ISSN 0853-7380
E-ISSN 2252-696X

Accredited by the Ministry of Research and Technology /
National Agency for Research and Innovation
Decree Number: 85/M/KPT/2020

Jurnal Ilmu Ternak dan Veteriner

IJAVS *Indonesian Journal of Animal and Veterinary Sciences*

Volume 26
Number 1
March 2021

PUSAT PENELITIAN DAN PENGEMBANGAN PETERNAKAN
BADAN PENELITIAN DAN PENGEMBANGAN PERTANIAN
KEMENTERIAN PERTANIAN

JITV	Volume 26	Number 1	Page: 1-48	Bogor, March 2021	ISSN 0853-7380

Jurnal Ilmu Ternak dan Veteriner

IJAVS *Indonesian Journal of Animal and Veterinary Sciences*

JITV	Volume 26	Number 1	Page 1-48	Bogor, March 2021	ISSN 0853-7380 E-ISSN 2252-696X

Published by:
Indonesian Center for Animal Research and Development
Indonesian Agency for Agricultural Research and Development, Ministry of Agriculture

Collaborated with:
Indonesian Society of Animal Science

Secretariat of IJAVS:
Jalan Raya Pajajaran Kav. E-59, Bogor 16128 - Indonesia
Telephone +62-251- 8322185
Fax +62-251-8380588
E-mail: criansci@indo.net.id; jitvnak@yahoo.com
Website: http://medpub.litbang.pertanian.go.id/index.php/jitv

Indonesian Journal of Animal and Veterinary Sciences is published four times a year in March, June, September and December.

PREFACE

In this edition, Volume 26 No 1, we proudly present articles from animal and veterinary sciences including genetics; reproduction, feed technology, and veterinary. The articles published in this edition are:

"Ultrasound Imaging to Estimate Carcass Quality of Pasundan Cattle based on Body Condition Score"; "Molecular Profile of *Trichophyton mentagrophytes* and *Microsporum canis* based on PCR-RFLP of Internal Transcribed Spacer"; "Pathogenicity of Philippine and Indonesian *Trypanosoma evansi* Isolates in Mice and Their Responses to Trypanocides"; "Immunomodulatory Effect of Virgin Coconut Oil in Wistar Rats Infected with *Staphylococcus aureus*"; and "Effect of Dietary Modified-Banana-Tuber Meal Substituting Dietary Corn on Growth Performance, Carcass Trait and Dietary-Nutrients Digestibility of Coloured-Feather Hybrid Duck".

We extent high appreciation to all peer reviewers who make this journal academically high value. Hopefully, these articles would offer any benefit to readers and the end-users of technological innovation, and attract interests from scientists to contribute their papers to the Indonesian Journal of Animal and Veterinary Sciences.

Chief Editor

Bogor, March 2021

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Acknowledgement

Ultrasound Imaging to Estimate Carcass Quality of Pasundan Cattle based on Body Condition Score

Khairunnisa S[1], Novelina S[1], Hilmia N[2], Hadi DN[3], Rahmat D[2], Ulum MF[1,4,*]

[1]*Faculty of Veterinary Medicine, IPB University, Jl. Agatis, Dramaga, Bogor 16680 – Indonesia*
[2]*Faculty of Animal Husbandry, Padjadjaran University, Jl. Raya Bandung-Sumedang KM. 21, Jatinangor, Sumedang 45363 – Indonesia*
[3]*Center for Artificial Insemination and Artificial Insemination for Beef Cattle Development (UPTD BPPIB), Ciamis 46271 – Indonesia*
[4]*Veterinary Teaching Hospital, Faculty of Veterinary Medicine, IPB University, Jl. Agatis, Dramaga, Bogor 16680 – Indonesia*
**Corresponding Email: ulum@ipb.ac.id*

(received 11-11-2020; revised 16-01-2021; accepted 18-01-2021)

ABSTRAK

Khairunnisa S, Novelina S, Hilmia N, Hadi DN, Rahmat D, Ulum MF. 2021. Pencitraan ultrasonografi untuk pendugaan kualitas karkas pada Sapi Pasundan berdasar nilai kondisi tubuh. JITV 26(1): 1-9. DOI:http//dx.doi.org/10.14334/jitv.v26i1.2584.

Penelitian ini bertujuan untuk mengestimasi kualitas karkas pada Sapi Pasundan menggunakan citra ultrasonografi (USG) berdasarkan nilai kondisi tubuh (NKT) yang berbeda. Sapi Pasundan betina total sebanyak 31 ekor sapi berumur 4-7 tahun dari Unit Pelaksana Teknis Daerah Balai Perbibitan dan Pengembangan Inseminasi Buatan Ternak Sapi Potong Ciamis Jawa Barat Indonesia dengan NKT 1,0-4,0 digunakan dalam penelitian ini. Skor marbling, *intramuscular fat* (IMF), tebal lemak punggung (LP), dan tebal otot dari *m. longissimus dorsi* (LD), *m. psoas major* (PM), *m. psoas minor* (PMN), serta *m. gluteus medius* (GM) diambil menggunakan pencitraan USG pada 3 lokasi berbeda yaitu pada tulang rusuk 12-13 (thorax), lumbal 4-5 (lumbal) dan diantara *tuber coxae* dan *tuber ischii* (gluteal) dengan frekuensi 5 MHz dari transduser convex. Hasil analisa menunjukkan bahwa peningkatan NKT berbanding lurus dengan peningkatan tebal LP, ketebalan otot, skor marbling dan skor IMF dari m. LD, m. PM, m. PMN, dan m. GM. Sapi Pasundan memiliki rentang skor marbling 1-5 dan rentang persentase IMF 2,62%-4,82%. Nilai kondisi tubuh sapi pasundan memengaruhi kualitas karkas pada parameter seperti ketebalan otot, skor marbling dan persentase *intramuscular fat* dari citra ultrasonografi m. LD, m. PM, m. GM dan m. PMN.

Kata Kunci: NKT, Sapi pasundan, Karkas, Ultrasonografi

ABSTRACT

Khairunnisa S, Novelina S, Hilmia N, Hadi DN, Rahmat D, Ulum MF. 2021. Ultrasound imaging to estimate carcass quality of Pasundan cattle based on body condition score. JITV 26(1): 1-9. DOI:http//dx.doi.org/10.14334/jitv.v26i1.2584.

This research aimed to estimate carcass quality of Pasundan cattle using ultrasound imaging based on Body Condition Score (BCS). Total 31 head of female cattle with age ranging from 4 to 7 years from Regional Technical Implementation Unit of the Center for Artificial Insemination and Artificial Insemination for Beef Cattle Development at Ciamis West Java, Indonesia with BCS ranging from 1.0-4.0. The marbling score, intramuscular fat (IMF), backfat thickness (LP), and thickness musculus of m. longissimus dorsi (LD), m. psoas major (PM), m. psoas minor (PMN), m. gluteus medius (GM) and m. biceps femoris (BF) were scanned using ultrasound on 3 different locations, i.e. on 12^{th}-13^{th} ribs (thorax), lumbar 4^{th} - 5^{th} (lumbar), and between tuber coxae and tuber ischii (gluteal) with 5 MHz frequency of convex transducer. The results showed that BCS increased when LP, marbling score and IMF from m. LD, m. PM, m. PMN, and m. GM was rising. Pasundan cattle showed marbling scores ranging from score 1 to 5 and percentage IMF ranging from 2.62% to 4.82%. Body Condition Score affected carcass quality of Pasundan cattle on parameters such as musculus thickness, marbling score, and intramuscular fat (IMF) from ultrasound imaging of m. LD, m. PM. m. PMN, m. GM, and m. BF.

Key Words: BCS, Carcass, Pasundan cattle, Ultrasound

INTRODUCTION

Pasundan cattle are the result of adaptation and crossing of more than ten generations of Balinese cows with Ongole crossbreeds, Sumba Ongole, and Madura cows (Kementerian Pertanian 2014). Pasundan cattle are known as the genetic resources of local livestock belonging to West Java based on the Decree of the Minister of Agriculture No: 1051/Kpts/SR.120/10/2014 at 13 October 2014 concerning the determination of the Pasundan cattle breed. The advantages of Pasundan cattle are efficiency in maintenance, relatively simple housing, disease/parasite resistance, thirst resistance, resistance to weather changes, short reproductive cycles, high feed efficiency, and a high percentage of carcasses (Indrijani et al. 2012). Pasundan cattle can contribute to meeting the demand for beef, especially in West Java by 20 percent of the 515 thousand head per year (Dinas Peternakan Jawa Barat 2016).

Factors taken into account in assessing meat and carcass quality in cattle according to USDA standards include carcass weight, carcass length, backfat

thickness, longissimus dorsi muscle thickness, pelvic fat percentage, marbling score, and fat color (Nold et al. 1992). Ultrasound imaging has been used since 1960 to measure muscle fat thickness in beef cattle which is a parameter in the Quality Beef Contest at the International Livestock Exposition (Stouffer 2004). Ultrasonography (USG) is a technique that is widely used for *in vivo* prediction of carcass or body composition in cattle, pigs, goats, and also sheep (Stouffer 2004). Ultrasound imaging can be used to determine meat and fat characteristics in live animals, specifically intramuscular fat percentage and marbling score (Gupta et al. 2013). The characteristics of the carcass and the quantity and quality of meat can be evaluated periodically, routinely, and more accurately by using ultrasound (Bugiwati et al. 2000). Furthermore, ultrasound imaging is non-distracting to livestock, is relatively inexpensive, and is a useful method for evaluating the condition of subcutaneous muscle and adipose tissue (Stouffer 2004).

The body condition score (BCS) is a method for interpreting body fat reserves using senses of sight and touch, subjectively (Edmonson et al. 1989). These reserves of body fat were used to cover the lack of energy from feed during delivery and milk production. The BCS assessments have been accepted as a simple and inexpensive method that is used for estimating body fat used in both commercial livestock and research activities (Otto et al. 1991). Two scale methods have been used in determining BCS, namely, a scale of 1-9 (America) and a scale of 1-5 (UK and Commonwealth country), and in Indonesia, BCS assessment generally use a scale of 1-5 (M'hamdi et al. 2012). Assessment of the ideal BCS for livestock is highly dependent on the purpose of raising the livestock. For cattle that are raised as livestock producing or fattening meat, the greater the value of their BCS will be better and very profitable the livestock business (Syaifudin 2013). This study aims to estimate the carcass quality of Pasundan cattle using ultrasound images based on different BCS. This research is expected to provide information regarding the estimation of carcass quality in Pasundan cattle using ultrasound images based on BCS as one of the considerations and evaluations in the breeding program of livestock breeds.

MATERIALS AND METHODS

The research was conducted at the Regional Technical Implementation Unit of the Center for Artificial Insemination and Artificial Insemination for Beef Cattle Development (UPTD BPPIB) at Ciamis, West Java. The 31 female Pasundan cows with natural conditions from various ages ranging from 4-7 years used in this study were then grouped based on BCS through direct visual observation with a scale of 1.0-5.0 (Roche et al. 2009). The variables observed were body condition score (BCS), backfat thickness (LP), m. longissimus dorsi thickness (LD), m. psoas major thickness (PM), m. psoas minor thickness (PMN), m. gluteus medius thickness (GM), m. biceps femoris thickness (BF), marbling score (MS), and percentage of intramuscular fat (IMF). These variable data were obtained by using an ultrasound image console (Sonodop S-3X, PT Karindo Alkesteron) with a convex transducer having a frequency of 5 MHz on a scanning area depth of 11.4 cm.

Ultrasound is performed to scan LP, m. LD, m. PM on the chest following the method of Jakaria et al. (2017) on 12^{th}-13^{th} ribs, and the lumbar part above the flank at 4^{th}-5^{th} lumbar bone, which is more backward than Tait (2016) has done. Thickness measurement m. BF, m. GM, m. LD, and m. PM in the gluteus follows the method used by Silva et al. (2012) between the ischium and ilium bones (Figure 1). Ultrasound scans were taken transversally and longitudinally against the axis of the body for each part imaged (Figure 1). Ultrasound scan is performed transcutaneously on the skin with previously shaved hair using a hair razor and cleaned with soapy water. The ultrasonic gel is used as a contact agent to facilitate the interface between the probe and the skin to produce a good image. After the scan was complete, an antibiotic and anti flies spray (Limoxin 25 spray, PT. TMC Indonesia) was applied to the image location to prevent flies from settling on.

The results of ultrasound imaging were stored in BMP format and then presented descriptively and the data were analyzed quantitatively by using Microsoft Excel and ImageJ applications (NIH, USA) software. Quantitative data assessment was carried out by measuring skin thickness, subcutaneous thickness, muscle thickness, area, and intensity of gray color m. LD, m. PM, m. GM, m. BF and m. PMN, and then assessing the marbling score and the IMF percentage based on AUS-MEAT Standard (AUS-MEAT 2020).

RESULTS AND DISCUSSION

Figure 2 shows the ultrasound image of the muscle in Pasundan cattle. Three different image scanning locations (thorax, lumbar and gluteal) wherein one location was imaged in two transducer positions namely transverse and longitudinal. The carcass composition in the thorax (Figure 2A and 2B) consists of skin, back fat (LP), and m. LD. The lumbar part (Figure 2C and 2D) of carcass composition consists of skin, subcutaneous fat, m. LD, m. PM, and m. PMN. The composition of the rump / gluteal carcass (Figure 2E and 2F) consists of skin, subcutaneous fat, m. BF, m. GM, m. LD, and m. PM. Muscles imaged on the sonogram display as gray color or areas of less echogenicity.

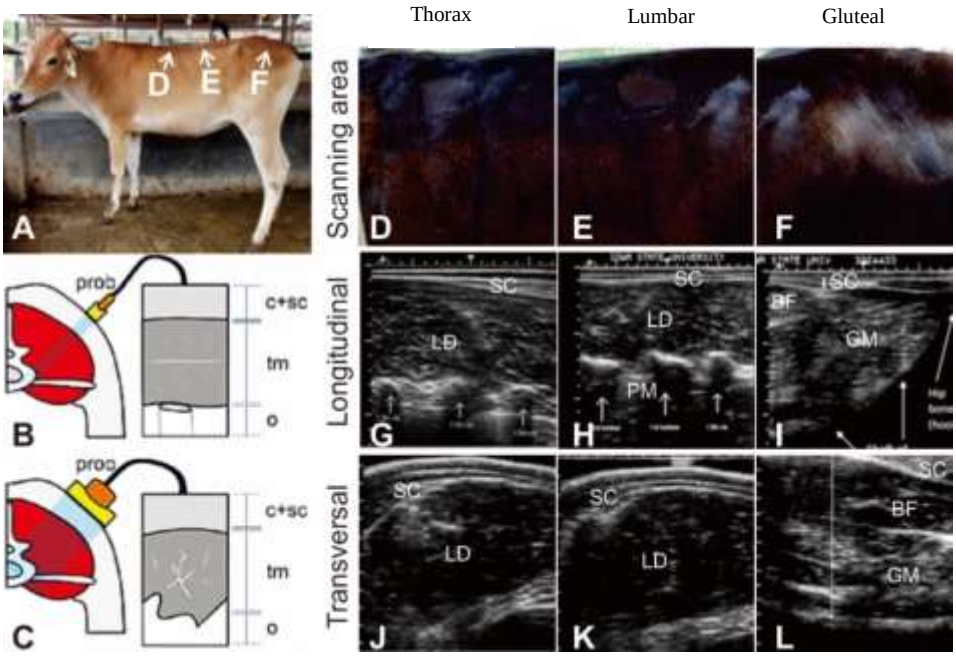

Figure 1. (A) Location of ultrasound imaging of Pasundan cattle; transducer display at (B) longitudinal and (C) transversal view, prob= transducer, c= cutan, s=subcutan, tm=muscle thickness, o= bone (adapted from Ulum *et al*. 2014). Scanning area (D) thorax, (E) lumbar, (F) gluteal; image of the ultrasound imaging at the image scanning view (G) thorax longitudinal, (H) lumbar longitudinal, (I) gluteal longitudinal, (J) thorax transversal, (K) lumbar transversal, (L) gluteal transversal (adapted from Tait 2016); LD= *m. longissimus dorsi*, PM= *m. psoas major*, BF= *m. biceps femoris*, GM= *m. gluteus medius*

Sonogram of skin, LP, muscle fat, muscle collagen, and bone shows white color or shows higher echogenicity than the surrounding tissue. The image pattern produced from the 3 locations is the same, namely, the live carcass composition is directly proportional to BCS.

The thickness of LP, m. LD, m GM, m. PM, and m. PMN in Pasundan cattle with different BCS can be seen in Figure 3. The thickness of LP and m. LD in Figure 3A-F shows that the thickness is in line with the increase in BCS. Imaging positions in the gluteal area (Figure 3E and 3F) showed that m. LD thickness increased dynamically with increasing BCS, but decreased at BCS 3.5-4.0. The location of the image that shows the thickest m. LD with the smallest standard deviation is at the position of the thorax imaging (Figure 3A and 3B). The thickness of m. GM and m. BF (Figure 3E and 3F) showed the same results as the thickness of m. LD where muscle thickness was directly proportional to BCS. The thickness of m. PM at the lumbar imaging position (Figure 3C and 3D) showed a different graph pattern with the gluteal imaging positions (Figure 3E and 3F). The lumbar imaging position (Figure 3C and 3D) shows that the greater the BCS, the greater the m. PM thickness. The gluteal imaging positions (Figure 3E and 3F) showed m. PM thickness is only seen in cows with BCS 2.0–4.0 where each increase in BCS coincided with increasing m. PM thickness. PMN muscles were only imaged in the lumbar imaging position (Figure 3C and 3D) in cows with BCS 2.0-3.5 where each increase in BCS showed a decrease in m. PMN thickness.

The marbling score of Pasundan cattle with different BCS can be seen in Figure 4. Figure 4A-D shows the marbling score at m. LD increases from 1 to 5 along with the increase in BCS. The marbling score at m. LD from the gluteal imaging position (Figure 4E and 4F) showed a dynamic increase with increasing BCS but then a decrease occurs in BCS 3.5-4.0. The highest marbling score of m. LD was 5 at BCS 3.5-4.0 (Figure 4C and 4F). The marbling score for m. PM can be seen in Figure 4C-F. The PM muscle in Figure 4C shows an increase from the 1 to 3 marbling score, then a decrease in BCS 2.5-3.0 to score 2 until it increases again and remains at score 3 at BCS 3.0-4.0. Whereas in Figure 4E-F the m. PM marbling score is only seen in cows with a BCS of 2.0-4.0. Each increase in BCS was accompanied by an increase in the m. PM marbling score (Figure 4D-F). The marbling score for m. PMN was only seen at BCS 2.0-3.5, where each increase in BCS also increased the marbling score (Figure 4C and 4D). The 2 marbling score is the highest marbling score of m. PMN at BCS 3.0-3.5 (Figure 4C). The m. GM and m. BF which were located in the gluteal section showed that each increase in BCS was also followed by an increasing marbling score with the highest score of 4 at BCS 3.5-4.0 (Figure 4E and 4F).

The IMF value based on the AUS-MEAT carcass quality standard in Pasundan cattle can be seen in Figure 5. The results obtained are not much different

from Figure 4. The IMF values on m. LD, m. GM, m. BF, m. PM, and m. PMN has increased along with the increase in BCS. Figure 5A-F shows the percentage of IMF in m. LD. Cows with a BCS of 1.0-1.5 have the smallest IMF percentage range at m. LD of 2.62% - 2.80%. The percentage of IMF in m. LD with BCS 2.0-3.0 and 3.0-3.5 are 2.77% -3.32% and 3.36% -3.88%, respectively. The highest percentage of IMF in m. LD was 3.39% -4.09% with BCS 3.5-4.0. The greatest percentage of m. PM was 4.00% in BCS 3.5-4.0 (Figure 5D). The m. GM and m. BF showed the greatest percentage of IMF in each muscle, respectively 3.76% and 3.84% (Fig. 5E and 5F). The percentage of IMF m. PMN is only seen in BCS 2.0-3.5, which shows the smallest percentage range of IMF from other muscles in the same image is 0.60% -1.73% (Figure 5C and 5D).

Ultrasound imaging is an objective method of measuring the carcass composition of live cattle that is effective, fast, and accurate (Lambe et al. 2010). Muscle imaged on the sonogram looks hypoechoic whereas LP, muscle fat, muscle collagen, and bone appear more hyperechoic. Soft tissue such as muscle will weaken some of the ultrasound waves transmitted in this way, so that it will show a gray or hypoechoic

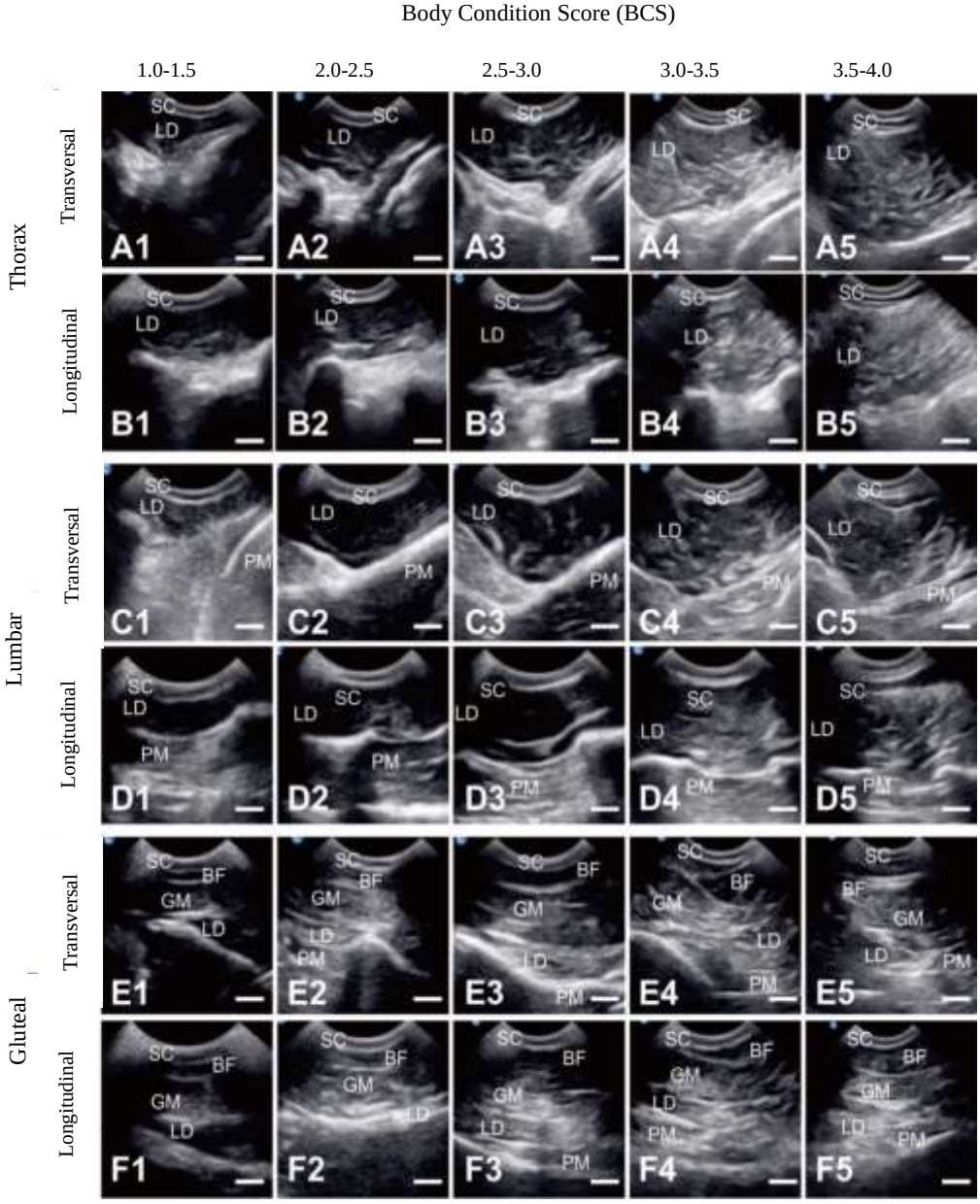

Figure 2. Sonogram of live carcass composition in Pasundan cattle with different BCS in scanning view of (A) Thorax transversal, (B) Thorax longitudinal, (C) Lumbar transversal, (D) Lumbar longitudinal, (E) Gluteal transversal, and (F) Gluteal longitudinal. Scale bar = 2 cm

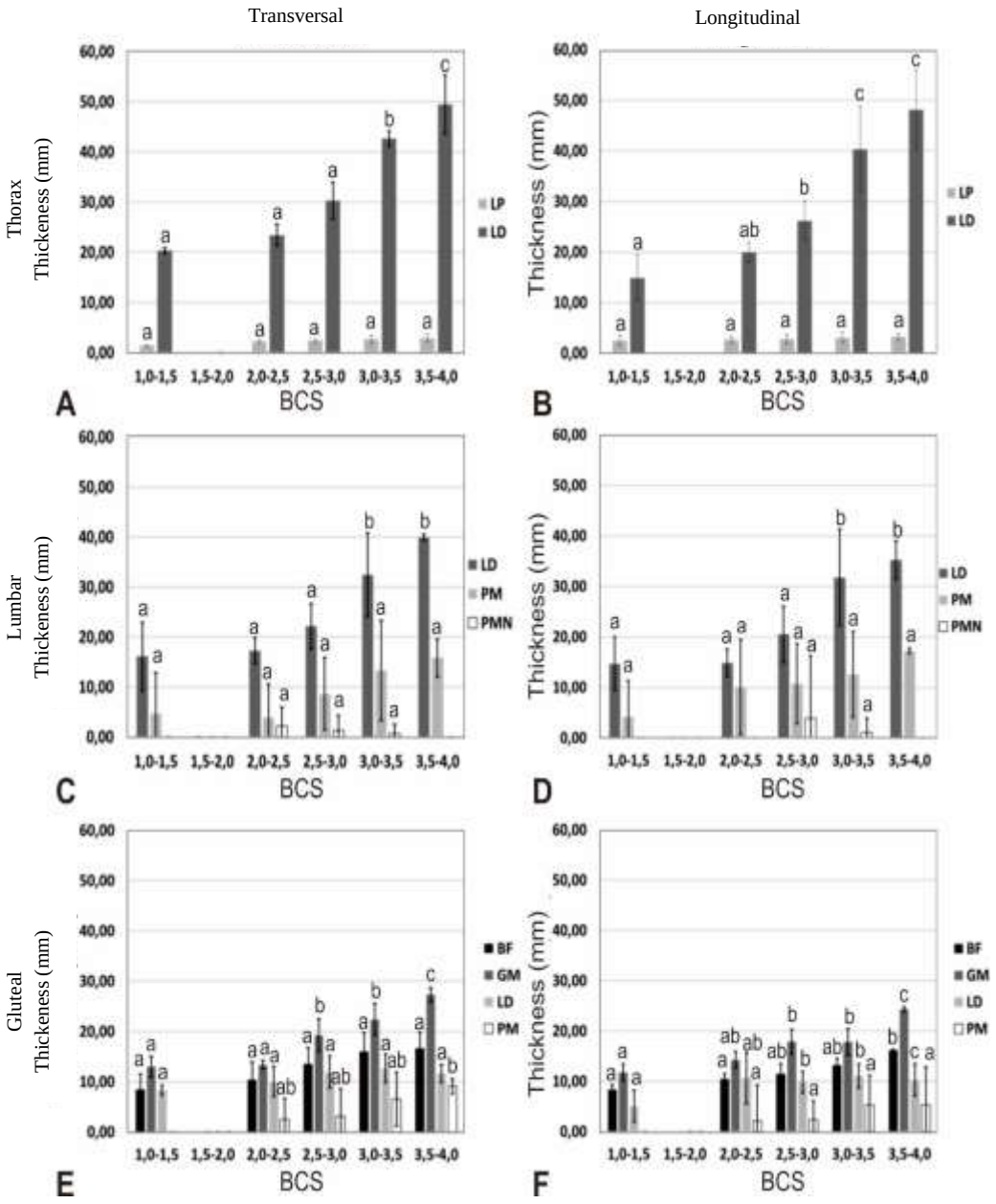

Figure 3. The thickness of back fat (LP), m. longissimus dorsi (LD), m. gluteus medius (GM), m. biceps femoris (BF), m. psoas major (PM), and m. psoas minor (PMN) in Pasundan cattle with different of BCS. A) Thorax transversal, B) Thorax longitudinal, C) Lumbar transversal, D) Lumbar longitudinal, E) Gluteal Transversal, and F) Gluteal longitudinal. Different superscript letters in the same muscle showed significant differences ($p<0.05$) between BCS in Pasundan cattle

color on the sonogram (Cruz-Arámbulo & Wrigley 2003). Muscle, fat, and bone collagen will show a highly-reflective interface so that it will appear white or hyperechoic on the sonogram (Noviana et al. 2018). The composition of the carcass of Pasundan cattle that is imaged shows various results along with the varied BCS. BCS 3.5-4.0 (fat) presented a sonogram with the largest live carcass composition compared to low BCS (thin) (Figure 2). This is following the opinion of Juandhi et al. (2019) stated that carcass weight and composition will increase with increasing BCS of livestock.

Research has been conducted by Bergen et al. (2005) has shown that m. LD and LP thickness in cattle can be estimated using ultrasound at 12^{th}-13^{th} rib imaging sites. According to Tait (2016), ultrasound imaging of tissue at the 12^{th}-13^{th} ribs with a transverse position can measure the thickness of m. LD and LP. This is following the results obtained in this study, the thickness of m LD and LP can be imaged in the thorax imaging location on 12^{th}-13^{th} ribs (Fig. 3A and 3B). The m. LD is linked originally in the procesus spinous bone of the 1^{st}-3^{rd} sacrum, lumbar vertebrae,

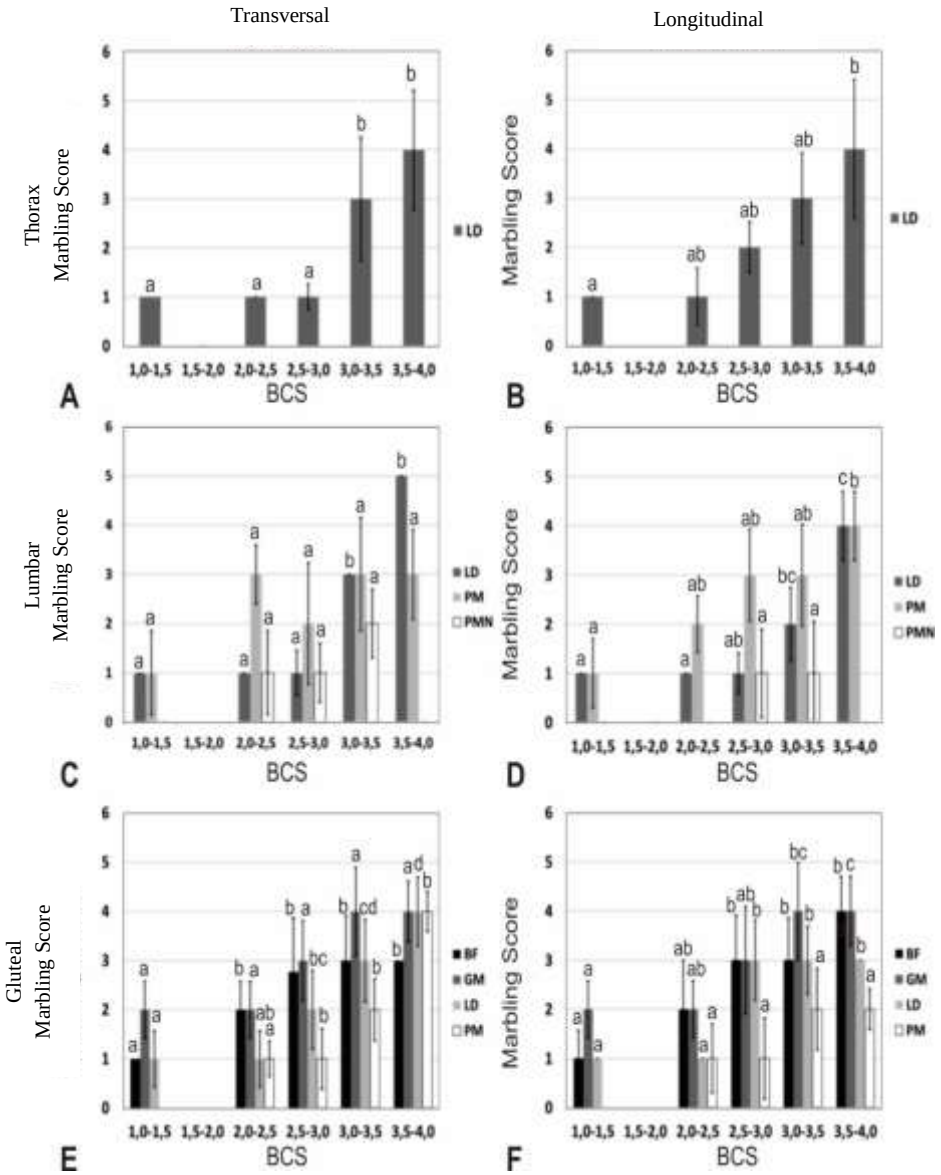

Figure 4. The marbling score is based on AUS-MEAT standard of m. longissimus dorsi (LD), m. gluteus medius (GM), m. biceps femoris (BF), m. psoas major (PM), and m. psoas minor (PMN) in Pasundan cattle with different of BCS. A) Thorax transversal, B) Thorax longitudinal, C) Lumbar transversal, D) Lumbar longitudinal, E) Gluteal Transversal, and F) Gluteal longitudinal. Different superscript letters in the same muscle showed significant differences ($p<0.05$) between BCS in Pasundan cattle

and 13th thoracicae vertebrae, as well as in the tuber coxae, tuber sacrale, and iliac crista from os ilium, then inserts into the lumbar vertebrae, ossa vertebrae thoracicae and fascia lata from ossa costales (Nurhidayat et al. 2016). Ultrasound imaging of tissue at the 12th-13th ribs will show a thicker m. LD in comparison to images posterior to the 13th rib and between 1st-2th lumbar Tait (2016).

The results in this study showed a slight difference in thickness of m. LD, m. GM, m. BF, m. PM, and m. PMN with three different scan locations. The m. PM at the lumbar scan sites (Fig. 3C and 3D) showed easier imaging results when compared to the gluteal scan sites (Figure 3E and 3F). According to (Budras et al. 2003) m. PM origin at the cranial border of the last rib (12th-13th os costales) and the transverse process of the vertebral vertebrae lumbales, meanwhile m. PM insertion in the trochanter minor os femur. M PMN was seen only in the lumbar imaging position (Figure 3C and 3D). The m. PMN origin in ossa vertebrae 12th-13th thoracic, ossa vertebrae 1st lumbales, and crura diaphragm then m. PMN insertion in psoas tubercle os ilium (Budras et al. 2003). Position ultrasound scans to obtain m. GM and m. BF images were performed on the rump section of the cow, where the coxaeal tubers and ischial tubers were oriented in the scan (Tait 2016). According to Nurhidayat et al. (2016) GM origin on the gluteal surface of the os ilium and insertion on the

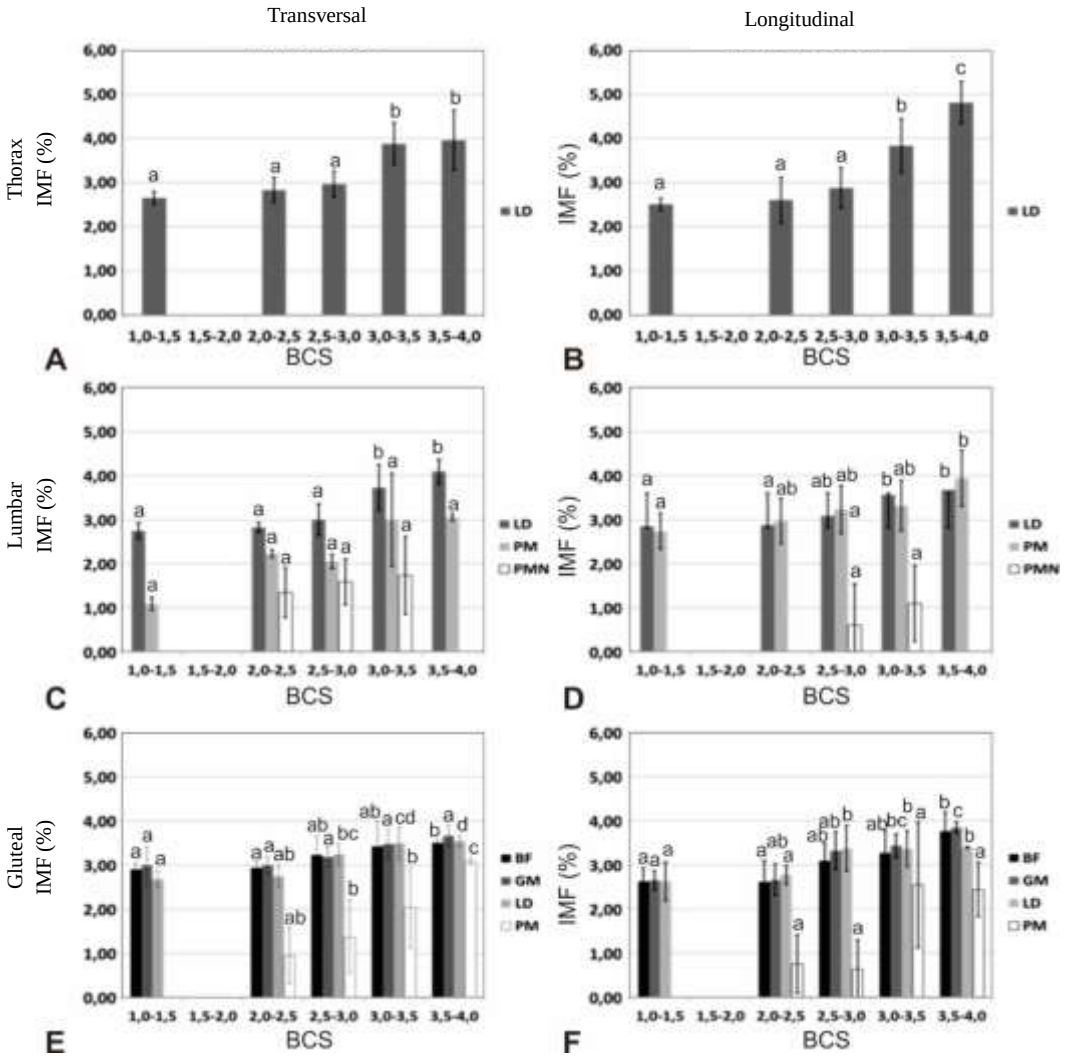

Figure 5. The value of Intramuscular Fat (IMF) based on AUS-MEAT standard m. longissimus dorsi (LD), m. gluteus medius (GM), m. biceps femoris (BF), m. psoas major (PM), and m. psoas minor (PMN) in Pasundan cattle with different of BCS. A) Thorax transversal, B) Thorax longitudinal, C) Lumbar transversal, D) Lumbar longitudinal, E) Gluteal Transversal, and F) Gluteal longitudinal. Different superscript letters in the same muscle showed significant differences ($p<0.05$) between BCS in Pasundan cattle.

greater trochanter of the femur. The m. BF or also known as m gluteobiceps that origin on the tuber ischii and median sacral crest of the sacrum then inserts into the patellar bone, tibia (fascia lata and fascia cruris), and calcaneus tendons (Budras et al. 2003).

The ultrasound imaging method can be used in determining the marbling score and the percentage of intramuscular fat as a basis for estimating the quality of meat and fat in living livestock (Gupta et al. 2013). According to Drake (2004), the quality of beef carcass is determined by the marbling score which can be measured in the ribeye between the 12^{th}-13^{th} ribs. Based on the results obtained, m. LD shows the highest marbling score and IMF percentage followed by m. PM, m. GM, m. BF, and m. PMN (Figure 4 and 5). The marbling score on m. LD is in the score range 1-5 and the IMF percentage range from 2.62%-4.82%. Pasundan cattle with BCS 3.5-4.0 had the highest marbling score and IMF percentage at m. LD compared to low BCS (Figure 4 and 5). Based on these results, m. LD has been used as the standard for calculating the marbling and IMF scores by the AUS-MEAT (2020). In addition to the AUS-MEAT standard, the ribeye section m. LD has also been used as a standard for scoring the marbling score by the Beef Marbling Standard (BMS) Japan (Gotoh et al. 2018), US Department of Agriculture (USDA) beef grades (Emerson et al. 2013), and Indonesian National Standard (SNI) 3932: 2008 concerning the quality of carcass and beef (BSN 2008).

The higher marbling and IMF scores of m. LD than other back muscles can be caused by differences in the function and fat content of each muscle. As stated by Nuraini & Hafid (2006), the function of each muscle will determine the distribution of fat and collagen levels in muscles. The location of m. LD and m. PM in the spine (spinal column) is likely to experience very rare contractions so that both muscles have lower collagen levels than m. GM and m. BF. This is also supported by

Lawrie (2003) statement where collagen levels in underactive muscles such as m. LD and m. PM has lower collagen levels than m semimembranosus, m semitendinosus, and bicep femoris. Collagen m. PM levels are smaller than m. LD so m. PM has a higher level of tenderness than other muscles (Carmack et al. 1995).

CONCLUSION

The Pasundan cattle body condition score influenced carcass quality on parameters such as muscle thickness, marbling score, and percentage of intramuscular fat on ultrasound images of m. LD, m. PM, m. GM, and m. PMN. The m. LD has the highest marbling score and percentage of intramuscular fat followed by m. PM, m. GM, m. BF, and m. PMN based on the location of ultrasound imaging on 12^{th}-13^{th} ribs, 4^{th}-5^{th} lumbar, and between the tuber coxae and tuber ischii.

ACKNOWLEDGMENTS

The researcher would like to thank the General Director of Research and Development at the Ministry of Research, Technology and Higher Education through the Higher Education Excellence Research Grant (PTUPT) through the Institute for Research and Community Services (LPPM) at the IPB University with a contract number (3/E1/KP.PTNBH/2019) and to Padjajaran University through the 2019 Lecturer Competency Grant Research (Hikom). Thank you to the Regional Technical Implementation Unit of the Center for Artificial Insemination and Artificial Insemination for Beef Cattle Development (UPTD BPPIB) at Ciamis, West Java, for providing and granting permission to use Pasundan cattle in this research. The author also thanks PT Karindo Alkesteron for providing the ultrasound device (Sonodop S-3X) used to take images in this study.

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Molecular Profile of *Trichophyton mentagrophytes* and *Microsporum canis* based on PCR-RFLP of Internal Transcribed Spacer

Endrawati D, Kusumaningtyas E

Indonesian Research Institute for Veterinary Science, Jl RE Martadinata 30 Bogor, Indonesia
E-mail: enikusuma@yahoo.com

(received 11-08-2020; revised 04-02-2021; accepted 05-01-2021)

ABSTRAK

Endrawati D, Kusumaningtys E. 2020. Profil molekuler *Trichophyton mentagrophytes* dan *Microsporum canis* berdasarkan PCR-RFLP dari *internal transcribed spacer*. JITV 26(1): 10-21. DOI: http://dx.doi.org/10.14334/jitv.v26i1.2546.

Trichophyton mentagrophytes dan *Microsporum canis* merupakan kapang dermatofit yang biasa menginfeksi hewan maupun manusia. Metode konvensional dan molekuler telah digunakan untuk mengidentifikasi kapang tersebut. Daerah *internal transcribed spacer* (ITS) mempunyai peluang besar untuk digunakan sebagai dasar dalam mengidentifikasi fungi. PCR-RFLP dilaporkan sebagai metode yang berguna untuk membedakan kapang dermatofit. Tujuan dari penelitian ini adalah membandingkan profil molekuler *T. mentagrophytes* dan *M. canis* berdasarkan hasil digesti fragmen ITS dengan enzim restriksi Dde I, Hinf I dan Mva I. Kapang diisolasi dari kerokan kulit 18 ekor hewan yang menunjukkan lesi dermatofitosis. Kapang ditumbuhkan pada media agar selama 14 hari pada suhu 37^{o}C kemudian diidentifikasi secara morfologi makro dan mikroskopik. Amplifikasi gen c*hitin synthase* digunakan untuk mengkonfirmasi dan memisahkan kapang dermatofit dari kapang-kapang yang lain. Fragmen ITS diamplifikasi dan selanjutnya dipotong menggunakan enzim restriksi Dde I, Hinf I dan Mva I. Hasil menunjukkan bahwa produk digesti fragmen ITS dari *T. mentagrophytes* dan *M. canis* berbeda. Fragmen 159 bp dari Dde I, 374 bp dari Hinf I dan 89 bp dari Mva I ada pada *T. mentagrophytes* tetapi tidak ditemukan di *M. canis*. Berdasarkan hasil tersebut, profil spesifik RFLP dari digesti daerah ITS oleh Dde I, Hinf I dan Mva I dapat digunakan sebagai *marker* spesifik untuk spesies dari fungi dermatofit.

Kata Kunci: Dermatofit, *Internal transcribed spacer*, PCR-RFLP

ABSTRACT

Endrawati D, Kusumaningtys E. 2020. Molecular profile of *Trichophyton mentagrophytes* and *Microsporum canis* based on PCR-RFLP of internal transcribed spacer. JITV 26(1): 10-21. DOI: http://dx.doi.org/10.14334/jitv.v26i1.2546.

Trichophyton mentagrophytes and *Microsporum canis* are dermatophytes fungi which commonly infect animal and human. Conventional and molecular methods were used for identification of the fungus. The region of internal transcribed spacer (ITS) has a high probability for fungal identification. PCR-RFLP was reported as a useful method to differentiate dermatophytes fungi. The objective of the study was to compare molecular profile of *T. mentagrophytes* and *M. canis* based on the result of ITS fragment digestion using Dde I, Hinf I and Mva I. The molds were isolated from skin scrapping of 18 animals which showed dermatophytosis lesion. The isolated molds were grown on agar plate for 14 days of incubation at 37^{o}C and then identified based on macro and microscopic morphologies. Amplification of chitin synthase gene was used for confirmation and separation of dermatophytes from other fungi. ITS fragment was amplified and then digested using restriction enzymes Dde I, Hinf I and Mva I. The result showed that digestion products from ITS fragment of *T. mentagrophytes* and *M. canis* were different. The fragment 159 bp from Dde I, 374 bp from Hinf I and 89 bp from Mva I were present in *T. mentagrophytes* but absent in *M. canis*. Based on these results, specific RFLP profile of digestion ITS region by Dde I, Hinf I and Mva I can be used as a specific marker for species of dermatophytes fungi.

Key Words: Dermatophytes, Internal transcribed spacer, PCR RFLP

INTRODUCTION

Dermatophytes are the fungus commonly invading *stratum corneum* of epidermis and keratinized tissues such as skin nails and hair of humans and animals. Cats and dogs are natural hosts which most infected by the fungus (Pasquetti et al. 2017). The fungus is commonly transmitted to human and cause *tinea capitis* and *tinea corporis* (Brillowska-Dabrowska et al. 2013). As dermatophytes transmitted from animal to animal, from animal to human and from human to human, identification and differentiation of the related species is important from an epidemiological point of view (Rezaei-Matehkolaei et al. 2012).

In the conventional identification methods, long incubation (7-14) is needed for characteristic traits to appear making the fungi difficult to be identified. Microscopic examination is limited because of the absence of macro or microconidia and the production of hyphae with prominent cross-walls. Identification was

difficult because of similarities among colonies of variant *Microsporum canis* (Rezaei-Matehkolaei et al. 2012). In addition, clinical isolates with similar geographical conditions of nature may show different phenotypes making identification even more complicated (Brillowska-Dabrowska et al. 2013; Katiraee et al. 2016).

A variety of molecular techniques, such as polymerase chain reaction (PCR) need to be considered. Other methods, such as mitochondrial DNA restriction fragment length polymorphism (RFLP) pattern and Chitin synthase 1 nucleotide sequence analysis has reported an as simple, fast and accurate method for identification (Jung et al. 2014). This research used ITS primers (ITS1 and ITS4) because the primers are universal and allow selective amplification of fungal sequences. ITS region is in a ribosomal cistron. The nuclear rRNA cistron has been used for fungal diagnostic and phylogenetics for more than 20 years (Begerow et al. (2010). The eukaryotic rRNA cistron consists of the 18S, 5.8S and 28S rRNA genes transcribed as a unit RNA polymerase I. Posttranscriptional processes split the cistron, removing two internal transcribed spacers. These spacers are the 5.8S which is referred as internal transcribed spacer (ITS) and the 18S nuclear ribosomal small subunit rRNA gene (SSU).

The ITS region has the highest probability of accurate identification for fungi. ITS was referred to a candidate of fungal barcode (Schoch et al. 2012). However, amplification of the internal transcribed spacer (ITS) region representing organism diversity was still unsatisfactory as the sequences of *T mentagrophytes*, *T tonsurans*, *T rubrum* and *Microsporum gypseum* are very similar (Jung et al. 2014). PCR-RFLP of ITS fragment is a method that combines PCR and enzymatic digestion of the PCR products. The method was reported to be a rapid and accurate technique for fungal identification by generating band patterns on agarose gel electrophoresis, which takes only 5 hours to be carried out (Mohammadi et al. 2015). ITS PCR and RFLP have also been used for differentiation of brewing yeast and brewery wild yeast contaminant (Pham et al. 2011). Mirzahoseini et al. (2009) reported that PCR-RFLP was a reliable tool to identify dermatophytes from a clinical specimen.

Application of the Mva I and the Dde I restriction enzyme to the ITS amplicon resulting good, stable and reproducible in the identification of the dermatophytes species (Elavarashi et al. 2013). Previously, it used one or two restriction enzymes to compare molecular profile of dermatophytes fungi. This research used three enzymes to produce fragments of profile from digestion products. It was hoped that application of more enzymes produces more specific molecular profile. In addition, the data would provide information about the most suitable enzyme which used for species identification. Therefore, differentiation among dermatophytes species are more accurate. As dermatophytes fungi infect human and animals such as pets, wild and livestock, the samples were taken from cat and dog which represent pet animals and cattle which represent livestock. This research was conducted to compare molecular profile of *Microsporum canis* and *Trichophyton mentagrophytes* based on the result of ITS fragment digestion using Dde I, Hinf I and Mva I. *T. mentagrophytes* and *M. canis* produced different digestion product which can be used to distinguished both species

MATERIALS AND METHODS

Clinical isolate

Scrapping skin sample was collected from infected cat and dog patients which came to animal hospital around Bogor, Jakarta and Sukabumi, Indonesia. The scrap was inoculated in Sabouraud dextrose agar (SDA) with chloramphenicol 0.05 mg/mL and cycloheximide 0.5 mg/mL (Pal & Dave 2013), to inhibit bacteria and spreading mold. The plates were incubated at 37^{o}C for 7-14 days. Dermatophytes fungi were purified by picking selected single colony and inoculated in new agar plate.

Conventional identification

The fungus was identified by colonies observation and microscopic direct examination using KOH 10%. The scraping skin was put in object-glass, KOH dropped in surface, press using cover glass. Fixation was done by trough the glass up the flame. Microscopic morphology was examined under microscope. Identification was performed based on mycelia and conidia form.

DNA extraction

DNA extraction was conducted according to White et al. (1990) with some modification. Mycelium of dermatophyte fungi was placed into microtube 1.5 mL. Two grams of mycelia were ground using micro pestle to form small particles. Amount of 500 µL sodium deodecyl sulphate (SDS) was added, then incubated at 65^{o}C for 30 minutes. The mixture was let until cold, added with 500 µl chloroform isoamyl (CI 24:1) and centrifuged at 10.000 *x g* for 20 minutes. Supernatant was placed into a new tube and 500 µL phenol-chloroform isoamyl (PCI 25:24:1) was added and centrifuged at 10.000 *x g* for 10 minutes. The supernatant was placed into new tube and 100 µl Na acetate 2 M (pH 5,2) and 500 µl ethanol 100% were

added. The mixture was frozen at -20°C for 8 hours and then centrifuged at 10,000 *x g* for 30 minutes. Supernatant was discarded and pellet was dried using vacuum concentrate plus (Eppendorf) for 30 minutes. The dried pellet was added with *nuclease free water* and 5 µL RNase then incubated at 37°C for 10 minutes continued with additional incubation at 70°C for 10 minutes (for RNAse inactivation). Purity and percentage of DNA were measured using NanoDrop spectrophotometer at λ 260/280.

Polymerase chain reaction and electrophoresis

Primer *Chytin Synthase* (*CHS1*: forward 5′-GAA GAA GAT TGT CGT TTG CAT CGT CTC-3′ dan reverse 5′-CTC GAG GTC AAA AGC ACG CCA GAG-3′) (Putty *et al*. 2018) were used to amplify dermatophytes specific sequence gene from mold. Primer ITS 1 (forward: (5′-TCC GTA GGT GAA CCT GCG G-3′) and ITS 4 (reverse: 5′-TCC TCC GCT TAT TGA TAT GC-3′) were used to amplify ribosomal DNA (rDNA) and produced 600– 700 bp (Zhang et al. 2011; Tartor et al. 2016). Amplification was performed using HotStarTaq@ Master Mix Kit (Invitrogen). Total volume was 50 µl (25 µl HotStarTaq@ Master Mix, 1 µl for each primer (10 µM), 10 µl platinum™ GC enhancer, 10 µl DNA template (10 ng) and Nuclease free water until 50 µl). Polymerase chain reaction (PCR) process was conducted in pre denaturation at 95°C, 5 minutes, denaturation 95°C for 3 seconds, annealing 56°C for 60 seconds, extension 3 seconds, followed by final extension 72°C for 5 minutes. The PCR product was kept at -20°C until used. Electrophoresis for PCR product was performed using agarose 1,5 % and SYBR™ safe staining, run at voltage 100 Volt. The bands were visualized using UV transilluminator.

DNA sequencing and analysis

PCR products from amplification of ITS region were sequenced and identified. The PCR product was sent to First Base Laboratories Sdn Bhd All Right Reserved, Selangor, Malaysia for sequencing. DNA sequences were analyzed using Bioedit and Mega-X and aligned with Gene Bank database using BLAST program (www.ncbi.nlm.nih.gov) and clustalw2 (https://www.ebi.ac.uk/Tools/msa/clustalo/). Open Reading Frame was determined using https://www.ncbi.nlm.nih.gov/orffinder/

Polymerase Chain Reaction-Restriction fragment length polymorphism (PCR-RFLP)

Restriction fragment length polymorphism (RFLPs) for PCR products were performed following Mohammadi et al. (2015) using enzymes Dde I, Hinf I and Mva I (Thermo Fisher Scientific Inc). The procedure for enzymes treatment was conducted according to the protocol of each enzyme from the company. Ten µl of ITS PCR product were mixed with 18 µl nuclease-free water (NFW), 2 µl 10x Tango buffer (composed by 33 mM Tris-acetate pH 7.9, 10 mM magnesium acetate, 66 mM potassium acetate, 0.1 mg/mL BSA) and 1 µl Dde I (10U/µL) (Thermo Fisher Scientific Inc). The mixture was incubated at 37°C for 1 hours. The reaction was stopped by incubation in 65°C water bath for 20 minutes. For Hinf I, ITS PCR product 10 µl, was mixed with 17 µl NFW, 2 µl 10x green buffer and 1 µl Hinf I (Thermo Fisher Scientific Inc) then incubated at 37°C for 5 minutes. The reaction was stopped by incubation in 65°C water bath for 20 minutes. For Mva I, ITS PCR product 10 µl, was mixed with 17 µl NFW, 2 µl 10x green buffer and 1 µl Mva I (Thermo Fisher Scientific Inc) then incubated at 37°C for 5 minutes. The reaction was stopped by incubation in 65°C water bath for 20 minutes. All digestion products were stored at -20°C until used. Electrophoresis for PCR digested product was performed using agarose 1.5 % and SYBR™ safe staining, run at voltage 100 Volt. The bands were visualized using UV transilluminator.

RESULTS AND DISCUSSION

Isolation of dermatophytes fungi

Dermatophytes fungi were isolated from cat, dog and cattle which came to animal hospital, pet clinics and animal husbandry around Jakarta, Bogor and Sukabumi city. There was a total of 18 patients which showed clinical signs of dermatophytosis such as itchy, red, scaly, circular rush and some hair loss as showed in Figure 1. The fungi infect certain organs or even around the body.

Table 1 provides information regarding the animal and the organ which had suspected dermatophytosis in this study. The patients were dominated by cats. It may due to less dog population compare to cat. Besides cats and dogs, dermatophytes such as *Microsporum canis* and *Trichophyton mentagrophytes* were also infected calves (Pal & Dave 2013). In this research, only one from 100 examined cattle were infected. Intensive husbandry with good sanitation reduced the possibility to be infected by the dermatophytes fungi. As shown in table 1, there was no organ or breeds preference. Age ≤ 12 months more frequently infected by the fungi. Aneke et al. (2018) reported that in dogs and cats, male and young individuals develop more frequently clinical lesions. Ilhan et al. (2016) found no significant association between genders in cats. The most likely risk factor for dermatophytes infection were seasons and age of animals. Winter and spring are the

(a) **(b)**

Figure 1. Dermatophytosis lesi detected in cat (a) and dog (b)

Tabel 1. Frequency of clinical sign of *dermatophytosis* based on the animal, age, infected organ and breeds

No	Animal	Breeds	Age (month)	Infected organ	Origin of sample
1	Cat	Local	36	back	Sindangbarang, Bogor
2	Cat	Local	24	Back, neck	Sindangbarang, Bogor
3	Cat	Local	24	almost all body	Loji, Bogor
4	Cat	Persia	24	tail, neck	Darmaga, Bogor
5	Cat	Local	4	tail	Sindangbarang, Bogor
6	Cat	Local	18	tail base	Darmaga, Bogor
7	Cat	Persia	9	abdomen, elbow, tail	Pasirkuda, Bogor
8	Dog	Golden retreiver	>24	back, neck	Pasirkuda, Bogor
9	Cat	Local	12	neck, tail	Gunungbatu, Bogor
10	Dog	Local	8	tail	Gunungbatu, Bogor
11	Cat	Local	12	tail	Ciomas, Bogor
12	Cat	Local	12	abdomen	Darmaga, Bogor
13	Cat	Local	12	neck	Sindangbarang, Bogor
14	Cat	Local	12	tail base	Darmaga, Bogor
15	Cat	Local	6	neck, head	Sindangbarang, Bogor
16	Cattle	FH	±15	Face, neck, leg	Sukabumi
17	Cat	Local	18	Tail	Animal hospital, Jakarta
18	Cat	Local	24	Head	Animal hospital, Jakarta

seasons when cases of dermatophytes were higher. *Microsporum canis* is the most common dermatophyte in cat (90-100%) globally (Torres-Guerrero et al. 2016).

Table 1 was only performed on the animal which showed the clinical sign of dermatophytoses, but the fungi had not been yet identified. In some cases, the sign leads to dermatophytoses, but the dermatophytes fungi failed to be isolated in culture and not detected in native preparation or molecular identification. The scrapping of infected skins was then observed under a microscope using KOH 10% and some were inoculated in agar medium. Colonies and microscopic of dermatophytes fungi are shown in Fig 2. Identification was performed based on the macro and microscopic morphology and confirmed by molecular identification.

As shown in Figure 2, colony of *Microsporum canis* is coarsely fluffy, furrier on top and darker in the underside of the growth medium than that of

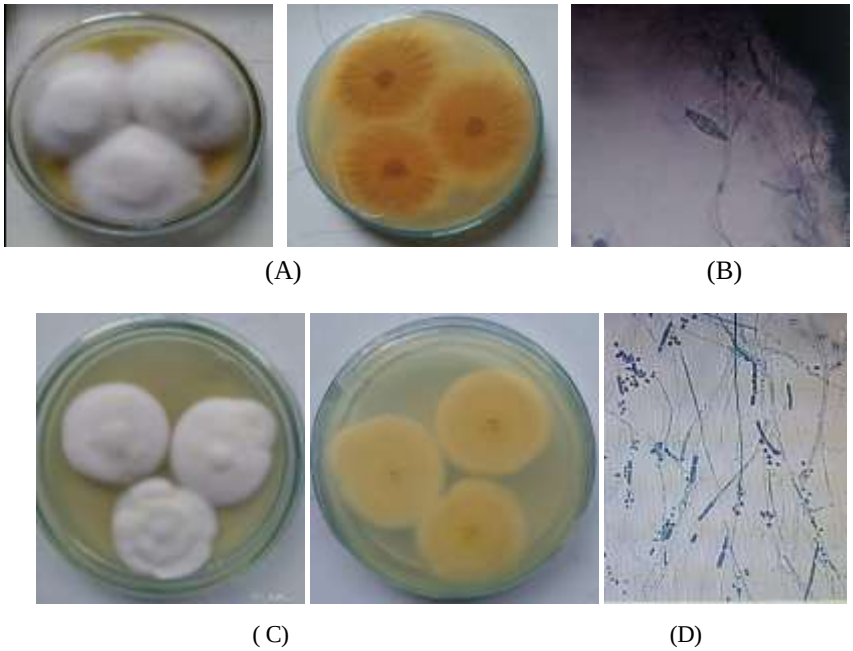

Figure 2. Colonies and microscopic morphologies of *Microsporum canis* (A, B) and *Trichophyton mentagrophytes* (C, D) at 37^{o}C for 14 days incubation

Trichopyton mentagrophytes. The dark yellow pigment was absent in some strains of *M canis due* to failure to develop macroconidia and retardation of colony growth. Macroconidia divided into compartments which are separated by coss-wall. Microconidia *M. canis* also resemble other dermatophytes therefore it is not useful for diagnostic or identification

Spora of *Trichophyton mentagrophytes* was more abundant therefore easily recognized. On the contrary for *Microsporum canis*, even with prolonged 14 days incubation, the conidia were still hardly present. As consequence, molecular identification is a necessity.

In this research, internal transcribed spacer and chitin synthase were amplified for fungal identification and characterization. According to Cafarchia et al. (2013), the first and second internal transcribed spacers (ITS1 and ITS2) of nuclear ribosomal DNA and the part the *chitin cynthase* gene (*p*chs1) have shown promise as markers for specific identification of dermatophytes.

Internal Transcribed Spacer (ITS)

ITS primers were used for amplification DNA region specific for fungi. The ITS bands were detected in fungi such as *Candida sp*., *Fusarium sp*. and dermatophytes but absent in bacteria (Elavarashi et al. 2013). Therefore, the ITS amplification products can be used as a fungal marker. This research use primers ITS 1 and ITS 4 to amplify both of marker specific and conserved sequence. These primer pairs are universal primers and are commonly used for fungal molecular diagnostic and identification (Ferrer et al. 2001; Aala 2012). The length sizes were various among genus and species. Amplification of the region using primer ITS 1 and ITS 4 in some dermatophytes from the previous research indicated that the region was conserved among dermatophytes fungi. PCR product using ITS1 and ITS4 primers is shown in Figure 3. PCR products were then sequenced for identification.

As shown in Figure 3 some of the fungi sequenced identified as dermatophytes fungi. One isolate was identified as *Trichophyton mentagrophytes* (Tm) and 7 isolates were *Microsporum canis* (Mc). However, not all fungi isolated from skin sent for sequencing. Mostly the fungi which had been confirmed as dermatophytes as the chitin synthase amplicon was detected (Figure 3 and 4), or the fungi which genus identified from macro and microscopic morphology.

PCR product using ITS 1 and ITS 4 primer was reported producing 690-720 bp for *T. mentagrophytes* and *M. canis* (Abdel-Fatah et al. 2013). ITS amplicon of *Microsporum* genus was also reported varied in size from ~851 bp in *Microsporum gypseum* to ~922 bp in *Microsporum canis* and ITS region of *M. canis* being ~50 bp longer than that of other dermatophytes (Cafarchia et al. 2013).

In this research, both *T. mentagrophytes* and *M. canis* produced 686-739 bp. Using the same primers pairs, the *M. canis* amplicon shorter than that reported by Zhang et al. (2019) (760 bp). Confirmable result was reported by Dhieb et al. (2014), 700bp. Elavarashi et al. (2013) reported that *T. mentagrophytes* ATCC 9533

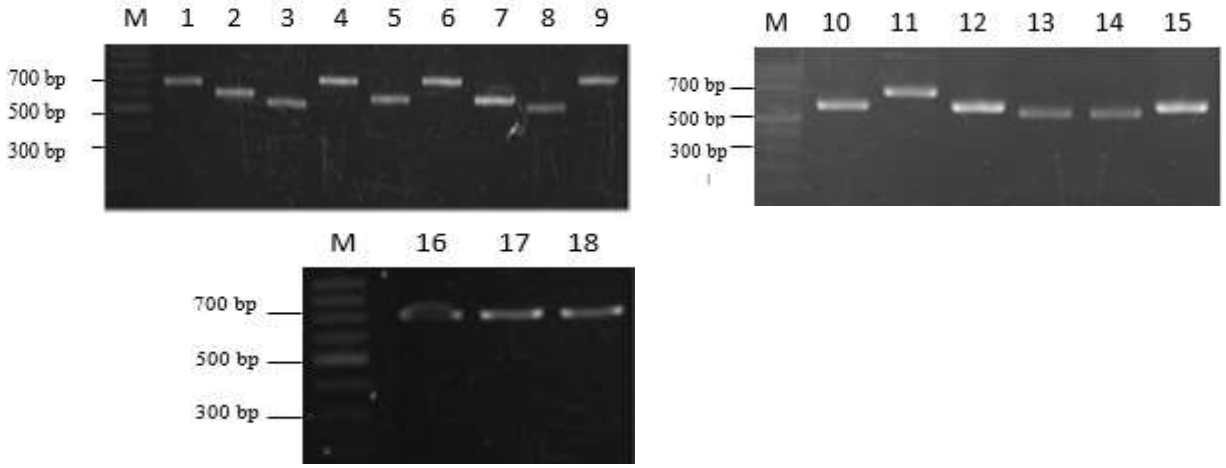

Figure 3. Internal transcribed spacer (ITS) gene of fungi isolated from skin scrapping. Dermatophytes fungi M: molecular mass DNA marker, 1, 4, 6, 9, 11, 17, 18 : *Micropsorum canis* (650 bp) and 16: *Trichopyton mentagrophytes* (650bp), 2, 3, 5, 7, 8, 10, 12, 13, 14, 15: other fungi which was isolated from skin scrapping samples.

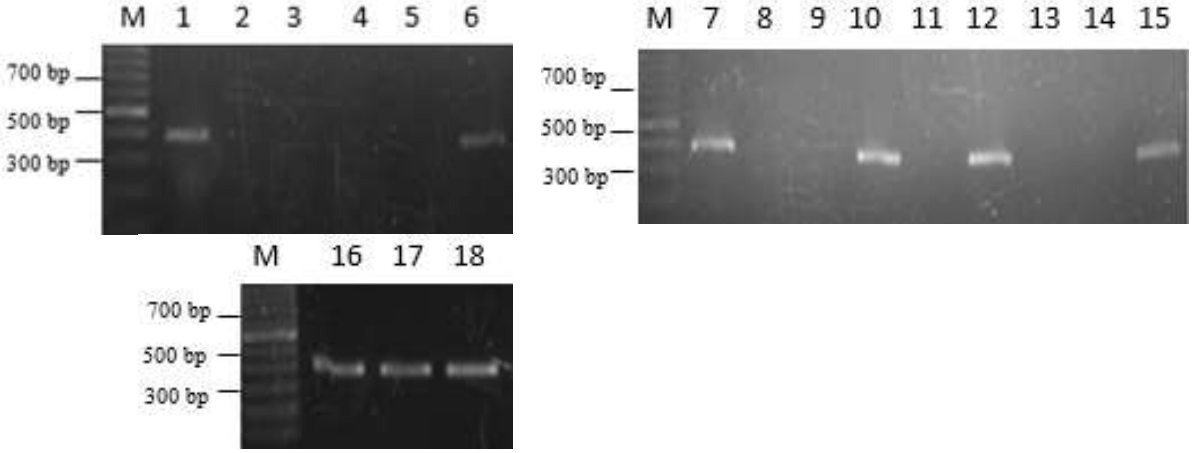

Figure 4. PCR products of chitin synthase *1* gene of fungi isolated from skin scraping. Dermatophytes fungi produced chitin synthase I band (350-400 bp) for *Microsporum canis* [6 (400bp), 7 (400 bp), 10 (350bp), 11 (350 bp), 12 (350bp), 17 (400 bp), 18 (400)] and *Trichophyton mentagrophytes* [1, 16 (400bp)]. M: marker. The bands were absent in non-dermatophytes fungi

produced almost similar amplicon, 690 bp. ITS fragments, produced by non-dermatophytes fungi (Table 2) such as *Aspergillus niger* (600bp), *Aspergillus bridgeri* (600 bp), yeast (around 500bp) and *Chaetomium pachypodiodes* (around 500 bp), were shorter than that of dermatophytes fungi. A similar result was reported by (Elavarashi et al. 2013) who revealed that ITS 1 and ITS 4 pairs primer produced around 550-600 bp PCR products in *Candida sp* and *Fusarium sp*. This result showed that PCR products of ITS 1 and ITS 4 pair primers can be used to distinguish dermatophytes and non-dermatophytes fungi.

Chitin synthase

Amplification of chitin synthase region was aimed to confirm that the isolate was dermatophytes fungi. The existence of chitin synthase band indicated a dermatophytes fungi. Saprophytic fungi isolated from skin scrapping did not produce this band. PCR for chitin synthase 1 gene was powerful to identify the presence of dermatophytes fungi from clinical isolate such as skin scraping and hair. Sharma et al. (2017) found 10 samples that were negative on the fungal culture but were positive for dermatophytes by PCR of chitin synthase indicating that PCR was more sensitive than culture.

Putty et al. (2018) reported that amplification of chitin synthase I gene resulting in 288 bp product size. They added that amplification of the gene was able to be considered as a rapid test for dermatophytosis to decided appropriate antifungal therapy. In this research, the same primer pairs produced longer PCR products, around 400 bp. According to Emam & Abd El-salam (2016) PCR products may be varied among the dermatophyte genus and amplicon size 288 bp was

Table 2. Similarity of dermatophytes in this research with database in GeneBank

Dermatophyte fungi in this research	Percent similarity	Acession number
Microsporum canis (Mc-1)	99.54%	*Microsporum canis* MT487816.1
	99.54%	*Microsporum canis* MT423728.1
Microsporum canis (Mc-2)	99.35%	*Microsporum canis* MT487816.1
	99.35%	*Microsporum canis* MT423728.1
Microsporum canis (Mc-3)	99.40%	*Microsporum canis* MT487816.1
	99.40%	*Microsporum canis* MT423731.1
Microsporum canis (Mc-4)	100%	*Microsporum canis* MT633048.1
	100%	*Microsporum canis* MT632638.1
Microsporum canis (Mc-5)	99.68%	*Microsporum canis* MT487816.1
	99.68%	*Microsporum canis* MT423728.1
Microsporum canis (Mc-6)	99.86%	*Microsporum canis* MT361863.1
	99.29%	*Microsporum canis* KF733019.1
Microsporum canis (Mc-7)	99.59%	*Microsporum canis* MT423731.1
	99.59%	*Microsporum canis* MT423730.1
Trichophyton mentagrophytes (Tm)	99.56%	*Trichophyton mentagrophytes* LC317435.1
	99.56%	*Trichophyton mentagrophytes* LC317440.1

found mostly in genus *Trichopyton*. However, as shown in Figure 4, both *Trichophyton mentagrophytes* and *Microsporum canis* produced amplicon around 400 bp. Hryncewicz-Gwóźdź et al. (2011) use the same primer to amplify chitin synthase gene of dermatophytes fungi. The result showed that both *Trichophyton tonsurans* and *T. mentagrophytes* produced 366 bp, almost similar to the PCR product in this research. Based on the result, amplification of the chitin synthase using primer CHS 1 was powerfull to differenciate dermatophytes and non dermatophytes but did not able to distinguish among genera within dermatophytes. This result also indicates that primer CHS 1 can be used for determination of dermatophytes fungi from clinical samples such as skin scraping from the animals suspected suffer from dermatophytosis.

Identification of dermatophytes fungi

Sequence analysis of PCR product of ITS genes showed that they were confirmed as *Trichophyton mentagrophytes* and *Microsporum canis*. The similarity percentage of both fungi with sequence database in GeneBank is more than 99% (Table 2).

Seven dermatophytes fungi were identified based on the characteristic colonies, microscopic morphologies and their nucleotide sequences of ITS PCR product. The fungi were identified as *M. canis and T. mentagrophytes*. Sequencing result of ITS 1 to ITS 4 regions of Mc1-7 showed that they had similarity almost 100% with ITS regions of *M. canis* from GeneBank. Conventional identification using macro dan microscopic morphology of *T. mentagrophytes* was also confirmed by sequencing result of the ITS region. Identification of dermatophytes and non-dermatophytes fungi isolated from cats, dogs and cattle suspected dermatophytosis as displayed in Table 3.

Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP)

For further molecular profile, PCR products of ITS fragment were then digested using restriction enzymes Dde I, Hinf I and Mva I. The enzymes cut the ITS sequence in their cleavage site. ITS sequence affects the cleavage site position resulting difference in fragment size of digestion products. The digestion product was presented at Figure 5. *T. mentagrophytes* (Tm) and *M. canis* (Mc) were digested by Dde I produced different fragments. Fragment 159 bp in Tm and 201 bp in Mc was able to differentiate both genera. *Microsporum canis* (Mc) 1-5 isolated from Bogor has a different pattern with Mc 6-7 which was isolated from Jakarta. This different pattern may represent different strains circulating between both regions, although it needs further examination to prove it.

Digestion ITS sequence using Hinf I showed that there was almost no difference pattern among *M. canis*. *T. mentagrophytes* revealed 374 bp at Hinf I and 89 bp at Mva I digestion products which were absent in

Tabel 3. Isolated fungi from skin scrapping of animal suspected dermatophytosis

No	Animal	Fungi
1.	Cat	Yeast
2.	Cat	White colony mold
3.	Cat	Yeast, *Aspergillus* sp
4.	Cat	*Aspergillus niger*
5.	Cat	Yeast
6 .	Cat	Yeast, *Microsporum canis* (Mc-1)
7.	Cat	White colony mold
8.	Dog	White colony mold, *Microsporum canis* (Mc-2)
9.	Cat	Yeast
10.	Dog	*Aspergillus bridgeri*, *Microsporum canis* (Mc-3)
11.	Cat	White colony mold
12.	Cat	Yeast, *Microsporum canis* (Mc-4)
13.	Cat	*Microsporum* (Mc-5)
14.	Cat	Yeast
15.	Cat	White colony mold
16.	Cattle	*Trichophyton mentagrophytes*
17.	Cat	*Chaetomium pachypodiodes*, *Microsporum canis* (Mc-6)
18.	Cat	*Aspergillus bridgerii*, *Microsporum canis* (Mc-7)

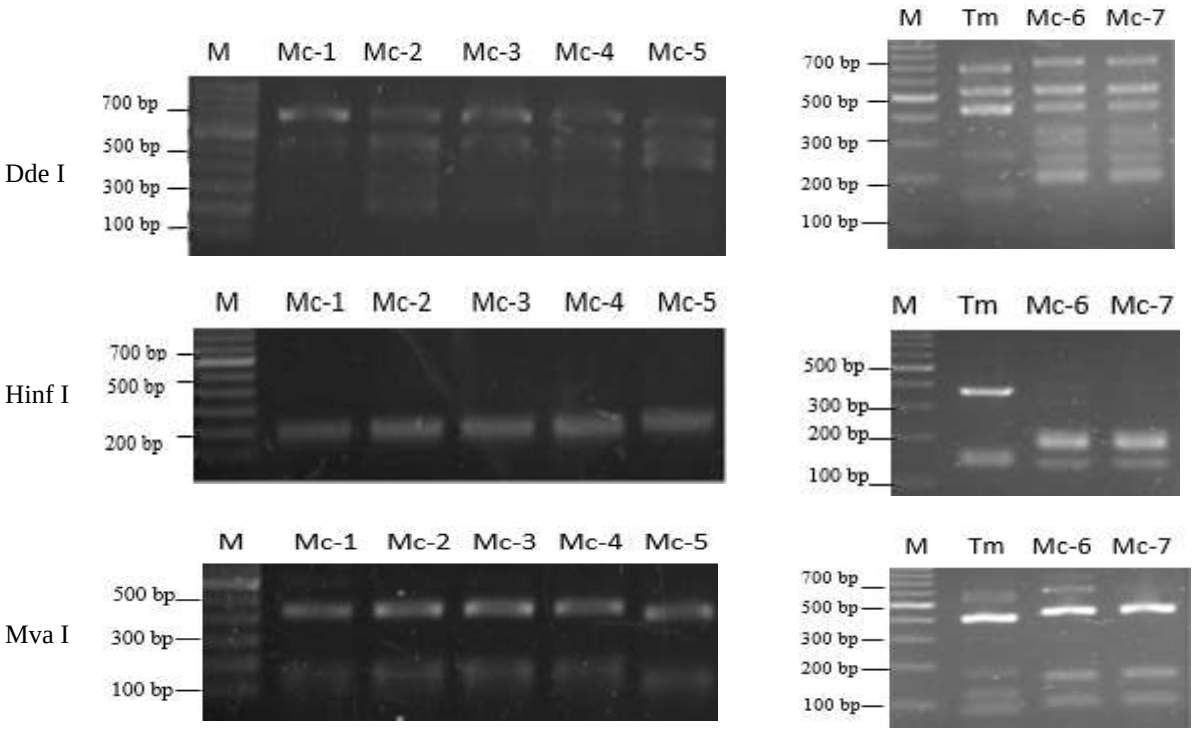

Figure 5. ITS fragment PCR product after digestion with Dde I, Hinf I and Mva I. M: molecular marker, Mc: *Microsporum canis*, Tm: *Trichophyton mentagrophytes*. Specific fragments for Tm are 159 bp at Dde I, 374 bp at Hinf I and 89 bp at Mva I digestions

Table 4. In silico analysis of ITS sequence digested using Dde I, Hinf I and Mva I restriction enzymes

Dermatophytes species	ITS size	Dde I (CTNAG) (bp)		Hinf I (GANTC) (bp)		Mva I (CCWGG) (bp)	
	(bp)	Cut site	In silico of RFLP fragments	Cut site	In silico of RFLP fragments	Cut site	In silico of RFLP fragments
T. mentagrophytes	686	159/160 260/261	**100,159,260, 425, 526**	374/375, 382/383, 540/541	7, 145, **157**, 165,303,311, **374**,382, 540	407/409, 531/533, 581/583, 595/597	12, 48, 62, **89**,103,**122**, 153,172,**186,**277 ,**407**,531,581, **595**
M. canis (Mc-1)	738	205/206, 296/297,5 42/543	90,**195**,205, 245,296,**336**, **441**,532, **542**.	213/214, 407/408, 415/416, 595/596	7, 142, 179, 187,193,**201**213, 322,330,381,407 ,415, 524, 595	440/442, 605/607, 633/635	26,103,131, **163**,191,296, **440**,605,633
M. canis (Mc-2)	738	207/208, 298/299, 544/545	90,**193**,207, 245,298,**336, 440**,530, **544**	215/216, 409/410, 417/418, 597/598	7, 142, 179, 187,193,**201,**213 ,322,330,381, 407,415, 524, 595	442/444, 607/609, 635/637	26,101,129, **163**,191,294,**442** ,607,635
M. canis (Mc-3)	738	205/206, 296/297, 542/543	90,**195**, 205, 245,296,**336, 441**,532,**542**	213/214, 407/408, 415/416, 595/596	7, 144, 179, 187,193,**201,** 213,322,330,381 ,407,415,524, 595	440/442, 605/607, 633/635	26,103,131, **163**,191,296,**440** ,605,633
M. canis (Mc-4)	739	205/206, 296/297, 542/543	89,**196**, 205, 245,295,**336, 442**,533,**542**	213/214, 407/408, 415/416, 595/596	7, 143, 179, 187,193,**201,**213 ,323,331,381, 407,415,525, 595.	440/442, 605/607, 633/635	26,104, 132, **163**,191,297,**440** ,605,633
M. canis (Mc-5	739	207/208, 298/299, 543/544	90,**195**,207, 244,298**,335, 440**,531,**543**	215/216, 338/339, 408/409, 416/417, 596/597	7, 69, 77, 122,142,179,187 ,192,200,**215**,25 7,322,330,338, 380,400,408,416 523, 596	441/443, 606/608, 634/636	26,**103**, 131, 163,191,296,**441** ,606,634
M. canis (Mc-6)	738	205/206, 296/297, 542/543	90,195, **205**, 245,**296**,336, **441**,**532**,542	213/214, 407/408, 415/416, 594/595	7, **143**, 178, 186,193,**201,**213 ,322,330,380, 407,415,524, 594	440/442, 604/606, 632/634	26,**104**, 132, **162**,190,296,**440** ,**604**,632
M. canis (Mc-7)	738	206/207, 297/298, 543/544	90,194, **206**, **245**,**297**,336, **440**, **531**	214/215, 408/409, 416/417, 596/597	7, **141**, 179, 187,**201**,214,193 ,321,329,381, 408,416, 523, 596	441/443, 606/608, 634/636	26,**102**, 130, **163**,191,295,**441** ,606,634

ITS size was determined from sequence between 5' forward and 3' reverse primer annealing position in this study. Bold: the fragments present in electrophoresis gel

M. canis. Therefore, 374 bp Hinf I and 89 bp Mva I digestion product of ITS were as a marker for *T. mentagrophytes* which can be used to distinguish it from *M. canis*. The different pattern between *M. canis* from Bogor and Jakarta was also showed by digestion using Mva I. *M. canis* isolated from Jakarta (Mc 6,7) produce more bands compared to that from Bogor. The pattern of *M. canis* isolated from Jakarta was almost similar to what was reported by Rezaei-Matehkolaei et al. (2012) (Table 5).

As shown in Table 4 and 5, not all fragments in silico analysis present on an electrophoresis gel. The absent fragments concentration might be very low, and they were not detected in electrophoresis gel. It was also still leaving uncut fragments 686-738 bp in Dde I digestion which may be caused by the condition or

Table 5. Size of PCR and RFLP products of dermatophytes fungi present on electrophoresis gel

Dermatophytes species	PCR product	ITS fragments after digested by enzyme (bp)		
	ITS	Dde I	Hinf I	Mva I
T. mentagrophytes	686	159, 260, 425, 526, 686	157, 374	89, 122, 186, 407, 595
M. canis (Mc-1)	738	195, 336, 441, 542, 738	201	163, 440
M. canis (Mc-2)	738	193, 336, 440, 544, 738	201	162, 442
M. canis (Mc-3)	738	195, 336, 441, 542, 738	201	163, 440
M. canis (Mc-4)	739	196, 336, 442, 542, 739	201	163, 440
M. canis (Mc-5	739	195, 335, 440, 543, 739	215	103, 441
M. canis (Mc-6)	738	205, 245, 296, 441, 532, 738	143, 201	104, 162, 440, 604
M. canis (Mc-7)	738	206, 245, 297, 440, 531, 738	141, 201	102, 163, 441
T. mentagrophytes (Abdel-Fatah et al 2013)	680	240, 200, 190, 90	375, 158, 81, 65, 8	-
M. canis (Dhieb et al 2014)	700	-	140, 200, 240, 260	-
M. canis (Rezaei-Matehkolaei et al 2011)	737	-	-	441, 165, 103, 28
M. canis (Didehdar et al 2016)	737	-	-	441, 165, 103
M.canis (Abdel-Fatah et al 2013)	720	No cutting pattern	-	-

digestion time was not optimum. Ratio enzyme and DNA might not suitable as the concentration of PCR product was not measured. As in enzyme protocol mentioned the reaction is placed 1-16 hours. It is possible that the digestion processes needed further optimization. In another case, Abdel-Fatah B, et al. (2013) also reported that no cutting pattern in ITS fragments digested by Dde I.

As shown in Table 3, 4, 5 and Figure 5, digestion products from Dde I, Hinf I and Mva I has similar RFLP profile among *M. canis* from cats and dogs. The digestion profile from the three enzymes was able to differentiate between *T. mentagrophytes* and *M. canis* and also *M. canis* from Jakarta and Bogor. Based on these results, it is possible that specific RFLP profile of digestion ITS region using Dde I, Hinf I and Mva I are used as a specific marker, especially to distinguish among species and strain. However, it still needs further research to compare more samples isolated from more region. Recently, PCR RFLP is commonly used to generate species-specific DNA which used for dermatophytes identification (Mohammadi et al. 2015). Amplification of ITS region and digestion using Mva I and Dde I was also reported equally good for RFLP analysis and identification of dermatophytes directly from clinical material (Elavarashi et al. 2013).

CONCLUSION

Molecular profil from PCR_RFLP using Dde I, Hinf I and Mva I was different between *Trichophyton mentagrophytes* and *Microsporum canis.* Digestion product 159 bp from Dde I, 374 bp from Hinf I and 89 bp from Mva I were present in *T. mentagrophytes* but absent in *M. canis*. Based on these differences, it is possible that specific RFLP profile of digestion ITS region using Dde I, Hinf I and Mva I are used as a specific marker to differentiate among species, especially between *T. mentagrophytes* and *M. canis* local isolate from Indonesia.

ACKNOWLEDGEMENT

This work was supported by Grant from the Indonesian Agency for Agricultural Research and Development, Ministry of Agriculture APBN 2019 No 1806.202.052.B.

AUTHOR CONTRIBUTIONS

Endrawati D and Kusumaningtyas E had full access to all data in this study and contributed equally to this work.

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Pathogenicity of Philippine and Indonesian *Trypanosoma evansi* Isolates in Mice and Their Responses to Trypanocides

Dargantes AP[1,2], Wardhana AH[3,4], Abella JAC[1], Sequito MR[1], Reid SA[2], Copeman DB[5](deceased), Dargantes KAT[1]

[1]*College of Veterinary Medicine, Central Mindanao University, Musuan 8710, Bukidnon, Philippines*
[2]*School of Veterinary and Biomedical Sciences, Murdoch University, South Street, Murdoch, Western Australia 6150, Australia*
[3]*Department of Parasitology, Indonesian Research Centre for Veterinary Science (IRCVS), Bogor 16114, Indonesia*
[4]*Department of Veterinary Parasitology, Faculty of Veterinary Medicine, Airlangga Univdersity, Surabaya, 60115, Indonesia*
[5]*Australian Institute of Tropical Veterinary and Animal Science, James Cook University, Townsville 4811, Queensland, Australia*
E-mail: alanpdargantes@gmail.com

(received 15-05-2020; revised 18-01-2021; accepted 22-01-2021)

ABSTRAK

Dargantes AP, Wardhana AH, Abella JAC, Sequito MR, Reid SA, Copeman DB, Dargantes KAT. 2020. Patogenitas isolat *Trypanosoma evansi* asal Philippina dan Indonesia pada mencit serta responnya terhadap trypanosidal. JITV 26(1): 22-30. DOI: http://dx.doi.org/10.14334.jitv.v26i1.2508.

Patogenitas sepuluh isolat *Trypanosoma evansi* asal Filipina (Mindanao) dan satu isolat asal Indonesia (Jawa Timur) dipelajari dan dibandingkan. Disamping itu, studi ini juga menguji kepekaan isolat-isolat tersebut terhadap beberapa trypanosidal, yaitu diminazene aceturate, melarsomine dihydrochloride, suramin and quinapyramine sulphate/chloride. Sebanyak 25 mencit diinfeksi secara interperitoneal dengan masing-masing isolat dan 20 mencit diberi perlakuan dengan 4 obat (5 mencit/obat), sementara itu kelompok yang lain terdiri dari 5 ekor per isolat sebagai kelompok kontrol diinfeksi tanpa pengobatan dan 7 ekor per isolat sebagai kelompok yang tidak diinfeksi. Pengobatan dilakukan pada 24 jam pasca infeksi dan parasitemia diamati setiap hari selama 35 hari. Mencit yang diinfeksi dengan isolat Filipina secara nyata mengalami kematian lebih awal (hari ke 5 -11) dibandingkan dengan isolat Indonesia (hari ke 14 -16). Periode prepaten isolat Filipina juga secara nyata lebih pendek (3 – 8 hari) daripada isolat Indonesia (11 – 13 hari). Trypanosoma tidak terdeteksi di dalam darah mencit yang diinfeksi isolat Filipina setelah diobati dengan quinapyramine sulphate/chloride, melarsomine dihydrochloride atau suramin. Dua dari sepuluh isolat baik yang diinfeksi dengan isolat Filipina (C4 atau A9) yang kemudian diobati dengan diminazene aceturate masih menunjukkan parasitemia pada hari 29 dan 31. Disimpulkan bahwa isolat *T. evansi* dari Mindanao, Filipina lebih patogen dibandingkan dengan isolat dari Jawa Timur, Indonesia. Quinapyramine sulphate/chloride, melarsomine dihydrochloride dan suramin efektif terhadap *T. evansi* yang diteliti. Informasi ini sangat penting untuk meningkatkan strategi pengendalian Surra di Filipina dan Indonesia.

Kata Kunci: Jawa Timur, Mindanao, Patogenitas, Resisten, *Trypanosoma evansi*

ABSTRACT

Dargantes AP, Wardhana AH, Abella JAC, Sequito MR, Reid SA, Copeman DB, Dargantes KAT. 2020. Pathogenicity of Philippine and Indonesian *Trypanosoma evansi* isolates in mice and their responses to trypanocides. JITV 26(1): 22-30. DOI: http://dx.doi.org/10.14334.jitv.v26i1.2508.

Pathogenicity of 10 isolates of *T. evansi* collected from Mindanao, Philippines, and one isolate from East Java, Indonesia was determined and compared. The susceptibility of these isolates against diminazene aceturate, melarsomine dihydrochloride, suramin and quinapyramine sulphate/chloride was also tested. Twenty-five mice were infected intraperitoneally with each isolate and 20 were treated with the 4 drugs (5 mice/drug) while 5 infected and 7 uninfected mice served as infected-untreated and uninfected controls, respectively. Treatment was carried out 24 hours post-infection and parasitemia was monitored for 35 days. Mice infected with Philippine isolates significantly died earlier (5-11 days) than those infected with the Indonesian isolate (14-16 days). The prepatent period for Philippine isolates (3-8 days) was significantly shorter than the Indonesian strain (11-13 days). Trypanosomes were not observed in the blood of mice infected with any of the Philippine isolates when treated with quinapyramine sulphate/chloride, melarsomine dihydrochloride or suramin. Two of 10 mice infected with either C4 or A9 Philippine isolates and treated with diminazene aceturate had parasitemia on days 29 and 31, respectively. It is concluded that isolates of *T. evansi* from Mindanao, Philippines, are more pathogenic than the isolate from East Java, Indonesia. This study also indicated that quinapyramine sulphate/chloride, melarsomine dihydrochloride and suramin are effective against these *T. evansi* isolates from Mindanao, Philippines and East Java, Indonesia, while two of the Mindanao isolates are resistant to diminazene. This information is valuable in the enhancement of the control strategy against surra in the Philippines and Indonesia.

Key Words: East Java, Mindanao, Pathogenicity, Resistance, *Trypanosoma evansi*

INTRODUCTION

Trypanosomosis (Surra) caused by a protozoan parasite, *Trypanosoma evansi*, remains a major problem in livestock and wild animals. The parasite is widely distributed in Central and South America, Africa and South-east Asia including the Philippines and Indonesia (Boushaki et al. 2019). In some countries, the parasite has been also reported to infect humans (zoonotic) (Desquesnes et al. 2013; Chau et al. 2016; Wardhana & Sawitri 2018). Dewi et al. (2020) reported that the economic losses of surra outbreak in East Sumba of Indonesia were 25.7 billion rupiahs during 3 years (2010 – 2012) while in Mindanao, Philippines, a village with moderate to high surra in its livestock loses about US$158,000 annually (2.3 billion rupiahs) (Dobson et al. 2009).

Severe outbreaks of surra in the Philippines were reported in South Cotabato and Agusan del Sur, Mindanao between 1989-1994 (McInnes et al. 2012). The disease spread to other provinces in the following years. The strain of *T. evansi* causing the outbreaks was suspected to be highly virulent, previously not present in the country (Lazaro et al. 2019). In Indonesia, *T. evansi* was first identified from an outbreak of the disease amongst horses in Semarang in 1897 (Nuryady et al. 2019). Furthermore, some outbreaks occurred subsequently in cattle and water buffaloes in East Java, then surra rapidly spread to other islands in Indonesia (Dewi et al. 2019; Sawitri et al. 2019). The parasite can easily spread between provinces through infected water buffaloes, horses and cattle brought by transmigrating people or dispersed by the government as part of its animal dispersal project (Dargantes et al. 2009; Desquesnes et al. 2013; Sawitri et al. 2019).

Mekonnen et al. (2018) stated that control of surra normally relies on drug treatment and vector control, including chemoprophylaxis. The common drugs used for surra treatment are suramin, diminazene aceturate, quinapyramine and melarsomine (Gressler et al. 2015; Shiferaw et al. 2015; Gillingwater 2018). However, only two drugs, isometamidium and diminazene aceturate, are officially marketed in Indonesia (Dewi et al. 2019; Dewi et al. 2020).

Differences in pathogenicity and drug sensitivity amongst isolates of *T. evansi* have been documented elsewhere but not in Mindanao (Jatau et al. 2010; Kamidi et al. 2018). Gressler et al. (2015) stated that the pathogenicity of *T. evansi* may vary depending on the strain, host species and some non-specific factors such as stress, concurrent infections, or epizootiological local conditions.

During the past decade, several outbreaks of surra have occurred in Mindanao, Philippines, that caused high morbidity and mortality in horses, buffaloes, cattle and small ruminants. This pattern seems to be different from that described in the neighboring Indonesian archipelago where outbreaks only occur sporadically and are often localized. This raises the suspicion that strains of the parasite with different levels of pathogenicity may be present in Mindanao.

The present study was undertaken to provide some of this information. The pathogenicity of 10 isolates of *T. evansi* collected from animals in different areas in Mindanao, Philippines, and one isolate from East Java, Indonesia was determined and compared. In addition, efficacy of four different trypanocides was also tested. The information generated from this endeavor is valuable for the enhancement of the control strategy against surra in Mindanao, Philippines and Indonesia.

MATERIAL AND METHODS

Study site and ethical approval

The laboratory trial was done at the Animal Disease Diagnostic Laboratory of the College of Veterinary Medicine, Central Mindanao University, Philippines, with approval of the research protocol by the Animal Ethics Subcommittee of James Cook University, Australia (Permit no. A779-02; 2003).

Isolates of *T. evansi*

The origin of Philippine and East Javanese isolates of *T. evansi* used in this study are presented in Table 1. All isolates were passaged in mice twice then maintained in guinea pigs until tested.

Preparation of the inoculum

The inoculum for each isolate was prepared from the blood of an infected mouse. After counting the parasite in the blood, when parasitemia was high, a drop of tail blood was diluted with phosphate-buffered saline glucose (PBSG; pH=8.0) and viable trypanosomes were counted using a Neubauer hemocytometer. Blood was then diluted with PBSG to provide 15 mL of inoculum with 5,000 trypanosomes per mL (Sawitri & Wardhana 2017).

Infection of laboratory mice

A total of 352, 6-9 week-old DDY mice each weighing an average of 20 g were used in the trial. The mice were purchased from a local colony and examined to ensure freedom from blood parasites prior to infection. They were acclimatized for two weeks before the trial (Sawitri & Wardhana 2017).

For each isolate, 32 mice were randomly selected, 25 of which were each infected intraperitoneally (ip) with 0.2 mL of inoculum containing 1,000 *T. evansi*. For the infected mice, 4 groups of 5 were treated with 4 trypanocides and one group of 5 served as untreated, infected controls. The seven uninfected mice served as untreated uninfected controls for each isolate. The trial was carried out in three batches.

The selected mice were housed in plastic cages separated according to isolates, and provided with clean

Table 1. Origin and dates of collection of 11 isolates stock of *T. evansi* used in the present study

Code	Origin		Collection
	Species	Place	Date of the Stock
C1	Horse	Cacub, Marbel, South Cotabato, Mindanao, Phl	15 January 2002
C2	Buffalo	Cacub, Marbel, South Cotabato, Mindanao, Phl	15 January 2002
C3	Buffalo	Cacub, Marbel, South Cotabato, Mindanao, Phl	16 January 2002
C4	Buffalo	Cacub, Marbel, South Cotabato, Mindanao, Phl	16 January 2002
C5	Buffalo	Cacub, Marbel, South Cotabato, Mindanao, Phl	16 January 2002
A8	Buffalo	Sta. Josefa, Agusan del Sur, Mindanao, Phl	16 January 2002
A9	Buffalo	Talacogon, Agusan del Sur, Mindanao, Phl	15 January 2002
A10	Buffalo	Talacogon, Agusan del Sur, Mindanao, Phl	15 January 2002
A11	Buffalo	Talacogon, Agusan del Sur, Mindanao, Phl	15 January 2002
A13	Buffalo	Veruela, Agusan del Sur, Mindanao, Phl	16 January 2002
EJ1	Buffalo	Tuban, East Java, Indonesia	5 September 1984

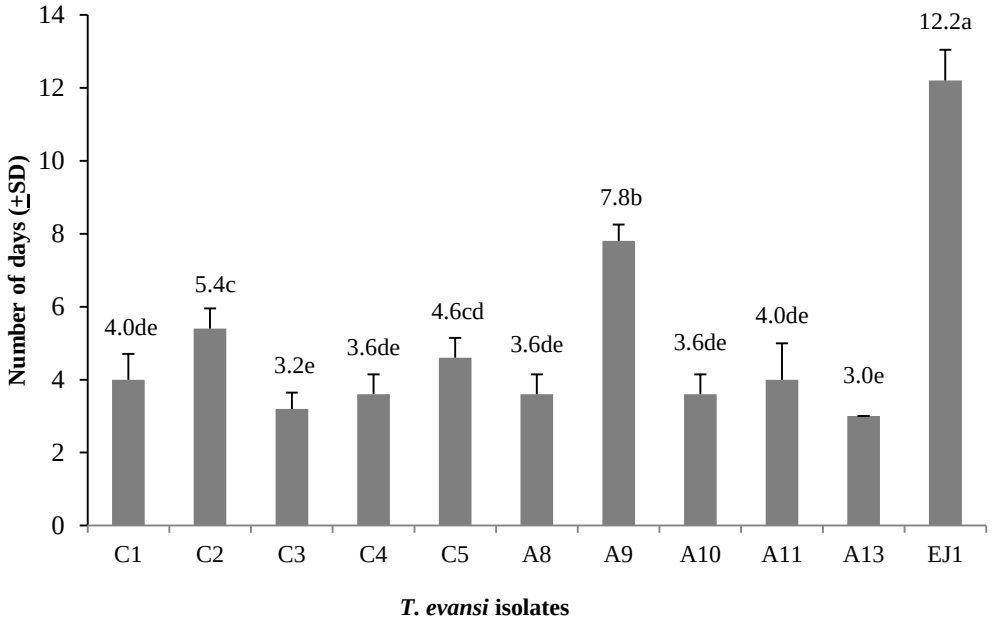

Figure 1. Mean pre-patent periods (days ± SD) of 10 Philippine isolates (C1-A13) and an Indonesian isolate (EJ1) of *T. evansi* in mice. Means with different letters are significantly different ($p<0.05$).

water and commercial pelletized feed, *ad libitum*. The cages were arranged in an open metal cabinet in a well-ventilated room. The litter material of sterile wood shavings was changed every 4 days during the trial.

Treatment with trypanocides

Four trypanocides were tested against *T. evansi* infection in mice using the following dose regimens: diminazene aceturate (Berenil®, Hoechst, Germany) ip at 7 mg kg^{-1} bw; melarsomine dihydrochloride (Immiticide®, A Webster, NSW, Australia) ip at 2 mg kg^{-1} bw; suramin (Naganol®, Bayer, Germany) ip at 10 mg kg^{-1} bw; and quinapyramine sulphate/chloride (Triquin™, Sanjivani Paranteral Limited, India) subcutaneously at 5 mg kg^{-1} bw. The drugs were diluted separately with sterile distilled water to a working concentration of 1-2 mg mL^{-1} and refrigerated (not more than 48 hours) until used. Each mouse was weighed using a digital weighing scale (Mettler, USA) and the amount of drug was calculated based on the dose regimen. Treatment was administered 24 hours after infection.

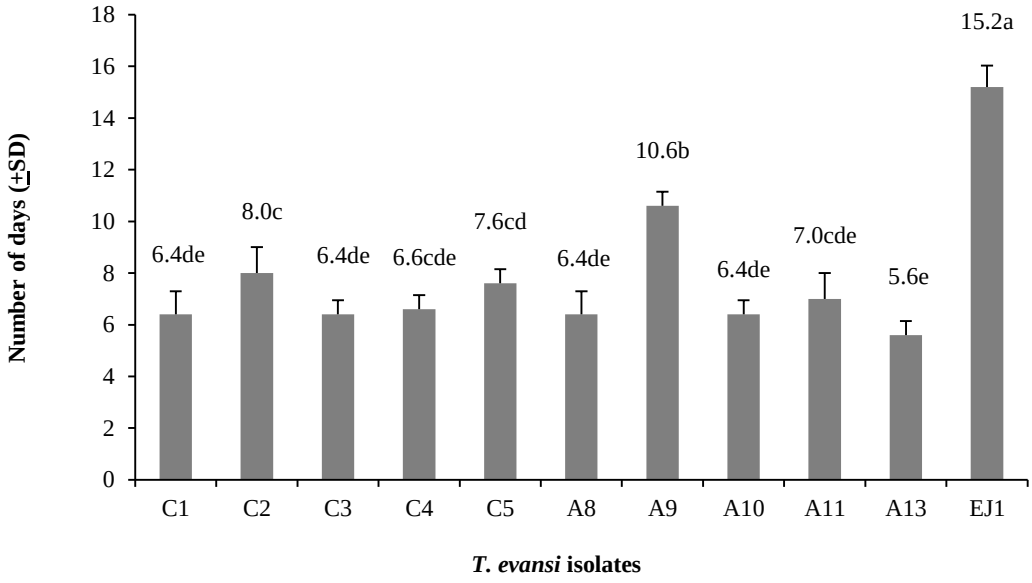

Figure 2. Mean duration of survival (days ± SD) of mice after infection with 10 Philippine isolates (C1-A13) and an Indonesian (EJ1) isolate of *T. evansi*. Means with different letters are significantly different ($p<0.05$).

Monitoring of parasitemia and pathology

Untreated-infected mice and the uninfected controls were examined daily for presence of trypanosomes in the blood by wet blood film taken from the tail tip (Villareal et al. 2013; Garba et al. 2017). The mice were monitored for clinical signs after infection. Treated mice were similarly monitored every 2 days, for 35 days after treatment. Heart smears were made from each mouse that died and examined for trypanosomes. Necropsy was conducted of every dead mouse and gross lesions were noted.

Pre-patent period and the duration of survival of infected, untreated mice were used as indices of pathogenicity. Absence of parasitemia among treated mice was regarded as an indicator of drug efficacy against *T. evansi.* Any surviving mouse after being treated using any of the four trypanocides was considered cured (Begolo et al. 2018).

Analyses of data

The statistical significance of differences ($p<0.05$) in duration of survival and pre-patent period between groups was determined by one-way analysis of variance (ANOVA) and Tukey's honestly significantly different test (Tukey's HSD) using SPSS statistical program.

RESULTS AND DISCUSSION

Pathogenicity

All mice infected with any of the isolates and not treated with a trypanocide developed a disease and died subsequently. The pre-patent period and duration of survival varied significantly ($p<0.05$) amongst isolates (Figures 1 and 2). Pre-patent period and duration of survival were correlated. Mice infected with Mindanao isolates died earlier (5-11 days) than those infected with the Indonesian isolate (14-16 days) ($p<0.05$). The pre-patent period for the Mindanao isolates varied between 3-8 days and was significantly shorter ($p<0.05$) than that of the Indonesian strain (11-13 days). Compared to all 10 isolates from Mindanao, the East Javanese isolate had lower pathogenicity. There was also heterogeneity in pathogenicity of the Mindanao isolates but only one (A9) stood out as being significantly different (lower pathogenicity; $p<0.05$) than the others. Before death, mice showed signs of general weakness and recumbency and appeared to be tilted on one side. Gross lesions observed at necropsy were splenomegaly, hepatomegaly, petechial hemorrhages in the intestines, and congested spleen, kidneys and lungs. No differences in gross lesions between isolates were observed.

This is the first attempt to compare the pathogenicity and drug sensitivity of different isolates of *T. evansi* from Mindanao, Philippines and an isolate from East Java, Indonesia. All isolates induced a fatal infection in mice. However, pre-patent period and duration of survival were significantly longer with the East Javanese isolate indicating that it is less pathogenic in mice than those taken from Mindanao, Philippines. The present finding on the differences of virulence of *T. evansi* isolates in Mindanao supports the observation of Verdillo et al. (2012) and Mekata et al. (2013) on the varying levels of pathogenicity among *T. evansi* isolates in the Philippines in mice. The pathogenicity of the 10

Mindanao *T. evansi* isolates can be considered as moderate to high while that of the East Javanese isolate as low to moderate based on the classification by Sawitri & Wardhana (2017). Using 25 *T. evansi* isolates (collected from livestock in various regions in Indonesia) to infect mice and confirmed by molecular tools using ESAG 6/7 gene as marker, Sawitri & Wardhana (2017) classified the virulence levels of the Indonesian isolates into three, namely: high (death of mice less than 7 days, post-infection, pi), moderate (death of mice between 7–15 days, pi) and low virulence (death of mice more than 15 days, pi).

Regional differences in the pathogenicity of *T. evansi* in mice have been also documented by others. Sawitri & Wardhana (2017) demonstrated that isolates of *T. evansi* collected from the same region in Indonesia had different levels of virulence in mice. In Pantanal, Brazil, different *T. evansi* isolates collected from domestic and wild animals showed marked heterogeneity in their morphology and pathogenicity in rodents despite demonstrating homogeneity in their biochemical and molecular properties (Parreira et al. 2016). Kamidi et al. (2018) also provided additional evidence regarding regional differences in the virulence of *T. evansi*.

The various pathogenicity patterns have been also reported among *Trypanosoma vivax, T. congolense* and *T. brucei* which are distributed widely among cattle in Africa and Latin America. Ramirez-Barrios et al. (2019) showed that *T. vivax* showed moderate to high pathogenicity in experimentally-infected sheep while varying virulence was observed for *T. congolense* isolates in mice, nude rats, and bovine (Bengaly et al. 2002; Giordani et al. 2016). Likewise, differences in virulence was also detected among *T. brucei gambiense* isolates in Balb/c mice (Holzmuller et al. 2008).

Queiroz et al. (2000) indicated that pathogenicity as measured by survival time after infection could be regarded as a biological marker for the heterogeneity of isolates of *T. evansi*. However, whether heterogeneity in mice is a reflection of similar heterogeneity of pathogenicity in domestic animals is unknown. Furthermore, there is probably sufficient variation in resilience between individual domestic animals and between species that minor differences in pathogenicity of strains in domestic animals in different regions may not be apparent clinically. Such seems to be the case in Mindanao where the isolate, which was significantly less pathogenic in mice than the other isolates from Mindanao, was taken from a buffalo with surra that was not noticeably clinically different from the other cases from which *T. evansi* isolates were taken.

On the other hand, the lower pathogenicity in mice of the East Javanese isolate as compared to all isolates from Mindanao paralleled the pathogenicity of surra in domestic animals in Indonesia and Mindanao. Thus, the extent to which measurable variations in pathogenicity of different isolates of *T. evansi* in mice reflect a biologically significant difference in domestic animals is still unresolved. Results from this study implied that a two-fold difference in pathogenicity in mice may indicate a similar trend in domestic animals. However, the measurement of minor pathogenicity variation, which is common in mice due to their uniform susceptibility, may result in greater variability between individual domestic animals due to their resilience to *T. evansi* infection.

Drug efficacy

No trypanosomes was found in the blood of mice infected with any of the *T. evansi* isolates of Mindanao during the 35-day observation period and treated with quinapyramine sulphate/chloride, melarsomine dihydrochloride or suramin (Table 2). In contrast, two of 10 mice infected with either C4 or A9 isolates (5 mice each) and treated with diminazene aceturate (Berenil®) had detectable parasitemia (relapsed infection) on days 29 and 31, respectively. Both mice died 5 days after parasites were detected in their blood. On the other hand, parasites were not detected in mice infected with the East Javanese isolate and treated with any of the drugs. All infected, untreated mice developed infection and died subsequently. *Trypanosoma evansi* infection usually results to 20–100% fatality in mice (Perrone et al. 2018).

Gillingwater et al. (2009) stated that a dosage of 1 mg kg^{-1} bw of quinapyramine administered for 4 consecutive days was able to cure all mice infected with *T. evansi* of Chinese origin. Another study reported that 50% of mice infected with *T. evansi* from the same origin were cured after treatment with quinapyramine at a dose rate of 5 mg kg^{-1} bw (Zhou et al. 2004). However, Macaraeg et al. (2013) revealed that *T. evansi* isolates from the Philippines were more susceptible to more than 3–10 mg kg^{-1} bw of quinapyramine. In addition, the authors suggested that *T. evansi* isolates from Luzon island required a higher dose to cure the infection (10 mg kg^{-1} bw) compared to *T. evansi* from Visayas and Mindanao islands (3 - <10 mg kg^{-1} bw). Accordingly, the dosage of quinapyramine used in the present study was slightly higher than that of the previous study (5 mg kg^{-1} bw) following the dose regimen recommended by the manufacturer. The fact that all infected mice were cured in this study indicated that the drug is effective for surra.

Melarsomine dihydrochloride, as shown in the present study, is effective against the 10 Philippine and Indonesian *T. evansi* isolates. This drug is not used in the Philippines and Indonesia for surra thus explains the sensitivity of these isolates to the drug (Dobson et al. 2009; Dewi et al. 2019; Dewi et al. 2020). The drug is also effective to treat experimental surra in buffaloes at

Table 2. Efficacy of different trypanocides against infection with 10 Mindanao (Philippines) isolates and one East Javanese (Indonesia) isolate of *T. evansi* in mice

Code	Origin	Mortality due to *T. evansi* ; % Efficacy*					
		Uninfected-untreated (7 mice per isolate)	Infected-untreated (5 mice per isolate)	Diminazene aceturate (Berenil®) 7 mg kg^{-1} bw	Melarsomine dihydrochloride (Immiticide®) 2 mg kg^{-1} bw	Quinapyramine Sulphate/Chloride (Triquin^{TM}) 5 mg kg^{-1} bw	Suramin (Naganol®) 10 mg kg^{-1} bw
C1	Philippines	0 ; -	5 ; -	0 ; 100	0 ; 100	0 ; 100	0 ; 100
C2	Philippines	0 ; -	5 ; -	0 ; 100	0 ; 100	0 ; 100	0 ; 100
C3	Philippines	0 ; -	5 ; -	0 ; 100	0 ; 100	0 ; 100	0 ; 100
C4	Philippines	0 ; -	5 ; -	1 ; 80 **	0 ; 100	0 ; 100	0 ; 100
C5	Philippines	0 ; -	5 ; -	0 ; 100	0 ; 100	0 ; 100	0 ; 100
A8	Philippines	0 ; -	5 ; -	0 ; 100	0 ; 100	0 ; 100	0 ; 100
A9	Philippines	0 ; -	5 ; -	1 ; 80 ***	0 ; 100	0 ; 100	0 ; 100
A10	Philippines	0 ; -	5 ; -	0 ; 100	0 ; 100	0 ; 100	0 ; 100
A11	Philippines	0 ; -	5 ; -	0 ; 100	0 ; 100	0 ; 100	0 ; 100
A13	Philippines	0 ; -	5 ; -	0 ; 100	0 ; 100	0 ; 100	0 ; 100
EJ1	Indonesia	0 ; -	5 ; -	0 ; 100	0 ; 100	0 ; 100	0 ; 100

- not applicable, *The number of mice tested for each drug and for each isolate was five, ** Relapsed infection occurred on day 29, ***Relapsed infection occurred on day 31

0.5 mg kg^{-1} bw in Mindanao, Philippines (Dargantes et al. in preparation). However, in Africa where the drug is used to treat surra in camels, melarsomine is no longer effective at 0.125 mg kg^{-1} bw dose (Kabi et al. 2009). In addition, Hébert et al. (2018) reported that melarsomine is unable to kill *T. equiperdum* in the central nervous system of infected horses and therefore discourages the use of this drug to treat animals suffering from a nervous type of dourine and perhaps of surra.

All infected mice treated with suramin in the present study were cleared of the parasite. Comparable results were reported by Al-Mohammed (2008) and concluded that a dosage of 10 mg kg^{-1} bw of suramin is effective against *T. evansi* isolates in Saudi Arabia. In addition to being effective in clearing parasitemia in mice, the drug also did not have any detectable toxic effects. The present study also supports the findings of Gillingwater et al. (2007) that suramin is effective against several stocks of *T. evansi* from Brazil, Columbia, Kazakhstan, Philippines, Indonesia, Kenya, China and Vietnam. Nevertheless, the drug is no longer available in the market (Desquesnes et al. 2013).

Relapse of parasitemia in mice (C4 on days 29 and A9 on days 31) treated with diminazene aceturate at a dose of 7 mg kg^{-1}, indicates that this drug is no longer effective in treating the infection with some of the *T. evansi* isolates in Mindanao. This is in line with the result of a previous study with Ethiopian *T. evansi* type A and type B treated with diminazene aceturate + antypirine (DIM) and diminazene aceturate + phenazone granules (DIM-SEQ) wherein more than half of the *T. evansi*-infected mice relapsed on the 4th-week post-treatment (Mekonnen et al. 2018). Interestingly, water buffaloes experimentally infected with *T. evansi* in Mindanao were not also cured by diminazene (Dargantes et al. in preparation). Kabi et al. (2009) also reported that diminazene aceturate is no longer suitable for the treatment of surra amongst camels in Uganda.

The most likely explanation of this result in the present study is that the two relapsing isolates had a degree of resistance to diminazene aceturate whereas the other isolates tested are susceptible. Nevertheless, it cannot be deduced as to what extent resistance is likely to be demonstrated in domestic animals, causing

treatment to be ineffective. This is because the isolates of *T. evansi* screened in the present study were not random selection of *T. evansi* strains in Mindanao and the comparative plasma levels and pharmacokinetics of diminazene aceturate in mice dosed at 7 mg kg^{-1} with those achieved in domestic animals dosed at 3.5 mg kg^{-1} are unknown. However, the use of diminazene aceturate for treatment of surra among animals in Mindanao should be discontinued to prevent further development of resistance among *T. evansi* isolates on the island.

Another possible explanation for the relapsing *T. evansi* infection in mice as observed in this study was the failure of diminazene to reach trypanosomes invading reserved tissues particularly the brain (Al-Mohammed 2008; Jatau et al. 2010). Relapsing parasitemia was also observed in mice infected with tissue-invading *T. brucei* and *T. vivax* and treated with diminazene aceturate (Qadeer et al. 2013).

Diminazene has been widely used in Mindanao for more than two decades at the recommended dose rate of 3.5 mg kg^{-1} bodyweight. It is widely accepted that prolonged usage of the same drug, especially at sub-therapeutic doses, leads to drug resistance which may also be aggravated by immunosuppression. Animals may be underdosed in the field where bodyweights are often estimated roughly, with the consequence that the computed dose is usually below the recommended therapeutic dose. Immunosuppression may be induced by heavy parasite burden, stressful conditions or trypanosomiasis (Yayeh et al. 2018). Drug resistance of trypanosomes to diminazene aceturate is common in African and Asian countries. Evidence of resistance was also provided in *in vitro* and *in vivo* trials with isolates collected from China, Ethiopia and the Philippines (Zhou et al. 2004; Nuryady et al. 2019).

CONCLUSION

It is concluded that the *T. evansi* isolates from Mindanao, Philippines, tested in this study, are more pathogenic than the isolate from East Java, Indonesia, with minimal pathogenic heterogeneity among the Philippine *T. evansi* isolates. Quinapyramine sulphate/chloride, melarsomine dihydrochloride and suramin are effective against the *T. evansi* isolates tested while the use of diminazene against surra in Mindanao should be re-evaluated. This information is valuable in the enhancement of the control strategy against surra in the Philippines and Indonesia.

ACKNOWLEDGEMENT

Dargantes AP and Wardhana AH are equal authors in the manuscript production. The authors would like to thank Dr. R. Mercado, Dr. L.T. Simborio, Dr. J. Elevazo, Dr. J. Dargantes and J. Gamboa for their invaluable help; Dr. E. Lim, Dr. E. Igsoc, Dr. P. Calo, Dr. M. Samar, staff of Mindanao Unified Surra Control Approach (MUSCA), Regional Disease Diagnostic Laboratories (RDDL) of Regions XI and Caraga, and Provincial Veterinary Offices (PVO) of Agusan del Sur and South Cotabato for their assistance during the collection of samples; Dr. P.K. Sanyal for the supply of Triquin®; M. Palaca for technical help; and, the Australian Institute for Tropical Veterinary and Animal Science (AITVAS), Australian Centre for International Agricultural Research (ACIAR), Australian Agency for International Development (AusAID), International Foundation of Science (IFS) and Central Mindanao University (CMU) for the financial support.

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Immunomodulatory Effect of Virgin Coconut Oil in Wistar Rats Infected with *Staphylococcus aureus*

Widianingrum DC[1], Salasia SIO*[2]

[1]*Department of Animal Science, Faculty of Agriculture, University of Jember, 68121 Jember, Indonesia*
[2]*Department of Bioresources Technology and Veterinary, Vocational College, Universitas Gadjah Mada, 55281 Yogyakarta, Indonesia*
**E-mail:* isrinasalasia@ugm.ac.id

(received 30-11-2020; revised 04-02-2021; accepted 11-04-2021)

ABSTRAK

Widianingrum DC, Salasia SIO. 2021. Peran Imunomodulator *Virgin Coconut Oil* pada Tikus Wistar yang diinfeksi dengan *Staphylococcus aureus.* JITV 26(1): 31-38. DOI: http://doi.dx.org/10.14334/jitv.v26i1.2670.

Virgin coconut oil (VCO) mengandung bahan aktif yang dapat meningkatkan imunitas tubuh terhadap agen penyakit. Penelitian ini bertujuan untuk mengetahui efek imunomodulator VCO berdasar tinjauan aktivitas *superoxide dismutase* (SOD), proliferasi limfosit, gambaran histopatologik organ hati dan ginjal tikus yang diinfeksi *Staphylococcus aureus* . Perlakuan VCO diberikan pada tikus dengan dosis 250 µL secara oral selama 1 minggu. Infeksi *S. aureus* dilakukan secara intraperitoneal menggunakan kanul bengkok dengan dosis $5x10^2$ sel bakteri. Penelitian ini menggunakan 20 ekor tikus *Wistar* betina umur 1 bulan dan dibagi menjadi 4 kelompok. Kelompok kontrol negatif (C-): tikus kontrol tanpa perlakuan; Kelompok AV: tikus diinfeksi dengan *S. aureus* dan diberi VCO; Kelompok VA: tikus diberi VCO terlebih dahulu kemudian diinfeksi dengan *S. aureus*; dan Kelompok kontrol positif (C+): tikus diinfeksi *S. aureus* tanpa pemberian VCO. Pada akhir percobaan semua tikus dieutanasi dan dinekropsi sesuai kode etik kesejahteraan hewan. Sampel plasma masing-masing perlakuan diambil untuk dilihat aktivitas SOD dan limfosit diisolasi dari limpa untuk diketahui kemampuan proliferasinya. Jaringan hati dan ginjal dikoleksi untuk pengamatan histopatologi. Hasil penelitian menunjukkan kelompok VA memiliki aktivitas SOD minggu ke-4 (41.50 ± 3.56 %) dan proliferasi limfosit (0.3018) lebih tinggi dibanding semua perlakuan. Hati pada kelompok tritmen menunjukkan infiltrasi leukosit, tidak ada hemoragi (VA); sel hepatosit normal (VA). Ginjal pada kelompok tritmen menunjukkan infiltrasi leukosit (AV); epitel glomerulus dan tubulus normal, masih terdapat hemoragi (VA). Dari hasil penelitian ini menunjukkan bahwa VCO berpotensi sebagai imunomodulator, hepatoprotektan maupun nephroprotektan.

Kata Kunci: *Virgin coconut oil*, *Staphylococcus aureus*, Proliferasi limfosit, *Superoxide dismutase*, Hati, Ginjal

ABSTRACT

Widianingrum DC, Salasia SIO. 2021. Immunomodulatory effects of virgin coconut oil in wistar rats infected with *Staphylococcus aureus* JITV 26(1): 31-38. DOI: http://doi.dx.org/10.14334/jitv.v26i1.2670.

Virgin coconut oil (VCO) contains bioactive that induce immunity against infectious diseases. This study aim to determine the immunomodulatory effects of VCO based on the activity of superoxide dismutase (SOD), lymphocyte proliferation, and histopathological examination in liver and kidney of rats infected with *Staphylococcus aureus*. The VCO was given intragastrically to rats with a dose of 250 µL for one week. The rats were infected with *S. aureus* at $5x10^2$ bacterial cells intraperitoneally. Twenty (20) female *Wistar* rats of one month old were divided into four groups. The negative control group (C-): without treatment, AV group: infected with *S. aureus* followed by VCO treatment; VA group: pretreated with VCO followed by *S. aureus* infection, and positive control group (C+): were infected with *S. aureus* without VCO. All rats were euthanized and necropsied based on the animal ethic standard. Plasma samples were taken to evaluate SOD activity, and lymphocytes were isolated from the spleen to determine their proliferative ability. Livers and kidneys were collected for a histopathology examination. Results showed that the VA group had the highest SOD activity on the 4^{th} week (41.50 ± 3.56 %) and lymphocyte proliferation (0.3018) compared to all treatments, indicating immunomodulatory effects of VCO. Liver of treatments group showed leucocytes infiltration, no hemorrhages (VA); the hepatocytes with normal cells (VA). Kidney of treatments group showed leucocytes infiltration (AV); normal epithelial glomerulus and tubulus cells, still found hemorrhage (VA). These studies indicated that VCO has a potential role as an immunomodulator, hepatoprotectant, and nephroprotectant.

Key Words: Virgin coconut oil, *Staphylococcus aureus*, Superoxide dismutase, Lymphocyte proliferation, Liver, Kidney

INTRODUCTION

Virgin coconut oil (VCO) is produced by natural processes either by fermentation, enzymatic (Silaban et al. 2014), centrifugation (Wong & Hartina 2014), salination (Aziz et al. 2017), oil addition, step-wise heating, etc. (Pontoh et al. 2008). Elimination of refining, bleaching, and deodorizing stages preserve the

essential content of VCO (Mansor et al. 2012). Medium-chain fatty acid (MCFA) consists lauric, caprylic, myristic, palmitic, palmitoleic, capric, stearic, and linoleic acids which are known as the essential content of VCO. The use of MCFA as antibacterial, antiviral, and antiprotozoal has been reported in several studies (Widianingrum et al. 2019a; Shilling et al. 2013; Yassen & Khelkal 2015; Tangwatcharin & Khopaibool 2012). The variety of MCFA in VCO depends on the material used. VCO contains lauric acid at 45.45 to 57.89% (Novarianto & Tulalo 2020), 17% myristic acid, 48% lauric acid, 8% caprylic acid, and 10% capric acid and may induce immunity (Fauzi et al. 2012). While the phenolic compounds of VCO around 49.56 to 59.88 µg/ mL are known as antioxidants (Pulung et al. 2016).

Immunomodulatory effect of VCO could be identified by lymphocyte proliferation and enzymatic activity of superoxide dismutase (SOD) (Yuniwarti et al. 2012; Abujazia et al. 2012). Immune system reacted and responded simultaneously following the invasion or infection of foreign materials (Tizard 2000). Activity of free radical molecules, which are highly reactive, may cause an oxidative stress condition characterized by inactivation of antioxidant enzymes such as the SOD (El-Tohamy 2012). Usunomena et al. (2012) stated that there were three types of SOD in the body, namely: 1. Copper-Zinc Superoxide Dismutase (Cu-ZnSOD), the main extracellular SOD found inside the cells; 2. Manganese Superoxide Dismutase (Mn-SOD), synthesized in the cytoplasm, then sent into the mitochondria; 3. Extracellular Superoxide Dismutase (EC-SOD), a primary extracellular SOD found outside of the cells.

The essential oils of VCO will be absorbed and metabolized in the liver and excreted through the kidneys (Famurewa et al. 2019). The excretion of VCO is commonly through feces, sweat, and urine (Jim 2013). Abnormalities of liver and kidneys indicate the capability performance of these organs in metabolic process (Wang et al. 2013). Disruptions of both organs are commonly classified in several stages depend on the intensity of exposure (Lip et al. 2011). Low exposure may cause little or no morphological and functional changes, but higher intensity of exposure may significantly show pathological changes (Galus et al. 2013). Infection of *Staphylococcus aureus* (*S. aureus*) induces platelet aggregation and may retain in the hepatic sinusoids and glomeruli of kidneys. (Deppermann & Kubes 2018; Surewaard et al. 2018). Accumulation injuries of these organs may lead to multi organs dysfunction (Guebre-Egziabher et al. 2013). Pathological changes could be caused by a single injury or multiple injuries and also by an acute to chronic damage (Ferenbach & Bonventre 2016). Fibrosis is a pathological change in the liver characterized by progressive scar tissue formation in the liver parenchyma in response to wound healing (Pellicoro et al. 2014). Pathogenesis of fibrosis is initiated by parenchymal cell damage due to chronic injury and inflammation (Hernandez-Gea & Friedman 2011). Prolonged fibrosis may develop to the formation of cirrhosistic liver. If the primary cause of injury is elliminated, the fibrosis is generally reversible (Koyama & Brenner 2017). Several factors that determine the reversibility of fibrosis include the total amount of collagen, the duration of fibrosis, and the presence of extracellular matrix destroying enzymes (Albanis & Friedman 2006). The presence of congestion may also be seen in fibrosis characterised by reddish colour and dilation of capillaries containing blood cells (Sujono et al. 2015). Mild congestion is generally reversible but it may become irreversible when the injury is getting worse (Chung et al. 2016).

On the other hand, cirrhosis is irreversible due to the cross-linking of collagen by tissue transglutaminase, which is difficult to degrade the extracellular matrix scar tissue (Pellicoro et al. 2012; Sohrabpour et al. 2012). Cirrhosis is known as the 14th most common cause of death worldwide. The early therapeutic is a new concept to prevent complications of cirrhosis to avoid or delay clinical decompensation reduce mortality (Tsochatzis et al. 2014).

The infection of *S. aureus* may affect various organs, including liver, spleen, kidneys and skin. (Felistiani 2017; Lucas et al. 2012; Fauzi et al. 2012). Kidneys are commonly vulnerable to *S. aureus* infections (Pollitt et al. 2018) that may cause abscess (Rauch et al. 2012), fatty degeneration, hydropic degeneration and necrosis (Suhita et al. 2013). The previous study reported that the VCO could be used as a ruminant supplement to protection an undegradable agent in rumen as formaldehyde (Widianingrum et al. 2019b). The purpose of this study is to determine the immunomodulatory effect of VCO based on the activity of SOD, lymphocyte proliferation, and histopathological examination of liver and kidney of rats infected with *S. aureus*.

MATERIALS AND METHODS

This experiment was approved by The Ethics Committee of Central Laboratory LPPT UGM with a number of 333/ KEC-LPPT/X/2015. A total number of 20 female Wistar rats of 1 month old, was provided by the UP2KH UGM and was adopted for 1 week before treatment. Rats were infected with *S. aureus* of $5x10^2$ bacteria intraperitoneally (Fauzi et al. 2012). The VCO (MIPA UGM) was given orally with a dose rate of 250

µL/ rat daily for 1 week consecutively. The treatment consists of four groups with 5 replications: 1) Group C- (negative control): rats were given orally with phosphate buffer saline (PBS) for a week without *S. aureus* infection; 2) Group AV: rats were infected with *S. aureus* followed by VCO; 3) Group VA: rats were previously treated with VCO followed by *S. aureus* infection; 4) Group C+ (positive control): rats were infected with *S. aureus* followed by PBS orally for a week consecutively in daily basis.

At the end of experiment, all rats were euthanized and necropsied for pathological examination. Lymphocytes were isolated from the spleen of each experimental rat at the end of the study. Spleen were injected with 3 mL of RPMI and then chopped in a sterile tube. The solution was centrifuged at 3,000 rpm for 5 minutes, 2 mL of NH_4Cl was added to lyse erythrocytes. After centrifugation at 3,000 rpm for 5 minutes, the pellets were taken and added with 1 mL of complete media. Lymphocytes were counted with a hemocytometer and determined their viability with trypan blue ($1x10^6$/ mL). A total of 100 µL of lymphocyte cells were added with 100 µL of complete media then placed on the microplate and incubated at 37°C for 72 hours with 5% CO_2. The solution was added with10 µL MTT and then incubated at 37°C for 4 hours with 5% CO_2. The suspension was added with 100 µL SDS and incubated overnight at room temperature. The proliferation of lymphocytes was measured using an ELISA reader at 550-600 nm (ACTG Laboratory Technologist Committee 2000).

Plasma samples were obtained to determine superoxide dismutase (SOD) activity by collecting the blood of treated rat with 10% ethylene diamine tetraacetic acid (EDTA). The SOD activity was tested using a commercial kit according to the standard instructions for use (StressMarq, Biosciences, Canada). The principle of determining the antioxidant activity of SOD is known as the ability of SOD to catalyze superoxide anion (O_2-) into molecular hydrogen peroxide and oxygen (Khachatryan et al. 2011). All samples were incubated at room temperature for 20 minutes, and then the absorbance was calculated using a UV spectrophotometer (Shimidzhu UV-Visible 1601 Type) at a wavelength of 450 nm.

$$\% \text{ Inhibiton} = \frac{Mean\ OD\ sampel}{mean\ OD\ zero\ standard} \text{ x } 100\%$$

Samples of liver and kidneys were collected from each rat, fixed in formalin 10%, and stained with hematoxylin-eosin for histopathological examination based on Suhita et al. (2013) method. The tissue was observed under a Zeiss binocular microscope at a magnification of 40x. The histopathological changes of the organs were reported descriptively.

RESULTS AND DISCUSSION

Based on the lymphocyte proliferation (Figure 1), the rat treated with VCO had higher lymphocyte proliferation. The group of rats given VCO before *S. aureus* infection (0.3018) had the most significant increased lymphocyte proliferation compared to rats given VCO after *S. aureus* infection (0.2641) and the control group (C+: 0.2170; C-: 0.2317).

The VCO-pretreated rats (VA) appeared to induce highest lymphocyte proliferation compared to other groups of rats. Results showed that VCO may induce

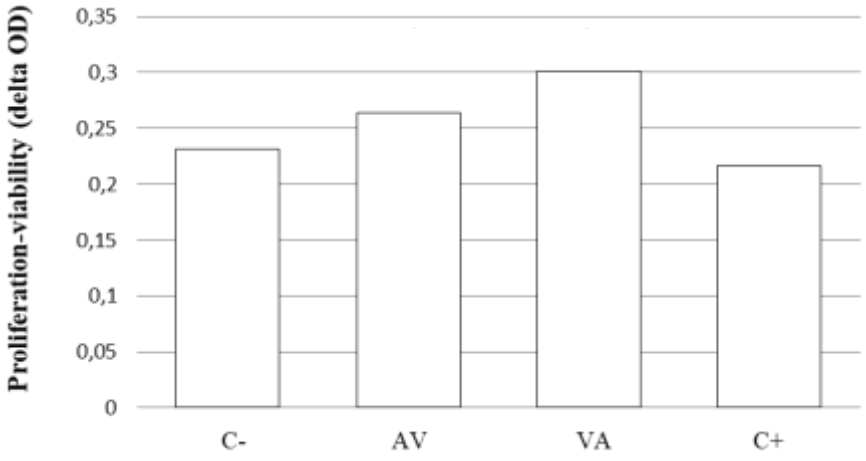

Figure 1. Lymphocyte proliferation in treated rats. Lymphocyte proliferation was seen by the color changes that are observed under the ELISA reader, the higher the absorbance value indicated that the higher activity the lymphocyte proliferation. The negative control group (C-): without treatment, AV group: infected with *S. aureus* followed by VCO treatment; VA group: pretreated with VCO followed by *S. aureus* infection, and positive control group (C+): were infected with *S. aureus* without VCO

Table 1. Percent inhibition of SOD activity in treated rats

SOD Activity	C-	AV	VA	C+
	..... %			
Week I	36.10±0.79	37.81±0.64	38.21±1.19	35.44±2.28
Week II	38.27±1.21	36.63±1.16	38.14±2.59	44.93±7.03
Week III	34.19±0.79 [b]	31.36±1.09 [ab]	29.58±1.68 [a]	41.37±0.82 [c]
Week IV	29.78±1.40 [a]	30.69±1.79 [a]	41.50±3.56 [b]	31.75±0.99 [a]

The difference in notation shows a significant difference at the 5% confidence level ($p < 0.05$).
The negative control group (C-): without treatment, AV group: infected with *S. aureus* followed by VCO treatment; VA group: pretreated with VCO followed by *S. aureus* infection, and positive control group (C+): were infected with *S. aureus* without VCO.

the infection of *S. aureus* has better effect on lymphocyte proliferation than that of post infection. These results indicated that the VCO may induce immunomodulation. The treatment could modulate lymphocyte proliferation (Yuniwarti et al. 2012), increasing antibody titers, macrophage phagocytosis activity, lymphocyte count, CD_4, CD_8, heterophils, and body weight (Yuniwarti et al. 2012).

Results of SOD activity (% inhibition) of each treatment are presented in Table 1. Based on the observations, the SOD activity at week 1 and week 2 were not significantly different among the treated rats. At week-1 of treated rats shows that the SOD responses were homogenous and the activity of SOD was not significantly different. A significant increase of SOD activity was noted when VCO was given prior to *S. aureus* infection (VA) at week 4 (41.50%). This result confirms that VCO treatment has best immunomodulation response in rats when VCO was given before *S. aureus* infection.

Dauqan et al. (2012) reported that the activity of SOD of coconut oil increased from week 4 and remained high until week 8. The coconut oil appear to have highest activity of SOD compared to both palm oil and corn oil. Nevin & Rajamohan (2006) also reported that antioxidant level of rats pretreated with VCO showed an increase in activity of catalase (CAT) and superoxide dismutase (SOD). In their study, the lipid peroxide levels of the liver and kidneys in the VCO treatment were significantly lower than other pretreatments. They knew that VCO increases the total glutathione (GTN), which is known as a sensitive indicator of antioxidant status. Both enzymes are known to induce the mechanisms of defense against reactive oxygen species (ROS) and to prevent lipid peroxidation (Bolin et al. 2010).

Microscopic changes of liver and kidney of treated rats are shown in Figure 2 and 3. There were no changes of liver in the negative control (untreated rats) (Figure 2A). Liver of rats infected with *S. aureus* showed nucleus enlargement with chromatin defragmentation, infiltration of leucocytes, hemorrhages (Figure 2B). Liver of rats treated with VCO at 250 µL / day after *S. aureus* infection showed leucocytes infiltration, however there were no hemorrhages (Figure 2C). Liver of rat pretreated with VCO followed by *S. aureus* infection showed the hepatocyte with normal cells (Figure 2D). The microscopic changes reveal that the VCO may induce immune system against infectious agents and modulate the cell damages (Senin et al. 2018). Bartz et al. (2011) reported that intraperitoneal dosing of *S. aureus* to rats may affect liver oxidative mtDNA and mtDNA content as well as increase mitochondrial OGG1 protein and enzymatic activity. Increasing or OGG1 indicates mitochondrial response during sepsis.

The present study shows that pretreatment of VCO prior to *S. aureus* infection (VA) may prevent or reduce liver damage. Microscopically, hepatic regeneration of VA developed better than AV group. VCO may also increase immunity against bacterial infection as shown in lymphocyte proliferation. Essential oil of fatty acids increases immunity capacity by improving metabolism and increasing the SOD level. The MCFA content has also been reported to inhibit complications in diabetic patients (Sheela et al. 2017), reducing risks in cardiovascular diseases and cancer (Narayanankutty et al. 2018), increasing high-density lipoprotein (HDL) (Chinwong et al. 2017). The mechanisms of regeneration in VCO treatment could be due to lymphocyte proliferation by releasing opsonin to induce macrophages, increase superoxide dismutase (SOD) activity, antibacterial effect by breaking the cell walls of *S. aureus* (Widianingrum et al. 2017).

Kidneys of the negative control (untreated rats) were normal with glomerular cells surrounded by squamous epithelial cells and tubules were surrounded by cuboid epithelial cells (Figure 3A). Kidneys of rats infected with *S. aureus* showed infiltration of leucocytes, hemorrhages (congestion), tubular necrosis

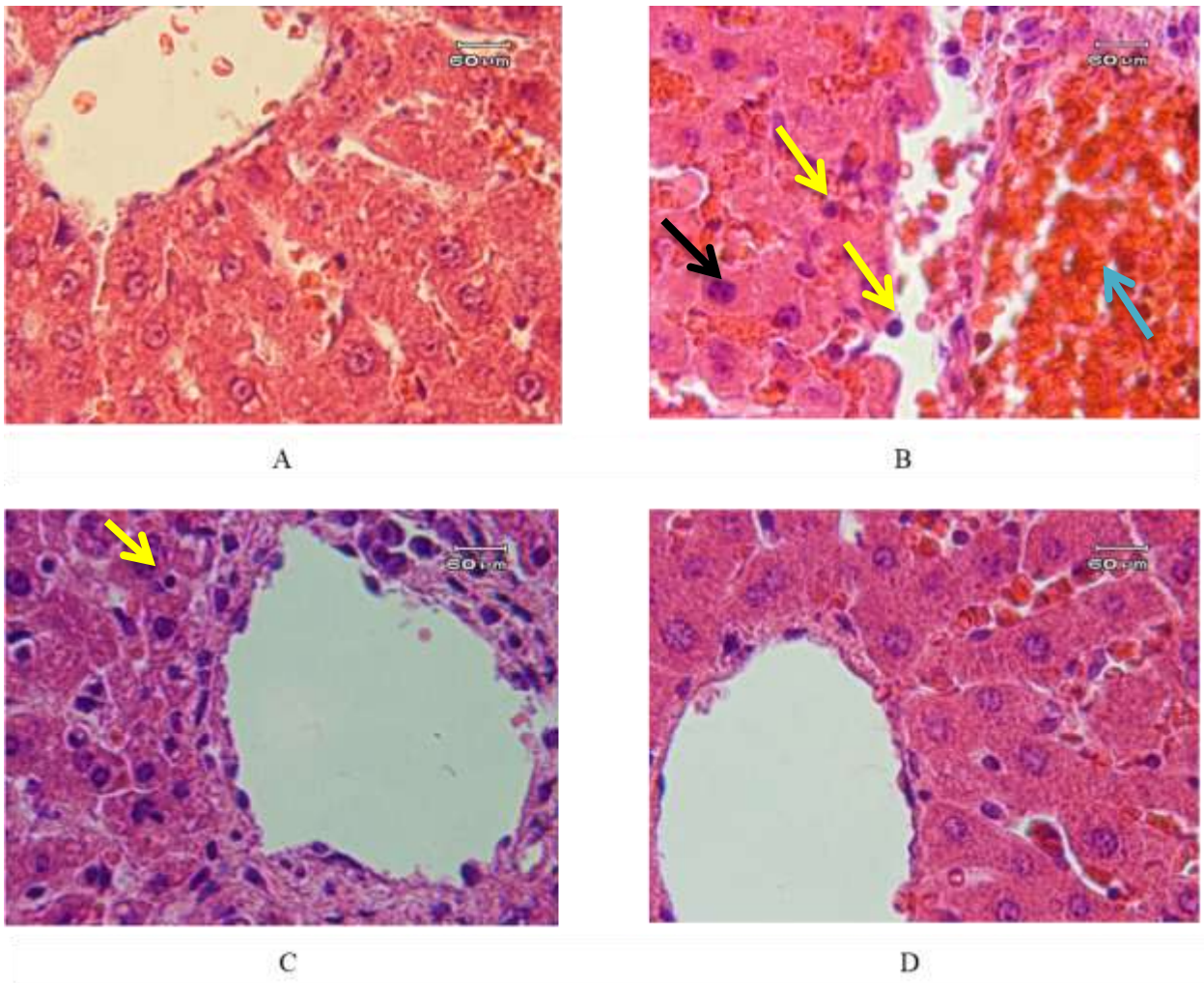

Figure 2. Histopathology of the liver (H&E, 40x magnification). A. Normal liver of the untreated rat, polyhedral hepatocytes with nucleus centrally and narrowed sinusoids; B. Liver of rat infected with *S. aureus* enlarged nucleus with chromatin defragmentation (black arrow), infiltration of leucocytes (yellow arrow), haemorrhages (blue arrow); C. Liver of rat treated with VCO at 250 µL / day after *S. aureus* infection showed vacuolitation cell; D. Liver of rats pretreated with VCO followed by *S. aureus* infection showed the hepatocytes with normal configuration.

immunity responses. The pretreatment of VCO prior to (Figure 3 B). Kidney of rat treated with VCO at 250 µL/day after *S. aureus* infection showed leucocytes infiltration (Figure 3C). Kidney of rats pretreated with VCO followed by *S. aureus* infection showed normal epithelial glomerulus and tubulus cells, however still found hemorrhage (Figure 3D). Regeneration of kidneys was mediated by a combination of direct VCO treatment and a complex metabolic mechanisms (Fernando et al. 2015; Rajamohan & Archana 2018). Rats that were dosed with monolaurin known as an essential substance of VCO for one week before *S. aureus* infection caused 50% survival rate of rats (Manohar et al. 2013). The most powerfull contain of VCO is lauric acid (Novarianto & Tulalo 2020). Monolaurin is monoglycerin which is obtained from lauric acid from VCO (Tangwatcharin & Khopaibool 2012). In the Manohar et al. (2013) study, the highest survival rate was found when VCO treatment was mixed with vancomycin. The bacterial infections were not detected in kidneys of the surviving rats after 30 days. The VCO treatments were also reported to affects kidney by increasing glutathione reductase and decreasing catalase (Đurašević et al. 2019). The degenerative and destructive effects in kidneys may be prevented, regenerated, and restored by providing VCO (Akinnuga et al. 2019). Restoration of kidneys is a modulation mechanism between antioxidants and anti-inflammation of VCO (Kamisah et al. 2016; Famurewa et al. 2017).

Leucocytes like neutrophils contain enzymes that digest proteins. A high number of activated neutrophils may cause damage to the surrounding tissue, not just the site of the original damage. Inflammation of vessel

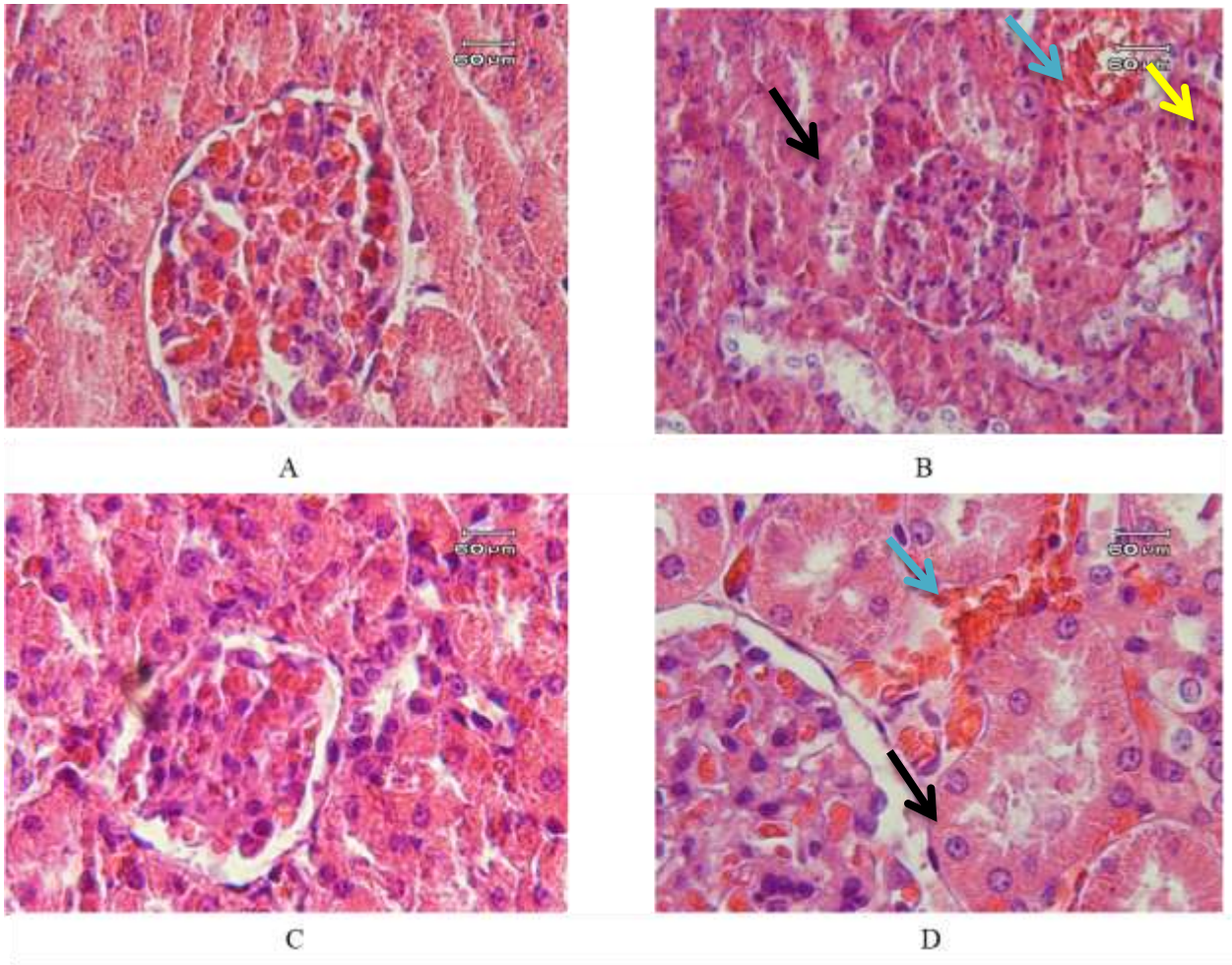

Figure 3. Histopathology of the kidneys (H&E, 40x magnification). A: Normal kidney of the untreated rat, B. Kidney of rat infected with *S. aureus* showed infiltration of leucocytes (yellow arrow), hemorrhages (blue arrow), tubular necrosis (black arrow); C. Kidney of rat treated with VCO at 250 µL / day after *S. aureus* infection, tend to normal; D. Kidney of rats pretreated with VCO followed by *S. aureus* infection showed hemorrhages (blue arrow), normal epithelial tubulus and glomerulus cells (black arrow)

walls (vasculitis), one of the harmful effect of acute inflammation, can cause excessive leakiness of blood vessels, hemorrhage, and can impair blood supply to tissue, causing ischaemia and infarction (Brooks 2010).

CONCLUSION

VCO has shown a potential role in immunomodulation and modulation mechanisms to repairing lesions of liver and kidneys due *to Staphylococcus aureus* infection.

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Effect of Dietary Modified-Banana-Tuber Meal Substituting Dietary Corn on Growth Performance, Carcass Trait and Dietary-Nutrients Digestibility of Coloured-Feather Hybrid Duck

Sjofjan O, Adli DN, Natsir MH, Nuningtyas YF, Wardani TS, Sholichatunnisa I, Ulpah SN, Firmansyah O

Department of Feed and Animal Nutrition, Faculty of Animal Science, Universitas of Brawijaya, Jl. Veteran, Malang, East Java 65145 - Indonesia
E-mail: osofjan@yahoo.com

(received 30-01-2021; revised 18-03-2021; accepted 06-04-2021)

ABSTRAK

Sjofjan O, Adli DN, Natsir MH, Nuningtyas YF, Wardani TS, Sholichatunnisa I, Ulpah SN, Firmansyah O. 2021. Pengaruh pakan tepung bonggol-pisang-modifikasi (TBP-M) sebagai bahan pengganti jagung pada kinerja pertumbuhan, karakter karkas dan daya cerna pakan itik hibrida (Peking x Khaki Campbell). JITV 26(1): 39-48. DOI: http://dx.doi.org/10/14334/jitv.v26i1.2686.

Penelitian bertujuan untuk mengetahui pengaruh penggunaan tepung bonggol pisang modifikasi (TBP-M), hasil perlakuan tepung bongkol pisang dengan enzim selulase, dan di stabilkan nilai nutrisinya menggunakan tepung daging-tulang, DL-methionin, dan lisyne terhadap nilai nutrisi, performans produksi, kualitas karkas dan kecernaan pakan itik hibrida (Peking x Khaki Campbell). Materi dalam penelitian ini 196 itik pedaging hibrida dengan rataan bobot hidup (421.31 ± 0.183g). Metode penelitian yang digunakan adalah Rancangan Acak Lengkap dengan 5 perlakuan dan 4 ulangan. Pakan percobaan terdiri dari kontrol (tanpa substitusi TBP-M), TBP-M25 (Pakan basal + TBP-M 25% substitusi jagung), TBP-M50 (Pakan basal + TBP-M 50% substitusi jagung), TBP-M75 (Pakan basal + TBP-M 75% substitusi jagung), TBP-M100 (Pakan basal + TBP-M 100% substitusi jagung). Data hasil penelitian dianalisis secara statistik dengan analisis keragaman (Anova, *analysis of variance*). Dilanjutkan dengan uji Duncan's Multiple Range Test untuk mengetahui perbedaan rataan antar perlakuan dengan menggunakan perangkat lunak SAS. Hasil penelitian menunjukkan bahwa substitusi jagung dengan TBP-M berpengaruh nyata (P<0,05) terhadap kecernaan bahan kering dan protein kasar pakan. Disimpulkan bahwa TBP-M dapat diberikan sebagai pengganti jagung tanpa menimbulkan efek negatif pada itik hibrida.

Kata Kunci: Karkas, Kecernaan gizi pakan, Itik hibrida, Bongkol pisang modifikasi

ABSTRACT

Sjofjan O, Adli DN, Natsir MH, Nuningtyas YF, Wardani TS, Sholichatunnisa I, Ulpah SN, Firmansyah O. 2021. Effect of dietary modified-banana-tuber meal (M-BTM) substituting corn on growth performance, carcass traits and dietary-nutrients digestibility of coloured-feather hybrid duck (Pekin x Khaki Cambell). JITV 26(1): 39-48. DOI: http://dx.doi.org/10/14334/jitv.v26i1.2686.

In this experiment, we investigate the effect of modified banana tuber meal (M-BTM) to substitute dietary maize in growing-finisher colored-feathered hybrid duck. One hundred and ninety six hybrid ducks (Pekin x Khaki Campbell) with 421.31 ± 0.183g body weight (BW) were allotted to 5 dietary treatments with 9 ducks (unsexed) per pen and 4 replications per treatment. These dietary treatments were: NC (negative control; maize-soyabean-meal based diet), BTM25 (25% maize was replaced by M-BTM-), BTM50 (50% maize was replaced by M-BTM), BTM75 (75% maize was replaced by M-BTM), and BTM100 (100% maize was replaced by M-BTM). The experimental design applied using completely randomize design (CRD). Data of this experiment were statistically analysed by one-way-analysis-of-variance of SAS University version 4.0 red hat (64-bit) University Online Edition. Result demonstrated that M-BTM improved significantly ($p< 0.05$) digestibility of dry matter and crude protein. It is concluded that M-BTM enhanced apparently growth performaces and digestibility parameters of colored-feathered hybrid duck (Pekin x Khaki Campbell).

Key Words: Carcass, Digestibility of energy, Hybrid ducks, Modified banana tuber meal

INTRODUCTION

Demand for poultry products have raised every year, according to Sjofjan et al. (2021) the poultry protein needed raised year to year. Poultry, especially broiler contribute more than 50% protein requirement in Indonesia. One of secondary meat protein source can be fulfilled from waterfowl (Sjofjan et al. 2021). One of the popular waterfowl as meat in Indonesia were Hybrid duck. Hybrid ducks are the offspring of a crossing between a male Peking duck and female Khaki Champbell duck. Peking ducks have rapid weight gain,

while Khaki Champbell ducks have a high body weight and high egg production compared to other local ducks (Sjofjan et al. 2021). Duck has a considerable high feed consumption compared to chicken; therefore feed has to be considered seriously. At least 65-75% of total productivity in poultry industry is allocated for feed (Ali et al. 2014).

The Indonesian government imposed regulation Permendag/10/2020 to anticipate the COVID-19, the Indonesian government has undertaking strict quarantine measures on the importation of live animal species and imported feed material originating from China or transiting into Indonesian territory (Badan Pusat Statistik 2020; Sjofjan et al. 2021). Thus, regulation causes reducing supply of raw material for poultry feed such as maize (Sjofjan & Adli 2021). Fourthly quarter (Q4) data from Badan Pusat Statistik (2020) reported that Indonesia imported maize at the amount of 911.194 tonnes/year or equally to a total rate of US$ 233.47 million. The maize is a main feed ingredient for poultry industries in Indonesia. Later, the industrial of feed mill, institution, academic, researcher, and farmers in Indonesia took an alternative to solve this problem by using potential local material in area scopes. The used of tuber meal in poultry diet are in partial replacement of maize. This was expected since the cost of tuber meals is much lower than that of maize (Beckford & Bartlett 2015; Achilonu et al. 2018).

One potential feed to replace maize were banana tuber meal since this root meal had similar energy content and low protein but high in crude fiber. The Indonesia has potential agro-industrial waste from banana plant. According to Hapsari et al. (2017), Indonesia is homeland of banana both wild species and cultivars. Moreover, banana is most famous fruit plant through worldwide. Being part of primary data of origin and biodiversity of banana, Indonesia has played a crucial role in supporting availablity of banana (Hapsari et al. 2017). Banana plant had tuber or rhizome, which is a basal part of banana stem. Rhizome grew after reproductive organ were formed as modification of the peduncle, white in color, with smooth surface. Rhizome consists of epidermis, periphery zone and center zone (Sumardi & Wulandari 2010; Libatique 2020).

There is a negative effect of utilizing banana tuber which causes gizzard erosion, low palatability, and low nutrient content. Thus, tuber meal needs to be modified in accordance to overcome the weakness. To improve its nutrient content, the modification utilize β-cellulose enzyme. The tuber contents 50% undigestible non-starch polysaccharide (NSP) component for poultry. The local resources as an alternative feed ingredient for poultry was reported to stimulate the releasing of volatile fatty acid in the caecum of ducks and reduced crude fiber in the gut of waterfowl (Sharmila et al. 2014). However, its use in conventional feed is limited by some factors and need to be well managed before using it as commercial livestock feed (Dei et al. 2011).

Accordingly, this study was carried out to investigate the effect modified banana tuber meal to promote replacement of maize in growing-finisher rations of colored-feathered hybrid. Then, choosing the most suitable level of treatment applicable in duck farm is considered to be a novelty of the result of the experiment.

MATERIALS AND METHODS

Ducks rearing condition

One hundred and ninety six colored-feathered hybrid ducks (Pekin x Khaki Campbell) with average 421.31 ± 0.183 g of body weight were allotted to 5 dietary treatments with 9 ducks (unsexed) per pen and 4 replications each treatment. Furthermore, the second experiment for digestibility, 20 hybrid ducks (Pekin x Khaki Campbell) aged 64 days (unsexed) were used. The total 20 metabolic cages were used. One cage for one duck. The experiment was conducted at conventionally-farm-controlled environmental, at Batu, East Java, Indonesia (latitude 7°55′06″S, longitude 112°34′35″E, elevation 813 m). The climate is tropical wet season and average rainfall around 3230 mm. The average room temperature and humidity were 26°C and 71%, respectively. Rice hull was used as litter floor pens. The lighting program was set at 16 hours light. Ducks were reared under supervision of a veterinarian and was approved by the University of Brawijaya Animal Ethics Committee. The hybrid duck were taken from commercial farmer from Blitar Regency, East Java, Indonesia.

Feeding treatment programmed

The ducks were given free access (*ad libitum*) to fresh water and feed throughout the study period twice a day at 07; 30 AM and 15; 30 PM. Experimental diets were formulated according to growing phase of day 24 to day 38 for first phase, day 39 to day 52 for second phase and day 53 to day 64 for finisher phase. The Composition of Feed in the experiment is presented in Table 2, 3 and 4, respectively for first, second and finisher phases.

Preparation of modified banana tuber meal

The banana tuber meal (BTM) were taken from local merchant at the wet-local-market, in Trenggalek city, East Java, Indonesia. The tuber meal was sifted to

Table 1. Nutrient composition of diet ingredient (g/kg, as-fed basis)

Item	Maize	Soybean meal	Banana tuber meal
Dry Matter, (g/kg)	91.4	91.3	86.3
Crude protein, (g/kg)	8.3	41.5	36.1
Fat, (g/kg)	40	16	45
Crude fibre, (g/kg)	17	34	16.7
Ash, (g/kg)	25	58	6.7
Nitrogen free extract, (g/kg)	554	300	435

separate the meal from the remaining stem-foliage, then placed on the floor that had been covered with trash bags and plastic. Suspensions of β-Cellulose are homogenized in a blender with 0.5% of meat-bone-meal then added at 0.010% per 1 kg of BTM. The treated BTM was the named as modified TBM (M-TBM). The last step was formulation with DL-Meth and Lysine on total feed formulation that are presented in Tables 2,3, and 4. The dietary treatments were: NC (negative control; maize-soya bean-rice bean-meal based diet), M-BTM25 (25% of maize was replaced with M-BTM), M-BTM50 (50% of maize was replaced with M-BTM), M-BTM75 (75% of maize was replaced with M-BTM), M-BTM100 (100% of maize was replaced with M-BTM). Diets were formulated to contain 2900 kcal metabolizable energy (ME)/kg, 18% crude protein, 0.75% total methionine and 0.28% total lysine. The other nutrients were formulated to meet or slightly exceed the nutrient requirement as suggested by Badan Standardisasi Nasional (2018). Experimental diets were formulated using Universitas Brawijaya (UB) Feed Formulation Software for Poultry following Badan Standardisasi Nasional (2018) requirement.

Data collection and sampling procedures

Daily feed consumption was recorded by measuring daily weight difference between feed offered and feed left. The weekly live weight of the duck was measured weekly at 06:00 AM. The feed efficiency was then calculated by dividing the total feed consumption by the live weight gain of the ducks during experiment (Abel et al. 2015). Duck mortality was recorded per flock from the beginning until the end of the experiment (Sjofjan et al. 2021). Twenty-four ducks from each pen which had weight to the nearest final live weight gain were taken to be sacrificed for carcass analyses.

Digestibility's analyses

The digestibility method was carried out by sampling the homogenized feces collected and stored in plastic trays. Then, immediately placed mixture of liquid of NA_2PO_4 2%; $NA_2H_2PO_4$ 2%, 24% formaldehyde; and 900 ml reverse osmosis water for digestibility's analysis following Sjofjan et al. (2021). Data were used to calculate digestibility parameters according to the following formulae:

AME=IE-FE
TME=AME+FEL

Where IE=ingested energy; FE=fecal energy voided by the fed birds; while FEL=fasting energy loss by the unfed birds, apparent metabolizable energy (AME), nitrogen-corrected apparent metabolizable energy (AMEn), total metabolizable energy (TME), nitrogen-corrected total metabolizable energy (TME). The values corrected to zero N balance, AMEn and TMEn, were calculated as follows:

AMEn=AME-(8.22×ANR/FI)
TMEn=TME-(8.22×FNL/FI) - (8.22×ANR/FI)

Where ANR=apparent N retention; FI=feed intake; and FNL=fasting N loss by the unfed bird; the factor 8.22 kcal/g for N retained in the body has been used according to Mustafa et al. (2004) and Sjofjan et al. (2021). Continually, the analyses of proximate of the feed sample was carried out to determine dry matter, ash, crude fibre, fat, and crude protein contents (Sjofjan et al. 2021). Crude protein was determined using Kjeltech analyses of Foss Detector, Switzerland and gross energy was determined using Parr Oxygen Bomb 1108; USA (Sjofjan et al. 2021).

Data analyses

The experimental design applied was completely randomize design (CRD). Data of experiment were statistically analysed by the one-way-analysis-of-variance of SAS University version 4.0 red hat (64-bit) University Online Edition (Sjofjan & Adli 2021). The differences among treatment means were determined at level of $p < 0.05$, using Duncan's multiple range test (Steel & Torrie 1990).

Table 2. Composition of dietary treatments (as fed basis) fed to second growing phase of colored-feathered hybrid ducks (Pekin x Khaki Campbell)

Ingredients	Treatments				
	NC	M-BTM 25	M-BTM50	M-BTM75	M-BTM100
Maize , (g/kg)	540	500	490	465	440
Rice bran, (g/kg)	150	150	150	150	150
Soybean meal, (g/kg)	120	120	120	120	120
Meat bone meal 50, (g/kg)	50	50	50	50	50
Fish Meal. 60, (g/kg)	50	50	50	50	50
M-BTM, (g/kg)	-	25	50	75	100
Limestone Powder, (g/kg)	30	30	30	30	30
Grit, (g/kg)	41	41	41	41	41
DL-Methionine, (g/kg)	1	1	1	1	1
Palm oil, (g/kg)	10	10	10	10	10
β-cellulase, (g/kg)	-	1	1	1	1
L-lysine , (g/kg)	5	5	5	5	5
Analyzed nutrients composition					
Dry matter, (%)	87.05	87.25	87.30	87.43	87.55
Crude protein, (%)	18.01	18.11	18.19	17.38	17.56
Fat, (%)	6.41	6.53	6.36	6.23	6.17
Crude Fiber, (%)	3.63	3.78	3.60	4.60	4.93
Ash, (%)	6.10	6.25	6.13	6.39	6.45
Nitrogen Free extract,(%)	48.33	46.66	45.88	47.05	46.63
Metabolizable energy, (Kcal/kg)	2,912	2,917	2,905	2,941	3,412
Lysine, (%)	1.07	1.10	1.11	0.93	1.15
Methionine, (%)	0.41	0.45	0.48	0.53	0.55
Met. + Cystine, (%))	0.68	0.67	0.66	0.65	0.56
Calcium, (%)	3.71	3.71	3.71	3.71	3.71
Total Phosphorus, (%)	0.85	0.87	0.85	0.86	0.88
Available Phosphorus, (%)	0.39	0.39	0.39	0.34	0.44
Bulk density, (g / L)	383	381	381	345	356

NC= negative control; maize-soyabean-meal based diet; M-MBTM25 = 25% of Modified Banana Tuber Meal replaced maize; M-MBTM50 = 50% of Modified Banana Tuber Meal replaced maize; M-MBTM75 = 75% of Modified Banana Tuber Meal replaced maize; M-MBTM100 = 100% of Modified Banana Tuber Meal replaced maize

Table 3. Composition of dietary treatments (as fed basis) fed to finisher phase of colored-feathered hybrid ducks (Pekin x Khaki Campbell)

Ingredients	Treatments				
	NC	M-BTM 25	M-BTM50	M-BTM75	M-BTM100
Maize, (g/kg)	550	515	500	475	450
Rice bran, (g/kg)	170	170	170	170	170
Soybean meal, (g/kg)	90	90	90	90	90
Meat bone meal 50, (g/kg)	50	50	50	50	50
Fish Meal. 60, (g/kg)	50	50	50	50	50
M-BTM, (g/kg)	-	25	50	75	100
Limestone Powder, (g/kg)	30	30	30	30	30
Grit, (g/kg)	41	41	41	41	41
DL-Methionine, (g/kg)	1	1	1	1	1
Palm oil, (g/kg)	10	10	10	10	10
β-cellulose, (g/kg)	-	1	1	1	1
L-lysine, (g/kg)	5	5	5	5	5
Analyzed nutrients composition					
Dry matter, (%)	86.96	87.09	87.21	87.34	87.46
Crude protein, (%)	15.81	15.99	16.17	16.35	16.54
Fat, (%)	6.66	6.60	6.54	6.47	6.41
Crude Fiber, (%)	3.76	4.08	4.41	4.73	5.06
Ash, (%)	6.15	6.21	6.28	6.34	6.40
Nitrogen Free extract, (%)	48.91	48.48	48.06	47.63	47.21
Metabolizable energy, (Kcal/kg)	2696	2648	2600	2552	2510
Lysine, (%)	0.84	0.85	0.85	0.86	0.86
Methionine, (%)	0.50	0.50	0.51	0.51	0.52
Methionine + Cystine, (%)	0.75	0.76	0.76	0.77	0.78
Calcium, (%)	3.71	3.71	3.71	3.72	3.72
Total Phosphorus, (%)	0.86	0.87	0.88	0.89	0.89
Available Phosphorus, (%)	0.39	0.40	0.41	0.42	0.44
Bulk density, (g / L)	585	584	580	569	565

NC= negative control; maize-soyabean-meal based diet; M-MBTM25 = 25% of Modified Banana Tuber Meal replaced maize; M-MBTM50 = 50% of Modified Banana Tuber Meal replaced maize; M-MBTM75 = 75% of Modified Banana Tuber Meal replaced maize; M-MBTM100 = 100% of Modified Banana Tuber Meal replaced maize

RESULTS AND DISCUSSIONS

Performance of hybrid-duck

Experimental results are presented in Table 4 and Table 5, it shows that there were no significant difference ($p > 0.05$) on parameters observed. Initially, there were curve linear decrease in the growing phase 1, 2, and finisher phase in feed intake as the level of modified banana tuber meal increased in formulated diet (Figure 1). This was incontrast from the finding of Atapattu & Senevirathne (2013) who reported that the feed intake or feed conversion ratio was not affected by the type of the banana meal. In addition from Sugiharto et al. (2020) using banana peel meal up to 15% had no deleterious effect on the feed intake of the poultry.

The result of daily weight gain are presented on the Table 4, at the beginning phase were 39.1; 38.3; 37.4; 35.9 vs. 34.5 g/bird (control). The result continued at the second growing phase Table 4 were 59.2; 58.7; 57.8; 58.9 vs. 56.5 g/bird (control). For the last, at the finisher phase (Table 4) were 59.3; 58.6; 58.3; 56.1 vs. 54.3 g/bird (control).

The trends continued positive on the body weight increase, even though the data were not significant difference ($p > 0.05$). Table 4 showed the body weight at the beginning phase were 714; 736; 723; 747 vs. 733 (control), then followed on the growing phase 978; 976; 985; 987 vs. 988 (control), at the end phase showed that 1570; 1674; 1562; 1542 vs. 1558 (control).

The number of mortalities also decreased in accordance with increase in the day of rearing. The increased of daily gain may be in correlation with the modified banana tuber meal, the β-cellulose enzyme successfully reduced crude fiber and bind some non-starch polysaccharides (NSPs) and it helped reducing NSP content of the banana tuber meal. Abouelezz et al. (2018) reported that treatment failed to bind the NSP content on the cassava extraction residue treatment showing that the result did not give significant difference ($p > 0.05$). Moreover, the cooked and uncooked banana meal that consisted of NSP could have negative effect on the animals (Atapattu & Senevirathne 2013). Sjofjan et al. (2021) stated the used of enzyme would inhibit the negative effect of NSP in the duck intestinal. The enzyme could be useful as an endogenous enzyme in the cell wall that supported in breaking dawn the NSP of feedstuff (Sjofjan et al. 2021).

Results of this study showed that β-cellulose banana tuber meal improved feed conversion ratio (FCR) in growing 1, 2 and finishing phases in comparation to control diet. FCR of growing phase 1 were respectively for M-BTM25, M-BTM50, M-BTM75 and M-BTM100 of 3.81; 3.54; 3.12, 3..55 *vs*. NC (control diet) of 3.87, and of growing phase 2, that were 3.31; 2.97;2.96; 2.95 *vs*. 3.34 of NC diet and finishing phase of 3.33; 3.97; 3.44; 3.13 *vs*. 3.24 NC diet. However, since we

Table 4. Growth performance of colored-feathered hybrid duck (Pekin x Khaki Campbell), fed modified banana tuber meal (M-BTM) diets at growing phase

Performance	NC	M-BTM 25	M-BTM50	M-BTM75	M-BTM100	SEM
First growing phase (age of 24-38days)						
Initial body weight, (g/bird)	444	447	446	445	448	0.32
Body live weight at age of 38 days, (g/bird)	733	714	736	723	747	0.22
Daily body weight gain, (g/bird)	34.5[b2)]	39.1[a]	38.3[ab]	37.4[b]	35.9[ab]	1.22
Feed conversion ratio (FCR)	3.87[a]	3.81[a]	3.54[b]	3.12[b]	3.55[b]	4.55
Mortalities, (%)	0.00	0.00	0.00	1.26	0.00	0.12
Feed consumption, (g/bird)	1,622	1,614	1,523	1,512	1,563	0.11
Second growing phase (age of 39-52days)						
Body live weight at age of 52 days, (g/bird)	988	978	976	985	987	0.22
Daily body weight gain, (g/bird)	56.5[b]	59.2[a]	58.7[ab]	57.8[b]	58.9[ab]	4.55
Feed conversion ratio	3.34[a]	3.31[a]	2.97[b]	2.96[b]	2.95[b]	3.32
Mortalities, (%)	0.00	1.26	0.00	1.26	1.26	0.13
Feed consumption, (g/bird)	1,813	1,834	1,717	1,652	1,543	0.14
Finisher (age of 53-64days)						
Body live weight at age of 64 days, (g/bird)	1558	1570	1674	1562	1542	24.53
Daily body weight gain, (g/bird)	54.3[b]	59.3[a]	58.6[ab]	58.3[b]	56.1[ab]	2.11
Feed conversion ratio	3.24[a]	3.33[a]	3.97[b]	3.44[b]	3.13[b]	0.12
Mortalities, (%)	4.26	3.43	0.00	1.26	3.43	0.23
Feed consumption, (g/bird)	2,033	2,003	2,118	2,534	1,592	0.19

NC= negative control; maize-soyabean-meal based diet; M-MBTM25 = 25% of Modified Banana Tuber Meal replaced maize; M-MBTM50 = 50% of Modified Banana Tuber Meal replaced maize; M-MBTM75 = 75% of Modified Banana Tuber Meal replaced maize; M-MBTM100 = 100% of Modified Banana Tuber Meal replaced maize; SEM = Standard error of mean. Values with different superscript in the same row are significantly difference ($p<0.05$)

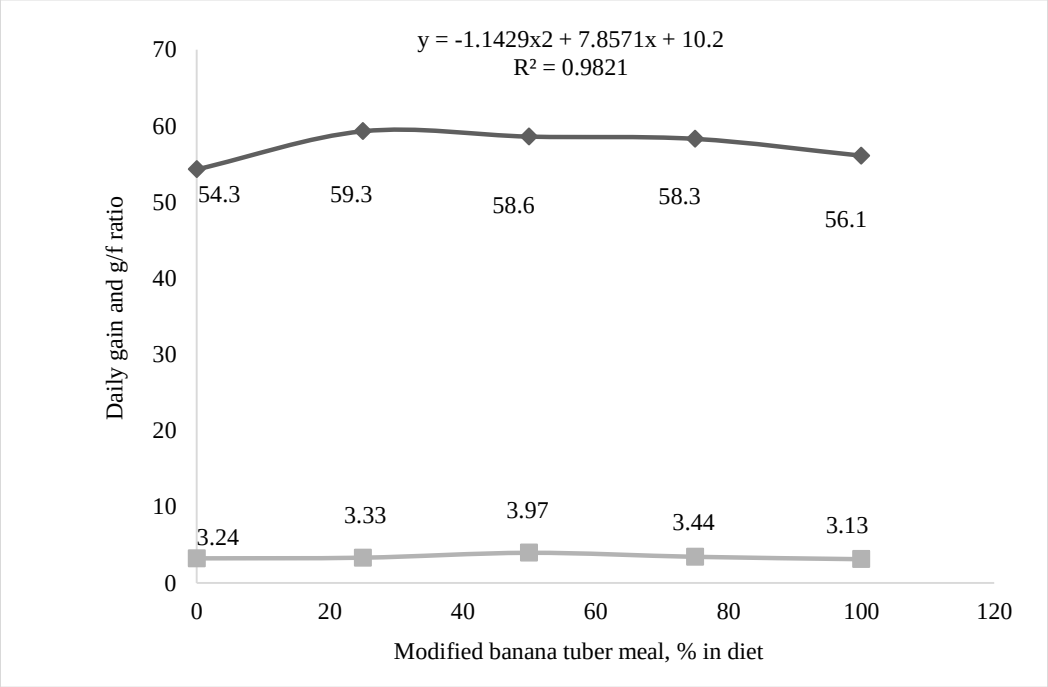

Figure 1. Growth performance of colored-feathered hybrid ducks (Pekin x Khaki Campbell), fed modified banana tuber meal at the age of 64 days. The symbols represent daily gain (●) and feed conversion ratio (■).

managed controlled-trial environment the response of moderate variable value means were probably expressing the good result achievement. In addition, the breed and environmental factors were considerable evidence in modern rearing strains which had relatively similar in physiological and genetic potential. The hybrid duck used in this study were actually kept in relatively adaptive environment with relatively lower temperatures of 24.11^{0}C in the morning and 27.12^{0}C in the afternoon and humidity of 61.12% in morning and 74.22% in afternoon, had supported sufficient daily feed consumption.

Experimental Diets Digestibility

The digestibility reflected the impact of Feed conversion ratio (FCR) (Table 5). The result showed used of modified banana tuber meal improved digestibility of dry matter and crude protein significantly ($p<0.05$). The digestibility of crude protein of M-BTM diets were 68.22%, 67.22%, 62.40% and 66.43% respectively for M-BTM25, M-BTM50, M-BTM75 and M-BTM100 *vs* 52.57 % was in control diet. In line with nutrient digestibility of dry matter were also increasing, showing of 43.18%; 41.22%; 43.12%; 40.14% respectively for 25%, 50%,75%, 100% of M-TBM diets, *vs*. 41.11% for control diet. In this study discrepancy was most probably caused by the protein quality (adequate and balanced amino acid composition) and the presence of anti nutrient components in the diets. There problems in the agro industrial origin were due to anti-nutrients contents such as lectins, polyphenol, anti-nutritional amino acid, saponins, cyanogen glycoside substances, protease inhibitors, and relatively oxalate (Anwar et al. 2016). To reduce anti-nutritional factors there are several technique to increase digestibility using heat, chemicals, machineries, enzyme supplementation, or fermentation (Anwar et al. 2016; (Najoan et al. 2020a; Tien et al. 2014). Compared to Sugiharto et al. (2020) protein digestibility did not vary between banana meal treatment and control, this inference noticed better protein digestibility when feeding banana meal to poultry (Sugiharto et al. 2020).

The modified banana tuber meal for the hybrid ducks did not showed significant effect on the AME, AMEn, TME, and TMEn (Table 6). The AME mean values were 1,247; 1,158; 1,086; 1,987 kcal/kg respectively for 25%, 50%, 75%, 100% M-TBM *vs*. 1,280 kcal of control treatment, followed by AMEn of dietary treatments of 1,523; 1,456; 1,625; 1,647 kcal/kg *vs*. 1,666 kcal AMEn/kg of control. In addition the TME mean values were 2,122; 2,089; 2,071; 2,074 kcal/kg respectively for 25%, 50%, 75%, 100% M-TBM vs. 2,173 kcal TME of control treatment, followed by TMEn of dietary treatments of 1,544; 1,666; 1,752; 1,666 kcal/kg for 25%, 50%, 75%, 100% M-TBM *vs*. 1,777 kcal TMEn/kg of control treatment. Reported from Mohammed et al. (2020) the tuber meal containing bitter toxic called gum later called terpenes.

Table 5. Nutrients digestibility of experimental diets of growing-finisher colored-feathered hybrid duck (Pekin x Khaki Campbell), fed modified banana tuber meal (M-BTM) diets at age of 64 days

Nutrient digestibility	NC	M-BTM 25	M-BTM50	M-BTM75	M-BTM100	SEM
Dry matter, (%)	41.11[b]	43.18[a2)]	41.22[b]	43.12[a]	40.14[a]	0.13
Crude protein, (%)	52.57[b]	68.22[a2)]	67.22[a]	62.04[ab]	66.43[a]	2.11
AME (kcal / kg)	1,280	1,247	1,158	1,086	1,937	12.11
AMEn (kcal/kg)	1,666	1,523	1,456	1,625	1,647	10.13
TME (kcal/kg)	2,173	2,122	2,089	2,071	2,074	4.3
TMEn (kcal/kg)	1,777	1,544	1,666	1,752	1,666	4.5

NC= negative control; maize-soyabean-meal based diet; M-MBTM25 = 25% of Modified Banana Tuber Meal replaced maize; M-MBTM50 = 50% of Modified Banana Tuber Meal replaced maize; M-MBTM75 = 75% of Modified Banana Tuber Meal replaced maize; M-MBTM100 = 100% of Modified Banana Tuber Meal replaced maize; SEM = Standard error of mean. Values with different superscript in the same row are significantly difference ($p<0.05$)

Table 6. Carcass traits and organs weight of colored-feathered hybrid duck (Pekin x Khaki Campbell), fed modified banana tuber meal at the age of 64 days

Meat quality	NC	M-BTM 25	M-BTM50	M-BTM75	M-BTM100	SEM
Gizzard (%)	2.59	2.85	2.90	2.94	3.27	0.21
Heart (%)	2.50	2.47	2.84	2.40	2.13	0.15
Liver (%)	1.92	2.40	2.54	2.56	2.71	3.11
Spleen (%)	0.036	0.030	0.332	0.286	0.265	0.01
Pancreas (%)	1.22	1.33	1.17	1.33	1.22	0.14
Abdominal fat (%)	8.47	7.55	7.85	7.66	7.56	4.11
Caeca length (%)	2.88	3.33	3.28	3.44	3.22	0.07
Caeca width (%)	1.88	1.70	1.56	1.86	1.75	0.11
Carcass weight (%)	45.22	46.11	47.22	46.13	45.33	4.5
Carcass (%)	41.12	41.22	43.10	43.13	41.12	0.22
Breast meat (%)	11.11	12.12	14.12	14.45	15.14	0.33
Gizzard (g)	55.99	58.05	54.26	54.60	53.67	2.33
Heart (g)	13.03	12.63	12.59	13.53	12.59	6.13
Liver (g)	38.78	36.89	41.25	38.78	40.25	3.41
Spleen (g)	1.27	1.65	1.38	1.4	1.23	0.22
Pancreas (g)	5.38	5.64	5.73	5.93	6.04	3.1
Abdominal fat (g)	35.97	36.80	40.29	41.26	40.33	3.2
Caeca length (cm)	12.25	13.10	12.75	13.08	13.10	5.44
Caeca width (cm)	1.96	1.80	1.85	2.15	1.35	0.22
Carcass weight (g)	1204	1302	1260	1293	1192	235
Carcass (g)	61.33	62.11	61.00	63.11	62.12	0.12
Breast meat (g)	25.11	26.12	26.12	26.13	24.14	0.12

NC= negative control; maize-soyabean-meal based diet; M-MBTM25 = 25% of Modified Banana Tuber Meal replaced maize; M-MBTM50 = 50% of Modified Banana Tuber Meal replaced maize; M-MBTM75 = 75% of Modified Banana Tuber Meal replaced meize; M-MBTM100 = 100% of Modified Banana Tuber Meal replaced maize; SEM = Standard error of mean

These anti-nutritional factors reduce the digestibility when the raw feed made from tuber given to animals (Mohammed et al. 2020).

Carcass traits

Carcass traits and relative organ weight emphasis the result of the influence of the modified the banana tuber meal whether it causes damaged or not. The result did not show negative effect on the relative organ weight, and the difference of the treatment means were not significant ($p>0.05$). Apparently, there were no negative effect detected even in the gizzard. The growth factors correlate with age, while the poultry uses in the relative age cause the same internal organ's growth (Sjofjan et al. 2021). In agreement with Blandon et al. (2015) who stated that the used of banana peels meal replacing maize, were no significant difference ($p>0.05$) both carcass trait and relative organ weight. The factors affected this result might be due to banana tuber meal contains secondary compounds like terpenoids, flavonoids and others phenolic compounds with a important physiological activity as reported by Blandon et al. (2015) and Fitroh et al. (2018) in banana peels. The terpenoids and flavonoids are anti-nutritional factors that are obstacle in poultry feed. This phenolic compounds are hard to balance with other raw materials as a feed (Blandon et al. 2015). There was only slight reduction ($p>0.05$) in abdominal fat of the duck fed modified banana tuber meal (7.55%; 7.85%; 7.66%; 7.56%, respectively for M-BTM25, M-BTM50, M-BTM75 and M-BTM100 *vs*. 8.47% for control. The use of plantain plant in poultry has been limited because of possibility deleterious effects arising from the presence of tannin (Blandon et al. 2015). Blandon et al. (2015) stated that tannin existed in two forms, namely; (a) free active form caused bitter taste and (b) bound tannin form which are insoluble, supposedly inert and has little or no effect on the palatability but can be useful to reduce abdominal fat (Blandon et al. 2015). In other hand, the report from Najoan et al. (2020a) stated that the flavonoid act can eliminated the abdominal fat which bound into glycine and taurine. The next step is forming glycine and taurine into bile salt and secreted to duodenum which is degraded by microbes (Najoan et al. 2020b).

CONCLUSION

Result of this study demonstrated the enhancement apparently growth performances and digestibility parameters of colored-feathered hybrid duck (Pekin x Khaki Campbell) after fed modified banana tuber meal (M-BTM) diets.

ACKNOWLEDGMENT

The author extend appreciation to LPPM, University of Brawijaya for Research Grant flagship for granted Number: 437.18/UN.10.C10/PN/2020 and Non-Tax Revenue (PNBP) UB SP DIPA023.17.2.677512/2020.

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Phone: 0251 - 8322185 | Fax: 0251 - 8380588
e-mail: jitvnak@yahoo.com/jitvnak@litbang.pertanian.go.id
http://medpub.litbang.pertanian.go.id/index.php/jitv/index

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Jurnal Ilmu Ternak dan Veteriner

Indonesian Journal of Animal and Veterinary Sciences

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Acknowledgement

Editorial board and executive editor of Indonesian Journal of Animal and Veterinary Sciences (IJAVS) extent high appreciation to the expertises of peer reviewer of IJAVS (Volume 26 No. 1 2021).

1.	Prof (R). Dr. Ir. Sofjan Iskandar, M.Rur.Sc	:	Animal Feed and Nutrition-IRIAP
2.	Dr. drh. Sumarningsih, M.Sc	:	Virology and Bacteriology-IRCVS
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Jurnal Ilmu Ternak dan Veteriner

IJAVS *Indonesian Journal of Animal and Veterinary Sciences*

Volume 26, Number 1, March 2021 ISSN 0853-7380 E-ISSN 2252-696X

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