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PREFACE

In this edition, volume 22 no. 2 June 2017, we proudly present articles from animal and veterinary sciences including feed and nutritive technology, microbiology, physiology, and pharmacology. The articles published in this edition are: "Supplementation of inorganic and organic zinc mixtures in feed of Boerka goats fed by oil palm fronds"; "Ultrasonography of udder parenchymal tissue of Murrah and Swamp buffalo calves"; "The growth of local white muscovy growth during starter and grower periods"; "Qualitative and quantitative characteristics of SenSi-1 Agrinak chicken"; "Evaluation of LipL32 ELISA for detection of bovine leptospirosis in West Java"; and "Serotype detection, molecular characterization and genetic relationship study on Pasteurella multocide local isolate".

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

Chief Editor;

Bogor, June 2017

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Supplementation of Inorganic and Organic Zinc Mixtures in Feed of Boerka Goats Fed by Oil Palm Fronds

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ABSTRAK

Ginting SP, Antonius, Simanihuruk K. 2017. Suplementasi campuran Zn anorganik dan organik pada kambing Boerka yang diberi pakan pelepah kelapa sawit. JITV 22(2): 51-56. DOI: http://dx.doi.org/10.14334/jitv.v22i2.1798

Seng (Zn) merupakan elemen pada berbagai jenis enzim maupun hormon yang memiliki peran fisiologis sangat penting di dalam tubuh, yang mempengaruhi produksi maupun reproduksi ternak termasuk aktivitas mikroba rumen dalam pemecahan serat pakan. Penelitian bertujuan untuk meningkatkan performa kambing yang diberi pakan dasar pelepah kelapa sawit yang mendapat suplementasi 35 ppm Zn dalam bentuk Zn anorganik (ZnO) dan organic (Zn-metionin). Tiga puluh ekor kambing jantan persilangan Boer x Kacang (Boerka) umur 9-11 bulan digunakan dalam penelitian ini dan yang dibagi menjadi lima kelompok perlakuan pakan sebagai berikut: P1 (kontrol): pakan komplit berbasis pelepah sawit, P2: P1 + 35 ppm Zn (100% ZnO), P3: P1 + 35 ppm Zn (75% ZnO + 25% Zn-metionin), P4: P1 + 35 ppm Zn (50% ZnO + 50% Zn-metionin), P5: P1 + 35 ppm Zn (25% ZnO + 75% Zn-metionin). Rancangan percobaan yang digunakan adalah Rancangan Acak Lengkap dengan enam ulangan. Peningkatan proporsi Zn organik meningkatkan konsumsi pakan dan konsumsi paling tinggi terdapat pada kelompok yang diberi suplementasi Zn anorganik/Zn organik dengan rasio 25/75. PBBH hanya berbeda (P<0,05) pada kelompok yang mendapat suplementasi Zn dengan proporsi Zn-metionin paling tinggi (75%). Konsentrasi Zn dalam darah meningkat secara nyata (P<0,05) dengan peningkatan proporsi Zn-metionine, namun komposisi asam lemak terbang tidak berbeda antar perlakuan (P>0,05). Disimpulkan bahwa performa kambing yang diberi pelepah sawit sebagai pakan dasar dapat ditingkatkan dengan pemberian suplemen Zn dalam bentuk campuran Zn anorganik (ZnO) dan Zn organik (Zn-metionin)

Kata Kunci: Kambing, Seng, Suplementasi, Pelepah Sawit

ABSTRACT

Ginting SP, Antonius, Simanihuruk K. 2017. Supplementation of inorganic and organic zinc mixtures in feed of Boerka goats fed by oil palm fronds. JITV 22(2): 51-56. DOI: http://dx.doi.org/10.14334/jitv.v22i2.1798

Zinc is an element of many enzymes and hormones having very important physiological functions in the body so that it influences the production and reproduction of animals including the activity of the rumen microflora in degrading fiber in a diet. The aim of this study was to improve the performances of goats offered oil palm fronds based diets through the supplementation of 35 ppm of Zn in the form of inorganic (ZnO) and organic zinc (Zn-methionine). Thirty mature male crossing Boer x Kacang (Boerka) goats were divided into five groups and randomly allocated to one of the five feed treatments as follows: P1: complete feed based on the palm oil fronds (Control), P2: P1 + 35 ppm Zn (100% Zn0), P3: P1 + 35 ppm Zn (75% Zn0 + 25% Zn-methionine), P4: P1 + 35 ppm Zn (50% Zn0 + 50% Zn-methionine), P5: P1 + 35 ppm Zn (25% Zn0 + 75% Zn-methionine). The experiment was conducted in a Completely Randomized Design of six replications. Increasing the proportion of Zn methionine in the mixtures elevated feed consumption, and the highest feed intake was observed in goats received 75% Zn-methionine/25% ZnO. Daily body weight gains was only affected (P<0.05) by the 75% Zn-methionine/25% ZnO supplement. The concentration of Zn in the blood increased significantly (P<0.05) when Zn methionine was added and it increased steadily as the proportion of Zn methionine greater in the mixtures, but the VFA compositions of the rumen were not affected (P>0.05) by Zn supplementation. It is concluded that the performances of goat fed complete diets based on the oil palm fronds could be improved by supplementation of inorganic and organic Zn mixture.

Key Words: Goats, Zinc, Supplementation, Palm Oil Fronds

INTRODUCTION

Zinc (Zn) as a component of various enzymes or hormones plays crucial physiological role in the body influencing production and reproduction of animal (Supriyati 2013). It is also an enzyme component, plays a role in antioxidant system of body to eliminate volatile radicals resulted from metabolism process (Flora 2009; Zhao et al. 2014). Zinc is the second most element of micro mineral in the body, but cannot be saved in the body tissue, so that it should be available every time through the diet to meet physiological requirement of the animal (Zalewski et al. 2005; Swain et al. 2016). Maost Zn in the forage is distributed to cell

wall (Whitehead et al. 1985; Cheng et al. 2012), so that influencing its availability for ruminant (Spears 2003). The assurance of adequate Zn intake is crucial in order to optimize the activity of fiber breaker enzyme in the rumen as the Zn function as cofactor. This role is more relevant in feeding system which uses oil palm frond as a basic diet consisting of high fiber with cellulose content of 49.8% (Izzuddin 2008).

Inorganic Zn in the form of oxide zinc (ZnO) or sulfate zinc ($ZnSO_4$) has been used as supplement for ruminant and those two inorganic zincs forms were reported having relatively comparable with the availability level (Jia et al. 2009; Jia et al. 2008). Organic Zn in the form of Zn-methionine as methyl (CH_3) contributor is important in the DNA transcription and translation process. However, the use of organic Zn in ruminant diet is relatively limited due to higher price compared to the inorganic Zn. On the other hand, the inorganic Zn availability is limited and indicating environmental pollution resulted from high dose requirement (Feng et al. 2009). So, this study was aimed to improve the performance of goat offered oil palm frond-based diet through Zn supplementation.

MATERIALS AND METHODS

Experimental animal and diet

As much of 30 mature males crossing Boer x Kacang (Boerka) goats aged 9-11 months with the average body weight of 24.56±3.01 kg were used in this study. Those goats were weighed then divided into five groups of five treatments by supplementing 35 ppm Zn that consisted of inorganic Zn (ZnO) and organic Zn (Zn-methionine) as below:

P1: ZSAZxzspalm oil frond-based complete feed (Control)
P2: P1 + 35 ppm Zn (100% ZnO)
P3: P1 + 35 ppm Zn (75% ZnO + 25% Zn-methionine)
P4: P1 + 35 ppm Zn (50% ZnO + 50% Zn-methionine)
P5: P1 + 35 ppm Zn (25% ZnO + 75% Zn-methionine)

The goats were reared in the individual metabolic cage and treated with anti-parasite worm medicine. The complete feed consisted of Crude Protein by 17.9% and Digested Energy by 2630 Kcal/kg Dry Material (Table 1). Palm oil frond was used as fiber source in the complete feed by 30%. The wheat of *Indigofera zollingeriana* leaf and soybean meal used as protein source. The ingredients were evenly mixed as complete feed. Zn supplement was processed by mixing the inorganic Zn (commercial) and organic Zn (Zn-methionine) produced in Balai penelitian Ternak, Ciawi in the different ratio and same Zn concentration (35 ppm). Zn supplement was then mixed with corn wheat as carrier (25 g) to ease the way of consumption. Zn supplement was fed in the morning and must be sure to be consumed. Complete feed then was provided *ad libitum* in the morning (08.30 am) and evening (02:00 pm)

The goat was fed with the diet and the left over diet were weighed every day to calculate daily consumption. The drinking water was given *ad libitum*. Goats were weighed every two weeks for 10 weeks to evaluate its body weight gain.

Table 1. Completed diet used in this research

Feed ingredients	Proportion (% BK)
Indigofera zollingeriana leaf meal	20.0
Cake palm cake	19.0
Molasses	5.0
Soybean meal	24.0
Bone meal	1.0
Palm oil frond	30.0
Chemical composition[a]	
Crude Protein (% DM)	17.9
Digested energy (Kkal/kg DM)	2630

[a]Counted.

Sample collection and analysis

To evaluate the volatile fatty acid in the rumen, rumen liquid sample was collected using a tube inserted into rumen through esophagus of each goat. Samples were collected five hours after feeding at the end of feed test. Rumen liquid then was filtered using 4 layers of filter cloth and directly centrifuged (10,000 x *g*) for five minutes. The filtrate was then stored in a refrigerator (-20°C) for further analysis. The VFA was analyzed using a Gas Chromatography. Blood sample (10 ml) was collected from jugular vessel at the time rumen liquid collection. The sample then was separated by centrifuging at 1500 x g 40°C for 20 minutes. The plasma was then transferred into labeled tube and stored at -20°C until further analysis.

Statistical analysis

This study was conducted under the Completely Randomized Design with five treatments and six repetitions. Data were analyzed using analysis of variance by SAS (2001). When there was a treatment effect, then the Duncan test was applied (Gomez & Gomez 1984). The mathematical model used was:

$$Y_{ij} = \mu + \tau_i + e_{ij}$$

Where:
Y_{ii} is variable response measured by the treatment-i (i=1-5), μ was general average value and the e_{ij} is error random

RESULTS AND DISCUSSION

Feed consumption and body weight gain

Feed consumption (dry material) with inorganic Zn supplementation was not different (P>0.05) from the control (P1 vs. P2), even increased numerically (Table 2). Increasing organic Zn proportion tended to increase feed consumption and the highest consumption was on the administration of inorganic and organic zinc mixtures by 25/75 of ration. The increase of feed consumption was suspected to relate to more Zn availability in rumen needed for enzymatic fiber degradation. One of restriction factors of consumption is the number and level of digestible fiber (NDF) in the rumen (Harper & McNeill 2015). Consumption level of DM on the whole treatments was around 3.46-4.2% of body weight and was normal. This shows that complete feed can be used to improve palatability of oil palm fronds when it is supplemented as low basal diet (*cafetaria style*). The oil palm frond as fiber source is crucial to assure optimal rumen function.

The effect of Zn supplementation to daily weight gain (DWG) of goat was shown in the Table 2. The highest DWG (P<0.05) was from P5, while the DWG was not significantly different (P>0.05) among the control (P1) and P2, P3 and P4. This shows that increase of consumption of diet supplemented by Zn is expressed linearly with the body weight gain only on

Table 2. Consumption of completed diet and daily body weight gain of Boerka goat offered by inorganic and organic zinc mixtures with different ratio

Parameter	P1	P2	P3	P 4	P5
Consumption:					
DM (g/d)	857.8±78.5^{a}	906.5±85.1^{ab}	913.3±65.7^{b}	1025.6±81.5^{bc}	1098.3±89.7^{c}
DM (g/kg BW)	34.61±4.7^{a}	37.27±4.67^{ab}	38.13±9.59^{b}	38.47±0.44^{bc}	42.11±0.37^{c}
DM (% BW)	3.46±0.37^{a}	3.73±0.23^{ab}	3.81±0.84^{b}	3.84±0.78^{bc}	4.2±0.51^{c}
Body Weight:					
Initial (kg)	23.78	23.40	23.44	25.64	24.94
Final (kg)	25.78	25.24	24.46	27.68	27.22
PBBH (g)	54.57±2.19^{a}	52.57±2.37^{a}	57.71±2.87^{a}	58.29±3.21^{a}	65.14±2.93^{b}

P1 = Control (without supplementation)
P2 = P1 + 35 ppm Zn (100% Zn0)
P3 = P1 + 35 ppm Zn (75% Zn0 + 25% Zn-methionine)
P4 = P1 + 35 ppm Zn (50% Zn0 + 50% Zn-methionine)
P5 = P1 + 35 ppm Zn (25% Zn0 + 75% Zn-methionine)
BW= Body Weight

the diet with the highest organic Zn composition. The average body weight gain of goat on the whole treatments was around 52-65g and categorized into moderate. Jia et al. (2009) fed Z-methionine on goat by 20 ppm and resulted daily weight gain by 42.7 kg. Zn-methionine supplementation on lamb as much as 30 and 60 ppm resulted in daily weight gain each by 45 g and 55 g (Haryanto et al. 2005). Compared other study, the daily weight gain in this study indicated that the inorganic and organic zinc mixtures supplementation in the form of Zn-methionine and ZnO could be added without reducing the growth response.

Blood Zn Concentration and Volatile Fatty Acid of Rumen Liquid

The effect of Zn supplementation on blood Zn content was shown in Figure 1. Blood Zn concentration of the control group without Zn supplementation (P1) was not different (P>0.05) compared to the one on the 100% inorganic Zn (ZnO) supplementation (P2). Supplementation of inorganic and organic zinc mixtures increased Zn content in the blood (P<0.05). This is proved that organic increased Zn availability was better than the inorganic Zn. Supplementation of 25%

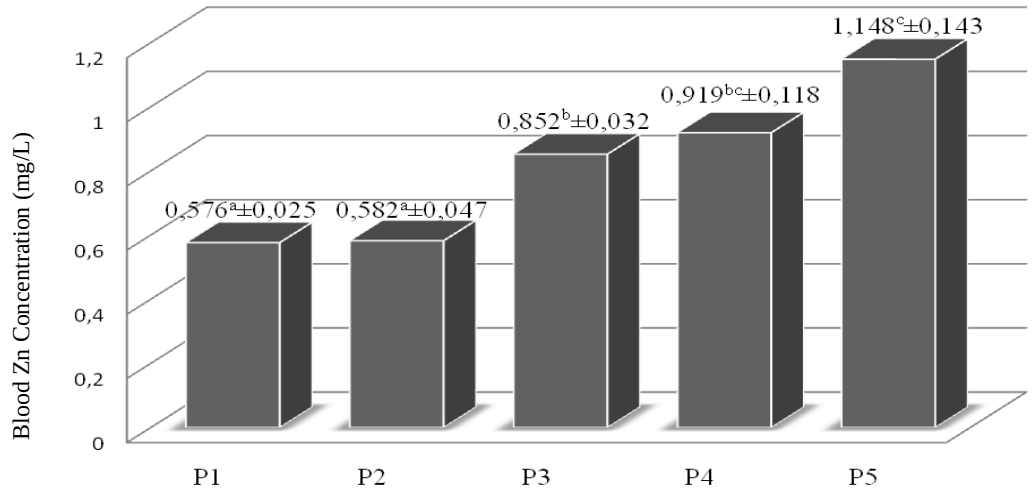

Figure 1. Effect of organic/inorganic ZN to Boerka goat blood Zn concentration offered by oil palm frond as basal diet.
P1 = Control (without supplementation);
P2 = P1 + 35 ppm Zn (100% Zn0);
P3 = P1 + 35 ppm Zn (75% Zn0 + 25% Zn-methionine);
P4 = P1 + 35 ppm Zn (50% Zn0 + 50% Zn-methionine);
P5 = P1 + 35 ppm Zn (25% Zn0 + 75% Zn-methionine).
Different superscripts in the same column show significant (P<0.05).

Table 3. Volatile amino acid concentration of goat (mM) without Zn or mixed-organic and inorganic Zn with different proportion

Volatile amino acid (mM)	P1	P2	P3	P 4	P5
C_2	25.50±4.63	27.37±5.04	27.65±4.38	21.08±2.51	24.34±1.88
C_3	9.78±1.46	9.73±2.42	11.93±3.34	8.73±0.76	7.06±2.50
iC_4	1.65±0.08	1.61±0.25	1.92±0.64	1.98±1.11	1.21±0.35
nC_4	5.12±0.67	5.93±1.82	6.85±1.21	4.31±0.27	4.0±1.71
iC_5	2.28±0.47	1.84±0.56	2.43±1.21	2.07±1.08	1.44±0.43
nC_5	0.72±0.09	0.71±0.15	0.79±0.19	0.60±0.16	0.50±0.17

P1 = Control (without supplementation)
P2 = P1 + 35 ppm Zn (100% Zn0)
P3 = P1 + 35 ppm Zn (75% Zn0 + 25% Zn-methionine)
P4 = P1 + 35 ppm Zn (50% Zn0 + 50% Zn-methionine)
P5 = P1 + 35 ppm Zn (25% Zn0 + 75% Zn-methionine)

inorganic Zn and 75% organic Zn resulted in the highest content of Zn in the blood (1148 mg/L) and it is in accordance with the study of Jia et al. (2012) namely 1.17 mg/L Zn in the goat blood supplemented by 100% Zn-methionine (20 ppm) or is lower compared to study of Aditia et al. (2014): 2.97 mg/L of Zn by supplementing 200 mg of Zn.

The effect of Zn supplementation to the concentration of some volatile fatty acids of goat rumen was shown in the Table 3. There was no effect (P>0.05) of treatments on the volatile fatty acid. Numerically, fatty acid (C2) concentration on the P1 was equal to the other groups. Numerically, concentration of propionate acid (C3), isobutirate acid (iC_4) and iso-valerate acid $i(C_5)$ were comparable with all treatments. Composition of acetic acid, propionate acid and butyrate acid were 21.08-25.5, 7.06-11.93 and 1.21-1.98 mM, respectively.

A research conducted by Supriyati et al. (2012) showed an increase on fiber digestibility (neutral detergent fiber and acid detergent fiber) by addition of bio-complex Zn into the goat diet. Jia et al. (2012) reported that the effect of Zn-methionine only influenced digestibility of acid detergent fiber and not on the neutral detergent fiber or dry material digestibility. In this study, there was no difference in volatile fatty acid composition that indicates the Zn supplementation used did not influence fiber digestibility.

CONCLUSION

Supplementation of 35 ppm Zn on oil palm frond for goat based diet did not increase the goat performance. Inorganic and organic zinc mixtures supplementation increased consumption, body weight gain and Zn concentration in the blood but did not affect the volatile fatty acid composition in rumen. An improvement of goat performance was in line with the higher Zn-methionine proportion in the Zn mixture supplement.

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Ultrasonography of Udder Parenchymal Tissue of Murrah and Swamp Buffalo Calves

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ABSTRAK

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Pemeriksaan organ ambing melalui parameter berupa parenkim ambing (PAR) dan bantalan lemak ambing (MFP) dapat dilakukan untuk memantau status kesehatan dan perkembangan kelenjar ambing. Penelitian ini dilakukan untuk memantau PAR dan MFP pada ambing ternak kerbau jenis Murrah dan Rawa menggunakan ultrasonografi mode-brightness secara transkutan. Sebanyak 8 ekor kerbau berjenis Murrah (n= 4) dan Rawa (n= 4) berumur 6 bulan yang dipelihara di Balai Penelitian Ternak (Balitnak) Ciawi Bogor digunakan dalam penelitian ini. Pencitraan ultrasonografi dilakukan pada pangkal puting ambing dan dilakukan pada ke empat kuartir ambing yaitu kiri depan, kiri belakang, kanan depan, dan kanan belakang. Citra ultrasonografi di evaluasi dan di nilai pada skor, ekogenitas dan luasan PAR dalam MFP. Hasil pencitraan menunjukkan bahwa sonogram PAR tampak anekoik hingga hipoekoik, sedangkan MFP tampak hipoekoik hingga hiperekoik. Meskipun terdapat variasi diantara kuartir ambing, nilai skor dan ekogenitas serta luas PAR dalam MFP pada kerbau Murrah memiliki nilai yang lebih tinggi dibandingkan dengan kerbau Rawa (P>0.05).

Kata Kunci: Ambing, Anak Kerbau, Parenkim Ambing (PAR), Bantalan Lemak Ambing (MFP), Ultrasonografi

ABSTRACT

Ulum MF, Raudlowi H, Krisnan R. 2017. Ultrasonography of udder parenchymal tissue of Murrah and Swamp buffalo calves. JITV 22(2): 57-62. DOI: http://dx.doi.org/10.14334/jitv.v22i2.1629

The examination of udder through a parameter such as parenchymal tissue (PAR) and mammary fat pads (MFP) can be used to evaluate health status and the development of mammary gland. This research was conducted to evaluate the PAR and MFP of Murrah (n=4) and Swamp (n=4) buffalo heifer calve udder using brightness-mode ultrasonography transcutaneously. Eight buffalo consisted of Murrah (n=4) and Swamp (n=4) buffalo aged of six months old reared at Indonesian Research Institute for Animal Production, Ciawi, Bogor were used this study. Ultrasonography imaging was performed on the udder nipple for the four quarters: left front, left back, right front, and right back. The ultrasonography image was evaluated and then assessed for score, echogenicity and PAR area on MFP. The results showed that sonogram PAR was aniconic to hypoechoic, while the MFP was hypoechoic to hyperechoic. Even though there was a variation in the four quarters of udder, Murrah buffalo calve had the highest (P>0.05) value of score, echogenicity, and PAR area compared to the Swamp buffalo.

Key Words: Udder, Buffalo Calve, Udder Parenchymal (PAR), Mammary Fat Pads (MFP), Ultrasonography

INTRODUCTION

Milk production in Indonesia needs to be increased to fulfill milk protein requirement. Domestic milk production only meets 30% (0.9 million ton) of total requirement, therefore an importation is conducted to overcome the shortcomings (DGLAH 2017). Generally, milk is produced by milk producer livestock such as dairy cattle, buffalo, dairy goat, dairy sheep, horse and camel. The main milk producer livestock in Indonesia is crossed Friesian Holstein (FH) cattle. Other livestock animal that can be used as milk producer is buffalo. The current milk production of buffalo is 0.5-2.25 kg/head/day (Wirdahayati 2007), while the milk production of FH cattle reaches 14.08 kg/head/day (Awan et al. 2016).

Indonesia has buffalo rearing center in many areas with the population of 1.3 million heads and the growth rate by 3.14% per year (DGLAH 2014). Almost 95% of the population is the swamp buffalo with a varied diversity on color, size, and behavior; and the 5% is the river buffalo (Dudi 2007). Commonly, the swamp buffalo is dairy buffalo, but its development is limited leads to the low production. The Province of West Sumatera is one of the areas that produces milked and processed buffalo milk for a long time (Ibrahim 2008).

However, its rearing in conventional technique. Therefore, a study examination of buffalo as milk producer needs to be conducted.

Selection of the superior cow and bull has a crucial role in improving the productivity of dairy buffalo. Suhardono (2004) suggested that the best selection of cow and bull respectively was 50% and 20% of the offspring to create seedlings with good milk production. Milk is produced by mammary gland consisting of udder parenchymal (PAR) and mammary fat pads (MFP). Udder examination for the PAR and MFP can be done to evaluate the health and development of udder. The PAR and MFP parameters can be examined using ultrasonography imaging since early stage at the 2 months of age (Esselburn et al. 2015). The early evaluation of udder quality needs to be conducted to evaluate the potential quality of mammary gland. However, there is no report of ultrasonography evaluation on the buffalo mammary in Indonesia. This study was conducted to evaluate the PAR and MFP of Murrah and Swamp heifer calves mammary using brightness ultrasonography method.

MATERIALS AND METHODS

Experimental animals

As much as eight of six months Murah (n= 4) and Swamp (n= 4) heifer calves buffalo reared at Indonesian Research Institute for Animal Production, Ciawi, Bogor were used in this research.

Ultrasonography imaging

Ultrasonography imaging of mammary gland transcutant was performed using ultrasonography portable Chison Q8® (PT Mega Utama Medica, Indonesia). The calves were handled manually in the cage without sedation (Figure 1). Ultrasonography gel was applied evenly on the udder surface. The transducer was firmly affixed to the udder to result in the good image. Ultrasonography imaging was performed started from the back of the nipple base on the four quarters. Imaging method followed the procedure of Nishimura et al. (2011) and Albino et al. (2015) for dairy cattle. The sonogram was caught from the transversal point of view using transducer multi linear frequency of 7.5-15 MHz. The imaging angle was conducted in the ventrodorsal direction of the body so that the skin, PAR and MFP tissue were well visible and could be clearly observed in sonograms. The sonogram was saved in the form of BMP for further analysis using ImageJ® software (NIH, USA).

The sonogram obtained was assessed descriptively for PAR and MFP. Further analysis of the score, echogenicity and area of each PAR and MFP used ImageJ® software (NIH, USA). One way ANOVA and Duncan post hoc test with 95% of the confidential interval used SPSS v. 16.0 SPSS Inc., USA) software to determine the difference of the parameter assessed. The value of $P<0.05$ showed a significant difference between the group.

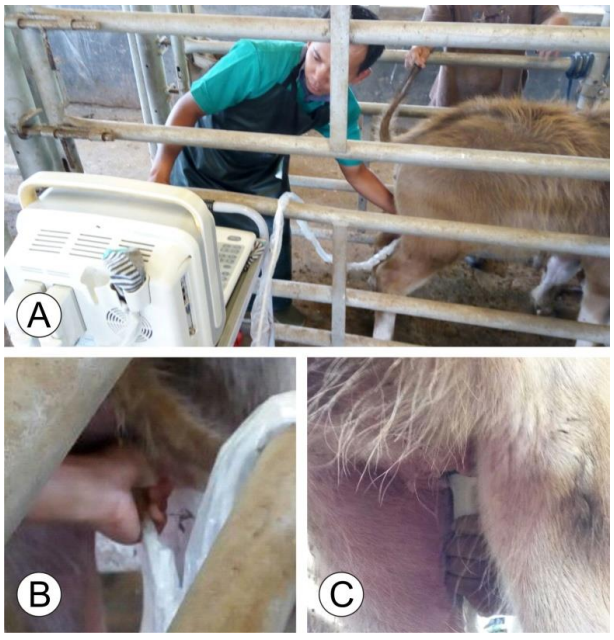

Figure 1. Ultrasonography imaging of parenchymal tissue (PAR) and mammary fat pads (MFP) of buffalo calve udder. (A) Side view, (B) back view, (C) front view.

RESULTS AND DISCUSSION

The sonogram showed different udder from each individual or each quarter (Figure 2). The structure of tissue showed on the sonogram from the outside to the inside was skin, subcutaneous, parenchyma, fat pads, and peritoneal cavities. Echogenicity of the skin was hypoechoic to hyperechoic. Then the subcutaneous tissue under the skin has the similar echogenicity: hypoechoic to hyperechoic. The barrier between subcutaneous and muscle was fascia that looks like hyperechoic line. Udder parenchyma was in the subcutaneous area surrounded by the mammary fat pads. Udder parenchyma was anechoic to hypoechoic, while the mammary fat pad was hypoechoic to hyperechoic. The deeper image of sonogram was anechoic as a peritoneum space.

Figure 3 shows udder parenchymal (PAR) and mammary fat pad (MFP) scores of each quarter of buffalo calve udder. The PAR and MFP scores on the four quarter of those two buffalo calve udders had a various score. The Murrah buffalo calve had the highest average PAR scores than the Swamp buffalo, while the highest MFP scores was on the Murrah buffalo calve.

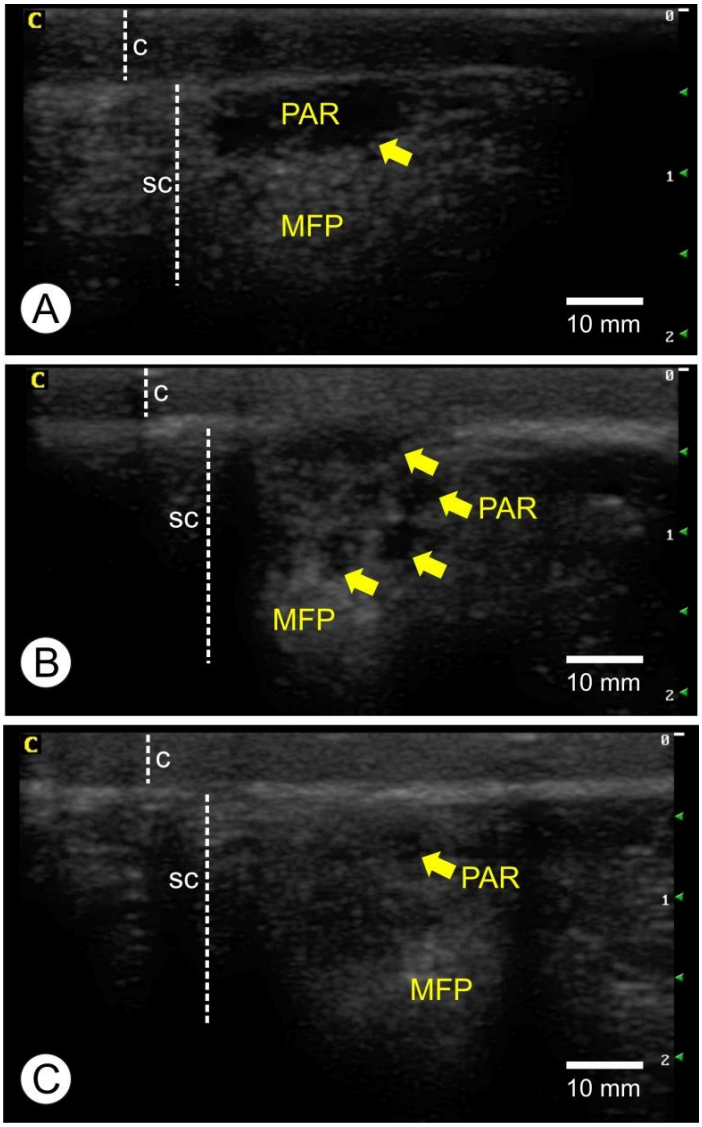

Figure 2. Sonogram of buffalo calves udder with the parenchyma tissue (PAR) showed by the arrow and mammary fat pads (MFP) under skin © in the subcutaneous area (sc).
(A) Murrah calves udder of the front left quarter with wide PAR
(B) Rawa calve udder of right back quarter with medium PAR size
(C) Swamp calve udder of right back quarter with small PAR size.
Note: the arrow (➡) shows the PAR in the MFP..

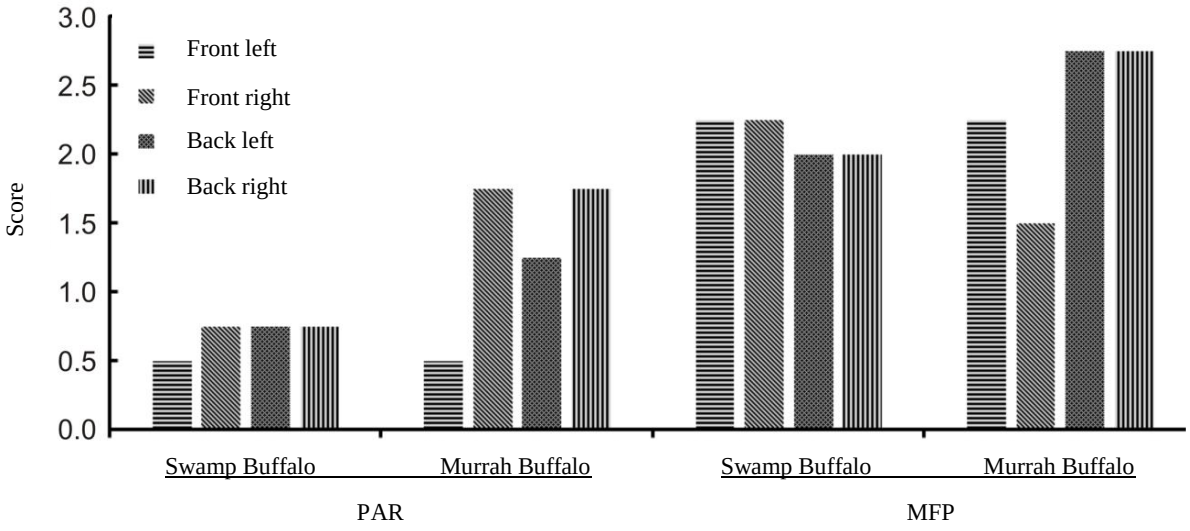

Figure 3. The average score of udder parenchymal (PAR) and mammary fat pads (MFP) of each quarter of buffalo calve udder. Score: <1=very small PAR size/thin MFP, 1-<2=small PAR/medium MFP, ≥2= medium PAR/thick MFP.

Table 1. Sonogram echogenicity of parenchyma (PAR) and mammary fat pads (MFP) of each quarter of buffalo calve udder

Buffalo	Quarter of Udder			
	Front left	Front right	Back left	Back right
	Parenchymal (PAR) (a.u.)			
Swamp	18.44 ± 3.48[a]	21.96 ± 2.30[a]	24.34 ± 6.14[a]	24.70 ± 4.11[a]
Murrah	22.89 ± 10.60[a]	29.64 ± 9.23[a]	33.00 ± 12.75[a]	20.09 ± 5.12[a]
	Mammary Fat Pads (MFP) (a.u.)			
Swamp	44.39 ± 10.63[a]	47.21 ± 2.46[a]	49.84 ± 4.75[a]	40.87 ± 6.25[a]
Murrah	46.46 ± 8.26[a]	51.00 ± 9.18[a]	48.33 ± 17.33[a]	47.73 ± 6.93[a]

Data are presented in the form of average and standard deviation (x ± SD). The different superscript shows a significant difference (P<0.05).

Table 1 shows the echogenicity of the sonogram of the PAR and MFP of each quarter of udder. The PAR echogenicity was lower than the MFP both on the Swamp and Murrah buffalo claves. The Murrah buffalo calve had higher PAR average and MFP echogenicity than the Swamp buffalo clave. The PAR and MFP of each quarter of udder had various echogenicity even though the statistical test showed that the PAR and MFP echogenicity of each quarter did not significantly different (P>0.05).

Analysis of the PAR and MFP area of each quarter was presented in Table 2. The PAR and MFP area were similar in each quarter and two breeds. The Murrah buffalo calve had wider PAR area than the Swamp buffalo calve on each quarter. The back quarter of udder had wider average PAR than the front quarter. Whereas, the right and left quarter had similar PAR area. Statistical test showed that the PAR area of each quarter of the two breeds did not significantly different (P>0.05).

This research successfully showed the difference of sonogram of each quarter (Figure 1). The tissue structure of superficial ultrasonography imaging was: skin, subcutaneous, fat pads, parenchyma and peritoneal space. The skin consists of 2 layers: epidermis that is hyperechoic and dermis that is hypoechoic (Wortsman & Navarrete 2017). The echogenicity of epidermis depends on the thickness of the stratum corneum, while the dermis echogenicity depends on the number of collagen fibers and the intracellular matrix (Szymanska et al. 2000). The subcutaneous layer appeared on the sonogram under the skin (Figure 2). The echogenicity of subcutaneous layer of udder was hyperechoic, because it consists of soft tissue and lots of fat tissue (Szymanska et al. 2000; Nishimura et al. 2011).

Table 2. Parenchymal tissue (PAR) area and mammary fat pads (MFP) of each quarter of buffalo calve udder

Buffalo	Quarter of Udder			
	Front left	Front right	Back left	Back right
	Parenchymal (PAR) (cm^2)			
Swamp	0.267 ± 0.079^a	0.253 ± 0.146^a	0.201 ± 0.040^a	0.256 ± 0.130^a
Murrah	0.273 ± 0.150^a	0.297 ± 0.225^a	0.419 ± 0.042^a	0.416 ± 0.234^a

Description: Data were presented in the form of average and standard deviation (x ± SD).
The different superscript shows a significant difference (P<0.05).

The mammary gland consists of parenchymal tissue as the main milk producer tissue and fat pad that supports the position of parenchymal tissue (Esselburn et al. 2015). Nishimura *et al.* (2011) reported that the mammary gland of two months calves was clearly oval and hypoechoic, while the PAR of five months calves was irregular in shapes and sometimes just looked like an anechoic canal and the increasing of echogenicity. This study shows that the development of mammary gland can be evaluated through the increase of the PAR echogenicity (Table 1). The older the animal, the PAR shape will expand, thicken and create branches (Akers et al. 2000). The increase of MFP echogenicity shows the thickness of fat tissue of the udder (Esselburn 2012). The thickness of MFP shows the improvement of the udder quality (Meyer et al. 2006).

The ultrasonography imaging score showed that the PAR and MFP of Murrah buffalo calve was higher than that of Swamp buffalo calve (Figure 3). Further analysis of the PAR area showed that the Murrah buffalo calves had wider area than the Swamp buffalo (Table 2). The higher score and wider area can be concluded that the quality and production of Murrah buffalo milk will be higher than the Swamp buffalo. Murrah buffalo is a dairy buffalo and is one of the river buffalos, while the Swamp buffalo is reared as working animal and meat producer, even in several areas it is also milked (Sutama 2008). The wide PAR area of buffalo calve can be predicted that it will result in development of huge cisterna udder. The huge cisterna udder will result in high milk production (Ayadi et al. 2002). The PAR area of the back udder was wider than the front. This result is similar to the research conducted by Esselburn et al. (2015) who reported that the ratio of front and back quarter area was 47:53.

CONCLUSION

An evaluation of parenchymal tissue (PAR) and mammary fat pads (MFP) of buffalo calve is easy to be performed using ultrasonography. The difference of parameter on the score, echogenicity and PAR area in MFP may be used to predict the potential of the quality of udder. Ultrasonography imaging showed a better udder quality of Murrah calve compared to the Swamp calve.

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The Growth of Local White Muscovy during Starter and Grower Periods

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ABSTRAK

Susanti T, Purba M. 2017. Pertumbuhan entog putih lokal periode starter dan grower. JITV 22(2): 63-67. DOI: http://dx.doi.org/10.14334/jitv.v22i2.1615

Ternak dengan pertumbuhan relatif cepat dan bobot badan yang besar memiliki potensi sebagai penghasil daging. Di Indonesia, entog lokal, terutama yang berbulu putih, merupakan salah satu ternak penghasil daging. Namun analisis terhadap pertumbuhannya masih jarang dilakukan. Oleh karena itu, tujuan penelitian ini adalah untuk menentukan titik infleksi sebagai peubah pertumbuhan, sehingga mempermudah penyusunan program pengembangan entog putih lokal. Sebanyak 168 ekor entog putih unsex diamati pertumbuhannya sejak umur DOD sampai 112 hari. Data pertumbuhan adalah bobot badan per individu yang diperoleh dari hasil penimbangan setiap 2 minggu. Analisa data dilakukan menggunakan kurva pertumbuhan model Gompertz. Hasil penelitian yang diperoleh adalah persamaan pertumbuhan entog putih lokal berdasarkan model Gompertz. Persamaan tersebut adalah Y=2591,3*exp(-3,8636*exp-0,0272*t). Berdasarkan persamaan tersebut, titik infleksi entog putih lokal terjadi pada umur 50 hari dengan bobot 953,29 g. Pertambahan bobot badan maksimal yang dapat dicapai adalah 2591,30 g. Dari penelitian ini dapat disimpulkan bahwa pertumbuhan entog putih lokal relatif lambat, namun bobot badannya relatif tinggi.

Kata Kunci: Entog Putih Lokal, Pertumbuhan, Periode Starter, Periode Grower

ABSTRACT

Susanti T, Purba M. 2017. The growth of local white muscovy growth during starter and grower periods. JITV 22(2): 63-67. DOI: http://dx.doi.org/10.14334/jitv.v22i2.1615

Livestock animals with relatively fast growth and great body weight are potential as a producer of meat. In Indonesia, the local muscovy, especially the feathered white is one of the meat-producing livestock. However, an analysis of the growth on local white muscovy is still rarely done. Therefore, the purpose of this study was to determine the point of inflection as growth variables, thus simplifying the preparation of development programs of local white muscovy. A total of 168 of the local unsex white muscovy were examined for their growth since the DOD until 112 days of age. The data of growth i.e. body weight individually examined in every two weeks. The data were analyzed using Gompertz model. The result obtained was the growth equation of local white Muscovy based on the model of Gompertz: Y=2591.3*exp (-3.8636*exp-0.0272*t). Based on these equations, the point of inflection of the local white muscovy occurred at the age of 50 days with a weight of 953.29 g. The maximum body weight gain achieved was 2591.30 g. It is conclude that the growth of local white muscovy was relatively slow, but the body weight was very heavy.

Key Words: Local White Muscovy, Growth, Starter Period, Grower Period

INTRODUCTION

Generally, local muscovy widely developed in Indonesia has various feather color of black and white. White muscovy relatively has high economy value in producing clean white meat as the preference of the consumer. White muscovy have the potential to be developed as meat-producing livestock.

Muscovy is well known as meat producer due to its relatively heavy body weight compared to other waterfowl. Baeza et al. (2002) reported that at 10 weeks of age, male muscovy was 1.700 ± 101.55 g/head of body weight with FCR value by 3.03 ± 0.21. Besides, the quality of muscovy meat is relatively unchanged on the population selected for body weight increases on 12 weeks of age. Even though, the muscovy have high potential of body weight, however its development program is rarely conducted, so it leads to decrease the population.

Growth is one of basic characteristic of biological system. It is an important characteristic that describe the genetic potential of an individual. Growth can be defined as the development of body size per time unit or also called as growth rate (Tompić et al. 2011). Based on the rate, the growth is divided into two phases: acceleration and retardation phases. On the acceleration phase, the growth is fast, so that the rate is high. In this case, metabolic process is more intensive than the catabolism. While, the growth on the retardation phase is slow due to the catabolism more active than the

anabolism. Those two phases are bordered by inflection point which is a condition where the acceleration growth phase is stop and start to the retardation phase. Consequently, growth curve will be sigmoid with the inflation point that describes the highest growth rate (maximum) and the time of the puberty (Brody 1945; Sengul & Kiraz 2005). Growth is highly influenced by species, breed and environment (Inounu et al. 2007).

Growth curve is very useful for analysis of production lifetime production efficiency which is useful for selection program to determine market strategy by optimizing the management and efficiency of livestock production related to body weight at the slaughtering age and feed management like administration of energy, protein and mineral at the right time (Vitezica et al. 2010; Darmani Kuhi et al.2010). A growth function must be able to describe data well and consisting of valuable biological parameter (France et al. 1996).

To describe sigmoid growth curve, non-linear regression can be used, such as Von Bertalanffy or Gompertz logistic model. A models to predict the growth pattern have been used on various different species: duck (Suparyanto et al. 2004; Schinckel et al. 2005; Vitezica et al. 2010), chicken (Rizzi et al. 2013), pig (Schinckel & Craig 2002), Garut sheep (Inounu et al. 2007) and turkey (Porter et al. 2010). Among those models, Gompertz model has the highest accuracy and good biological interpretation, especially on presuming the point and value of inflection (Rizzi et al. 2013; Darmani Kuhi et al. 2010; Sengul & Kiraz 2005; Nahashon et al. 2006; Roush et al. 2006). The evaluation of growth on body weight of local white muscovy starting from hatching to the adult age in this study used Gompertz model. The aim of this study was to determine the inflection point as growth variable of local white muscovy to do further breeding and raising program .

MATERIALS AND METHODS

The materials used in this study were 168 heads of unsexed local white muscovy. Those muscovy were confined in the same cage and feed conditions at the Indonesian Research Institute for Animal Production, Ciawi, Bogor. Brooder cages were used for the DOD until 4 weeks of age and litter by 1.5 x 2.5 m cages with covered barn were used from 4 weeks to age of reproduction.

Observation and analysis were performed to body weight of muscovy on the starter and grower period during April to August 2015. Starter period was started from the day old duck (DOD) until 56 days. Meanwhile, grower period was started from 57 days to 112 days of age. Body weight was examined by weighing individually muscovy every week of starter period and every 2 weeks in the grower period.

Data analysis was conducted on age and body weight. Suparyanto et al. (2004) hypothesized the growth of duck using non-linear regression equation of Gompertz model by Marquardt procedure, which predicts easily the value on every iteration process can be determined easily.

The equationtion of Gompertz model was as follows:

$$Y = A*Exp^{[-B*Exp(-kt)]}$$

where:

A = Body weight (asymptote), ie at the value of t approaching infinity.

Exp= Basic logarithm (2,71828)

k = Average growth rate until adult age.

Y = Animal body weight at t time

t = Time unit (day)

Statistical analysis was conducted using SAS program (2002). To obtain estimated value when the inflection point happen of a growth curve, it was used a equation of Blasco et al. (2002) who suggested ~~said~~ that inflection point is the second derivative of a nonlinear equation. The notation to estimate the inflection point of age was t_i and for the inflection point of body weight was y_i. Its mathematical equation (Suparyanto et al. 2001) was:

$$t_i = \ln B/k$$

$$y_i = A\ e^{-1}$$

Inflection point is maximum estimation point of life weight development. At that point, there is a shift in change from the growth acceleration to the retardation growth, which means a point where the animal experiences puberty (Brody 1945). The time of inflection is the most economic period, because at this point the mortality value is the lowest with fastest growth. Inflection point is difficult to be determined biologically, however, with the non-linear of growth curve it can be solved (Inounu et al. 2007).

RESULT AND DISCUSSION

Analysis results of body weight using the Gompertz equation and the value of each notation on that equation was presented in the Table 1. Those notations consisted of asymptote value that means maximal life weight increase reachs by the white muscovy, the value of B as integral constantan and the value of k that showed growth rate to adult weight (Suparyanto et al. 2004).

Table 1. Asymptote value (A), B and k as growth variables of local white muscovy

Variable	Value
Asymptote (A)	2591.30 ± 75.0330
B	3.8636 ± 0.1537
K	0.0272 ± 0.00133
Equation	$Y=2591.3*\exp(^{-3.8636*\exp-0.0272*t})$

Description:
B = integral constantan
K = parameter shows growth rate to the adult weight

According to the value of the growth variable, the growth equation of the local white muscovy was: $Y=2591.3*\exp(^{-3.8636*\exp-0.0272*t})$. Asymptote value means maximum llife weight gain that can be reach by the local muscovy. According to that asymptote value, a maximal body weight gained by the local muscovy was 2591.3 g. This maximum body weight was relatively high compared to PMp duck that is also are the meat producer waterfowl. Susanti & Prasetyo (2014) reported that maximum body weight of PMp duck was 2083.3 g. However, the maximum body weight gain of local white muscovy was under the Peking duck by 3052.6 g (Susanti 2015). It is allegedly to be caused by the different waterfowl breed observed, because the A value is a property that is highly influenced by genetic factor (Brody 1945).

The B value was used to describe the relation of Y0 (initial weight) and t, especially on the growth curve of the Brody model (Inounu et al. 2007). Meanwhile, on the other models such as Gompertz model is only as an integral constant. The k value is not a genetic affects, but highly affected by the environment. Inounu et al. (2007) suggested that k value for sheep is influenced by the year of born, the age of parent and breeding season.

The asymptote B and k values showed the variable growth was an inflection point consisting of the age and weight of inflection from the derivative of equation function of the Gompertz curve. According to the Gompertz curve, it was obtained that the age and weight were the inflection point values that was used to estimate growth rate of local white muscovy (Table 2).

Table 2. Weight and time of inflection, and growth rate of local white muscovy

Variable	Value
Inflection weight (g)	953.29
Inflection age (day)	50.00
Growth rate (g/e/h)	19.07

Table 2, shows that inflection age of local white muscovy is 50 days with inflection weight of 953.29 g. The inflection age was relatively slow compared to local ducks. Susanti & Prasetyo (2014) reported that inflection age by 23 days for PMp duck as the meat producer duck, and 33 days for Alabio and Mojosari duck as layer duck, and 45.66 days for Peking duck (2015). Nahashon et al. (2006) reported that inflection age of mutiara chicken was 40.25 days for the male, and 40.18 days for the female. The infection weight of local white muscovy by 953.29 g was relatively heavy compared to inflection weight of Peking duck of 1122.99 g (Susanti 2015).

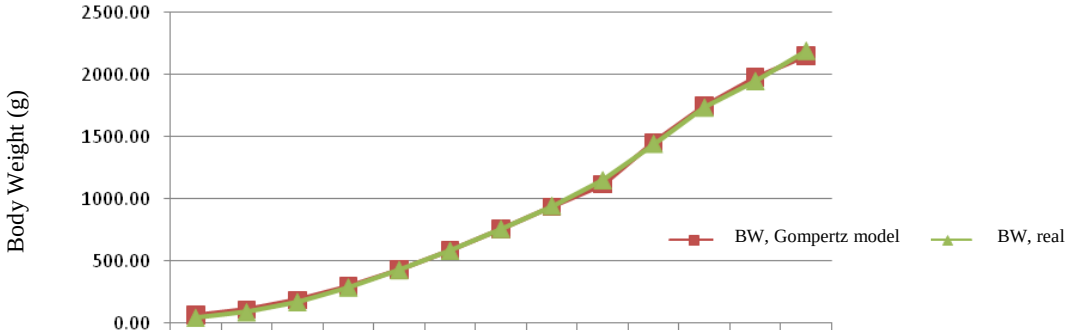
According to that inflection point, estimation of growth rate was 19.07 g/head/day. The growth rate of local white muscovy was relatively slow compared to the PMp or Peking ducks as meat producing animals. Susanti & Prasetyo (2014) reported the growth rate of PMp duck was 33.32 g/head/day. While, Susanti (2015) reported that growth rate of Peking duck was 24.60 g/head/day. Pingel (1993) suggested the selection of livestock animal that has a fast growth rate for optimum slaughtering weight in a relatively short period as expected by most farmers. Considering that fast growth livestock animals have good feed conversion and relatively low body fat content.

A determination value (R^2) showed a reliability of the an obtained model. The higher R2, the more the ability means the model to describe Y variable behavior (Mattjik & Sumertajaya 2000). In this case, Y is a growth value of estimation according to Gompertz model. The R^2 value in this study was 0.91079 and can be used to estimate a growth curve of local white muscovy according to Gompertz value.

The comparison of growth curve of Gompertz model and weighing data of this study is presented in the Figure 1. Figure 1 shows that growth curve estimation is sigmoid. There was a little difference in body weight at the beginning of growth, as shown that the body weight during day 1-7 was lighter than the Gompertz model. Whereas, the growth from the age of day 8 to 112 was similar.

CONCLUSION

High determination value (R^2) of 0.91079 proved that Gompertz model was able to predict the real growth of local white muscovy. The development of local white muscovy observed was relatively slow, but produced heavy body weight. Development of local white muscovy as meat producing animals obtained by crossing with other birds that have relatively fast growth, even though its body weight is relatively light.



	1	7	14	21	28	35	42	49	56	70	84	98	112
BW, Gompertz model	60.3	106.	184.	292.	426.	583.	755.	935.	1116	1457	1748	1980	2156
BW, real	44.5	88.9	171.	288.	428.	583.	758.	944.	1150	1444	1736	1950	2193

Age (days)

Figure 1. Growth rate of life body weight of local white muscovy in accordance to the Gompertz model and the real weighing data.

In accordance with the growth variables of local white muscovy, the inflection point at 50 days of age with body weight of 953.29 g, the growth rate was 19.07 days. A maximal body weight gain can reached to 2591.30 g.

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Qualitative and Quantitative Characteristics of Sensi-1 Agrinak Chicken

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ABSTRAK

Hasnelly, Iskandar S, Sartika T. 2017. Karakteristik kualitatif dan kuantitatif ayam SenSi-1 Agrinak. JITV 22(2): 68-79. DOI: http://dx.doi.org/10.14334/jitv.v22i2.1605

Salah satu galur ayam lokal pedaging yang dihasilkan Balai Penelitian Ternak (Balitnak) adalah Ayam Lokal SenSi-1 Agrinak. Galur baru ayam pedaging lokal ini telah dilepas dengan Surat Keputusan Menteri Pertanian No. 39/Kpts/PK.020/1/2017 pada tanggal 20 Januari 2017. SenSi-1 Agrinak diseleksi dari rumpun ayam lokal Sentul yang berasal dari Kabupaten Ciamis Jawa Barat. Kriteria seleksi berupa warna bulu abu polos, warna bulu pucak (putih bercak hitam) yang diaplikasikan terhadap jantan dan betinanya. Kriteria seleksi lain berupa jengger kacang (pea) dan bobot tubuh tertinggi 25% dari populasi per generasi ayam-ayam jantannya. Seleksi berlangsung selama enam generasi pada kondisi pemeliharaan intensif dengan ransum tunggal berkadar sekitar 17% protein kasar dan 2850 kkal ME/kg; kadar gizi lainnya mengikuti kadar yang disarankan untuk ayam ras White Leghorn. Pengukuran karakteristik kualitatif dan kuantitatif dilakukan pada sebanyak 499 ekor jantan dan 492 ekor betina SenSi-1 Agrinak Abu dan sebanyak 497 ekor jantan dan 492 ekor betina SenSi-1 Agrinak Pucak berumur 10-12 minggu dan 12 bulan. Hasil penelitian menunjukkan bahwa pada SenSi-1 Agrinak Abu, sebaran warna bulu abu pada jantan 55,51%, betina 60,77%; sebaran warna shank kuning jantan 52,51%, betina 33,33%; bentuk jengger kacang (pea) jantan 90.98 %, betina 89,23%. Pada SenSi-1 Agrinak Pucak, sebaran warna bulu pucak jantan 75,65%, betina 83,30%; sebaran warna shank kuning jantan 51,91%, betina 36,59%; sebaran jengger kacang jantan 91,55%, betina 92,28%. Rataan bobot badan SenSi-1 Agrinak berbulu Pucak Jantan 908,76+ 130,98 g/ekor betina 750,53+ 110,56 g/ekor. Rataan Bobot bobot badan SenSi-1 Agrinak berbulu Abu Jantan 886,38+142,93 g/ekor betina 739,17+ 118,87 g/ekor. Rataan bobot badan jantan terseleksi SenSi-1 Agrinak berbulu Pucak mencapai 1051+76g/ekor dan SenSi-1 Agrinak berbulu Abu 1015+107 g/ekor. Hasil penelitian ini dapat dijadikan sebagai dasar standarisasi ayam SenSi-1 Agrinak (Abu dan Pucak) sebagai *male line* ayam lokal pedaging.

Kata Kunci: Karakter, Sensi-1 Agrinak, Kualitatif, Kuantitatif

ABSTRACT

Hasnelly, Iskandar S, Sartika T. 2017. Qualitative and quantitative characteristics of SenSi-1 Agrinak chicken. JITV 22(2): 68-79. DOI: http://dx.doi.org/10.14334/jitv.v22i2.1605

One of local chicken breeds develop in Indonesian Research Institute for Animal Production (IRIAP) is local SenSi-1 Agrinak chicken. This new improved local-meat-type breed was released with Ministry Agriculture Decree Number 39/Kpts/PK.020/1/2017 on 20th January 2017. SenSi-1 Agrinak was originally selected from native Sentul chicken breed obtained from Ciamis district in West Java Province. Selection criteria were two feather colors of grey or black spotted white (pucak), which were applied to both males and females. Pea-comb type was also one criterion for males, applied at the age of 10 weeks. Live weight at the age of 10 weeks with the selection intensity of 25% was applied to each generation of males chicken. Selection proceeded for six generations. Selection program was carried out under standard feed formulae containing around 17% crude protein with 2850 kcal ME/ kg, and containing other nutrients following the ones recommended for modern chicken of White Leghorn. Observation was conducted on each of about 2000 young chickens of grey and of Pucak SenSi-1 Agrinak both males and females age of 10 and 84 weeks. Results showed that grey SenSi-1 Agrinak chicken in total population, had: i) Grey feather color distribution of 55.51% in males, and 60.77% in females; ii) Yellow shank color of 52.51% in males, and 33.33% in females; iii) Pea type comb of 90.98% in males, and 89.23% in females; iv). Ten weeks live weight of 886.38+142.93 g/bird in males, and 739.17+ 118.87 in females. Pucak SenSi-1 Agrinak chicken in total population, had: i) Pucak feather color of 75.65% in males, and 8330% in females; ii) Yellow shank color of 51.91% in males, and 36.59% in females; iii) Pea type comb of 91.55% in males, and 92.28% in females; iv) Ten weeks live weight of 908.76+ 130.98 g/bird in males, and 750.53+ 110.56 g/bird in females. Whilst for grey SenSi-1 Agrinak male chicken after selection had live weight at 10 weeks old of 1015+107 g/bird, and for Pucak SenSi-1 Agrinak male chicken was 1051+76 g/bird. This initial performance information for those two breeds of considerably improved local chicken can be used as the base of information for SenSi-1 Agrinak breed for male line of meat type of local chicken breeding.

Key Words: Qualitative Character, Quantitative Character, SenSi-1 Agrinak Chicken

INTRODUCTION

Indonesian native chicken apparently have species physical characteristic are grouped into at least 34 breeds or distinct groups of local chicken namely: Ayunai, Balenggek, Banten, Bangkok, Burgo, Bekisar, Cangehgar, Cemani, Ciparage, Gaok, Jepun, Kampung, Kasintu, Kedu (Black and White Kedu), Pelung, Lamba, Maleo, Melayu, Merawang, Nagrak, Nunukan, Nusa Penida, Olagan, Rintit or Walik, Sedayu, Sentul, Siem, Sumatera, Tolaki, Tukung, Wareng, Sabu, and Semau. Some of them are used for non-food purposes such as offerings for religious rituals, beauty – voice and feathers, and cock – fighting and indeed Kampung chickens are the most popular and kept almost throughout the entire country (Henuk & Bailey 2014). Among them, 18 breeds are most popular in Indonesia while 11 breeds are good meat and eggs producers (Agriflo 2013; Han 2014).

Sentul chicken is an Indonesian native genetic source from Ciamis District, Province of West Java. The chicken has high growth performance and egg production (dual-purpose) and unique phenotypic uniformity compared to another original and local chickens.

The Indonesian Research Institute for Animal Production (IRIAP) has created broiler native chicken through selection of the Sentul chicken since 2010. In 2016, the SenSi-1 Agrinak 6th generation chicken has produced superior breeding stock for male line of native chicken called SenSi-1 Agrinak through Decree of Minister of Agriculture of Indonesia Number 39/Kpts/PK.020/1/2017 on 20 January 2017. In the selection process, the chickens were divided into 2 groups based on their feather color: uniform grey color from the light gray to the dark grey and the Pucak feather (white feather with black spots by about 10-20%).

For the commercial scale, standardized qualitative and quantitative properties of the SenSi-1 Agrinak chicken are required. This study was conducted establish a baseline data on the characteristics of the SenSi-1 Agrinak chicken that may be used as a standard of the Sensi-1 Agrinak chicken.

MATERIALS AND METHODS

Released SenSi-1 Agrinak chicken in the 6th generation was sorted by their feather color:

1. SenSi-1 Agrinak with gray feather (499 heads male and 492 heads female)
2. SenSi-1 Agrinak with Pucak feather (497 heads male and 492 heads female)

The determination qualitative characteristic was based on the criteria described by Kusuma (2002) and Mansjoer (1981). Qualitative characteristics observed were the color of feather, the color of shank and shape of comb at the age of 10 weeks.

The body weight at 0-9 weeks was observed through the weighing 10% of the population every month. For the purpose of selection to the market demand (700-1000 g/head), individual weighing weight was conducted at the tenth week.

The quantitative characteristics (body size) observed were: adult body weight, length of beak, width of beak, length of head, width of head, length of neck, length of back, chest circumference, length of chest, length of upper thighs, length of lower thighs, length of shank of 12 months old hens.

The data of qualitative characteristics were analyzed using relative phenotypic frequency formula (percentage), while the data of quantitative characteristics were analyzed by simple descriptive statistics (average, maximum, minimum, standard deviation, coefficient of variant).

RESULTS AND DISCUSSION

Qualitative characteristics

Qualitative characteristics are crucial for the breeders as a trademark, so it is often considered in a breeding program. Those characteristics are controlled by one or more gens and slightly or not at all influenced by environment factor (Hardjosubroto 2002). Sidadolog (2011) suggested that qualitative characteristics such as the color of feather, type of comb and color of the shank are determined by one or more pair of major gen and will be inherited to its offspring. The qualitative inheritance always follows Mendel's law. Proportion variance was determined by the number of gen pairs and gen properties (dominant, intermediary, recessive, hypostatic epistasis, interaction) forming that property. Qualitative characteristics are unique traits used as a standard in dividing livestock animal type. The qualitative characteristics observed of the SenSi-1 chicken Agrinak in this study as male line of broiler chicken were the color of feather, type of comb and color of the shank.

Color of the feather

The color of feather of chicken is formed by pigment, physical structure or the combination of both (Natawiharja 2003). Colored feather is formed by gen (i), while the white feather of bird other than formed by inhibiting genes (I) toward the color pigment, is also formed by the absence of color pigmentation and does not have color gen (c) such as the albino chicken with white feather which is recessive to the colored feather gen (Hutt 1949).

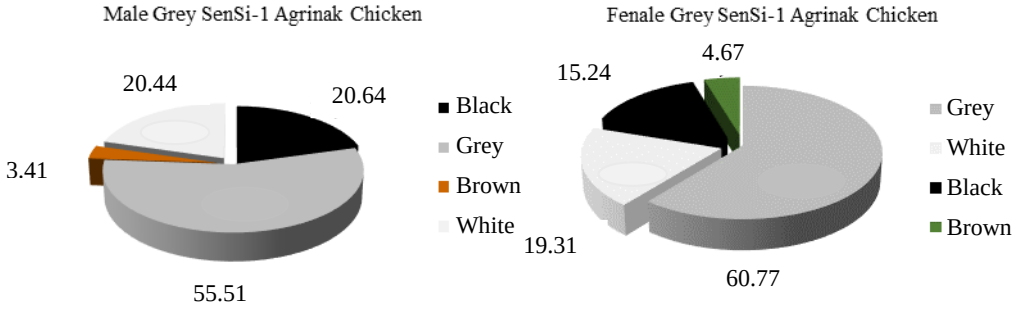

Figure 1. Color distribution of feather of male and female Grey SenSi-1 Agrinak chicken (%).

Figure 2. Grey SenSi-1 Agrinak chicken

The selected SenSi-1 Agrinak chickens were divided into two groups based on the color of feather: grey feather as Grey SenSi-1 Agrinak chicken and the white feather with blcak dots by 10-20% as Pucak SenSi-1 Agrinak chicken. Analysis results and the color of feather of SenSi-1 Agrinak chicken (Grey and Pucak) based on sex observed at 10 weeks old is presented in Figures 1-4.

Figure 1 shows that from 499 chickens observed, there are four feather colors of male Grey SenSi-1 Agrinak chickens: grey, black, golden brown and white with the percentage of 55.51%; 20.64%; 3.41% and 20.44% respectively. Meanwhile, from the 492 chickens observed in female Grey SenSi-1 Agrinak chicken, there are also four feather colors grey, black, golden brown and white with the percentage of 60.77%; 15.24%; 4.67% and 19.31% respectively.

The appearance of the four colors of feather in the Grey SenSi-1 Agrinak chicken is caused by certain pigments during their growth process. The pigments contained in the feather are lipoxrome and melanin (Natawiharja 2003). The golden old color of feather (s) is recessive to the silver color (S), plain feather (b) is recessive to the patterned feather (B). The S and s genes are adrift on the sex chromosome, as well as the B and b gens (Hutt 1949).

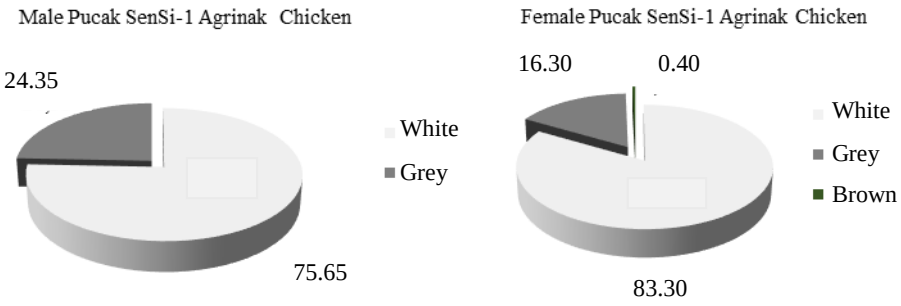

Figure 3. Color distribution of feather of male and female Pucak SenSi-1 Agrinak chicken (%).

Figure 4. Pucak SenSi-1 Agrinak Chicken.

Color of shank

Observation of the shank color was also performed at the 10 weeks old. Observation results of the color shank of the Grey and Pucak SenSi-1 Agrinak chicken based on sex are presented in Figure 5 and Figure 6.

Figure 5 shows that shank color from 499 male Grey SenSi-1 Agrinak chickens is yellow, black, green, grey, white and brown with the percentage of 52.51%; 24.05%; 9.42%; 15.14%; 7.24%; and 2.20% respectively. The shank color of 492 female grey SenSi-1 Agrinak chicken is yellow: 33.33%, black: 33.33%, grey: 17.89%, green: 12.60% and white: 2.85%.

Figure 6 shows that shank color of 497 male Pucak SenSi-1 Agrinak is yellow: 51.91%, green: 24.14%, grey: 15.14%, white: 7.24% and black: 0.20%. Then, from 492 female Pucak SenSi-1 Agrinak chickens, there are five shank colors: yellow: 36.79%, green: 39.02%, grey: 18.29%, white: 5.49%, and black: 0.41%.

The distribution of shank color of Grey and Pucak SenSi-1 Agrinak chickens both in the male and female was still varied (yellow, grey, black, green, brown and white). The large variation of shank color of the Grey and Pucak SenSi-1 Agrinak chicken has the great similarities with the most shank of the native chickens.

As the percentage of shank color distribution showedthat the yellow color was dominant. Yellow

shank in the American chicken and other breed is formed by fat or lipoxrom pigment in the epididymis layer and no melanin pigment in the epididymis and dermis layers. Melanin gen in the dermis layer is recessive (id) to the melanin pigmentation inhibitor gen, lipoxcrom (Id). The existence of the gen B in chicken may decrease the number of melanin pigment in the shank.

The yellow color of the shank of female chicken may be used to estimate egg production rate by looking at color change of shank. The lipoxcrop pigment in the shank is identical with the yellow pigment in the egg. So that, the shank color may be used as an indication of egg production rate of a chicken. Therefore, the shank color change may be used for layer chicken culling (Jull 1951).

The meat color of the Grey and Pucak SenSi-2 Agrinak chicken was dominant light. Skin meat color is closely related to the meat color. The light color of the Grey and Pucak SenSi-1 Agrinak chicken meat is m[illegible]y preferred by the consumer.

Comb type

The type of comb also becomes one unique characteristic of the SenSi-1 Agrinak chicken as the male line of native broiler chicken. The comb type of the SenSi-1 Agrinak chicken is a dominant pea. The Figure 7 and 8 shows the comb type of the SenSi-1 Agrinak chicken in this study.

Figure 7 shows the variance of the comb type of male Grey SenSi-1 Agrinak chicken 90.98% of pea and 9.02% of scomb. Whereas, in the female grey SenSi-1 Agrinak chicken, the pea type is 89.23%, and the scomb type is 10.77%. Figure 8 shows the dominant comb type of male Pucak SenSi-1 Agrinak chicken is the pea type of 91.55% with the recessive one is scomb of 8.45%. Whereas, the dominant comb type of the female Pucak SenSi-1 Agrinak chicken is the pea type as well of 92.28%, and 7.72% is the scomb type as the recessive

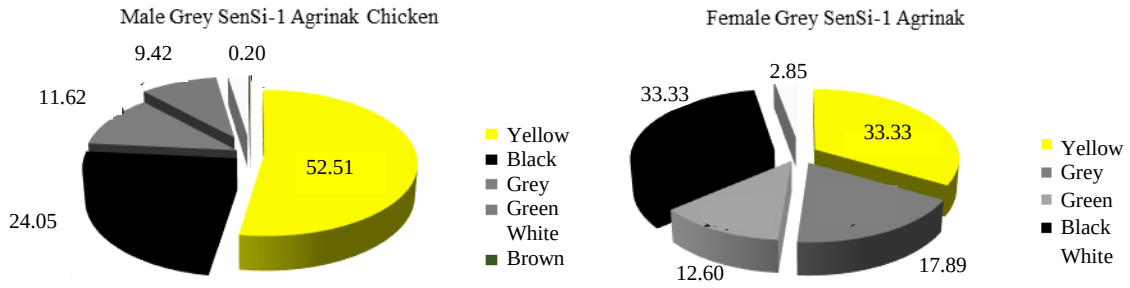

Figure 5. Color distribution of shank of male and female Grey SenSi-1 Agrinak chicken (%).

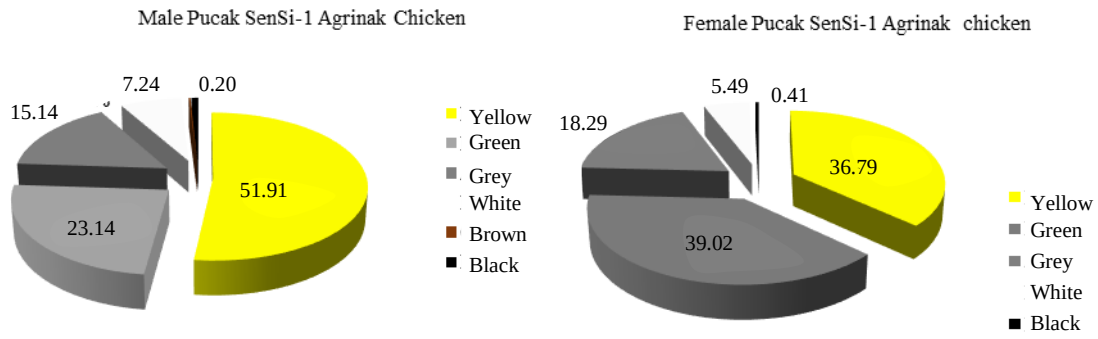

Figure 6. Color distribution of shank of male and female Pucak SenSi-1 Agrinak chicken (%).

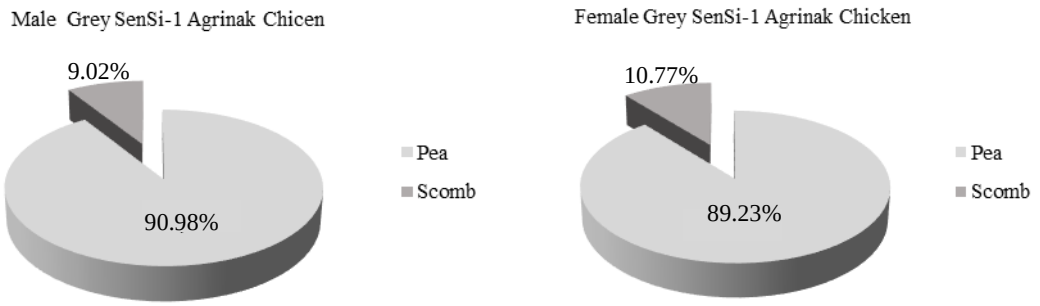

Figure 7. Distribution of comb type of the male and female Grey Sensi-1Agrinak chicken.

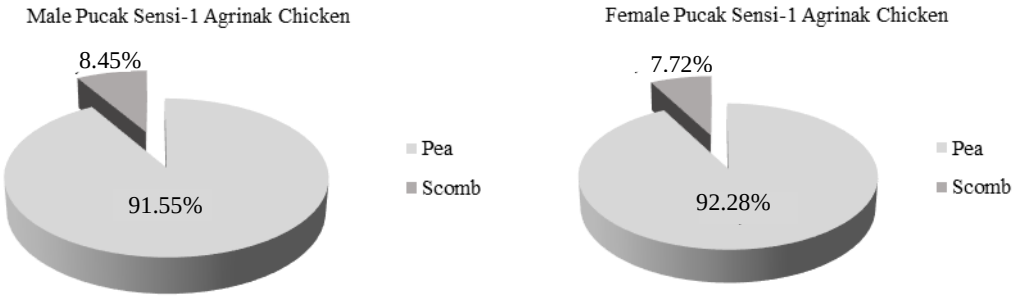

Figure 8. Distribution of comb type of the male and female Pucak Sensi-1Agrinak chicke

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Quantitative characteristics

Body weight at 0-9 weeks old

Average live weight at 0-9 weeks old is presented in Table 1, and based on the sex in Table 2.

The live weight observation was conducted for 10% of total population. Table 1 shows a normal growth performance indicated by the increase of live weight along with the age. The average live weight of Pucak SenSi-1 Agrinak chicken was higher of 745.66±68.75 g/head than the Grey SenSi-1 Agrinak chicken of 731.57±75.61 g/head.

High value of the coefficient of variance of the Grey SenSi-1 Agrinak chicken indicating the absence of uniformity on the growth in the population. Different from Grey SenSi-1 Agrinak chicken, the coefficient of variance value of Pucak Sensi-1 Agrinak (fewer than 15%) shows that uniformity on the growth of the male and female SenSi-1 Agrinak chicken appeared at 10 weeks old is uniform. Nasoetion (1992) said that uniform livestock animal population has coefficient of variance value of 5-15%. Then, Sidadolog (2011) suggested that common coefficient variance of native chicken is 25%. The body weight of Grey and Pucak SenSi-1 Agrinak chicken as the male line at the 6th generation is presented in Table 3.

Table 3 shows that the average live weight of selected male at 10 weeks old based on feather color variance was >1 kg. The average live weight of Pucak SenSi-1 Agrinak was 1051±107g/head from 103 head chicken, while the average live weight of Grey SenSi-1 Agrinak chicken was 1015±76g/head from the same number population with a coefficient of a variant of 7.22%-10.58%.

Table 1. Live weight of Grey and Pucak SenSi-1 Agrinak chicken at 0-9 weeks old

Variable	Age (week)									
	0	1	2	3	4	5	6	7	8	9
Live Weight of Grey SenSi-1 Agrinak										
Average (g/head)	29.72	51.42	86.38	128.79	182.14	266.66	366.52	467.40	612.27	731.57
Standard of Deviation (g/head)	2.97	9.18	22.71	15.78	20.87	46.15	44.51	57.06	58.38	75.61
Coefficient of Variance (%)	9.98	17.85	26.29	12.26	11.46	17.31	12.14	12.21	9.54	10.34
Minimum (g/head)	38.00	31.20	59.17	96.50	149.70	96.20	279.10	367.20	521.40	593.09
Maximum (g/head)	23.00	64.00	171.40	146.30	221.60	327.90	489.10	591.60	738.30	889.30
Live Weight of Pucak SenSi-1 Agrinak										
Average (g/head)	30,49	51.04	60.69	118.46	178.43	258.87	368.12	459.11	607.53	745.66
Standard of Deviation (g/head)	3,15	8.68	12.79	9.76	27.95	37.55	47.34	65.47	50.41	68.75
Coefficient of Variance (%)	10,33	17,00	21.07	8.24	15.66	14.51	12.86	14.26	8.30	9.22
Minimum (g/head)	39,00	38,63	46.50	98.50	125.10	192.20	301.22	342.80	523.00	653.10
Maximum (g/head)	22,00	67,00	87.80	130.30	229.90	327.22	473.30	587.20	728.10	933.10

Table 2. Live weight of Grey and Pucak SenSi-1 Agrinak chicken at 10 weeks old

Variable	Male	Female
Grey SenSi-1 Agrinak Chicken		
Population, (head)	499	492
Live Weight		
Average (g/head)	886.38	739.17
Standard of Deviation, (g/ head)	142.93	118.81
Coefficient of Variance (%)	16.12	16.07
Minimum (g/ head)	323.00	339.00
Maximum (g/ head)	1330.00	1075.00
Pucak SenSi-1 Agrinak Chicken		
Population (head)	497	492
Live Weight		
Average, (g/ head)	908.76	750.53
Standard of Deviation, (g/ head)	130.98	110.56
Coefficient of Variance, (%)	14.41	14.73
Minimum, (g/ head)	515.00	323.00
Maximum, (g/ head)	1251.00	1088.00

Feed consumption

Feed consumption of Grey and Pucak SenSi-1 Agrinak chicken in Table 4 shows normal consumption increasing with increasing age and body weight. Both factors are closely related and interact with each other. The potential of high growth performance leads to high consumption of diet (in the ad libitum administration).

As consequence, the body weight will highly be influenced by feed consumption. Until 10 weeks old, the Grey and Pucak SenSi-1 Agrinak chickens consumed similar diet by 70.99 gr/head and 78.31 g/head respectively. Cumulatively, the average amount of diet consumed until 10 weeks old of Grey and Pucak SenSi-1 Agrinak chicken was ± 2.5 kg/head.

Carcass

The carcass is a crucial factor in assessing meat production as an edible body part (Moran 1977). The carcass of the broiler chicken is part of live broiler chicken after slaughtered, and removal of the feather, viscera, abdominal fat, head, neck and the both legs. Table 5 shows carcass quality of Grey and Pucak SenSi-1 Agrinak chickens.

Average carcass weight of the Grey and Pucak SenSi-1 Agrinak both male and female was quite high by 75%. This percentage is higher than the carcass percentage of crossed KUB with Sentul and Gaok chickens by 64.10-66.73% (Hasnelly 2013). Hasnelly (2013) stated that carcass production may be determined from cutting weight. The heavier cutting weight produces higher carcass. The common color of carcass of Grey and Pucak SenSi-1 Agrinak is yellowish white, besides gray carcass, especially of the grey SenSi-1 Agrinak chicken

Table 3. Live weight of selected male SenSi-1 Agrinak chicken at 10 weeks old

Variables	Grey SenSi-1 Agrinak	Pucak SenSi-1 Agrinak
Population, (head)	103	103
Live Weight		
Average (g/head)	1015	1051
Standard of Deviation (g/ head)	107	76
Coefficient of Variance (%)	10.58	7.22
Minimum (g/ head)	1330	1251
Maximum (g/ head)	839	903

Table 4. Feed consumption of Grey and Pucak SenSi-1 Agrinak at 0-10 weeks old

Variables	Age (week)									
	1	2	3	4	5	6	7	8	9	10
Feed consumption of Grey SenSi-1 Agrinak										
Average (g/head)	6.65	11.09	17.30	22.48	26.89	37.69	62.49	59.87	67.30	70.99
Standard of Deviation (g/ head)	0.95	1.32	1.95	6.98	6.79	4.22	14.51	12.53	15.38	21.16
Coefficient of Variance (%)	14.22	11.91	11.26	31.05	25.27	11.19	23.23	20.93	22.85	29.81
Minimum (g/ head)	5.24	9.16	15.01	18.78	20.03	3194	40.16	42.55	51.26	51.01
Maximum (g/ head)	11.90	13.83	27.74	68.77	45.52	46.87	98.78	99.71	112.20	118.43
Feed consumption of Pucak SenSi-1 Agrinak										
Average (g/head)	6.93	11.38	17.79	21.49	29.63	41.12	60.88	66.09	70.79	78.31
Standard of Deviation (g/ head)	0.39	1.72	2.16	6.75	8.31	8.53	17.41	13.77	19.28	17.28
Coefficient of Variance (%)	5.62	15.08	12.13	31.43	28.06	20.73	28.60	20.83	27.24	22.07
Minimum (g/ head)	5.89	9.37	15.33	18.21	19.19	31.40	37.29	51.97	55.63	51.01
Maximum (g/ head)	8.24	15.20	26.07	67.50	48.55	71.29	111.52	103.41	112.68	106.65

Table 5. Carcass and carcass pieces of SenSi-1 Agrinak chicken at 10 weeks old

Breed	Live Weight (g/head)	Whole Carcass		Liver (g/head)	Rempela (g/head)	Both Legs (g/head)	Head (g/head)	Abdominal Fat (g/head)
		(g/head)	(%)					
Male Grey SenSi-1 Agrinak Chicken								
Average (g/head)	969.50	699.30	7865	23.10	27.40	49.00	89.70	4.40
Standard of Deviation (g/ head)	103.15	76.38	0.79	4.07	6.65	6.31	8.22	2.63
Coefficient of Variance (%)	10.64	10.92	1.00	17.61	24.28	12.87	9.16	59.84
Minimum (g/ head)	813.00	579.00	76.86	17.00	20.00	39.00	80.00	1.00
Maximum (g/ head)	1129.00	809.00	79.47	30.00	40.00	57.00	104.00	9.00
Female Grey SenSi-1 Agrinak Chicken								
Average (g/head)	794.80	537.20	76.94	22.60	26.80	40.30	71.10	4.70
Standard of Deviation (g/ head)	73.66	43.38	1.89	3.06	5.43	4.90	9.90	4.32
Coefficient of Variance (%)	9.27	8.08	2.46	13.55	20.27	12.16	13.93	91.95
Minimum (g/ head)	668.00	447.00	73.98	17.00	19.00	31.00	59.00	1.00
Maximum (g/ head)	898.00	607.00	79.89	26.00	38.00	46.00	94.00	12.00
Male Pucak SenSi-1 Agrinak Chicken								
Average (g/head)	971.10	686.40	78.65	23.60	27.00	49.10	83.60	2.80
Standard of Deviation (g/ head)	91.43	80.55	1.83	2.37	3.50	8.95	13.47	2.66
Coefficient of Variance (%)	9.42	11.73	2.33	10.03	12.95	18.23	16.11	94.94
Minimum (g/ head)	843.00	568.00	76.24	18.00	21.00	31.00	51.00	1.00
Maximum (g/ head)	1111.00	831.00	82.36	26.00	34.00	59.00	95.00	9.00
Female Pucak SenSi-1 Agrinak Chicken								
Average, (g/head)	786.20	561.70	78.35	19.60	24.20	38.30	70.70	2.22
Standard of Deviation, (g/ head)	108.29	76.81	1.20	2.55	3.77	5.96	9.86	1.86
Coefficient of Variance (%)	13.77	13.67	1.54	13.00	15.56	15.57	13.94	83.52
Minimum (g/ head)	602.00	427.00	76.62	16.00	17.00	32.00	56.00	1.00
Maximum (g/ head)	954.00	678.00	80.85	24.00	29.00	49.00	88.00	8.00

.

Table 6. Body weight and size of adult Grey and Pucak SenSi-1 Agrinak chicken

Variable	Male (N=25 head)					Female (N=25 head)				
	Maxim	Minim	Average	StDev	CoVar (%)	Maxim	Minim	Average	StDev	CoVar (%)
Grey SenSi-1 Agrinak Chicken										
Live weight (g)	3706.00	2466.00	3050.17	323.21	10.60	2623.00	1560.00	2032.48	252.34	12.42
Beak length (cm)	37.34	16.41	28.01	5.22	18.62	34.89	21.01	30.78	3.76	12.23
Beak width (cm)	15.76	9.36	12.82	1.86	14.55	19.51	10.89	16.23	3.01	18.54
Head length (cm)	57.45	36.23	48.26	5.43	11.25	51.87	34.63	45.28	3.86	8.53
Head width (cm)	39.52	24.92	32.91	3.01	9.14	34.09	27.56	31.64	1.38	4.35
Neck length (cm)	18.00	13.00	16.04	1.49	9.26	13.50	11.00	12.50	0.60	4.76
Back length(cm)	35.00	21.00	24.19	2.90	11.98	25.00	21.00	22.58	1.04	4.60
Chest circumference (cm)	43.00	33.00	38.92	2.63	6.75	36.00	28.50	32.20	1.69	5.25
Chest length (cm)	19.00	13.00	14.76	1.51	10.22	19.00	16.00	17.78	0.87	4.88
Upper thighs length (cm)	14.00	11.00	12.56	0.96	7.65	12.00	8.00	10.24	0.83	8.11
Lower thighs length (cm)	17.00	13.00	14.34	1.21	8.46	13.00	11.00	12.08	0.62	5.16
Shank length (cm)	14.00	9.00	10.68	1.03	9.64	9.00	7.00	7.94	0.55	6.88
Pucak SenSi-1 Agrinak Chicken										
Live weight (g)	3808.00	2764.00	3190.80	219.53	6.88	2631.00	1538.00	2200.04	254.80	11.58
Beak length (cm)	42.35	30.84	36.97	2.76	7.47	35.52	23.46	29.27	3.00	10.27
Beak width (cm)	20.54	13.77	17.55	1.56	8.88	14.76	10.07	11.72	1.25	10.64
Head length (cm)	58.21	46.26	51.72	2.98	5.76	45.91	31.07	40.44	3.90	9.65
Head width (cm)	41.39	32.67	36.21	2.35	6.49	33.44	23.08	28.11	2.10	7.48
Neck length, (cm)	17.50	14.00	15.58	0.96	6.19	14.00	11.00	12.92	0.86	6.67
Back length (cm)	29.50	25.00	26.94	1.38	5.12	23.00	16.00	19.88	1.94	9.78
Chest circumference (cm)	41.00	35.00	37.12	1.58	4.26	37.00	28.00	33.92	2.08	6.13
Chest length (cm)	27.00	20.00	22.66	1.50	6.61	17.00	12.00	15.32	1.35	8.78
Upper thighs length (cm)	14.00	12.00	12.80	0.61	4.78	14.00	10.00	11.08	0.76	6.85
Lower thighs length (cm)	17.50	14.00	15.28	0.87	5.67	14.00	11.00	12.48	0.77	6.17
Shank length (cm)	15.00	9.50	10.88	1.08	9.95	9.00	7.00	7.96	0.35	4.41

Body size

Body size observation was conducted at 12 months of age (adult) as a consideration that there is no change in body size of the adult. The body size also describes the uniqueness of the SenSi-1 Agrinak chicken as male line of native broiler chicken. Table 6 presents adult body weight and body size of Grey and Pucak SenSi-1 Agrinak chickens both the male and female.

Average live weight of the adult Grey and Pucak SenSi-1 Agrinak chickens of 3.050 kg/head and 3190.80 g/head respectively was higher than body weight of adult Sentul chicken of 2.24 kg (Munggaran 2004), and higher than another native chickens such as Arab chicken of 2.04 kg (Rukmana 2003), and Merawang chicken of 2.36 kg (Hasnelly et al. 2006). The supposition of live weight increase is a selection response showed in the male Grey SenSi-1 Agrinak chicken of 25.55 g/head/generation or 37.41

g/head/generation and in the Pucak SenSi-1 Agrinak chicken of 30.23 g/head/generation, or 40.94 g/head/generation. However, the actual and real selection response was higher by 43.50 g/head/generation or 55.33 g/head/generation and 38.50 g/head/generation or 47.67 g/head/generation (Iskandar et al. 2015)

Body size of adult Grey and Pucak SenSi-1 Agrinak chicken may become unique characteristics of SenSi-1 Agrinak chicken. The body firm may also vary as an influence of the quality and quantity of the diet. In reverse, the length of neck, legs and chest is not affected by the quality and quantity of diet. The abnormal body shape of the adult chicken may appear due to malnutrition during its growth (Iskandar et al. 2016). Therefore, the body size of Sentul chicken presented was normal body size. When the body appearance is combined with the feather color, the unique Sentul chicken will be appeared. The body size, in many ways, is specific. This size will form a unique stereotype of the Sentul chicken. Starting from the shape of head, the shape of body and leg, harmoniously will deliver a specific impression (Sartika et al. 2007). Change of body weight shows body development of the young chicken, while the change of body size shows the growth and development of body parts (Sasimowski 1987). The average bone development increases at 4-12 weeks of age and decreases at 12-20 weeks of age. There are only a few changes of bone in the adult bird, so the measurement of bone to determine body size at the adult age gives the more accurate results (Hutt, 1949). Therefore, the body size may be used to observe quantitative characteristics of chicken.

CONCLUSION

Grey SenSi-1 Agrinak chicken had dark to light grey color of feather, while the Pucak SenSi-1 Agrinak chicken had white feather with black dots by 10-20%. The SenSi-1 Agrinak chicken also had dominant pea comb type and dominant yellow shank. The body weight of selected male SenSi-1 Agrinak chicken at 10 weeks might reach 1.015-1.051 kg/head. The body weight and size of adult male and female SenSi-1 Agrinak chickens was uniform with average coefficient of variance was lower than 15%.

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Evaluation of LipL32 ELISA for Detection of Bovine Leptospirosis in West Java

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ABSTRAK

Sumarningsih, Susanti, Tarigan S. 2017. Evaluasi pada LipL32 ELISA untuk deteksi bovine leptospirosis di Jawa Barat. JITV 22(2): 80-90. DOI: http://dx.doi.org/10.14334/jitv.v22i2.1610

Uji diagnosa Leptospirosis yang saat ini digunakan, yaitu micro Agglutination Test (MAT) dan isolasi, merupakan uji yang mahal, sulit dan membutuhkan keahlian khusus. Studi ini bertujuan mengembangkan ELISA berbasis protein rekombinan LipL32 sebagai uji yang mudah dan murah untuk mendeteksi Leptospirosis. DNA pengkode LipL32 dimurnikan dari bakteri Leptospira Pomona, dimasukkan ke dalam plasmid pRSET-C, kemudian diekspresikan sebagai protein poly-histidine-tagged pada sel E.coli BL21. Protein LipL32 berhasil dimurnikan dari supernatan sel lisis dengan Ni-NTA column sebanyak 1mg/l kultur. LipL32 tersebut digunakan sebagai antigen untuk koting dengan konsentrasi final 5µg/ml. Akurasi dari ELISA dievaluasi berdasarkan analisa ROC, dengan membandingkan hasil ELISA dan MAT dari 517 serum sapi. Pada studi ini diketahui bahwa area dibawah kurva (area under curve) sebesar 0.853, sehingga ELISA LipL32 dikategorikan sebagai uji dengan akurasi sedang (moderately accurate), dan menunjukkan bahwa ELISA mampu membedakan serum positive dan negative Leptospirosis. Selain itu, hasil yang diperoleh juga menunjukkan bahwa ELISA LipL32 mampu mendeteksi serum positive MAT spesifik terhadap serovar Hardjo, Grippotyphosa, Tarrasovi, Rachmati and Bataviae. Optimum ambang batas (cut off) untuk ELISA yang ditentukan berdasarkan kurva ROC adalah 0.504, dan diperoleh sensitivitas dan spesifisitas untuk ELISA sebesar 86.0% dan 69.5% berturut-turut. Secara keseluruhan dapat disimpulkan bahwa ELISA LipL32 pada penelitian ini dapat digunakan sebagai uji cepat untuk identifikasi antibody anti-Leptospira pada sapi.

Kata Kunci: ELISA, LipL32, Leptospirosis, Bovine

ABSTRACT

Sumarningsih, Susanti, Tarigan S. 2017. 2017. Evaluation of LipL32 ELISA for detection of bovine leptospirosis in West Java. JITV 22(2): 80-90. DOI: http://dx.doi.org/10.14334/jitv.v22i2.1610

The current diagnosis of leptospirosis, micro Agglutination Test (MAT) and isolation, is expensive, impractical and technically demanding. This study was aimed at developing an ELISA based on recombinant LipL32 as a practical, inexpensive test for Leptospirosis. The DNA encoding LipL32 was isolated from *Leptospira pomona*, inserted into pRSET-C plasmid then expressed in E.coli BL21 as a poly-histidine-tagged protein. The amount of LipL32 protein, which was purified from the supernatant of lysed cells by a Ni-NTA column, was 1mg/l culture. This purified LipL32 was used as the coating antigen at 5µg/ml. The accuracy of ELISA was evaluated based on ROC analysis, by comparing the ELISA and MAT results of 517 bovine sera. Result in this study showed that the area under curve (AUC) was 0.853, which categorised the LipL32 ELISA as a "moderately accurate" test and indicates that the ELISA was able to differentiate positive and negative Leptospirosis serum. The result also showed ELISA LipL32 could detect serum positive MAT to Hardjo, Grippotyphosa, Tarrasovi, Rachmati and Bataviae. The optimal cut off for OD ELISA determined based on ROC curve was 0.504, and it showed sensitivity and specificity of ELISA LipL32 relative to MAT were 86.0% and 69.5%, respectively. Overall, the result in this study showed that ELISA LipL32 can be used as a rapid test for identification of anti-Leptospira antibodies in bovine.

Key Words: ELISA, LipL32, Leptospirosis, Bovine

INTRODUCTION

Leptospirosis is one of the most important diseases both in human and animals. In Indonesia, the outbreak of this disease has been reported in human since the early 1900's and still occurring (Rahmawati 2013). The serological prevalence in animal, especially bovine, is high (Susanti et al. 2008). Although being endemic in Southeast Asia especially Indonesia, the disease is often neglected or under-reported (Costa et al. 2015, Rajapakse et al. 2015). Human as accidental host can get infected from infected animal and contaminated environment. Common maintenance hosts for Leptospira are rodents, bovine and pig. Previous study in ICRIVS using MAT as serology test has reported high seropositivity of Leptospirosis in bovine and other mammals (Kusmiyati et al. 2005). Further, numbers of

cases was also occurred among livestock farm and abattoir workers (Fang et al. 2015).

The reason for the under-reported cases of leptospirosis is the difficulty in recognizing clinical signs for Leptospirosis, which is not specific and can vary from mild flu-like to severe illness. The standard diagnosis for leptospirosis, MAT and isolations, were impractical and technically demanding. Consequently, only limited laboratories are able to perform these two tests to confirm Leptospirosis infection. The drawbacks of MAT include the continuous maintenance culture of life Leptospira, time consuming, the difficulty in maintaining free-contaminating culture, requirement of high technical skill to perform the assay and difficulty for interpretation of the MAT results (Musso & La Scola 2013). Despite the fact that MAT has been recommended as a golden standard test, some studies proved the deficiency of MAT as a golden standard test because its sensitivity is too low (Limmathurotsakul et al. 2012). The isolation of Leptospira can be used as a definitive diagnostic method, which demonstrating the presence of Leptospira in clinical sample. However, this method is time consuming, technical demanding and not sensitive compared to other available method. Therefore, many diagnostic methods have been developed as alternative tests to overcome these problems, including immunological and molecular tools (Rajapakse et al. 2015).

LipL32 is the major outer membrane protein of Leptospires, which is highly conserved among pathogenic Leptospira species (Haake et al. 2000). Previous studies indicated that LipL32 protein as a potential candidate for rapid and accurate diagnosis of Leptospirosis (Bomfim et al. 2005). Furthermore, ELISA using recombinant LipL32 has been commercially available for detection of canine leptopsirosis. However the suitability of such test for bovine leptospirosis is yet to be confirmed.

This study was aimed to develop indirect ELISA using recombinant protein LipL32 for detection of anti-Leptospira antibodies in bovine, and evaluate the performance by comparing this ELISA LipL32 to the MAT results.

MATERIALS AND METHODS

Preparation of recombinant LipL32 protein

Recombinant LipL32 protein was produced according to previous study (Sumarningsih et. al. 2016) with some modification on expression method. Briefly, a single colony of *E. coli* BL21 cells containing plasmid- pRSET-C-LipL32 was propagated in 10 ml LB broth containing ampicillin (50 µg/ml) and chloramphenicol (35 µg/ml). After overnight incubation at 37°C, this culture was added into 250 ml fresh LB broth containing 2% glucose, ampicillin (50 µg/ml) and chloramphenicol (35 µg/ml). Incubation was continued at 37°C until the density (OD600) reached 0.4-0.6, then the culture was induced by addition of 1 m*M isopropyl-beta-D- thiogalactopyranoside* (IPTG). After another incubation at 37°C for 4 hours, the culture was centrifuged at 7000 x*G* for 30 minutes. The supernatant was discarded and the pellets was collected and kept at -20°C until further processing. The cells were disrupted by sonication for 3 minutes at maximum amplitude. The LipL32 was purified from the disrupted cells using a native Ni-NTA purification kit (Fast Start, Qiagen). The purity of recombinant LipL32 protein was analyzed with SDS PAGE and the protein concentration was measured by Bradford assay using bovine serum albumin as standard.

Sequence Analysis

The plasmid of pRSET-C-LipL32 was purified from *E.coli* BL21 cells and sent to 1st base (http://www.base-asia.com/dna_sequencing/) for sequencing as described in previous study (Sumarningsih et al. 2016). Amino acid sequences of LipL32 from Leptospira serovar Pomona, Hardjo and Grippotyphosa were obtained from GeneBank with accession number AY609326, AY442332 and AY609327, respectively. All these LipL32 sequences were analyzed using bioinformatics software Geneious (version 7.1.2) and aligned against sequences P1 (AAKAKPVQKLDDDDDGDDTYKEERHNK) and P2 (LTRIKIPNPPKSFDDLKN-IDTKKL) that has been identified as important epitopes for LipL32 (Lottersberger et al. 2009).

Production of positive and negative control sera for LipL32 ELISA

Ten calves from a nearby farm were bled and tested for leptospirosis using MAT. After the testing, one calf which was negative for leptospirosis was purchased. About 20 ml bloods were drawn, serum was prepared, aliquoted, kept at -20 and used as negative control serum. The calf was immunized with 1 mg of recombinant LipL32 and montanide ISA 70 M VG (SEPPIC) as adjuvant. The second immunization was carried out with $6x10^8$ formol-inactivated Leptospira hardjo and 2mg Quil A in 3.2ml PBS. Four weeks after second immunization, the serum was collected and tested with MAT and ELISA using recombinant LipL32 as coating antigen. After confirming that the serum was positive in both tests, 20 ml of blood was drawn, serum was prepared, aliquoted and used as a positive control serum.

Optimization of ELISA LipL32

ELISA optimization was performed to determine the optimal concentration of recombinant LipL32 protein as coating antigen for ELISA. Briefly, recombinant LipL32 protein was serially diluted by twofold dilution; start at 12.5 µg/ml to 0.195 µg/ml in carbonate-bicarbonate buffer pH 9.6 and 100 µl of this solution was added into microtitre plate. Negative and positive serum control were included as standard for optimization of ELISA LipL32 in this study. Both serum were used with dilution of 1/100 and 1/200 in PBS pH 7.4. HRP-goat anti bovine IgG (Jackson Immuno Research) as secondary antibody was used with dilution 1/3000 and 1/6000 in PBS pH 7.4. After adding the substrate solution and ABTS, the absorbance at wavelength 420 nm was measured with Spectrophotometer microtitre plate reader.

Sample collection

Blood samples were collected from 517 bovine (dairy and beef cattle) of various ages in West Java in 2016. The blood samples were handled and processed carefully to prevent hemolysis. The serum was then carefully prepared and stored at -20°C until used.

Micro Agglutination Test (MAT)

MAT for bovine serum was performed as described in previous study (Sumarningsih et al. 2016). Briefly, panels of antigens were added into serum samples that had been diluted in PBS, pH 7.4 at 1 : 50, 1 : 100, 1 : 400, 1 : 1600. The panels of antigens consisted of 14 *Leptospira* serovars including: *L. icterohaemorrhagiae, L. javanica, L. celledoni, L. canicola, L. ballum, L. pyrogens, L. cynopteri, L. rachmati, L. australis, L. pomona, L. grippotyphosa, L. hardjo, L. bataviae,* and *L. tarrasovi*. After incubation for 2 hours at 30°C, the mixture was examined under a dark-field microscope. The MAT titer was determined as the highest serum dilution that agglutinated at least 50% the life leptospires.

ELISA LipL32 for field bovine sera

ELISA LipL32 for field sera was performed using microtitre plates (NUNC Maxisorb). Briefly, the plate were coated with diluted recombinant LipL32 protein (5 µg/ml) in carbonate-bicarbonate buffer and incubated overnight at 4°C. This coating solution was removed and plate was washed twice with PBS-Tween 0.05%. The well was blocked by adding 150 µl of blocking buffer (5% normal goat serum in PBS) and incubated for 2hours at room temperature. After washing the plate twice, 100 µl of diluted bovine serum (1/100) was added into each well and incubated for 1.5 hours at room temperature. The solution was discarded and the plate was washed four times. Goat anti-bovine IgG-HRP was added at 1/6000 dilution in PBS (100 µl/well) and incubated for 1.5 hours at room temperature. The solution was discarded and plate was washed four times. Substrate and chromogenic (ABTS) solution) was added, 100 µl/well. After incubation at room temperature for 10 minutes, the absorbance (OD ELISA) was read using Spectrophotometer microtitre plate reader at wavelength 420 nm.

Statistical analysis

Data were analyzed using descriptive statistics. The accuracy of LipL32 ELISA as a serological test was analyzed using a ROC-curve based on MAT as the golden standard. A MAT titre of 1/100 was used as the cut-off for negative and positive for leptospirosis. The Area under curve (AUC) is used as the discriminating power of LipL32 ELISA for positive and negative leptospirosis. The cut-off of the ELISA was determined as the OD at which the test gave the highest sensitivity and specificity. All statistical analyses were carried out using a commercial statistical package.

RESULTS AND DISCUSSION

Quantity and quality of recombinant LipL32 protein

The concentration of recombinant LipL32 measured by Bradford assay was 1mg/l culture. The quantity of LipL32 protein produced in present study was as low as ⅛ as that in previous study (Sumarningsih et al. 2016). As in previous study, the purity of recombinant protein LipL32 produced in present study was high (see Figure 1.

Recombinant protein LipL32 in this study was successfully purified using a native buffer system. Similar to the previous study which using pRSET-B to express recombinant protein LipL32 as a solubilized protein and revealed that recombinant protein LipL32 was located in the cytoplasm of *E. coli* (Chalayon et al. 2011). This is in contrast to Leptospira since in this bacteria LipL32 is located in the outer membrane as a dominant lipoprotein (Cullen et al. 2005)

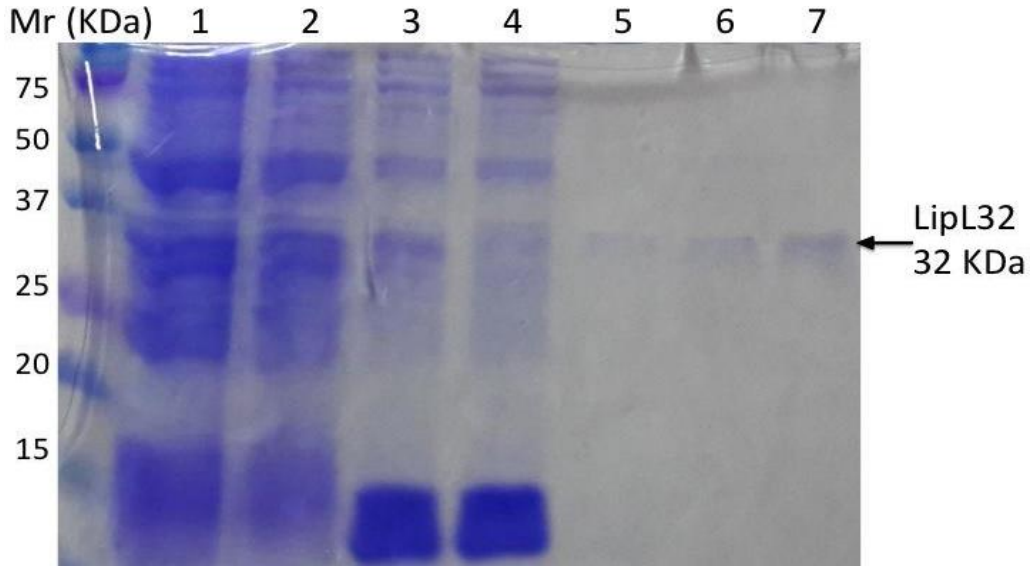

Figure 1. SDS PAGE of recombinant LipL32 protein at different stages of purification. Line 1: uninduced cell, Line 2: induced cell, Line 3: supernatan of lysed cell, Line 4: Flow through, Line 5: Wash buffer, Line 6: LipL32 elution fraction 1, Line 7: LipL32 elution fraction 2. (arrow ← : LipL32 protein 32kDa).

Optimization of ELISA LipL32

In preliminary experiment, the optimum concentration of recombinant protein Lipl32 was determined by checkerboard titration (Figure 2). The optimum dilutions of secondary antibody and coating antigen were determined based on the OD differences between positive and negative serum for anti-Leptospira. Serum anti-Leptospira generated by immunised the bovine using inactivated Leptospira serovar Hardjo was included in this study for comparison.

The OD for positive serum control in ELISA LipL32 using conjugate diluted 1 in 3000 was higher than that when using conjugate 1 in 6000 (Figure 2a vs Figure 2b). However, the background ELISA with the 1 in 3000 diluted conjugate was high as indicated by the increased (above 0.5) OD for negative serum. To obtain a better specificity, a dilution of 1 in 6000 of secondary antibody was used in all assays. Based on the OD difference between positive and negative serum was shown in Figure 2.b, the optimum concentration of LipL32 protein for ELISA coating was determined to be 5 µg/ml.

Identification of bovine Leptospirosis using MAT

MAT was used in this study to determine the status of Leptospirosis. Bovine sera from the field were tested with MAT using panels of 14 *Leptospira* serovars. The result of MAT summarized in Table 1 showed that 164 of a total 571 serum samples were positive MAT and the titers ranged from 100 to 1600. This 28.7% seroprevalence was comparable with that of previous study performed in Indonesia and other country (Kusmiyati et al. 2005, Subharat et al. 2012; Alonso-Andicoberry et al. 2001).

According to previous study on seroprevalence of bovine Leptospirosis in Indonesia, bovine sera that were positive on MAT were dominated against serovar *Hardjo* (Kusmiyati et al. 2005). However, in this study as shown in table 2, the majority of MAT positive sera were against serovar *Grippotyphosa* (50%). Of 164 serum positive MAT, 82 to serovar *Grippotyphosa*, 71 to serovar *Hardjo*, 3 to serovar *Batavaie*, 3 to serovar *Rachmati*, 1 serum to *Tarrasovi* and 1 serum to *Icterohaemorrhagiae.* Two sera were positive for both serovars *Grippotyphosa* and *Bataviae*, and 1 serum was positive for *Grippotyphosa* and *Rachmati*.

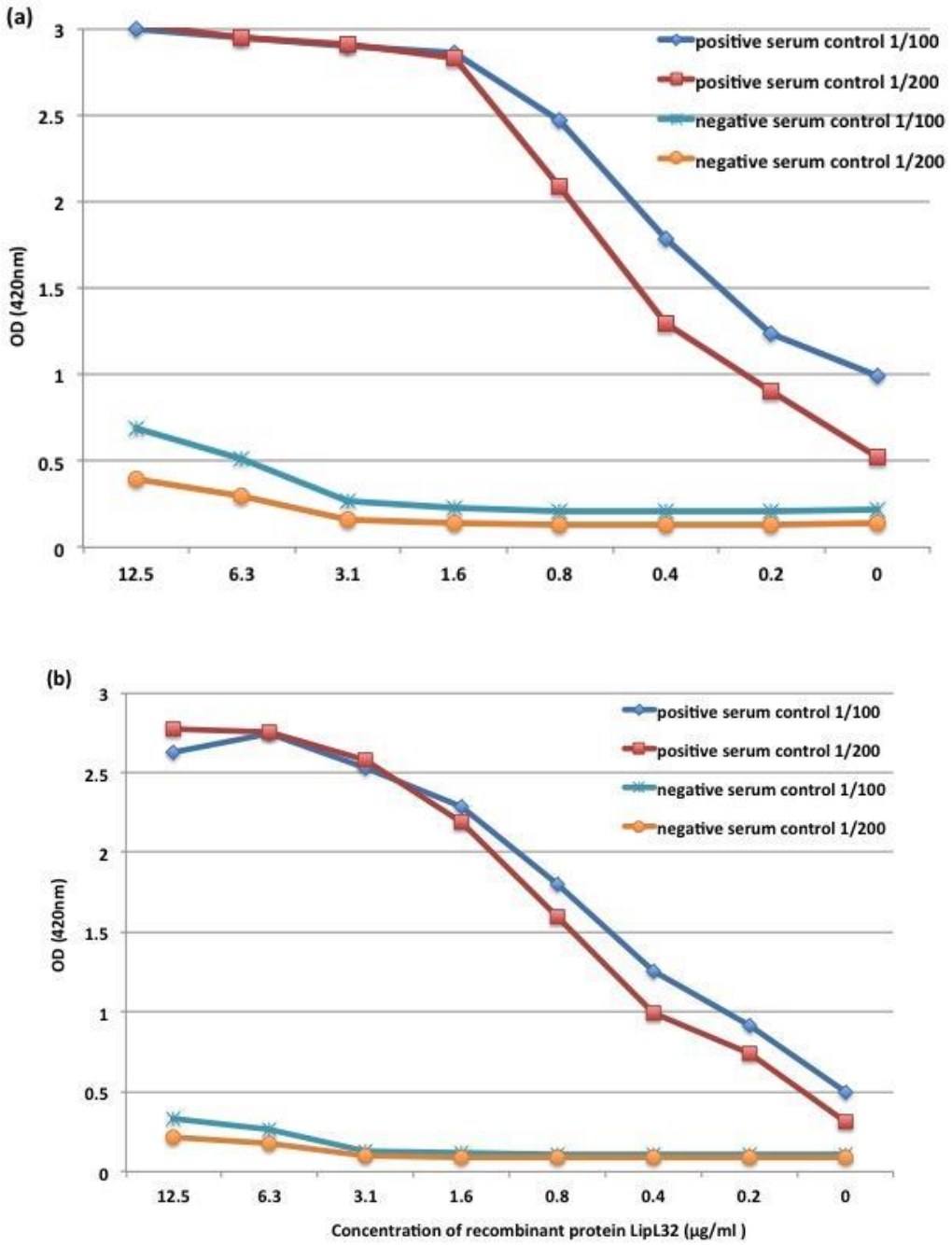

Figure 2. Optimisation of ELISA LipL32; Titration of recombinant protein LipL32 for ELISA coating: (a) ELISA using Goat-anti-Bovine-IgG-HRP diluted in 1/3000, and (b) ELISA using Goat-anti-Bovine-IgG-HRP diluted in 1/6000. Twofold dilution of LipL32 start at 12.5 µg/ml to 0.195 µg/ml in carbonate-bicarbonate buffer pH 9.6.

.

Table 1. MAT result of field bovine sera

MAT results	Number of samples	Percentage (%)
Positive	164	28.7
Negative	407	71.3
Total	571	100

Table 2. Serospecific of bovine sera positive in MAT

Serovar	Number of sample	Percentage (%)
Grippotyphosa	82	50.00
Hardjo	71	43.29
Bataviae	3	1.83
Rachmati	3	1.83
Tarrasovi	1	0.61
Icterohaemorrhagiae	1	0.61
Grippotyphosa and Rachmati	1	0.61
Grippotyphosa and Bataviae	2	1.22
Total	164	100

Performance of ELISA LipL32 compared to MAT

The LipL32 ELISA developed in this study was able to differentiate between bovine serum that were positive and negative Leptospirosis. As shown in Figure 3, 95% confidence intervals of the MAT-negative serum OD was well-separated with that of MAT-positive serum, even with the lowest titer. The mean OD of MAT-negative serum was 0.451. The mean OD of the positive sera with titer of 100, 400 and 1600 were 0.742, 0.881 and 0.986, respectively. Although, the OD was increased by the increasing MAT titer, the differences were not significant.

The ELISA based on recombinant protein is relatively easier and safer to perform compared to MAT. Bomfim et al. (2005) evaluated LipL32 ELISA for detection of bovine Leptospirosis and reported that the agreement was perfect with MAT. The LipL32 ELISA was reported to have 100% of sensitivity and specificity. LipL32 ELISA was also available commercially from IDEXX, but it is intended to be used for Leptospirosis detection in canine and it need to be imported with high cost for application in Indonesia. This problem motivated us to develop another LipL32 ELISA suitable for detection of bovine Leptospirosis in Indonesia.

The Receiver Operating Characteristic (ROC) curve was used to measure the performance of LipL32 ELISA in this study by comparing the ELISA's OD and MAT results. Results (Figure 4) showed that the area under curve for the ROC curve, which indicates the discriminating power of the test, was 0.853. A test with $0.7<AUC<0.9$ is categorized as a moderately accurate (Greiner et al. 2000).

ROC curve was also used to determine the cut off, sensitivity and specificity of LipL32 ELISA. Figure 4 and Table 4 showed the optimum cut off OD for ELISA LipL32 in this study was 0.504. Based on this cut off values, the sensitivity and specificity were 86.0% and 69.5%, respectively.

Carcass

Table 4 presented the distribution of OD ELISA LipL32 based on MAT titer. The OD for MAT negative serum was found mostly less than 0.5, and the OD for bovine serum positive to MAT varied and mostly

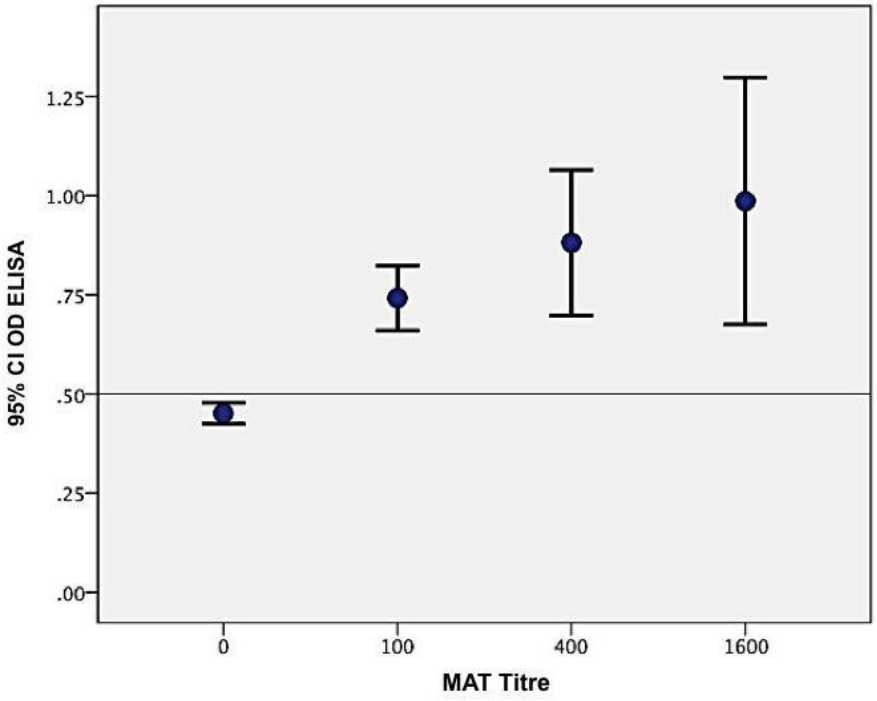

Figure 3. ELISA LipL32 evaluation (with 95% Confidence Intervals). Graph was generated by plotting the OD values based on the peak titre of MAT (100, 400 and 1600), and peak titre 0 was indicated for MAT negative serum.

Table 3. Analysis of OD_ELISA based on the peak titre of MAT

MAT result (peak titre)	N	Mean	Std. Dev	Std. Error	95% Confidence Interval for Mean		Min	Max
					Lower Bound	Upper Bound		
Negative MAT	407	.451	.206	.0102	.431	.471	.115	1.823
Positive MAT : 100	94	.742	.301	.031	.680	.803	.343	2.045
400	49	.881	.479	.068	.743	1.019	.367	2.669
1600	21	.986	.501	.109	.758	1.215	.506	2.089
Total	571	.555	.320	.013	.529	.581	.115	2.669

ranged between 0.5-1.0. Some serum with MAT titer 100 and 400 had low OD (less than 0.5). The OD ELISA for all bovine serum with MAT titer 1600 was above 0.5. The sensitivity of bovine serum in ELISA LipL32 was higher as the titer of MAT increased. Similar finding has been reported by previous study that evaluated the ELISA LipL32 for leptospirosis canine (SNAP Lepto), which is available commercially from IDEXX. The study showed the agreement percentage between MAT and ELISA LipL32 (SNAP Lepto) for serum with MAT titer 100, 200, 400, 800 and 1600 was 62.5%, 55%, 72.4%, 69.8% and 73.5%, respectively (Curtis et al., 2015). The sensitivity of ELISA LipL32 developed in this study is apparently higher than that study. However, the specificity of our ELISA is lower. These results indicate that LipL32 ELISA is potential to be used as a rapid test for identification of leptospirosis especially as a screening test for anti-Leptospira antibodies in bovine. For a screening approach, the sensitivity of ELISA LipL32 can be improved by lowering the cut off OD. It would decrease the specificity, but it can be overcome by follow-up confirmatory test (Table 5).

In this study, LipL32 ELISA was able detected sera that were positive against various serovars (hardjo, grippotyphosa, tarrasovi, rachmati, bataviae, Tarrasovi and Icterohaemorrhagiae). This is in agreement with fact that LipL32 is highly conserved among serovars of

pathogenic Leptospira. The ELISA OD was presented in table 6, for sera that were MAT-positive against serovar *Grippotyphosa, Hardjo, Bataviae, Rachmati, Tarrasovi*, and *Icterohaemorrhagiae*. One serum which was MAT-positive against both serovar *Grippotyphosa* and *Rachmati* has 0.92 OD, and the other two sera were MAT-positive against both serovar *Grippotyphosa* and *Bataviae* with the mean OD of 0.61. This findings was also supported previous study that reported mice sera anti recombinant LipL32 was successfully recognised and reacted to 32kDa band (protein LipL32) from whole cell lysate of Leptospira from 17 different serovar (Chalayon et al. 2011). The fact that LipL32 ELISA can detect antibody to different serovars provides further support the usefulness of LipL32 ELISA as a screening test for Leptospirosis

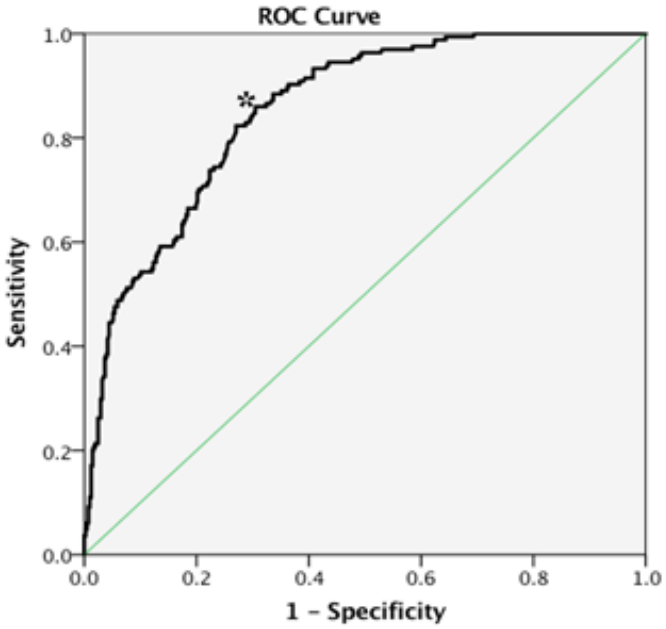

Figure 4. Receiver Operating Characteristic (ROC) Curve of ELISA LipL32. Analysis of ELISA LipL32 using bovine sera in which 164 were MAT positive and 407 were MAT negative. Optimal cut off point is indicated (*) for OD 0.504 with 86% sensitivity and 69.5% specificity, and area under curve of 0.853 (95% CI).

Table 4. Distribution of OD ELISA LipL32 based on MAT titre of bovine serum

MAT result	N	Frequency of serum sample (f) based on OD ELISA				
		(0.1 – 0.5)	(0.5 – 1.0)	(1.0 – 1.5)	(1.5 – 2.0)	(> 2.0)
Negative MAT	407	278	119	7	3	0
Positive MAT						
100	94	15	60	17	1	1
400	49	8	29	5	6	1
1600	21	0	13	4	3	1

Table 5. Comparison between the percentage of sensitivity-specificity and cut off OD ELISA LipL32 based on ROC curve analysis

Cut Off for OD ELISA	% Sensitivity (95% CI)	% Specificity (95% CI)
0.400	97.0	42.8
0.451	93.3	57.7
0.477	90.2	63.1
0.504*	86.0	69.5
0.532	75.0	75.7
0.544	72.6	77.6

* Optimal Cut off for OD ELISA LipL32 with 86% and 69.5% of sensitivity and specificity, respectively.

Table 6. Analysis of OD_ELISA based on the Leptospira serovar specific

MAT result (serovar specific)	N	Mean	Std. Dev	Std. Error	95% Confidence Interval for Mean		Min	Max
					Lower Bound	Upper Bound		
-ve MAT	407	.451	.206	.010	.430	.471	.115	1.823
+ve MAT: Grippotyphosa	82	.883	.475	.052	.779	.988	.367	2.669
Hardjo	71	.712	.254	.030	.652	.773	.343	1.503
Bataviae	3	1.345	.621	.358	-.196	2.887	.860	2.045
Rachmati	3	.881	.189	.109	.409	1.351	.769	1.100
Tarrasovi	1	.911	.	.	.	.	.912	.912
Icterohaemorrhagiae	1	.830	.	.	.	.	.830	.830
(Grippo and Rach)	1	.917	.	.	.	.	.918	.918
(Grippo and Bat)	2	.614	.331	.234	-2.356	3.583	.380	.847
Total	571	.555	.320	.013	.529	.581	.115	2.669

Table 7. Amino acid sequence of important epitope (P1 and P2) from LipL32 protein

Name	Amino acid sequence of LipL32	Source
P1 (151-177)	AAKAK PVQKL DDDDD GDDTY KEERH NK	Lottersberger et al. 2009
rLipL32_4f2	AAKAK PVQKL DDDDD GDDTY KEERH NK	Present study
L. Pomona	AAKAK PVQKL DDDDD GDDTY KEERH NK	Genebank AY609326
L. Hardjo	AAKAK PVQKL DDDDD GDDTY KEERH NK	Genebank AY442332
L. Grippotyphosa	AAKAK PVQKL DDDDD GDDTY KEERH NK	Genebank AY609327
P2 (181-204)	LTRIK IPNPP KSFDD LKNID TKKL	Lottersberger et al. 2009
rLipL32_4f2	LTRIK IPNPP KSFDD LKNID TKKL	Present study
L. Pomona	LTRIK IPNPP KSFDD LKNID TKKL	Genebank AY609326
L. Hardjo	LTRIK IPNPP KSFDD LKNID TKKL	Genebank AY442332
L. Grippotyphosa	LTRIK IPNPP KSFDD LKNID TKKL	Genebank AY609327

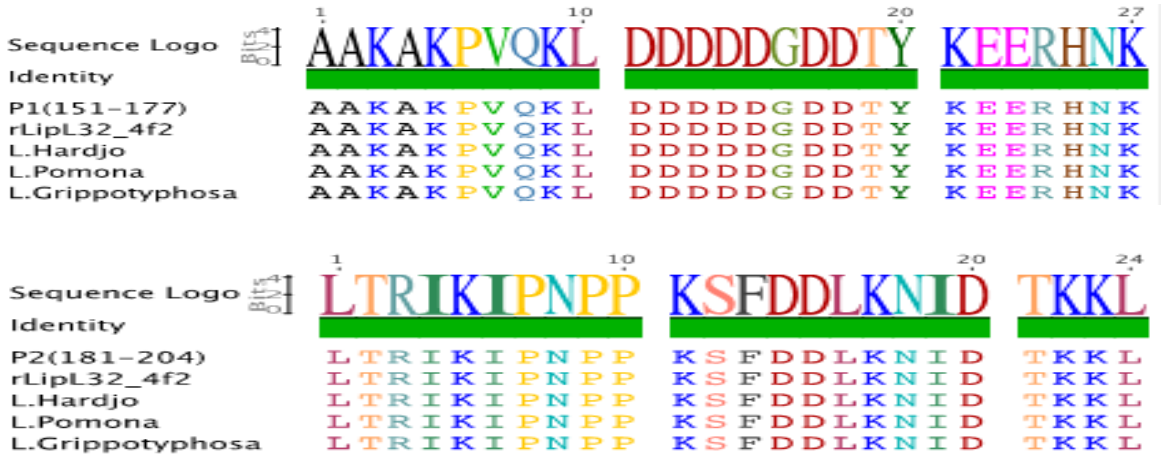

Figure 5. Sequence alignments for the important epitope of LipL32 protein located in amino acids number 151 to 177 (P1) and 181-204 (P2). LipL32 protein in this study (rLipL32_4f2) was aligned to P1, P2 and other LipL32 sequences obtained from genebank.

Previous studies performed using simultaneous and parallel solid phase peptide synthesis on derivatives cellulose membranes has been identified two important epitopes of LipL32, P1 and P2 which located in two different regions 151–177 and 181–204, respectively. Both epitopes were highly conserved between pathogenic Leptospira (Lottersberger et al. 2009). Sequences analysis showed 100% homologous for both peptides (P1 and P2) with recombinant LipL32 protein produced in this study (rLipL32_4f2) and LipL32 protein from Leptospira serovar Pomona, Hardjo, and Grippotyphosa obtained from gene bank with accession number AY609326, AY442332 and AY609327, respectively (Table 7) .

MAT is a gold standard for Leptospirosis and used in this study to determine the true positive and true negative status of Leptospirosis (OIE 2013). However, previous studies found that MAT has many limitation and was imperfect as gold standard for evaluation of the new diagnostic test (Limmathurotsakul et al. 2012). Beside serology assay, PCR targeting LipL32 gene has been developed in order to increase the sensitivity of Leptospirosis detection. The PCR had higher sensitivity and spesificity compared to MAT (De Abreu Fonseca et al. 2006; Ooteman et al. 2006). Thus, bovine serum that positive in ELISA LipL32 can be confirmed by PCR test for eliminating false positive.

CONCLUSION

The result showed ELISA LipL32 in this study can be used as rapid test for identification of leptospirosis and for screening anti-Leptospira antibodies in bovine. This ELISA LipL32 was able to detect serum MAT-positive to hardjo, grippotyphosa, tarrasovi, rachmati, and bataviae. The optimal cut off for OD ELISA determined based on ROC curve was 0.504, and its sensitivity and specificity of ELISA LipL32 were 86.5% and 69.5%, respectively.

ACKNOWLEDGMENT

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Serotype Detection, Molecular Characterization, and Genetic Relationship Study on *Pasteurella multocide* Local Isolate

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ABSTRAK

Prihandini SS, Noor SM, Kusumawati A. 2017. Deteksi serotipe, karakterisasi molekuler dan studi kekerabatan genetik isolat lokal *Pasteurella multocide*. JITV 22(2): 91-99. DOI: http://dx.doi.org/10.14334/jitv.v22i2.1630

Pasteurella multocide merupakan bakteri penyebab penyakit ngorok atau Haemorrhagic Septicaemia (HS) di Indonesia dengan tingkat mortalitas dan morbiditas yang tinggi pada heterogenus spesies tak terkecuali sapi sebagai sumber produk hewani yang memiliki nilai ekonomis tinggi. Kompleksitas identifikasi secara konvensional dan biokimia menjadi kendala utama dalam deteksi penyakit ini terutama karena *P. multocide* memiliki lima golongan serotipe yaitu A, B, D, E dan F, meskipun serotipe B merupakan penyebab utama kasus HS di Asia termasuk Indonesia. Untuk itu perlu dilakukan suatu penelitian yang dapat mempermudah penentuan serotipe. Tujuan penelitian ini selain untuk deteksi serotipe lima isolat *P. multocide* yang berasal dari Lampung dan Kupang, juga untuk melakukan karakterisasi molekuler dan studi kekerabatan genetik dengan teknik Polymerase Chain Reaction (PCR) dan sekuensing. Setelah dilakukan PCR terhadap gen spesifik, gen kapsular, gen 16S rRNA, sekuensing dan analisis menggunakan Bioedit, BLASTn, CLUSTALW dan MEGA7.0.25, diketahui bahwa kelima isolat terbagi menjadi dua kelompok serotipe yaitu A dan B. Isolat *P. multocide* (kode PMc) asal Lampung memiliki homologi tinggi dengan isolat ATCC 12945, sehingga dapat digunakan sebagai kontrol positif serotipe A dalam deteksi isolat *P. multocide* lain dengan PCR. Sedangkan isolat *P. multocide* asal Kupang dapat digunakan sebagai kontrol positif serotipe B karena identik dengan *P. multocide* PMTB2.1 (CP007205.2) asal Malaysia yang diisolasi dari kerbau terinfeksi HS.

Kata Kunci: *Pasteurella multocide*, Ngorok, Deteksi, Polymerase Chain Reaction (PCR), *Sekuensing*

ABSTRACT

Prihandini SS, Noor SM, Kusumawati A. 2017. 2017. Serotype detection, molecular characterization and genetic relationship study on *Pasteurella multocide* local isolate. JITV 22(2): 91-99. DOI: http://dx.doi.org/10.14334/jitv.v22i2.1630

Pasteurella multocide is a bacteria that causes snoring disease or Haemorrhagic Septicaemia (HS) in Indonesia with high mortality and morbidity in heterogeneous species including cattle as a source of animal products with high economic value. The complexity of conventional and biochemical identification is a major obstacle in the detection of this disease because *P. multocide* has five serotypes A, B, D, E and F, while serotype B is the leading cause of HS cases in Asia including Indonesia. Therefore, it is necessary to conduct a research that can determine the serotype and molecular characterization and genetic study of five isolates of *P. multocide* from Lampung and Kupang by Polymerase Chain Reaction (PCR) technique. After PCR was performed on specific genes, capsular genes, 16S rRNA genes, sequencing and analysis using Bioedit, BLASTn, CLUSTALW and MEGA7.0.25, it was found that the five isolates were divided into two serotype groups: A and B. Isolate *P. multocide* (code: PMc) from Lampung is high homolog with ATCC isolate 12945, so it can be used as a positive control serotype A in the detection of other *P. multocide* isolates with PCR. Whereas, isolate *P. multocide* from Kupang can be used as positive control of serotype B because it is identical to *P. multocide* PMTB2.1 (CP007205.2) from Malaysia that is isolated from buffalo infected by HS.

Key Words: *Pasteurella multocide*, Snoring Disease, Detection, Polymerase Chain Reaction (PCR), Sequencing

INTRODUCTION

Pasteurella multocide (*P. multocida*) is a pathogen gram-negative bacteria for livestock animals and causes specific pasteurellosis with the main symptoms: fever and respiratory disorder causing sound like snoring due to the neck swelling that extends to chest area resulting in suppression of respiratory cavity. Therefore, pasteurellosis on cattle and buffalo or Septicaemia Epizootic (SE)/ Haemorraghic Septicaemia (HS) in Indonesia is well known as snoring disease. In addition to being acute, its high morbidity and sepsis are extremely deadly to the infected animals. HS is a mayor epizootic disease on cattle and buffalo in the developing countries with high average morbidity and mortality (El-Jakee et al. 2016). HS can acutely infect cattle or buffalo that leads to economic losses in the Southeast Asia including Indonesia. Therefore, this disease is a

crucial disease from the economy point of view (Moustafa et al. 2015). The first infection in Indonesia was reported in Balaraja, Tangerang in 1884 and last several years, it was reported to extend to the South Kalimantan in 2003; South Bengkulu, South Tapanuli, Riau, Jambi and Kaur in 2005; East Nusa Tenggara and Muko-muko Bengkulu in 2006.

P. multocide is divided into five capsule serotypes: A, B, D, E, and F. The serotype causes HS to cattle or buffalo is serotype B:2 or Asian serotype and serotype E:2 or African serotype (Moustafa et al. 2015). *P. multocide* with Serotype A and some serotypes D capsule leads to fowl cholera in poultry (Dziva et al. 2008). *P. multocide* along with the *Bordetella bronchiseptica* causes acute atrophic rhinitis to pig related to high strain toxigenicity, because the *P. multocide* play a dominant role leading to the development of anthropic rhinitis lesi (Magyar et al. 2013; Kubatzky 2012; Shayegh et al. 2009) as well as it is related to pneumonia and septicemia diseases (De Oliveira et al. 2015). Liu et al (2017) reported that prevalence of *P. multocide* Serotype A is the most common in China before 1990 followed by serotype B and D.

Those five different *P. multocide* capsule serotypes causing the detection and differentiation processes are a different from indirect haemagglutination test developed. A conventional method to detect and diagnose Pasteurella infection on the bacterial observation uses microscopy with the staining. Moreover, isolation using in vitro on the selective medium followed by phenotypic and serology characteristic are conducted (Wilson & Ho 2013) that takes a long time. Method of detection and differentiation of serotype A and D of *P. mulcotide* widely used in the veterinary laboratory, especially in the developing countries is a non-serology test (Arumugam et al. 2011b). Some DNA-based methods that have been developed to determine the type of molecular are PCR-based, Restriction endonuclease analysis/REA, ribotyping, colony hybridization test, Filled Alternation Gel Electrophoresis (FAGE) and Real-Time PCR (Ranjan et al. 2011). The other DNA-based methods that are also developed are DNA hybridization (Mutters et al. 1985), endonuclease restriction analysis (Kim & Nagaraja 1990), pulsed-field gel electrophoresis (PFGE) and Capsular Polymerase Chain Reaction (PCR) (Townsend et al. 2001). The PEGE is determined as the golden standard, but it requires complicated planning and expensive equipment compared to the Capsular PCR which is simpler to be applied. Therefore, in this study, it was developed a technique of detection and differentiation of *P. mulcotide* using Capsular PCR which can detect capsular gen of *P. multocide* to four isolates from Lampung and one isolate from Kupang.

MATERIALS AND METHODS

Bacteria isolation

P. multocide isolate used in this study were five sample isolates from Lampung (4 isolates) and Kupang (1 isolate). Those five isolates have been re-detected morphologically and bio-chemically at Laboratory of Bacteriology, Indonesian Research Center for Veterinary Science (IRCVS), Bogor. Isolate of *P. multocide* ATCC12945 (serotype A) (Furian et al. 2014) and NCTC 11668 (serotype B) from America and IRCVS collection, respectively were used as control positive, while the *Brucella sp* isolate as a negative-control.

Primer

Standard OIE primer was used to detect specific species (kmt gene) and capsular gene (*hya*D-*hya*C and *bcb*D) to those five *P. multocide* isolates in this study. CAPA is a pair of primer to detect amplicons of the capsular gene of *hya*D-*hya*C and *bcb*D (serotype A), while the CAPB is a pair of primer to detect B/bcbD capsular amplicon (serotype B). Amplification of 16S rRNA gene was conducted using a pair of 16S PM1 primer designed from five gene references from GenBank on online devices of Primer3Plus (http://www.bioinformatics.nl/primer3plus). Structure of nucleotide base of forward primer of 16S PM1 was 5'-AGG-CCT-TCG-GGT-TGT-AAA-GT-3 and reverse: 5'-CCA-TGC-AGC-ACC-TGT-CTC-TA-3'with the size of the product by 642 base pairs. Before the construction, specivity of primer was tested first using the same online software device (Thornton & Basu 2011).

Detection and characterizing of specific species molecular (kmt Gene) of *P. multocide* using PCR

Phenotypic and bio-chemical detection of those five *P. multocide* isolates was performed. Nuclear acid-based detection was started using extraction of *P. multocide* DNA as template PCR using specific primer of KMT1T7: 5'-ATC-CGC-TAT-TTA-CCC-AGT-GG-3' and KMT1SP6: 5'-GCT-GTA-AAC-GAA-CTC-GCC-AC-3' (Townsend et al. 2000).

Reconstitution of *P. multocide* isolate was conducted by dissolving frozen dry isolates in the Brain Herat Infusion (BHI) then was incubated at 38°C for 24 hours. Isolates culture from incubation in the blood agar medium was then harvested for DNA isolation using Qiagen® kit.

Concentration of isolated DNA was then measured using NanoDrop 1000 *Spectrophotometer* $OD_{260/280nm}$ and stored in the micro tube at 20°C as stock template

of each PCR reaction. The kmt gene was detected by reacting a mixture of 22.5 µl Platinum PCR SuperMix High Fidelity Invitrogen, 0.5 µl primer KMT1T7, 0.5 µl primer KMT1SP6, and 1.5 µl DNA samples. PCR reaction was performed using these following programs: pre-denaturation (at 94°C for 2 minutes); denaturation (at 94°C for 30 seconds); annealing (at 55°C for 30 seconds); elongation (at 68°C for 30 seconds); post elongation (at 68°C for 1 minute). The reaction was performed for 35 cycles and then the PCR product was electrophoresed in the gel agarose 2% at 100 volt, 200 mA for 70 minutes and showed the tape at around 460 base pairs under UV exposure and was documented using the geldoc.

Detection and characterization of capsular gene molecule of *P. multocide* using PCR

Once the isolate in previous PCR reaction was confirmed as *P. multocide*, then further detection of the capsular serotype was performed. Mix reaction consisting of 22.5 µl of Platinum PCR SuperMix High Fidelity invitrogen, 0.5 µl primer of capsular forward, 0.5 µl primer of capsular reverse and 1.5 µl DNA samples. PCR condition and the electrophoresis on agarose gel were similar to the detection program of the kmt gene. Amplification of capsular gene of A/*hya*D-*hya*C used primer of CAPA: forward 5'-TGC-CAA-AAT-CGC-AGT-CAG-3' and reverse 5'-TTG-CCA-TCA-TTG-TCA-GTG-3' with product 1044 bp (Townsend et al. 2001). Amplification of capsular B/bcbD gene used primer of CAPB: forward 5'- CAT-TTA-TCC-AAG-CTC-CAC-C-3' and reverse 5'- GCC-CGA-GAG-TTT-CAA-TCC -3'with the product at around 760 bp.

Detection and characterization of molecule gene 16S rRNA of *P. multocide* using PCR

Amplification of 16S rRNA gene was performed to determine the genetic relationship (Mizrahi-Man et al. 2013) of six local isolate of *P. multocide* from various countries at the GenBank. PCR reaction was performed by mixing 12.5 µl Hot Star Taq, 1 µl primer forward, 1 µl primer reverse, 1 µl DNA and 9.5 µl RNAse free water in micro tube. PCR condition was programed to pre-denaturation (at 95°C for 15 minutes); denaturation (at 94°C for 1 minute); annealing (at 53°C for 45 seconds); elongation (at 72°C for 1 minute); and post-elongation (at 72°C for 10 minutes). The reaction was conducted as 32 cycles and the PCR product was then electrophoresed in the agarose gel 2% at 100 volt, 200 mA for 50 minutes and the desired result was 642 bp under UV exposure or gelDoc camera (Lee at al. 2014).

16S rRNA gene sequencing

Amplification products of 16S rRNA were sent to 1st Base Singapore to be sequenced using Sanget method.

Data analysis

Data of sequencing were analyzed using BIODET program to determine consensus and contig sequences, then blast nucleotide was done using BLASTn program (NCBI) to determine the identity structure and sequence homology resulted to other isolates in the GenBank. The sequence was compared to five highest isolates of BLASTn with multiple alignments using ClustalW program. Phylogenic tree was built using Neighbor joining program MEGA 7.0.25 method (Peng et al. 2017).

RESULTS AND DISCUSSION

Specific gene of *P. multocide* was detected using electrophoresing the PCR result of those five isolates in agarose gel. DNA of those five isolates was isolated using Qiagen® kit and then PCR was performed in accordance with the formula and condition described in the materials and methods. Amplification of specific gen (kmt) of *P. multocide* using showed the amplicons at around of 460 bp (Figure 1). It showed molecularly that those five isolates in this study were *P. multocide*. Solongo et al. (2015) performed genetic analysis of *P. multocide* isolated from local cattle of Mongolia and showed similar result. PCR condition was performed in accordance to the properties of forward and reverse oligonucleotide. Before the PCR process, melting temperature checking of forward and reserve primers was performed according to master mix that will be used in order to obtain the optimal result (Lorenz 2012).

Capsular gene of those five local isolates of *P. multocide* was detected using primer of CAPA and CAPB. Amplification result showed that two of five sample isolates were *P. multocide* serotype A that coded capsular gene A (PMa and PMc) showed with amplicons appearance at around 1044 base pair (Figure 2). Whereas, those three other isolates were isolate *P. multocide* serotype B (PMb, PMd, and PMk) that showed amplicons at 750 base pair (Figure 3).

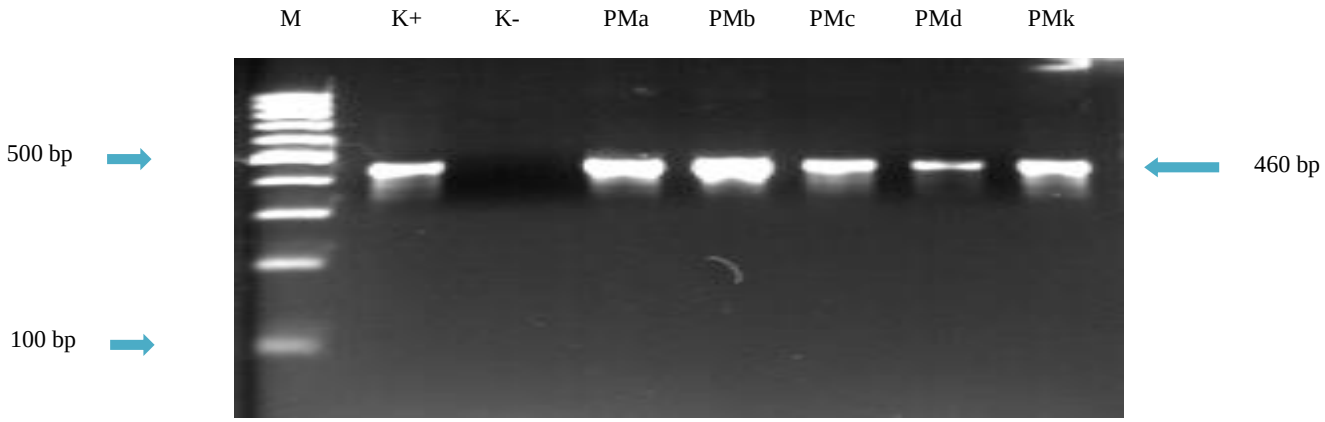

Figure 1. Detection of kmt gene of *P. multocide* using PCR.
PCR product of 460 *base pairs* (bp) specific to *P. multocida*
M: Marker, **K+:** Control positive (NCTC11668), **K-:** Control negative (*B. abortus*),
PMa, PMb, PMc, PMd: *P. multocide* Isolate from Lampung,
PMk: *P. multocide* Isolate from Kupang.

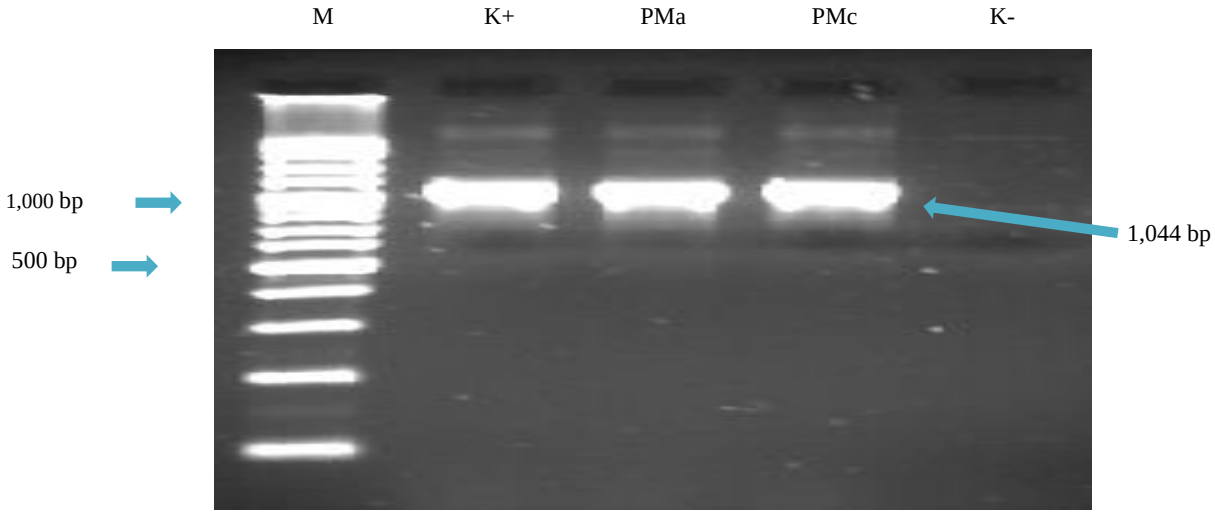

Figure 2. Detection of A capsular gene (*hyaD-hyaC*) of *P. multocide* using PCR.
Amplicons of serotype B of *P. multocide* on around 1.044 *base pairs* (bps)
M: Marker, **K+:** Control Positive (ATCC12945), K-: Kontrol Negative (*B. abortus*)
PMa, PMc: *P. multocide* Isolate from Lampung.

The two PCR processes informed that *P. multocide* in this study were serotype A and B. all isolate samples from cattle in Lampung and Kupang. Serotype A on cattle generally related to pneumonia (Frank 1989), while the serotype B related to the hemorrhagic septicemia/HS (Ranjan et al. 2011). Kupang as the capital of East Nusa Tenggara province known as beef cattle production center in eastern Indonesia, is an endemic area of HS case or known as snoring disease. The snoring disease on cattle happens almost every year in Kupang (Berek et al. 2015) leading to decrease in cattle population in latest several years (Priyanto 2016). While the HS outbreak in South Lampung was reported in 1984 and 1989 (Putra 2006). However, this study has not been able to determine that *P. multocide* from Kupang always serotype B. It shows that it needed further studies with more samples from more area in East Nusa Tenggara, except Kupang.

Amplification of 16S rRNA gene using PCR method to *P. multocide* isolate was performed to determine the relationship of local *P. multocide* with several isolate from abroad. Subunit gene of ribosome

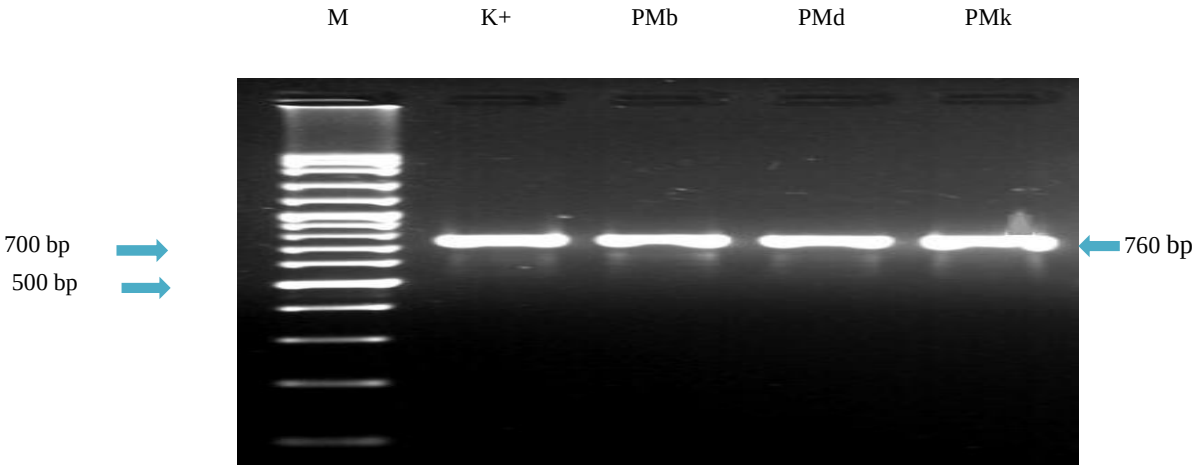

Figure 3. Detection of B capsular gene (*bcb*D) of *P. multocide* using PCR. Amplicons of B *P. multocide* serotype on around 760 *base pairs* (bps)
M: Marker, **K+:** Control positive (NCTC11668),
PMb, PMd: *P. multocide* Isolate from Lampung,
PMk: *P. multocide* Isolate from Kupang.

16S (16S rRNA), especially has been widely used in studying and characterizing bacteria community coverage on various ecology including the host related to the community like human microbiome endogen (Arumugam et al. 2011a) or the host that unrelated to humans. rRNA sequence, especially 16S rRNA represents the most important targets in the study of bacterial and ecological evolution, including to determine the phylogenic among taxa and exploration of environment bacteria diversity. The 16S rRNA gene was selected because that gene is distributed universally that allow phylogenic relationship analysis between the distant taxa (Větrovský & Baldrian 2013).

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Amplification result of 16S rRNA gene of *P. multocide* local isolate was 642 base pairs (Figure 4) which then was sequenced and analyzed using BIODET program to determine the contig sequence. The BLASTn program on NCBI site was used to blast to determine isolate homolog to the other isolates at the GenBank.

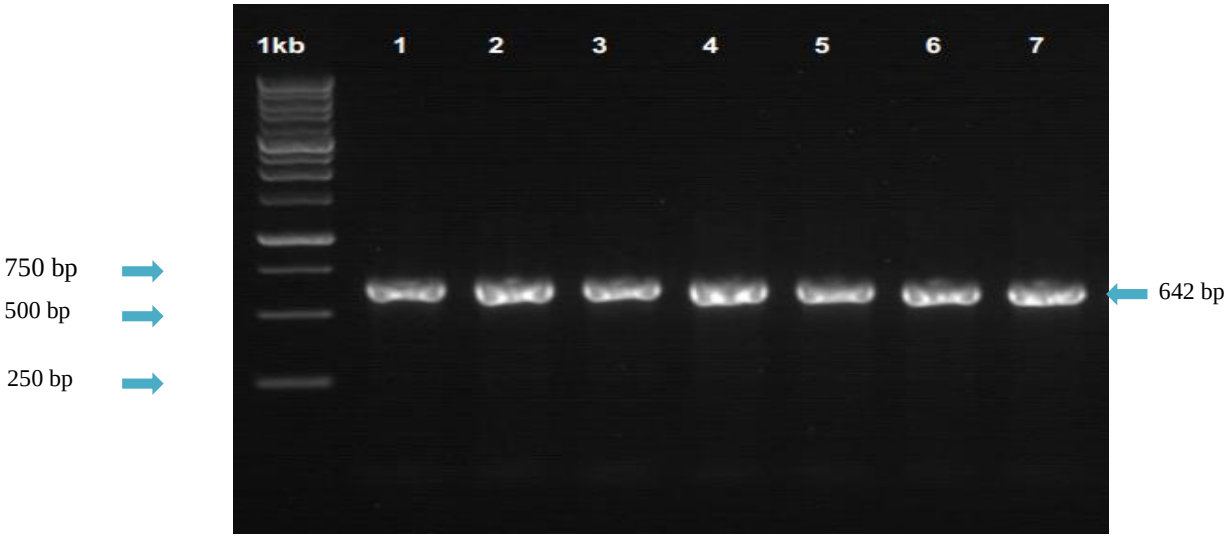

Figure 4. Detection of 16S rRNA gene of *P. multocida*
Amplicons of 16S rRNA gen of *P. multocide* on around 642 *base pairs* (bps)
1kb: Marker, 1: NCTC11668, 2: ATCC12945
3: PMa, 4: PMb, 5: PMc, 6: PMd, (Isolate from Lampung) 7: PMk (Isolate from Kupang).

Table 1. Matrix of nucleotide difference (642 nucleotides) of 16S rRNA gene using MEGA 7.0.25 program

Name	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
PM_797_India																
PM_FUP9_Egypt	0.000															
PM_Japan	0.000	0.000														
PM_NCTC10322_Scotland	0.000	0.000	0.000													
PM_GDZQ201401_China	0.004	0.004	0.004	0.004												
PM_TB2.1_Malaysia	1.190	1.190	1.190	1.190	1.213											
PM_Razi_Iran	1.190	1.190	1.190	1.190	1.213	0.000										
PM_NCTC10322_Canada	1.190	1.190	1.190	1.190	1.213	0.002	0.002									
PM_ATCC12945	1.190	1.190	1.190	1.190	1.213	0.002	0.002	0.000								
PM_NCTC11668	1.190	1.190	1.190	1.190	1.213	0.002	0.002	0.000	0.000							
PM_a_Lampung	1.179	1.179	1.179	1.179	1.202	0.004	0.004	0.002	0.002	0.002						
PM_b_Lampung	1.179	1.179	1.179	1.179	1.202	0.004	0.004	0.002	0.002	0.002	0.000					
PM_c_Lampung	1.190	1.190	1.190	1.190	1.213	0.002	0.002	0.000	0.000	0.000	0.002	0.002				
PM_d_Lampung	1.190	1.190	1.190	1.190	1.213	0.000	0.000	0.002	0.002	0.002	0.004	0.004	0.002			
PM_k_Kupang	1.190	1.190	1.190	1.190	1.213	0.000	0.000	0.002	0.002	0.002	0.004	0.004	0.002	0.000		
Brucella_abortus	0.220	0.220	0.220	0.220	0.225	1.195	1.195	1.195	1.195	1.195	1.184	1.184	1.195	1.195	1.195	

Description: PM: *Pasteurella multocide*; a,b,c,d, k: code for samples (local isolate).

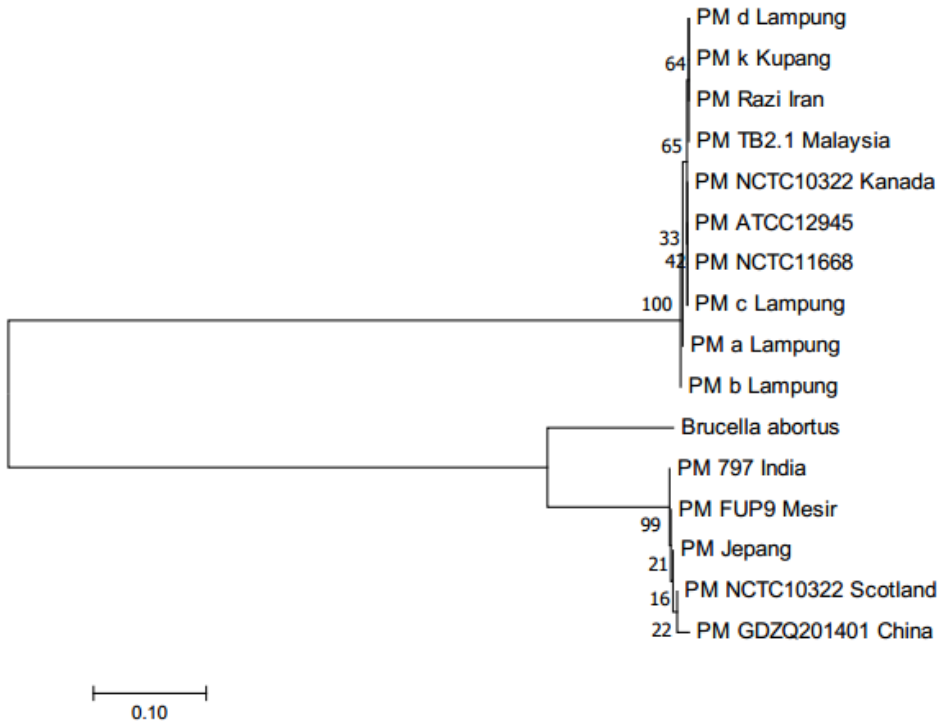

Figure 5. Phylogram of Evolutionary History of 16S rRNA (642 nt) gene nucleotide of local isolate P. mulcotide compared to isolates from other countries from GenBank using *Neighbor-joining* that counting by 2 parameters Kimura method on MEGA 7.0.25 program.

The multiple alignment of the sequence of 16S rRNA gene nucleotide of *P. multocide* of sequencing resulted in this study with the sequencing from China, Japan, India, Scotland and Egypt from the GenBank using ClustalW program showed the existence of the nucleotide.

Genetic distance counting by 2 parameters Kimura method (Table 1) showed genetic distance difference between the *P. multocide* isolates from Lampung and Kupang and *P. multocide* isolates from several countries in the world. The average genetic distance between *P. multocide* species using neighbor joining method of 642 bases of nucleotides of the 16S rRNA gene showed that level of the nucleotide of *P. multocide* isolate in the results of the study have no relationship with isolates from India, Egypt, Japan, Scotland and China. *P. multocide* isolate from Lampung and Kupang had relationship with the *P. multocide* isolate from Malaysia, Iran and Canada (Figure 5).

Matrix value of the genetic distance between each isolate presented in Table 1 was used to build phenogram tree (Figure 5). That phenogram tree shows the genetic distance between all isolates. In other words, all *P. multocide* isolates aligned is divided into two main branches, so that the *P. multocide* isolate from Lampung and Kupang is in the same branch with the *P. multocide* from Malaysia, Iran, and Canada, Pascoe et al. (2017) said that genetic structure of bacteria population can be associated with geographical location of isolation. On several species, there is a strong correlation between geographic distance and genetic distance that may be caused by different evolutionary evolution. Sheppard et al. (2014) stated that frequency of certain genotype isolated from the different host is a basic of host identification related to clonal complex. This indicates that nucleotide sequence is different in two main groups of phenogram tree that shows the thing that may occur due to a different host, geographic and isolate. It does not rule out the possibility of the different nucleotide sequence was affected by lineage development due to deviations of a single or multiple genes on Multi Locus Sequence Typing gene (Bisgaard et al. 2013).

CONCLUSION

This study and analysis informed that five bacteria isolates have been identified as *P. multocide* serotype A and serotype B. Four isolates from Lampung consisted of each two isolates either serotype A or serotype B. While, isolates from Kupang was *P. multocide* serotype B. PMc isolate had high homologue with the ATCC 12945, so that it can be used as positive control of serotype A. On the other hand, *P. multocide* isolate

from Kupang can be used as positive control of serotype B, because it is identic to *P. multocide* PMTB2.1 (CP007205.2) isolated from buffalo infected with HS in Malaysia.

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