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PREFACE

In this edition, for last volume 21, we proudly present articles from animal and veterinary sciences including feed and nutritive technology, animal breeding, genetic, paracitology, and micology. The articles published in this edition are: "Characteristic morphology and genetic variability of Benggala grass (*Panicum maximum cv Purple guinea*) through gamma ray irradiated on acid land"; "Productivity of *Brachiaria decumbens* in a Mixed-culture with *Pueraria phaseloides* in Different Manure and Dolomite Administration level into Suboptimal land"; "Study of BMP15 gene polymorphism in Boer, Kacang, and Boerka goats"; "Management strategies to improve reproductive performance of Brahman cross cattle (*Bos indicus*) in East Java, Indonesia"; "Application of BS4-enzyme on the methane production from mixture of cattle manures and waste paper" and "Bioactivities and analysis of peptides from Sumbawa horse milk generated by *Bacillus thuringiensis* protease".

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, December 2016

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LIST OF CONTENT

Characteristic Morphology and Genetic Variability of Benggala Grass (*Panicum maximum* cv Purple guinea) through Gamma Ray Irradiated on Acid Land

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ABSTRAK

Fanindi A, Sutjahjo SH, Aisyah SI, Purwantari ND. 2016. Karakter morfologi dan keragaman genetis rumput Benggala (*Panicum maximum* cv Purple guinea) melalui iradiasi sinar gamma pada lahan masam. JITV 21(4): 205-214. DOI: http://dx.doi.org/10.14334/jitv.v21i4.1635

Mutasi induksi menggunakan sinar gamma adalah salah satu cara untuk meningkatkan keragaman rumput Benggala (*Panicum maximum*). Keragaman merupakan modal utama dalam melakukan seleksi untuk menghasilkan varietas rumput Benggala pada lahan masam. Penelitian bertujuan untuk mengetahui karakter morfologi dan keragaman genetik rumput Benggala yang mendapat perlakuan sinar gamma. Penelitian dilakukan di lahan masam yaitu di daerah Tenjo Kab. Bogor yang memiliki pH 4,9-5,5 dan kandungan Al-dd 2,26 cmol/kg dengan kejenuhan Al 10,68%. Penelitian dimulai dengan mencari radio sensitivitas rumput Benggala, selanjutnya dari radio sensitivitas tersebut ditentukan dosis perlakuan radiasi yaitu 200 Gy, 250 Gy, 300 Gy dan 350 Gy. Perlakuan dosis radiasi dilakukan secara acute dan fractionated, sehingga terdapat 8 dosis perlakuan ditambah kontrol (tanaman tanpa di radiasi). Pengamatan variabel yang diukur adalah tinggi tanaman, tinggi batang, diameter batang, panjang ruas, panjang daun, leber daun, bobot segar, bobot kering dan jumlah anakan. Hasil penelitian menunjukkan bahwa radio sensitivitas rumput Benggala yang dihasilkan berupa letahal doses (LD) LD 20 dan LD50 yaitu 176,83 Gy dan 358,23 Gy. Dengan persamaan matematikanya: Y= 44,22 + 45,91 X Cos (0.0042x -0.07). Karakter morfologi pada M1 menunjukkan bahwa dosis radiasi mempengaruhi semua variabel karakter tanaman. Dosis 300 Gy fractionated (150 Gy x 2) menghasilkan bobot segar tanaman yang lebih tinggi. Sementara itu, pada generasi M1V1 dosis radiasi yang memberikan bobot segar hijauan tinggi adalah dosis 250 Gy. Heritabilitas sedang sampai tinggi dengan nilai koefisien keragaman genetik (KKG) cukup tinggi sampai tinggi diperoleh pada karakter jumlah anakan, bobot segar dan bobot kering. Sehingga memungkinkan untuk dilakukan seleksi selanjutnya pada ketiga karakter ini.

Kata Kunci: *Panicum maximum*, Iradiasi, Mutasi, Morfologi, Keragaman Genetis

ABSTRACT

Fanindi A, Sutjahjo SH, Aisyah SI, Purwantari ND. 2016. Characteristic morphology and genetic variability of Benggala grass (*Panicum maximum* cv Purple guinea) through gamma ray irradiated on acid land. JITV 21(4): 205-214. DOI: http://dx.doi.org/10.14334/jitv.v21i4.1635

The induction mutation using gamma rays is one way to increase the diversity of Benggala grass (*Panicum maximum*). Diversity is the main base in selection to produce varieties of Benggala grass on acid land. The aim of this study is to know the morphological character and genetic diversity of grass that received gamma rays. The research was conducted in Tenjo district, Bogor which has pH 4.9-5.5 and the content of Al-dd 2.26 cmol/kg with Al saturation of 10.68%. The study was started by identifying for Benggala grass radio sensitivity, then from radio sensitivity determined radiation treatment dose was decided of: 200 Gy, 250 Gy, 300 Gy and 350 Gy. Treatment of radiation dose is acute and fractionated, so there are 8 treatment doses plus control (plants without radiation). The variables measured were plant height, stem height, stem diameter, length of segment, leaf length, leaf width, fresh weight, dry weight, and number of tillers. Results showed that the radio sensitivity of Benggala grass produced in the form of lethal doses (LD) LD 20 and LD50 was 176.83 Gy and 358.23 Gy. By the mathematical equation: Y = 44.22 + 45.91 X Cos (0.0042x -0.07). The morphological character of M1 shows that the radiation dose affected the measured variable. 300 Gy fractionated radiation dose (150 Gy x 2) resulted in higher fresh weight of the plant. While in the generation of M1V1 radiation dose of 250 Gy produced high fresh forage weight. Medium to high heritability with GVC value (criteria of coefficient of genetic variability) is high enough until high is obtained on the character of the number of tillers, fresh weight and dry weight. So it is possible to do next selection on these three characters.

Key Words: *Panicum maximum*, Irradiation, Mutation, Morfology, Genetic Variability

INTRODUCTION

Benggala grass is one of grasses widely used by farmer in Indonesia. The grass has production by 6.7-15 ton of DM/ha/year (Hare et al. 2009). The grass is easily harvested, may grow back after pruning, requires few pesticides and non intensive cultivation management. It is also resistant to dry land and grows well on various soil types, even tolerant to moderate shading which means it may grow under tree shade or bush. These are how the Benggala grass is potential for "ideal" forage as fiber source for ethanol production besides its low management cost and abundant production (Ratsamee et al. 2012). It also may be used as quality "cut and carry" grass for beef and dairy cattle (Aganga & Tshwenyane 2004; Nakamanee et al. 2008) and also grass for pasture with good quality (Carnevalli et al. 2006).

One of constrains of the Benggala grass cultivation is various tolerance to the acid land. A research result showed that the Benggala grass did not grow well on both oxisol and ultisol lands (Chen & Hutton 1992), while Almeida et al. (2000) reported that the most genotype of Benggala grass had low tolerance to the acid land (aluminum stress). All this time, in Indonesia, the approach for cultivation improvement was through fertilization or addition of lime, where the breeding approach to create Benggala grass varieties tolerant to acid land has not been conducted. Even though, the use of Benggala grass varieties tolerant to the suboptimal land may save production cost by 1 million/ha (Karti 2016). The forming of varieties tolerant to acid land through mutation may be performed by induction mutation using gamma ray radiation. The induction mutation on Benggala grass is expected to increase mutation frequency to increase its diversity. All this time, the diversity of Benggala grass in Indonesia is remain low since there is no breeding program besides its apomixis reproduction generating similar offspring to the parent plant.

The success of induction mutation using the gamma ray radiation on the plant has successfully created plant varieties tolerant to sub optimal land such as in soybean (Hanafiah et al. 2010), rice (Sobrizal 2016) and wheat (Nur et al. 2014). Many researches of the induction mutation on Benggala grass have been reported, such as quality improvement through tissue and protoplasm isolations (Akashi & Adachi 1991) and development of tolerance on acid land (Pongtongkam et al. 2005). The use of induction mutation on many plants, including the Benggala grass showed that the induction mutation using gamma ray radiation may be conducted to create Benggala grass cultivar tolerant to acid land. The forming of Benggala grass tolerant to acid land requires several steps, where the early stage is to determine radio-sensitivity and observation of morphology of generation M1 and M1V1. Therefore, this study was conducted to determine LD_{50}, observing morphology characteristics of Benggala grass irradiated using gamma ray to obtain mutative mutants tolerant to the acid land.

MATERIALS AND METHODS

Determination of radio-sensitivity of Benggala grass

Radio-sensitivity of Benggala grass is determined by irradiation using gamma ray from 0 doses (control) to 1000 Gy. The irradiation was performed at National Nuclear Energy Agency of Indonesia. Benggala grass seed used was cv Purple guinea. The seedling was radiated by gamma ray doses by 0 Gy, 50 Gy, 100 Gy, 150 Gy, 200 Gy, 250 Gy, 300 Gy, 350 Gy, 400 Gy, 450 Gy, 500 Gy, 550 Gy, 600 Gy, 650 Gy, 700 Gy, 750 Gy, 800 Gy, 850 Gy, 900 Gy, 950 Gy and 1000 Gy. The gamma ray source was Cobalt 60 delivered through *irradiator gamma chamber* 4000A. The number of seedling irradiated per doses was 500 seeds. Then, the seeds were planted into germination tub and were evaluated on the percentage of growth rate at three weeks after planting. Variables observed in this study were percentage of growth rate (1), the amount of leave (2), seedling height which measured from root to peak (3). The percentage data were used in determining the Lethal Dose 50 value (LD_{50}) analyzed using the Curve-fit Analysis program.

Identification of putative mutants of Benggala grass (M1) and the diversity of agronomy characteristics of M1V1 generation

The field study was conducted at Regional Technical Implementing Unit of dry land, District of Tenjo, Bogor Regency for 10 months. The research land has pH by 4.9-5.5 and Al-dd content by 2.26-4.52 cmol/kg with the Al saturation by 10.68%. This study was designed in Randomized Block Design (RBD) with three replications. The treatment performed in this study was radiation doses between the LD_{20}-LD_{50} consisted of four acute doses and four fractionated doses, and the control. The doses used were 200 Gy, 250 Gy, 300 Gy, 350 Gy and 100 x 2 Gy, 125 x 2 Gy, 150 x 2 Gy and 175 x2 Gy and the control. The irradiation was conducted at the National Nuclear Energy Agency of Indonesia of Pasar Jumat-Jakarta. The irradiated Benggala grass seed was 200 seeds each dose grew in polybag (one seed each), then transferred into the field after one month.

The observation of morphology characteristics of the M1V1 was conducted at the same location as the characteristic observation of M1. The crops material used was all crops in M1. A total of 2-3 seedlings were

collected from each individual of M1 to become M1V1. The number of crops would be planted in the M1V1 were around 2,700-3,000 plants. The maintenance and cultivation were similarly performed as in the M1.

The board-sense heritability value was counted following the Kalton et al. (1952) formula:

$$h^2 = \frac{\sigma^2 S1 - \sigma^2 S0}{\sigma^2 S1}$$

Where:
$\sigma^2 S0 = \sigma^2 c$ = The variance between the seedling;
$\sigma^2 S1$ = The derivative variance of the self clone or the seedling mutation result

The diversity was counted by:

$$\sigma^2 = \frac{\sum_{i=1}^{n} (X_i - \bar{X})^2}{n}$$

Where:
σ^2 = Variance;
x_i = Midpoint;
$\bar{x}$ = Average;
n = The number of data

Genetic variability was determined by the basis of genetic variability coefficient (GVC) using the following method by Singh & Chaudhari (1977):

$$KKG = \left(\sqrt{\sigma_g^2} / x \right) x100$$

RESULTS AND DISCUSSION

Radio-sensitivity of Benggala grass

Observation of radio-sensitivity in this study was conducted by observing the LD_{50} of the irradiated Benggala grass. The observation result was presented in Table 1. The data then were counted and analyzed using Curve-fit and then its mathematic formula was formed. The Curve-fit analysis result (Figure 1) showed that the LD_{50} and LD_{20} value of Benggala grass using the seed were **358.23 Gy** and **176.83 Gy** respectively. While, its mathematic formula was Model Sinusoidal Fit: **Y= 44.22 + 45.91 X Cos (0.0042x -0.07)**. The LD_{50} value may also be determined using crop height indication. The height will decrease along with the increase of radiation. The crop height at the 250-350 Gy decreased a half compared to the control. This shows that the crop height may be an indicator to determine the LD_{50} of Benggala grass irradiated by gamma ray. The research of use of the crop height and root length has been performed by Ambavane et al. (2015) to measure LD_{50} of millet plant.

The sensitivity rate may be observed from the response of crops both of the morphology, sterility or lethal doses 50 (LD_{50}). The lethal doses 50 LD_{50} is a dose causing the death of 50% irradiated population. From the mutation study done, commonly, the desirable mutation is around the LD_{50} or slightly under LD_{50}. And commonly, in this range, it is obtained high

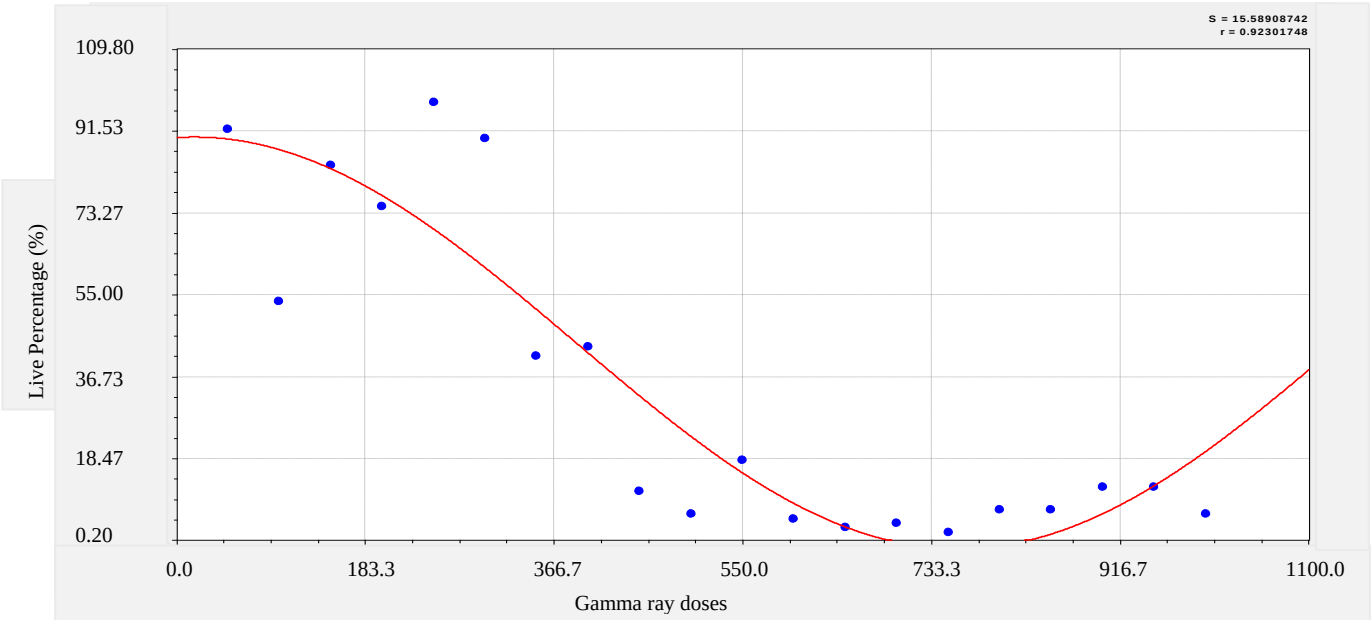

Figure 1. Graphic of lethal doses by 20 – 50% of Benggala grass irradiated by gamma ray by 0-1000 Gy.

Table 1. Live rate percentage, sprout height and the number of leave of Benggala grass at one month after planting

Doses (Gy)	Crop height (cm)	The number of leaves	Live rate percentage (%)
0	9.01±3.00	3.33±0.97	100.00
50	9.48±3.00	3.44±0.67	91.92
100	7.36±3.11	3.43±0.89	53.54
150	6.93±2.76	3.51±0.95	83.84
200	4.66±2.36	2.95±1.23	74.75
250	4.24±2.56	2.76±1.23	97.98
300	4.83±2.83	2.91±1.23	89.90
350	3.28±2.09	2.27±1.48	41.41
400	3.04±1.92	2.14±1.54	43.43
450	1.76±1.17	1.18±1.60	11.11
500	1.17±0.40	0.83±0.41	6.06
550	0.86±0.56	0.44±0.51	18.18
600	1.22±0.75	0.40±0.89	5.05
650	0.67±0.47	1.00±1.73	3.03
700	0.65±0.47	0.50±1.00	4.04
750	0.30±0.14	0.00±0.00	2.02
800	0.39±0.18	0.00±0.00	7.07
850	0.63±0.42	0.29±0.49	7.07
900	0.33±0.09	0.00±0.00	12.12
950	0.29±0.12	0.00±0.00	12.12
1000	0.22±0.04	0.00±0.00	6.06

diversity (Aisyah 2009). The LD_{50} is also obtained from the breeders to induce the mutation, where it is expected a balanced physiologies damage with the obtained genetic change (Sudrajat & Zanzibar 2009). The LD_{50} range doses may be used to both determine sensitivity rate against physical and chemical mutagens and estimate the appropriate dose or concentration to induce the mutation (Abdullah et al. 2009).

Morphology characteristic of M1 of Benggala grass at the acid land

The research result of the M1 is presented in Table 2. It shows that dose of gamma ray radiation affects leaves height and width. The highest crop was in the 150 x 2 Gy doses (300 Gy with divided doses) by 56.11 cm, while the lowest crop was in the 350 Gy by 37.65 cm. This shows that higher radiation dose will decrease the crops height. This is in accordance with the research performed by induction mutation of gamma ray radiation, where the addition of the gamma ray will prevent the growth as in the millet (Ambavane 2015) and soybean (Hanafiah 2012).

The leaves width was affected by dose of gamma ray radiation. The widest leaves was in the 150 x 2 Gy, while the lowest doses was in radiation of 300 Gy, 350 Gy and 175 x 2 Gy which was the highest doses in this study. This also shows that the increase of gamma ray doses will prevent the crop growth including the height of crops and the leaves width.

The fresh crop weight, dry crop weight and the number of seedling was presented in Table 3. The heaviest fresh crop weight was in the 150 x 2 Gy by 47.61 g/crop. The fresh crop weight at the 150 x2 Gy dose was also higher compared to the control. While, the lowest fresh crop weight was at 300 Gy and 350 Gy by 9.93 gr/crop and 13.18 gr/crop, respectively. The effect of radiation doses that leads to better productivity performance of crops at the sub-optimal land was also

Table 2. Average crop height, leaves width and leave length of Benggala grass (M1) irradiated by gamma ray for three months at the acid land

Dose of gamma ray (Gy)	Variable		
	Crop height (cm)	Leave width (cm)	Leave length (cm)
Control	45.61[ab]	1.00[ab]	30.71[a]
200	46.02[ab]	0.97[ab]	29.54[a]
250	44.84[ab]	0.95[ab]	29.46[a]
300	38.64[b]	0.86[b]	29.33[a]
350	37.65[b]	0.85[b]	26.04[a]
100x2	45.55[ab]	1.00[ab]	30.53[a]
125x2	45.20[ab]	1.03[ab]	29.86[a]
150x2	56.11[a]	1.17[a]	34.41[a]
175x2	40.18[b]	0.85[b]	25.83[a]

[a]The same numbers in the same column followed by same word means no significant difference at test degree of 5% (Duncan multiple range test).

Table 3. Average fresh crop weight, dry crop weight and the number of tiller of Benggala grass (M1) irradiated by gamma ray for three months at the acid land

Dose of gamma ray (Gy)	Variable		
	Fresh weight (g)	Dry weight (g)	The number of tiller (crop)
Control	26.47[ab]	9.17[ab]	13.91[bc]
200	20.00[b]	7.34[b]	14.67[bc]
250	26.23[ab]	8.79[b]	17.80[ab]
300	9.93[b]	4.14[b]	11.85[c]
350	13.18[b]	5.37[b]	17.08[ab]
100x2	25.26[b]	8.72[b]	14.70[bc]
125x2	23.06[b]	8.73[b]	15.29[bc]
150x2	47.61[a]	16.00[a]	20.81[a]
175x2	15.18[b]	5.65[b]	13.59[bc]

[a]The same numbers in the same column followed by same word means no significant difference at test degree of 5% (Duncan multiple range test)

reported in the soybean in the dry land (Hanafiah 2012); high temperature wheat (Setiawan 2015); soybean at the dry acidic land (Giono et al. 2014) and corn at the acid land (Lubis et al. 2014), whereas, the induction mutation in the Benggala grass resulted in a mutant tolerant to acid land (Pongtokam et al. 2005). The influence of the induction mutation (gamma ray) to the fresh weight is allegedly due to a mutation at the gen level causing a change in structure and position of gen in the chromosome. This leads to phenotype change of an organism. The change of the number of basic chromosome both when the increase or decrease of one set or part of chromosome causing the appearance or the disappearance of one character. After the established mutation in the gen or chromosome level in a population, it is followed by nature or Artificial selection (Ambavane et al. 2015).

This research result also showed that dry crop weight was influenced by gamma ray, where the heaviest dry crop production was at 150 Gy x 2 (300 Gy *fractionated*) by 16.00 gr/crop. The heavy dry weight of crop at the 150 Gy x 2 was in accordance with the heavy fresh crop weight at the 150 Gy x 2. It is also related to the high number of seedling in those doses. The highest number of seedling in this study was at the

150 Gy x 2 by 20.81 seedlings. This number was higher compared to the other doses, even than the control.

Morphology characteristic of M1V1 in the acid land

The crop height, leaves width and leaves length of M1V1 crop in the acid land was presented in Table 4. The highest crop was at the 250 Gy, while the lowest one was at the 125 Gy x 2 (250 Gy *fractionated*). The leaves width did not show a difference between the treatments. The longest leave was at 200 Gy and control, while the shortest one was at the 175 Gy x 2. The decrease of characteristic along with the increase of doses of gamma ray radiation, commonly occurred in the crops experiencing gamma ray radiation, as in the soybean crop (Hanafiah 2010) and paddy (Sobrizal 2016).

The fresh crop weight, dry crop weight and the number of seedling were presented in Table 5. The heaviest fresh weight was at 250 Gy and control, while the lightest fresh crop weight was at 125 Gy x 2 (250 *fractionated*). The heaviest dry crop weight was also at 250 Gy. While highest number of seedling was at 200 Gy and the lowest one was at the 125 x 2 Gy.

The stem diameter, stem height and segment length was presented in Table 6. The segment length and stem height did not show a difference between the treatments. The widest stem diameter was at 350 Gy by 5.23 cm, while the shortest one was in the 125 Gy x 2.

Table 4. Average crop height, leaves width and leaves length of Benggala grass (M1V1) irradiated by gamma ray for three months at the acid land

Dose of gamma ray (Gy)	Variable		
	Crop height (cm)	Leaves width (cm)	Leaves length (cm)
Control	133.84[a]	2.13[a]	57.62[a]
200	128.37[ab]	1.97[a]	57.43[a]
250	136.77[a]	2.06[a]	54.41[ab]
300	123 .80[ab]	1.86[a]	54.58[ab]
350	127.90[ab]	1.99[a]	54.80[ab]
100x2	125.47[ab]	2.00[a]	54.96[ab]
125x2	110.84[b]	1.57[a]	53.05[b]
150x2	127.99[ab]	1.80[a]	52.83[b]
175x2	117.86[ab]	2.08[a]	52.99[b]

[a]The same numbers in the same column followed by same word means no significant difference at test degree of 5% (Duncan multiple range test).

Table 5. Average fresh crop weight, dry crop weight and the number of seedling of Benggala grass (M1V1) irradiated by gamma ray for three months at the acid land

Dose of gamma ray (Gy)	Variable		
	Fresh weight (g/crop)	Dry weight (g/crop)	The number of seedling (crop)
Control	346.92[a]	84.77[ab]	14.64[ab]
200	313.91[abc]	77.43[ab]	15.47[a]
250	350.97[a]	87.48[a]	14.84[ab]
300	306.85[abc]	77.87[ab]	12.90[b]
350	313.98[ab]	83.19[ab]	14.39[ab]
100 x 2	327.46[ab]	80.63[ab]	13.74[ab]
125 x 2	215.17[c]	61.56[b]	12.79[b]
150 x 2	319.43[ab]	86.70[ab]	14.09[ab]
175 x 2	241.34[bc]	65.79[ab]	13.43[ab]

[a]The same numbers in the same column followed by same word means no significant difference at test degree of 5% (Duncan multiple range test).

Genetic variability and heritability of M1V1 in the acid land

The heritability and genetic variability of M1V1 in the acid land was presented in Table 7 and 8. The genetic diversity and heritability are used to estimate genetic development from selection of Barmawi et al. (2013). The genetic diversity itself is very difficult to study characteristic, therefore other genetic parameters such as heritability is required (Satoto & Suprihatno 1996). Heritability is genetic parameter used to assess the ability of a genotype in a crop population in inheriting their characteristics or estimation to assess how much the diversity of appearance of a genotype in a population, especially caused by genetic factors. The heritability of a characteristic is highly required to be assessed to determine the efficiency of a selection (Seyoum et al. 2012). High heritability value shows that genetic factor is more involved in determining those characteristics compared to the influence of environment, so that a selection on the characteristics is very possible.

The heritability and genetic variability value of Benggala grass for the crop height, stem height, segment length, leave length and leaves width at the acid land were presented in Table 7. The GVC value was counted from the basis of relative GVC from its absolute value where the absolute value of GVC in this study was 0-56.41% (Table 7 and 8). The value 56.41% was used as 100% relative value. The relative criteria values of the GVC were low (0-25%), medium (25-50%), quite high (50-75%) and high (75-100%) (Moedjiono & Mejaya 1994). It means that the absolute values in this study were 0% < x ≤14.10%; 14.10% < x ≤28.21%; 28.21% < x ≤42.31%; and 42.31% < x ≤56.41%.

The crop height, stem height, stem diameter and segment length had low to moderate heritability. The moderate heritability of crop height was at 100 Gy x 2, 300 Gy and 150 Gy x 2. The heritability of stem height was at 100 x 2 Gy, as well as the stem diameter, the moderate heritability value was at 100 Gy x 2, 150 Gy x 2, whereas the segment length had moderate heritability value at 300 Gy, 100 Gy x 2 and 150 Gy x 2. Narrow heritability value of crop height, leaves width and leaves length in the M2 generation is also reported by Poerba (2004) of *Talinum paniculatum* Jacq. (Gaertn).

Heritability value of leaves length had narrow to moderate value, where the moderate heritability was at 100 Gy x 2 dan 350 Gy. The genetic variability of leaves length low value. Heritability value of leaves width had high to low value range, where the high heritability value was at 300 Gy. Diversity of this characteristic was from low to rather low.

The number of seedling had moderate to high value, where the high value was at 250 Gy and 150 Gy x 2. The genetic diversity value of the number of seedling of Benggala grass was in medium to quite high value range. The high heritability value and quite high diversity is allowed it to do further selection on the number of seedling variable.

The fresh and dry crop weights are two indicators in determining forage production. The heritability of fresh crop weight (Table 8) was in narrow to high value range. Average dose had moderate heritability value. High heritability of fresh crop weight was at 100 Gy x 2 with high variability genetic which means a possibility to do selection in this characteristic. Dry crop weight had medium to quite high heritability value range.

Table 6. Stem diameter, stem height, and segment length of Benggala grass (M1V1) irradiated by gamma ray for 3 months in the acid land

Dose of gamma ray (Gy)	Variable		
	Stem diameter (cm)	Stem height (cm)	Segment length (cm)
Control	4.89[ab]	76.56[a]	19.01[a]
200	5.15[ab]	73.28[a]	19.20[a]
250	4.93[ab]	82.25[a]	19.16[a]
300	5.17[ab]	72.53[a]	19.11[a]
350	5.23[a]	77.01[a]	19.17[a]
100x2	5.09[ab]	78.83[a]	19.14[a]
125x2	4.85[b]	66.18[a]	18.49[a]
150x2	5.08[ab]	81.41[a]	19.89[a]
175x2	5.11[ab]	68.52[a]	19.59[a]

[a]The same numbers in the same column followed by same word means no significant difference at test degree of 5% (Duncan multiple range test)

Table 7. Genetic characteristic of crop height, stem height, stem diameter, segment length, leaves length and leave width of Benggala grass (M1V1)

Characteristic	Doses (Gy)							
	200	250	300	350	100 x 2	125 x 2	150 x 2	175 x 2
Hight								
σ^2p	746.60	1059.2	1338.2	928.2	1693.1	909.5	1318.5	946.7
σ^2g	0.00	132.9	412.00	1.93	766.83	0.00	392.25	20.44
h^2	0.00	0.13	0.31	0.00	0.45	0.00	0.30	0.02
GVC (%)	0.00	8.03	13.02	0.95	17.22	0.00	13.82	3.40
Criteria of GVC	low	low	low	low	rather low	low	low	low
Stem H.								
σ^2p	627.83	1140.14	1090.85	812.30	1290.51	651.54	1133.91	844.74
σ^2g	0.00	214.28	165.00	113.5	364.66	0.00	208.06	0.00
h^2	0.00	0.19	0.15	0.14	0.28	0.00	0.18	0.00
GVC (%)	0.00	17.80	17.71	0.00	24.23	0.00	17.72	0.00
Criteria of GVC	low	rather low	rather low	low	rather low	low	rather low	low
Stem D.								
σ^2p	0.92	0.92	1.04	0.81	1.07	0.81	0.97	1.06
σ^2g	0.16	0.17	0.29	0.05	0.32	0.05	0.21	0.30
h^2	0.18	0.18	0.28	0.07	0.30	0.06	0.22	0.29
GVC (%)	7.84	8.35	10.38	4.44	11.06	4.69	9.09	10.72
Criteria of GVC	Low	Low	Low	Low	Low	Low	Low	Low
Segment L.								
σ^2p	12.34	12.74	16.05	11.80	15.24	10.35	14.57	10.52
σ^2g	1.19	1.59	4.90	0.65	4.09	0.00	3.42	0.00
h^2	0.10	0.12	0.31	0.06	0.27	0.00	0.23	0.00
GVC (%)	5.67	6.59	11.59	4.21	10.57	0.00	9.30	0.00
Criteria of GVC	Low	Low	Low	Low	Low	Low	Low	Low
Leave L.								
σ^2p	70.68	72.36	73.02	97.14	88.44	68.80	83.84	71.94
σ^2g	19.44	21.11	21.77	45.89	37.19	17.55	32.59	20.69
h^2	0.27	0.29	0.30	0.47	0.42	0.39	0.39	0.29
GVC (%)	7.68	8.44	8.55	12.36	11.10	7.90	10.81	8.58
Criteria of GVC	Low	Low	Low	Low	Low	Low	Low	Low
Leaves W.								
σ^2p	0.27	0.32	0.39	0.24	0.35	0.19	0.27	0.28
σ^2g	0.09	0.13	0.20	0.05	0.17	0.00	0.08	0.09
h^2	0.31	0.42	0.52	0.21	0.47	0.01	0.31	0.32
GVC (%)	14.85	17.81	24.14	11.29	20.30	2.90	15.94	14.21
Criteria of GVC	Rather low	Rather low	Rather low	Low	Rather low	Low	Rather low	Rather low

σ^2p = Phenotype GVC = Genetic variability coefficient Segment L = Segment length
σ^2g = Genetic Stem H. = Stem height Crop H = Crop height
h^2 = Heritability Stem D. = Stem diameter
Leaves L. = Leaves length width (Leaves W.) of Benggala grass (M1V1)

Table 8. Variance of phenotypic (σ^2p), genetic (σ^2g), heritability (h^2) and genetic variability coefficient (GVC) of fresh crop weight (Fresh Weight), dry crop weight (Dry Weight) and the number of tiller of M1V1 Benggala

Characteristic	Doses (Gy)							
	200	250	300	350	100 x 2	125 x 2	150 x 2	175 x 2
Fresh Weight								
σ^2p	31732	45385	46423	56115	68443	17636	50706	34968
σ^2g	0.00	13652	12105	21797	34126	0.00	16388	650.77
h^2	0.00	0.30	0.26	0.39	0.50	0.00	0.32	0.02
GVC (%)	0.00	33.29	35.86	47.02	56.41	0.00	40.08	10.57
Criteria of GVC	Low	Quite high	Quite high	High	High	Low	Quite high	Low
Dry Weight								
σ^2p	2027.7	2585.5	2582.6	2913.4	3099.2	960.45	3221.2	1835.4
σ^2g	379.66	937.46	934.58	1265.4	1451.2	0.00	1573.2	187.39
h^2	0.19	0.36	0.36	0.43	0.47	0.00	0.49	0.10
GVC (%)	25.16	35.00	39.26	42.76	47.25	0.00	45.74	20.81
Criteria of GVC	Rather low	Quite high	Quite high	High	High	Low	High	Rather low
The Number of tiller								
σ^2p	35.68	41.30	30.43	44.91	36.68	28.04	46.81	37.55
σ^2g	16.57	22.19	11.32	25.79	17.56	8.93	27.70	18.44
h^2	0.46	0.54	0.37	0.57	0.48	0.32	0.59	0.49
GVC (%) (%)	26.32	31.75	26.07	35.30	30.49	23.36	37.36	31.96
Criteria of GVC	Rather low	Quite high	Rather low	Quite high	Quite high	Rather low	Quite high	Quite high

Σ^2p = Phenotypic
Σ^2g = Genetic
H^2 = Heritability
GVC = Genetic Variability Coefficient (Fresh Weight), Fresh Crop Weight Dry Weight), Dry Crop Weight
The Number of Tiller Of M1V1 Benggala

Moderate heritability value was at 100 Gy x 2 and 150 Gy x 2. Heritability and genetic diversity commonly will be linear with genetic diversity coefficient.

CONCLUSION

The LD_{50} and LD_{20} value of Benggala grass cv Purple guinea were 358.23 Gy and 176.83 Gy. This value may be used as a standard in determining gamma ray radiation doses to irradiate the Benggala cv Purple guinea grass. Morphological characteristic of M1 showed that the doses of radiation affected the crop height, leaves width, fresh crop weight and dry crop weight, where the 150 Gy x 2 resulted in the heaviest fresh crop weight. Morphological characteristic of the M1V1 was also influenced by radiation doses, where the heaviest fresh crop weight was at 250 Gy. Moderate to high heritability value was found in the characteristic of the number of tiller, fresh crop weight and dry crop weight; as well the genetic variability had quite high range value which shows a possibility to do selection on those characteristics then it creates Benggala grass which has high production in the acid land.

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Productivity of *Brachiaria decumbens* in a Mixed-Culture with *Pueraria phaseloides* in Different Manure and Dolomite Administration Level into Suboptimal Land

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ABSTRAK

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Peningkatan produktivitas hijauan di lahan suboptimal dapat dilakukan dengan berbagai cara salah satu diantaranya melalui perlakuan pemupukkan dan amelioran. Pupuk kandang dan dolomit sangat dibutuhkan oleh tanah di lahan suboptimal, karena kombinasi keduanya dapat menyuplai unsur hara dan memperbaiki biofisik tanah. Materi penelitian yang digunakan adalah rumput *B. decumbens*, *Pueraria phaseloides*, pupuk kandang (Pukan), dan dolomit. Penelitian menggunakan rancangan Acak Kelompok pola faktorial 3x3, dosis pupuk kandang merupakan faktor pertama, dan dosis super dolomit merupakan faktor kedua, masing-masing perlakuan mendapat 3 ulangan. Peubah yang diamati adalah produksi biomassa, kapasitas tampung, dan kandungan nutrisi. Hasil penelitian menunjukkan bahwa produksi biomasa pada taraf perlakuan pukan 10 ton/ha dan super dolomit 2 ton/ha, nyata (P<0,05) paling tinggi sebesar 1.638,94 g/m^2 dibanding perlakuan lain dan terendah dicapai tanpa perlakuan (Kontrol), sebesar 543,75 g/m^2. Kapasitas tampung campuran *B. decumbens* dan *P. phaseloides* pada pukan 10 ton/ha nyata (P<0,05) paling tinggi sebesar 0,08 UT, dibandingkan dengan perlakuan pukan lain. Berdasarkan perlakuan dolomit, perlakuan dosis 1 ton/ha nyata (P<0,05) menghasilkan kapasitas tampung paling tinggi yaitu sebesar 0,83 UT. Mixed crop between B. decumbens and P. phaseloides in sub optimal land showed the highest forages quality and production when it was created with combination of 10 t/ha of manure and 2 t/ha of ameliorant. The manure and ameliorant treatment was also increasing the nutrient content of B. decumbens and P. phaseloides as well as land cacity.

Kata Kunci: *Brachiaria decumbens*, *Pueraria phaseloides*, Pupuk Kandang, Super Dolomit

ABSTRACT

Herdiawan I. 2016. Productivity of *Brachiaria decumbens* in a mixed-culture with *Pueraria phaseloides* in different manure and dolomite administration level into suboptimal land. JITV 21(4): 215-223. DOI: http://dx.doi.org/10.14334/jitv.v21i4/1562

Productivity improvement of forage in suboptimal land can be done in various ways, such as through manure and ameliorant treatment. Manure and dolomite is required by soil on suboptimal land, a combination of both can supply nutrients and improve soil biophysics. The research materials used in this study were *B. decumbens*, *Pueraria phaseloides*, manure, and dolomite. Randomized Block Design (RBD) in 3x3 factorial was applied. The dose of manure and dolomite are the first and second factors respectively, each treatment has 3 replications times. Variables measured were biomass production, dry matter production, carrying capacity, and nutrient content of forage. Results showed that the highest (P<0.05) biomass production produced by the 10 t/ha manure and 2 ton/ha dolomite treatment by 1,638.94 g/m^2 compared to the other treatments, and the lowest was achieved in the control by 543.75 g/m^2. Carrying capacity of *B. decumbens* and *P. phaseloides* on 10 ton/ha manure was the highest (P<0.05) by 0.08 AU compared to the other treatments. Super dolomite administration by 1 ton/ha significantly (P<0.05) produced the highest carrying capacity by amount of 0.83 AU. Manure and dolomite administration significantly (P<0.05) improved the nutritional content of *B. decumbens* and *P. phaseloides*.

Key Words: *Brachiaria decumbens*, *Pueraria phaseloides*, Manure, Dolomite

INTRODUCTION

Fertilization and ameliorant administration are two kinds of attempt to improve the quality of soil in order to optimizeproductivity of crops. Organic fertilizer is a biological buffer to improve physical, chemical and biological properties of soil to provide balance nutrient (Kariada & Aribawa 2005). Administration of manure and super dolomite to the growing media is one attempt to improve biophysical properties of soil in the suboptimal area. Super dolomite consists of two macrominerals which highly required by crops; calcium (CaO) and Magnesium (MgO). Magnesium and Calcium serve as enhancer nutrient, pH neutralizer, improving physical; biological and chemical properties of soil to increase the production (Sumaryo & Suryono

2000). Fertilization using organic material is indispensable, especially to supply soil nutrition, improving soil texture and biophysical (Parman & Sarjana 2007). Kandil & Gad (2010) reported that in the low fertility soil, fertilization using chicken manure might increase vegetative growth and quality of the harvest. *Brachiaria humidicola* grass is responsive to chicken manure administration, even in the second planting season (Kusuma 2015). Chicken manure level used were generally 10, 20, and 30 ton/ha (Luthfyrakhman & Susila 2013). Cullison (1975) *in* Reksohadiprodjo (1994) described that carrying capacity is a pasture area, where it grows forage available for animal that grazed it as needed in a short time. So that, knowledge about the number of forage available in a pasture is important to determine its stocking. Assessment of total biomass of crop is a parameter used as an indicator of crop growth. The other reason in use of total biomass of crop is that DM of crop regarded as manifestation from all process and events occurred in the crop growth (Sitompul & Guritno 1995).

Carrying capacity of pasture reflects balance between available forage and the animal unit shepherded per time unit (Rusdin et al. 2009). Whereas, Reksohadiprodjo (1985) described that carrying capacity is the capability of pasture to produce forage required by the number of animal shepherded in one hectare, or capability of pasture to accommodate animal per hectare. Carrying capacity may also be described as capability of pasture in accommodating the number of animal which may be maintained per wide area of the pasture (Subagyo & Kusmartono 1988).

The carrying capacity is identic with the stocking rate as the number of animal (animal unit) per pasture area wide (Mudumi 1990). The carrying capacity is an analysis of the capability of a pasture area to accommodate a number of animals, so the grass requirement of animal for 1 year is fulfilled. The carrying capacity of pasture is closely related to kind of animal, grass production, season and pasture area. In other words, the carrying capacity varied depending on assessment of grass production. Grass production in the wet season will be higher than in the dry season which means there is more forage in the wet season. Damry (2009) said that wide area required to maintain one animal with 400 kg body weight was 14.4-15.5 ha/year and the ideal carrying capacity by 2.5 AU/ha/year.

MATERIALS AND METHODS

This study was conducted in Cibadak Sub-district, Lebak district, Province of Banten. Average rainfall was 1100 mm/year, temperature was 32-36°C, humidity was 56-66%, the soil type was acrisol, soil pH was 4.81 (BBSDLP 2015). Land area used was 5000 m^2 divided into 27 plots (±185 m^2 each) of mixed *B. decumbens* - *P. phaseloides*. Seed of *P. phaseloides* was from the seed sowed in the seedling for 4 weeks then was transferred into polybag until 8 weeks old and then was transferred to the field. Seed of *B. decumbens* was from pols planted in the polybag until 8 weeks old and then was transferred to the field. Simple soil processing: rotary plowing once, fertilizing, and super dolomite administration was conducted based on the treatment level. The watering was conducted from the beginning of planting until 6 weeks grew. Weedling was conducted every month. The first pruning of grass was conducted at 40 days after planting with harvest interval of 20 days , while at 60 days with harvest interval of 30 days . Observation was performed after the first pruning until the study ends.

Factorial Randomized Block Design (Gomez & Gomez 1984) was applied in this study. The first factor was 3 level (0, 5, and 10 ton/ha) manure administration. The second factor was 3 level (0, 1, and 2 ton/ha) super dolomite administration. These treatents were repeated 3 times. Variables observed were fresh biomass production, nutrient content and carrying capacity. The fresh biomass production was obtained by weighing fresh grass/legume in every harvest. Nutrient content was analyzed proximately in laboratory of Nutrient of IRIAP-Ciawi. The carrying capacity used quadrant method (Alikodra 1990; Reksohadiprodjo 1996). Fifteen plots were selected randomly. Sampling was done using grass scissor of 1 x 1 quadrant as close as to the ground (±1.5 cm). That grass was placed into envelope then weighed by 100 gr/m2. Those plots were selected by determining 1 point of the first plot, moved 10 steps to the right or left. Grass samples were put into paper bag to be analyzed in the laboratory later. Available forage production is total forage production produced by pasture (Dry Matter) reduced by production of weeds multiplied by proper use factor by 40-45% (Hariadi 2008). Land area requirement for 1 animal unit per year (ha/AU) was calculated by Voisin (1959) formula. Carrying capacity of pasture was calculated according to Abdullah (2009), assumed that 1 AU is equal to 500 kg cattle with diet requirement per day (DM) was determined by 2.3-3% of body weight, and the stay period (grazing) for 70 days (Susetyo 1980). The simplest carrying capacity was calculated using a formula of Thorne & Stevenson (2007):

$$\text{Animal Unit month} = \frac{\text{DM Prod. x pasture area}}{\text{DM requirement per month}}$$

RESULTS AND DISCUSSION

Fresh Biomass Production of *B. decumbens* in mixed-culture with *P. phaseloides* at different manure and ameliorant level

There was an interaction between manure and dolomite administration level to fresh biomass production of *B. decumbens* and *P. phaseloides*. At the 10 ton/ha manure and 2 ton/ha dolomite administration resulted in the highest production by 1,638.94 g/m^2, where the lowest production was the control by 543.75 g/m^2.

Table 1. Fresh biomass production of *B. decumbens* in mixed-culture with *P. phaseloides at* different manure and super dolomite (g/m^2) level

Treatment	Ameliorant (super dolomite) (ton/ha)		
Manure (ton/ha)	0	1	2
0	543.75^{e}	966.63^{d}	1,009.69^{d}
5	910.94^{d}	1,462.50^{b}	1,205.00^{c}
10	1,183.31^{c}	1,314.56^{bc}	1,638.94^{a}

Description: Different words show significant difference (P<0.05).

Increased of manure and super dolomite level administration significantly increased fresh production of *B. decumbens*, except in the 5 ton/ha and 2 ton/ha level of manure and super dolomite, respectively. This proves that the *B. decumbens* is very responsive to fertilizer and the ameliorant. The *gramineae*, generally requires a fairly high N, so the mixed culture system of legume with fertilizer and ameliorant administration will multiply N available in the soil. Manenetje & Jones (1992) stated that *Brachiaria brizantha*, *Brachiaria decumbens* and *Brachiaria humidicola* were highly tolerant to acid soil and responsive to fertilizer consisting of N; P; and K, even though cannot stand the low drainage land. Winarso (2005) said that balance use of fertilizer would increase crop production. Abdurachman et al. (2000) reported that administration of any kind of manure of cattle, goat and chicken by 5 ton/ha in the Ultisol Jambi significantly increased organic-C level of soil and corn and soybean production. The administration of dolomite and cattle manure was expected to increase soil fertility, especially its physical and chemical properties improvement which will increase peanuts production (Rahmadani et al. 2012).

Padmowidjoto (2006) stated that development of mixed culture between legume and forage may improve environment and ecosystem condition, and as a result, improving the growth and increasing production of the crops. Translocation of N from legume to the surrounding crops influences its fertilization and increases its weight (Sirajuddin et al. 2010). The translocation of nutrient from the legume to the surrounding crops decreases mass of legume to be lower level than the one from the monoculture system. Patel & Rajagopal (2003) said that the growth of legume planted intercropping was not better than legume planted monoculture, and on the contrary. This is allegedly that legume does not get additional nutrients from the host crop, indeed the nutrients required by legume are absorbed by the host crop, resulting in low production of legume.

Gardner et al. (2008) said that in the mixed culture system, legume donated N to the grass during its growth period resulting in higher biomass production compared to the monoculture system. Legume is crop that has ability to produce high organic material and may improve soil fertility. Its ability to fix/grab N from the air may increase nutrient supply for the surrounding crops, especially the N. Soedomo (1981) stated that there was a translocation of N from legume to grass by around 0-53% from the fixated N through fallen legume leaves, rotten root and nodule. Nulik et al. (2013) said that N was also available for the surrounding through the decomposition of root and leaves. The crops planted along with or after legume has potential of higher productivity due to higher chance to access N, however, this is highly dependent to management of legume and high biomass number. Symbiosis of legume and rhizombium is able to fixate N from the air to fulfill N requirement of the host and its surrounding crops (Islami & Utomo 1995; Hidayati et al. 2001).

Carrying capacity of mixed *B. decumbens* - *P. phaseloides* at different fertilizer and ameliorant level

Analysis of variance showed an interaction between manure and supernatant dolomite level to the carrying capacity of the mixed *B. Decumbens* - *P. phaseloides.*

Carrying capacity of 5 ton/ha manure plus 1 ton/ha administration and 10 ton/ha manure plus 2 ton/ha super dolomite showed significant different (P<0.05) compared to the other treatment level. The 0 ton/ha manure and super dolomite level showed the lowest carrying capacity. The carrying capacity closely connects with biomass production and dry material content of crops, as of in 5 ton/ha manure plus 1 ton/ha super dolomite level, and 10 ton/ha manure plus 1 ton/ha super dolomite showed biomass production and higher dry material compared to the other treatment level. As stated by Santosa (1995) that higher production level of crop per one unit area increased its ability to accommodate a number of animal. Increase of grass production per one unit area is not apart from effect of legume crops which was planted along,

because generally N content of legume may be transferred both into soil and crops directly. Besides, there was an increase of dry matter of crops in mixed-culture due to high dry matter contribution from legume. Dhalika et al. (2006) stated that legume played a critical role in increasing pasture productivity due to its capability in fixing a number of nitrogen in the air. Its direct contribution to crop productivity through provision of forage resource rich in nitrogen.

In the mixed-culture of grass and legume in one area, legume that may produce N_2 through fixation process of N_2 from the air with the help of *Rhizobium*, indirectly may supply N requirement of its surrounding crops. The N produced by the legume will be used by the surrounding for metabolic process which increases dry material level of the crops. As stated by Murbandono (2001) that manure fertilizer is crucial to enrich soil nutrient. Sutejo (2002) stated that nutrient in the soil in the form of N, P, and K, may be used in the form of anion and cation.

Nutrition content of *B. decumbens* in mixed culture with *P. phaseloides* at different manure and amelioran level

The statistical analysis showed no interaction between manure and ameliorant level toward dry material of *B. decumbens*. Combination of 5 ton/ha manure and 1 ton/ha ameliorant resulted in the highest dry matter by 22.51%. The lowest DM content of *B. decumbens* by 16% was in the control.

Fertilization is one-factor affecting nutrient value of the forage (McDonald et al. 2002). Ifradi et al. (1998) stated that manure administration would increase dry material, protein and decrease crude fiber. Rosmarkam & Yuwono (2002) stated that fertilization using nitrogen would increase production and total DM of crops. Besides, Reksohadiprodjo (1994) also described that mixed culture of grass and legume was better than grass planted monoculture, as in addition to having protein, legume also has higher phosphor and calcium. Susanti (2007) reported that DM content of elephant grass with fertilization was higher than without fertilization.

Research result showed that there was interaction between manure and ameliorant level toward crude protein of *B. decumbens*. Increase of manure and ameliorant level administration was followed by increase of crude protein content. The highest crude protein content was in the 10 ton/ha manure and 2 ton/ha ameliorant administration.

As reported by Sarief (1986) that the decrease of N in the soil would decrease protein level and increase ratio of protoplasm and cell wall thickening the cell wall resulting in hard and fibrous leaves. Minson (1990) also said that content and composition of crude protein in forage were influenced by nitrogen availability in the soil solution. Crude protein content of grass is highly dependent to the concentration of available N in the soil. Administration of manure means administrating optimum N which can be absorbed by crops. Kaunang (2005) stated that fertilization might increase protein content of forage. Protein of forage will be degraded and fermented into ammonia, volatile fatty acid, CO_2 and CH_4. Valentin et al. (1988) reported that mixed culture of elephant grass and *Arachis glabrata* without N fertilization might increase crop production more than 100% and increased protein about 200% compared to grass planted monoculture. Kaunang (2005) *in* Marliani (2010) stated that content and composition of crude protein in forage were influenced by availability of nitrogen in the soil that inhibiting synthesis process in the forage. Mixed culture system of grass and legume (legume were planted along with grass for the sake of the grass) increased nutrient of the pasture compared to the monoculture system, as a result of high content of nitrogen and calcium in the legume (Suharta 2010). Engelstad (1997) stated that optimal nitrogen administration might improve crop growth and increase protein synthesis. This is clarified by Crespo & Odurado (1986) who said that efficiency of nitrogen conversion increased along with increase of N

Table 2. Carrying capacity of mixed *B. decumbens* - *P. phaseloides* at different manure and super dolomite (UT) level

Treatment	Ameliorant super dolomite (ton/ha)			Average
Manure (ton/ha)	0	1	2	
0	0,27^{e}	0,57^{d}	0,57^{d}	0,47
5	0,58^{d}	0,99^{a}	0,80^{b}	0,79
10	0,77^{c}	0,92^{b}	0,94^{a}	0,88
Average	0,54	0,83	0,77	

Description: Different words show significant difference (P<0.05).

Table 3. Content of Dry Material (DM) of *B. decumbens* planted along with *P. phaseloides* at different manure and ameliorant level (%)

Treatment	Ameliorant (super dolomite) (ton/ha)			Average
Manure (ton/ha)	0	1	2	
0	16.00	17.72	17.40	17.04[c]
5	19.68	22.51	20.04	20.74[a]
10	19.69	20.51	19.50	19.90[b]
Average	18.46[c]	20.25[a]	19.23[b]	

Description: Different words show significant difference (P<0.05).

fertilization increasing crude protein content. Research result showed no interaction between manure and ameliorant treatment to the crude fiber content. The highest crude fiber content by 31.16% was in the control (without manure) and the lowest one was in the 5 ton/ha manure level by 27.96%. The highest crude fiber was in the control (without ameliorant) by 31.43% and the lowest was in the 1 ton/ha ameliorant administration by 27.83%.

Ifradi et al. (1998) said that manure administration would increase dry matter and protein and decreasing crude fiber. Sarief (1986) described that decrease of N in the soil would decrease protein level and increasing ratio of protoplasm and cell wall thickening cell wall resulting hard and fibrous leaves. DM and CF content tend to increase along with increase of age of the forage and decrease of fertilization level. Allegedly, as a result of the N which is highly mobile in the soil may facilitate the root to absorb water in the soil. That causes lots of water content in the crops that may hamper lignification in the crops. Then, aging leads crops to renaissance phase, where the crops are in the aging period producing high cellulose and lignin.

There was no interaction between treatment level and ameliorant to energy content of *B. decumbens* in this study. The highest energy content of *B. decumbens* was in the 10 ton/ha manure administration by 4011 Kkal/kg and the lowest was in the control (without manure) by 3999 Kkal/kg. Administration of ameliorant by 2 ton/ha produced the highest energy content by 4021 Kkal/kg and the lowest one was in the 1 ton/ha ameliorant administration by 3994 Kkal/kg. Nuhuyanan

Table 4. Crude Protein content of *B. decumbens* in mixed culture with *P. phaseloides* at different manure and ameliorant level (%)

Treatment	Ameliorant (super dolomite) (ton/ha)			Average
Manure (ton/ha)	0	1	2	
0	10.02d	11.82c	12.70b	11.51
5	11.05c	12.07b	13.76a	12.29
10	12.14b	13.52a	13.80a	13.15
Average	11,07	12,47	13,42	

Description: Different words show significant difference (P<0.05).

T**able 5**. Crude Fiber content of *B. decumbens* in mixed culture with *P. phaseloides* at different manure and ameliorant level (%)

Treatment	Ameliorant (super dolomite) (ton/ha)			Average
Manure (ton/ha)	0	1	2	
0	37.13	27.75	29.92	31.16a
5	25.94	26.13	31.80	27.96c
10	31.17	29.62	30.25	30.35ab
Average	31.43a	27.83c	30.66ab	

Description: Different words show significant difference (P<0.05).

(2010) reported that fertilization and legume introduction provided better effect to in vitro digestibility of DM, OM, TDN and EM of grass compared to the without introduction of legume and fertilizing.

Research results showed no interaction between manure and ameliorant level to Calcium (Ca) content of *B. decumbens*. The highest Ca content was in the 5 and 10 ton/ha manure by 24% each and the lowest was in the control (without manure administration) by 0.23%.

The administration of ameliorant by 2 ton/ha showed the highest Ca content by 0.25%, while the lowest one was in the control (without ameliorant administration) by 0.23%. Dhalika et al. (2006) stated that counterweight mixed culture of grass and legume significantly increased Ca content of forage. Legume, as calcium source is able to provide Ca for its surrounding crops (Reksohadiprodjo 1994). Hartadi et al. (1990) also said that calcium content in the Sentro was 1.19-1.26% depending on crops age. The older crops have higher calcium, while the calcium content of *Cynodon plectosthacyus* is only 0.3%. Ayub et al. (2004) stated that absorption rate of Ca and P by forage planted intercropping would be higher than the monoculture, impact on the production and nutrient quality of the mixed-forage.

The highest phosphor (P) content was achieved in the 10 ton/ha manure by 0.19% and the lowest one was without manure by 0.15%. The highest Phosphor content was in 2 ton/ha ameliorant by 0.19%, while the lowest one was without ameliorant by 0.16%. Hartadi et al. (1990) reported that phosphor content of African grass was 0.31%, while in the Centro legume was around 0.40%. Phosphor content of forage in the monoculture system of grass is 0.23%, whereas in the legume planted monoculture is 0.34%. It shows that phosphor content in the grass planted monoculture is.

Table 6. Energy content of *B. decumbens* planted along with *P. phaseloides* at different manure and amelioran level (Kkal/kg)

Treatment	Amelioran (super dolomite) (ton/ha)			Average
Manure (ton/ha)	0	1	2	
0	4005	3985	4007	3999[c]
5	3989	3978	4059	4009[b]
10	4019	4018	3996	4011[a]
Average	4004[b]	3994[c]	4021[a]	

Description: Different words show significant difference (P<0.05).

Table 7. Ca content of *B. decumbens* in mixed culture with *P. phaseloides* at different manure and ameliorant level (%)

Treatment	Ameliorant (super dolomite) (ton/ha)			Average
Manure (ton/ha)	0	1	2	
0	0.21	0.23	0.25	0.23[b]
5	0.24	0.23	0.26	0.24[a]
10	0.23	0.27	0.23	0.24[a]
Average	0,23[c]	0,24[b]	0,25[a]	

Description: Different words show significant difference (P<0.05)

Table 8. Phosphor (P) content of *B. decumbens* in mixed culture with *P. phaseloides* at different manure and ameliorant level (%)

Treatment	Ameliorant (super dolomite) (ton/ha)			Average
Manure (ton/ha)	0	1	2	
0	0.17	0.15	0.14	0.15[c]
5	0.15	0.16	0.22	0.18[b]
10	0.15	0.22	0.20	0.19[a]
Average	0.16[c]	0.18[b]	0.19[a]	

Description: Different words show significant difference (P<0.05).

very poor which allegedly has slightly contributed to the amount of phosphor of the mixed-culture crops

The increase of phosphor content of crops in the mixed legume and grass culture is the phosphor contributed by the legume. The increase of legume proportion in the mixed legume and grass culture produces higher phosphor. The highest phosphor content in the mixed-culture system was achieved by mixed 20% grass and 80% legume, but still lower than phosphor content of crop planted monoculture (Dhalika et al. 2006). Ayub et al. (2004), stated that quality, biomass production, and Ca, P consentration in intercropping forages were higher compared to monoculture.

Analysis of variance showed no interaction between manure and ameliorant to the digestibility of *B. decumbens* by in vitro. Manure level by 0 ton/ha showed the highest digestibility value by 59.65%, whereas manure level by 10 ton/ha showed the lowest digestibility value by 44.65%. Ameliorant level by 2 ton/ha showed the highest digestibility value by 58.74, whereas ameliorant level by 0 ton/ha showed the lowest digestibility value by 45.33%.

Dry matter digestibility consists of organic and inorganic materials which can be digested. Inorganic material consists of minerals, while the organic material consists of compounds of nitrogenous, carbohydrate, fat and vitamins. Digestibility of dry matter is influenced by lignin concentration (Purbajanti et al. 2011). Nitrogen plays role in growth promoting of crops in the vegetative period, chlorophyll forming and as component that forms fat, protein and other organic compounds (Mengel & Kirkby 2001). Nitrogen also specifically influences digestibility of forage. Research result of Ningsih (2007) showed that digestibility of elephant grass (*Pennisetum purpureum* cv. Taiwan) grew in the Ultisol soil was around 64.72% up to 65.33%. The amount of nutrients absorbed by crops will increase photosynthesis that increasing the amount of carbohydrate produced by crops which then supports stems and leaves forming (Lakitan 1996). This will influence the value and amount of digested dry matter. Digested dry matter, mostly consists of digested organic material (protein, fat and carbohydrate) (Ifradi et al. 2012).

Analysis of variance showed no interaction between manure and ameliorant level with organic matter digestibility value of the *B. decumbens* by in-vitro. Van Soest et al. (1982) stated that nitrogen (N) may increase the cell content due to the association of N with carbon chain to prevent cell wall forming as well as increasing cell content. This implicates increase of forage digestibility.

Coefficient value of digestibility of dry matter or organic matter shows digestibility level of diet in digestive tract and how much contribution of a diet for animal (McDonald et al. 2002). The organic matter digestibility related to chemical composition of forage (N, ash, eter extract, cell wall, ADF, ADL, silica, where

Table 9. Digestibility Value of Dry Material of *B. decumbens* in mixed culture with *P. phaseloides* at different manure and ameliorant level (%)

Treatment	Ameliorant (super dolomite) (ton/ha)			Average
Manure (ton/ha)	0	1	2	
0	63.50	63.86	51.59	59.65[a]
5	44.44	51.38	61.51	52.44[b]
10	-28.06	42.78	63.11	44.65[c]
Average	45,33[c]	52,67[b]	58,74[a]	

Description: Different words show significant difference (P<0.05)

Table 10.Digestibility Value of Organic Materil of *B. decumbens* in mixed culture *P. phaseloides* at different manure and ameliorant level (%)

Treatment	Ameliorant (super dolomite) (ton/ha)			Average
Manure (ton/ha)	0	1	2	
0	61.56	64.13	48.22	57.97[a]
5	42.44	44.28	61.23	49.32[b]
10	-23.60	-37.43	60.20	40.41[c]
Average	42,53[c]	48,61[b]	56,55[a]	

Description: Different words show significant difference (P<0.05).

the increase of percentage of those cell wall, ADL, silica, and eter extract will decrease digestibility of organic matter of forage (Muir et al. 2003). Suyitman (2003) stated that organic matter content of elephant grass (*Pennisetum purpureum)* cv. Taiwan was around 84.20 - 89.90% and will increase along with fertilizing. Sutardi (1980) said that the increase of dry matter increased organic matter digestibility since the dry matter is directly proportional to the organic matter digestibility.

CONCLUSION

Mixed crop between *B. decumbens* and *P. phaseloides* in sub optimal land showed the highest forages quality and production when it was created with combination of 10 t/ha of manure and 2 t/ha of ameliorant. The manure and ameliorant treatment was also increasing the nutrient content of *B. decumbens* and *P. phaseloides* as well as land capacity.

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Study of BMP15 Gene Polymorphism in Boer, Kacang, and Boerka Goats

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ABSTRAK

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Gen BMP15 atau sering disebut dengan FecX (fekunditas kromosom X) adalah gen yang mengatur sifat prolifik. Penelitian ini bertujuan untuk mengidentifikasi mutasi gen BMP15 dan menganalisa polimorfisme pada kambing Boer, Kacang dan Boerka. Kambing betina yang digunakan sebanyak 50 sampel, masing-masing 17 Boer, 16 Kacang dan 17 Boerka yang dianalisa keragaman genetiknya diidentifikasi menggunakan metode PCR-*Sequencing*. Amplifikasi gen BMP15 menghasilkan fragmen dengan panjang 141 bp. Penentuan genotipe gen BMP15 menghasilkan tiga genotipe. Hasil analisis menunjukkan bahwa pada gen BMP15 ditemukan 2 SNP polimorfik yang dianalisa berdasarkan frekuensi genotipe, frekuensi alel, heterozigositas dan uji keseimbangan Hardy-Weinberg ($\chi 2$). Hasil sekuens fragmen gen BMP15 menunjukkan adanya mutasi antara basa adenin (A) dengan guanin (G) dan penentuan dari genotipe gen BMP15 ditemukan tiga genotipe yaitu GG, GA dan AA. Dapat disimpulkan bahwa telah ditemukannya identifikasi dari mutasi gen BMP15 pada kambing Boer, Kacang dan Boerka dan bersifat polimorfik yang diidentifikasi menggunakan metode PCR-*Sequencing*.

Kata Kunci: Gen BMP15, PCR-*Sequencing*, Prolifik, Keragaman Genetik, SNP

ABSTRACT

Batubara A, Elieser S, Sumantri C. 2016. Study of BMP15 gene polymorphism in Boer, Kacang, and Boerka goats. JITV 21(4): 224-230. DOI: http://dx.doi.org/10.14334/jitv.v21i4.1636

The bone morphogenetic protein 15 (BMP15) gene or commonly called FecX (fecundity chromosome X) is a gene that controls the prolific properties. This study was aimed to identify the mutation of BMP15 gene and to analyze its polymorphism in Boer, Kacang, and Boerka goats. The total of 50 female goat bloods were identified using PCR-Sequencing method, 17 Boer, 16 Kacang and 17 Boerka respectively. BMP15 gene amplification resulted fragment with the length of 141 bp. Genotyping of BMP15 gene produced three genotypes. Result showed that two polymorphic SNP were found from BMP15 gene analyzed by genotype frequency, allele frequency, heterozygosis, and equilibrium of genotype in all population by the Hardy-Weinberg equilibrium test ($\chi 2$). Sequence analysis results of BMP15 gene showed that there were two mutation between adenine (A) and guanine (G) bases and determination of genotype BMP15 gene produced three genotypes there were GG, GA and AA. In conclusion, there was mutation of BMP15 gene in Boer, Kacang and Boerka goats and genetic polymorphism were identified using PCR-Sequencing method.

Key Words: BMP15 Gene, PCR-Sequencing, Prolific, Genetic Polymorphism, SNP

INTRODUCTION

Goats spread in different region with different climates and evolved in isolation from each other over long periods of time due to various selective pressures and genetic drift. The main breeding of goats is determined by geographic position, morphological character and production performance. Because of environmental factors and varying selection of treatment, the rate of genetic change is very diverse (Rout et al. 2008). Kacang goat is a local goat species with the highest population level in Indonesia. It has a high fertility rate with the percentage of prolific properties respectively, namely twin 52.2% and triplets 2.6% (Pamungkas et al. 2009). Boer goat is a good type of meat production and a famous meat purpose, excellent meat quality, great adaption, exceptional resistance to disease, high non-seasonal fertility and kidding percentage are known to have a fast growing rate compared to other goats breeds (Hua et al. 2009; Zhang et al. 2009; Haryono et al. 2011). Boerka goat is a population of superior goat breeding through crossbreeding Boer and Kacang under the Agricultural Research Program, The Ministry of Agriculture - Indonesia with the composition genotype between Boer goat and Kacang are 50% (Haryono et al. 2011). With reproduction rate found in Boer, Kacang and Boerka goat are 1,80; 1,80 and 1.82, respectively (Elieser et al. 2012).

Prolific trait is the reproductive trait or the ability to give birth to more than one lamb. The prolific trait of each individual goat varies due to genetic variation leading to an increase in ovulation rate and the number of litters (litter size). The number of lamb per birth determines the rate of increase in the population of goats (Hidayat et al. 2015). Identification of the genetic diversity of livestock is essential for the conservation of genetic resources and the development of more productive seedlings (Batubara et al. 2013). According to (Chu et al. 2007) patterns of gene control over prolific mechanisms indicate a difference between goat and sheep breads, but the prolific properties of goats and sheep are still controlled by the same gene, BMP15. The bone morphogenetic protein 15 (BMP15) gene or commonly called FecX (fecundity chromosome X) (Farhadi et al. 2013) is a gene that controls the prolific properties of various sheep types (Hidayat et al. 2015) and an X linked gene (FecX locus) of sheep belonging to TGFβ family with the protein product of BMP15 are paracrine factor, stimulates follicle growth, granulosa cell proliferation and cell-survival signaling (Demars et al. 2013).

Many studies have shown that the importance of BMP15 gene in regulating ovarian function has spurred the extensive studies in several species including rodents, sheep and human. In goats, information for these studies still limited (Cui et al. 2009) especially for Boer goats, Kacang and crossbreeding B) are non.

BMP15 has got important in terms of booroola phenotype and also termed as GDF-9B genetic code for protein synthesis in oocytes which enhance the formation of follicles and fecundity of sheep and goat. Role of BMP15 genes is not known how it works to manage granulose cell (Jalbani et al. 2017). Recently, researchers argued that 5 mutations influence the prolificacy in BMP15 gene by expressing amino acid sequences (vicFec^{XL}, Fec^{XB}, Fec^{XI}) or premature stop codons (Fec^{XG}, Fec^{XH}) (Monteagudo et al. 2009) on ovulation rate are considerably improved in heterozygosis (Bodin et al. 2007).

The objective of this study was to identify the diversity of Bone Morphogenetic15 (BMP15) fecundity gene in Boer, Kacang and Boerka goats using PCR-Sequencing method and analyzing BMP15 gene polymorphism.

MATERIALS AND METHODS

Sample sources

The total numbers of goat used in this study were 50 samples derived from three populations with 17 Boer, 16 Kacang and 17 Boerka from Goats Research Station Sei Putih, North Sumatera, Indonesia, Indonesia. Blood sampling was done with a venoject needle on the jugular vein. The venoject needle was connected to the vacutainer tube containing EDTA. The required blood is ± 5 mL and stored at ± 4 °C. Sample was analyzed for DNA extraction, DNA amplification and data analysis in Animal Molecular Genetics Laboratory, Faculty of Animal Science, Bogor Agricultural University.

DNA extraction

Genomic DNA was extracted from blood samples based on the modified PCR Bio systems Kit DNA extraction procedure. The first step was sample preparation as follow: 8 µl of blood sample in 1.5 mL micro centrifuge tube and as much as 10 µl 5x PCRBIO Rapid Extract Buffer A (1 µl/mL) was added as lysis buffer. 10 µl of 10x PCRBIO Rapid Extract Buffer B was added. A total of 70 µl of PCR Grade H_2O was added. Then homogenized with vortex. Samples were incubated at 75°C for 20 minutes and homogenized at every 5 minutes. And incubated back at 95°C for 15 minutes. Then 900 µl of PCR Grade H_2O was added. Conducted centrifuge at high speed, 13200 rpm for 1 minute. Supernantan was taken and stored at -20°C.

DNA amplification

BMP15 gene fragment was amplified by PCR technique. The PCR was carried out in a reaction volume of 50 µL containing 2 µL genomic DNA template, 0.6 µL primer of reverse and forward, 24.4 µL nuclease free water, 25 µL Master Mix (2x PCRBIO HS Taq Mix Red). Amplification was carried out with a thermal cycler machine GeneAmp® PCR System 9700 (Applied Bio system). The condition of thermal cycling consisted of predenaturation at 95°C for 5 min, followed by 40 cycles of denaturation at 95°C for 15 s, annealing at at 62°C for 15 s and extension at 72°C for 15 s. The final extension step was at 72°C for 2 min. The DNA amplification products were checked on 1.5% agarose gels in 0.5 TBE buffer containing with a 100 bp ladder as a molecular weight marker.

DNA sequencing and analysis

PCR products representing different genotype of each gene were sequenced with DNA sequencer machine provided by DNA sequencing service in Macrogen-Korea. Sequence results were analyzed by BioEdit (Hall 1999) and sequence alignment was performed by MEGA software version 6.0 (Tamura et al. 2013) in order to find nucleotide mutation. The BLAST (Basic Local Alignment Search Tool) program was used to search the NCBI GenBank database for reference and homologous sequences.

Table 1. Forward and reverse primer sequence for the amplification*

Gen	GenBank	Sekuens Primer	Product
BMP15	EU743938.1 Kromosom X	F: 5' CACTGTCTTCTTGTTACTGTATTTCAATGAC-3' R: 5'GATGCAATACTGCCTGCTTG 3'	141 bp

*Source: (Chu et al. 2007)

Data analysis

Genotype and allele frequencies

The genotype and allele frequencies as were described by (Nei & Kumar 2000) were analyzed using genotyping data from each gene and calculated based on the populations of boer, kacang and boerka goats. Genotype frequency was calculated by the following formula:

$$X_{ii} = \frac{x_{ii}}{N}$$

Allele frequency was calculated by the following formula:

$$X_i = \frac{2n_{ii} + \sum n_{ij}}{2N}$$

Description:

X_{ii} = iith genotype frequency
X_i = ith allele frequency
n_{ii} = Number of sample of ii genotype
n_{ij} = Number of sample of ij genotype
N = Total samples

Heterozygosis

Observed heterozygosis (Weir 1996) and expected heterozygosis (Nei & Kumar 2000) were tested by the following formula:

$$H_o = \sum_{i \neq j} \frac{n_{ij}}{N}$$

$$H_e = 1 - \sum_{i=1}^{q} x_i^2$$

Description:

H_o = Observed heterozygosis
n_{ij} = Number of heterozygous animal
N = Number of observed animal
H_e = Expected heterozygosis
X_i = Frequency of allele
Q = Total allele

Hardy-Weinberg equilibrium

Test of Hardy-Weinberg equilibrium (HWE) was conducted with chi-square test (Kaps & Lamberson 2004).

$$\chi^2 = \sum \frac{(obs-exp)^2}{exp}$$

Description:

χ^2 = Hardy-Weinberg equilibrium test
obs= Observed number of iith genotype
exp= Expected number of iith genotype

The number of degrees of freedom (df) is equal to the number of possible genotypes minus the number of (Allendorf et al. 2013) or as describe below:

df = (number of iith genotype) – (number of jth allele)

RESULTS AND DISCUSSION

Genetic polymorphism of the BMP15 gene

BMP15 gene fragments were successfully amplified by polymerase chain reaction technique for all samples. Amplification was successfully performed on the BMP15 gene with annealing temperature of 62°C 15 seconds and yielded a product of 141 bp while according to (Hidayat et al. 2015) the annealing temperature is 63°C 45 seconds. The success of the amplification is dependent heavily on the annealing temperature. The annealing temperature is the optimum temperature for the primer attachment process according to the target DNA sequence to be propagated during the PCR process. This difference is caused by the condition of the PCR machine and the mixture of PCR reagents. The primer attenuation temperature (annealing) ranges from 36 -72°C, but the usual temperature is 50 - 60°C (Muladno 2002). According to (Pelt-Verkuil et al. 2008) the annealing time required for the primer to be complementary and adhered to its target depending on the thermo cycler machine's

heating capacity used, the PCR mixed volume as well as the primer and targeted gene concentrations. Primer that has been attached to the next target undergoes elongation or extension at 72°C for 15 seconds. Then proceed with the final elongation at the same temperature for 2 minutes. The three stages of PCR are denaturation, annealing and elongation are the stages for 1 thermal cycle. In this study 40 cycle was done.

Homology and detection of mutation gene BMP15

The results of BMP15 gene sequence and alignment sequence with GenBank (access code EU74393.1) using forward and reverse primer pair indicated that the genotype frequency of BMP15 gene based on Boer goat, Kacang of samples was found by two SNP, SNP c.38A>G, SNP c.49G>A.

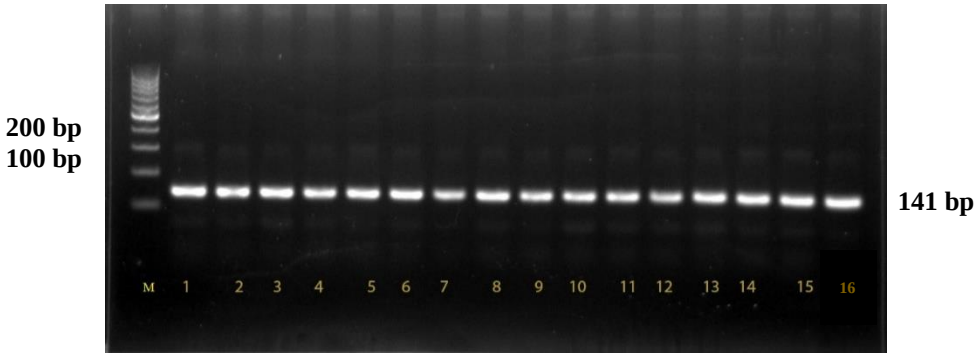

Figure 1. Visualization of PCR product amplified from Boer (1-5), Kacang (6-10), Boerka goat (11-16) samples (141 bp).

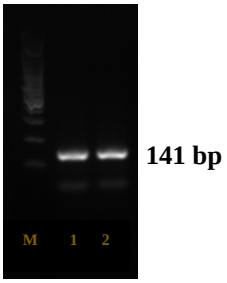

Figure 2. Visualization of PCR product amplified with annealing temperature of 62°C 15 seconds and yielded a product of 141 bp while according to (Hidayat et al. 2015) from Boer (1) and Boerka (2) samples (141 bp).

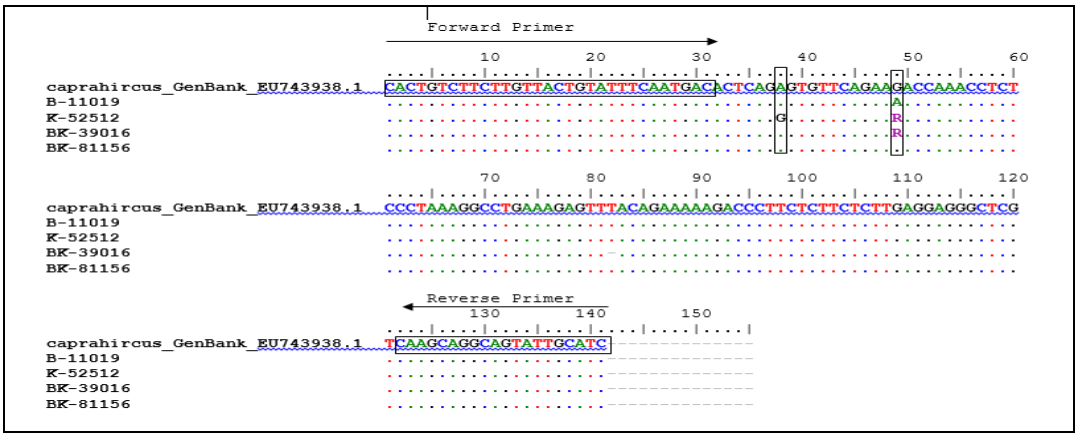

Figure 3. Nucleotide sequences alignment result of BMP15 gene. GenBank of BMP15 sequences (accession number EU 743938.1) were used for reference to find nucleotide mutation. = homologous sequences.

Genotype frequency and frequency of Allele of BMP15 fragment

The result of genotype frequency on SNP found in Table 2 explained that the genotype frequency of BMP15 gene based on samples of Boer, Kacang and Boerka goats used in this study yields three genotypes found is genotype GG, GA and AA. The genotype for Boer goat found in BMP15 SNP c.49 gene were GG, GA and AA with genotype frequency of 0.471; 0.235 and 0.294. Genotypes for Kacang goat found in BMP15 SNP c.49 gene were GG, GA and AA with genotype frequency of 0.125; 0.438 and 0.438. The genotypes for Boer goat found in BMP15 SNP c.49 gene were GG, GA and AA with genotype frequencies of 0.235; 0.235 and 0.529. SNP is said to be polymorphic if it has an allele frequency of ≤0.99 for large populations and ≤0.95 for a smaller population (Allendorf et al. 2013). So it can be said that BMP15 gene in boer, kacang and boerka goats.

In previous study of BMP15 gene in another goat breed, (Chu et al. 2007) found two genotypes: AA and AB in Jining Gery goats. Genotype AA was found in low fecundity goat breeds and AB genotype had 1.3 kids more than homozygous AA. In another study on different goat breeds, Feng et al. (2009) found three genotypes AA, AG and GG in Jining Grey goats, and only AA genotype was found in both Liaoning Cashmere and Inner Mongolia Cashmere goats. Boer goat found two genotypes AG and GG, while only AA genotype in both Angora and Inner Mongolia Cashmere goats. In a recent study of Wang et al. (2011), three genotypes (AA, BB and AB) were detected in Funiu White goats and their frequency was 0.071, 0.715 and 0.214, respectively. Two genotypes (AB and BB) were detected in Taihang black goats and their frequency was 0.342 and 0.658, respectively. The Funiu white goat with genotype BB had 0.91 or 0.82 kids more than those with AB or AA, respectively. However, these results preliminarily showed that BMP-15 gene is a genetic marker and closely linkage to the litter size trait and consequently, can be used as a marker-assisted selection (MAS) for high litter size productivity in goat (Abdel-Rahman et al. 2013). The genotype frequency shows the ratio of the number of genotypes to a population by computing the ratio between the numbers of specific genotypes in each population whereas the allele frequency is the ratio of an allele to the overall allele to an SNP in the population (Noor 2010). SNP is said to be polymorphic if it has an allele frequency of ≤0.99 for large populations and ≤0.95 for a smaller population (Allendorf et al. 2013).

Heterozygosis and Hardy-Weinberg equilibrium

The result of Ho and He test showed that the observed heterozygosis (Ho) does not differ greatly from the heterozygosis of expectation (He). Tombasco et al. (2013) states that if the value of Ho (Heterozygosis observation) is lower than He (Heterozygosis expectation) then it may indicate an intensive selection process. According to Tambasco et al. (2013) the difference between the observed heterozygosis value (Ho) and heterozygosis (He) can be used as an indicator of the presence of genotype imbalance in the observed Boer goats, Kacang and Boerka population indicated that there is already a selection activity performed and the absence of random marriage.

The observed heterozygosis value (Ho) of the BMP15 gene was found in SNP c.49G>A in Boer, Kacang and Boerka goats respectively of 0.235; 0.438 and 0.235. For the highest heterozygosis value is on SNP c.49G>A of Kacang goats is 0.438. The heterozygosis value of SNP BMP15 gene is presented in Table 3. Marson et al. (2005) suggest that the genetic diversity of a population can be measured using heterozygosis values aimed at assisting the selection program. Heterozygosis expresses the genetic diversity of a population that can be used for selection programs. Noor (2010) explains that gene diversity can be used as a reference in determining breeding programs that are selected if diverse populations and crosses are performed when the population is uniform. An SNP is said to have high diversity if the heterozygosis value >0.50 (Allendorf et al. 2013).

Table 2. Allelic and genotypic frequencies values of BMP15 in Boer, Kacang and Boerka goats

Goats	Genotype frequency			Allele frequency	
	GG	GA	AA	G	A
Boer	0.471	0.235	0.294	0.588	0.412
Kacang	0.125	0.438	0.438	0.344	0.656
Boerka	0.235	0.235	0.529	0.353	0.647

Table 3. Heterozygosis and Hardy-Weinberg equilibrium (HWE) value

Goats	Number of samples	Total goats			He	Ho	X^2
		GG	GA	AA			
Boer	17	8	4	5	0.484	0.235	4.496
Kacang	16	2	7	7	0.451	0.438	0.015
Boerka	17	4	4	9	0.457	0.235	3.996

The balance of population can be seen through the Hardy-Weinberg Equilibrium presented in Table 2. The result show that BMP15 gene on SNP c.49G>A is in a balanced state. Factor affecting the balance in a population are non-random mating, selection, migration, mutation and genetic driff (Noor 2010).

CONCLUSION

In conclusion, the present study of BMP15 gene in Boer, Kacang, and Boerka are polymorphic. Two polymorphic SNP and three genotypes GG, GA, and AA were found with allele frequency of G and A are <0.99. Sequence analysis results confirm mutation A→G and G→A base in BMP15 gene.

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Management Strategies to Improve Reproductive Performance of Brahman Cross Cattle (*Bos indicus*) in East Java, Indonesia

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ABSTRAK

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Produksi ternak nasional belum dapat mencukupi kebutuhan dalam negeri sehingga pemerintah melakukan importasi sapi Brahman ke Indonesia. Banyak issue menyatakan bahwa sapi Brahman mempunyai banyak permasalahan reproduksi. Tujuan penelitian ini adalah mengetahui tampilan reproduksi sapi Brahman cross melalui perbaikan manajemen pemeliharaan, diantaranya: mempertahankan skor kondisi tubuh induk >3, deteksi birahi dan perkawinan yang tepat serta penyapihan pada umur 5-6 bulan. Penelitian dilakukan pada oktober 2011 – desember 2013 dengan menggunakan materi awal ternak berjumlah 189 ekor dengan total peternak yang terlibat 151 orang. Lokasi penelitian adalah 2 kecamatan di kabupaten Lamongan (Jawa Timur), yaitu: modo dan sambeng. Pakan yang diberikan adalah hasil samping pertanian (potensi lokal) dan suplementasi induk menggunakan pakan hijauan, dedak padi dan konsentrat (2 bulan akhir kebuntingan dan awal laktasi). Terdapat peningkatan tampilan reproduksi sapi brahman pada akhir tahun penelitian. Durasi estrus setelah beranak, siklus sampai perkawinan dan masa kosong lebih pendek pada akhir penelitian daripada kondisi sebelumnya, yaitu: 98 hari, 64 hari dan 118 hari. Kondisi ini berhubungan dengan kondisi induk yang baik saat melahirkan (dari 3,0 menjadi 3,3) dan didukung oleh masa laktasi yang lebih pendek (153 hari). Tampilan produksi pedet menunjukkan rataan bobot lahir 31-32 kg. Penyapihan dini dilakukan pada umur 153 hari dengan rataan bobot sapih 139,6 kg dan pertambahan bobot badan harian (PBBH) 0,7 kg/hari. Tingkat kematian pedet semakin menurun sepanjang tahun (6%). Dapat disimpulkan bahwa teknologi perbaikan manajemen, diantaranya: mempertahankan skor kondisi tubuh induk >3, deteksi birahi dan perkawinan yang tepat serta penyapihan pada umur 5-6 bulan dapat memperbaiki tampilan reproduksi sapi Brahman cross.

Kata Kunci: Reproduksi, Sapi, Brahman Cross

ABSTRACT

Ratnawati D, Indrakusuma DA, Affandhy L, Cowley F, Mayberry D, Poppi D. 2017. Management strategies to improve reproductive performance of Brahman cross cattle (*Bos indicus*) in East Java, Indonesia. JITV 21(4): 231-237. DOI: http://dx.doi.org/10.14334/jitv.v21i4.1512

The demand for meat in Indonesia cannot be supplied by domestic cattle production, and so government imported breeding stock from abroad to increase domestic production. A commonly imported breed is Brahman cross bred (Bx). Smallholder farmers have reported a wide range of problems associated with reproductive performance of Bx. The purpose of this study was to identify changes in the reproductive performance of Bx cows by providing advice to farmers on improved management strategies, such as: maintaining body condition score (BCS) of cow> 3, appropriate estrous detection and weaning at 5- 6 months. This study was conducted from October 2011 – December 2013 by using 189 Bx cows and 151 farmers. The research was conducted in two sub districts of Lamongan district (East Java), Modo and Sambeng. Cattle were fed diets based on crop residues and were supplemented with green feeds, by-products (rice bran) and concentrate during the last 2 months of pregnancy and early lactation. The reproductive performance of cows improved during the study, there were a shorter lactation length/earliery weaning (reduced from 188 days to 153 days) and improved BCS (from 3.0 to 3.3). The length of postpartum anestrous interval (PPAI) decreased from 198 days to 98 days. Cycling to conception decreased from 75 days to 64 days and days open decreased from 217 days to 118 days over the length of the study. This was associated with improvement in BCS of cows at calving (from 3.0 to 3.3) and a shorter lactation length (reduced from 188 days to 153 days). There was no change in the average birth weight of calves (31.8 kg). There was no change in average weaning weight of calves over the year and average daily gain of calves while suckling 0.7 kg/day. The pre weaning calf mortality rate was decreased during this study (from 13% to 6%). This project demonstrated that Brahman cross cows can successfully be kept by smallholder farmers in Indonesia if appropiate management is applied. It is concluded that improving management, such as maintaining body condition score of cow >3, estrous detection, appropiate mating and weaning at 5-6 months can improve reproduction performance of Brahman cross.

Key Words: Reproduction, Cattle, Brahman Cross

INTRODUCTION

The total beef cattle population in Indonesia has decreased in recent years from 12,686 million (2013) to 16,004 million heads (2016) (DGLAH 2017). High demand for beef, which is not balanced with supply of domestic cattle exacerbates this condition. Beef consumption was 6.413 kg in 2015 and increased by 5.69% in 2016 (6.778 kg). The government of Indonesia has implemented several policies, with the aim of helping Indonesia to become self-sufficient in beef production. Strategies developed to increase beef production include importing Brahman (*Bos indicus*) cattle from Australia, national breeding policy (refining local cattle, developing a commercial production of local cattle), strengthening national food security (availability of food and feed), increasing local cattle population, substitution of beef with poultry, fishery, and sheep products and specific institutional policy (improvement of coordination between institution, optimizing organization for rescue productive cows and proven breed) (Rusono 2011).

Brahman cattle have a large mature size and a faster growth rate (Tatman et al. 2004) than local Indonesian cattle (e. g. Bali (*Bos javanicus*) and Ongole (*Bos indicus*) breeds), when they are managed well and kept in good condition. However, Brahman cows also have higher maintenance energy requirements compared to local cattle (Antari et al. 2014a; Antari et al. 2014b), and many smallholder farmers have reported problems with low growth rates and long inter-calving intervals (Ciccioli et al. 2003). This means that farmers invest considerable amounts of time, labor and money before they have a calf ready for sale. Long calving intervals are driven by long post-partum anestrus interval (PPAI) and repeated, unsuccessful mating (Ciccioli et al. 2003). These problems may be caused by inadequate nutrition, lack of weaning management, poor estrous detection and poor mating management (Syarifuddin & Wahdi 2008).

Nutrition has a big influence on the reproductive performance of cows. The most critical time in the cow's reproduction cycle is during late pregnancy and early lactation when the foetus and calf place large energy demands on the cow. Body Condition Score (BCS) can be used as an indication of nutritional status. Body condition scre (BCS) ≥3 (1-5 scale) at time of calving increases the likelihood of the cow returning to oestrus. Ratnawati et al. (2014) reported that Brahman-cross cows in East java villages with BCS 3.1-3.3 have a PPAI of 110-135 days. PPAI is defined as the interval from calving to first oestrus after calving. A PPAI length of 110-135 days will allows cow to produce a calf every year. A longer PPAI reduces reproductive efficiency by delaying new calves and reducing the number of calves during her lifetime. Poor nutrition affects PPAI by suppressing hypothalamic secretion of GnRH (gonadothrophin releasing hormone) which stimulates the adenohypofise (anterior pituitary) to secrete LH (*Luteinizing Hormone*). Low secretion of LH, causes delayed ovulation (Nogueira 2004), extends the time to return to estrous after calving (Boland & Lonergan 2003) and extends calving interval.

Lactation is another cause of extended PPAI, especially in *Bos indicus* cattle breeds such as Brahmans. Suckling stimulates the pituitary gland to release prolactin, which inhibits the production of hormones necessary for ovulation (Goff 2015). Weaning calves earlier will therefore support the cow to start cycling by removing the suckling stimulus and reducing energy demands for lactation.

Poor estrous detection may also be affecting successful reproduction in Brahman cows in Indonesia (Tambunan et al. 2013). Most cows in East Java are mated using artificial insemination (AI). Accurate detection of estrous by the farmer and timely insemination are therefore crucial to improving the likelihood of successful mating. Inaccurate oestrous detection is caused by many factors, including: silent heat, insufficient time devoted by farmers to checking for sign of oestrus, or farmers observe inappropiate signs of oestrus. Ratnawati et al. (2014) reported that Brahman-cross bull in Lamongan district of East Java were given free access for oestrous detection and natural mating, was able to reduce the time from calving to conception from an average of 196 to 123 days .

An integrated village management system (IVMS) of nutritional, reproductive and health components was able to improve reproductive efficiency in Bali cows in Eastern Indonesia (Poppi et al. 2011). This system uses improved management strategies such as strategic supplementation of cows to maintain a BCS ≥3/5, timely detection of oestrous, properly mating management and weaning of calves at or before 6 months of age.

It was hypothesised that adapting the strategies of the IVMS to Brahman-cross cows in East Java will also improve their reproductive performance.

MATERIALS AND METHODS

Brahman-cross cows kept by smallholders in Sambeng and Modo sub-districts of Lamongan district (East Java), were monitored for their reproductive performance and management. The study was conducted between October 2011 and December 2013. The total number of farmer and cattle involved in this study is shown in Table 1. Different numbers of farmers and cattle were involved in the study each year because some farmers sold their cows for any reason.

Table 1. Number of farmer and cattle involved in this study from 2011-2013

	Year of study		
	2011	2012	2013
Number of farmers	151	148	105
Number of cow	189	183	126

Farmers received regular individual visits from project staff throughout the project. Project staff collected data on animal performance and provided advice on implementing the management strategies of the IVMS described above (weaning of calves, maintaining good BCS, oestrous detection and appropriate feeding). Interaction with project farmers included both formal farmer group meetings and farmer visit) and informally (regular visit at list once a month).

Feeding management

Cattle were fed diets based on crop residues (rice straw and corn stover), and were supplemented with green roughages (mostly grasses, but also tree legumes), by-products (rice bran) and a commercial concentrate (yellow feed SPT-01). The composition of the yellow feed SPT-01 concentrate (at the packet) were; water (max 12%), crude protein (min 16%), crude fat (min 4%), crude fiber (max 11%), ash (max 10%), TDN (min 67%), Ca (0.9-1.2%) and P (0.6-0.8%). Tree legumes (*Gliricidia sepium*, *Sesbania grandiflora* and *Leucaena leucocephela*) were planted by farmers and project staff around the village sites, and farmers were encouraged to cut and feed these forages to cows. The yellow feed SPT-01 concentrate was provided by the project for cows in the last two months of pregnancy and in early lactation (approximately 2 kg DM/head/day) in addition to their normal daily ration. Feed offered by the farmer was monitored and recorded (sub-sample 38 farmers/ month).

Mating management

Most cows were kept tethered in individual pens and mated using AI. Farmers monitored signs of estrous, and AI was conducted by the local inseminator. Timing of AI depended on availability of inseminator, but generally, if the farmer reported in the morning, AI would be done on the same day. However, if farmers noticed signs of estrous and reported in the afternoon, AI was conducted on the following morning.

One farmer kept their cattle untethered in communal pen with a bull, and it was assumed that cows were mated whenever the bull detected cow oestrus.

Weaning of calves

Farmers were encouraged to wean calves at 5-6 months of age. Calves were physically separated from the cows at weaning so they were not able to suckle. Many farmers chose to sell calves at weaning.

Measurements and data analyses

Cows and calves and BCS (visual assessment, 1-5 scale) were measured monthly during the first year of the project and every two months thereafter. Dates of oestrous detection, mating, calving and weaning were also recorded. Oestrous was detected by visual observation of restlessness, presence of clear mucus, standing to be mounted, swelling and reddening of vulva. This information was used to calculate average daily live weight gain (ADG) of calves, PPAI (days from calving to first observed oestrus), time from cycling to conception (days from first observed oestrus to conception), days open (days from calving to conception), length of lactation and incidence of lactation anoestrous (failure to show signs of oestrous while lactating). Average cow weight was corrected for pregnancy using the method of Silvey & Haydock (1978), which adjusts weight based on day of gestation and birthweight of calf. Daily climate data (temperature, rainfall) was collected from Lamongan district government weather station.

Data was analyzed using Microsoft excel and SPSS (one way anova). All procedures were reviewed and approved by the University of Queensland Animal Ethics Committee in accordance with the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes.

RESULTS AND DISCUSSION

Feeding management

The feeding system of Brahman cows in Lamongan depended on the availability of local feed sources. There were many kinds of local feed resourses used, which was categorized into three categories, there were: basal diet, medium quality greenfeed and high quality feed. On the average, cows in this study were offered 12.7 kg of basal diet; 25.1 kg of medium greenfeed and 3.1 kg of high quality of feed each day. The basal diet consisted of low quality feeds, mainly rice straw and corn stover. Medium quality greenfeed included native grasses elephant grass, tree legumes, and sugarcane tops. A small number of farmers fed,

Table 2. Availability of feeds during the study

Month	Feeds															
	RS		RB		G		Co		TL		TW		CS		Ca	
	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B
January	√	√	√	√	√	√				√		√	√	√		
February	√	√	√	√	√	√				√			√	√		
March	√	√	√	√	√	√		√		√		√		√		
April	√	√	√	√	√	√		√		√	√	√		√		
May	√	√	√	√	√	√	√	√		√	√	√	√	√		
June	√	√	√	√	√	√	√	√		√	√		√	√		
July	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
August	√	√	√	√	√	√	√	√	√	√	√		√	√		
September	√	√	√	√	√	√		√	√	√	√			√		
October	√	√	√	√	√	√			√	√	√		√	√		
November	√	√	√	√	√	√	√	√	√	√	√		√	√		
December	√	√	√	√	√	√	√	√	√	√	√		√			

RS = Rice straw
RB = Rice brand
G = Grass
Co = Concentrate
TL = Tree legume
TW = Tofu waste
CS = Corn stover
Ca = Cassava
A = Year 2012
B = Year 2013

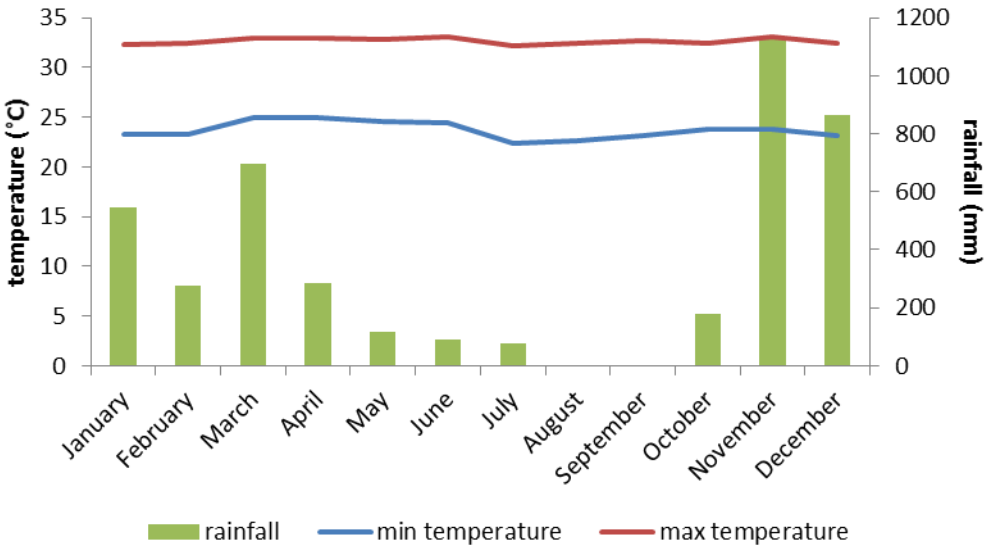

Figure 1. Average minimum and maximum temperature and rainfall in Lamongan district during the study (2011-2013).

their cattle high quality feeds such as chopped cassava, cassava peel, onggok (cassava by-product), tofu waste and grain concentrates (rice brand, yellow feed, concentrate etc).We recorded types and amount of feed given by 10-20 farmers to the cattle in every subdistrict. The availability of feed during the study shown in Table 2.

Farmer gave feeds to the cattle as a mixture of many feeds. In the wet season (November to March, Figure 1), the availability and digestibility of green feed is higher than in the dry season (April to October).

Table 2. Reproductive performance of Brahman cows in Lamongan, East Java (mean ± SD)

	Year of study		
	2011	2012	2013
Average post-partum anestrous interval (days)	198[a]±31	143[b]±9	98[c]±6
Average time from cycling to conception (days)	-	75±17	64±12
Average days open (days)	-	217±13	118±7
Average lactation length (days)	188[a]±7	169[b]±15	153[c]±3
Cows pregnant 1st mating (%)	6	42	88
Cows mated with AI (%)	86	86	92
Cows pregnant within 100 days of calving (%)	-	13	33
Cows with lactation anestrous (%)	50	39	3
BCS of cows at calving (1-5 scale)	3±0.1	3.3±0.1	3.3±0.1
Cows in BCS <3 at calving (%)	17	22	10
Average cow weight corrected for pregnancy (kg)	-	363	390

[a,b,c] Different letters within rows indicate significant differences at P<0.05

Table 3. Brahman calf production (mean ± SE)

	Year of Study		
	2011	2012	2013
Number calves born (%)	8	65	66
Average birth weight (kg)	31±2	31±0	32±0
Average weaning weight (kg)	164±13	139±4	140±5
Average age at weaning (days)	188[a]±15	169[a]±3	153[b]±3
ADG while suckling (kg/day)	0.7±0.1	0.7±0	0.7±0
Mortality rate (%)	13	5	6

[a,b] Different letters within rows indicate significant differences at P<0.05

During the dry season, additional supplementation from concentrates or by-products was necessary to maintain cow BCS. Planting tree legumes can increase the availability of green feeds during the dry season, when grasses reach maturity and growth slows (Shelton 2004). Farmers could also manage cattle so that periods of high energy requirements (e. g. late pregnancy and early lactation) coincide with the middle of the wet season when higher quality feed is available.

Adequate feed quantity and quality around the time of parturition is necessary to maintain BCS and support the reproductive performance of the cow. Body condition score has a significant impact to ovarían cyclicity and pregnancy rate of beef cows. Improved BCS and nutrition may also have improved corpus luteum viability and embryo survival. For smallholders, high quality green feeds (such as gliricidia, sesbania, leucaena) are a good source of protein (Antari et al. 2014a) and highly digestible energy to increase reproduction performance.

Reproductive performance of cows

Improvement in reproductive performance of cows was recorded during the periode of this study (Table 2). There was an increased percentage of pregnancy at first mating and decreased percentage of cows with lactation anoestrus between 2011 and 2013. The PPAI was shorter in 2013 than that in 2011 and 2012, and the percentage of pregnancy within 100 days of calving increased from 13 to 33%. The PPAI decreased by 100 days between 2011 and 2013. This shorter time from calving to first oestrous may be related to improvement in BCS of cows at calving (Ciccioli et al. 2003; Meikle et al. 2004). Although there was not a significant increase in the average BCS of cows at calving during

the study, there was decrease in the proportion of cows calving at BCS less than 3 (from 17% down to 10%). Cows with BCS <3 observed as: body outline bony; tail to head slightly recessed; backbones, shoulder, hip bone are visible. Days open and time from cycling to conception were not significantly different each year. As part of the interventions of IVMS, farmers were trained to improve their feeding practices, including: feeding more green feed and giving supplements at late pregnancy and early lactation. Lents et al. (2008) reported that supplementation of cows during early lactation improved milk production and prevented loss of body condition. Similar improvements in the number of Brahman-cross cows in BCS <3 at calving were demonstrated when the same management strategies were promoted to smallholder farmers in Lampung (Tambunan et al. 2013).

The reduced lactation length in 2013 (Table 2) may have assisted cows to return to oestrous quickly. The PPAI can be extended as a result of negative feedback from lactation during suckling, prompting lactational anoestrous. Brahman-cross cows lactating through the dry season in Northern Australia had lower liveweights at the end of the wet season than cows that had been weaned earlier, at the beginning of the dry season (Dollemore et al. 2013). This demonstrates the difficulty for cows to regain liveweight and BCS with extended lactation, and the importance of weaning before nutritional challenge and loss of BCS occurs. The improved management strategies applied in this study, resulted in substantial improvement in the proportion of cows with lactational anoestrous, falling from 50% in 2011 to 3% in 2013. There were more cows returning to oestrous while still lactating, resulting in a 2,5-fold improvement in the 100 day in calf rate. Similar improvements in 100 day in calf rate were demonstrated in Brahman-cross cows in Lampung (Tambunan et al. 2013). When a cow becomes pregnant within 100 days of calving, it will lead to producing calf every year and improving farmer income.

Throughout the Project, the majority of cows were mated using AI. The proportion of cows pregnant at first mating increased from 6% in 2011 to 88% in 2013. Low reproductive productivity of cattle in Lamongan is therefore likely to be impaired by farmers not detecting signs of oestrous properly. This problem could be caused by many factors, including: silent heat, detecting the wrong sign, and farmers busy with other activities. A long period of PPAI and days open contribute to long of inter-calving interval (Ciccioli et al. 2003).

Calf production

There was little change in calf production (weight at weaning) during this study (Table 4). The total range of birth weights (2011-2013) did not change during the study, with male calves born at 31-33 kg and female calves born at 28-31 kg. Average age at weaning decreased by over a month during this study. Average daily gain for calves while suckling was constant throughout this study (0.7 kg/day), indicating that cows were directing dietary energy towards maintaining their BCS and reconceiving, rather than increasing milk production for their calves. Calf mortality rate declined during the study. With the support and training provided by the project, farmers have better knowledge of how to manage their new born calves, making sure that calves suckling and getting feed to the cow to produce milk. Improving feeding management needed to increasing milk production so that ADG calves increased.

CONCLUSION

The improved management strategies of the IVMS have been shown to increase reproduction rates of Brahman-cross cows in Lamongan district, East Java. Strategies such as maintaining BCS of cows $\geq$3, appropriate detection of estrous mating management and early weaning at 5-6 months old increased the percentage of cows conceiving within 100 days of calving, and able to produce a calf every year, from 13% to 33%. The proportion of cows conceiving at first mating increased from 6% at the start of the study to 88% at the end. These outcomes are expected to have a significant impact on the economic performance of smallholder cow-calf enterprises.

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Application of BS4-Enzyme on the Methane Production from Mixture of Cattle Manures and Waste Paper

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ABSTRAK

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Koran bekas adalah sampah yang dapat diubah menjadi biogas melalui fermentasi anaerob. Tujuan dari penelitian ini adalah untuk membandingkan produksi gas total dan metana pada substrat campuran feses sapi dan koran bekas masing-masing sebagai inokulum dan substrat dengan penambahan enzim dan tanpa penambahan enzim. Enzim BS4 diproduksi di Balai Penelitian Ternak (Balitnak) dengan menumbuhkan Eupenicillium javanicum BS4 pada bungkil kelapa. Terdapat tiga perlakuan dalam penelitian ini, yaitu: 30% BK dari feses (M30); 15 % + 15% koran bekas (MP30); dan MW30- ditambahkan dengan 3 ml (0,42 U/g BK) enzim (MPE30) Persentase penambahan substrat koran diperhitungkan terhadap bobot kering feses dan setiap perlakuan diulang lima kali. Produksi gas total dan metana diukur tiap minggu. Kehilangan bahan kering (KBK) diukur selama 5 minggu fermentasi. Analisis pengaruh perlakuan terhadap produksi gas dan metana dilakukan dengan rancang acak lengkap pada setiap minggu. Uji beda nyata ditentukan dengan LSD. Penambahan enzim dan koran meningkatkan produksi gas. Produksi gas dan metana tertinggi secara nyata (P<0,05) didapat dari perlakuan MP30 dan MPE30 dibandingkan dengan M30 pada minggu kelima. Penambahan enzim secara sangat nyata meningkatkan produksi metana pada minggu pertama. Produksi gas total dan metana terhadap KBK paling efektif secara nyata ditunjukkan pada perlakuan MPE30. Enzim BS4 dapat digunakan untuk mempercepat dan meningkatkan efisiensi transformasi campuran koran dan feses menjadi metana yang dapat digunakan sebagai biogas.

Kata Kunci: Metana, Enzim-Fibernolitik, Koran Bekas, Feses Sapi

ABSTRACT

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Cellulose from abundant newspaper waste could be transformed into methane through anaerobic fermentation. This research was carried out to compare the gas production including methane between samples containing feces and waste paper mixture as inoculum and substrate, respectively and added with and without BS4 enzyme. The enzyme was produced in Indonesian Research Institute for Animal Production (IRIAP) by growing *Eupenicillium javanicum* BS4 in coconut meals. There were three treatments, i.e., 30% manure (M30); 15 % manure + 15 % paper waste (MP 30); MP30 + 3 mL BS4 enzyme equal to 0.42 U/g dry matter (MPE30) The percentage of waste papers addition in feces was calculated on dry matter (DM) basis and every treatment had five replications. Total gas and methane productions were measured weekly, while dry matter losses were determined during 5 week fermentation. Interactions between treatments and incubation time were analyzed using completely randomized design each week. Kind of substrates influenced both total gas and methane productions during incubation time. Both waste papers and enzyme addition enhanced gas production. The highest total gas and methane productions for five weeks incubation were highly significantly observed (P<0.01) in MP30 and MPE30 compared to M30. Addition of enzyme significantly increased total gas and methane productions in the first week. The highest methane and total gas yield/g dry matter were obtained by BS4 enzyme addition. It is concluded that BS4 enzyme is good in accelerating and increasing the transformation efficiency of waste paper and manure mixture for biogas production.

Key Words: Methane, Fibrenolytic-Enzyme, Waste Papers, Cattle Manures

INTRODUCTION

Waste paper, including newspapers, is one of the types of waste that are most commonly found in the environment. In 2005, the production of waste newspaper per capita was 0.61 kg/capita/year, and the amount continues to increase with the growing number of population (Wahyono et al. 2011). An effort that can be done to reduce the waste paper is to recycle the waste paper into methane biogas by anaerobic digestion

process using mixture of animal manure with waste paper as media.

Biogas is a renewal energy that can be used as an alternative to meet the fuel needs. The raw material of this energy source is a non-fossil material, generally from an organic waste such as animal manures. The use of methane biogas as a fuel substitute for LPG (Liquefied Petroleum Gas) is already quite extensive. The anaerobic digestion of organic waste materials also solve the pollution problem caused by abundant waste material.

In Mumbai, India, local household waste treatment with biogas reactor has been implemented since 1903 (Nijaguna 2002). The biogas is generally composed of methane (CH_4) 53-70%, CO_2 (30-47%), N_2 (0.2%), and other gasses such as ammonia, hexane and O_2 in very low concentrations. One source of methane emissions resulting from agriculture activity is livestock production activities. Beside rumen activity stacking of cow dung in the field allows the release of methane into the open air as a greenhouse gas that potentially involved in global warming. Its greenhouse effect is 24 times greater than CO_2. On the other hand, collecting the methane as biogas is preferred because the methane has a high energy density, i.e., about 400-600 BTU/ft^3 (Folkson 2014).

Waste paper consists of organic fiber materials such as amorphous and crystalline celluloses. The crystalline cellulose is stable and difficult to be degraded by bacteria and spoilage microbes. The fibers require pre-treatment processes by chemical, physically-chemical, or biological processes before it could be converted into biogas. Physical methods such as grinding and exposure to radiation; chemical methods such as addition of acids or bases; biological method such as enzymatic degradation have been reported as pretreatment methods (Baatar et al. 2014). Hydrolysis using enzymes or bio treatments has several advantages for low energy input, does not use hazard chemicals, and environmentally friendly (Sutaryo et al. 2014).

Cattle manure is usually used as a source of inoculum in the anaerobic production of methane biogas. Using newspapers waste as the substrate needs the inoculum which has some cellulolytic, hemicellulolytic and methanogenic bacteria. All types of bacteria originated from the intestinal tract of cattle are manifested in the manures. The cellulolytic and hemicellulolytic bacteria in the cattle manure transform the cellulose and hemicellulose into monosacharides such as glucose which was transformed by other bacteria into simple organic acids. These organic acids are then processed into methane by methanogenic microbes (Ofoefule et al. 2010).

BS4 enzyme a multi enzyme produced by *Eupenicillium javanicum* in coconut meal consist of β-D-mannanase, β-D-endoglucanase (CMCase), β -mannosidase, α-D-galactosidase, and β-D- glucosidase. The multi enzymes have been shown synergistically degraded cellulose and hemicellulose of plant cell walls of palm kernel cake into reducing sugars (Purwadaria et al. 2003; Mirnawati et al. 2013). It is possible that BS4 can degrade cellulose of waste papers. The synergistic activity of the entire enzymes in waste paper digestion can be detected by assaying filter paper-degrading activity (Fpase).

Beside pretreatments methane biogas production can be accelerated by addition of substrates continuously. The waste products or sludge from the digester can still be used as organic fertilizer (Haryati 2006).

The research reported in this paper explored the possibility to optimize methane production from waste papers and cattle manure as the substrates and inoculum respectively with the addition of enzyme.

A. B.

Figure 1. The total gas (A) and methane (B) measuring equipments. The total gas measurement was taken directly with measured syringe, while for the measurement of methane, the gas must pass through the 0.05 N NaOH solution flask.

MATERIALS AND METHODS

A fresh cattle manures used as a source of inoculum was obtained from IRIAP. Old newspapers were cut with a width of 5mm using a paper shredder and then chopped with a length of 10 mm. BS4 fibernolytic enzyme used in the experiment was produced using a mold *E. javanicum* grown on coconut meal in solid substrate fermentation. Gas production experiment was conducted in 500 ml glass bottles, incorporated with CO_2 and closed with rubber bottle caps and reinforced with aluminum sheet. The total gases of CO_2 and methane production were measured directly with measured syringe, while the methane was measured after the gas was reacted with 0.05 N NaOH (Figure 1).

The enzyme addition was determined based on the activity of the enzyme to the filter paper (FP-ase). The amount of enzyme added was 3 ml per bottle with the activity of 0.42U/g DM that suitable used for feed additive containing palm kernel meal (Sinurat et al. 2007). Each treatment consisted of 5 replications.

The experiment was conducted for five weeks and samples of total gas production and methane were collected and measured weekly. After the measurement the gas in the sample bottles was relatively empty. Dry Weight Loss (DWL), the yield of total gas and methane towards DWL of each treatment were determined at the end of experiment.

Determination of FPase activity assay was carried out using a modification of the method by Mandels & Sternberg (1976). A volume of 0.4 mL of enzyme sample was mixed with 1.1 mL of 0.05 mM Na-acetate buffer at pH 4.8. Rolled filter paper of Whatman no.1 with a size of 1 x 6 cm was added to a sample and the control solution. The sample solution was incubated at 60°C for 1 hour while the control solution was not incubated. DNS reagent (3 ml) was added to a solution of the sample after incubation and directly on the control to stop the enzymatic reaction and for detection the reducing sugars produced. Samples and controls then were heated in boiled water for 15 minutes. In each test tube 5 mL of distilled water was added. The absorbance of the samples and the control were then measured using a UV-Vis spectrophotometer with a wavelength of 540 nm. Spectrophotometer measurement results were compared with the control samples and the standard curve of D-glucose 300-1,800 µg/mL.

Dry matter (DM) contents were measured in the begining of the trial at the end of the experiment. Determination was conducted using gravimetry method after heating at 105°C oven for overnight.

The ratio of total gas and methane productions in five weeks with the dry matter loss (DML) were calculated to observe the efficiency of gas production for each treatment. Statistical analysis was performed to show the differences between treatments on the data obtained in each week during the five weeks of incubation. The analysis (ANOVA) was carried out using completely randomized design with 4 (four) treatments and 5 (five) replications. Least Significant Difference (LSD) test was used to evaluate significant differences between treatment when the ANOVA show a significant effect ($P<0.05$).

RESULTS AND DISCUSSION

The kind of substrates over a 5 week incubation were significantly influenced the total gas and methane productions (Table 1) in every week. Even though the dry matter of the three treatments were similar, the total

Table 1. Total gas (A) and methane (B) productions (mL/bottle) in every week during the course of fermentation

A. Gas total

Substrates	Incubation time (weeks)					Total
	1	2	3	4	5	1-5
M30	94.0^{b}	124.8^{b}	67.8^{b}	60.4^{c}	84.6	431.6^{b}
MP30	203.3^{a}	243.7^{a}	-164.3^{a}	115.7^{a}	65.7	783.7^{a}
MPE30	247.2^{a}	186.0^{ab}	165.6^{a}	76.4^{b}	58.4	733.6^{a}
P value	<0.01	0.02	<0.01	<0.01	0.23	<0.01

B. Methane

Substrates	Incubation time (weeks)					Total
	1	2	3	4	5	1-5
M30	25.3^{c}	37.3^{b}	22.5^{b}	19.5^{c}	27.7^{a}	132.3^{b}
MP30	55.3^{b}	60.2^{b}	43.0^{a}	30.7^{a}	21.3^{ab}	209.2^{a}
MPE30	80.6^{a}	55.0^{ab}	39.6^{a}	14.4^{b}	9.6^{b}	199.2^{a}
P value	<0.01	<0.01	<0.01	<0.01	0.02	<0.01

Different superscript in the same column shows significant difference. M30 was-30% DM of manure; MP30 was 15% manure +15% waste paper; MPE30 was MP30 + BS4 enzyme.

gas and methane productions of MP30 and MPE30 were highly significantly higher than that of M30. The gas production during fermentation caused by the decomposition of structural carbohydrates into methane and CO_2 by the activity of fibernolytic, sugars fermented and methanogenic bacteria (Ofoefule et al. 2010). The waste papers were much easier transformed into glucose and gasses than the cattle manures which already fermented and digested in the digestion track.

The pattern of gas production (Table 1) mimics the general growth curve of microbes, after adaptation the gas produced in logarithmic phase and after the maximum production is reached, the production is reduced. However, an exception was found in the enzyme supplementation treatment (MPE30) in which the highest total gas and methane production occurred in the first week due to acceleration by BS4 addition. BS4 showed filter paper activity or can transform paper into sugars. Other treatments showed the maximum total gas and methane productions occurred in the second week incubation. After that the gases were still produced, but with the lower amount. The gas production reduction occurred due to the less amount of substrate remained for further fermentation process. These results suggest that the addition of enzymes can accelerate the formation of gas so as to shorten the incubation time in the biogas production process. The cumulative amount of total gas and methane produced during incubation were calculated (Table 2) To understand how much the gas produced.

Similar to weekly gas production, the cumulative total gas and methane production were highly significantly ($P<0.01$) influenced by the treatments. Since the gasses were produced in every week resulted in the increase amount of cumulative gasses in the course of incubation. Waste papers showed significantly ($P<0.05$) better substrate for producing gasses compared to the cattle manure. The highest total gas and methane productions were observed when the BS4 enzyme was added (MPE30) or the mixture of waste paper and manures with BS4 addition on the first and second weeks, while the highest total gas was only observed in the first week. After that the effect of enzyme addition was not detected, since the cumulative amount of gasses between MPE30 and M30 were not significantly different ($P>0.05$).

The effectivity of dry matter transformation into gasses was observed by dividing the amount of products (gasses) towards dry matter loss (Table 3). The highest DML of substrate was obtained in M30 treatment with an average loss of 4.68 g for 5 weeks, and the lowest in MPE30 with 1.88 g for 5 weeks. In contrast to the DML, the highest yield of total gas and methane productions were highly significant ($P<0.01$) produced in the BS4 enzyme addition (MPE30) i.e., 391.6 mL/g and 106.3 mL/g respectively, and the lowest were in the M30 (92.30 mL/g and 28.31 mL/g respectively). These results indicated that the BS4 enzyme addition (MPE30) was the most effective in the transformation of substrate to gasses.

In the experiment the gas production was observed only for five weeks incubation, and it was still produced until the fifth week. In a review by Haryati (2008) it was reported that the process of anaerobic digestion in the continuous digester culture with the waste papers substrate takes 8 weeks at a temperature of 35ºC. A third of the biogas generated in the first week, a quarter in the second week, and the rest is produced by the third week until the eighth week. Our results showed that the decrease of gas production after three weeks of incubation was in agreement with the review.

MP30 and MPE30 produced total gas more than those produced from cattle feces substrate only at M30. Total gas and methane productions (Table 1 and 2) in the mixture of substrate treatments were higher than the cattle manure substrates because MP30 and MPE30 contained more soluble carbohydrates. Dry mass of waste papers consists of 58-95% carbohydrate (40-55% 18-40% cellulose and hemicellulose) (Sun & Cheng 2002), while the dry weight of cattle manures composed of 13% carbohydrates, in the form of glucose, cellulose and hemicellulose (Aslanzadeh et al. 2011). Similar results were obtained by Ofoefule et al. (2010), which concluded that cow dung and newspapers mixed media can be used as a good medium for the production of biogas. The cattle feces C/N ratio is 22:1. The addition of the waste papers which contains almost no nitrogen can increase the C/N ratio to close the optimum value of C/N ratio of gas production (25 : 1). The ratio range of carbon: nitrogen in the substrate to produce methane gas are 20 : 1 to 30 : 1 and the optimal ratio is 25 : 1 (Li et al 2011). If the ratio of carbon and nitrogen in the substrate is less than the optimal, it can affect an accumulation of short-chain organic acids or lead to the formation of ammonia gas. The accumulation of organic acid or ammonia excess can inhibit the total gas especially methane productions. Addition of an extra waste paper increased the carbon content of substrate and subsequently increasing the total gas and methane productions.

Although the addition of cattle manures reduced the C/N ratio, the feces was needed as the inoculum for cellulolytic, hemicellulolytic, and methagonic transformation processes, the nitrogen also is needed for the bacterial growth. Most of cellulolytic bacteria found in cattle feces comes from the genus *Bacteroides*, those are *Clostridium, Ruminococcus*, and *Bifidobacterium*, as well as other bacteria such as *Allistipes, Prevotella*, and *Enterococcus*. Most those bacteria are obligate anaerobes that difficult to grown in aerobic condition (Dowd et al. 2008).

Table 2. The cumulative total gas (A) and methane (B) production (mL/bottle) for 5 (five) weeks of fermentation

A. Total gas

Substrates	Incubation time (weeks)				
	1	2	3	4	5
M30	94. 0^{b}	218. 8^{b}	286. 6^{b}	347. 0^{b}	431. 6^{b}
MP30	203. 3^{a}	438.0^{a}	602.3^{a}	718.0^{a}	783.7^{a}
MPE30	247.2^{a}	433.2^{a}	598. 8^{a}	675.2^{a}	733.6^{a}
P value	<0.01	<0.03	<0.01	<0.01	<0.01

B. Methane

Substrates	Incubation time (weeks)				
	1	2	3	4	5
M30	25.3^{c}	62.8^{c}	85.1^{b}	104.6^{b}	132.3^{b}
MP30	55.3^{b}	115.7^{b}	158.5^{a}	189.2^{a}	209.2^{a}
MPE30	80.6^{a}	136.0^{a}	175.2^{a}	189.6^{a}	199.2^{a}
P value	<0.01	<0.01	<0.01	<0.01	<0.01

Different superscript in the same column shows significant difference.
M30 was-30% DM of manure
MP30 was 15% manure +15% waste paper
MPE30 was MP30 + BS4 enzyme

Table 3. Dry matter losses (DML) and Yield of gasses of different substrates after 5 (five) weeks of incubation

Treatment	DML (g)	Gas Yield (mL/g)	CH_4 Yield (mL/g)
M30	4.68^{a}	92.3^{c}	28.3^{c}
MP30	3.62^{b}	271.0^{b}	57.9^{b}
MPE30	1.88^{c}	391.6^{a}	106.3^{a}
P	<0.01	<0.01	<0.01

Different superscript in the same column shows significant difference.
M30 was-30% DM of manure;
MP30 was 15% manure +15% waste paper;
MPE30 was MP30 + BS4 enzyme.

Total gas and methane productions of MPE30 was higher in the first and the second week of incubation (Table 2), however their production rate declined from the third week until the end of incubation. The enzyme activity was rather not expressed or inhibited. Inhibition of the enzyme by the reaction product can be used as a major reason fibrenolytic enzymes work cessation (Kristensen et al. 2009). Especially in the first week of incubation the carbohydrates were fastly digested into monosaccharides, some were fermented and transformed into the gasses, but some inhibited BS4 activity. Therefore in the third week until the fifth week of incubation, the addition of the enzyme did not have any significant difference in the methane productions with the one without enzyme addition (MP30). The decline of production also may be due to less available fermentable substances.

The addition of the enzyme in the treatment MPE30 improves production efficiency and methane gas. Samples with treatment MPE30 able to produce the amount of gas in total (733.0 mL) and methane (199.2 mL) using the least substrate at 1.88 g (Table 2 and 3) producing the highest yield of 391.6 mL/g and 106.3 mL/g respectively. As a comparison, MP30 treatment required almost double the amount of dry substrate (3.62 g) to produce same amount of gas and methane. Increased yield of methane in the digestion is also found in the study of solid waste digestion by Roman et al. (2006). From these results, it is concluded that the addition of hydrolase enzymes accelerated the fermentation process and enhanced the effectivity of transformation. Although the formation of gas in the treatment with the addition of enzyme resemble the treatment without the addition of enzymes, fermented

waste organic materials can still be used for organic fertilizer. The time for addition of the enzyme may also noteworthy because report of Sutaryo et al. (2014) showed that the addition of multienzyme into the composite substrate dairy cattle manure (DCM) at the pre-treatment stage provide results that significantly increased (4.15%) methane production compared to the control without the addition of enzymes into anaerobic digester. In this experiment the addition of enzyme only arranged at one dose and it already accelerated the gasses production in the first week of incubation. Less dosage of enzyme activity might slower the transformation of the carbohydrates into monosaccharide just enough for the VFA fermentation and methane production resulted the protection of feedback inhibition occurred for more methane production. The addition of BS4 enzyme proved that the biogas production could accelerate or lesser time of fermentation is required to produce the gasses. Large scale operation should be designed for field application. The enzyme addition might be applied with continuous substrate feeding which may enhance the biogas production.

Utilization of dung cow manure with the addition of agricultural waste or agricultural residues has been widely applied to biogas production. The use of waste paper and the addition of enzymes can be added as an alternative to accelerate anaerobic digestion to obtain more efficient results.

CONCLUSION

Results in this study showed that newspapers waste can be used as a carbon source and a good substrate for biogas production of methane after mixed with cattle manures. The addition of fibrenolytic enzyme from *E. javanicum* BS4 into the substrate increased the production of methane in the first two weeks of incubation. The addition of the BS4 enzyme can enhance the gas yield towards substrate or dry matter loss or improve the efficiency of waste papers transformation into methane or biogas as renewable energy. The use of enzymes in the incubation process can accelerate the formation of biogas so as to shorten the incubation time.

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Bioactivities and Analysis of Peptides of Sumbawa Horse Milk Generated by *Bacillus thuringiensis* Protease

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ABSTRAK

Kusumaningtyas E, Widiastuti R, Kusumaningrum HD, Suhartono MT. 2016. Bioaktifitas dan analisis peptida dari susu kuda Sumbawa yang dihasilkan oleh protease *Bacillus thuringiensis*. JITV 21(4): 244-254. DOI: http://dx.doi.org/10.14334/jitv.v21i4.1627

Susu kuda Sumbawa dipercaya dapat menyembuhkan berbagai penyakit seperti asma, hipertensi, diabetes dan gastrointestinal, tetapi potensi peptida bioaktifnya belum dieksplorasi. Tujuan dari penelitian ini adalah untuk mengevaluasi bioaktivitas peptida hidrolisat protein susu kuda Sumbawa dan menganalisis sifat fisiko kimia peptida terpilih. Protein susu dihidrolisis dengan protease *Bacillus thuringiensis*, peptida yang diperoleh difraksinasi bertingkat kemudian dianalisis aktivitas antibakteri dan antioksidan. Fraksi peptida <3 kDa kemudian disekuensing menggunakan LCMS-MS dan dianalisis sifat fisiko-kimianya. Hasil penelitian menunjukkan bahwa fraksi peptida <3 kDa dari hidrolisis 30 menit paling aktif sebagai antibakteri dan lebih aktif terhadap bakteri Gram negatif. Untuk antioksidan, aktivitas antioksidan fraksi tersebut per µg protein/mL adalah 83% terhadap radikal ABTS dan 31% terhadap radikal DPPH. Nilai tersebut setara dengan vitamin C 12.5 mg/mL untuk ABTS dan 14.5 mg/mL untuk DPPH. Peptida HPYFYAPELLYYANK dengan prediksi berat molekul 1887.92 Da dengan titik isoelektrik 7.47 mempunyai indek terapi prediksi yang tinggi (64.75). Hasil tersebut menunjukkan bahwa peptida dari susu kuda Sumbawa yang dihidrolisis menggunakan protease *Bacillus thuringiensis* aktif sebagai antibakteri dan antioksidan. Peptida HPYFYAPELLYYANK dari fraksi <3 kDa berpotensi sebagai antibakteri.

Kata Kunci: *Bacillus thuringiensis*, Peptida Bioaktif, Susu Kuda

ABSTRACT

Kusumaningtyas E, Widiastuti R, Kusumaningrum HD, Suhartono MT. 2016. Bioactivities and analysis of peptides of Sumbawa horse milk generated by *Bacillus thuringiensis* protease. JITV 21(4): 244-254. DOI: http://dx.doi.org/10.14334/jitv.v21i4.1627

Sumbawa horse milk is claimed to cure some diseases such as asthma, hypertension, diabetes and gastrointestinal disorder but its potential bioactive peptide has not been explored. The aims of this study are to evaluate bioactivities of peptides from Sumbawa horse milk protein hydrolysate and to analyzethe physio-chemical properties of selected peptides. The milk protein was hydrolyzed by *Bacillus thuringiensis* protease, the peptide produced were sequential fractionated and then analyzed for antibacterial and antioxidant activities. The peptide fraction <3 kDa was then sequenced using LCMS-MS and the physio-chemical properties of the peptides were analyzed. Result showed that peptides fraction <3 kDa from the 30 min hydrolysis was the most active as antibacterial and more active to Gram negative bacteria. For antioxidant, scavenging activity of the fraction per µg protein/mL were 83% to ABTS and 31% to DPPH radicals. The values were similar with vitamin C 12.5 µg/mL for ABTS and 14.5 µg/mL for DPPH. Peptide HPYFYAPELLYYANK with molecular weight prediction 1887.92 Da and isoelectric point 7.47 has high therapeutic index prediction (64.75). The result showed that peptides from Sumbawa horse milk hydrolyzed by *Bacillus thuringiensis* protease was active as antibacterial and antioxidant. Peptide HPYFYAPELLYYANK from fraction <3 kDa was potential as antibacterial.

Key Words: *Bacillus thuringiensis*, Bioactive Peptide, Horse Milk

INTRODUCTION

Horse milk is minor importance in milk production in comparison to cow and goat milk. Resemblance with human milk in many respect make horse milk have been traditionally important and claimed to have special therapeutic properties. Overall, horse milk is considered to be highly digestible, rich in essential nutrient and possesses an optimum whey: casein protein ratio, making it suitable in pediatric dietetic (Potocnik et al. 2011). Nutritional and therapeutic properties are also beneficial for elderly diet. Around 30 million people consume horse milk regularly throughout the world. In Turks, Bashkirs, Kazaks, Mongol, Yakuts and Uzbeks they make lactic-alcoholic beverage called Koumiss (Potocnik et al. 2011). Domnez et al. (2014) reported

that triglyceride and cholesterol were decreased but high density lipoprotein (HDL) was increased significantly after consumption of Koumiss for 15 days. Administration of koumiss to cancer patients was able to decrease adverse effect of chemotherapy (Uniacke-Lowe et al. 2010)

In Indonesia, Sumbawa horse milk is claimed to cure some diseases such as asthma, hypertension, diabetes and gastrointestinal disorder. Sumbawa is one of the eastern island of Indonesia. Horse is widely grazing in the forest throughout the year. Fractionation of Sumbawa horse milk with different polarity showed that whey protein fraction, soluble in acetone exhibited significant inhibition on the growth of *S. agalactiae* and *S. pyogenes* (Detha et al. 2013). Sumbawa horse milk not only contains beneficial protein and good microorganism but may also contain bioactive peptides yielding by enzymatic hydrolysis of bacterial protease.

Bacillus thuringiensis is an endospore-forming bacteria which is commonly found in soil, water, plants, stored cereal, dead insect, animal feces (Argolo-Filho & Loguercio 2014), milk before or after pasteurization (Zhou et al. 2008), fresh fruit and vegetable (Frederiksen et al. 2006). Some researchers found *B. thuringiensis* as part of phyllo plane microbiota and has evolved to provide symbiotic protection (Argolo-Filho & Leguercio et al. 2014). Research on *B. thuringiensis* has been actively done in order to make sure the safety of this bacterium in vertebrata. Wilcks et al. (2008) reported that *B. thuringiensis* was able to colonize in intestinal germ free of rats at high concentration in stable form but were eliminated through their feces with no effect found to the animals. The presence of *B. thuringiensis* in the rectal sample from dairy cow indicated that multiplication of *B. thuringiensis* cells had occurred in the digestive tract (Ammons (2009). Not only safety indication was shown by *B. thuringiensis*, but also its protection to the healthy gut. Kweon et al. (2012) found that *B. thuringiensis* contaminating in fresh milk was able to act as probiotic by preventing fatal infection of *Escherichia coli* 055, *Salmonella typhimurium* 01D and *Staphylococcus aureus* 305 in mice. *B. thuringiensis* also produced bacteriocin-like inhibitor substances (Bt-BLIS) with high level of activity against *Bacillus cereus* and other Gram positive bacteria such as *Vibrio cholera* (Barboza–Corona et al. 2007). Bacteriocins produced by *B. thuringiensis* was also able to inhibit growth of multiple antibiotic resistant bacteria associated with subclinical mastitis in dairy goats (Gutierrez-Cavez et al. 2015).

B. thuringiensis produce alkaline protease that showed proteolytic activity at various culture condition. The enzyme was active to hair substrate keratin and was used for de-hairing (Agastya et al. 2013). Recently, Zhang et al. (2015) identified and characterized a novel alkaline thermolysin like protease from *B. thuringiensis* with possible use in therapeutic and biotechnological application. Kent et al. (2012) reported that fermentation of sodium caseinate using *B. thuringiensis* resulting in Caseicin A and Caseicin B that identified previously by Hayes et al.. (2006) as antimicrobial peptide. *B. thuringiensis* isolate in this study from horse milk that showed highly active protease, as indicated by large clear zone when the bacterium was grown in the medium containing skim milk. The protease was used to hydrolyze Sumbawa horse milk protein to produce bioactive peptides. this paper disscuses proof of the potent antibacterial and antioxidant activities of bioactive peptide fraction.

MATERIALS AND METHODS

Microorganisms

B. thuringiensis was isolated from horse milk from Bogor, Indonesia. *Escherichia coli* (ATCC 25922), *Salmonella Typhimurium* (ATCC 13311) *Listeria monocytogenes* (ATCC 15313) and *Staphylococcus aureus* (ATCC 25923) were used for bacterial assays.

Milk and enzyme preparation

Horse milk was collected from Sumbawa Island, Indonesia. The milk was centrifuged at 6000 ×g, 4°C for 15 min to discard the fat. Defatted milk was hydrolyzed immediately or stored at -20°C until used. Protease was produced by culturing *B. thuringiensis* in Luria Bertani Broth (Difco, Becton Dickinson and Co, US) containing 0.05 % (w/v) skim milk (Difco, Becton Dickinson and Co, US) and incubated at 37 °C for 24 h. The cells were separated by centrifugation 3500 ×g for 20 min. The crude enzyme was harvested by precipitation with cold (4°C) 50% (w/v) ammonium sulphate overnight and centrifuged at 10.000 ×g, 4°C for 20 min. The precipitate was air dried and stored at -20°C until used. For hydrolysis, crude enzyme was diluted in phosphate buffer saline 10 mM, pH 7.4 (1:2 (w/v)). Protein concentration was analyzed using Quick start ™ Bradford protein assay (Bio-Rad Inc). Standard curve was obtained from reaction between 5 µL of bovine serum albumin at serial dilution of 2.5-25 mg mL^{-1} and 95 µL Bradford solution. Deionized water was used as blank. Absorbance was measured at λ 600 nm in microtiter plate reader (Labsystems, original Multiscan Ex, Champaign USA).

Enzyme activity assay was conducted according to Bergmeyer et al. (1983). Reaction was performed by mixing 250 µL casein 2% (w/v) with 50 µL enzyme and 250 µL PBS 0.05 M pH 7 and incubated at 37°C. As much as 500 µL of TCA 0.2 M (Sigma-Aldrich, USA) was added, incubated at 37°C for 10 min and then

centrifuged at 2000 × g for 10 min. As much as 375 µL supernatant was mixed with 1250 µL Na_2CO_3 0,4 M and 250 µL reagent *Folin ciolcateau* (Sigma-Aldrich, USA) (1:2) and then incubated at 37°C for 20 min. Optical density was measured at λ 578 nm (spectrophotometer UV-Mini-1240 Shimadzu). One unit (IU) of enzyme was defined as the amount of enzyme needed to produce 1 µmol tyrosin per min.

Hydrolysis

Horse milk was hydrolyzed by *B. thuringiensis* protease (0.67 IU) with enzyme substrate ratio 1 : 20 (v/v). Hydrolysis was conducted at 55°C, pH 11 for 30 and 60 min. The reaction was stopped in boiled water 95°C for 5 min. Hydrolysates were centrifuged at 10.000 × g for 15 min to remove lipid and insoluble protein. The pH was adjusted to around 7. The supernatant was filtered using 0.45 nm membrane (Acrodisc LC 13 mm, 0.45 µm, PVDF, Pall Life Sciences, USA). Hydrolysate was sequentially filtered through membrane MWCO 30k, 10k and 3k (AMICON Ultra centrifugal units, Merck Millipore Ltd., Tullagreen, Carrigtwohill, Co). Retentate was diluted with the same volume of sterilized deionized water. Protein concentration of each fraction was determined using Quick start ™ Bradford protein assay (Bio-Rad Laboratories Inc). All fractions were evaluated for antibacterial and antioxidant activities.

Antibacterial assay

Minimum Bactericidal Concentration (MBC) assay was performed according to Keepers et al. (2014) with modification. Briefly, 100 µl of each peptide fraction was prepared in 2-fold dilution series in a 96-well round-bottom microplates. As much as 100 µl *S aureus, L monocytogenes, E. coli* or *S. typhimurium* suspensions of 10^6 CFU mL^{-1} was then added to the ependorf containing peptide fractions. Content of the well (100 µl) was grown onto Mueller Hinton agar plate (BD Difco™, Becton Dickinson and Co, USA) and incubated for 24 h at 37°C. Additional 24 h incubation was done to ensure no bacterial growth. The MBC was recorded as the lowest concentration of the peptide fraction which produced no visible bacterial growth. Each treatment was done in three replications.

Antioxidant assay.

2,2'-*azino-bis* (3-*ethylbenzthiazoline*-6-*sulphonic acid* (ABTS) solution (Sigma-Aldrich, USA) was made according to Thaipong et al. (2006). The working solution was prepared by reaction of ABTS stock solution of 7.4 mM and 2.6 mM potassium persulphate and incubated for 18 hours at room temperature in the dark. The solution was diluted with deionized water to obtain an absorbance of 1.1±0.05 at 405 nm. The fresh ABTS was then used for antioxidant assay. Sample of 100 µL (1 µg mL^{-1}) of each peptide fraction was mixed with 200 µL ABTS in microplate and incubated at room temperature for 15 min to allow the reaction. Absorbance of the resulting mixture was recorded at λ 405 nm using microplate reader (Labsystems, original Multiscan Ex, and Champaign, USA). The peptide control was made by substituting ABTS radical with deionized water, while ABTS control was made by substituting peptide fraction with deionized water. Serial concentrations of Vitamin C p.a (2.5-17.5 µg mL^{-1}) was used as standard.

Antioxidant assay using 2,2-diphenyl-1-picrylhydrazyl (DPPH) (Sigma-Aldrich, USA) was conducted by mixing ethanol 96% with DPPH to obtain absorbance 1.1 ±0.05 at λ=540 nm. As much as 100 µL of peptide fraction was added to 200 µL DPPH. After incubation for 30 min at room temperature, the absorbance of the mixture was measured at λ 540 nm. Vitamin C p.a (2-16 µg/mL) was used for generating standard curve (Thaipong *et al.* 2006). The scavenging activity of peptide fractions to ABTS and DPPH radicals was expressed using equation:

Scavenging activity (%) = 100 x (A0-A1)/A0

Where A0 was absorbance of ABTS/DPPH and A1 was the final absorbance of sample minus initial absorbance. Result from ABTS and DPPH assays was presented as means of experiments performed in three replications.

Peptide profiling by RP-HPLC.

Peptide fraction with highest antibacterial and antioxidant activities was run in reverse-phase high performance liquid chromatography (RP-HPLC). A preparative C-18 column (25 cm x 1.0 cm id); Xterra waters) was used with Hewlett-Packard 1050 series HPLC (Waters Corporation) equipped with diode array detector. Eluate was monitored at 215 nm. The solvents consisted of: (A) 0.1% (v/v) trifluoroacetic acid (TFA) (Sigma-Aldrich, USA) in deionized water and (B) 0.1% (v/v) TFA in acetonitrile (Sigma-Aldrich, USA), flow rate 1 mL/min. The HPLC system was equilibrated with 95% solvent A for 5 min, followed by linear gradient of 5-45 (v/v) solvent B over 15 min to elute peptide and a 5 min re-equilibration.

SDS-PAGE analysis

Peptide profile from the 30 and 60 min hydrolysis was analyzed by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) by using precast gradient polyacrylamide gel

electrophoresis 4-20% (Any kD™ Mini-Protean® TGX gel, Bio-Rad Laboratories Inc.) with coomassie blue staining (Sigma-Aldrich, USA).

Hemolysis assay

Hemolysis assays were performed using experimental procedure described by Lorenzon et al. (2012) and Nguyen et al. (2011). Freshly prepared chicken red blood cells (RBCs) were washed three times with 0.01 M Tris-HCl (pH 7.4) containing 0.15 M NaCl (Tris-saline). A suspension of 1% (v/v) erythrocytes was made by re-suspending cells in Tris-saline. A 100 µl peptide (0.1 mg mL^{-1}) was added with 100 µl RBCs, incubated for 1h, at 37°C. The samples were centrifuged at 3000 *x* g for 5 min. A 100 µl of the supernatant was transferred to 96-well microplate and the absorbance was determined at 540 nm. The assay was performed in triplicate. A 1% Triton X-100 (Sigma-Aldrich, USA) solution was used as positive control (100% lysis) and Tris-saline as a negative control.

Peptide sequencing

Peptide samples were trypsin digested and extracted according to standard procedures (Bringans *et al.* 2007). Peptides were analyzed by electrospray ionisation mass spectrometry using the Agilent 1260 Infinity HPLC system [Agilent] coupled to an Agilent 6540 mass spectrometer [Agilent]. Tryptic peptides were loaded onto a C18 column 300 SB, 5 cm [Agilent] and separated with a linear gradient of water/acetonitrile/0.1% formic acid (v/v). Spectra were analysed to identify proteins of interest using Mascot sequence matching software [Matrix Science] with Ludwig NR database.

Peptide analysis

Calculation of theoretical properties and primary structure were conducted using primary structure analysis www.expasy.org and possibility to antimicrobial peptide was analyzed using http://aps.unmc.edu/AP/prediction/prediction_main.php , therapeutic index was analyzed using http://split4.pmfst.hr/split/dserv1/?akcija=run, peptides structure predicted using http://bioserv.rpbs.univ-paris-diderot.fr/PEP-FOLD/ (*Maupetit et al. 2009; Maupetit et al. 2010; Thevenet et al. 2012; Shen et al 2014) and* and RasWin Molecular Graphic (http//www.rasmol.org), therapeutic index was analyzed using http://split4.pmfst.hr/split/dserv1/?akcija=run (Juretic *et al.* 2009).

RESULTS AND DISCUSSION

Antibacterial activities

The peptides produced from horse milk protein hydrolysis were fractionated into fraction >30, 10-30, 3-10 and <3 kDa. The antibacterial activities of those fractions was shown in Table 1. The minimum bactericidal concentration (MBC) of the peptides before fractionation was much higher compared to after fractionation. Result revealed that fractionation based on the size was able to separate, concentrate the active peptides and improve the antibacterial properties. From all fractions, fraction <3 kDa has the highest antibacterial activity as it has the lowest MBC value, especially for *E. coli* and *S. Typhimurium*.

The result showed that minimum bactericidal concentration (MBC) fraction <3 kDa from the 30 and 60 min hydrolysis was similar, suggesting that 30 min was sufficient time to produce potent antibacterial peptide. Fraction <3 kDa in this study was better in killing Gram negative bacteria, *E.coli* and *S. typhimurium* than Gram positive bacteria, *S. aureus* and *L. monocytogenes*. Similar result reported by Kent *et al* (2012) who found that fraction <5 kDa yielding from casein hydrolyzed by *B. thuringiensis* protease contain peptide Caseicin A and Caseicin B which were active to Gram-negative bacterium, *Cronobacter sakazakii*.

Table 1. Minimum bactericidal concentration of peptide fractions

Indicator microorganisms	Minimum bactericidal concentration of peptide fractions (mg protein mL^{-1})									
	30 min hydrolysis					60 min hydrolysis				
	Before Fractio-nation	After fractionation				Before fractio-nation	After fractionation			
		>30 kDa	10-30 kDa	3-10 kDa	<3 kDa		>30 kDa	10-30 kDa	3-10 kDa	<3 kDa
S. aureus	>10	>0.14	>0.14	>0.14	>0.14	>10	>0.14	>0.14	>0.14	>0.14
L. monocytogenes	10	0.14	0.07	0.14	0.14	10	0.14	0.14	0.14	0.14
E. coli	10	0.14	0.07	0.14	0.035	5	0.14	0.07	0.14	0.035
S. typhimurium	10	0.07	0.07	0.14	0.035	10	0.14	0.07	0.14	0.035

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Antioxidant activities

Two antioxidant assays used in this study were 2,2-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) and 2,2-diphenyl-1-picrylhydrazyl (DPPH). Scavenging activities of the peptides were determined by reduction of ABTS or DPPH absorbance after addition of peptide. Under the assay condition, 100% of scavenging activity correspond to complete scavenging of ABTS or DPPH radical. Scavenging activities to ABTS and DPPH radicals varied among the peptide fractions (Figure 1). Generally, peptide from the 30 minutes hydrolysis scavenged the ABTS radical better than the 60 min hydrolysis. Peptides from the 30 min hydrolysis, fraction <3 kDa, had the best antibacterial activity and also high scavenging activity (82%), almost similar to the scavenging activity of 12.5 µg mL^{-1} vitamin C (84%). As in antibacterial assay, fractionation increased scavenging activities to the ABTS radicals. However, it seems that size might not be important factor for the activities. For example, fraction 10-30 kDa for 30 and 60 min hydrolysis scavenged ABTS radical better than fraction 3-10 kDa.

For DPPH assay, the highest scavenging activity was shown by fraction <3 kDa from the 60 min hydrolysis (57%) (Fig. 1) almost similar to 14 µg mL^{-1} vitamin C (58%). Fraction 10-30 kDa from 60 min hydrolysis which has high activity in ABTS assay was only able to scavenge DPPH radical by 49%. The fraction whose good antibacterial and scavenging ABTS radical activity, fraction <3 kDa from the 30 min hydrolysis, scavenged DPPH radical by 22%. Different from that of ABTS assay, in DPPH assay, peptide from the 60 min hydrolysis resulted in better scavenging activity compared to that from 30 min hydrolysis.

The radical scavenging activities were assessed with the lipid soluble DPPH radical as well as the water soluble ABTS radical (Damgaard et al. 2014). The ABTS or DPPH assay is based on the ability of antioxidant to transfer electron or donate hydrogen atom to the radicals which result in a color change and reduce the absorbance. Generally, good scavenging activity to ABTS radical was obtained from the 30 min

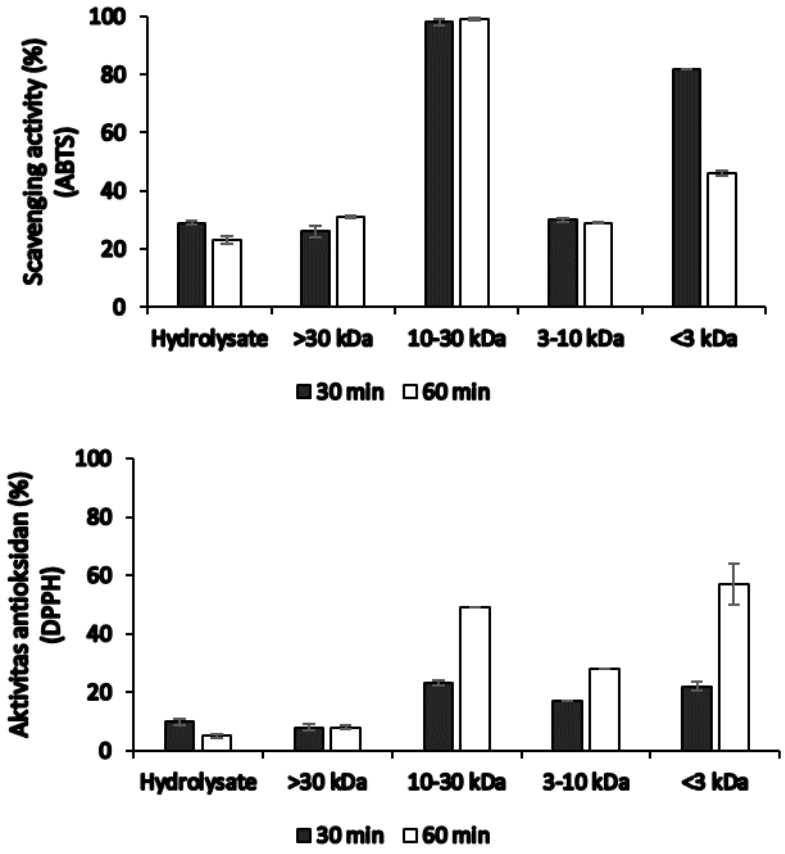

Figure 1. Scavenging activities of peptide before and after fractionation using membrane molecular cut off 30, 10 and 3 kDa against ABTS and DPPH radicals per µg protein mL^{-1}. Value represent the mean ± standard deviation (SD).

hydrolysis peptide, while that of DPPH was obtained from the 60 min hydrolysis peptide. The result indicated that the time of hydrolysis is determinant to scavenging activities. Amza et al. (2013) reported that hydrolysis of gingerbread plum (*Neocarya macrophylla*) more than 30 min decreased scavenging activity to DPPH radicals. Hydrolysis of ovine milk using protease *Bacillus sp.* P7 showed that antioxidant activity measured by ABTS method increased up to 2 h hydrolysis and stable for up to 4 h. (Correa et al. 2011). In addition, enzyme substrate ratio must also take into consideration along with hydrolysis time which determine hydrophobic and aromatic amino acid content in the peptide sequence produced which will affect the antioxidant capacity (Gao et al. 2014).

The result of ABTS and DPPH assay showed that scavenging activity and their mechanism were slightly different due to the difference of sensitivity and mechanism of action of ABTS and DPPH assay. DPPH was performed in organic solvent, thus is more suitable for lipophilic antioxidant compound whereas ABTS is compatible with both aqueous and organic system (Prior et al. 2005). It is possible that peptide hydrolysate generated from the 60 min hydrolysis contain more lipophilic peptides than the peptides from 30 min hydrolysis.

Hemolysis activities

Hemolysis assay is a simple method to evaluate possible toxicity of the active peptide to eukaryote cells. In spite of the many promising bioactive peptide, some may be cytotoxic to eukaryotic cells which hampered the peptide application. Expectation for antibacterial peptide application is that the peptide is able to kill pathogenic bacteria but does not harm eukaryotic cell. In this study, fraction 10-30 kDa had sufficient antibacterial and antioxidant activities but high in their hemolysis activity (17.21 and 17.28%) (Figure 2). Fraction <3 kDa which possess high antibacterial and antioxidant activities peptide demonstrated low hemolysis activity (2.27 and 2.26%) which indicate safety application. Thus, fraction <3 kDa was more promising as this fraction is highest in antibacterial activity and good antioxidant activity, but low in hemolysis activity.

Peptide profiling in RP-HPLC

Profiling of the fraction <3 kDa using RP-HPLC resulted in the chromatogram with major peaks as shown in Figure 3 at the hydrophobic condition. Peptide with retention time (R_t) 9.5 and 10 min were more hydrophobic as they were eluted in the hydrophobic mobile phase. Kim et al. (2005) demonstrated that hydrophobicity as indicated by the retention time is better related to the antimicrobial activity than hydrophobic calculation from the sequence analysis.

Based on the HPLC chromatogram, peptides from fraction <3 kDa appeared as a mixture of hydrophilic and hydrophobic molecules. Hydrophobicity and retention time in HPLC chromatogram of peptide may correlate with antimicrobial peptide activity. Nielsen et al. (2007) found that the compound with longest HPLC-retention time showed best minimum inhibitory concentration (MIC). They also reported that HPLC retention time is a better parameter for antibacterial activity than hydrophobicity and hydrophobic moment alone. Peptides with higher hydrophobicity on their nonpolar face generally showed stronger self-associating ability in solution, since dimers can be formed by interactions between the nonpolar faces of two molecules (Chen et al. 2007). Peptide with less hydrophobic in HPLC data has a weaker tendency to aggregate in aqueous solution and that it has lower propensity for interaction with hydrophobic surface than hydrophobic peptide (Abraham et al. 2014). Hydrophobicity of the peptides as indicates by HLPC retention time data also correlated well the activity

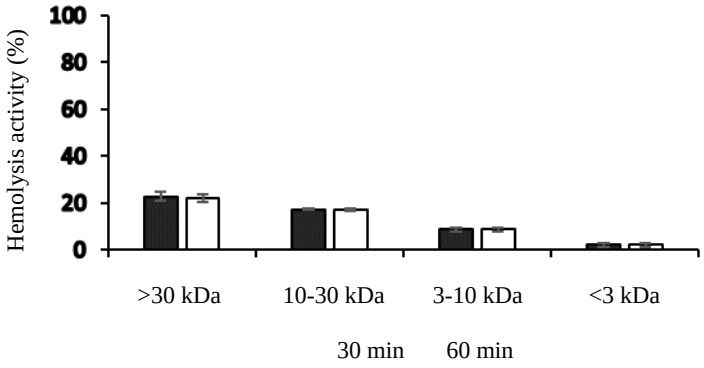

Figure 2. Hemolysis activities of peptide (0.1 mg protein mL^{-1}) before and after fractionation using membrane molecular cut off 30, 10 and 3 kDa. Value represent the mean ± standard deviation (SD).

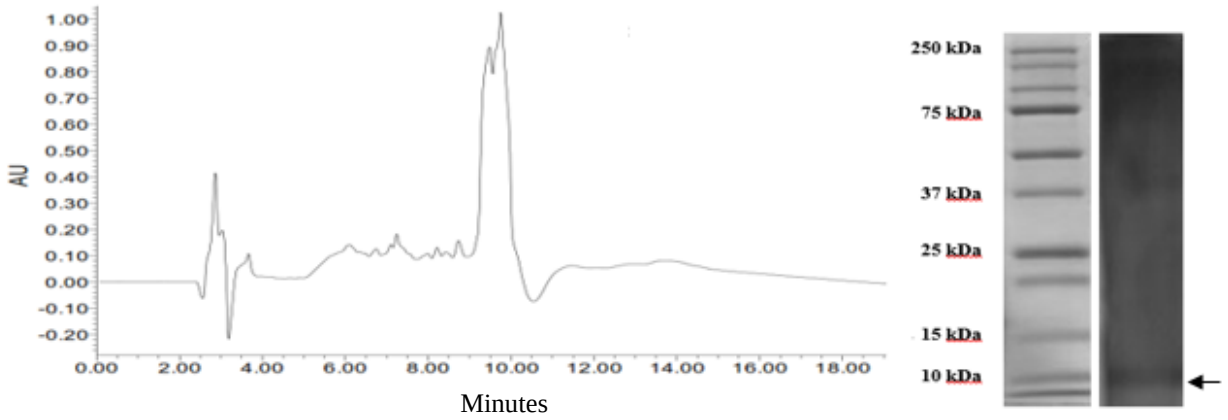

Figure 3. Profile of peptide fraction <3 kDa in HPLC chromatogram and SDS PAGE.

against artificial membrane and Gram positive bacterial species such as *Staphylococcus aureus*, *Staphylococcus epidermidis* and *Micrococcus luteus* rather than mean hydrophobicity that obtained from simple calculation of hydrophobicity of each constituent amino acid. Even though there are cases where antimicrobial activity against Gram negative bacteria such as *Escherichia coli* did not show correlation with analysis of HPLC retention time (Kim *et al.* 2005).

The fraction <3 kDa was detected in higher molecular weight in electrophoresis gel (around 10 kDa) (Fig 3), the peptides may form oligomer. Banerjee *et al.* (2015) reported that oligomer form of antibacterial peptide purified from hearth of *Capra hircus* was detected in electrophoresis gel. Some of antimicrobial peptides are able to form oligomer or supramolecular in the presence or without sodium dodecyl sulfate. Antimicrobial peptides generally adopted disordered structures and the presence of proline affect the supramolecular arrangement in peptide solution (Vermeer *et al.* 2012), beside possibility to form self-aggregation and protein or peptide association (Monteiro *et al.* 2015).

Oligomerization of antimicrobial peptide in lipid membrane are vital for their mode of action. Monomer or oligomer form of the bioactive peptide was reported of capable to affect the way of peptide insert to surface of target cells. Saravan and Bhattacharjya (2011) demonstrated that replacement of Tyr to Ala showed lack of oligomerization and reduced antibacterial activity of peptide VK22AA, a peptide derived from fowlicidin-1 and cathelicidin, a family of antimicrobial peptide from chicken. The tetrameric structure was stable and embedded into zwitterionic phosphocholine micelles such that the non-polar region is located inside the lipid acyl chains while the monomer form is localized at the surface of the micelles. Bai *et al.* (2012) reported that branched peptides (oligomer) have been shown considerable advantages over their monomeric form such as improved antimicrobial activity, maintaining high efficacy under physiological condition, enhanced bacterial surface binding affinity, decreased susceptibility to proteolytic degradation and low toxicity. The oligomer form, in case of protegrin antimicrobial peptide, increased stability and antimicrobial activity as well as cytotoxicity. Protegrin monomer interacts very weakly with the surface of zwitterionic membranes but absorbed well on the surface of anionic membrane, but oligomer could insert forming pores into both anionic and zwitterionic mamalian membrane (Lazaridis *et al.* 2013).

This phenomenon of higher molecular weight in electrophoresis gel may also explain the presence of anomalous SDS-PAGE migration of peptide due to peptide-peptide interaction and peptide-SDS interaction. Rath *et al.* (2009) found that hydropathy and helicity were able to cause gel shifting that produce slower migration which should not be considered as indicator of oligomeric state, compactness and degree of foldedness.

Peptides analysis

After tryptic digestion, analysis using Mascot software revealed that the peptides fraction composed of 13 tryptic peptides with molecular mass calculation of 921.48 – 1954.95 Da (Table 2).

The hydrophobicity of the peptides is 11.26-17.98 kcal /mol. Grau-Campistany et al. (2015) found that formation of transmembrane pores is only possible under the condition of hydrophobic matching: the peptide have to be a long enough to span the hydrophobic bilayer core to be able to induce vesicle leakage and kill the bacteria. Rosenfeld *et al.* (2010) reported that increasing the ratio between

Table 2. Physio-chemical properties of the possible peptide from fraction <3 kDa

Peptide identified	Length	Mass (Da)	PI	Net charge	Hidrophobicity (kcal/mol)	Therapeutic index
LVNELTEFAK	10	1162.62	4.09	-1	14.89	**28.69**
YLYEIAR	7	926.48	6.48	0	10.50	27.43
HPYFYAPELLYYANK	15	1887.92	7.47	0	11.74	**64.75**
AEFVEVTK	8	921.48	4.09	-1	16.08	6.40
DAIPENLPPLTADFAEDK	18	1954.95	3.39	-4	26.57	6.60
DAFLGSFLYEYSR	13	1566.73	4	-1	12.21	10.57
RHPEYAVSVLLR	12	1438.80	9.53	+1	14.45	5.53
HPEYAVSVLLR	11	1282.70	7.50	0	12.64	6.28
HLVDEPQNLIK	11	1306.71	5.19	-1	17.98	9.58
LGEYGFQNALIVR	13	1478.79	6.49	0	11.26	8.36
KVPQVSTPTLVEVSR	15	1638.93	9.77	+1	15.52	5.90
VPQVSTPTLVEVSR	14	1510.83	6.51	0	12.72	8.14
KQTALVELLK	10	1141.70	9.63	+1	14.44	7.33

hydrophobicity and the net positive charge increased both antimicrobial and bacterial lipopolysaccharide neutralization activities. Whereas antimicrobial activity increases linearly with the increase in the peptides hydrophobicity, peptides with different hydrophobicity are endowed with similar LPS neutralizing activities. Hydrophobicity side chain of peptide determined optimization of membrane selectivity or antibacterial potency (Henriksen et al. 2014). Bahnsen et al. (2013) added that charge and hydrophobicity alone cannot account for antimicrobial and cytotoxicity activities, but also influenced by conformational and secondary structure of peptide. It is important to maintain certain content of amphipathic secondary structure for a desirable biological activity (Mai et al. 2015).

Fraction <3 kDa from the 30 min hydrolysis were dominated by neutral and negatively charge peptides. Although antimicrobial peptide is commonly being cationic in nature, a number of anionic antimicrobial peptides have been reported, serving as important weapons in eukaryote innate immune system. Similar to their cationic counterpart, anionic antimicrobial peptides can adopt varying amphiphilic structure such α-helix and β-sheet conformations with interaction with the membrane key to activity (Laverty et al. 2011).

Beside hydrophobicity and peptide charge, amino acid composition of a peptides affects antimicrobial activity. Bahnsen et al. (2013) revealed that Arg in the peptide sequence is important as a presence of multiple guanidium groups facilitated both uptake and enhances antimicrobial activity. Amino acid composition also related to physicochemical properties of the peptide that would affect the activity. Change amino acid composition from GS10 [cyclo-(VKLdYPVKLdYP)], a synthetic analog of the naturally occurring antimicrobial peptide gramicidin (GS) in which the two positively charged ornithine (Orn) residues are replaced by two positively charged lysine (Lys) residues and the two less polar aromatic phenylalanine (Phe) residues are replaced by the more polar tyrosine (Tyr) residues, increased aqueous solubility and changed physicochemical properties and its capacity with both model and biological membrane (Abraham et al. 2014).

The peptide with the highest therapeutic index (TI) prediction to *E. coli* in our study, is HPYFYAPELLYYANK with TI=64.75. Although the charge of peptide HPYFYAPELLYYANK was neutral, the TI prediction was higher than previous report positively charges antimicrobial peptides, Casecidin 15, YQEPVLGPVRGPFPI with TI: 9.17 and Casecidin 17, YQEPVLGPVRGPFPIIV with TI: 11.98. Both casecidin 15 and Casecidin 17 have MIC value to *E. coli* DPC6053, 0.4 mg mL-1. TI is calculated from ratio 50% hemolytic activity (HC50) to minimum inhibitory concentration (MIC). Unfortunately, the information about hemolytic activity of both Casecidin 15 and Casecidin 17 are not available. More comprehensive data was revealed by peptide Piscidin and its substitution of hydrophobic amino acid. D-Piscidin 1 (FFHHIFRGIVHVGKTIHRLVTG) with TI prediction to *E. coli* 7.54 showed experimental hemolytic activity (HC50) and MIC were 1.8 µM and 2.8 µM respectively with TI to Acinetobacter baumannii was 0.6. Replacement of I at 9th amino acid to K (FFHHIFRGKVHVGKTIHRLVTG) with TI prediction to *E. coli* 9.44 showed experimental hemolytic activity

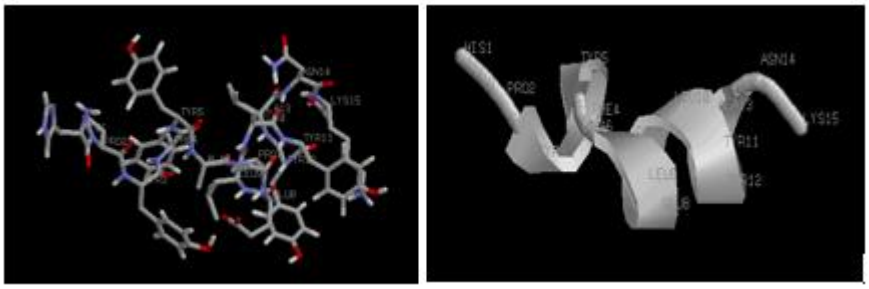

Figure 4. Predicted structure of the peptide from fraction of <3 kDa with high therapeutic index (TI), peptide HPYFYAPELLYYANK, TI: 64.75.

(HC50) and MIC were 98 µM and 3.0 µM respectively and TI to *Acinetobacter baumannii* was 33 (Jiang et al. 2014). Case of Piscidin showed that TI prediction was sufficiently accurate for antimicrobial activities prediction. Although we have not synthesized and examined the activity of peptide HPYFYAPELLYYANK alone, the peptide is promising to be antimicrobial peptide as TI prediction was 64.75 and higher than other reported peptides.

Although peptide HPYFYAPELLYYANK showed high in TI prediction but the peptide has not been confirmed as antibacterial activity determinant in the fraction <3 kDa. Interaction among peptide in the fraction may decrease or enhance the antibacterial activity. Hayes et al. (2006) reported that antimicrobial activity was not detected in casein fermented by *Lactobacillus acidophilus* before fractionation. Electrostatic interaction between charged peptides may have a negative effect to antimicrobial activity of the fermentate. On the contrary, Rahnamaeian et al. (2015) demonstrated that combination of two antimicrobial peptides from bumblebee, hymenoptaecin and abaecin resulting in a more potent effect at low concentration.

Peptide structure prediction

Structure prediction of the peptide HPYFYAPELLYYANK with high therapeutic index was in Figure 4. Structure prediction showed that peptide peptide HPYFYAPELLYYANK is able to form helix that important for antimicrobial activity (Fig. 4). Multiple studies demonstrated that helicity is important for toxicity, antimicrobial activity (Huang et al 2010) and interfacial binding (Fernandez-Vidal, 2007) beside physicochemical parameter such as hydrophobicity amphipaticity, charge and sequence.

CONCLUSION

B. thuringiensis that commonly contaminate milk was a source of protease enzyme which can be used to produce antibacterial and antioxidant peptide from horse milk. Time of hydrolysis, separation and processes are important in generating the desirable bioactive peptide. Considering safety of the peptide, sequentially filtrations were needed to obtain high activity and safe peptide. Fraction <3 kDa from the 30 min hydrolysis was safe fraction with good in antibacterial and antioxidant activities. The fraction consists of 13 peptides variety in physicochemical properties. Peptide HPYFYAPELLYYANK has the highest therapeutic index and potent to be antimicrobial peptide.

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Pengujian efektifitas enzim BS4 terhadap performan ayam petelur yang diberi jenis bahan pakan yang berbeda (Effectivity of BS4 enzyme complex on the performance of laying hens fed with different ingredients)

(Org: Eng)

JITV 21(1): 1-8

An experiment was carried out to evaluate the effectivity of an enzyme complex produced by Eupenicilium javanicum BS4 on the performance of laying hens fed with different feed ingredients. Three diets were formulated with similar nutrients (protein, ME, digestible amino acids, Ca and available P) value to meet the nutrient requirement of laying hens. Diets were formulated based on maize, palm kernel cake (PKC) or rice bran. The diets were either supplemented or not with BS4 enzyme complex and fed to Isa Brown pullet from 19 to 37 weeks old. Each diet was fed to 24 birds and the performances were recorded. Data were analysed statistically with 2 X 3 factorial design with six replicates. Results showed that supplementation of BS4 enzyme into laying hens diet did not influence body weight change, feed intake, mortalities, egg weight and egg shell thickness but significantly increased egg production ($P<0.05$) and the FCR ($P<0.01$) in any feedstuff tested. It also improved egg yolk color score on diet contained 20% PKC. Laying hens fed 20% PKC produced more eggs than those fed maize or rice bran. The egg shell of laying hens fed rice bran were thicker than those fed with maize or PKC. It is concluded that BS4 enzyme complex is effective when supplemented into laying hens diet.

(Author)

Key Words: BS4 Enzyme, Palm Kernel Cake, Rice Bran, Egg Production, Egg Quality

UDC: 633.3

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Kecernaan protein *in vitro* dan fermentabilitas pakan campuran hijauan murbei dan leucaena (*In Vitro* protein digestibility and fermentability of mulberry (*Morus alba*)-Leucaena foliage mixed feed)

(Org: Eng)

JITV 21(1): 9-18

This experiment was carried out to determine the effect of mulberry-leucaena foliage mixed feed on protein digestibility and VFA production using an in vitro gas production study. Mulberry was mixed with one of 2 leucaena varieties (*Leucaena leucocephala* hybrid and *Leucaena leucocephala* local) at 3 levels (0, 25 and 50%). Study was conducted in completely randomized design. Mulberry foliage, leucaena and mixtures of mulberry-leucaena were incubated for 24 hours in glass syringes. Parameter recorded were gas production, in vitro true dry matter digestibility (IVTDMD), in vitro N digestibility (IVND) and VFA production. Results of the study showed that supplementation of leucaena to mulberry decreased IVOMD, gas production and protein digestibility in the rumen buffered medium. The mixture of mulberry and leucaena hybrid at ratio 50% resulted in the lowest IVND than other treatment. However it increased protein digestibility in acid pepsin incubation as an estimate of protein availability in intestine. Gas production decreased in mulberry leucaena mixtures was followed by decreased total VFA production. Tannin derived from leucaena hybrid supplementation to mulberry at ratio 1 : 1 was most effective to decrease protein digestion in the rumen and to increase protein digestibility in acid pepsin incubation. In conclusion mixing of hybrid leucaena hybrid with mulberry foliage was able to protect protein degradation from mulberry in the rumen.

(Author)

Key Words: Mulberry, Leucaena, Protein Digestibility, *In Vitro*

UDC: 663.142

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Fermentasi anaerobik efektif menurunkan konsentrasi total tanin pada tumbuhan semak bunga putih (*Chromolaena odorata*) (Anaerobic fermentation effectively reduces concentration of total tannins in *Chromolaena odorata*)

(Org: Eng)

JITV 21(1): 19-25

Chromolaena odorata is a potential feed source but its usage is hampered by presence of various secondary metabolic compounds in plant's tissues. One group of them is tannin. This experiment was aimed to evaluate various pre-treatment methods on total tannin concentration and in vitro digestibility of dry- and organic-matter. An 8 x 3 completely randomized experimental design was employed to test 8 different treatments. The treatments were: Fresh = freshly-chopped chromolaena leaves as control, Sun-dried = sun-dried (3x 24 hours) chromolaena leaves, Oven-dried = oven-dried (60ºC for 24 hours) chromolaena leaves, Boiled = water-boiled (5 minutes) chromolaena leaves, RenWater = water-soaked (4 hours) chromolaena leaves, RenNaOH = NaOH-soaked (4 hours) chromolaena leaves, RenHCl = HCl-soaked (4 hours) chromolaena leaves, and Fermented = anaerobically-fermented (21 days) chromolaena leaves. Parameters measured were concentration of total tannins and nutrient content. The results showed that application of low heat (Sun-dried), hot water (Boiled), water soaking (RenWater), or unaeobic fermentation technique significantly reduced total tannin by 43% into 62% compared to control. The highest suppression (62%) was achieved by Fermented treatment. In the contrary, medium heat application (oven-dried at 60°C) or chemicaltreatments (HCl or NaOH) had no effect. Protein content of chromolaena was improved by 60% and crude fiber was reduced by 32% in Fermentaed treatment compared to the control. It could be concluded that anaerobic fermentation can be used an effective strategy to reduce tannin concentration in Chromolaena odorata without affecting its feeding value.

(Author)

Key Words: *Chromolaena odorata*, Tannins, Digestibility, NH_3, VFA

UDC: 599.735.51

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Dinamika folikel dan repeatabilitas pertumbuhan gelombang folikel pada sapi peranakan ongole (PO) (Follicular dynamic and repeatability of follicular wave development in Peranakan Ongole (PO) cattle)

(Org: Eng)

JITV 21(1): 26-33

Superovulation treatment on PO cattle (Bos indicus) was less responsive compared to Bos taurus breed. It might due to the difference of their follicular dynamic. This study was conducted to investigate the follicular dynamics and its repeatability in PO cattle. Follicular dynamics observations conducted on 9 cows trough ultrasound scanning every day. Observations of wave patterns repeatability were performed in 6 cows which its wave pattern already known on the next consecutive IOI. Research result indicated that PO cattle had 3 (66%) and 4-waves (34%) pattern. The first wave of 3 and 4-waves pattern emerged on day -0.4+0.9 and 1.4+1.1 respectively. The second wave of 3 and 4-wave pattern emerged on day 9.8+1.5 and 7.4+1.9 respectively. The pattern of 3 waves has a longer follicle dominant duration (11.6+1.5 day) in the first wave of estrous cycle, compared with 4 waves pattern (10+2.92 and 7+1.00 day respectively). The growth rate of dominant follicle was not different significantly between the 3 and 4-waves pattern (0.87+0.23 and 0.94+0.25 mm/day respectively). Similarly, ovulatory follicle diameter between 3 and 4-waves pattern was also not different significantly (12.24+12.34 and 12.30+12.23 mm respectively). Observation of wave patterns repeatability in 6 PO cows indicated that PO cattle had high repeatability in follicular wave pattern (0.88) and the number of growing follicle was 0.91. This study resulted data for dynamic of follicular development, wave pattern, its repeatability which be expected to design the protocol of superovulation treatment or other reproduction technologies based on follicular dynamic to improve its result in PO cattle.

(Author)

Key Words: PO Cattle, Follicular Wave, Repeatability, Interovulatory Interval

UDC: 636.2.034

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Nanopartikel chitosan hormon hCG (*Human Chorionic Gonadotrophin*) dalam meningkatkan induksi ovulasi pada sapi perah (Chitosan nanoparticle of hCG (Human Chorionic Gonadotrophin) hormone in increasing induction of dairy cattle ovulation*)*

(Org: Eng)

JITV 21(1): 34-40

degradation of hCG hormone in the blood, to avoid the use of multiple injections for ovulation induction and to enhance reproductive efficacy. This study aimed to prepare chitosan nanoparticles hCG (CS-NPh) and to determine its efficacy as nasal spray of CS-NPh. The observed parameters include physico-chemical characteristics of CS-NPh and the follicle size, corpus luteum, the time of ovulation and onset of estrus performed after administration of CS-NPh as a nasal spray compared with intramuscular hCG (control) at a dose of 1,000 IU in dairy cattles. The result showed that the formation of the hormone hCG nanoparticles is still in the size range of nanoparticles with a well and more stable molecular mass distribution, so it can be used as a carrier component of hormones. The result showed that the time of ovulation after hCG by intramuscular (day to 3.13±0.35) and CS-NPh as a nasal spray (days to 3.33±0.49) with the follicle size by 1.62±0.22 and 1.76±0.28 cm showed no significant

differences (p> 0.05), likewise the size of the corpus luteum and onset of oestrus. This indicates that administration of CS-NPh as a nasal spray can be used in enhancing the induction of ovulation in dairy cattles.

(Author)

Key Words: Nanoparticles, hCG, Nasal Spray, Ovulation

UDC: 636.2.033

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Pohon filogenetik sapi Kuantan menggunakan DNA barcode (Phylogenetic tree of Kuantan cattle by DNA barcoding)

(Org: Eng)

JITV 21(1): 41-48

Kuantan cattle is one of local beef cattle breed of Riau Province which its origin was unknown. Kuantan cattle are commonly found in Indragiri Hulu and Kuantan Singingi Regency. Based on phenotype characterizations, kuantan cattles are similar with pesisir cattle (West Sumatera beef cattle). Historically, kuantan cattle were pesisir cattle brought by "minang" immigrants (Immigrant from West Sumatera) to this region. The purpose of this study was to analyze the origin of the kuantan cattle through genetic diversity analysis using DNA barcode. DNA barcode used was Cytochrome oxidase subunit I gene which was found in the mtDNA. DNA isolation was done on 25 kuantan's blood samples and 18 pesisir blood samples. Amplification of COI gene segment used Polymerase Chain Reaction technique. The forward primer sequence used in this study was F'5 TTCTCAACCAACCATAAAGATATTGG-3' and the reverse primer sequence used was reverse 5'-TAGACTTCGGGGTGTCCAAAGAATCA-3. It squeezed kuantan and pesisir sequence 5711 - 6420 base (GeneBank accession number NC_005971) with length by 710 bp. Analysis result of sequence using MEGA 5.2 Program showed that there were 6 polymorphic sites establishing 7 haplotypes on kuantan cattle and 9 polymorphic sites establishing 12 haplotypes on pesisir cattle. Based on genetic distance and phylogeney tree, kuantan and pesisir cattle were in same group with *Bos indicus*. Mutation in the COI gene segment in this study was too small and was not able to distinguish the difference of those breeds. The result of neighbor joining analyze indicated that kuantan cattle origin was from *Bos indicus* just like pesisir cattle.

(Author)

Key Words: COI Gene, Polymorphic, Kuantan Cattle, Genetic Distance, Phylogenetic Tree

UDC: 636.39

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Hubungan jenis pengencer dan sistem pengemasan terhadap lama simpan dan kualitas semen cair kambing Boer (Relationship of extender and packaging system an the length of preservation and the quality of chilled semen of Boer goat)

(Org: Eng)

JITV 21(1): 49-54

The aim of this research was to compare the effectiveness of different extender (either Triladyl or Tris Egg Yolk extender) and different packaging method (pool and straw) of chilled semen an the length of preservation and the quality of chilled semen of Boer goat. Semen was collected using an artificial vagina from 3 two years old Boer bucks with body weight of 50-55 kg. It was evaluated under a microscope, then each was diluted either in Tris egg yolk extender (TEY) or Triladyl. Those diluted sperms were then packed either in pool or straw and preserved at 5^0C refrigerator. Sperm motility, viability and membrane integrity of each group were evaluated every 24 h for up to 5 days. Results showed that sperm motility in Triladyl of pool packaging system up to 3 days was higher than straw packaging system or TEY in pool or straw packaging system which were 45.8%, 26.1%, 32.1% and 9.1%, respectively (P<0.05). Percentage of sperm membrane integrity showed the same pattern to Triladyl both in pool and straw packaging system which was higher than TEY group (75.2% and 77,2%; P<0.05). Sperm viability in Triladyl both in pool or straw packaging system decreased (P<0.05) after 3 days of preservation (77.1% and 76.2%) but TEY significanly decreased after 4 days of preservation either in pool or straw packaging system (73.2% and 58.0%; P<0.05). It was concluded that sperm quality decreased with increasing of the length of preservation while Triladyl extender in pool packaging system showed the best quality.

(Author)

Key Words: Chilled Semen, Boer, Triladyl, Tris Egg Yolk, Straw

UDC: 636.58

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Penentuan kapasitas produksi *primordial germ cell* sirkulasi (PGC-sirkulasi) pada ayam KUB menggunakan metode buffer lisis *ammonium chloride potassium* (ACK) (Determination of production capacity of circulated primordial germ cells (circulated-PGCs) of KUB chicken using lysis buffer ammonium chloride potassium (ACK))

(Org: Eng)

JITV 21(1): 55-61

In poultry embryos, primordial germ cells (PGCs) are progenitor cells for gametes, which have unique migration pathway. Primordial germ cells arise from epiblast in germinal crescent and circulate through the bloodstream for a short period of time, then leave blood vessel to migrate toward gonads. The aim of this study was to determine the potential production capacity of circulated-PGCs of KUB chicken at different developmental stages of embryo using a rapid and simple method. Seventy five KUB chicken fertile eggs were divided into five groups and incubated at 38.5 0C with a humidity of 60%. Hatching was set to the embryonic development stage of 14-18. The blood was collected through dorsal aorta using micropipette under microscope. The collected blood was placed in a 1.5 ml eppendorf tube which was previously filled with 100 µl phosphate buffered saline without Ca2+ and Mg2+ (PBS-) mixed with fetal bovine serum (FBS) with a ratio of 90%:10%. The PGCs were purified using lysis buffer ammonium chloride potassium method. The results showed that average production of circulated-PGCs per embryo of KUB chicken were significantly affected by stage of embryonic development ($P<0.05$). The average production of circulated-PGCs at stage 14, 15, 16, 17, and 18 were 37.9; 53.5; 49.8; 38.3; and 33.5 respectively. The number of circulated-PGCs was not different among stages 14, 17 nor 18. The highest number of circulated-PGCs of KUB chicken was obtained at stage 15, so that the isolation and collection of PGCs through the blood circulation was recommended in stage 15.

(Author)

Key Words: KUB Chicken, PGCs, Embryonic Development Stage, Ammonium Chloride Potassium

UDC: 57.083.2

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Potensi tes antigen dan tes serologi yang didasarkan pada epitope linier CNTKCQTP pada haemagglutinin H5N1 untuk Avian Influenza (Potency of antigenic and serologic tests based on CNTKCQTP linear epitope on H5N1 haemagglutinin for Avian Influenza)

(Org: Eng)

JITV 21(1): 62-72

Rapid diagnostic tools or point-of-care (POC) test is needed in the effort to control and eradicate the high pathogenic avian influenza (HPAI) H5N1 in Indonesia. Accuracy of a POC test is determined by the specificity of antibodies, which is the main component of a POC test. Recently a linear epitope, CNTCKQTP epitope, located at 274-281 amino acid residue of H5 hemagglutinin has been confirmed to be present all clade of H5N1 viruses. This study aimed at producing and evaluating the reactivity of a monospecific, polyclonal antibody against the epitope. The Antibody was produced by immunizing a goat with the peptide in the form of multiple antigen peptide (MAP). The specificity of the antibody was estimated by assaying its reactivity against influenza virus subtypes H3N3, H4N4, H5N1, H6N5, H7N7, H9N2, H10N7 and H11N9; and recombinant hemagglutinins H1-H12, H14 and H15 with ELISA and immunoblot. The results of the assay showed that CNTKCQTP antibody was not specific for H5 haemagglitinin because it cross-reacted with other haemagglutinins especially H7, H8 and H9. The potential of the peptide containing the epitope, GNCNTKCQTPMGAINSS. as an ELISA reagent for assaying H5 antibodies in chickens previously vaccinated and challenged with the H5N1 virus was also evaluated in this study. In contrast the results of previous studies, the ELISA using GNCNTKCQTPMGAINSS as coating antigen was not sensitive in detecting antibody to haemagglutinin H5 in chickens.

(Author)

Key Words: AI Virus, Hemagglutinin H5, CNTKCQTP Epitope, MAP, Immunoassay

UDC: 636.32/.38

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Estimasi komponen varian dan nilai pemuliaan kriteria pertumbuhan pra-sapih pada domba Romney (Estimated variance components and breeding values for pre-weaning growth criteria in Romney sheep)

(Org: Eng)

JITV 21(2): 73-82

This study was carried out to investigate variance components, direct heritability, maternal genetic parameters, estimated breeding values (EBV) and factors affecting pre-weaning growth criteria of Romney sheep. Data were collected over the period from 2006 to 2012 with records of 4989 lambs descended from 76 rams and 2190 ewes of Romney sheep maintained at S. Island of New Zealand via Gene Marker Lab., Faculty of Agric. and Life Sci., Lincoln Univ., New Zealand. Results proved that genetic and non-genetic factors affecting studied criteria had significant effects ($P<0.05$). Genetic and environmental estimates of live body weights at birth (LBW), weaning (LWW) and Kleiber ratio (KR) were 0.20±0.074, 0.15±0.042 and 0.14±0.052 for direct heritability (h2a±SE); 0.59±0.219, 0.41±0.023 and 0.08±0.002 for maternal heritability (h2m±SE); 0.11684, 2.6378 and 0.27565 for additive variances (σ2a), 0.34596, 7.1179 and 0.14532 for maternal variances (σ2m); and 0.002395, 10.1262 and 0.509339 for permanent environmental variances (σ2e), respectively. EBV's of LBW, LWW and KR ranged from -0.555: 0.502, -1.554: 3.006 and -0.633: 0.242 direct, -0.863: 0.954, -4.942: 2.554 and -0.469: 0.179 maternal for rams, respectively; and -0.664: 0.830, -2.996: 4.586 and -1.651: 0.677 direct, 1.429: 1.142, -7.541: 4.920 and -1.223: 0.492 maternal for ewes, respectively. Results suggest the importance of considering the non-genetic factors in pre-weaning growth performance of lambs.

Moderate heritability and positive coefficients of phenotypic and genetic correlation for studied criteria indicate to the possibility of improving them using traditional selection.

(Author)

Key Words: Romney Sheep, Genetic Factors, Non-Genetic Factors, Heritability, Pre-Weaning Growth, EBV

UDC: 636.2

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Karakteristik rumen fermentasi secara in vitro dan mikroba domba ekor tipis yang diberikan biomassa ubi jalar (*In vitro* rumen fermentation characteristics and microbes of thin tail sheep given sweet potato biomass)

(Org: Eng)

JITV 21(2): 83-87

Sweet potato plant (Ipomoea batatas L) is produced in Indonesia in large quantities. Sweet potato leaves and stems can be used as a source of protein and the tuber can be used as an energy source. This study was aimed to analyze the effect of feeding of sweet potato biomass on the rumen fermentation characteristics, digestibility of dry matter and organic matter as well as rumen microbial population of thin tail sheep. This study used a randomized block design by applying four feed treatments, i.e., T0 (100% Napier grass), T1 (70% of Napier grass + 30% concentrate), T2 (50% of Napier grass + 50% sweet potato leaves), and T3 (70% sweet potato leaves + 30% sweet potato tuber) with three replicates. Rumen fluid samples were taken with stomach tube method using a vacuum pump. Results showed that the T3 diet has higher (P<0.05) digestibility of dry matter and organic matter, concentration of NH3 and VFA, and rumen bacterial population than those of T0 and T1diets but similar to that of T2 diet. Rumen pH and protozoa population was not different among the treatments. It can be concluded that the use of sweet potato biomass can improve the quality of rumen fermentation of sheep.

(Author)

Key Words: Rumen, Digestibility, Fermentation, Sheep, Sweet Potato Biomass

UDC: 636.293.2

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Pengaruh dari perbedaan tingkatan protein dan energi pakan konsentrat terhadap asupan nutrisi dan produksi susu pada kambing Saanen x Etawah silangan (Effect of different protein and energy levels in concentrate diets on nutrient intake and milk yield of Saanen x Etawah Grade goats)

(Org: Eng)

JITV 21(2): 88-95

Dairy goat contributes to food and nutrition security. However, information on nutrient consumption and milk yield, as well as milk composition of Saanen x Etawah (SAPERA) grade goat is limited. This experiment was done to evaluated nutrient intake, milk yield and its composition of lactating SAPERA goats fed with different levels of dietary energy and protein in concentrate diet. Thirty multiparous SAPERA goats were used in a randomized block design with three treatments (R1, R2 and R3) and ten replications for 12 weeks of lactation. The concentrate diets were formulated to contain: 18% CP and 72% TDN (R1), 17% CP and 75% TDN (R2), 16% CP and 78% TDN (R3). Those does were penned individually, and fed by basal diet (fresh chopped King Grass ad libitum, 500 g of fresh mixed forages) and 1 kg of experimental concentrate. Results showed that the treatments had significant (P<0.05) effects on CP, DIP, Ca, P intakes and FCR but had no significant (P>0.05) effects on DM and TDN intake. No significant differences were found in milk yield and milk composition between treatments. In conclusion, this trial suggested that the best feed for lactating SAPERA goats was the mixture of chopped grasses, mixed forages and concentrate diets (16% CP and 78% TDN) with 160 g/kg CP and 750 g/kg TDN of the total DM, produced a milk of 1.55 kg/d with 90 g/day of milk fat, 43 g/day of milk protein and 75 g/day of milk lactose.

(Author)

Key Words: Energy, Protein, Saanen X Etawah Grade Goat, Lactation, Milk Yield

UDC: 591.8

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Perubahan histologi jaringan hati dan hubungan serum biokimia pada kambing yang mengalami kebuntingan ketosis (Histological changes of liver tissue and serobiochemical relation in does with pregnancy ketosis)

(Org: Eng)

JITV 21(2): 96-100

Histological changes of liver in does with pregnancy ketosis were characterized. Twenty pregnant does at day 80 of pregnancy were used for this experiment. A total of 10 does were fed by grass (Napier) and goat concentrate with water ad libitum. Those 10 goats considered as healthy pregnant goat, and another 10 goats showing clinical and subclinical signs of ketosis considered as unhealthy pregnant does. Liver biopsies were performed when clinical signs appeared. Beta-Hydroxybutyrate (BHBA), free fatty acid (FFA), and glucose were dosed. Histological preparation revealed similar incidence and intensity of mild liver steatosis with lower cellular vacuolation in hepatocyte presence in healthy late pregnant does. Almost all of the pregnant does with ketosis state (n=8/10) had large amount of small lipid droplets in almost every hepatocyte over the whole liver acinus with higher number of cellular vacuolation, and related with higher BHBA and FFA levels while low in glucose level.

(Author)

Key Words: Beta-Hydroxybutyrate, Does, Free Fatty Acid, Liver Biopsies, Pregnancy Ketosis

UDC: 636.2.033

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Pengaruh emisi gas metana untuk fermentasi enterik pada sapi potong menggunakan metode Tier-2 IPCC di Indonesia (Methane emission factors for enteric fermentation in beef cattle using IPCC Tier-2 method in Indonesia)

(Org: Eng)

JITV 21(2): 101-111

Methane emission from enteric is a sub-category considered under the Agriculture sector greenhouse gas emissions by UNFCCC. Thus Indonesia developed calculation on enteric CH4 EF for ruminant using Tier-2 method as country-specific emission factors (EF). Indonesia has huge amount of beef cattle population, which contributes significant amount to national enteric methane emission. The aim of this study was to estimate enteric methane EF for beef cattle in Indonesia using IPCC Tier-2 method. The EF generated from this study is then used to estimate the methane emitted from beef cattle. Data on beef cattle population was obtained from CSA, data on energy content of feed, feed intake and digestibility were compiled from laboratory analysis and published paper. Equations were adopted and followed the instruction of IPCC 2006. Local cattle has different CH4 EF among each sub-category, which are ranging from 18.18 to 55.89 Kg head-1 yr-1, with the average of 36.75 head-1 yr-1. Imported beef cattle has lower CH4 EF (25.49 kg head-1 yr-1) than the average for local beef cattle. Overall, the national CH4 EF of beef cattle calculated by using IPCC Tier-2 method in Indonesia is 33.14 head-1 yr-1. The value is lower than default EF from IPCC for Asia country (47 kg head-1 yr-1). The conclussion is enteric CH4 EF for beef cattle in Indonesia calculated using Tier-2 method shows the real livestock system in Indonesia condition. Further researchs needed to be addressed are calculation of EFs for various breeds and feeding systems, since large variations of breeds and types of feed among provinces in Indonesia.

(Author)

Key Words: Methane Emission Factors, Enteric Fermentation, Beef Cattle, IPCC Tier-2

UDC: 636

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Emisi gas rumah kaca dari sektor produksi ternak di Korea Selatan (Greenhouse gas emissions from cattle production sector in South Korea)

(Org: Eng)

JITV 21(2): 112-123

South Korea has declared to reduce greenhouse gas emissions by 30% compared to the current level by the year 2020. The greenhouse gas emissions from the cattle production sector in South Korea were evaluated in this study. The greenhouse gas emissions of dairy cattle, Non-Korean native cattle and Korean native (Hanwoo) cattle production activities in 16 local administrative provinces of South Korea over a ten-year period (2005–2014) were estimated using the methodology specified by the Guidelines for National Greenhouse Gas Inventory of the IPCC (2006). The emissions studied herein included methane from enteric fermentation, methane from manure management, nitrous oxide from manure management and carbon dioxide from direct on-farm energy use. Over the last ten years, Hanwoo cattle production activities were the primary contributor of CH4 from enteric fermentation, CH4 from manure management, NO2 from manure management and CO2 from on-farm energy use in the cattle livestock sector of South Korea, which comprised to 83.52% of total emissions from cattle production sector.

Key Words: Methane, Nitrous Oxide, Carbon Dioxide, Cattle, Global Warming

UDC: 636.2.034

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Pengaruh penambahan vitamin C dalam media pengencer terhadap kualitas spermatozoa sapi perah setelah *thawing* (Effect of addition of vitamin C in diluent medium for quality of dairy cow spermatozoa after thawing)

(Org: Eng)

JITV 21(2): 124-134

The process of freezing and thawing of semen could lead spermatozoa death and low fertility for alive spermatozoa. This research was subjected to determine the optimum concentration of vitamin C in diluent media to improve the quality of non-sexed and sexed of thawed dairy cattle (Bos taurus) spermatozoa. The method used was completely randomized design with 3x4 factorial consisting of spermatozoa isolation and vitamin C concentration. Spermatozoa isolation factors were non-sexed and sexed (X and Y) spermatozoa. Vitamin C concentrations factors were 0% (K); 0.25% (P1); 0.50% (P2) and 0.75% (P3). Data were analyzed using analysis of variance (ANOVA) and Duncan's Multiple Range Test 95%. The optimum concentration of vitamin C in diluent media to improve the quality of non-sexed thawed dairy cattle (Bos taurus) spermatozoa was 0.25%. While the optimum concentration of vitamin C in diluent medium to improve quality of thawed dairy cattle (Bos taurus) spermatozoa both X and Y was 0.50%. Administration of vitamin C in diluent media could improve the quality of thawed non-sexed and sexed (X and Y spermatozoa) spermatozoa in dairy cattle (Bos taurus).

(Author)

Key Words: Spermatozoa, Vitamin C, Sexing, Diluent, Thawing

UDC: 633.3

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Produktivitas *Indigofera zollingeriana* pada berbagai taraf naungan dan kemasaman tanah di lahan perkebunan kelapa sawit (Productivity of *Indigofera zollingeriana* under different canopy and soil acidity level in oil palm estate)

(Org: Eng)

JITV 21(2): 135-143

Oil palm estate area in Indonesia is generally located in a sub-optimal land that has great opportunity for the development of forage supply. This study aims were to determine productivity of Indigofera zollingeriana under various canopy level. This research used factorial randomized block design with 3 canopy levels (under 2, 5, and 7 year oil palm canopy) and 2 levels of soil acidity (neutral and acid soil) treatments, where each treatment was repeated 4 times. Parameters observed were production and nutrient content of Indigofera zollingeriana. Research results showed that there was no interaction between the canopy levels and soil acidity on the production of fresh leaves, stems/branches, biomass, and leaves/stem ratio of I. zollingeriana. Production of fresh leaves, stems, biomass, and leaves/branches ratio of I. zollingeriana significantly ($P<0.01$) decreased along with increase of canopy level. Soil acidity significantly ($P<0.05$) decreased production of fresh leaves, stems, biomass, and leaves/branches ratio. Level of canopy treatment significantly ($P<0.05$) increased content of crude protein, crude fiber and energy, otherwise value of in vitro dry matter digestibility (IVDMD) and organic matter digestibility (IVOMD) were decrease. Soil acidity significantly ($P<0.05$) decreased calcium content, in vitro dry matter digestibility (IVDMD) and organic matter digestibility (IVOMD) of I. zollingeriana.

(Author)

Key Words: *Indigofera zollingeriana*, Oil Palm Estate, Canopy, Acid Soil

UDC: 612.128

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Penentuan aktivitas enzim pengurai serat kapang lapuk putih yang diisolasi dari pelepah sawit (Determination of fibernolytic enzyme activities of white rot fungi isolated from oil palm fronds)

(Org: Eng)

JITV 21(2): 144-150

Oil palm fronds (OPF) is widely used as the source of roughage for the farm animals. However, the full potential of OPF as animal feed is limited by their high lignin content which limits the rumen microbe’s access to the cellulose and hemicellulose. White rot fungi (WRF) are a group of fungi belonging to basidiomycete phylum and are commonly found in decaying woody plant. They possess the ability to degrade lignin. This experiment aims to identify the phylum of the best lignin decaying fungi based on their ratio ligninolytic to cellulytic and hemicellulytic activities. In this experiment, 11 fungi species were isolated from decaying oil palm fronds. They are labelled as WR1, WR 2, WR3, WR4, WR5, WR6, WR7, WR8, WR9, WR10 and WR11. Their fibernolytic enzyme activities which include laccase, manganese

peroxidase, lignin peroxidase, avicelase, carboxylmethylcellulase and xylanase are analysed from solid state fermentation method. It was found that 5 fungi species which are the WR1, WR2, WR4, WR7 and WR10 produced the highest ratio of lignin degrading enzyme to cellulose and hemicellulose degrading enzyme. The fungi are then analysed under microscope to determine the phylum of the fungi. From the observation, the fungi are identified to belong to the phylum of basidiomycetes due to presence of clamp connection.

(Author)

Key Words: Oil Palm Fronds, White Rot Fungi, Lignin, Enzyme

UDC: 633.31/.37

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Kontribusi tanaman legume dalam peningkatan serapan fosfor rumput *Panicum maximum* cv Riversdale dengan sistem tanam tumpang sari (Contribution of legumes on phosphoric absorption by *Panicum maximum* cv Riversdale in intercropping system)

(Org: Eng)

JITV 21(3): 151-158

Phosphorus availability in soil as a mobile mineral influences forage growth. The purpose of doing this research is to enhance the soil phosphorus availability and grass production of Panicum maximum cv Riversdale by intercropping system with legums. The experiment was conducted based on with randomized design with five treatments of mixcropping of: (i) Gliricidia sepium + P. maximum; (ii) Calliandra calothyrsus + P. maximum; (iii) Leucaena leucocephala cv Taramba + P. maximum; (iv) Calopogonium mucunoides + P. maximum; (v) P. maximum as negative control. Plants were grown in pots with split-root technique using partition with a whole to allow some legume roots grew in the grass side. After growing for three months, on the legume areas 32P isotop solution was injected for 50 ml (11.23 µci/ml). After 21 days incubation samples were collected from both soil areas and both plants. The translocation of 32P was determined using geiger counter from legumes into the grass and the concentration of 32P was also determined in all plants. Forage productions was determined both in the legumes and grass. Result showed that soil phosphorus concentration in the grass area was significantly improved by intercropping with G. sepium and C. callothyrsus, while the one with L. leucocephala was similar, and the one with C. mucunoides was significantly lower than that of negative control (without legume). Detection of 32P showed that there was P translocation in the system. P. maximum grass production depending on kind of legumes ($P<0.05$) those with G. sepium was significantly higher, L. leucocephala and C. callothyrsus were not significantly higher, while the one with C. mucunoides was 26.2% lower from the control although not significantly. However, C. mucunoides produced the highest forage from the legume plant. It is concluded that grass-legume intercropping had a positive impact on phosphorus soil concentration in the grass area and grass production. Kind of legumes influenced the effectivity.

(Author)

Key Words: Forage Production, Legume, *Panicum maximum*, Intercropping, ^{32}P Isotop

UDC: 633.31/.37

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Pengaruh dari teknik dan waktu menabur, tingkat benih dan pengelolaan gulma pada perusahaan kacang herba terpilih di Nusa Tenggara Timur, Indonesia (Effect of techniques and time of sowing, seed rate, and weed management on selected herbaceous legumes establishments in East Nusa Tenggara, Indonesia)

(Org: Eng)

JITV 21(3): 159-164

A series of experiments on techniques and time of sowing, and weed management of legumes Clitoria ternatea cv Milgarra, Centrosema pascuorum cv Cavalcade and cv Bundey, and Lablab purpureus cv Highworth, was conducted in East Nusa Tenggara (in the islands of Timor, Flores, and Sumba) in order to determine proper technique and time of sowing and weed management, which would be efficient in labor use and sufficient biomass production. Treatments of sowing techniques included dibble, furrow (covered and not covered), and broadcast (harrowed and not harrowed); while sowing time consisted of early wet season (December-January), mid of wet season (February-March) and the end of wet season (April-May), while weed managements consisted of weeded and unweeded treatments. The experiments employed block randomized design with four replications using plot sizes of 3 x 4 m to 4 x 5 m, depending on the availability of land for the experiments. The results of the experiments showed that the best sowing technique with the highest plant population at 4 weeks after planting was dibbling (42 plants/m²), followed by furrow, while the lowest plant population was obtained at the broadcast technique (9-20 plants/m²). Similarly, the highest biomass production was obtained in the dibbling technique (1.75 to 2.5 tons DM/ha per harvest at 12 weeks after planting in Ende, and 4-5 ton DM/ha in Nagekeo), followed by furrow technique covered or not covered (1-1.3 tons DM/ha in Ende and 3.5-4 tons DM/ha in Nagekeo), and the lowest in broadcast technique (0.3-1 ton DM/ha in Ende and 2-2.5 ton DM/ha in Nagekeo). However, considering the labor requirement and cost, it was recommended that furrow technique to suit the small farmer practices in the region. Weed management showed that weeded treatment (keep legume cleaned of weeds) gave significantly better ($P<0.05$) biomass production compared with to that of unweeded treatment. It can be seen also that weed had more suppressing effects on Clitoria ternatea, compared to that of Lablab purpureus, especially when the plants were sown in the early wet season.

(Author)

Key Words: Herbaceous Legumes, Clitoria ternatea, Centrosema pascuorum, Lablab purpureus, NTT, Planting Technique, Planting Time, Weed Management, Plant Establishment

UDC: 636.32/.38

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Respon domba yang diberi pakan dasar silase tongkol jagung atau rumput gajah dengan atau tanpa suplementasi Kaliandra (Response of sheep fed on corn cob silage or elephant grass basal diet with or without Calliandra leaf meal supplementation)

(Org: Eng)

JITV 21(3): 165-173

Limited availability of forage diet throughout the year could be overcome by utilization of crop by-products. Corn cob, a by-product from maize production is potential fiber source for grass replacement. The objective of this study was to compare effect of two different basal diets (basal grass diet and corn cob silage) with or without Calliandra supplementation on feed intake, nutrient digestibility, nitrogen utilization, rumen fermentation and growth of sheep. Twenty male sheep of St Croix breed were used in this study. The sheep were divided into 5 groups based on body weight. Each group was fed with one of four diet treatments for 13 weeks. The treatments were grass basal diet + concentrate, Corn cob silage (CCS) + concentrate, Grass basal diet + concentrate + 5% Calliandra leaf meal, CCS + concentrate + 5% Calliandra leaf meal. The ratio of basal diet (grass or CCS) to concentrate was 40 : 60% and was formulated in iso protein (crude protein content 14%). The diet was offered in total mix ration. The experiment was conducted in a randomized complete block design and arranged in factorial 2 x 2 (2 type basal diets and 2 Calliandra supplementation levels) with 5 replications. Results showed that there was no interaction between basal diet and Calliandra supplementation on feed consumption, average daily gain (ADG), nutrient digestibility, rumen fermentation, except for crude protein (CP) intake. Feed consumption was not affected by basal diets or Calliandra supplementation. Feed conversion ratio and protein conversion ratio were better in grass basal diet than CCS. CP digestibility was higher in corn cob silage than basal grass diet without Calliandra supplementation. N retention was higher in corn cob basal diet than basal grass diet. Rumen fermentation was significantly affected by basal diet in which rumen ammonia and VFA concentrations were higher in grass basal diet. Grass basal diet had higher propionic acid production than CCS basal diet. It is concluded that in iso protein diet, basal grass diet was comparable to corn cob basal diet as revealed by average daily gain was similar in both diets with average 107.5 g/head/day. Calliandra supplementation at 5% in the grass or CCS basal diet did not improve sheep performance.

(Author)

Key Words: Corn Cob, Silage, Calliandra, Sheep, Elephant Grass

UDC: **636.58**

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Respon pertumbuhan galur ayam lokal terseleksi terhadap ransum dengan kadar energi metabolis dan protein berbeda (Growth response of improved native breeds of chicken to diets differed in energy and protein content)

(Org: Eng)

JITV 21(3): 174-181

The aim of doing this experiment was to observe the growth response of improved native breeds of chicken under diets differed in energy and protein content. Three groups of improved native breeds, obtained from mating of ♂KUB to ♀KUB (Line 1), of ♂SenSi to ♀KUB (Line 2) and of ♂Gaok x ♀KUB (Line 3), were subjected to three experimental diets, differed in metabolizable energy (ME) and crude protein (CP) content. The experimental diets consisted of Diet 1 (2,800 kcal ME/ kg with 17,81% CP), Diet 2 (2,950 kcal ME/ kg with 18,61% CP) and Diet 3 (3,100 kcal ME/ kg with 19,25% CP). The experiment was designed as factorial 3 x 3 with 7 replications of each treatment combination, consisted of 5 birds per treatment combination. The chickens were raised up to 10 weeks of age. Results of the experiment showed that Line 2 (♂SenSi mated to ♀KUB) had highest body weight at 10 weeks of age ($P<0.05$), lowest feed conversion ratio (FCR) and highest European Production Efficiency Factor (EPEF), compared to other two lines. The appropriate diet for Line 2 was Diet 2. It could be concluded the crossbred line that was resulted from crossing of ♂SenSi to ♀KUB (Line 2), had potential to be used as improved native chicken for the industry in Indonesia supported by appropriate diet containing 2,950 kcal ME/kg with 18.61% crude protein.

(Author)

Key Words: KUB Chicken, SenSi Chicken, Gaok Chicken, Diets

UDC: 616-093/-098

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Uji aktivitas eksopolisakarida bakteri asam laktat sebagai imunomodulator (Exopolysaccharide (EPS) activity test of lactic acid bacteria (LAB) as immunomodulatory)

(Org: Eng)

JITV 21(3): 182-189

Immunomodulatory activity assay and characterization of exopolysaccharide (EPS) from Lactic Acid Bacteria (LAB) was done in Bogor. Bacteria used in this study was LAB strains of Lactobacillus delbrueckii subsp. bulgaricus and Streptococcus thermophilus. Exopolysaccharide was extracted from L. delbrueckii subsp. bulgaricus and S. thermophilus then characterized with FT-IR spectrophotometer to determine the functional group. IR spectrum analysis using Fourier Transform-Infra Red (FT-IR) showed that EPS from both LAB isolates were carbohydrate compounds. Immunomodulatory activity in vivo from EPS was measured using phagocytic activity and phagocytic capacity macrophage cells from mice peritoneal cavity fluid. Exopolysaccharide were given orally to mice in concentrations of 100 µg/ml, 200 µg/ml and 300 µg/ml for 14 days then the mice were infected with Staphylococcus aureus. Result showed that EPS from both LAB isolate enhanced either phagocytic activity and phagocytic capacity macrophage cell from mice peritoneal fluid. EPS from L. delbrueckii subsp. bulgaricus concentration 300 µg/ml showed the highest phagocytic activity of macrophage cells and EPS from S. thermophilus concentration 300 µg/ml showed the highest phagocytic capacity. It is concluded that EPS potency tested as immunomodulatory derived from a culture of L. delbrueckii and S. thermophilus subsp.bulgaricus are able to increase the activity and phagocytosis murine peritoneal macrophages.

(Author)

Key Words: Exopolysaccharide, Lactic Acid Bacteria (LAB), Immunomodulatory, Yoghurt, Fourier Transform-Infra Red (FT-IR)

UDC: 636.293.2

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Tingkat pengetahuan, sikap, kebiasaaan (KAP) dan kerugian finansial peternak Kerbau akibat penyakit Surra di desa terpilih di Filipina Selatan (Knowledge, attitudes, practices (KAP), and financial losses of buffalo raisers due to Surra among selected villages in Southern Philippines)

(Org: Eng)

JITV 21(3): 190-203

The study was conducted to assess the knowledge, attitudes, practices (KAP), and financial losses of buffalo raisers due to Trypanosoma evansi infection (surra) and its control in Agusan del Sur Province. One-hundred and sixty (160) buffalo raisers from eight villages in four municipalities (towns) in Agusan del Sur, Mindanao, Southern Philippines were personally interviewed. Majority (63.65%) of respondents provided information about surra. Mean knowledge score of 12.54 was quite low to consider the respondents well informed about surra. Financial losses from mortalities among livestock in eight villages (in four towns) in Agusan del Sur amounted to 9.3 million Philippine Pesos (PHP) (US$ 0.2 M) with additional losses for treatment and diagnosis amounting to PHP 657,000 and PHP 229,500, respectively. The estimated mass treatment and diagnostic costs were PHP 2.4 and PHP 1.1 million, respectively. The estimated overall total financial losses was PHP 13.7 million, averaging PHP 1.7 million per village, and an estimated PHP 538 million (US$ 10.7 M) of total financial losses among livestock in Agusan del Sur due to surra. In conclusion, buffalo raisers in Agusan del Sur Province lack adequate knowledge, attitudes and practices to effectively control surra, a disease that has caused high financial losses among livestock in the province.

(Author)

Key Words: Buffalo Raisers, Surra, Philippines, KAP, Losses

UDC: 633.2.031

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Karakter morfologi dan keragaman genetis rumput Benggala (*Panicum maximum* cv Purple guinea) melalui iradiasi sinar gamma pada lahan masam (Characteristic morphology and genetic variability of Benggala grass (*Panicum maximum* cv Purple guinea) through gamma ray irradiated on acid land)

(Org: Eng)

JITV 21(4): 205-214

The induction mutation using gamma rays is one way to increase the diversity of Bengggala grass (*Panicum maximum*). Diversity is the main base in selecting to produce varieties of Benggala grass on acid land. The aim of this study is to know the morphological character and genetic diversity of grass that received gamma rays. The research was conducted in the Tenjo district, Bogor which has pH 4.9-5.5 and the content of Al-dd 2.26 c mol/kg with Al saturation of 10.68%. The study started by looking for Benggala grass radio sensitivity, then from radio sensitivity determined radiation treatment dose of 200 Gy, 250 Gy, 300 Gy and 350 Gy. Treatment of radiation dose is acute and fractionated, so there are 8 treatment doses plus control (plants without radiation). The variables measured were plant height, stem height, stem diameter, length of segment, leaf length, leaf width, fresh weight, dry weight, and number of tillers. The results showed that the radio sensitivity of Benggala grass produced in the form of lethal doses (LD) LD 20 and LD50 is 176.83 Gy and 358.23 Gy. By the mathematical equation: Y = 44.22 + 45.91 X Cos (0.0042x -0.07). The morphological character of M1 shows that the radiation dose affects the measured variable. 300 Gy fractionated radiation dose (150 Gy x 2) results in higher fresh weight of the plant. While in the generation of M1V1 radiation dose of 250 Gy produces high fresh forage weight. Medium to high heritability with GVC value (criteria of coefficient of genetic variability) is high enough until high is obtained on the character of the number of tillers, fresh weight and dry weight. So it is possible to do next selection on these three characters.

(Author)
Key Words: *Panicum maximum*, Irradiation, Mutation, Morfology, Genetic Variability

UDC: 633.31/.37

Herdiawan, I. (IRIAP, Bogor)

Produktivitas *Brachiaria decumbens* dalam kultur campuran dengan *Pueraria phaseloides* dalam berbagai tingkat pupuk kandang dan dolomit di lahan suboptimal (Productivity of *Brachiaria decumbens* in a mixed-culture with *Pueraria phaseloides* in different manure and dolomite administration level in the suboptimal land)

(Org: Eng)

JITV 21(4): 215-222

Productivity improvement of forage in suboptimal land can be done in various ways, such as through manure and ameliorant treatment. Manure and dolomite is needed by the soil on suboptimal land, because the combination of the two can supply nutrients and improve the soil biophysical. The research materials used were B. decumbens, Pueraria phaseloides, manure, and dolomite. The study used Randomized Block Design (RBD) in 3x3 factorial. The dose of manure was the first factor and dose of super dolomite was the second factor, each treatment received 3 times replications. Variables measured were biomass production, dry matterial production, carrying capacity, and nutrient content of forage. The results showed that the highest ($P<0.05$) biomass production produced by the 10 t/ha manure and 2 ton/ha dolomite treatment by 1,638.94 g/m2 compared to other treatments, and the lowest was achieved in the control by 543.75 g/m2. Carrying capacity of B. decumbens and P. phaseloides on 10 ton/ha manure was the highest ($P<0.05$) by 0.08 AU compared to others treatment. Super dolomite administration by 1 ton/ha significantly ($P<0.05$) produced highest carrying capacity by amount of 0.83 AU. Manure and dolomite administration significantly ($P<0.05$) improved the nutritional content of B. decumbens and P. phaseloides.

(Author)
Key Words: *Brachiaria decumbens*, *Pueraria phaseloides*, Manure, Dolomite

UDC: 636.39

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Studi tentang Gen Polimorfisme BMP15 pada Kambing Boer, Kacang dan Boerka (Study of BMP15 gene polymorphism in Boer, Kacang, and Boerka goats)

(Org: Eng)

JITV 21(4) 223-229

The bone morphogenetic protein 15 (BMP15) gene or commonly called FecX (fecundity chromosome X) is a gene that controls the prolific properties. This study aims to identify the mutation of BMP15 gene and to analyze its polymorphism in Boer, Kacang, and Boerka goats. The total of 50 female goat bloods were identified using PCR-Sequencing method, 17 Boer, 16 Kacang and 17 Boerka respectively. BMP15 gene amplification resulted fragment with the length of 141 bp. Genotyping of BMP15 gene produced three genotypes. The result showed that BMP15 gene found two polymorphic SNP were analyzed by genotype frequency, allele frequency, heterozygosis and equilibrium of genotype in all population was detected by the Hardy-Weinberg equilibrium test ($\chi 2$). Sequence analysis results of BMP15 gene showed that there were two mutation between adenine (A) and guanine (G) bases and determination of genotype BMP15 gene produces three genotypes showed that there were GG, GA and AA. In conclusion, there was found the mutation of BMP15 gene in Boer, Kacang and Boerka goats and genetic polymorphism were identified using PCR-Sequencing method.

(Author)
Key Words: BMP15 Gene, PCR-Sequencing, Prolific, Genetic Polymorphism, SNP

UDC: 636.2.033

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Affandhy, L. (BCRS, Pasuruan)
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Poppi, D. (School of Agriculture and Food Sciences, The University of Queensland, Gatton, Queensland, Australia)

Strategi manajemen untuk meningkatkan perfomans produksi reproduksi sapi Brahman Cross (*Bos indicus*) di Jawa Timur, Indonesia (Management strategies to increase production reproductive on performance of Brahman cross cattle (*Bos indicus*) in East Java, Indonesia)

(Org: Eng)

JITV 21(4): 230-236

The demand for meat in Indonesia cannot be supplied by domestic cattle production, and so breeder cattle stock are imported from abroad overseas to increase domestic production. A commonly imported breed is Brahman cross bred (Bx). Smallholder farmers have reported a wide range of problems associated with Bx cow reproductive performance. The purpose of this study was to identify changes in the reproductive performance of Bx cows by providing advice to farmers on improved management strategies, such as: maintaining body condition score (BCS) of cow> 3, appropriate estrous detection and weaning at 5- 6 months. This study was conducted from October 2011 – December 2013 by using 189 Bx cows and 151 farmers. The research was conducted in two sub districts of Lamongan district (East Java), Modo and Sambeng. Cattle were fed diets based on crop residues and were supplemented with green feeds, by-

products (rice bran) and concentrate during the last 2 months of pregnancy and early lactation. The reproductive performance of cows improved during the study, there were a shorter lactation length/early weaning (reduced from 188 days to 153 days) and improved BCS (from 3.0 to 3.3). The length of postpartum anestrous interval (PPAI) decreased from 198 days to 98 days. Cycling to conception decreased from 75 days to 64 days and days open decreased from 217 days to 118 days over the length of the study. This was associated with improvement in BCS of cows at calving (from 3.0 to 3.3) and a shorter lactation length (reduced from 188 days to 153 days). There was no change in the average birth weight of calves (31.8 kg). There was no change in average weaning weight of calves over the year and average daily gain of calves while suckling 0.7 kg/day. The pre weaning calf mortality rate was decreased during this study (from 13% to 6%). This project demonstrated that Brahman cross cows can successfully be kept by smallholder farmers in Indonesia if appropiate management is used. It can be concluded that improving management, such as maintaining body condition score of cow >3, estrous detection, appropiate mating and weaning at 5-6 months can improve reproduction performance of Brahman cross.

(Author)

Key Words: Reproduction, Cattle, Brahman Cross

UDC: 636.2.033

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Purwadaria, T. (IRIAP, Bogor)

Aplikasi enzim-BS4 dalam produksi biogas media campuran kotoran sapi dan koran bekas (Application of BS4-enzyme on the methane production from mixture of cattle manures and waste paper)

(Org: Eng)

JITV 21(4): 237-242

Cellulose from abundant newspaper waste could be transformed into methane through anaerobic fermentation. This research was carried out to compare the gas production including methane between samples containing feces and waste paper mixture as inoculum and substrate, respectively and added with and without BS4 enzyme. The enzyme was produced in Indonesian Research Institute of Animal Produce (IRIAP) by growing *Eupenicillium javanicum* BS4 in coconut meals. There were three treatments, i.e., 30% manure (M30); 15 % manure + 15 % paper waste (MP 30); MP30 + 3 mL BS4 enzyme equal to 0.42 U/g dry matter (MPE30) The percentage of waste papers addition in feces was calculated on dry matter (DM) basis and every treatment had five replications. Total gas and methane productions were measured weekly, while dry matter losses were determined during 5 week fermentation. Interactions between treatments and incubation time were analyzed using completely randomized design each week. Kind of substrates influenced both total gas and methane productions during incubation time. Both waste papers and enzyme addition enhanced gas production. The highest total gas and methane productions for five weeks incubation were highly significantly observed ($P<0.01$) in MP30 and MPE30 compared to M30. Addition of enzyme significantly increased total gas and methane productions in the first week. The highest methane and total gas yield/g dry matter were obtained by BS4 enzyme addition. It was concluded that BS4 enzyme is good in accelerating and increasing the transformation efficiency of waste paper and manure mixture for biogas production.

(Author)

Key Words: Methane, Fibrenolytic-Enzyme, Waste Papers, Cattle Manures

UDC: 636.1

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Suhartono, M.T. (Department of Food Science and Technology, Faculty of Agricultural Technology, Bogor Agricultural University)

Bioaktifitas dan analisis peptida dari susu kuda Sumbawa yang dihasilkan oleh *Bacillus thuringiensis* protease (Bioactivities and analysis of peptides from Sumbawa horse milk generated by *Bacillus thuringiensis* protease)

(Org: Eng)

JITV 21(4): 243-253

Sumbawa horse milk is claimed to cure some diseases such as asthma, hypertension, diabetes and gastrointestinal disorder but its potential bioactive peptide has not been explored. The aims of this study are to evaluate bioactivities peptides from Sumbawa horse milk protein hydrolysate and to analyzethe physio-chemical properties of selected peptides. The milk protein was hydrolyzed by *Bacillus thuringiensis* protease, the peptide produced were sequential fractionated and then analyzed for antibacterial and antioxidant activities. The peptide fraction <3 kDa was then sequenced using LCMS-MS and the physio-chemical properties of the peptides were analyzed. The result showed that peptides fraction <3 kDa from the 30 min hydrolysis was the most active as antibacterial and more active to Gram negative bacteria. For antioxidant, scavenging activity of the fraction per µg protein/mL were 83% to ABTS and 31% to DPPH radicals. The values were similar with vitamin C 12.5 µg/mL for ABTS and 14.5 µg/mL for DPPH. Peptide HPYFYAPELLYYANK with molecular weight prediction 1887.92 Da and isoelectric point 7.47 has high therapeutic index prediction (64.75). The result showed that peptides from Sumbawa horse milk hydrolyzed by *Bacillus thuringiensis* protease was active as antibacterial and antioxidant. Peptide HPYFYAPELLYYANK from fraction <3 kDa was potential as antibacterial.

(Author)

Key Words: *Bacillus thuringiensis*, Bioactive Peptide, Horse Milk

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(i) Primary scientific manuscript of unpublished research results.
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   a. Lawrence TLJ, Fowler VR. 2002. Growth of farm animals. 2nd ed. New York (USA): CABI Publishing.
   b. Bamualim A, Tiesnamurti B. 2009. Konsepsi sistem integrasi antara tanaman padi, sawit, dan kakao dengan ternak sapi di Indonesia. In: Fagi AM, Subandriyo, Rusastra IW, penyunting. Sistem integrasi ternak tanaman padi, sawit, kakao. Jakarta (Indones): LIPI Press. p. 1-14.
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Proceeding:
Umiyasih U, Antari R. 2011. Penggunaan bungkil inti sawit dan kopra dalam pakan penguat sapi betina berbasis limbah singkong untuk pencapaian bobot badan estrus pertama >225 kg pada umur 15 bulan. Prasetyo LH, Damayanti R, Iskandar S, Herawati T, Priyanto D, Puastuti W, Anggraeni A, Tarigan S, Wardhana AH, Dharmayanti NLPI, editors. Proceeding of National Seminar on Livestock Production and Veterinary Technology. Bogor (Indones): Indonesian Center for Animal Research and Development. p. 192-199.

Thesis:
Krisnan R. 2008. Kombinasi penggunaan probiotik mikroba rumen dengan suplemen katalitik pada pakan domba (Thesis). [Bogor (Indones)]: Institut Pertanian Bogor.

Electronic magazines:
Wina E, Tangendjaja B, Dumaria. 2008. Effect of *Calliandra calothyrsus* on *in vitro* digestibility of soybean meal and tofu wastes. Livest Res Rural Develop. Vol. 20 Issue 6. http://www.lrrd.org/lrrd20/6/wina 20098.htm.

Institution:
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b. [CDC] Centers for Disease Control. 2006. Standard operating procedure for the direct Rapid Immunohistochemistry Test (dRIT) for the detection of rabies virus antigen. [accessed December 20th 2011]. http://www.rabiesblue print.com/IMG/pdf/DRIT_SOP.pdf.

Patent:
Blanco EE, Meade JC, Richards WD. 1990. Ophthalmic ventures, assignee. Surgical stapling system. United States patent US 4,969,591. 1990 Nov 13.

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