress. The Opening Ceremony will offer you the Congress Program at a glance. In addition, participants will also join at a warm Welcome Dinner gathering at Keraton Yogyakarta. Sri Sultan Hamengku Buwono X, His Majesty of The Royal Palace of Yogyakarta will give you the most memorable moment during this event.

Moreover, cultural night offers us an opportunity to introduce significant culture from participants’ countries and gives a spectacular performance to enjoy in order to strengthen our friendship and future cooperation. Field trip, on the other hand, provides a wonderful sightseeing to the most valuable ancient heritage around Yogyakarta, such as Borobudur and Prambanan Temples, and more other interesting places to visit. I do hope that you enjoy your stay in Yogyakarta and not miss all of these spectacular opportunities.

Closing Ceremony will be held on November 14, 2014 immediately after the last session of presentation. During this great moment we will welcome the next host of the 17th AAAP Congress to deliver a brief message. The AAAP Congress Award will provide and announce some participant who receive appreciation for their valuable research.
With all of our hospitality, we will try our best to make your brief visit to Yogyakarta and our beautiful country Indonesia, become a wonderful experience and memorable moments.

I wish you all a very pleasant and most enjoyable stay in Yogyakarta, Indonesia.

Terima kasih (Thank you).

Sincerely Yours
Mr. Yudi Guntara Noor
President
The 16th AAAP Congress
PREFACE

The proceedings of the 16th Congress of the Asian-Australasian Association of Animal Production Societies (AAAP) held on 10-14 November 2014 at Grha Sabha Pramana, Universitas Gadjah Mada, Yogyakarta, Indonesia, consist of two volumes. Those are Volume I of Plenary and Invited Papers and Volume II of Abstracts Contributed Papers. This is the second volume of the proceedings that contains a total of 754 abstracts, consist of 368 papers for oral presentation and 386 papers for poster. Papers were categorized into various disciplines, such as Nutrition and Feed Technology; Genetics and Reproduction; Physiology, Animal Welfare and Health Management; Product Technology and Food Safety; Waste and Environmental issues; Forage Agrostology; as well as Agribusiness, Marketing, Extension and Community Development. The scientific committee has initially received a total of 1,028 abstracts from 42 countries. After reviews have been made, 60 of them were rejected and 74 were cancelled by the authors. The reviewers consist of 4 international and 71 internal reviewers from 6 universities and 1 research institute in Indonesia. In the interest of time limitation for proceedings publication, we apologize for not including 140 submitted abstracts in the proceedings since they were not being followed up with full manuscripts until the extended due date we offered.

The scientific committee would like to thank all the reviewers and appreciate their effort to make significant contribution in reviewing the full manuscripts. Similarly, we would also like to thank supporting staffs at the secretariat office of the Faculty of Animal Science, Universitas Gadjah Mada as well as of the Indonesian Center for Animal Research and Development who have helped in the preparation of the proceedings. Finally, we would like to thank all the authors for their valuable contribution to the congress and make it useful for our societies.

Editorial Team
## CONTENTS

### ORAL PRESENTATION

<table>
<thead>
<tr>
<th>Code</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Genetic and Reproduction</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Large Ruminants</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A 15 ID</td>
<td>Effects of Estrous Synchronization of Bali Cattle Using PGF2α</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td><em>Indira P N, Ismaya and Kustono</em></td>
<td></td>
</tr>
<tr>
<td>A 34 IN</td>
<td>Prediction of 305 Days Lactation Milk Yield from Fortnightly Test Milk Yields in Hill Cattle under Field Conditions</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td><em>R K Pundir</em></td>
<td></td>
</tr>
<tr>
<td>A 42 ID</td>
<td>Development of Technology Production of Frozen of Swamp Buffalo (<em>Bubalus bubalis</em>) in the Kampar Regency</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td><em>Yendraliza, C. Arman and J. Handoko</em></td>
<td></td>
</tr>
<tr>
<td>A 116 ID</td>
<td>Analysis of Reproductive Efficiency in Peranakan Ongole (PO)- and its Crosses with Limousin (LIMPO) Cattle in East Java, Indonesia</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td><em>S. Suyadi and H. Nugroho</em></td>
<td></td>
</tr>
<tr>
<td>A 135 ID</td>
<td>Performance Test and Genetic Potency of Bali Cattle Using Animal Recording Software</td>
<td>17</td>
</tr>
<tr>
<td></td>
<td><em>Luqman Hakim and V.M. Ani Nurgiartiningisih</em></td>
<td></td>
</tr>
<tr>
<td>A 141 ID</td>
<td>Application of Genetic Marker Technology for Predicting Twinning Trait in Ongole Cattle</td>
<td>21</td>
</tr>
<tr>
<td></td>
<td><em>Endang Tri Margawati, Indriawati and Muhamad Ridwan</em></td>
<td></td>
</tr>
<tr>
<td>A 201 ID</td>
<td>Membrane Status, Acrosome and Sperm Quality of Ongole Cross Bred Bull after Sexing Using Percoll Density-Gradient Centrifugation and Albumin Separation</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td><em>Trinil Susilawati, Sri Rahayu, Herni Sudarwati, Eko Nugroho, Setiabudi Udrayana and Lieyo Wahyudi</em></td>
<td></td>
</tr>
<tr>
<td>A 246 ID</td>
<td>Phylogenetic Analysis of Simeulue Buffalo Breed of Indonesian through Mitochondrial D-loop Region</td>
<td>29</td>
</tr>
<tr>
<td></td>
<td><em>Eka Meutia Sari, M. Yunus and Mohd. Agus Nashri Abdullah</em></td>
<td></td>
</tr>
<tr>
<td>A 339 JP</td>
<td>Genetic Polymorphisms and Their Association with Growth and Carcass Traits in Japanese Black Steers</td>
<td>33</td>
</tr>
<tr>
<td></td>
<td><em>F.N. Jomane, T. Ishida, K. Morimoto, T. Tokunaga and H. Harada</em></td>
<td></td>
</tr>
<tr>
<td>A 413 ID</td>
<td>The Effect of Straw Position in Nitrogen Vapour During Equilibration on Post-Thawing Motility and Membrane Integrity Following Quick Freezing in Maduran Cattle Sperm</td>
<td>37</td>
</tr>
<tr>
<td></td>
<td><em>H. Ratnani, MN. Ihsan, G. Ciptadi and S. Suyadi</em></td>
<td></td>
</tr>
<tr>
<td>Code</td>
<td>Title</td>
<td>Page</td>
</tr>
<tr>
<td>--------</td>
<td>-----------------------------------------------------------------------</td>
<td>------</td>
</tr>
<tr>
<td>G 906 ID</td>
<td>Effect of Indigenous Probiotics Lactic Acid Bacteria on the Intestinal Histology Structure and the Expression of Tight Junction Molecule Claudins in the Ileum of Broiler Chickens <em>Sri Harimurti and Bambang Ariyadi</em></td>
<td>1210</td>
</tr>
<tr>
<td>G 1110 ID</td>
<td>Toxicological Effects of Aflatoxin B1 on Liver Function of Broiler <em>Merry Muspita Dyah Utami and Ali Agus</em></td>
<td>1214</td>
</tr>
<tr>
<td><strong>Others</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G 73 ID</td>
<td>Using Designated Dunging Areas and Feeding Papaya Fruit and Betel Nut to Reduce Parasite Burdens in Confined Pigs <em>Aris Triono Syahputra, I Made Putra, Sukendra Mahalaya, Luther Kossay, and Colin Cargill</em></td>
<td>1221</td>
</tr>
<tr>
<td>G 76 ID</td>
<td>Isolation of <em>Streptococcus suis</em> in Confined Pigs Versus Free Range Scavenging Pigs in Eastern Indonesia <em>Mitra Slipranata, Aris Triono Syahputra, Luther Kossay, Alberth Soplanit, Nakeus Muuid, Sukendra Mahalaya, I Made Putra, Siti Isrina Oktavia Salasia, and Colin Cargill</em></td>
<td>1229</td>
</tr>
<tr>
<td><strong>Products Technology and Food Safety</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Large Ruminant</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I 105 ID</td>
<td>Chemical and Microbiological Quality of Buffalo Meat Paste (Petis) at Different Concentration of Lactic Acid Bacteria <em>W. Ningrum, D. R. Malini, B. Kuntoro, W. N. H. Zain, and E. Purnamasari</em></td>
<td>1233</td>
</tr>
<tr>
<td>I 206 ID</td>
<td>Ultrastructure and Amino Acid Profile of Crossbred Ongole Cattle Hide Products <em>Dedes Amertaningtyas, Trinil Susilawati and Hari Purnomo</em></td>
<td>1237</td>
</tr>
<tr>
<td>I 456 ID</td>
<td>Physicochemical Quality and Stability of Low Fat Mayonnaise Using Rice Bran Oil <em>Herly Evanuarini, Nurliyani, Indratiningsih and Pudji Hastuti</em></td>
<td>1241</td>
</tr>
<tr>
<td>I 644 ID</td>
<td>Powdered Yoghurt Probiotic Quality Produced by Foam-Mat Drying Method with Different Drying Temperature and Albumen Level <em>Ari Surya Sukarno, Nurliyani and Indratiningsih</em></td>
<td>1244</td>
</tr>
<tr>
<td>Code</td>
<td>Title</td>
<td>Page</td>
</tr>
<tr>
<td>--------</td>
<td>----------------------------------------------------------------------</td>
<td>------</td>
</tr>
<tr>
<td>I 1126 KR</td>
<td>Monthly and Seasonal Variation of Yield Grade Frequency of Eight Years in Korean Cattle Steer Carcasses</td>
<td>1248</td>
</tr>
<tr>
<td></td>
<td><em>Min Yu Piao and Myunggi Baik</em></td>
<td></td>
</tr>
<tr>
<td></td>
<td><strong>Small Ruminant</strong></td>
<td></td>
</tr>
<tr>
<td>I 259 ID</td>
<td>Natural Antioxidant Properties and Physico-Chemicals of Kefir Prepared by Combination of Local Honey and the Time of Fermentation of Goats Kefir</td>
<td>1251</td>
</tr>
<tr>
<td></td>
<td><em>Firman Jaya, Dedes Amertaningtyas, Djalal Rosyidi, Manik Eirry Sawitri and Eny Sri Widyastuti</em></td>
<td></td>
</tr>
<tr>
<td>I 596 ID</td>
<td>Microbiological, Chemical and Physical Properties of Mare, Goat and Cow Milk During Cold Storage</td>
<td>1255</td>
</tr>
<tr>
<td></td>
<td><em>Nurliyani, Yuni Suranindyah, and Feny Prabawati</em></td>
<td></td>
</tr>
<tr>
<td>I 629 TW</td>
<td>Heat Intensity of Market Milk in Taiwan: Part II. α-Lactalbumin, β-Lactoglobulin and Furosine Concentrations in Fresh Goat Milk</td>
<td>1260</td>
</tr>
<tr>
<td></td>
<td><em>M. J. Lin and E. E. Liang</em></td>
<td></td>
</tr>
<tr>
<td>I 673 ID</td>
<td>Characteristics and Composition of Cheese Manufactured from Goat Milk Containing Probiotic <em>Lactobacillus casei</em> and <em>Bifidobacteria sp</em> During Storage</td>
<td>1263</td>
</tr>
<tr>
<td></td>
<td><em>Juni Sumarmono, Triana Yuniastuti, Triana Setyawardani, Singgih Sugeng Santososo, and Yusuf Subagyo</em></td>
<td></td>
</tr>
<tr>
<td>I 877 ID</td>
<td>Physical and Sensory Quality of Sheep Meat Sate Grilled with Different Time and Fuel</td>
<td>1267</td>
</tr>
<tr>
<td></td>
<td><em>Setiyono, Edi Suryanto, Rusman and Jamhari</em></td>
<td></td>
</tr>
<tr>
<td>I 878 ID</td>
<td>Chemical Composition and Food Safety of Sheep Meat Sate Grilled with Different Time and Fuel</td>
<td>1270</td>
</tr>
<tr>
<td></td>
<td><em>Edi Suryanto, Setiyono, Rusman and Jamhari</em></td>
<td></td>
</tr>
<tr>
<td>I 988 ID</td>
<td>Antimicrobial Activity of Indigenous Probiotic <em>L. plantarum</em> Tw 14 from Goat Milk as Natural Preservative Candidate</td>
<td>1273</td>
</tr>
<tr>
<td></td>
<td><em>Triana Setyawardani, Kusuma Widayaka dan Triana Yuni Astutti</em></td>
<td></td>
</tr>
<tr>
<td></td>
<td><strong>Poultry</strong></td>
<td></td>
</tr>
<tr>
<td>I 503 KR</td>
<td>Bacteria Counts and Oxidative Properties of Chicken Breast Inoculated with <em>Salmonella typhimurium</em> Exposed with Gaseous Ozone Exposure</td>
<td>1276</td>
</tr>
<tr>
<td></td>
<td><em>Muhlisin, Youngiae Cho, Ji Hye Choi, Chung Su Park, Tae-Wook Hahn and Sung Ki Lee</em></td>
<td></td>
</tr>
<tr>
<td>I 551 ID</td>
<td>Firmness and Microstructure Properties of Chicken Meatball Fortified with Eggshell Calcium Powder</td>
<td>1280</td>
</tr>
<tr>
<td></td>
<td><em>Edi Suryanto, Setiyono, Rusman and Agus Hadi Prayitno</em></td>
<td></td>
</tr>
<tr>
<td>Code</td>
<td>Title</td>
<td>Page</td>
</tr>
<tr>
<td>----------</td>
<td>-----------------------------------------------------------------------</td>
<td>------</td>
</tr>
</tbody>
</table>
| K 727 ID | Agronomic Performance of *Leucaena leucocephala* cv. Tarramba in Tropical Environment of Sumbawa  
*Tanda Panjaitan, Muhammad Fauzan, Dahlanuddin, Michael Halliday, and Max Shelton* | 1365 |
| K 745 ID | Productivity and Species Diversity of Domestic Forage Based on Altitude in Malang Regency, East Java  
*Iwan Prihantoro, Fransiska Rahmadani, Agustinus Tri Aryanto and M. Agus Setiana* | 1369 |
| K 885 ID | Effects of Land Type on Vegetative Character (Germination, Leaves, Stems) and Rooting (Heavy, Long, Nodule) of Peanut (*Arachis hypogaea*)  
*Bambang Suwignyo, S. Al - Kautsar and Bambang Suhartanto* | 1373 |
| K 941 ID | The Effect of Legumes Mulch as Fertilizer on Growth Characteristics and Production of *Rumput Benggala* (*Panicum maximum*)  
*Lizah Khairani and Iin Susilawati* | 1377 |

**POSTER PRESENTATION**

<table>
<thead>
<tr>
<th>Code</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><strong>Genetic and Reproduction</strong></td>
<td></td>
</tr>
<tr>
<td>Large Ruminant</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
| A 63 BT  | Effect of Traditional Inter-Species Crossing (*Bos indicus* x *Bos frontalis*) on Cattle Productivity in Bhutan  
*Nar B Tamang, Tashi Samdup and John Perkins* | 1383 |
| A 107 KR | Molecular Genetic Evaluation of Korean Native Cattle Breeds Using Microsatellite Markers  
*Sangwon Suh, Mi-Jeong Byun, Chang-Yeon Cho, Seong-Bok Choi, Young-Sin Kim, Yeoung-Gyu Ko and Jae-Hwan Kim* | 1387 |
| A 163 ID | Reproductive Performance of Brahman Cows Kept in Individual or Group Pens in East Java, Indonesia  
*D. Ratnawati, L. Affandhy, D.A. Indrakusuma, D.E. Mayberry and D.P. Poppi* | 1390 |
| A 167 LK | Genetic Parameters and the Effect of Production and Type Traits on Productive Life of Korean Holsteins at First Lactation  
*Nidarshani Wasana, Gwang Hyun Cho, Su Bong Park, Si Dong Kim, Jae Gwan Choi, Byung Ho Park and Chang Hee Do* | 1394 |
| A 171 KR | An Analysis of Monthly Measured Acetone and β Hydroxybutyrate Acid in Milk of Holstein Cows  
*Yang Shin Chul, Gwang Hyun Cho, Chan Hyuk Park, Hyung Jun Song and Chang Hee Do* | 1398 |
<table>
<thead>
<tr>
<th>Code</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
</table>
| G 977 JP | Effect of Thyroidectomy on Blood Parameters in Chicks under Restricted Feeding Schedule  
*Takashi Bungo, Hiroshi Tanizawa and Takahiro Nikki* | 2459 |
| G 978 JP | Effect of Early Restraint Treatment on Responses to Subsequent Restraint in Chicks  
*Natsuki Fukano, Eriko Nakasai, Hiroshi Tanizawa and Takashi Bungo* | 2462 |

**Others**

<table>
<thead>
<tr>
<th>Code</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
</table>
| G 79 ID | The Effect of ETEC Vaccination for Pigs Breeding in Kintamani Bali Province Maintained by the Farmer  
*Anastasia Sischa Jati Utami and Ida Ayu Parwati* | 2466 |
*Sonia Tabasum Ahmed, Hong-Seok Mun and Chul-Ju Yang* | 2470 |
| G 205 KR | Effects of Group Housing Period before Furrowing on the Reproductive Performance and the Behavior of Sows  
| G 961 EG | Antioxidant Effects of Garlic, Ginger and Their Combination on Semen Quality of Rabbits  
*H. S. Zeweil, K. Kamel, M. Ahmed, S. Zahran, Yasmin El-Gendy and A. Abdo* | 2478 |
| G 964 KR | Comparison of Meat Quality Traits among Duroc Breeding Stock Lines in Korea  
*Jungseok Choi, Yangil Choi, Sora Ha and Sangkeun Jin* | 2482 |

**Products Technology and Food Safety**

**Large Ruminant**

<table>
<thead>
<tr>
<th>Code</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
</table>
| I 153 KR | Effect of Packaging and Additives on the Quality of Irradiated Restructured Meat Rolls  
*D. G. Lim, D. U. Ahn, J.-S. Cha, H.-C. Kim and K.C. Nam* | 2485 |
| I 180 ID | Characteristics of Garut Lamb Fed Ration Containing Sunflower Seed Oil  
*Lilis Khotijah, Suryati T and Disa AA* | 2489 |
| I 181 ID | Correlation of Browning Intensity and Antioxidant Activity in Dendeng  
*Tuti Suryati, Astawan M, Lioe HN and Wresdiyati T* | 2493 |
| I 211 KR | Effect of Breed on the Contents of Flavor and Functional Compounds in Freeze-Dried Soup  
*Dinesh D. Jayasena, Sun Hyo Kim, Samooel Jung, Kang Nyeong Heo, Hee Bok Park, Jun Heon Lee and Cheorun Jo* | 2497 |
<table>
<thead>
<tr>
<th>Code</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
</table>
| I 215 KR | Effects of Electron Beam Irradiation and Different Packaging Methods on the Safety and Quality of Egg Powder during Ambient Storage  
Hyun Jung Lee, Hyun-Joo Kim, Amali U. Alahakoon, Samooel Jung, Ki Chang Nam and Cheorun Jo | 2501 |
| I 217 KR | Effect of Thin Layer Dielectric Barrier Discharge Plasma on Inactivation of Aspergillus flavus and Quality Changes in Beef Jerky  
Hyun-Joo Kim, Hae In Yong, Amali U. Alahakoon, Sanghoo Park, Kijung Kim, Wonho Choe and Cheorun Jo | 2505 |
| I 268 ID | Effect of Citrus Aurantifolia Extract and Schleichera oleosa Liquid Smoked on Quality of Se’i Gemini E.M. Malelak, Geertruïda M Sipahelut and Pieter R Kale | 2509 |
| I 377 ID | The Effect of Ginger (Zingiber officinale Linn. Var. Rubrum) Addition and Soaking Time on Chemical Composition and Total Microbial of Goat Meat  
Setiyono, Edi Suryanto, Rusman, R. Sasongko Adi Nugroho, and Lucky Zulkarnain | 2513 |
| I 464 JP | Sensory Research of Soup of Goat Meat in Okinawa  
T Hirayama, S Tasaki, M Hirakawa, T Oikawa, SG Roh and K Katoh | 2516 |
| I 504 KR | Effect of Ozone Exposure on Bacteria Counts and Oxidative Properties of Beef Inoculated with Escherichia coli O157:H7  
Sung Ki Lee, Muhlisin, Youngjae Cho, Ji Hye Choi, Seung Gyu Lee and Tae-Wook Hahn | 2518 |
| I 630 TW | Heat Intensity of Market Milk in Taiwan: Part I. α-Lactalbumin, β-Lactoglobulin and Furosine Concentrations in Fresh Cow Milk  
M. J. Lin and E. E. Liang | 2523 |
| I 832 ID | Chemical Characterization of Oligosaccharides in the Milk of Water Buffalo (Bubalus bubalis)  
Epi Taufik, Rarah Ratih Adjie Maheswari, Robiyanto Hendro Susanto, Kenji Fukuda and Tadasu Urashima | 2527 |
| I 838 TW | Effect of Dry Aging on the Quality of Beef Short Loin  
Y. C. Kuo, S. C. Huang and R. S. Lin | 2531 |
| I 894 ID | Effect of Soy Protein Hydrolysate Addition on Peroxide Value and Sensory Properties of Beef  
Jamhari, Rusman, Resty Tarwiyatul Falah and Anggista Luthfiana Senja Pratiwi | 2534 |
Proceedings of the 16th AAAP Animal Science Congress Vol. II
10-14 November 2014, Gadjah Mada University, Yogyakarta, Indonesia

<table>
<thead>
<tr>
<th>Code</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
</table>
| I 763 TW | Influence of Cooking Methods on the Qualities of Chicken Breast Meat  
Wanwisa Chumnqoen, Hsin-Yi Chen, Chih-Feng Chen, Deng-Cheng Liu and Fa-Jui Tan | 2622 |
| I 812 KR | Quality Characteristics of Marinated Chicken Breast Meat by Addition of Grapefruit Seed Extract  
HyunSu Choi, HyunJin Lee, HyungGyu Choi and YangIl Choi | 2626 |
| I 813 KR | Effect of Sodium Chloride Replacement on Quality Characteristics of Low-Sodium Frankfurter Sausage  
HyungGyu Choi, HyunJin Lee, HyunSu Choi and YangIl Choi | 2629 |
| I 923 ID | The Potency of Bioactive Peptide of Native Chicken Leg as an Anti-hipertency Agent  
Yuny Erwanto, Arif Ismanto, Jamhari, Amrih Prasetyo and Ragil Yulianto | 2632 |
| I 1017 TW | Effect of Yolk as Emulsifiers on Physical Properties and Sensory Evaluation of Ice Cream  
M. J. Lin, P. S. He and Y. C. Huang | 2636 |

**Others**

<table>
<thead>
<tr>
<th>Code</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
</table>
| I 288 ID | The Potency of Curcuminoid Tumeric Extract in Preventing Low Density Lipoprotein (LDL) Oxidation Process on Rat with Atherosclerosis  
Trini Susmiati, Prabowo Purwono Purto, Triwahyu Pangestiningsih, Rini Widayanti and Claude Mona Airin | 2640 |
| I 662 TW | Antioxidative Properties of *Pleurotus eryngii* Fruiting Body Base Extract and Its Application to Pork Patties  
Meng-Shiun Ho, Wanwisa Chumnqoen, Deng-Cheng Liu, Ming-Tsao Chen and Fa-Jui Tan | 2644 |
| I 806 ID | The Characteristics of Edible Film From Pigskin Gelatin  
M. Sompie, S. Triatmojo, A. Pertiwiningrum and Y. Pranoto | 2648 |
| I 966 KR | Difference of Meat Quality Characteristics between Duroc and Crossbred Pigs  
Sora Ha, Jungseok Choi, Yangil Choi and Sangkeun Jin | 2652 |

**Waste and Environmental Issues in Livestock**

**Large Ruminant**

<table>
<thead>
<tr>
<th>Code</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
</table>
| J 240 ID | The Analysis Life Cycle Assessment (LCA) on Dairy Farming Production System  
A. Atabany, B.P. Purwanto, S. Purwanto and W. Al Zahra | 2656 |
The Potency of Bioactive Peptide of Native Chicken Leg as an Anti-hypertency Agent

Yuny Erwanto¹, Arif Istimanto², Jamhari¹, Amrih Prasetyo³ and Ragil Yulianto¹
¹Department of Animal Products Technology, Faculty of Animal Science, Gadjah Mada University, Indonesia 55281, ²Department of Animal Science, Mulawarman University, East Kalimantan, Indonesia, ³Agricultural Technology Research and Application Agency, Semarang, Indonesia
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ABSTRACT
Hypertension is the most common serious chronic health problem in adults and carries a high risk factor for arteriosclerosis, strokes, and end-stage renal disease. The objective of this research was to investigate the potency of chicken leg protein for the inhibition of angiotensin converting enzyme (ACE) activity. Protein of chicken leg expected as a source of bioactive peptides that can be used as antihypertensive agent. The stages of research were preparation chicken leg protein, digestion of chicken leg protein by pepsin and trypsin, determination of ACE inhibitory activity, and purification of ACE inhibitory peptides. Digestion process was measured by soluble protein concentration and molecular weight profile of native protein fraction as well as after pepsin and trypsin digestion. The result showed concentration of soluble protein using biuret test was 2.54 ± 0.62 mg/ml while after pepsin and trypsin were 43.65 ± 0.89 and 37.78 ± 0.84 mg/ml, respectively. Molecular weight (kDa) of chicken leg consecutive was 84.45; 76.31; 53.50; 43.67, and 41.52 kDa, after pepsin digestion was 43.67, 30.62; and 22.02 kDa and after trypsin digestion was 33.89; 23.76; and 14.31 kDa. Inhibitory activity of chicken leg protein on ACE was 65.24% and the IC₅₀ value was 2.98 mg/ml. ACE Inhibitory activity of chicken leg protein hydrolyzed after purification using SEP-PAK Plus Cartridge C-18 increased to 70.05 % and the IC₅₀ value was 2.03 mg/ml.In conclusion, native chicken leg has a high potential to be utilized and produced ACE inhibitory peptides.

Key Words: Chicken leg, Bioactive peptides, Hypertension, ACE inhibition

INTRODUCTION
Recently, many biologically active peptides from food proteins have been investigated. Inhibitors of angiotensin I-converting enzyme (ACE) have attracted particular attention for their ability to prevent hypertension (Katayama et al., 2003). Blood pressure is controlled by various regulatory factors in the body. Angiotensin I-converting enzyme (ACE) is one such factor. Angiotensin I-converting enzyme (ACE) converts decapeptide (angiotensin-I) into octapeptide (angiotensin-II), a pressure hormone for hipertency. Thus, inhibition of ACE results in inhibition of the conversion of angiotensin I into angiotensin II and is one of the effective methods for surprising rises in blood pressure (Saiga, et al., 2008). Previous research reported that chicken breast muscle and collagen hydrolysate posseses an anti-hypertensive effect and collagen peptide was isolate as the effective entity. Saiga et al. (2008) then used commercial chicken legs as sourced of collagen active peptide. ACE inhibitory peptides derived from poultry (poultry muscle and ovalbumin) were investigated and showed a potential activity for inhibiting ACE (Fujita et al., 2000). Research on the ACE inhibitory peptides derived from pork myosin light protein (Katayama et al., 2007) were also investigated.

Chicken leg is less desirable part for consumer, it consist some components that were: skin, bone, muscle, and collagen. So far, chicken leg only used as a soup and chicken leg crackers. Therefore this research aims was to investigate the potency of Indonesia native chicken leg as a source of bioactive peptides as antihypertensive agent.
MATERIALS AND METHODS

Preparation of Chicken Leg Protein
The chicken leg proteins were digested for 2 hours by pepsine and trypsin (Sigma Chemical Co. St. Louis, MO, USA) 1:50 at pH 7.0 and 37°C. The enzymatic hydrolysis by trypsin was stopped after 2 hours by boiling at 100°C for 10 min and the hydrolysates were collected. After incubation the hydrolysates were filtered through a 0.45 mm-pore sized filter paper, lyophilized and stored at -20°C.

Determination of Chicken Leg Composition and SDS-PAGE
The moisture, crude fat, crude protein and ash were determined by standard methods of the Association of Official Analytical Chemists (AOAC 1990). The protein concentrations of the crude extracted proteins and their hydrolysates were measured by the biuret method at 540 nm. The hydrolysates were analyzed by SDS-PAGE using 15-17.5 % gradient gels. Electrophoresis was carried out as described by previous research with a constant voltage of 150 V for 1.5 h. For experiment under reducing conditions The proteins were stained with 0.1 % Coomassie Brilliant Blue R-250 in 50% methanol and 10% acetic acid and de-stained with the same solvent system without dye. SDS-PAGE molecular weight standards proteins myosin were used as molecular weight markers.

ACE Inhibitory Assay
Analysis of ACE inhibition were employed the previous assays with slight modifications (Katayama et al., 2007; Muguruma et al., 2009). Protein hydrolyzate separated using SEP-PAK Plus C 18 Cartridge (Waters Co., Milford, MA, USA) with 10, 20 and 50% CH3CN. The ACE inhibitory then also was measured after the filtration by SEP-PAK.

RESULTS AND DISCUSSION
The proximate compositions of native chicken leg were measured as a basic data of the composition of the chicken leg extract. The results of the chemical composition are shown in Table 1.

<table>
<thead>
<tr>
<th>Table 1 Proximate compositions of native chicken leg</th>
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</thead>
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<tr>
<td>Content (%)</td>
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<tr>
<td>----------------</td>
</tr>
<tr>
<td>63.69</td>
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</table>

Breast meat contained about 20-22 % crude protein whereas higher crude protein content (32.54%) was found in chicken leg of this research. The presence of bone marrow and connective tissue cause the high crude protein content found in chicken leg extract (Cheng et al., 2008) and previous research founded ash content of the chicken leg was very high (11.80%) and the current result showed ash content around 0.57%. The differences of ash contents in this current research than previous due to this research separated chicken leg between connective tissue and bone. The bone was discarded and the connective tissue and a little meat were used for this experiment.

Previous studies showed the sequence of ACE an inhibitory peptide from various sources such as porcine muscle protein are Met-Asn-Pro-Pro-Lys and Ile-Thr-Thr-Asn-Pro (Arihara et al., 2001). Some of these peptides showed not only high activity in inhibiting ACE in vitro, but also in in vivo antihypertensive activity in spontaneus hypertensive rate (SHR). Amino acid formulations sequence of native chicken leg, possible to be used as a source of peptide inhibitors of an ACE. Based on research, have known that digestion process by pepsin and trypsin against chicken leg protein was optimally running, this could be seen based on the parameters levels of dissolved protein and molecular weight before and after digestion.
Numbers of dissolved protein in chicken leg protein using biuret assay were 17.2, 22.3 and 37.8 mg/mL, respectively. The enzymatic hydrolysis of proteins by pepsin and trypsin have degraded the proteins into amino acids and other simpler components, during hydrolysis it was possible that the peptide bond are splitting, so it would give fewer substances that would be detected by biuret.

Based on calculations of SDS-PAGE results showed the molecular weight of the protein native chicken leg, after pepsin digestion process and after trypsin digestion were (84.45; 76.31; 53.50; 43.67 kDa); (43.67, 30.62; 22.02 kDa) and 33.89; 23.76; 14.31 kDa. The larger the molecular weight indicates the length of the polypeptide chain make it easier to interact with other proteins. The molecular weight of proteins prior to digestion chicken leg protein showed the highest rate in the amount of 60 kDa, it’s similar with chicken bone protein molecular weight before digestion by Cheng (2008) it’s 66 kDa. This suggests that the protein prior to digestion chicken leg protein polypeptides that there is still a long leash.

![Figure 1: SDS-PAGE patterns of chicken leg protein before and after enzyme digestion. A: before hydrolysis; B: pepsin digestion; C: pepsin and trypsin digestion and M: molecular weight marker.](image)

The band at 30 kDa protein found in protein hydrolysates chicken leg protein using the enzyme pepsin after hydrolysis for 2 hours. The molecular weight of the protein hydrolyzed with pepsin was smaller than the molecular weight before hydrolysis. Figure 1 also showed smaller molecular weight of sample after trypsin digestion (Figure 1 lane B) and this result indicated that the chicken leg protein can be digested by pepsin and trypsin enzyme. Research using pepsin was also carried out by Cheng et al. (2008), to hydrolyze proteins from chicken bones. The results were slightly different compared with chicken bones protein hydrolizate, and showed the results of molecular weight in the range around 66 kDa.

Angiotensin converting enzyme inhibitory activity of chicken leg proteins hydrolysates was 65.21 % and IC₅₀ value was mg/ml. Angiotensin converting enzyme of chicken leg proteins hydrolysates after purification using Sep-Pak Plus C-18 Cartridge was 70.05 % and the IC₅₀ value of 0.433 mg/ml.

**CONCLUSION**

Higher activities of ACE inhibitor were obtained by after SEP-PAK Plus C-Catridge, the results of this study showed that by-products such as native chicken leg had a high potential to be utilized and produced ACE inhibitory peptides. The active peptide may be further applied as an ingredient of functional food for anti-hypertension.
REFERENCES


This is to certify that

YUNY ERWANTO

has participated as a

POSTER PRESENTER

at the 16th Asian-Australasian Association of Animal Production Societies Congress

"Sustainable Livestock Production in the Perspective of Food Security, Policy, Genetic Resources and Climate Change"

Universitas Gadjah Mada, Yogyakarta - Indonesia

10th - 14th November 2014

President
Asian-Australasian Association
of Animal Production Societies

Mr. Yudi Guntara Noor

Chairman
Organizing Committee

Budi Guntoro, Ph.D.
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INTRODUCTION
Recently, many biologically active peptides from food proteins have been investigated. Inhibitors of angiotensin I-converting enzyme (ACE) have attracted particular attention for their ability to prevent hypertension (Katayama et al., 2003). ACE is one of various factors that controlled blood pressure. ACE converts decapptide (angiotensin-I) into octapeptide (angiotensin-II), a pressure hormone for hypertension. Thus, inhibition of ACE is one of the effective methods for surprising rises in blood pressure. Saiga et al. (2008) then used commercial chicken legs as sourced of collagen active peptide and as ACE inhibitory peptides.

Chicken leg is less desirable part for Indonesian consumer. So far, chicken leg only used as a soup and chicken leg crackers. Chicken leg contents 22.98% of protein (Purnomo, 1992). Therefore this research aims was to investigate the potency of Indonesia native chicken leg as a source of bioactive peptides as antihypertensive agent.

MATERIALS AND METHODS

Preparation of Chicken Leg Protein
Determination of Chicken Leg Composition (AOAC, 1990)
Determination of protein (Biuret Method)
SDS PAGE Analysis
ACE Inhibitory Assay

RESULTS AND DISCUSSION

Composition of Native Chicken Leg

<table>
<thead>
<tr>
<th>Constituent</th>
<th>(%)</th>
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<tr>
<td>Moisture</td>
<td>63.69</td>
</tr>
<tr>
<td>Crude protein</td>
<td>32.54</td>
</tr>
<tr>
<td>Crude fat</td>
<td>2.63</td>
</tr>
<tr>
<td>Ash</td>
<td>0.57</td>
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</tbody>
</table>

Evaluation of Protein Hydrolysis of Chicken Leg

Fig. 1. The soluble protein after enzymatic digestion (mg/mL). 1: native chicken leg protein; 2: After pepsin digestion; 3: After trypsin digestion

Molecule weight analysis using SDS PAGE

Fig. 2. SDS-PAGE patterns of chicken leg protein. A: before hydrolysis; B: after hydrolysis by pepsin; C: after hydrolysis by pepsin and continued with trypsin and M: molecular weight marker.

Potency of Chicken Leg Protein as an ACE Inhibition

IC50 Before Filtration: 2.98 mg/ml
IC50 After filtration: 0.43 mg/ml

CONCLUSION
Higher activities of ACE inhibitor were obtained by after SEP-PAK Plus C-Catridge
The results of this study showed that by-products such as native chicken leg had a high potential to be utilized and produced ACE inhibitory peptides
The active peptide may be further applied as an ingredient of functional food for anti-hypertension