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PREFACE

In this edition, volume 21 no. 3 September 2016, we proudly present articles from animal and veterinary sciences including feed and nutritive technology, microbiology, physiology, and pharmacology. The articles published in this edition are: “Contribution of legumes on phosphoric absorption by *Panicum maximum* cv Riversdale in intercropping system”; “Effect of techniques and time of sowing, seed rate, and weed management on selected herbaceous legumes establishments in East Nusa Tenggara, Indonesia"; "Response of sheep fed on corn cob silage or elephant grass basal diet with or without Calliandra leaf meal supplementation"; "Growth response of improved native breeds of chicken to diets differed in energy and protein content"; “Exopolysaccharide (EPS) activity test of lactic acid bacteria (LAB) as immunomodulatory" and “Knowledge, attitudes, practices (KAP), and financial losses of buffalo raisers due to Surra among selected villages in Southern Philippines".

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, September 2016

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LIST OF CONTENT

Contribution of Legumes on Phosphoric Absorption by *Panicum maximum* cv Riversdale in Intercropping System

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ABSTRAK

Sajimin, Purwantari ND, Sugoro I. 2016. Kontribusi tanaman legume dalam peningkatan serapan fosfor rumput *Panicum maximum* cv Riversdale dengan sistem tanam tumpang sari. JITV 21(3): 151-158. DOI: http://dx.doi.org/10.14334/jitv.v21i3.1520

Ketersediaan fosfor (P) dalam tanah sebagai unsur hara yang sifatnya mobil mempengaruhi pertumbuhan tanaman. Tujuan dari penelitian ini untuk meningkatkan ketersediaan fosfor dan produksi rumput *Panicum maximum* cv Riversdale yang ditanam tumpang sari dengan tanaman leguminosa. Percobaan dirancang secara acak lengkap dengan lima perlakuan dan lima ulangan yaitu: A. *Gliricidia sepium* + *P. maximum*; B. *Calliandra calothyrsus* + *P. maximum*; C. *Leucaena leucocephala cv Taramba* + *P. maximum*; D. *Calopogonium mucunoides* + *P. maximum*; E. *P.maximum* (kontrol negatif). Tanaman tumpang sari ditumbuhi pada pot dengan diberi pembatas yang dilubangi untuk pertumbuhan akar tanaman leguminosa di media rumput. Setelah tiga bulan tumbuh pada area tanaman leguminosa isotop ^{32}P diinjeksi sebanyak 50 ml (11,23 µci/ml). Setelah inkubasi selama 21 hari sampel tanah pada kedua area dan kedua tanaman dikumpulkan untuk analisis kadar isotop pada tanaman dan translokasi fosfor dideteksi dengan menggunakan perunut isotop ^{32}P. Produksi hijauan juga diamati pada ke dua tanaman. Hasil penelitian menunjukkan kadar fosfor tanah rumput dipengaruhi jenis legum, *G. sepium* dan *C. callothyrsus* nyata lebih tinggi terhadap kontrol, sedangkan *L. leucocephala* tidak berbeda nyata, dan kadar P nyata lebih rendah pada *C. mucunoides*. Deteksi ^{32}P menunjukkan akar legum yang terintegrasi di daerah rumput memindahkan P. Produksi rumput *P. maximum* dengan sistem tumpang sari secara nyata meningkat pada *G. sepium*, secara tidak nyata pada *L. leucocephala* dan *C. callothyrsus* dibandingkan perlakuan kontrol, sedangkan *C. mucunoides* turun 26,2% walaupun tidak berbeda nyata. Data dari tumpangsari *C. mucunoides* menunjukkan produksi hijauan legum tertinggi. Dapat disimpulkan bahwa ketersediaan fosfor dan produksi rumput dapat ditingkatkan dengan sistem tumpang sari dengan legum. Jenis legum mempengaruhi efektivitas.

Kata Kunci: Produksi Hijauan, Leguminosa, *Panicum maximum*, Tumpang Sari, Isotop ^{32}P

ABSTRACT

Sajimin, Purwantari ND, Sugoro I. 2016. Contribution of legumes on phosphoric absorption by *Panicum maximum* cv Riversdale in intercropping system. JITV 21(3): 151-158. DOI: http://dx.doi.org/10.14334/jitv.v21i3.1520

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 ^{32}P 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 ^{32}P was determined using geiger counter from legumes into the grass and the concentration of ^{32}P 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 ^{32}P 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.

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

INTRODUCTION

Developing forage plantations in the marginal areas such as acid areas is not optimal for crop production, but potentially important for feed forage. Horst et al. (2006) reported that acid soil reaches 1.7 billion hectars and 43% is in tropical area. In Indonesia, acid land reaches 102.8-107.4 million ha and has not been used optimally (Mulyani et al. 2004; Agus et al. 2015). Farmers in Indonesia used excessive phosphorus chemical fertilizer to enhance the crop production for 100 kg TSP/ha of paddy field. In the long term excessive P application might form cementing layer on land surface (Karama et al. 1991; Saidu & Abayomi 2015).

Phosphorus (P) is absorbed as orthophosphate ions is an important element for growing plant cells for phospholipid in the cell membrane, for accumulation and releasing cell energy in metabolism and as sugar-phosphate in nucleotides for genetic information (Franzini et al. 2009). P deficiency showed retarded growth in crops and reddish leaves due to the increase of anthocyanin. P is important for metabolism including cell division, respiration, and photosynthesis (Richardson et al. 2009). Hakim et al. (1986) suggests that in high acidity soil (pH <5), phosphate ions are easily binded with Al, Fe or Mn forming insoluble compounds that reduce the P availability. Together with N, P is important for forage quality and the availability are influenced by microbial activities surrounding the roots (Guo et al. 2000; Alan et al. 2009).

Good combination of intercropping or mixed cropping system of legumes and grass increases the grass production as well as reduce the use of N and P inorganic fertilizers or results friendly ecosystems (Exner et al. 1999). Intercropping of legumes and maize increases N in the soil (Li et al. 2003; Eskandori et al. 2009; Belel et al. 2014). The intercropping of legumes and elephant grass in the contour system also reduce the erosion and increase the production and forage quality (Anantawiroon et al. 2006; Mutegi et al. 2008).

Intercropping of cowpea-maize improves soil phosphorus availability and maize yields (Latati et al. 2014). Other researchers also reported that the non-legume plants get phosphorus from legumes (Elgersma et al. 2000). Transportation of phosphorous from the legumes to the *Panicum* grass may be traced using radioisotope of ^{32}P injected in the legume areas. The intercropping system might be carried out using partition to separate both plants except that some of legume roots were in the grass area. The roots may translocate the radioisotope from the legume areas. Therefore, the transportation of ^{32}P may be traced.

This research was aimed to increase the yield of the *Panicum maximum* (*Panicum* grass) in acid soil by intercropping with kinds of legumes incorporated with ^{32}P.

MATERIALS AND METHODS

Kinds of soils

This study was conducted in the greenhouse of IRIAP, Ciawi-Bogor using red-yellow podsolit soil collected from experimental garden. Soil was dried and sieved by 2 mm and then its nutrient content was analyzed. The results showed that soil consisted of 7% sand texture, 64% ash, and 29% clay at pH of 4.6. The organic and inorganic elements were 2.39% C, 0.097 % N, 61.67 ppm P, 0.08 ppm K, 5.72 ppm Ca, 1.09 ppm Mg and 0.31 ppm Na.

Design of experiment

This study employed completely randomized design with five treatments and five repitations as follows:

a) *Gliricidia sepium* + *Panicum maximum* cv Riversdale
b) *Calliandra calothyrsus* + *P. maximum* cv Riversdale
c) *Leucaena leucocephala* cv Taramba + *P. maximum* cv Riversdale
d) *Calopogonium mucunoides* + *P. maximum* cv Riversdale
e) *P. maximum* cv Riversdale only as negative control

If there were significant difference in analyses of variance, data were further analyzed with Duncan (Gomez & Gomez 1984).

Intercropping system

Intercropping system used in this study was Split-Root Technique (Catchpoole 1988) in combination with method by Xiao et al. (2004). One pol grass and one pol of each legume were planted in a pot containing 32 kg dry weight of soil and divided by a diagonally fiber partition. The partition had a hole so that the legume root partially passed through the hole in the area of the grass plant (Figure 1).

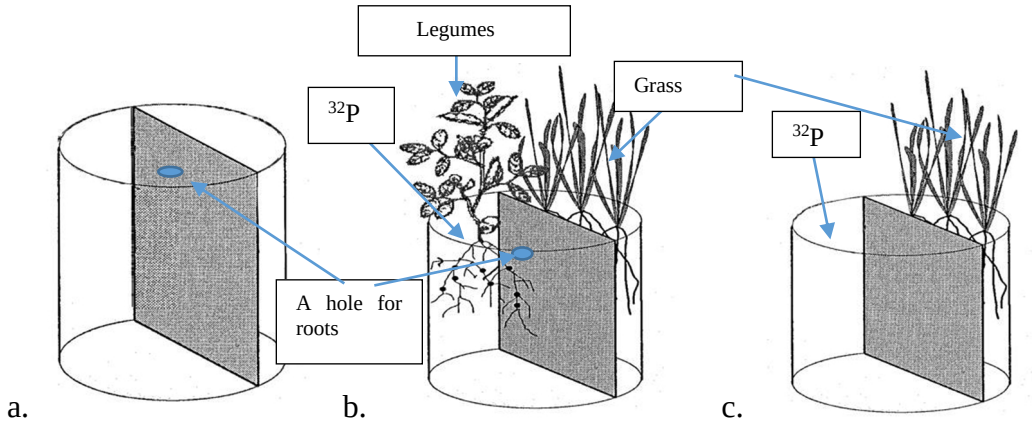

Figure 1. Position of the plants and ^{32}P injection in the pots with the fiber partition. (a) Pots before the plants growing; (b) Pots after the plants growing, some of legume roots grew through the hole to the grass area; (c) Control pots without legumes.

Aplication and detection of ^{32}P

Radioisotope ^{32}P as $KH_2{}^{32}PO_4$ solution was injected 50 mL (561.5 µci/pots) in the soil area of legumes at three months growth. The transportation of the ^{32}P was traced using Geiger Muller all on the surface soil and part of the plants in IRIAP, while the concentration of ^{32}P was determined in the laboratory of National Nuclear Energy Agency of Indonesia. Distribution of the radio isotope in the stem pit and leaves of legumes was detected after the injection for 14 days. All data were collected for phosphorus recovery determination.

Total phosphorus content in the soil before and after the experiment was determined in Indonesian Center for Agricultural Land Resources Research and Development. Variables determined for forage production were plant height, number of shoots and bundles respectively for the legumes and the *Panicum* grass, dry weight of nodules, dry weight of roots, and forage production.

RESULTS AND DISCUSSIONS

Soil phosphorus availability

P-availability of soils for grass or legumes was determined after the plants grew, but before P-isotope was introduced (Table 1). The soil planted only with *P. maximum* (control) at 64.0 ppm was lower than those integrated with legume roots of *G. sepium* (74.3 ppm) and *C. calothyrsus* (70.7 ppm), similar of *L. leucocephala* (65.7 ppm), but higher than the one with *C. mucunoides* (56.7 ppm). The P-availability in soils of *G. sepium* and *C. calothyrsus* were not significantly different. P-availability of soils planted with legume seemed higher than that of grass only (negative control). The P-availability in soils were influenced by legume intercropping and also kinds of legumes incorporated, the lower P in the legume area affected the lower P in the grass area. The highest P was observed from *G. sepium* and *C. calothyrsus*, followed by *L. leucocephala cv. Tarramba*, and negative control. P in the grass area intercropped with *C. mucunoides* was significantly lower than that of the negative control.

The increase of P-availability in the grass area was in agreement with the one reported by Latati et al. (2014) that discover the increase of P in maize soil area integrated with cowpea. The lowest P in the legume soil area might be related with the P- consumption for the leave production. Leave production of *C. mucunoides* is relatively high. The lowest P-availability in the grass area integrated with *C. mucunoides* was not only affected by the low P in the legume area, but might be also influenced the availability of its root in the grass area. The effectiveness of P-transportation from legume soil area to the grass area was then detected by the transportation of ^{32}P isotop.

Recovery of ^{32}P on the legumes and grass

Data of ^{32}P tracers in each repeatation were similar, therefore detection of ^{32}P using Geiger Muller counter was used to detect the P transportation from legumes soils to grasses's and part of both plants (Table 2). The isotop was transported from the legume soils in the whole parts of legumes and transported to grass soils and parts of grass. The transportation from legumes to grass areas was more influenced by kind of legumes than those from the concentration of ^{32}P in the soils like the one observed in *L. leucocephala*.

The injection of ^{32}P was carried out after the legumes producing roots in the grass areas. The ^{32}P was more detected in the legumes than that in grass except for *C. mucunoides*. The stem of *L. leucocephala* had the

highest traced, while from other parts and other legumes, as well as grass. The highest trace was observed in *G. sepium* dan *C. mucunoides*.

Data of tracers showed that ^{32}P *G.sepium* was higher in its stem than that in its leave tips. ^{32}P has more mobility in the stem than in the leaves. After pruning plants usually will grow again and the ^{32}P will be spread out following the cell division (Kalaivanan et al. 2014). The Geiger Muller counter is more to detect the isotop transportation in the plant parts, therefore to observe the effect of the ^{32}P injection to the legumes and grass the concentration of ^{32}P of all plants were detected (Table 3).

Table 1. P availabilities on soils grown with legumes and on grass areas integrated with roots of legumes

Kinds of legume roots integrated with *P. maximum*	P on areas of legume soil (ppm)	P on areas of grass soils (ppm)
G. sepium	118.3^{a}	74.3^{a}
L. leucocephala cv. Tarramba	74.7^{c}	65.7^{b}
C. calothyrsus	107.3^{b}	70.8^{a}
C. mucunoides	78.7^{c}	56.7^{c}
Control	ND	64.0^{b}

Different superscript letters in the same column show significant difference (P<0.05); ND was not determined.

Table 2. Distribution of 32P on legumes and grasses and their soil surface

Kind of legumes	Recovery of ^{32}P on legume soils and plants (μci/g.min)			
	Soil surface	Stem	Leaves	Leave tips
G. sepium	3391.7^{ab}	366.7^{ab}	216.7^{a}	158.3^{a}
L. leucocephala	8000.0^{a}	583.3^{a}	158.3^{b}	100.0^{b}
C. callohyrsus	1116.7^{b}	283.3^{b}	183.3^{b}	133.3^{a}
C. mucunoides	1250.0^{b}	350.0^{ab}	337.5^{a}	150.0^{a}
Control	2333.3^{ab}	ND	ND	ND
	Recovery of ^{32}P on grass soils and grass (μci/g.min)			
G. sepium	91.7^{b}	216.7^{b}	208.3^{ab}	233.3^{a}
L. leucocephala	91.7^{b}	258.3^{b}	250.0^{ab}	183.3^{b}
C. callohyrsus	166.7^{a}	275.0^{b}	166.7^{b}	175.0^{b}
C. mucunoides	150.0^{a}	350.0^{a}	300.0^{a}	275.0^{a}
Control	ND	ND	ND	ND

Different superscript letters in the same column show significant difference (P<0.05);
ND was not determined. Traced of 32P was carried out by Geiger Muller.

Table 3. Trace of 32P (μci/g plants) on the legumes and grasses

Kind of legumes	Legumes	Grasses
G. sepium	839^{d}	11133^{a}
L. leucocephala	4945^{b}	9107^{a}
C. calothyrsus	2361^{c}	8043^{a}
C. mucunoides	14540^{a}	9673^{a}
Control	No plants	71^{b}

Different superscript letters in the same column show significant difference (P<0.05).

Each legume had significant difference of ^{32}P concentration. The highest concentration of ^{32}P on the legumes was observed at *C. mucunoides* followed by *L. leucocephala, C. calothyrsus*, while *G. sepium* had the lowest concentration (Table 3). Although no legume was grown in the control, ^{32}P was detected in the grass with very low concentration. This very low concentration resulted in none significant different at ^{32}P concentration in each grass intercropped, although there was 28 % different from the one integrated with *G. sepium* vs with *C. calothyrsus*. The highest ^{32}P concentration in the grass was observed at the one intercropped with *G. sepium* root (11,133 μci/g), followed by *C. mucunoides* (9,673 μci/g), *L. leucocephala* (9,107 μci/g) and *C. calothyrsus* (8043 μci/g). Contribution of ^{32}P from the legumes into the grass depended on kinds of legumes. The grass integrated with *G. sepium* showed certain condition that the ^{32}P was low in the legume but it was high in the grass. The relation of the P concentration toward the grass production will be discussed in the grass production paragraph. Anantawiroon et al. (2006) reported that kinds of legumes in the intercropping system resulted in different production and quality of Napier grass.

Compared to the control the grass with legume root integration had higher ^{32}P. This result is in agreement with that reported by Richardson et al. (2009) that ^{32}P from legume areas is trans located to barley. The translocation is influenced by the legume root amount in the barley areas and legume morphology. The ^{32}P in the grass areas influences the grass phosphoric absorbtion.

Root nodules and weights

Each legume function in nitrogen fixation had different root nodules in shapes, location in the roots and numbers (Table 4). Numbers of the nodules was expressed in weight, higher weight shows higher numbers. The highest weight nodules were observed in *L. leucocephala*, however, they were only in the center (primary root). Therefore, the effectivity of the noodles only functioned for the legume not for the grass. Nodules of *G. sepium* spread over primary and secondary roots in high number including in grass area, therefore it would influence better for the grass production. The root nodules of *C. mucunoides* were quite a lot in the grass area, however, their quality was not as good. The color was black. The best quality of root nodules for nitrogen fixation is when they are pink and large.

Data of Table 5 shows that legume roots grew together with grass roots. This rhizofer system helps the translocation of nutrients including P from legume areas to grass area. The root nodules of the legumes especially those in the grass area will also help the nitrogen fixation for the grass growth. Rhizosfer zone in the grass areas will be influenced by the root legume structure (Fustec et al. 2010), while root activity significantly influences the physical, chemical and

Table 4. Root nodules of the legumes

Kind of legumes	Weight (g/plants)		Shape and colours	Position
	Legume areas	Grass areas		
G. sepium	1.38^{b}	0.45^{b}	Round, cream	Spread
L. leucocephala	3.94^{a}	0.06^{c}	Large, pink	Centre
C. calothyrsus	0.74^{c}	0.10^{c}	Spherical, pink	Centre
C. mucunoides	1.51^{b}	0.71^{a}	Small, round, black	Spread

Different superscript letters in the same column show significant difference (P<0.05). Centre position means the noodles were only observed in the primary roots.

Table 5. The weight of legume and grass roots

Kind of legumes	Weight of legume roots (g)		Weight of grass roots (g)
	Legume areas	Grass areas	
G. sepium	81.2^{a}	9.0^{c}	135.0^{a}
L. leucocephala	60.0^{b}	27.5^{b}	150.5^{a}
C. calothyrsus	14.0^{c}	2.8^{d}	34.6^{c}
C. mucunoides	15.5^{c}	35.0^{a}	60.0^{b}

Different superscript letters in the same column show significant difference (P<0.05).

biological condition of the plants and then affects the plant growth and production. Walzi et al. (2012) showed that more legume roots enhance nutrient distribution for their companion. Surpricely our data showed that the highest weight of legume roots in the legume area and quite low in grass area observed in *G. sepium* produced heighest weight of grass root. In the opposite observed in *C. mucunoides* which had high legume root weight in legume area produced low weight of grass root. The weight root or structural of roots infleuence the grass production will be discussed later in the production paragraph.

Grass heights and shoots

The grass height was significantly influenced by kind of legumes intercropped, while number of shoots in clumps was not significantly influenced by the treatments (Table 6). The number of shoots was not significantly influenced eventhough in control, the one was not intercropped which had the highest number, 30 % than the lowest. The highest *P. maximum* was observed in the one intercropped with *G. sepium* (160.7 cm) followed with *C. callothyrsus* (140.0 cm). Grass that grew without any legume integrated showed lowest height (98.3 cm). The same result has been reported by Sajimin et al. (2005) and Sajimin & Jarmani (2014) that the height of *P. maximum* is 118.2 cm/clump in monocultures, while intercropping with *Clitoria ternatea* it reaches 156.0 cm/clump. Intercropping with legume roots produced more grass due to the transportation of nutrients from legume areas. The same result was also reported by Ojo et al. (2013) that *P. maximum* grown faster if intercropped with a legume of *Lablab purpureus* compared with the one without. Onyeonogu & Asiegbu (2013) the highest tiller number per meter square was obtained in *P. maximum* intercropping with the legumes *Stylosanthes hamata*.

Forage production

Legume forage production was significantly influenced by kind of legumes (Table 7). The highest forage production of the legumes was observed in *C. mucunoides* followed by the others. This plant is a shrub legume, while others are tree legumes. This experiment was more to see the grass production, however, since the legumes also share the forage production. The legume production should be noticed. Each legume species significantly produced different forage amounts due to the difference in morphology and genetics. This legume had the highest root weight in legume area (Table 4) which may take part in using the nutritional elements from the grass soil for the legume growth resulted the reduction of grass production.

Table 6. Heights and shoot numbers per clumps of grass grown integrated with legumes

Kind of legumes	Height of grass (cm)	Number of shoots per clumps
G. sepium	160.7^{a}	9.8
L. leucocephala	135.5^{b}	12.3
C. calothyrsus	140.0^{ab}	9.5
C. mucunoides	125.5^{b}	10.8
Control	98.3^{c}	15.0

Different superscript letters in the same column show significant difference (P<0.05).

Table 7. Forage production of legumes and *P. maximum* cv Riversdale intercropped with the legume roots

Kind of legumes	Forage production (g DM/pot)	
	Legumes	*P. maximum*
G. sepium	21.0^{c}	54.7^{a}
L. leucocephala	21.1^{c}	50.7^{ab}
C. calothyrsus	25.4^{bc}	47.4^{ab}
C. mucunoides	51.9^{a}	30.0^{b}
Control	No plants	40.7^{ab}

Different superscript letters in the same column show significant difference (P<0.05).

Except for *C. mucunoides* the productions of grass intercropped with legumes were significantly higher than the control. The highest production of grass was observed at the one intercropped with *G. sepium* (54.7 g/plant) followed by the one with *L. leucocephala* (50.7 g), *Caliandra* (47.4 g), while the one with *C. mucunoides* had the lowest (30.0 g) (Table 7). The production of *L. leucocephala* was not significantly different to those of *G. sepium* and *C. calothyrsus.* The lowest grass production in the one with *C. mucunoides* might be related with short grass due to limited nutrient caused by poor quality of legume root nodules (Table 4 and 6), and the least weight of grass root. The intercropping system was not affected for the grass production, however it produced the highest legume forage.

All the effected intercroppings for grass productions were from tree legumes, those were *G. sepium*, *L. leucocephala*, and *C. calothyrsus*. The leaves of the height plants might drop to grass area and result the increase of nutritional elements in the grass soil, opposite to *C. mucunoides* which was quite short and only dropped its leaves in legume areas. All tree legumes gave higher P translocation higher than the negative control (Table 1). In the top of that the *Rhizobium* in their nodules which have the ability to fix the nitrogen will take part in improving the growth. The same results those grass biomass productions were increased by legume intercropping have been reported by Baba et al. (2011) and Abdullah et al. (2014). Darmadeh (2013) also reports that integration of peanut increases the biomass production of maize.

Intercropping with *G. sepium* gave the highest production, since it translocated the highest P to the soil grass, produced more effective root nodules, as well as the tallest grass and heighest grass root weight. Although the grass production was the highest, the legume production was quite low. Therefore, the total amount of the forage production from grass and legume was comparable with the one intercropped with *C. mucunoides*.This experiment was carried out in the pot or in the limited area. However, it already showed the positive effect of legume intercropping. Scaling up should be evaluated in the farm and the evaluation also should consider the forage production of the legumes.

CONCLUSION

It can be concluded from the experiment that intercropping *Panicum* grass with the legume roots increase P availability in the grass area. The P translocation was proved by the detection of ^{32}P radio isotop injected in the legume area. The increase of the P in the grass area and the activity of *Rhizobium* in nitrogen fixation enhanced the production of the grass. The best grass production was observed when the grass was intercropped with *G. sepium*.

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Effect of Techniques and Time of Sowing, Seed Rate, and Weed Management on Selected Herbaceous Legumes Establishments in East Nusa Tenggara, Indonesia

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ABSTRAK

Kana-Hau D, Nulik J. 2016. Pengaruh dari teknik dan waktu menabur, tingkat benih dan pengelolaan gulma pada perusahaan kacang herba terpilih di Nusa Tenggara Timur, Indonesia. JITV 21(3): 159-164. DOI: http://dx.doi.org/10.14334/jitv.v21i3.1586

Suatu seri penelitian cara dan waktu tanam dan manajemen gulma bagi beberapa species tanaman pakan leguminosa herba terpilih (*Clitoria ternatea* cv Milgarra, *Centrosema pascuorum* cv Cavalcade dan cv Bundey, dan *Lablab purpureus* cv Highworth) telah dilakukan di Nusa Tenggara Timur (Timor dan Flores) untuk mendapatkan cara dan waktu tanam dan manajemen gulma terbaik, yaitu antara lain menghemat tenaga kerja dan memberikan produksi biomassa yang memadai sesuai dengan potensi spesies yang diteliti. Perlakuan cara tanam meliputi tugal, garit (ditutup dan tidak ditutup), dan sebar (digaru dan tidak digaru); dan waktu tanam yang meliputi awal musim hujan (Desember-Januari), pertengahan musim hujan (Februari-Maret) dan akhir musim hujan (April-Mei), serta manajemen gulma dengan menyiang atau tanpa menyiang. Penelitian menggunakan rancangan acak berblok dengan 4 ulangan dirancang dalam plot-plot ukuran 3 x 4 m s/d 4 x 5 m, bergantung pada ketersediaan lahan untuk penelitian. Hasil penelitian menunjukkan bahwa cara tanam terbaik dengan populasi tanaman tertinggi (42 tanaman/m² pada minggu ke 4 setelah tanam) dan produksi biomassa terbesar dipeoleh pada cara tugal (1,75 s/d 2,5 ton BK/ha per panen pada 12 minggu setelah tanam di Ende dan 4-5 ton BK/ha di Nagekeo), diikuti oleh cara tanam digarit tutup (1-1,3 ton BK/ha di Ende dan 3,5-4 ton BK/ha di Nagekeo), dan terendah pada cara sebar (9-20 tanaman/m² dan produksi biomassa 2-2,5 ton BK/ha di Nagekeo dan 0,5-1 ton BK/ha di Ende pada panen tanaman umur 12 minggu). Manejemen gulma pada leguminosa menunjukkan bahwa penyiangan nyata memberikan produksi biomasa yang lebih tinggi dibandingkan dengan tanpa penyiangan. Nampak pula bahwa pengaruh gulma lebih besar pada spesies *Clitoria ternatea* dibandingkan dengan pada spesies *Lablab purpureus*, terutama pada penanaman awal musim hujan.

Kata Kunci: Leguminosa Herba, *Clitoria ternatea*, *Centrosema pascuorum*, *Lablab purpureus*, NTT, Cara Tanam, Waktu Tanam, *Establishment*

ABSTRACT

Kana-Hau D, Nulik J. 2016. Effect of techniques and time of sowing, seed rate, and weed management on selected herbaceous legumes establishments in East Nusa Tenggara, Indonesia. JITV 21(3): 159-164. DOI: http://dx.doi.org/10.14334/jitv.v21i3.1586

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.

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

INTRODUCTION

Legumes as fodder plants in general, including herbaceous legumes, have higher nutritive values compared to grasses (native an introduction), especially in protein content (±5-10% vs >15-30%) (Kana-Hau 2014; Tuna et al. 2004; Evitayani et al. 2004). Herbaceous legumes can be planted in monoculture and or in mixture (in rotation or relay with grasses or food crops of cereal). Integrating with maize crops can be done by planting when the maize has reach its flowering period (anthesis) or earlier when maize plant has just reached a man's knee hight (Nulik et al. 2013; Abera 2012). Legumes can contribute to the farming systems through their ability in fixing free N_2 from the air, prevent soil erosion, reducing evaporation through soil cover (Gourley et al. 2014; Pipai et al. 2014), and provide high quality fodder and even food for human consumptions (Kana-Hau 2014; Nulik et al. 2013).

Several problems are faced when cultivating herbaceous forage legumes in East Nusa Tenggara (ENT), either in monoculture or in mixture with cereals, i.e. competition with weeds and the shortage of labor during the planting season for planting and weeding. Weeding may need upto 45% of the labor time in dryland farming systems in East Nusa Tenggara (Ngongo et al. 2005). To achieve proper forage plant establishment, several factors need to be considered and to be properly understood, i.e. technique and time of sowing, seed rate, and weed management which all are important to be understood in the dryland farming systems in ENT province.

Good establishment would expect to have good number of plant population with even distribution in the plot, which will determine good biomass production and good contribution of N fixation to be available for the forage itself and to the subsequent food crops planting in the integrated systems (Nulik et al. 2013). In order to determine proper techniques and time of sowing, and weed management to achieve excellent plant establishment of several selected adapted herbaceous legume species in ENT a series of experiments has been conducted between 2014/2015 and 2015/2016 in the two of three major islands in the province, i.e. Timor, and Flores. Good plant establishment is expected to provide quick land surface cover, and thus able to suppress the growth of weeds and can provide optimum biomass production in a relatively short time (3-4 months) (Nulik et al. 2013). Herbaceous legume species such as *Lablab purpureus* with its quick initial growth, was found to be significantly suppressed weed growth, which was grown to overcome weed invasion in coffee plantations (Santos et al. 2016).

MATERIALS AND METHODS

This experiment was conducted in a range of trials separately in several places in ENT (West Timor, and Flores), Indonesia (Table 1.), employing randomized block design, having 4 replications in the years 2015/2015 and 2015/2016.

Selected adapted herbaceous legumes were used in the experiments included: *Clitoria ternatea* cv Milgarra, *Centrosema pascuorum* cv Cavalcade dan Bundey, and *Lablab purpureus* cv Highworth (*Dolichos lablab*). Treatments consisted of sowing techniques: (i) dibble, (ii) furrowed rows, (iii) furrowed rows covered, (iv) and broadcast combined with treatment for seed rates: (i) low (5-6 kg/ha), and (ii) high (10-12 kg/ha). Treatments of sowing time: (i) Early wet season (November-December), (ii) mid-season (February-March), and (iii) end of wet season (April-May). Sites: West Timor (Naibonat-Kupang, and So'e-TTS), and Flores (Wologai and Wolofeo in Ende, and Mbay in Nagekeo), and treatment of weed management: (i) weeded, and (ii) unweeded.

The plants were planted in plot sizes of 3 x 4 m to 4 x 5 m depending on the availability of land for experiments at the locations stated above.

Data collected included plant population at 4 and 8 weeks after planting, and biomass production harvested at about $\geq$12 weeks. Collected data were then analyzed using ANOVA using the software of QUASP (Queensland University Agriculture Statistical Package), at which further test of *Lsd* (*Least significant difference test*) was conducted for the difference of means.

Table 1. Sites details, species, and experiment year

Location	Soil type	Species	Research	Planting year
Kupang	Sandy Vertisol	*Lablab purpureus*	Planting time	2015, 2016
TTS	Inceptisol	*Lablab purpureus*	Planting time	2014, 2015, 2016
Nagekeo	Inceptisol	*Clitoria ternatea*, *Centrosema pascuorum*	Sowing techniques	2015, 2016
Ende	Volkanik (Molisol)	*Clitoria ternatea* *Centrosema pascorum*	Sowing techniques	2015, 2016

RESULTS AND DISCUSSIONS

Planting techniques and seed rates

Plant population

Plant population, especially at 4 weeks after planting (in Ende), was highly significantly ($P<0.001$) affected by planting techniques (Table 2), having the highest plant population in the dibbling technique treatment (40 plants/m²), followed by furrowed technique and uncovered/unharrowed (28 plants/m²), then furrowed covered/harrowed or compacted (21 plants/m²), while the lowest plant population was obtained in the broadcast technique (9 plants/m²). This finding was similar to those obtained in Nagekeo experiment, where the highest plant population was achieved in the dibbling technique treatment (42 plants/m²), followed by furrowed and unharrowed technique (34 plants/m²), then furrowed and harrowed (27 plants/m²), and the lowest was observed in the broadcast and harrowed (20 plants/m²). Nicodemo et al. (2015) obtained that highest plant population was encountered in the broadcast on land treated with harrow before and after seeds were sown (28.5 plants/m²) while the lowest plant number (9.4 plants/m²) was obtained in the broadcast only technique without harrowing the land. At this we observed that the harrowed land provides good seed contact with the soil, and thus better seedling emergences.

The current findings in East Nusa Tenggara were actually often investigated in many occasions that the dibbling technique was always the best planting technique for herbaceous legumes for seedlings emergence and establishments. However, there is another important factor to be considered in forage legumes planting in the region, that is the shortage of labor during the planting time, as much of labor (hired and or in the family) was concentrated in the cultivation of food crops during the season. The thought to conduct planting techniques experiment was related to the shortage of labor for planting the legumes during the planting season in the region especially if dibble planting technique is tube operated. Other reason for conducting these experiments was based on previous experience with the success of broadcasting mungbean seed for establishment after the harvest of rain fed rice in Kupang, which gave excellent results in terms of even distribution of plants and grain production (Tony Basuki, personal communication), and experience in broadcasting seeds of *Centrosema pascuorum* and *Macroptilium atropurpureum* into the land after corn was harvested (personal experience), having still some residual plant materials. Expecting that these experiences can also be achieved from the current experiments for forage legumes, having similar seed characters. However, differences in the condition of the current land preparation and water erosion (surface run off), results were significantly different to that of the mungbean. The current land was cleaned from any plant materials (weeds and plant residues) thus perfect clean, while at the broadcast plating of mungbean, the land was still occupied by the stumps of the rice after harvest and some residual of the plants (straw). At mungbean broadcast trials seeds were positioned under the plant residues of rice straw or near to the stump of harvested rice plants which were able to prevent erosion by water (surface run off) and allowed proper contact with soil which all resulted in better seed germination and emergence. The plant residues and stumps also prevent the seed from the direct sunlight and thus prevent seed desiccations, as also obtained in many other experiments of seed sowing into available plant residuals i.e. in alfalfa (*Medicago sativa*) broadcast. While the current herbaceous legumes experiment lands were prepared by proper tillage's and proper cleaning of the lands from plant materials, which may have caused the failure of even distribution of plants as it may be affected by water erosion. Further work may be needed to broadcast into residual plants, such as rice or maize.

Actually, plant population of 9-20 seedlings/m² obtained in the broadcast treatments in Nagekeo should be sufficient to have good soil cover and thus biomass production, provided that the distribution was even, however because the effect of water erosion (surface run off), the distribution was not even and thus growth was not properly achieved (Figure 1) as well as biomass production. Furrowed treatment gave quite good plant

population and distribution, and thus for the purpose of labor shortage problem can be recommended besides dibbling technique (when labor is of no restriction).

Biomass Production

Biomass production (Table 2) was best achieved when sowing was conducted in the technique of dibbling the seed ($P<0.05$) with plant population of >40 plants/m², followed by furrowed planting technique with plant population of >22 plants/m² (covered or not covered with soil), and the lowest plant population was given by the broadcast technique with plant population of 9 to 20 plants/m². Actually, the population of 9 to 20 plants/m² would be sufficient for having proper land cover and biomass production, provided that the distribution was even for the species (*Clitoria ternatea* and *Centrosema pascuorum*), however the current experiment obtained uneven plant distribution on the broadcast technique (Figure 1). As the sowing technique experiment was to obtain effective and efficient planting to quickly achieve soil cover and good biomass production, especially with less labor use, where dibbling technique needs labor intensive during the planting season (December to February) when most family labor is concentrated in tending and taking care for food crops cultivation (planting and weeding).

Figure 1. Plant population, distribution and soil cover in dibble sowing techniques (left: above and below), furrowed (middle: above and below) and broadcast techniques (right: above and below) of *Clitoria ternatea* in Nagekeo, Flores.

Table 2. Effects of sowing techniques and seed rate on the production of biomass (kg/ha) of *C. pascuorum* and *C. ternatea* in Ende (left) planted in 2015 and Nagekeo (right) in 2016

Location	Species	Seed rate	Sowing Techniques				Lsd (5%)	
			Dibbling	Furrow	Broadcast harrowed	Broadcast		
Ende	*C. pascuorum*	Low	981	650	1008	331	Rate	615.7
		High	1746	846	1157	562	Sowing	870.8
	C.ternatea	Low	2386	1225	924	558	Species	615.7
		High	2603	1396	1640	504		
Nagekeo	*C.ternatea*	Low	4154	3572	2634	2563	Rate	930.6
		High	5086	4156	3750	2246	Sowing	1316.1

Sowing time

Research conducted in Naibonat-Kupang and Soe-TTS found that the best planting time for herbaceous legumes (*Lablab purpureus* and *Clitoria ternatea*) is in the early wet season (December-January). The reason for farmers in ENT to plant grain legumes (mungbean, peanut) when rain get lessen (around February to March), because the fact that when planting conducted in early wet season it will produce more vegetative materials compared to production of grain. High rainfall during vegetative growth may reduce seed production of peanut (Rahmianna et al. 2015). Mung bean is normally planted in relay method when corn is about to be harvested in the region (Murdolelono 2011). However as herbaceous forage legumes, the important part is to produce forage (leaf material) so the best planting time was in the early wet season, though planting in the mid of the wet season will also give reasonable biomass and seed production too. This will happen to the planting of legumes in the time when the maize plants have reach the adult's knee height or when maize has reached anthesis phase, which will be about the mid toward the end of the wet season (February to March), which however will produce reasonable forage amount for the purpose of effectively make use of the land and remaining soil moisture (Nulik et al. 2013).

Weed Management

Result indicated that weed investation significantly ($P<0.05$) decreased biomass production (Figure 2), especially for *Clitoria ternatea* than that of for *Lablab purpureus*. Despite that weed can affect legume biomass production, legume with quick growth (Santos et al. 2016) and of tall species (Storkey et al. 2011) can also suppress weed growth, such as *Lablab purpureus*, when it was used in the coffee plantation to overcome the growth of weeds.

Early wet season planting and proper seed rate were expected to give good plant establishment thus quick land cover to prevent weed growth and therefore less work for weeding. However, planting time and seed rate were conducted separately, thus the certainty of the combination effects on weed investation could not be concluded yet and need further experiment. Santos et al. 2016 found that *Lablab purpureus* showed quick establishment growth in the first year compared with *Macroptilium atropurpureum* (Siratro) and *Arachis pintoi* and thus significantly suppressed weed growth in coffee plantation, and the latter two species started to suppressed weed in the second year of their growth. Cutting management may also improve *C. ternatea* competition with weeds (Gomez & Kalamani 2003). Collins & Grundy 2005 suggested to incorporate grasses into *C. ternatea* in the second year to make use of the improved availability of N which could otherwise be taken by invaded weeds and meanwhile forcing the legume to fix more N, as the legume will tend to stop fixing N in the second year when soil N is high. The quick growth of *Lablab purpureus* in the first establishment year is related to its nature characters (Valenzuela & Smith 2002) which can produce biomass of up to 6 tons DM/ha when harvested in 3-4 months after planting (Amole et al. 2013), even up to 8 tons DM/ha under favorable condition (good rain and soil) in the current experiment in Naibonat-Kupang.

CONCLUSION

The best panting technique for the legumes *C. ternatea* and *C. pascuorum* is by dibbling the seed, however considering the labor shortage problem during the planting season (December – March), the practical planting technique would be by planting the seed in the rows of furrow, which should sufficiently give proper plant population. While the best planting time for forage legumes, was at the early wet season, especially if planting in monoculture for the purpose of rotational planting with cereal crops in the subsequent planting season.

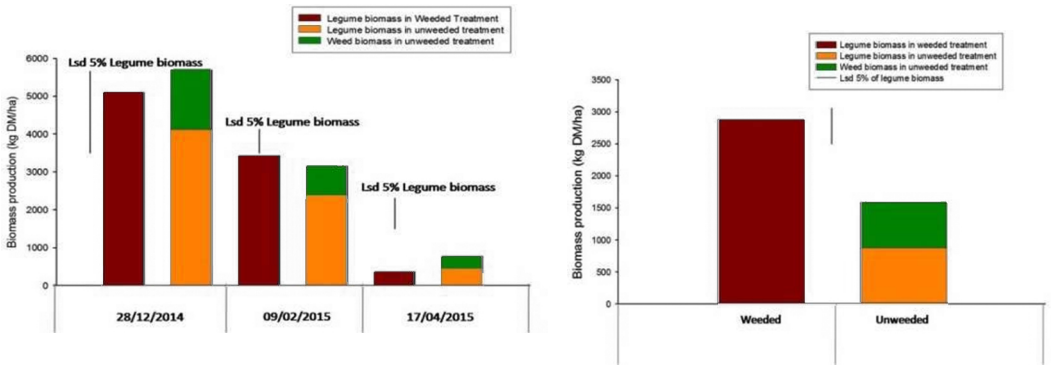

Figure 3. Effect of weed management and planting time on *Lablab purpureus* (left) and weed management on *Clitoria ternatea* (right) on biomass production.

In order to obtain good herbaceous forage legume growth, having high biomass forage production, it is important to do weeding properly. Dibble sowing techniques gave the best plant population and biomass production in the *establishment* of *C. ternatea* and *C. pascuorum* with higher plant population number at 4 weeks and 8 weeks after sowing, however considering labor shortage during the planting season, it would be better to recommend furrow planting technique (harrowed and not harrowed). Moreover there is a need to pursue more on the broadcast technique under condition of land having plant residues for its benefit in better seed contact with soil and prevention of water erosion (surface run off) that may wash away the seed after sowing.

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Response of Sheep Fed on Corn Cob Silage or Elephant Grass Basal Diet with or without Calliandra Leaf Meal Supplementation

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ABSTRAK

Yulistiani D. 2016. Respon domba yang diberi pakan dasar silase tongkol jagung atau rumput gajah dengan atau tanpa suplementasi Kaliandra. JITV 21(3): 165-173. DOI: http://dx.doi.org/10.14334/jitv.v21i3.1574

Terbatasnya ketersediaan hijauan pakan sepanjang tahun dapat diatasi melalui penggunaan hasil samping pertanian. Tongkol jagung, hasil samping produksi jagung potensial untuk digunakan sebagai sumber serat pengganti rumput. Tujuan dari penelitian ini untuk membandingkan pengaruh dua jenis pakan dasar (rumput dan silase tongkol jagung) dengan dan tanpa suplementasi tepung daun kaliandra terhadap konsumsi, kecernaan pakan, penggunaan nitrogen, fermentasi rumen dan pertumbuhan domba. Penelitian menggunakan 20 ekor anak domba St Croix jantan lepas sapih. Domba dikelompok menjadi 5 kelompok berdasarkan bobot badan. Tiap ekor domba dalam tiap kelompok diberikan salah satu dari 4 pakan perlakuan selama 13 minggu. Pakan perlakuan yang diberikan adalah: pakan dasar rumput + konsentrat, pakan dasar silase tongkol jagung (CCS) + konsentrat, pakan dasar rumput + konsentrat+5% tepung daun kaliandra, CCS + konsentrat+5% tepung daun kaliandra. Imbangan pakan dasar (rumput atau silase tongkol jagung) dengan konsentrat adalah 40 : 60% dan diformulasikan secara iso-protein (kandungan protein kasar 14%) pakan diberikan secara total mixed ration. Penelitian dilakukan menggunakan rancangan acak kelompok pola faktorial 2x2 (2 tipe pakan dasar dan 2 level suplementasi kaliandra) dengan 5 ulangan. Hasil penelitian menunjukkan tidak ada interaksi antara pakan dasar dan suplementasi kaliandara terhadap konsumsi pakan, pertambahan bobot badan, kecernaan pakan dan fermentasi rumen, kecuali pada konsumsi protein kasar. Konsumsi pakan tidak dipengaruhi oleh pakan dasar dan suplementasi kaliandra. Ratio konversi pakan dan ratio konversi protein lebih baik pada pakan dasar rumput dibanding silase tongkol jagung. Kecernaan protein kasar di pakan dasar silase tongkol jagung lebih tinggi dibanding pakan dasar rumput yang tanpa diberi suplementasi tepung daun kaliandra. Retensi nitrogen lebih tinggi di pakan dasar CCS dibanding pakan dasar rumput. Fermentasi rumen dipengaruhi oleh jenis pakan dasar dimana konsentrasi rumen ammonia, total VFA dan proporsi propionat lebih tinggi pada pakan dasar rumput. Dari penelitian ini dapat disimpulkan bahwa pada pakan iso protein pakan dasar rumput sebanding dengan pakan dasar silase tongkol jagung seperti yang ditunjukkan dengan pertambahan bobot badan harian yang sama (107,5 g/ekor/hari) pada kedua jenis pakan tersebut. Suplementasi tepung dau kaliandra pada level 5% tidak meningkatkan tampilan domba.

Kata Kunci: Tongkol Jagung, Kaliandra, Domba, Rumput Gajah

ABSTRACT

Yulistiani D. 2016. Response of sheep fed on corn cob silage or elephant grass basal diet with or without Calliandra leaf meal supplementation. JITV 21(3): 165-173. DOI: http://dx.doi.org/10.14334/jitv.v21i3.1574

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.

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

INTRODUCTION

Ruminant productivity in Indonesia is constrained by the shortage of feed availability either in quantity or quality, particularly in a dry season when the availability of forage is limited. This condition becomes critical due to the priority of land use is for food crop planting as well as the conversion of grazing area for settlement and infrastructure. Limited availability of forage diet could be overcome by utilization of crop by-products. One of the potential crop by products is a by-product from corn production. Corn is one of food crops targeted by Indonesian Government to be self-sufficient domestically. The increase of corn production will be followed by the increase in corn by-products, one of them is corn cob. Corn cob is potential to be used as a roughage source for grass replacement. Ground corn cob can be used as a basal diet for sheep up to 40% (Yulistiani & Puastuti 2012). Wanapat et al. (2012) reported that ground corn cob was able to replace cassava chip in concentrate diet for swamp buffaloes, while Wachirapakorn et al. (2014) used corn cob mixed with rice straw as a roughage source in the total mixed ration for lactating dairy cow. Corn cob is not palatable and easily contaminated by fungi *Aspergillus flavus* which is very toxic to the animal. Ensiling is able to increase corn cob palatability as indicated by the high intake of corn cob silage (Yulistiani & Puastuti 2012) and able to prevent fungal contamination.

High productivity state of ruminants animal (growth, pregnancy, lactation) their nutrient requirement are higher than for maintenance. Their Protein requirement can not be met from rumen microbial supply. Therefore, to achieve the optimal productivity ruminants must be offered diet containing rumen undegradable protein sources that are digestible (Mustafa et al. 2000; Saricicek 2000) such as legume forage containing tannin.

Calliandra is one of the legumes containing condensed tannin (CT) which is able to grow in infertile soil and can be used as bypass protein supplement. CT content in Calliandra tends to be higher in infertile soil. The range of CT content in Calliandra was 19.9-24.7% (Tiemann et al. 2010). Besides its potential in supplying bypass protein, Calliandra was able to reduce enteric methane emission by 24% when non-tanniniferous legume was replaced by Calliandra at 30% (Tiemann et al. 2008).

Corn cob silage was potential to be used as a roughage source to replace a freshly chopped grass diet. However, its productivity was still lower than basal grass diet (Yulistiani & Puastuti 2012). Therefore, an attempt should be made to increase sheep growth rate in corn cob basal diet by supplementing with by-pass protein. To the best of the author knowledge, no information is available on the Calliandra supplementation in corn cob silage basal diet for sheep. The objective of the study was to compare the 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.

MATERIALS AND METHODS

Feeds, animal feeding, and experimental design

Corn cob silage was prepared by mixing ground corn cob (90% DM) and fine ground corn grain at 2% of corn cob (w/w). The mixture was then sprayed with water to obtain DM 40% and kept in air tight plastic bag for at least 21 days. After 21 days, the silage was ready to be used for sheep feeding.

Calliandra leaf meal was prepared by drying the Calliandra foliage under the sun for 3 days or until it was dry (DM 85%), and ground using hammer mill with a sieving size of 5 mm.

Animal management and feeding experiment: In this study used 20 male St Croix sheep with an average initial body weight of 15.93±3.0 kg were used. In growth trial, sheep were kept in an individual pen for 12 weeks (including 1 week adaptation period) and fed on a diet mixture of chopped elephant grass (*Pennisetum purpureum*) or corn cob silage with concentrate. The diet was given in total mix ration. The ratio of basal diet (grass or corn cob silage) with concentrate was 40: 60%. The ration was formulated in iso-nitrogenous (CP 14%). Diets were fed twice daily in equal portion (09.00 am and 15.00 pm). The nutrient content of the feed used in the study is presented in Table 1. The sheep were divided into 5 groups based on body weight, where each lamb in each group was fed on one of four dietary treatments. The dietary treatments were as follows:

1. Elephant Grass + Concentrate
2. Elephant Grass + Concentrate + 5% Calliandra leaf meal.
3. Corn cob silage + Concentrate
4. Corn cob silage + Concentrate + 5% Calliandara leaf meal.

Before the study, sheep were dewormed using commercial de-wormer (valbazen). For estimating nutrient digestibility at the end of growth trial, sheep

Table 1. Chemical composition of the feeds used in experimental diet

Parameter	Chemical composition (% DM)			
	OM	CP	NDF	ADF
Elephant grass	87.5	6.5	68.3	48.6
Corn cob silage	96.6	3.5	75.7	42.7
Concentrate for grass based diet mixture	92.5	19	21.7	9.09
Concentrate for corn cob silage based diet mixture	91.9	22	14.9	9.74
Calliandra leaf meal	82.8	14.5	52.4	41.29

OM, organic matter; CP, crude protein; NDF, neutral detergent fibre, ADF, acid detergent fiber.

were moved to metabolism cages for 2 weeks: comprising of a week each for adaptation and collection periods.

The experiment was conducted in a randomized complete block design and arranged in factorial 2 x 2 consisted of 2 different basal diets of elephant grass and corn cob silage and that 2 supplementation levels with or without Calliandra. Calliandra leaf meal (5%) was added to the ration.

Parameters recorded

Parameters recorded were feed consumption, growth rate, nutrient digestibility, feed efficiency, N balance and rumen characteristics. Feed consumption was measured daily by calculating the difference between feed offered and refused.

Daily feed offered was weighed, and refusal was also weighed in the morning before the feed was given. Feed digestibility was measured using total collection method by daily weighing feed offered, refusal and fecal excreted for 7 days. For measuring nitrogen (N) balance, N in the feed, feces and in urine was analyzed. Urine production was measured daily: urine was collected in a bucket containing 5 ml concentrated H_2SO_4. Feces, feed offered, and refusal was sub-sampled (100 g) and dried in an oven for 7 days. Samples from each animal in each day were taken 10% and bulked for each animal and ground for chemical analysis. After 8 days of the collection period, rumen fluid of each sheep was collected using a stomach tube. Rumen fluid was collected at 3 hours after morning feed. Rumen fluid was analyzed for pH and ammonia (NH_3-N) and VFA concentration. Growth rates of sheep were obtained by bi-weekly weighing in the morning before feeding. Nitrogen balance was measured by calculating the difference between N intake and N in urine and feces.

Sample analysis

Crude protein, dry matter, organic matter analyses were done according to the method of AOAC (1990), while NDF and ADF analyses were carried out according to the method of Van Soest et al. (1991). Rumen ammonia was analyzed using Conway's method, while VFA was analyzed using gas chromatography.

Statistical analysis

Data were analyzed using ANOVA of SAS program 9.1 (SAS 2004) and that differences among means were compared using Duncan's multiple range test. All the differences were stated at 5%.

RESULTS AND DISCUSSION

DM, CP intake, average daily gain (ADG) and feed conversion are shown in Table 2. There was no interaction effect of basal diet and Calliandra supplementation or main effect of basal diet and supplementation on feed consumption (DMI and CP intake), ADG and feed conversion. The average DMI intake of all treatment was 3.89 %. This level of DM intake was higher than the level suggested by (Kearl 1982) for sheep or NRC (2007) for growing male sheep. The consumption of corn cob silage basal diet in the current study was similar to the previous study reported by Yulistiani & Puastuti (2012).

Table 2. Feed consumption and performance of lamb fed on different feeding treatments

Variables	Main Factor				P values		
	Basal diet (B)		Supplement (%, S)				
	Grass	CCS	0	5	B	S	B x S
Dry matter intake							
Total DMI (g/head/day)	759	773	748	784	0.6367	0.4974	0.4901
DMI g/kg BW	37.6	39.3	37.6	39.3	0.3721	0.6272	0.8872
DMI/ $BW^{0,75}$	79.3	82.5	79.0	82.7	0.3424	0.5326	0.8311
% DMI/BW	4.03	3.76	3.75	3.93	0.3716	0.6286	0.8887
CP intake (g/head/day)	121.2	135.5	126.2	130.5	0.1963	0.6962	0.3797
CP intake g/kg BW	6.06[b]	6.83a	6.34	6.55	<0.0001	0.0872	0.0272
ADG (g/head/day)	111.5	104.2	103.2	113.2	0.3588	0.2034	0.7635
Feed conversion ratio	6.85[a]	8.20[b]	7.71	7.31	0.0233	0.4898	0.3210
Protein conversion ratio	1.09[b]	1.44[a]	1.32	1.22	0.0022	0.3049	0.1149

Different letter in one row indicated significant different (P<0.05); CCS, corn cob silage; DMI, dry matter intake; CP crude protein; ADG, average daily gain.

Intake of CP was significantly (P<0.05) affected by the interaction of basal diet and Calliandra supplementation. Calliandra supplementation was able to increase CP intake in a grass basal diet. Calliandra supplementation was not able to increase CP intake in the CCS basal diet, due to the CP content (14.5%) of Calliandra used in the current study was low status. The low protein content of Calliandra used in present study was relatively to a very mature and mixed with stem during pruning. Hence, supplementation of 5% Calliandra did not increase the CP content of the diet. Acero-Camelo et al. (2009) also reported low CP content of Calliandra (14.3%). The CP content of Calliandra decreased with increasing plant maturity from 21% at 6 weeks to 18% at 12 weeks cutting intervals (Abqoriyah et al. 2015) and from 23.2 to 15.6% from six to 16 weeks cutting intervals (Kabi & Bareeba 2008).

The ADG in all treatments was similar in a current experiment with average 107.8 g/head/day. Kearl (1982) recommended that the CP required by sheep with a body weight of 15 kg getting a daily body gain of 100 g/day was estimated to be 100 g CP/day and digestible intake protein (DIP) of 80 g/day. NRC suggested that lower amount of digestible protein intake (100 g LW/day) was required for growth which was 70 g/day. The average DIP in the present study was 84.5, 109.7, 97.0 and 95.3 g/day respectively for grass, corn cob, 0 and 5% Calliandra supplementation. Those values of DIP intake in the present study were higher than suggested by Kearl (1982) and NRC (2007). In this study, only grass basal diet showed similar DIP intake to Kearl (1982) recommendation and the ADG obtained was 111.5 g/head/day. The ADG obtained in the present study was higher than that previously reported for sheep fed on CCS and grass basal diets of Sumatera Cross Bred sheep having ADG of 59.4 in CCS diet and 80.3 g/head/day in grass basal diet (Yulistiani & Puastuti 2012). This difference could be associated with the breed of sheep in the study. Results of previous study (Yulistiani et al. 2015) with similar breed of sheep (St Croix) showed DMI (3.7% BW) and CP intake (4.5 g/kg BW) were lower and resulted low in ADG (72.8 g/day) compared to the current study ADG (107.5 g/day). The higher nutrient intake of the present research resulted in better performance of sheep than the previous study.

No response to Calliandra supplementation on goat growth rate was also reported by Acero-Camelo et al. (2009), where the supplementation of 20% fresh Calliandra leaves did not significantly increase the body weight of goat grazed on native grass pastures *(Dichanthiumannulatum, Heteropoguncontortus, Cynodondactylon).*

Feed conversion ratio and protein conversion ratio were affected by the type of basal diets. The feed conversion and protein conversion ratio of grass basal diet is better (P<0.05) than CCS basal diet. Although the ADG and feed consumption were not affected (P>0.05) by treatments, feed conversion ratio, and protein conversion ratio was better in grass basal diet

Table 3. Nutrient digestibility of sheep fed on different diet treatments

Variables	Main Factor				P values		
	Basal diet (B)		Supplement (%, S)				
	Grass	CCS	0	5	B	S	B x S
Dry matter	62.0	64.1	63.7	62.1	0.2843	0.4091	0.7902
Organic matter	67.1	68.9	70.4	65.7	0.4599	0.0636	0.2784
Crude protein	69.7b	81.0a	77.4x	73.3y	0.0001	0.0094	0.1800
Neutral detergent fiber	49.4	55.8	54.4	51.1	0.0686	0.1988	0.8067
Acid detergent fiber	49.5	54.9	55.6	50.2	0.2370	0.2764	0.6990

Different letter in one row indicated significant different (P<0.05); CCS, corn cob silage

Table 4. Nitrogen (N) utilization in sheep fed on different diet treatments

Variables	Main Factor				P values		
	Basal diet (B)		Supplement (%, S)				
	Grass	CCS	0	5	B	S	B x S
N intake (g)	18.91b	23.0a	20.93	20.98	0.0003	0.9527	0.3200
N feses (g)	5.75a	4.35b	4.57x	5.52b	0.0050	0.0382	0.5246
N urine (g)	5.31	5.37	5.31	5.37	0.8736	0.8283	0.5187
N absorbtion (g)	13.16b	18.64a	16.35	15.45	0.0001	0.1395	0.0795
N retention (g)	7.85b	13.2a	11.0	10.1	0.0001	0.1242	0.0634
% N feses/ intake	30.30a	19.0b	22.6x	26.6y	0.0001	0.0094	0.1800
% N urine/ intake	28.1a	23.3b	25.3	25.6	0.0437	0.9726	0.2192
% N absorbn/intake	69.7b	81.0a	77.4x	73.3y	0.0001	0.0094	0.1800
% N retention/ intake	41.5b	57.7a	52.8x	48.1y	0.0001	0.0219	0.1626

Different letter in one row indicated significantly different (P<0.05); CCS, corn cob silage

than CCS basal diet. This is attributed by feed conversion ratio, and protein conversion ratio which was calculated from feed intake (DMI and CPI) divided by ADG. Therefore, the higher ADG and lower DMI and CPI in grass basal diet resulted in better feed conversion ratio and protein conversion ratio. This indicates that basal grass diet was more efficient than CCS basal diet.

Nutrient digestibility of DM, OM, NDF, ADF, and CP from treatment diet was not affected by either interaction of basal diet and Calliandra supplementation or by the main effect of basal diet and supplementation, except for CP (Table 3). CP digestibility was affected by the type of basal diets and levels of Calliandra supplementation. CP digestibility of corn cob basal diet was higher than grass basal diet. Lima et al. (2011) reported that CP digestibility of sorghum silage was higher than the fresh sorghum forage.

CP digestibility with Calliandra was lower than without supplementation. This lower CP digestibility in Calliandra supplemented diet could be related to tannin content in Calliandra. The presence of condensed tannin CT) in the feed formed CT-protein complexes during mastication which is resistant to degradation in the rumen (Makkar 2003).This CT-protein complexes pass through the rumen and then partially broken down in the abomasums and duodenum at pH 2-3 resulted in higher fecal N (Tiemann et al. 2008). In the case of tannin in Calliandra, it was reported that its protein-tannin complex had the lowest digestibility from abomasums through feces than other legumes (*Leucaena* sp) (Kariuki & Norton 2008). Thus resulted

in the higher N content in feces or lower CP digestibility of Calliandra supplemented the diet.

Nitrogen (N) utilization of sheep fed on treatment diet was not affected by the interaction between type of basal diet and Calliandra supplementation (Table 4). All N utilization parameters recorded (in g/day) were affected by the type of basal diets except for N excreted through urine. However, when the parameter was expressed in percentage of N intake, all the parameters were affected (P<0.05) by basal diets and supplement. N intake in CCS basal diet was higher than in grass basal diet (P<0.05). However, N excretion in feces of grass basal diet was higher than in CCS basal diet. The higher N excretion of grass basal diet could be due to N bound to undigested cell wall was excreted through feces. CP content in the grass is higher than CCS (Table 1) in which partly bound to the cell wall. N retention of CCS basal diet was higher than grass basal diet. This result indicates that CCS basal diet is better than grass basal diet.

Fecal N in Calliandra supplemented diet was higher than unsupplemented diet (Table 4). The increased of fecal N in Calliandra supplementation diet indicates that tannin-protein complex could be incompletely digested in the lower digestive tract, the increase of N feses was about 25% compared to unsupplemented diet. Result in the present study was supported by the result of Kai et al. (2016) who reported that the increased level of tannic acid supplementation caused in increased N excretion in feces by 25.3% at level supplementation tannic acid 2.6% in the diet. Moreover, Kai et al. (2016) reported that tannic acid supplementation caused shifting N excretion from urine to feces. Therefore, they found no difference in N retention between with or without tannic acid supplementation. In the present study, N retention was significantly higher in a diet without Calliandra supplementation. The non-significant differences of N excretion between supplemented and unsupplemented diet in the urine indicated that protein degradation of both diets was similar. It seems that supplementation of Calliandra which is expected to play an important role in protecting protein and supplying by pass protein did not occur, even more protein was excreted in the feces there by tannin protein complex in Calliandra which was not dissociated in the abomasum and lower digestive tract. It was also shown that the un-supplemented diet had 10% higher N retention. Katuromunda et al. (2012) also reported that elephant grass diet supplemented by Calliandra excreted more N in feces compared to control or to Centrosema or Desmodium. Further, Korrir et al. (2016) reported although Calliandra supplementation to wheat straw diet increased N retention, but fecal N excretion was also high.

Rumen fermentation in sheep fed on diet treatments showed there is no interaction effect of type basal diet and Calliandra supplementation on rumen fermentation (Table 5). Rumen pH was similar in all diet treatments with an average of 6.56. This pH was in the range (6.5-7.0) of ideal condition for microbial activities in the rumen for fiber and protein digestion (Wanapat & Cherdthong 2009; Calabro et al. 2008). Oelker et al. (2009) also reported similar ruminal pH of cows fed on

Table 5. Average of ruminal pH, ammonia nitrogen (NH3-N) and VFA concentration in the rumen fluid of lamb fed on diet treatments

Variables	Main Factor				P values		
	Basal diet (B)		Supplement (%, S)				
	Grass	CCS	0	5	B	S	B x S
Rumen pH	6.59	6.53	6.50	6.62	0.4805	0.1757	0.8599
Rumen NH3-N (mg/100ml)	15.4a	9.8b	11.6	12.6	0.0008	0.4125	1.000
Total VFA mMol	127.8a	104.8b	120.0	112.2	0.0001	0.1740	0.2553
Proportion VFA (%)							
Acetate	63.5b	68.2a	66.3	65.3	0.0082	0.5060	0.8637
Propionate	26.4a	20.9b	23.2	24.0	0.0048	0.6367	0.8718
Butyrate	10.01	8.74	9.14	9.61	0.1320	0.5661	0.3892
Others VFA	1.34a	0.93b	1.25	1.02	0.047	0.2324	0.3406
Ratio acetat/propionat	2.46b	3.30a	2.94	2.81	0.0024	0.5488	0.9717

Different letter in one row indicated significantly different (P<0.05); CCS, corn cob silage; VFA, volatile fatty acid.

corn silage or chopped alfalfa hay. Rumen ammonia (NH_3-N) was significantly higher in grass basal diet than the CCS diet. Feed protein in the rumen degraded into an amino acid, NH_3-N formed in the rumen from amino acid breakdown. The excess of NH_3-N which is not incorporated during microbial synthesis is absorbed from the rumen and converted into urea in the liver and excreted in the urine (Patra & Saxena 2011). The high NH_3-N in grass basal diet was due to feeding fresh forages, its protein became rapidly soluble during mastication, releasing 56-65% of protein concentration as a soluble protein in the rumen. This soluble protein degraded in the rumen and resulted in the increased rumen NH_3-N concentration (Ullyatt et al. 1975).

The similarity in rumen NH_3-N concentration between un-supplemented and Calliandra supplemented diets indicates that protein in Calliandra was not degraded in the rumen which was caused by a tannin-protein complex that resistant to microbial degradation in the rumen (Makkar 2003). It is generally agreed that tannin in feed reduced the rate of protein degradation in the rumen. The reduction of protein degradation could lower NH3-N level in the rumen (Patra & Saxena 2011). The average concentration of rumen NH_3-N in this study for un-supplemented and Calliandra supplemented diets was 12.1 mg/dl. The optimum concentration of NH_3-N suggested by Satter & Slyter (1974) was 5 mg NH_3-N/dl for rumen microbial synthesis, but higher values (10-20 mg/100 ml) had also been recommended (Preston & Leng 1987) to optimize degradation of fibrous feed. It seems that the rumen NH_3-N concentration in this study in all treatment diets was in the ideal conditions for optimizing grass or CCS basal diet. Therefore, all diet treatments had similar fiber digestibility (Table 2).

Total volatile fatty acid (VFA) production was significantly ($P<0.05$) affected by the type of basal diets (Table 5). However, Calliandra supplementation did not significantly affect total VFA production and molar proportion of VFA. Similarly, Tiemann et al. (2008) also observed that substitution of none-tanniniferous legume (Vigna) with tannin-rich legumes Calliandra or Flemingia did not change total VFA and proportion of VFA.

Total VFA production in grass basal diet was higher by 21% compared to CCS diet. The higher total VFA production was followed by the higher propionate proportion in this diet consequently the acetic to propionic acid ratio decreased. In contrast to the present study, Lima et al. (2011) reported that feeding sorghum and soybean forage mixture silage produced a higher propionic acid than fresh forage. The higher propionic acid in silage diet was due to the lactic acid formation during ensiling was converted to propionic acid in the rumen. Lactic acid originated from the fermentation of water soluble carbohydrate (McDonald et al. 2002). In the current study, CCS diet produced higher acetic acid proportion, which was related to corn cob containing high fiber (NDF content 75.7%, Table 1). The higher NDF content of CCS produced a higher proportion of acetic acid.

In the current study, grass basal diet was more efficient either in FCR or in crude protein conversion ratio. In fact, the net protein utilization (Nretained/N intake) or protein biological value (N retention/N absorption) was higher in the CCS basal diet. However, this diet was less efficient compared to a basal grass diet. Biological value is the proportion of feed protein utilized by the animal for synthesizing body tissues and compounds (McDonald et al. 2002). Animals have low ability to store amino acid in a free state when the amino acid is not required for protein synthesis it will be broken down used as an energy source. The higher total VFA production might be matched with protein availability, consequently feed conversion ratio, and protein conversion ratio in grass basal diet was better than CCS diet. More over higher production of propionic acid which is a precursor for meat production in grass basal diet caused better performance sheep in grass diet. Although ADG and feed consumption were not significantly different between treatments, the grass basal diet was more efficient than CCS diet as indicated by better feed conversion ratio and protein conversion ratio (Table 2).

In the case of Calliandra supplementation, it was expected to be able to supply bypass protein due to its tannin content protecting protein of feed from rumen microbial degradation thereby more protein was available to be absorbed in the intestine and used for building body tissue, which in turn increased lamb growth rate. However, in the present study Calliandra supplementation was not able to improve lambs growth rate as indicated the similarity of ADG in the unsupplemented diet. Although the supplemented diet protein did not degrade in the rumen, the Calliandra protein diet supplementation was excreted in feces. This could be the reason for no performance improvement of lambs fed on supplemented by 5% Calliandra leaf meal.

CONCLUSION

Corn cob silage could be used as an alternative roughage source, especially in a dry season to replace grass basal diet, since the body weight gain was similar between grass basal diet and corn cob silage diet in iso-protein diet for sheep. In addition, corn cob silage is also better in CP and N utilization. Five percents supplementation of Calliandra in the diet was not improved sheep performance significantly.

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Growth Response of Improved Native Breeds of Chicken to Diets Differed in Energy and Protein Content

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ABSTRAK

Hidayat C, Iskandar S, Sartika T , Wardhani T. 2016. Respon pertumbuhan galur ayam lokal terseleksi terhadap ransum dengan kadar energi metabolis dan protein berbeda. JITV 21(3): 174-181. DOI: http://dx.doi.org/10.14334/jitv.v21i3.1397

Penelitian ini bertujuan untuk mengetahui respon pertumbuhan galur ayam lokal hasil seleksi terhadap ransum dengan kadar energi metabolis dan protein ransum berbeda.. Tiga kelompok anak ayam hasil perkawinan galur ayam lokal terseleksi, yaitu Line 1 (♂KUB dengan ♀KUB), Line 2 (♂SenSi dengan ♀KUB) dan Line 3 (♂Gaok dengan ♀KUB), diberi tiga ransum perlakuan berbeda, yang terdiri dari ransum Diet 1 (2800 kkal ME/ kg dengan 17,81% protein kasar), Diet 2, (2950 kkal ME/ kg dengan 18,61% protein kasar) dan Diet 3 (3100 kkal ME/ kg dengan 19,25% protein kasar). Percobaan ini menggunakan rancangan percobaan faktorial 3x3. Setiap kombinasi perlakuan diulang sebanyak 7 kali dengan jumlah anak ayam umur sehari (DOC) sebanyak 5 ekor/ulangan, yang dipelihara sampai dengan umur 10 minggu. Hasil penelitian menunjukkan bahwa galur Line 2 (♂SenSi x ♀KUB) menunjukkan bobot hidup umur 10 minggu yang lebih tinggi (P<0,05), feed conversion ratio (FCR) yang rendah dan European Production Efficiency Factor (EPEF) yang tinggi, dibandingkan dengan kedua galur lainnya. Ransum yang optimum untuk galur Line 2 adalah ransum Diet 2, maka diambil kesimpulan bahwai galur ayam hasil perkawinan ♂SenSi dengan ♀KUB berpotensi untuk dimanfaatkan sebagai ayam lokal tipe pedaging komersial di Indonesia dengan menggunakan ransum optimum yang mempunyai kadar energi 2950 kcal ME/kg dengan 18,61% protein kasar.

Kata Kunci: Ayam KUB, Ayam SenSi, Ayam Gaok, Ransum

ABSTRACT

Hidayat C, Iskandar S, Sartika T , Wardhani T. 2016. Growth response of improved native breeds of chicken to diets differed in energy and protein content. JITV 21(3): 174-181. DOI: http://dx.doi.org/10.14334/jitv.v21i3.1397

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.

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

INTRODUCTION

The increase in demands of native chicken meat in the last decade in Indonesia has been followed by the finding of native KUB chicken as moderately improved native chicken breed. The KUB chicken was selected for egg production subjected to be female line in supporting the production of native day-old chick (DOC) for national meat production (Iskandar & Sartika 2014; Hidayat et al. 2011; Hidayat et al. 2014). To create KUB as female line, the IRIAP (Indonesian Research Institute for Animal Production) has also produced SenSi-chicken (Iskandar et al. 2012; Hidayat et al. 2014) and Gaok-chicken (Sartika et al. 2007) as the candidate of male lines in the IRIAP's research project to create grand parent or parent stocks of improved meat type of native chicken.

In order to have an optimum performance as an expression of genetically improved breed, the appropriate supports, such as optimum and appropriate feeding management, including nutrient requirements, is needed to increase biological and economic

efficiency. As strongly stated by Leeson (2011), the changing in commercial goals impinge on nutrient needs and feeding programs. Applegate & Angel (2014) reported that scientific community has begun to embrace the concept of return on investment of nutrient used for compositional growth or egg production. The concept has inspired our experiment to find out the optimum level of dietary energy and protein for new improved breeds of native chicken (KUB, SenSi, and Gaok chickens).

Therefore, this experiment was aimed to observe the growth response of improved breeds of native chicken raised with rations differed in dietary energy and protein content.

MATERIALS AND METHODS

Experimental animal

Three hundred and fifteen-day old chickens (DOC) of improved native breeds obtained from mating of ♂KUB-1 to ♀KUB-1 (Line 1), of ♂SenSi-1 to ♀KUB-1 (Line 2) and of ♂Gaok to ♀KUB-1 (Line 3), were subjected to three experimental rations, differed in dietary ME and dietary CP contents. Each treatment combination was replicated by seven and the experimental design used was randomized complete design in factorial arrangement 3 x 3. In each treatment combination there were five unsexed-DOCs, randomly picked and confined in a colony wire cage with floor space of 35 cm x 35 cm and height of 40 cm. Heating light bulb was constructed to provide proper heat during brooding age. Feed in mash form and drinking water were provided *ad libitum*.

Health programs such as vaccination with proper vaccines and cage sanitation were also applied following the health and sanitations programs for modern broiler, up to ten weeks of age. Immunization program consisted of: i) At four days old the DOC were vaccinated against NDIB (new castle disease and infectious bronchitis); ii) At seven days old the chickens were vaccinated against IBD (gumboro); iii) The IBD vaccine was repeated in day 21; iv) Newcastle disease vaccine was repeated at the age of 28 days. There was no more vaccination applied afterward until the experiment was ended when the chicks were 10 weeks of age.

Table 1. Experimental diets given to the unsexed improved breeds of native chicken up to 10 weeks of age

Ingredients	Diet 1 [3)]	Diet 2	Diet 3
Fish meal, (%)	5.00	5.00	7.83
Corn, (%)	44.65	41.16	57.15
Soybean meal, (%)	16.07	18.66	19.61
Rice bran, (%)	30.00	30.00	10.00
Vegetable oil, (%)	1.00	2.17	3.00
CaCO3, (%)	1.51	1.19	0.99
Dicalcium phosphate, (%)	0.68	0.67	0.39
NaCl, (%)	0.25	0.25	0.20
Top Mix[1)], (%)	0.30	0.30	0.30
L-Lysine, (%)	0.17	0.19	0.13
DL-Methionine, (%)	0.17	0.21	0.20
Chlorin Cloride, (%)	0.10	0.10	0.10
Sodium bicarbonate, (%)	0.10	0.10	0.10
Total, (%)	100	100	100
Nutrient content:			
Crude protein, (%)	17.81	18.61	19.25
Energy, (kcal ME [2)]/kg)	2800	2950	3100
Energy/protein ratio, (kcal ME/kg protein)	15.72	15.85	16.10
Calcium, (%)	1.31	1.12	1.24
Total phosphorus, (%)	0.72	0.89	0.62
Calcium/Phosphorus ratio	1.82	1.26	2.00
Lysine, (%)	0.36	0.36	0.38
Methionine, (%)	0.19	0.16	0.17
Crude fiber, (%)	4.99	5.13	4.42

1) Every kg of pack of Top Mix contained 1,200,000 iuVit.A; 200,000 iu Vit.D3; 800 iuVit.E; 200 mg Vit.B1; 500 mg Vit.B6; 1,200 mcg Vit.B12; 200 mg Vit. K; 2,500 mg Vit.C; 600 mg CaD-phantothenate, 4,000 Niacine; 1,000 mg Choline Chloride; 3.000 mg Lysine; 12,000 mg Mn; 2,000 mg Fe; 20 mg I; 10,000 mg Zn; 20 mg Co; 400 mg Cu; 1,000 Santoquin (antioxidant) dan 21,000 mg Zn-bacitracin

2) ME: Metabolizable Energy.

Experimental rations consisted of common ingredients (Table 1) and formulated to contain 2,800 kcal ME/kg with 17.81% CP (Diet 1), 2,950 kcal ME/kg with 18.61% CP (Diet 2) and 3,100 kcal ME/kg with 19.25% CP (Diet 3). The experimental diets were given in mash form throughout the experimental period.

Live weight and feed consumption were measured every week from each group of replications. Mortality was recorded at any time when the loss happened. At the age of ten weeks from each replication, one male and one female were taken randomly for carcass and carcass cuts analysis. Slaughter process applied was with the Islamic slaughter method (Hafiz et al. 2015; Ali et al. 2011). Feed conversion ratio was calculated by feed consumed divided by the total live weight gain of bird (g feed consumption/ g live weight gain). European Production Efficiency Factor (EPEF) was calculated using the following formula (Marcu et al. 2013):

Viability: Percent of the number of chickens that live in each replication.
Body live weight: Body live weight at the time of measurement (g/bird).
Age: Age at the time of observation was stopped (day)
FCR: Feed Conversion Ratio

$$EPEF = \frac{\text{Viability (\%) x Body Live Weight (kg) x 100}}{\text{Age (day) x FCR}}$$

Data were subjected to ANOVA (analysis of variance) using the SAS 9.13 statistical software, after tested their normal distribution using quadratic or inverse transformations. The mean values of the treatments were then tested using Duncan's multiple range tests at the 5% degree of confident.

RESULTS AND DISCUSSION

Body live weight

Performance responses of body live weight, feed consumption, and mortality are presented in Table 2. ANOVA results on these variables did not show any significant interaction effect (P>0.05). Therefore, the analysis was applied separately on each treatment factors.

Significantly (P<0.05) higher body live body weight of Line 2 (830 g/bird) than the other two lines showing that the increase of about 8.07% higher than its female parents of Line 1 (♂KUB-1 x ♀KUB-1), which was genetically selected for egg production. Crossing between SenSi (of the sixth generation) as male line and KUB-1 as female line lead us to find prospective formula of parent stock to produce meat type of Indonesian native chicken. While crossing Gaok chicken as male line to female KUB-1 (Line 3) did not show improvement. This might be due to lower (third) generation of Gaok chicken compared to SenSi chicken.

This early attempt of studying of crossing two selected native chickens (SenSi-1 and KUB-1 chickens) was the promotion of the concept in developing National native chicken industry. The attempt might inspire Indonesian breeder in developing the concept of modern chicken breeding that can be applied appropriately in Indonesian native chickens. Selection criteria for KUB-1 chicken was based on egg production resulted in GGP (Great Grand Parent) for female line, while SenSi-1 and Gaok chicken which was selected based on live weight for GGP has to be further observed to find out more effective even more efficient breeding program to create the meat type of native chicken. These three GGP candidates have to be further built up to find out more effective and more

Table 2. Body live weight, feed consumption, and mortality of unsexed improved breeds of native chicken given experimental diets up to 10 weeks of age

Factors	Body live weight (g/bird)	Feed consumption (g/bird)	Mortality (%)
Lines (L)			
Line 1 (♂KUB-1 x ♀KUB-1)	768[b 3)]	2517[a]	4.76[a]
Line 2 (♂SenSi-1 x ♀KUB-1)	830[a]	2,502[a1)]	0.95[a]
Line 3 (♂Gaok x ♀KUB-1)	732[c]	2341[b]	1.90[a]
SE [1)]	27	68	3.24
Diets (D)			
Diet 1 [2)]	771[x]	2,429[y]	0.95[x]
Diet 2	840[y]	2,560[x]	0.95[x]
Diet 3	720[z]	2,372[y]	5.71[x]
SE	25	70	3.07
Interaction			
L x D	NS [4)]	NS	NS

1) SE = Standard Error
2) Dietary treatment consists of; Diet 1 contained 2,800 kcal ME/kg with 17.81% crude protein (CP), Diet 2 contained 2,950 kcal ME/kg; with 18.61 % CP; Diet 3 contained 3,100 kcal ME/kg with 19.25 % CP
3) Values in the same column and factor, with difference superscript are significantly different (P<0.05)
4) NS: Not statistically significant (P>0.05).

efficient breeding program in order to produce final lines for improved meat type of native chicken.

In the purpose of improving performance of Indonesian native chicken, although it does not have a high body weight especially at early age like modern broiler, the native chicken is the breed with tolerance to hot and humid climate. Attempts of breeding the birds with tolerance breed to specific nutritional deficiencies as indicated by Pym (2011) may considerably be overcome by the use of available commercial feed ingredients and complete feed for modern chicken industry.

Live weight at 10 weeks of age was influenced by nutrition as shown in Table 2. Diet 2 containing 2,961 kcal ME/kg with 18.61% crude protein, offered better feed to chicks with the highest (P<0.05) live weight (840 g/bird) compared to Diet 1 (771 g/bird or Diet 3 (720 g/bird). Lowest (P<0.05) live weight of the chicken in Diet 3, containing 3,100 kcal ME/kg with 19.25% crude protein, showing that this diet was not much suitable for the chicken. The dietary energy of Diet 3, somehow may probably increase in order to balance the appropriate ratio, however, Diet 2 with lower crude protein and energy would be more efficient.

Feed consumption and efficiency

There was no effect of interaction of breed and nutrition (P>0.05) on feed consumption. Line 3 (♂Gaok x ♀KUB-1) consumed less feed (2,341 g/bird; P<0.05) compared to the other two breeds (2,502 g/bird for Line 2 and 2,517 g/bird for Line 1) (Table 2). It seemed that improving performance of native chicken decreased feed consumption as it was shown by the early selected Sentul chicken, which is the grand parent of SenSi chicken, consumed the same quality feed more (2,602 g/bird) at the same age (Iskandar et al. 2012), however, it was slightly more when compared to SenSi chicken fed with different quality of ration (2,400 g/bird, Iskandar et al. 2015).

There was significant effect (P<0.05) of interaction between breeds and quality of experimental rations on feed conversion ratio (FCR). Table 3 show that chicken in ration Diet 2 significantly (P<0.05) had the best FCR than the other two experimental diets. Feed formulation of Diet 2, containing 2,950 kcal ME/kg with 18.61 % crude protein was probably the optimum for all lines. Line 2 (♂SenSi-1 x ♀KUB) feed utilization was more efficient than the Line 1 or Line 3, although the value of feed conversion ratio slightly higher (3.09 kg/kg) than that of reported by Iskandar et al. (2015) of about 2.5 kg/kg. In term of FCR Line 2 converted feed more efficiently into body on Diet 1, which contained 2800 kcal ME/kg with 17.81% CP, while the other two lines were on Diet 2. Chicken of the Line 1 would actually be expected to consume more feed to grow a kilo gram of its live weight than other lines, as it was selected for egg production.

Economic efficiency in term of EPEF (European Production Efficiency Factor) suggested by Marcu et al. (2013) was also used to measure the economic efficiency. There was significant (P<0.05) effect of interaction between lines and diets. Table 3 shows inverse pattern of FCR and confirms that line S2 chicken was more efficient than that of other two lines fed diet Diet 2. The highest value (EPEF of 411) was achieved by Line 2 (♂SenSi-1 x ♀KUB) on diet Diet 2, while the lowest (EPEF of 296) was achieved by Line 1 on Diet 3. So, in term of EPEF young chicks of crossbred of ♂SenSi-1 x ♀KUB-1 (Line 2) would perform much better on Diet 2.

Mortality

Mortality values were presented in Table 2, there was not any significant (P>0.05) difference between

Tabel 3. Feed Conversion Ratio (FCR), European Production Efficiency Factor (EPEF) of unsexed improved breeds of native chicken given experimental diets up to 10 weeks of age

Variable	Line 1 (♂KUB-1 x ♀KUB-1)			Line 2 (♂SenSi-1 x ♀KUB-1)			Line 3 (♂Gaok x ♀KUB-1)			SE [3]
	Diet 1 [1]	Diet 2	Diet 3	Diet 1	Diet 2	Diet 3	Diet 1	Diet 2	Diet 3	
FCR (kg feed consumption/ kg live weight gain)	3.44^{b} [2]	3.14^{cde}	3.74^{a}	3.02^{e}	3.0^{de}	3.29^{bcde}	3.41^{bc}	3.28^{bcde}	3.34^{bcd}	0.03
EPEF	319^{c}	387^{ab}	237^{d}	385^{ab}	411^{a}	332^{bc}	296^{c}	332^{bc}	296^{c}	8.63

1) Dietary treatments consisted of; Diet 1 contained 2,800 kcal ME/kg with 17.81% crude protein (CP), Diet 2 contained 2,950 kcal ME /kg with 18.61 % CP; Diet 3 contained 3,100 kcal ME /kg with 19.25 % CP
2) Values in the same row with difference superscript are significantly different (P<0.05)
3) SE = Standard Error.

experimental factor either breeds factor nor diets factor. It could not be concluded as it had higher standard error of mean. The high mortality of 4.76% happened in Line 1 compared to 0.95% in line Line 2. There was no satisfactory explanation why such mortality figures appeared in the experiment.

Nutrients consumed

Nutrients consumption in term of g/kg body live weight (BLW) was presented in Table 4. The nutrients consumption was affected by the interaction (P<0.05) of breeds and diets. Therefore, the figures show that each breed of chicken should have its own optimum level, depended on the genetic capacity.

Dietary ME (metabolizable energy) consumed by the birds, varied with breeds and diet factors. Dietary ME was utilized efficiently to a kilo gram (kg) of BLW was by crossbred ♂SenSi-1 x ♀KUB-1 on Diet 1 (8,150 kcal ME/kg BLW), meanwhile ♂Gaok x ♀KUB-1 (9,147 kcal/kg BLW) was on Diet 1, which was not significantly (P>0.05) from the other two lines on the same diet. The figures, which are the amount of energy consumed per kg BLW, could be interpreted as efficiency of particular nutrient in part of formation one kg BLW of chicken. The lower dietary energy consumed, the more efficient the bird in utilizing diet.

From point of genetic capacity, all lines did not show response of increasing consumption of dietary energy when fed low energy diet. It is unlike the old concept which stated that bird will consumed more diet on low energy density diet. This phenomenon was also discussed by Mbajiorgu et al. (2011) on native Venda chicken, due to the loss sensitivity to regulate feed intake according to dietary energy level, although the physiological explanation was not clear and merited further investigation.

The pattern of crude protein and lysine consumptions (g/kg LW) followed pattern of their energy consumption (Table 4), leading to indication that crossbred ♂SenSi-1 x ♀KUB-1 chicken was more efficient in utilizing protein and amino acid lysine in Diet 1, whilst all breeds utilized dietary protein and energy of Diet 2 was fairly similar. Diet 3 was not quite appropriate in having energy, protein and lysine levels available for supporting the maximum growth of the three breeds. As a result of that occurs in Diet 3 allegedly associated with an imbalance of nutrients that occurs on Diet 3. Dairo et al. (2010) revealed that increase of energy content of the experimental diets indicated to a large extent adequacy of supply of other nutrients even at the minimal dietary level, hence formulation of animal feed must take into consideration the nutrient density with energy as the prime factor of the particular feed to facilitate production objectives.

Methionine and calcium consumptions were having much the same pattern, showing that all lines utilized amino acid methionine and calcium more efficient on Diet 2 in comparison to the other two experimental diets. However, contrary to the response of lines to those two nutrients, phosphorus consumption was more on Diet 2 compared to other two diets. This case is likely due to the ratio of Ca/P are different ratio of Ca/P in all three treatment diets and not ideal for chicken since the ideal ratio of Ca/P is 3 : 1.

Table 4. Nutrients consumed of unsexed improve breeds of native chickens given experimental rations up to 10 weeks of age

Consumption	Line 1 (♂KUB-1 x ♀KUB-1)			Line 2 (♂SenSi-1 x ♀KUB-1)			Line 3 (♂Gaok x ♀KUB-1)			SEM [5]
	Diet 1 [3]	Diet 2	Diet 3	Diet 1	Diet 2	Diet 3	Diet 1	Diet 2	Diet 3	
Energy, (kcal ME [1]/ kg BLW [2])	9,251^{bc} [4]	8,931^{c}	11,073^{a}	8,150^{d} [4]	8,818^{cd}	9,796^{b}	9,147^{bc}	9,295^{bc}	9,910^{b}	125
Crude Protein, (g/kg BLW)	588^{bcd}	563^{cde}	687^{a}	518^{e}	556de	608^{bc}	581^{bcd}	586^{bcd}	615^{b}	7.41
Lysine, (g/Kg BLW)	11.89^{b}	10.90^{cd}	13.57^{a}	10.48^{d}	10.76^{d}	12.01^{b}	11.76^{bc}	11.34^{bcd}	12.14^{b}	0.14
Methionine, (g/kg BLW)	6.27^{a}	4.84^{d}	6.07^{a}	5.53^{b}	4.78^{b}	5.37^{bc}	6.20^{a}	5.04^{cd}	5.43^{bc}	0.08
Calcium, (g/kg BLW)	43.28^{a}	33.90^{d}	44.29^{a}	38.13^{bc}	33.48^{d}	39.18^{b}	42.79^{a}	35.29^{cd}	39.64^{b}	0.58
Phosphorus, (g/kg BLW)	23.78^{b}	26.94^{a}	22.14^{bc}	20.95^{cd}	26.60^{a}	19.59^{d}	23.52^{b}	28.04^{a}	19.82^{d}	0.41

1) ME : Metabolizable Energy
2) BLW : Body Live Weight
3) Dietary treatments consisted of; Diet 1 contained 2,800 kcal ME/kg with 17.81% crude protein (CP), Diet 2 contained 2,950 kcal ME/kg with 18.61 % CP; Diet 3 contained 3,100 kcal ME/kg with 19.25 % CP
4) Values in the same row with different superscript are significantly different (P<0.05)
5) SEM : Standard Error of Mean.

The phenomenon also mentioned by Mbajiorgu et al. (2011) in their discussion paper that chickens will increase intake in response to marginal level of first limiting feed nutrient and independent of dietary energy level (Boorman 1979) since appetite was assumed to depend on nutrient requirements of the animal and the contents of those nutrients in the feed (Emmans & Fisher 1986). However, contrary to the fact that phosphorus level in Diet 2 was higher than that level in Diet 1 or Diet 3. The increase in feed consumption was not clearly due to phosphorus level in the diet. Adamu et al. (2012) reported that dietary Ca : P ratio of 3 : 1 was favorable for finishing broiler chickens under semiarid environment. In this experiment Ca : P ratios were 1.86, 1.26 and 2.00 respectively for Diet 1, Diet 2 and Diet 3, which might not at proper ratios. The finding suggested us to do further investigation, particularly with native chickens.

Carcass

Carcass in term of dressing percentage and carcass cuts are presented in Table 5. There was no significant (P>0.05) effect of the interaction between breeds and quality of experimental diets on carcass. The data were analyzed according to each factor. Line 1 chicken had slightly higher (P<0.05) dressing percentage (64.94%) compared to the other two breeds performing only up to about 63% dressing percentage. There were no significant (P>0.05) different in breast, gizzard and fat pad percentage among the three breeds. Slightly higher thighs percentage (10.58%) and liver percentage (2.43%) of Line 1 chicken revealed in the experiment. The carcass performance of the all lines of the chicken for some extent was not much different from other Indonesian native chicken as reported by Iskandar et al. (2012) on Sentul native chicken of having dressing percentage of 62% for female and 71% for male, and of having breast percentage of about 17% for both female and male at 10 weeks of age. There was obviously, lower than dressing breast, thighs percentage of exotic broiler chickens (Beg et al. 2016).

However, it is interesting to note in native that the abdominal fat pad was much lower, showing that the native chicken meat would be much healthier than exotic broiler chicken for human consumption (Ewald 2015). Furthermore, Guan et al. (2013) reported that native chickens produced better quality meat as far as inosine-5' monophosphate (IMP) content, fiber diameter, and shear forces were concerned.

Table 5. Percentage of dressing, breast, thighs, drumstick, liver, gizzard, abdominal fat pad of 10 weeks old of unsexed improved breeds of native chicken given diets differed in nutrients content.

Factors	Dressing (%) [3]	Breast (%)	Thighs (%)[1]	Drumstick (%)	Liver (%)	Gizzard (%)	Abdominal fat pad (%)
Lines (L)							
Line 1 (♂KUB-1 x ♀KUB-1)	63.00^{b} [4]	16.06^{a}	11.06^{b}[2]	10.19^{b}	2.38^{b}	2.62^{a}	0.52^{a}
Line 2 (♂SenSi-1 x ♀KUB-1)	63.18^{b}	15.44^{a}	11.34^{ab}	10.49^{a}	2.56^{a}	3.72^{a}	0.42^{a}
Line 3 (♂Gaok x ♀KUB-1)	64.94^{a}	16.06^{a}	11.58^{a}	10.42^{ab}	2.43^{ab}	2.85^{a}	0.17^{a}
SE [1]	0,86	0,39	0,25	0,16	0,09	0,50	0,22
Diets (D)							
Diet 1 [2]	63.93^{x}	15.59^{y}	11.47^{x}	10.40^{x}	2.45^{xy}	2.85^{x}	0.24^{x}
Diet 2	64.07^{x}	16.26^{x}	11.27^{x}	10.23^{x}	2.36^{y}	2.86^{x}	0.38^{x}
Diet 3	63.12^{y}	15.71^{xy}	11.24^{x}	10.47^{x}	2.56^{x}	3.48^{x}	0.48^{x}
SE	0.92	0.39	0.26	0.17	0.09	0.52	0.23
Interaction							
L x D	NS [5]	NS	NS[5]	NS	NS	NS	NS

1) SE = Standard Error
2) Dietary treatments consisted of; Diet 1 contained 2,800 kcal ME/kg with 17.81% crude protein (CP), Diet 2 contained 2,950 kcal ME/kg with 18.61 % CP; Diet 3 contained 3,100 kcal ME /kg with 19.25 % CP
3) As the ratio to live weight
4) Values in the same column and factor with difference superscript are significantly different (P<0.05)
5) NS = Not Significant (P>0.05).

The influence of experimental diets on carcass percentage was statistically significant (P<0.05), showing that Diet 2 facilitated better to produce more dressing, and breast percentage. Whilst the other carcass cuts, except liver, were not influenced by the level of dietary energy and protein. Abdominal fat pad varied from 0.24% to 0.48% with varying in protein or energy content of the diets. The gap of figures was actually wide, but it was not any statistically different. This was due to the high SEM (0.23%) as the result of high variation of the individual bird fat pad and constant energy/protein ratio (about 158 kcal ME/kg crude protein). However, the level of fat pad of the native chicken breeds was again much lower than of exotic broiler chicken (Azizi et al. 2011). The absence of effect on dressing percentage and cut carcass composition associated with the similar protein energy ratio among all treatment diets, Rosa et al. (2007) said that some of the results of the experiment showed that the content of energy and protein in the diet had no influence on the composition of cut carcass composition of chicken.

CONCLUSION

It is concluded that Line 1 (♂KUB-1 x ♀KUB-1) and its crossbreeds, Line 2 (♂SenSi-1 x ♀KUB-1) and Line 3 (♂Gaok x ♀KUB-1) had slightly difference biological response to rations differ in nutrient content. Meanwhile, indigenous crosses breed ♂SenSi-1 x ♀KUB-1 potential for commercial native meat chicken in Indonesia with diet contained 2,950 kcal ME/kg with 18.61% crude protein raised up to 10 weeks of age.

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Exopolysaccharide (EPS) Activity Test of Lactic Acid Bacteria (LAB) as Immunomodulatory

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ABSTRAK

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Penelitian ini menguji aktivitas imunomodulator Eksopolisakarida (EPS) dari bakteri asam laktat (BAL) dan mengkarakterisasi gugus fungsinya. Strain BAL yang digunakan yaitu *Lactobacillus delbrueckii* subsp. bulgaricus dan *Streptococcus thermophilus*. EPS diekstraksi dari kedua kultur BAL tersebut. Analisis spektrum FT-IR EPS dari kedua strain BAL menunjukkan puncak serapan senyawa karbohidrat. Uji aktivitas imunomodulator dari EPS secara *in vivo* dihitung berdasarkan aktivitas dan kapasitas fagositosis sel makrofag cairan peritonium mencit. EPS diberikan secara oral pada mencit dengan konsentrasi 100 µg/ml, 200 µg/ml, 300 µg/ml selama 14 hari dan tikus diinfeksi dengan *Staphylococcus aureus*. Hasil menunjukkan bahwa EPS berasal dari kedua strain BAL dapat meningkatkan aktivitas dan kapasitas fagositosis sel makrofag cairan peritonium mencit. EPS dari kultur *L. delbrueckii* subsp. bulgaricus pada konsentrasi 300 µg/ml menunjukkan aktifitas fagositosis sel makrofag dan EPS dari kultur *S. thermophilus* konsentrasi 300 µg/ml menunjukkan kapasitas fagositosis tertinggi. Kesimpulan uji potensi EPS sebagai imunomodulator berasal dari kultur *L. delbrueckii* subsp. bulgaricus dan *S. thermophilus* menunjukkan bahwa EPS dari kedua strain tersebut mampu meningkatkan aktifitas dan fagositas sel makrofag peritonium mencit.

Kata Kunci: Eksopolisakarida, Bakteri Asam Laktat (BAL), Imunomodulator, Yoghurt, Fourier Transform-Infra Red (FT-IR)

ABSTRACT

Kusmiati, Kukihi FE, Afiati F. 2016. Exopolysaccharide (EPS) activity test of lactic acid bacteria (LAB) as immunomodulatory. JITV 21(3): 182-189. DOI: http://dx.doi.org/10.14334/jitv.v21i3.1414

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.

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

INTRODUCTION

Immuno system balance disorder is the one causing such an inflammation, infection and autoimmune diseases. One of prevention attempts of the disorder is restoring the immune system balance through providing immunomodulatory. Immunomodulatory is a compound that may change immune response by preventing/ normalizing an abnormal immune reaction (Suhirman & Winarti 2007).

Yoghurt is a healthy drink especially in the gastrointestinal tract containing lactic acid bacteria (LAB) of *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus* (Mazahreh & Ershidat 2009). Many studies showed that LAB in yogurt has potential has therapy effect including as the

immunostimulatory (Weerathilake et al. 2014; Patel et al. 2012).

Exopolysaccharide (EPS) is a sugar residue secreted by microbes to its surrounding. EPS is secreted by LAB in the form of mucus (De Vuyst & Degees 1999). Several lactic acid bacteria produce EPS playing role as nature texture producer in yogurt, cheese and milk-based foods manufacturing (Harutoshi 2013). EPS also has profitable biological characteristics as immunostimulator, antitumor, antiulcer and cholesterol-reducer (Welman & Maddox 2003). EPS is beneficial in food, health and cosmetic industry and need further studies. Potential of the EPS as immunomodulatory study is excessively studied.

EPS compound isolated from *Sarcodon aspratus* mushroom is able to intensify macrophage cells activity in producing cytokines and intensifying its phagocytosis (Im 2010). Biological activities of EPS depend on composition and its monomer bond, so it is crucial to characterize EPS generated both chemically and biologically (Madhuri & Prabhakar 2014).

One of methods may be used to characterize functional group of a compound is by spectrophotometry Fourier Transform Infra Red (FT-IR) (Sunil et al. 2013). This study was aimed to measure the potential of EPS compound resulted from *L. delbrueckii* subsp. *bulgaricus* and *S. thermophiles* isolated from yogurt as immunomodulatory and determine functional group in the EPS compound.

MATERIALS AND METHODS

Preparation and characterization of exopolysaccharide (EPS)

Preparation of Lactic Acid Bacteria (LAB)

Bacteria used in this study were *L. delbrueckii* subsp.*bulgaricus* and *S. thermophiles* which are the collection of Indonesian Culture Collection (InaCC) – Indonesian Institute of Sciences. One ose each from culture stock was transferred into compact de Mann Rogossa Sharpe (MRS, Merck) media and incubated for 48 hours at 37°C.

Observation of the LAB morphology

Preparate swabs and Gram staining were conducted for the fresh *L. delbrueckii* subsp.*bulgaricus* and *S. thermophilus* which then were observed under Leica microscope with 1000x magnification.

Inoculation of the LAB into the pre-culture

One ose LAB from regeneration media was collected aseptically and then inoculated into 20 mL pre-culture media and incubated in shaker incubator at 37°C for 48 hours.

Production of exopolysaccharide on fermentation media

As much as 2% LAB culture from pre-culture media were inoculated into 250 mL liquid MRS aseptically. Then it was incubated in the shaker incubator at 37°C for 72 hours.

Exopolysaccharide extraction

Exopolysaccharide was extracted from 72 hours old LAB culture and boiled at 100°C for 15 minutes (Feldman et al. (2013). The boiling was aimed to inactivate enzyme degrading the EPS. The culture was chilled into room temperature, and then centrifuged in 6000 rpm for 10 minutes. Tricloroacetate 85% was added then chilled at 4°C and centrifuged in 6000 rpm for 10 minutes in 4°C. Supernatant was accommodated and added by cold ethanol 96% by ratio 1 : 3 and idled for 48 hours at 4°C. That fusion then was centrifuged in 6000 rpm for 10 minutes at 4°C. The sediment EPS was washed by hot water twice. Biomass from the extraction was dried in the vacuum oven at 50°C. It then was weighed as dry weight of crude EPS (mg).

Total sugar analysis of EPS samples using Phenol Sulfate method

Phenol Sulfate method with the glucose as a standard was applied to measure the EPS content (Chaplin 1986). The EPS was hydrolyzed into monomer D-glucose. Carbohydrate and phenol in the strong acidic environment, followed by heating process, experienced dehydration producing furfural and furfural methyl hydroxy which condensed with the phenol turned into yellow-orange. The absorbability of the color was measured using spectrophotometer UV-Vis in the wavelength by 490 nm.

Protein analysis of EPS samples using Lowry method

Protein level was measured by Lowry method using *Bovine Serum Albumin* (BSA) (Copeland 1994). In the divalent ion copper alkali formed complex with peptide bond which then was reduced into monovalent ion. Monovalent copper ion and radical group of tyrosine, tryptophan and cysteine reacted with foline solvent producing unstable compound which reduced into blue tungsten molybdenum. That color absorption was measured by light spectrophotometer showed on maximum wave length by 750 nm.

Characterization of exopolysaccharide using Spectrophotometry Fourier Transform Infra Red (FT-IR)

Exopolysaccharide of LAB was characterized using spectrophotometer FT-IR (Shimazu) to determine its functional group. One part of EPS extract was mixed with 99 parts of dry Kalium bromide (KBr) then compressed into 3 mm diameter slab. The slab then was analyzed in infrared spectrum in frequency range by 400-4,000 cm^{-1} (Sunil et al. 2013).

Measurement of *In Vivo* phagocytosis activities and capacity of macrophage

Macrophage activity and capacity were observed under a 10x100 magnificent light microscope (Won et al. 2011; Yim et al. 2005). Animal experimental was performed in the Laboratory of Veterinary of Bogor Agricultural University-Dramaga, Bogor. The experimental animal used was male mice (*Mus musculus*) strain DDY, in 6-8 weeks old, with 18-21 g of body weight. The mice were acclimated for 7 days ahead. The mice was divided into 9 experimental groups: (1) normal control, (2) positive control, (3) negative control, (4) EPS *L. delbrueckii* subsp. *bulgaricus* (LB) 100 µg/ml, (5) EPS *L. delbrueckii* subsp.*bulgaricus* (LB) 200 µg/ml, (6) EPS *L. delbrueckii* subsp. *bulgaricus* (LB) 300 µg/ml, (7) EPS *S. thermophilus* (ST) 100 µg/ml, (8) EPS *S. thermophilus* (ST) 200 µg/ml and (9) EPS *S. thermophilus* (ST) 300 µg/ml. The positive control used standard β-glycan (Takeda) 200 µg/ml. There were 3 mice in each experimental group.

The experiment was conducted for 14 days, where in the 15^{th} day, those experimental animals were injected by intraperitoneal suspension of *Staphylococcus aureus* (1 x 10^6 cfu/ml). Those experimental animals were euthanatized 1 hour after infection and then their peritoneal liquid were collected. Swab preparation was made for all samples and then fixated using methanol for 5 minutes, stained with Giemsa and left for 20 minutes and then washed using distilled water.

Phagocytosis activity

Phagocytosis activity value is the number of macrophage cells that actively phagocyte *Staphylococcus aureus* in 100 macrophage cells. Phagocytosis activity was presented in percent, with the formulation (Ranjith et al. 2008) as the following:

$$\% \text{ Activity} = \frac{\text{The number of active macrophage}}{\text{The number of whole macrophage}} \times 100\%$$

Phagocytosis Capacity

Phagocytosis capacity value is the number of bacteria ingested by 50 active macrophages (Ranjith et al. 2008). This parameter was observed by staining with Giemsa and then the bacteria number was counted under microscope. Phagocytosis is a devouring process on bacteria or strange objects by enfolding those things using macrophage cytoplasm.

Data Analysis

Determining of immunomodulatory activity of crude EPS from *L. delbrueckii* subsp. *bulgaricus* (LB) and *S. thermophilus* (ST) was through macrophage activity and capacity induced by *Staphylococcus aureus* bacteria by *in vivo*. Data were analyzed by one-way ANOVA with 3 replications using SPSS ver 22.0 with P=0.05. This analysis was then followed by Duncan Multiple Range Test.

RESULTS AND DISCUSSION

Exopolysaccharide (EPS) Characterization

Morphology of Lactic Acid Bacteria (LAB)

The morphology of LAB isolate grew in the regeneration media was observed using Gram staining (Figure 1). It shows that *L. delbrueckii* subsp. *bulgaricus* and *S. thermophilus* isolates are Gram positive bacteria. *L. delbrueckii* isolate formed rod shape, meanwhile, the *S. thermophilus* isolate formed cocus chain shape.

Sugar and protein content of the exopolysaccharide (EPS)

Bacteria isolate was fermented in the liquid MRS, that is a selective media for LAB growth. Fermentation temperature was kept at 37°C, which is the optimum temperature for LAB growth. In the 72 hours fermentation, bacteria growth reached stationer phase, where in this phase, the culture was being collected due to secunder accumulation of EPS.

Table 1 shows that LAB isolate produced more EPS (65.40 mg per L) than the LAB LB isolate (61.40 mg er L). This is in accordance with a study conducted by Cerning (1995) that reported LAB ST produced EPS by 50-350 mg/L, meanwhile the LAB LB produced 60-250 mg/L. EPS amount produced is influenced by composition of media such as concentration of carbon and nitrogen or growth condition of bacteria such as the temperature, pH, incubation time and genetic factor. Different LAB species tends to have different

preference to certain carbon resource. Effect of using of different carbohydrate was observed by Mataragas et al. (2004), where showed that glucose could not increase total biomass production of *Leuconostoc mesenteroides* and *Lactobacillus curvatus*. On the other hand, Liew *et al*. (2005) reported that there was an effect of glucose on the amount of biomass of *Lactobacillus rhamnosus* cel.

EPS amount is also influenced by extraction method applied. Heating stage in the begining of the extraction (100°C) may increase EPS recovery, so that increase EPS amount produced. This is allegedly that enzimes afford to degrade EPS have been inactivated. The addition of trichloroacetate 85% to precipitate protein causes the decrease of EPS amount produced, as a result of EPS precipitation led by the trichloroacetate (Rimada & Abraham 2003).

The LAB LB in this study had more glucose than LAB ST isolate. It was resulted by use of more glucose as carbon resource in the LAB LB compared to the LAB ST. Weigher dry weight of EPS within lower glucose in the LAB ST than in the LAB LB was allegedly due to contamination of other compound.

The EPS produced by LAB LB contained lower protein than the LAB ST synced with the high glucose content resulted. Lower protein content shows purer EPS. As well known that protein and salt are contaminant (Yadav et al. 2011).

Analysis of EPS extracted from *L. delbrueckii* subsp. *bulgaricus* and *S. thermophilus* cultures with Fourier Transform Infra Red (FTIR).

Fourier Transform Infra Red (FT-IR) was used to determine functional group of the compounds within the EPS. As it shows in the Figure 2 and 3, both isolate had similar infra red IR and typical uptake of polysaccharides showed by its wave number. Table 2 shows that EPS produced by both isolates, each had hydroxyl (-OH), methyl and methylene (-CH), carbon (-C=O) in the carboxylate and C-C vibration. This shows that the EPS produced is carbohydrate compound.

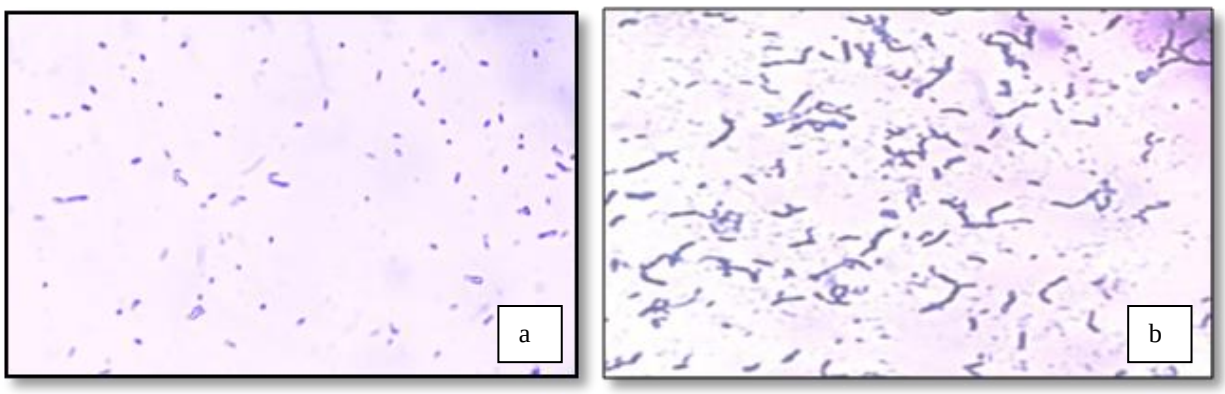

Figure 1. Morphology of *L. delbrueckii* subsp. *Bulgaricus* (a) and *S. thermophilus* (b) under microscope with the magnificaton by 10 x 100.

Table 1. Dry weight of crude EPS, sugar and protein of EPS from *L. delbrueckii* subsp. *bulgaricus* and *S. thermophilus*

Bacteria	DW of crude EPS (mg/250 ml media)	Level of EPS equivalent to Glucose (%)	Protein Level (%)
L. delbrueckii subsp. *Bulgaricus* (BAL LB)	15.35±0.29	49.96±0.25	1.20±0.03
S. thermophiles (BAL ST)	16.35±0.32	24.17±0.06	7.19±0.12

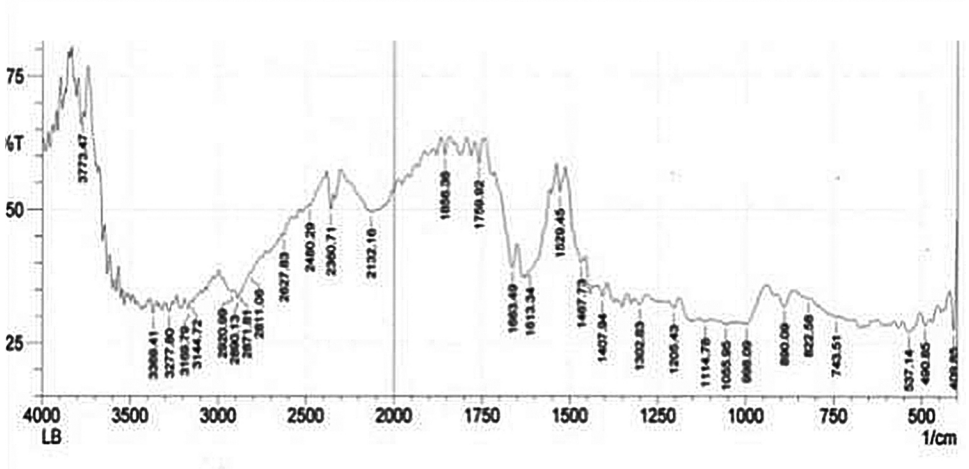

Figure 2. FTIR Spectrum of EPS *L. delbrueckii* subsp. *Bulgaricus.*

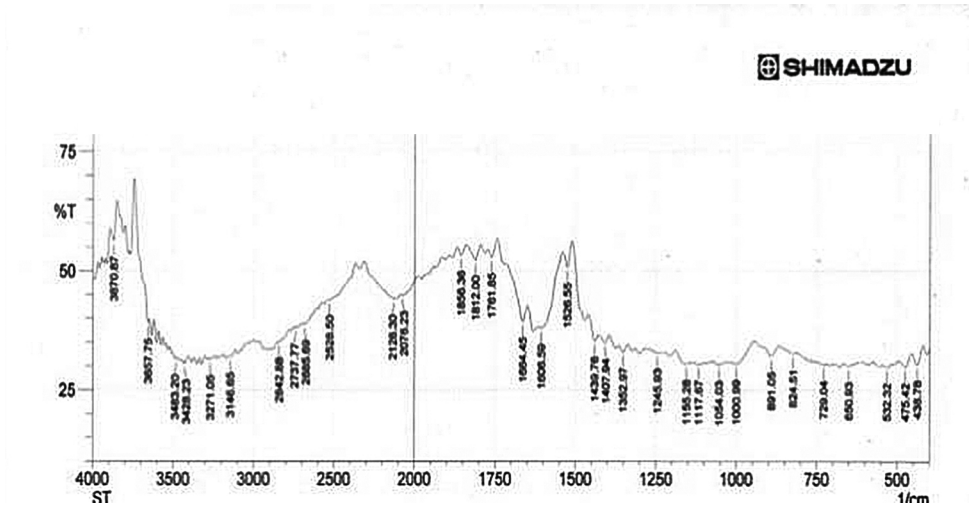

Figure 3. FTIR Spectrum of EPS *S. thermophilus.*

Table 2. Spectrum interception result *Fourier Transform Infra Red* (IR) exopolysaccharide

Bacteria	Wave Number (cm^{-1})	Functional Groups
L. delbrueckii subsp. *bulgaricus*	3,144-3,369	–OH band
	2,811-2,920	C-H (CH_2, CH_3) band
	1,613-1,663	–C=O band
	1,407	–C=O vibration
	1,055	C-C dan C-O vibration
S. thermophilus	3,146-3,483	–OH band
	2,842	*Stretching* –CH band
	2,076-2,126	alkuna group
	1,664	–C=O band
	1,606	*Stretching* enol and amida
	1,054	C-C and C-O Vibration

EPS Immunodulatory assay

Activity of EPS of LAB as immunomodulatory was measured based on its capability on increasing activity and capacity of phagocytosis of macrophage cells in the peritoneum liquid of mice under a microscope (Figure 1). Data were analyzed using one way ANOVA followed by Duncan Test.

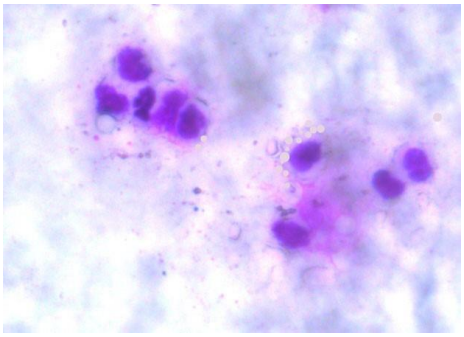

Figure 4. Macrophage cell actively phagocytes *S. aureus* bacteria.

Phagocytosis activity measurement using macrophage cells collected from peritoneum liquid of 9 mice groups was presented in the Table 3. The normal control had the lowest phagocytosis activity by 10.33% than all experimental groups. The negative control had phagocytosis activity score by 43.67%, which higher than the normal control. This shows that infection of the *Staphylococcus aureus* bacteria may lead macrophage cell as an immune system response. The positive control had the highest phagocytosis activity by 74.67%. EPS from LB of 300 µg/ml had the highest phagocytosis activity by 61% than the other. It may be concluded that EPS compound extracted from LAB LB and ST cultures has immunomodulator activity by increasing macrophage activity in the mice peritoneum. This is in line with LeBlanc et al. (2002) who said that several strains of LAB are able to increase immune response of mediator by T-lymphocyte proliferation, increasing phagocytosis capacity of mononuclear cell and increasing antitumor NK cell activity (natural killer). The LAB may increase immunomodulatory function through macrophage and lymphocyte cell activity (Kirjavainen et al. 1999). Immunomodulatory activity becomes mediator of interaction between immune cell and whole LAB cell or the cells components such as peptidoglycan, tamaroic acid and exopolysaccharide (Amrouche et al. 2006).

Phagocytosis capacity assay was performed by randomly selecting 50 macrophage cells containing minimal 3 *Staphylococcus aureus* from active macrophage cells. Table 3 shows the normal control group had lowest phagocytosis capacity by 35.33 cells bacteria per 50 active macrophage cells. Positive control group had the highest phagocytosis capacity among all the experimental groups by 574.67 cells bacteria per 50 active macrophages. Experimental group provided EPS from LAB ST 300 µg/ml had the highest macrophage capacity by 556.67 cells bacteria per 50 active macrophage cells among the experimental groups.

Test results show that oral providing of EPS isolate LB and ST for 14 days was able to increase phagocytosis activity and capacity of peritoneum macrophage cells. This proves that EPS is a compound that may influence immune system response.

Mechanism of EPS compound in increasing phagocytosis activity and capacity of macrophage is not well known. Several studies reported that EPS

Table 3. Phagocytosis activity of macrophage cell (per 100 cells) and phagocytosis capacity (the number of bacteria devoured per 50 active macrophage cells)

Test Group	Phagocytosis Activity (%)	Phagocytosis Capacity (%)
Normal	10.33±2.08^{a}	35.33±3.06^{a}
Negative	43.67±0.58^{b}	177.33±5.13^{b}
Positive	74.67±1.15^{h}	574.67±4.16^{i}
EPS LB 100 µg/ml	53.33±1.53^{d}	239±3.61^{c}
EPS LB 200 µg/ml	56.33±1.53^{e}	341.33±8.14^{e}
EPS LB 300 µg/ml	61±1.00^{g}	540±2.65^{g}
EPS ST 100 µg/ml	47±1.73^{c}	266.67±11.50^{d}
EPS ST 200 µg/ml	53.33±1.53^{d}	489±6^{f}
EPS ST 300 µg/ml	59±1.00^{f}	556.67±6.03^{h}

Different letter in the same column means significant different (P<0.05).

may be recognized by one of many C-type lectin receptor (CLRs) involved in intercepting antigen by macrophage cells (Abbas et al. 2015). CLRs receptor is located in phagocyte plasma membrane. EPS will be bind with a receptor changing the receptor of signal cells inducing several cellular responses including cell proliferation, differentiation, migration of immune cells and phagocytosis.

CONCLUSION

EPS is substantial extracellular bioactive molecule playing a role in biologic and therapeutic activities. Results showed that EPS from strains *L. delbrueckii* subsp. *bulgaricus* and *S. thermophiles* cultures is able to increase phagocytosis activity and capacity of macrophage cells of mice's peritoneum. *S. thermophiles* produced higher dry EPS extract than the *L. delbrueckii* subsp. *bulgaricus*. EPS produced by those both bacteria generated typical spectra infra red from polysaccharide. EPS from *L. delbrueckii* subsp. *bulgaricus* 300 µg/ml showed the highest macrophage activity, while the EPS from *S. thermophilus* 300 µg/ml showed the highest macrophage capacity. Those results scientifically provide preliminary information that LAB may be used as active ingredient for medicine or supplement for human being.

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Knowledge, Attitudes, Practices (KAP), and Financial Losses of Buffalo Raisers due to Surra among Selected Villages in Southern Philippines

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ABSTRAK

Escarlos JAJr, Cane JF, Dargantes AP. 2016. Tingkat pengetahuan, sikap, kebiasaaan (KAP) dan kerugian finansial peternak Kerbau akibat penyakit Surra di desa terpilih di Filipina Selatan. JITV 21(3): 190-203. DOI: http://dx.doi.org/10.14334/jitv.v21i3.1417

Penelitian ini dilakukan untuk mengukur tingkat pengetahuan, sikap, kebiasaan dan kerugian finansial peternak kerbau karena infeksi *Trypanosoma evansi* (surra) dan pengendaliannya di Provinsi Agusan del Sur. Sebanyak 160 peternak kerbau dari 8 desa di 4 kota di Provinsi Agusan del Sur, Mindanau, Filipina diwawancarai secara perorangan. Sebagian besar (63,65%) responden mengetahui tentang penyakit surra dengan nilai tengah pengetahuan sebesar 12,54. Nilai tersebut tergolong masih rendah dibandingkan responden yang mengetahui tentang penyakait surra dengan baik. Kerugian finansial yang disebabkan oleh kematian ternak di 8 desa tersebut tercatat sebanyak 9,3 juta peso (US$ 0.2 M) dengan penambahan kerugian dari proses pengobatan dan diagnosis berturut-turut sebanyak 657.000 peso dan 229.500 peso. Perkiraan kerugian pengobatan dan diagnosis secara keseluruhan berturut-turut adalah sebesar 2,4 dan 1,1 juta peso. Perkiraan kerugian finanasial secara keseluruhan adalah sebesar 13,7 juta peso dengan rataan sebesar 1,7 juta peso per desa. Dan diperkirakan terjadi kerugian sebesar 538 juta peso (US$ 10,7) dari total kerugian finansial pada peternakan di Provinsi Agusan del Sur karena penyakit sura. Sebagai kesimpulan, peternak kerbau di Provinsi Agusan del Sur kurang memiliki pengetahuan, tingkah laku dan kebiasaan yang efektif dalam mengendalikan penyakit sura.

Kata Kunci: Peternak Kerbau, Surra, Filipina, KAP, Kerugian

ABSTRACT

Escarlos JAJr, Cane JF, Dargantes AP. 2016. Knowledge, attitudes, practices (KAP), and financial losses of buffalo raisers due to Surra among selected villages in Southern Philippines. JITV 21(3): 190-203. DOI: http://dx.doi.org/10.14334/jitv.v21i3.1417

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 were 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.

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

INTRODUCTION

Surra is caused by the blood protozoan parasite *Trypanosoma evansi* (Brun et al. 1998; Desquesnes et al. 2013b). Typically, it is a wasting disease that affects a wide range of hosts resulting to chronic weight loss, anemia, subcutaneous edema, icterus, neurological abnormalities, reproductive problems, and causing deaths among thousands of animals each year (Dargantes et al. 2009; Dobson et al. 2009; Desquesnes et al. 2013b). In the last two decades, notable large epidemics have occurred in the Islands of Visayas and Mindanao in the Philippines, having high morbidity and mortality rates in horses, cattle, goats, buffaloes, and

pigs (Manuel 1998; Dargantes et al. 2009). Agusan del Sur, a province in Eastern Mindanao, had experienced a number of these surra outbreaks particsularly in buffaloes (*Bubalus bubalis*), animals that are important in the farming activities in the province (Reid 2002; Dargantes et al. 2009).

In Agusan Province, surra control endeavors had been centered to the identification and treatment of infected animals with very little attention given to the socio-economic impact of the disease, and the knowledge, attitudes and practices of the farmers towards the disease. Nevertheless, the level of understanding of the community about surra may greatly affect the concerted efforts towards the control of this disease**.** Education of farmers on the general features and impact of surra and its control is one important component in the effective and holistic approach to control the disease in the aforesaid province. Hence, there is a need to obtain this information which will definitely serve as a guide in formulating strategies to prevent further losses from surra and eventually eradicate the disease from the province.

This study was carried out to assess the knowledge, attitudes and practices of buffalo raisers in Agusan del Sur Province regarding surra (*Trypanosoma evansi*) and the financial losses caused by the disease among their livestock.

MATERIALS AND METHODS

Time of the study and study areas

The survey was conducted from March to December 2015 among buffalo raisers in four pre-selected municipalities of Agusan del Sur (Bunawan, Esperanza, San Francisco, Talacogon) in Mindanao, Southern Philippines. The municipalities were chosen since surra outbreaks occurred in these areas in previous years. Two villages were randomly chosen from each of the identified municipality.

Research design and respondents

This cross-sectional study utilized a total of randomly selected 160 buffalo owners from eight villages in four above mentioned municipalities as respondents. Twenty buffalo raisers were randomly selected and interviewed from each selected village. Respondents were personally interviewed using a pre-tested questionnaire. A focus group discussion (FGD) was done on selected (4-6) farmers in each village to validate the responses in the interviews. Farmers were requested to come to a common site in each village for the interview. They were also requested to bring their buffaloes for free veterinary consultation, deworming and vitamin supplementation services.

Government permits for the survey were sought from the Provincial Government of Agusan del Sur, the aforementioned municipalities and barangays through the Provincial Veterinary Office.

Interview with respondents

From every village, each of the 20 randomly selected buffalo owners was personally (one-on-one) interviewed using a pre-tested questionnaire with five parts, namely: Part I: Respondent's demographic information (age, education, livelihood, place of origin, and economic status) as well as some questions regarding the number of buffaloes and other animals he/she owns; Part II: Questions that assessed the respondent's level of knowledge of the disease (causative agent, transmission, clinical signs, and treatment); Part III: The respondent's attitudes towards the problems caused by surra. This part of the survey used the Likert's Scale for beliefs and feelings scoring system, consisting of a range between 1 to 5, with 5 assuming that the farmers totally agree to the statement, and 1 assuming that the farmers totally disagree with the statement; Part IV: The respondent's prevention and control practices for surra. This part of the survey used practice frequency terms, consisting of three choices: (1) Always; (2) Sometimes; and (3) Never, indicating how frequent a respondent does a certain surra preventive activity; and Part V: Questions related to necessary information for the computation of economic losses due to surra. The interview was done in the local dialect within 5-7 minutes by 4-5 properly trained interviewers.

Focus group discussion

After the survey, an focus group discussion (FGD) was conducted in each village, to resolve any issues or any conflicting answers in the personal interviews. Four to six (20-30% of the interviewed farmers) farmers who were interviewed were requested to participate in the FGD which was facilitated by two of the interviewers.

Estimating the financial losses due to surra

To be able to calculate the economic losses caused by surra, the following data were collected from the survey respondents: (1) Total number of animals that died of surra per species; and (2) Average selling cost of mature animals per species. Data pertaining to the total animal population in the municipalities surveyed was also retrieved from the Provincial Veterinary Office of Agusan del Sur. Basic costs (in PHP) of the following items were also applied as a constant to the computation (Table 1 and 2).

Table 1. Estimated treatment cost of animals with surra per species

Species	Cost of surra treatment (PHP)
Cattles	1,500
Buffaloes	1,500
Goats	500
Pigs	1,000
Horses	1,500

Table 2. Estimated diagnostic costs for animals with surra per species

Species	Cost of surra treatment (PHP)
Cattles	500
Buffaloes	500
Goats	500
Pigs	500
Horses	500

The study utilized the formulae as presented below. Only horses, pigs, buffaloes, cattles, and goats were considered in the computation since they are the only animal species that the local government are currently treating and diagnosing for surra.

Data interpretation and statistical analysis

Data were analyzed using the Statistical Package for Social Sciences (SPSS). Descriptive statistics was used in tabulating and describing the gathered data. Frequencies, totals and percentages were used to present the results of the study. Chi-square test was used to determine significant association between the respondents' profile and their KAP answers.

RESULTS AND DISCUSSION

Demographic characteristics of respondents

The result of the demographic characteristics of the buffalo owners who participated in the survey is presented in Table 3. The respondents were mostly males (73.8%) as the males of the household are usually the ones who take care of their draft animals. Females participated in the survey (26.25%) because their husbands were either working in the fields or already deceased. Majority (90%) of the participants were married, while the rest were either single or widowed. As to age profile, majority of the respondents (24.38%) were 55 years old or older while a small proportion (2.5%) of the respondents were aged 26-30 years old. Most of the buffalo owners have between 1-3 children (40.63%) while the least proportion of respondents had no children at all (7.5%).

Out of 160 respondents, 54.38% had only elementary education and only few had obtained college education (9.38%). A high number (63.75%) of respondents had a monthly income of less than US$<100 (considered in the very low-income range), while 32.5% of the respondents had an income range between US$ 100-200 per month. Few of the respondents (3.75%) had an income between US$>200-300 per month. Majority (85.63%) of the respondents owned or had leased an area of farmland while the rest (14.38%) solely depended upon hired and paid draft work using their buffaloes for income.

Figure 1 shows the ethnic origins/groups of the respondents. Only 85% of respondents out of 160 had an idea about their ethnic origins, and only 30.4% of these respondents are members of the indigenous tribe of Agusan del Sur (Manobo). The other respondents originated from various provinces in the Philippines with various ethnic backgrounds.

Overall economic losses = Total mortality losses + Total treatment losses + Total diagnostic losses + Mass treatment and diagnostic costs

$$\text{Average economic losses per barangay} = \frac{\text{Overall economic losses}}{\text{8 (number of villages surveyed)}}$$

Total economic losses for Agusan del Sur = Economic losses per barangay × 314 (number of barangays in Agusan del Sur)

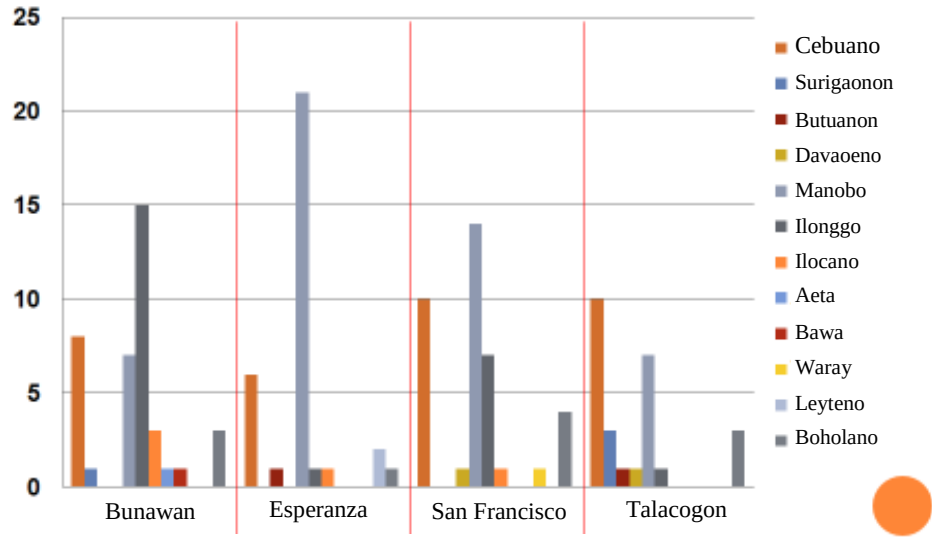

Figure 1. Ethnic group/origin of buffalo raiser respondents (n = 160) in Agusan del Sur, Southern Philippines.

Knowledge, attitudes, and practices of survey respondents

Knowledge

Figure 3 and Table 4 present the knowledge scores of respondents regarding etiology, transmission, hosts, clinical signs, diagnosis, treatment, control, and age by which an animal is affected by surra. Only 60.63% of respondents claimed to have heard of surra or have obtained information about surra, while 39.37% of them have not received any information at all. This distribution has severely affected the respondent scores, as those who had not heard of surra had problems in identifying the right answers to most of the questions pertaining to their knowledge about the disease. Major sources of information about surra came from their local veterinarians (25%).

Etiology

Regardless of the respondents having heard about surra or not, only 18.12% of buffalo owners had correctly identified surra's causative agent as a parasite (Desquesnes et al. 2013b). The rest believed that it was caused by either a virus or by a bacterium, since they claimed to have not seen the physical evidence of the parasite in the feces or in the digestive tract of dead animals, which is understandable since *T. evansi* is a blood protozoan parasite (Brun et al. 1998; Desquesnes et al. 2013b). The respondents have also claimed to have never been given clear explanations on the cause of the disease, only knowing it by name and some of its clinical manifestations.

Transmission

A total of 90 (64.28%) survey respondents correctly identified tabanids as the main vectors for surra (Vale 1974). They claimed to have observed that whenever a flood occurs in their area, the flies are very abundant in the muddy grazing fields where they tether their animals, and not long after seeing them around their buffaloes, their animals get sick with surra. The rainy season can be considered as a potentially higher risk of transmission of the disease since tabanid populations generally peak during these times, for tabanid larval habitats are usually muddy, aquatic or semi-aquatic in nature (Desquesnes et al. 2013a).

Hosts

Surra has a wide variety of hosts (Brun et al. 1998; Manuel 1998; Dobson et al. 2009; Desquesnes et al. 2013b), but the survey respondents regarded the buffalo as one of the main animals affected by the disease, as 86.87%. It was believed that buffaloes can get sick with surra. Results for other species varied greatly, with none having approximations as high as for buffaloes. This could be because the respondents have only claimed to have heard of the term surra being used on their buffaloes, as their local dialect has many different local names for various, non-specific diseases for all their livestock animals.

Table 3. Demographic characteristics of buffalo raisers (n = 160) in Agusan del Sur Province who participated in the survey

Demographic characteristics	Number of respondents	Percentage (%)
Sex		
Male	118	73.75
Female	42	26.25
Civil status		
Married	144	90.0
Single	10	6.25
Widowed	6	3.75
Age		
<25	5	3.13
26-30	4	2.50
31-35	13	8.13
36-40	25	15.63
41-45	28	17.50
46-50	25	15.63
51-55	21	13.13
>55	39	24.38
Number of children		
None	12	7.50
1-3	65	40.63
4-6	53	33.13
7 or more	30	18.75
Educational attainment		
Elementary	87	54.38
High school	58	36.25
College	15	9.38
Monthly income		
<PHP 5,000 (<US$ 100)	102	63.75
PHP 5,000 - PHP 10,000 (US$ 100-200)	52	32.50
>PHP 10,000- PHP 15,000 (>US$ 200-300)	6	3.75
Owned or leased farmland		
Yes	137	85.63
No	23	14.38

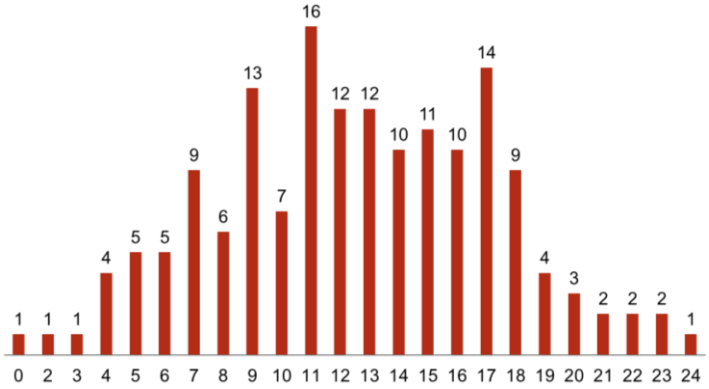

Figure 3. Knowledge scores (perfect score = 32) on surra among surveyed buffalo raisers (n = 160) in Agusan del Sur, Sourthern Philippines.

Clinical signs

Surra has a variety of nonspecific clinical signs that could easily be manifested in other diseases of buffaloes (Dargantes et al. 2009). The farmer respondents regarded weight loss (78.13%) as one of the main clinical signs of surra in combination with loss of condition (55.63%) and weakness (53.75%). Abdominal edema (Manuel 1998; Desquesnes et al. 2013b), one of the signs to look out for when one suspects surra, was only mentioned by 23.13% of respondents. Most of the respondents had a hard time distinguishing the clinical signs of surra from the clinical signs of other diseases and therefore had a hard time associating the disease directly to the symptoms they would observe.

Diagnosis

Only 56.25% of respondents knew that laboratory tests were needed to confirm cases of surra (Konnai et al. 2009; Sengupta et al. 2010; Desquesnes et al. 2013a). The rest of the respondents believed that the observation of clinical signs was enough, or by simply looking at the general appearance of the animal. Since the farmers claimed that they usually report suspected cases of surra to the local government for diagnosis when the animal is already dead or dying, there is little that the local veterinarians can do to diagnose the animal properly for the cause of death, often basing diagnosis for these animals by the clinical signs relayed by the owner. Blood collection to check animals of surra is not regularly done due to budget constraint in the government (Personal Communication Dr. Primo Calo 2014).

Treatment

Majority of the farmers (87.5%) knew that there is available treatment for surra, yet only 3.13% have correctly identified the drugs used for treatment. From the respondents' accounts, they claimed to usually either fail to ask for the name of the medication used for treatment or are simply not told. They usually only get their animals medicated by veterinarians, and have not tried to give medication to their animals by themselves. The limited availability of the drugs in the Philippine market (Dobson et al. 2009) could also be one reason why the respondents have never heard of the drugs before. Only 59.38% of respondents knew that suspected surra cases have to be treated as soon as possible, and only 53.75% knew the average duration of treatment (within one month) (Desquesnes et al. 2013a).

Control and prevention

Among the buffalo raisers surveyed, only 53.12% believed that prophylactic drugs can help in preventing surra. The rest of the population think that treatment is only used once the animal is already sick, and are mostly afraid to have their animals treated via injections, fearing fatigue due to the medicine given or even death after medication. Lighting fires (43.13%) and the use of fly traps (20.63%) were also chosen as effective preventive measures, yet were not practiced effectively, particularly in the use of fly traps, as the respondents do not have access to purchase the said fly control tools. Fly traps can be purchased easily online or abroad, and can even be made, but information about it is still limited in most regions of the Philippines, and are therefore not so widely used for fly control.

Surra can affect buffaloes at any age, but the most common age answered by respondents was adulthood (66.88%) and old age (67.5%). In the study of Dargantes et al. (2009), higher mortalities have been noted in young buffaloes aged 2-8 years old, but from the experience of the survey respondents, their older animals are usually the first ones to get sick, although this could be because farmers usually buy their buffaloes needed for draft services at around five years of age, wherein the animal has already been trained for

Table 4. Scores for knowledge about surra among buffalo raiser in Agusan del Sur, Southern Philippines

Questions	Correct answers	Percentage (%)
Etiology	29	18.12
Transmission	90	64.28
Hosts		
Buffalo	139	86.87
Cattle	79	49.38
Horse	64	40.00
Goat	35	21.88
Pig	29	18.13
Dog	10	6.25
Sheep	5	3.13
Clinical signs		
Weight loss	125	78.13
Loss of condition	89	55.63
Weakness	86	53.75
Fever	44	27.50
Edema	37	23.13
Calf mortality	30	18.75
Abortion	20	12.50
Anemia	17	10.63
Orchitis	14	8.75
Jaundice	13	8.13
Diagnosis	90	56.25
Treatment		
Treatable	140	87.50
Urgency	95	59.38
Duration	86	53.75
Drugs used	5	3.13
Control		
Prophylactic drugs	85	53.12
Light fires	69	43.13
Fly trap	33	20.63
Age affected		
Old age	108	67.5
Adult	107	66.88
Young adult	92	57.5
Newborn	81	50.63
Juvenile	80	50

different commands and is already able to plow land properly. Also, neonatal and calf deaths are more commonly blamed on other factors, such as malnutrition, trauma, gastrointestinal parasites, and extreme weather conditions.

Overall, the respondents' mean score in knowledge on surra was only 12.54 out of 32 possible correct answers, equating to 39.19% correct answers, which is 30.81% short compared to the 70% score needed to consider a respondent well informed about surra. With this, it is quite clear that the buffalo raisers in Agusan del Sur still lack sufficient knowledge about surra for them to be able to protect their animals against it, to possibly detect it earlier among their livestock and to help eradicate the disease in the province.

Attitudes

Figure 4 presents the answers of the respondents for surra attitude statements. Based on the median (m) answers for each question, the respondents strongly agreed with the following statements: Surra is a serious and life-threatening disease (m = 5), as these farmers have already either experienced or heard about the effects of surra on buffaloes during outbreaks, it would be reasonable enough for them to believe that it is a damaging disease. They need to consult a veterinarian if they think their animal has surra (m = 5) since the farmers failed in self-medicating their animals for surra, they always try to consult and seek treatment from the nearest veterinarian. Controlling the occurrence of surra is a problem for them (m = 5). The municipalities surveyed had already experienced surra outbreaks before, and even though there are methods available for detection of the parasite in sick animals, as well as medication, sporadic outbreaks still continuously occur (Dargantes et al. 2009; Dobson et al. 2009), this is a big problem for buffalo raisers. Downtime for sick animals, not to mention deaths of their animals that have surra, greatly affects the respondents' income and relatively, their way of life. Information about surra from the government is helpful (m = 5), The respondents had explained that although the Provincial Veterinary Office was quick to diagnose and treat surra cases in their respective municipalities, they were not given any information about the disease itself, only truly knowing the illness by its name and some commonly observed clinical signs. It might be a greater risk if they leave their animals untreated (m = 5), The respondents have already seen or experienced having several untreated animals die from the disease, and therefore believe that it should be treated as soon as it is diagnosed. Animals with surra are hard to manage (m = 5), The farmers believed that management of a sick animal is difficult, may it be sick with surra or even any other disease. Weakness, loss of condition and other clinical signs make it difficult for the owners to use their animals for draft services (Desquesnes et al. 2013b).

There should be control of movement of animals and checking for surra before they are moved (m = 5), The respondents believed that the movement of animals should be controlled to prevent the spread of contagious diseases, surra included. In several accounts during the survey, the farmers associated buffaloes given to them for free during dispersals to the occurrence of a surra outbreak not long after being given these animals. A total of 121 beneficiaries from all around the province had received buffaloes from several government and non-government organizations (DAMRDP 2015). There is a need for a local ordinance to implement this (m = 5), The buffalo raisers surveyed believed that a local ordinance focused on the treatment and eradication of surra would be beneficial for the whole province. They believed that any laws that would help to control and eradicate the disease would help them greatly with their livelihood. The occurrence of the disease would definitely decrease if there is better control of animal movement, treatment programs and better diagnostic tools that could be used to identify sick animals in the field.

Respondents slightly disagreed on the following statements:

1. They are well informed about this disease (m = 2)
   Respondents believed that they need more information about surra in order to fully understand it. Better understanding about the etiology, transmission, clinical signs, and treatment of the disease will help the buffalo raisers to prevent, detect and treat surra effectively.

Respondents strongly disagreed on the following statements:

1. They can diagnose their animals suffering from surra without the help of a veterinarian (m = 1)
   The respondents had a hard time knowing whether their animals have surra or not. The non-specific clinical signs and chronic nature of surra in buffaloes make it hard for them to detect surra without professional diagnosis. They highly depend on the veterinarians of the government to diagnose surra in their animals.

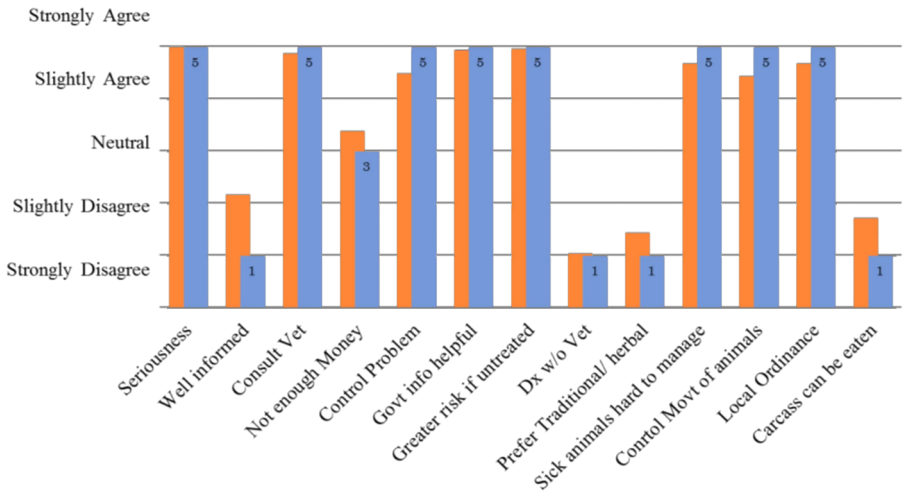

Figure 4. Attitude answers for surra of buffalo raisers in Agusan del Sur, Southern Philippines.

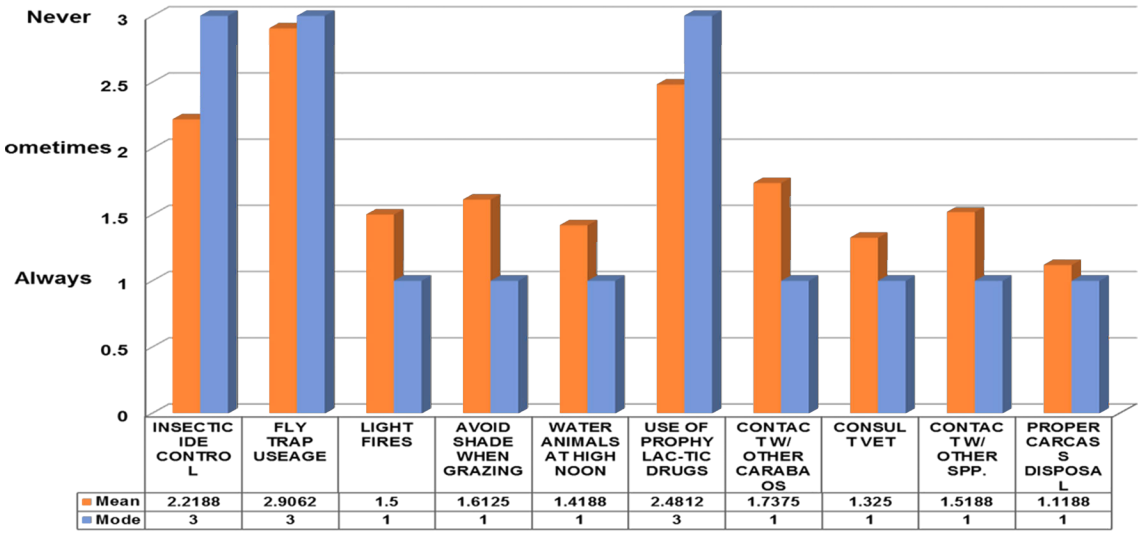

Figure 5. Surra prevention practices of farmers raising buffaloes in Agusan del Sur Province, Southern Philippines.

2. The carcass of an animal that died from surra can be eaten (m = 1)
   Respondents do not believe that the meat from an animal that has died of surra should be eaten. They believed that eating the meat may cause problems like sickness or even intoxication.

The respondents neither agreed nor disagreed with the following statements:

1. They do not have enough money to buy drugs that can treat surra (m = 3)
   The respondents reasoned out that having enough money for treatment was a matter of good timing.
2. They prefer traditional methods/herbal remedies to cure their animals suffering from surra (m = 3.5)
   The respondents have very little trust on traditional and herbal remedies to treat surra. They have seen that this does not work on the disease from past experiences and experiences of their neighbors who had animals that died from surra before. Although they will use traditional medicine as emergency treatments whenever help cannot immediately be sought, they think that it is a temporary solution that does not really target the problem itself.

Practices

Figure 5 presents the practices of farmers raising buffaloes in Agusan del Sur Province on dealing with surra. The most common answers (mode) for each question are also presented in the Table 6.

Table 5. Responses of buffalo raisers to attitude statements on surra

Statement	Number of respondents				
	Strongly disagree	Slightly disagree	Neither agree/ disagree	Slightly agree	Strongly agree
Surra is a serious and life-threatening disease	1	2	8	15	134
I am well informed about this disease	79	21	32	11	17
I should consult a veterinarian if I think my animal has surra	3	0	2	4	151
I do not have enough money to buy drugs that can treat surra	12	19	64	21	44
Controlling the occurrence of surra is a problem	2	11	12	16	119
Information about surra from the government is helpful	0	0	2	6	152
It might be a greater risk if I left my animal untreated	0	0	1	4	155
I can diagnose my animal suffering from surra without the help of a veterinarian	92	19	18	13	18
I prefer traditional methods/herbal remedies to cure my animal suffering from surra	62	18	47	21	12
Animals with surra are hard to manage	1	3	11	15	130
There should be control of movement of animals and checking for surra before they are moved	5	5	15	24	111
I think we need a local ordinance to implement this	1	3	12	14	130
The carcass of an animal that died from surra can be eaten	112	11	18	8	11

Table 6. Practices on dealing with surra among farmers raising buffaloes in Agusan del Sur Province, Southern Philippines

Practices	Respondents' answers		
	Always	Sometimes	Never
Insecticide control	35 (21.88%)	53 (33.13%)	72 (45%)
Fly trap usage	3 (1.88%)	9 (5.63%)	148 (92.5%)
Light fires	95 (59.38%)	49 (30.63%)	16 (10%)
Avoid shade when grazing	13 (8.13%)	72 (45%)	75 (46.88%)
Water animals at high noon	107 (66.88%)	39 (24.38%)	14 (8.75%)
Use of prophylactic drugs	11 (6.885)	61 (38.13%)	88 (55%)
Contact with other carabaos	27 (16.88%)	63 (39.38%)	70 (43.75%)
Consult veterinarian	116 (72.5%)	36 (22.5%)	8 (5%)
Contact with other spp	20 (12.5%)	44 (27.5%)	96 (60%)
Proper carcass disposal	144 (90%)	13 (8.12)	(1.88%)

Based on the median (m) answers for each question, the survey respondents always practiced the following:

1. Light fires (m = 1)
   The respondents would usually light fires in the afternoon, to keep away mosquitoes, tabanids, and other insects.
2. Water animals at high noon (m = 1)
   The farmers would often leave their animals to wallow in mud and drink water at high noon so that they will no longer be thirsty for the rest of the day and will have a protective layer of mud against biting flies and mosquitoes.
3. Consult a veterinarian when an animal is sick (m = 1)
   Since access to the local government veterinarians is attainable, most of the respondents would always consult a veterinary doctor when their animals are sick.
4. Contact with other animal species (m = 1)
   Just like with other buffaloes, contact between the respondents' buffaloes and other species of animals is unavoidable, especially with cattle, cats and dogs.
5. Proper carcass disposal (m = 1)
   The respondents always properly buried their dead animals to avoid getting sick themselves and to prevent other animals from getting sick.

The respondents sometimes did the following practices:

1. Insecticide control (m = 2)
   The use of insecticide for pest control is not a common practice for the buffalo raisers in Agusan del Sur.
2. Contact with other buffaloes (m=2)
   Respondents said that contact with other buffaloes within or outside of their respective barangays is unavoidable.

Most respondents never did the following practices. 1) Fly trap usage (m = 3), fly traps are not well known in the survey areas and therefore have not been used by the respondents to control flies in their respective farmlands. 2) Avoid shade when tethering their buffaloes (m = 3), buffalo raisers would usually keep their animals in the shade to avoid heatstroke while being tethered. 3) Use of prophylactic drugs (m = 3) Since the respondents do not have access to drugs being used to prevent surra, they have never tried giving prophylactic treatment to their animals on their own.

Association between respondent's demography and KAP scores

Only age has a significant association on the surra knowledge scores of the survey respondents. Female respondents tend to have higher knowledge scores than males. Scores on attitudes and practices for surra were unaffected by the buffalo raiser's demography. This findings conforms with the previous findings in other country (Cheraghli et al. 2014). The scores were highest among housekeepers since majority of housekeepers are female, related to housewives spending more time obtaining health information from different sources, as well as increased possibility of women in the community communicating and sharing this information to others. Similarly, the women in the towns surveyed are housewives who stay at home or tend to gardens and their farms, leaving the draft work to their male counterparts. Information, confirmation and discussion about the disease whenever an outbreak occurs are done more by females than males.

Financial losses

Tables 7 to 11 present the different financial losses due to surra in Agusan del Sur Province. The overall estimated economic losses for the four municipalities surveyed reached 13.7 million Philippine Pesos (PHP), with estimated losses per barangay of PHP 1.7 million and a calculated economic loss of PHP 538 million for the whole province (from a total of 314 barangays). IThe study including the same area reported in 2009 (Dobson et al. 2009), an estimated PHP 7.9 million (US$ 158,000) per annum is lost in every village without effective surra control. However, the previous study had also included losses from abortion, calf mortality and costs of replacement animals, thus the big difference. It can also be taken into consideration that the financial losses in the municipalities surveyed has decreased due to several factors, including the unrecorded cases of surra outbreaks or undiagnosed isolated cases, as well as the subtraction of mass treatment and diagnostic costs for pigs and animal mortalities unaccounted for by the survey respondents. Another factor could be the continuous efforts of the Provincial Veterinary Office to do mass treatments and early detection of the disease, as mortalities have greatly reduced. Sustainability of these efforts would affect the losses to a greater extent, and regular monitoring and treatments could eventually eradicate surra from Agusan del Sur Province.

Table 7. Total financial losses of buffalo raisers (n = 160) in eight villages (in four towns) of Agusan del Sur Province, Southern Philippines from surra mortality among their livestock

Village	Losses (PHP)					
	Cattle	Buffalo	Goat	Pig	Horse	Per village
Poblacion, Bunawan	0	65,684	0	39,722	0	105,406
San Andres, Bunawan	44,000	331,667	0	0	0	375,667
Borbon, San Francisco	0	868,000	0	0	20,000	888,000
Ladgadan, San Francisco	606,625	524,400	0	0	312,500	1,443,525
Mahagkot, Esperanza	573,067	2,778,000	0	0	0	3,351,067
Cubo, Esperanza	0	580,500	9,341	86,032	0	675,873
Labnig, Talacogon	33,454	620,000	26,818	13,626	0	693,898
Kauswagan, Talacogon	19,083	1,725,105	0	0	0	1,744,188
Total per species	1,276,229	493,356	36,159	139,380	332,500	
Total losses, mortality						9,277,624

Table 8. Total treatment costs for surra among livestock spent by buffalo raisers (n = 160) in eight villages (in four towns) of Agusan del Sur Province, Southern Philippines

Village	Treatment costs (PHP)					
	Cattle	Buffalo	Goat	Pig	Horse	Per village
Poblacion, Bunawan	0	4,500	0	10,000	0	14,500
San Andres, Bunawan	3,000	22,500	0	0	0	25,500
Borbon, San Francisco	0	52,500	0	0	3,000	55,500
Ladgadan, San Francisco	34,500	34,500	0	0	37,500	106,500
Mahagkot, Esperanza	42,000	180,000	0	0	0	222,000
Cubo, Esperanza	0	40,500	2,000	22,000	0	64,500
Labnig, Talacogon	3,000	46,500	5,000	3,000	0	57,500
Kauswagan, Talacogon	1,500	109,500	0	0	0	111,000
Total treatment cost per species	84,000	490,500	7,000	35,000	40,500	
Treatment cost per species	1,500	1,500	500	1,000	1,500	
Total treatment cost						657,000

Table 9. Diagnostic costs for surra among livestock spent by buffalo raisers (n = 160) in eight villages (in four towns) of Agusan del Sur Province, Southern Philippines

Village	Diagnostic costs (PHP)					
	Cattle	Buffalo	Goat	Pig	Horse	Per village
Poblacion, Bunawan	0	1,500	0	5,000	0	6,500
San Andres, Bunawan	1,000	7,500	0	0	0	8,500
Borbon, San Francisco	0	17,500	0	0	1,000	18,500
Ladgadan, San Francisco	11,500	11,500	0	0	12,500	35,500
Mahagkot, Esperanza	14,000	60,000	0	0	0	74,000
Cubo, Esperanza	0	13,500	2,000	11,000	0	26,500
Labnig, Talacogon	1,000	15,500	5,000	1,500	0	23,000
Kauswagan, Talacogon	500	36,500	0	0	0	37,000
Total diagnostic cost per species	28,000	163,500	7,000	17,500	13,500	
Diagnostic cost per species	500	500	500	500	500	
Total diagnostic costs						229,500

Table 10. Total mass treatment costs among livestock against surra in eight villages (in four towns) of Agusan del Sur Province, Southern Philippines

Village	Costs (PHP)					
	Cattle	Buffalo	Goat	Pig	Horse	Per village
Poblacion, Bunawan	9,000	322,500	67,500	0	0	399,000
San Andres, Bunawan	9,000	60,000	12,500	0	7,500	89,000
Cubo, Esperanza	15,000	105,000	30,000	0	0	150,000
Labnig, Talacogon	10,500	127,500	48,500	0	0	186,500
Borbon, San Francisco	34,500	487,500	196,500	0	3,000	721,500
Ladgadan, San Francisco	21,000	54,000	63,000	0	37,500	175,500
Mahagkot, Esperanza	195,000	180,000	30,000	0	0	405,000
Kauswagan, Talacogon	42,000	165,000	68,500	0	0	275,500
Total mass treatment cost per species	336,000	1,501,500	516,500	0	48,000	
Total mass treatment cost						2,402,000

Table 11. Total mass diagnostic costs for surra among livestock in eight villages (in four towns) of Agusan del Sur, Southern Philippines

Village	Costs (PHP)					
	Cattle	Buffalo	Goat	Pig	Horse	Per village
Poblacion, Bunawan	3,000	107,500	67,500	0	0	178,000
San Andres, Bunawan	3,000	20,000	12,500	0	2,500	38,000
Borbon, San Francisco	11,500	162,500	196,500	0	1,000	371,500
Ladgadan, San Francisco	7,000	18,000	63,000	0	12,500	100,500
Mahagkot, Esperanza	65,000	60,000	30,000	0	0	155,000
Cubo, Esperanza	5,000	35,000	30,000	0	0	70,000
Labnig, Talacogon	3,500	42,500	48,500	0	0	94,500
Kauswagan, Talacogon	14,000	55,000	68,500	0	0	137,500
Mass diagnostic cost per species	112,000	500,500	516,500	0	16,000	
Total diagnostic cost						1,145,000

Table 12. Total financial losses (PHP) due to surra among livestock in eight villages (in four towns) of Agusan del Sur, Southern Philippines

Type of loss	Amount
Total losses from mortalities	9,277,624
Total losses from treatment costs	657,000
Total losses from diagnostic costs	229,500
Total mass treatment costs/losses	2,402,000
Total mass diagnostic costs/losses	1,145,000
Overall total losses among eight survey villages	13,711,124
Average loss per village	1,713,890
Estimated total losses for Agusan del Sur	538,161,617

Table 12 shows the total financial losses from surra among livestock for the four barangays surveyed, as well as the total losses for each barangay and the estimated losses for the whole province, assuming that each of the 314 barangays in Agusan del Sur has the same economic losses as the barangays included in the study.

CONCLUSION

This research results support the conclusions that (1) buffalo raisers in Agusan del Sur, Southern Philippines lack adequate knowledge, attitudes and practices that are necessary for an effective surra control; and (2) these farmers suffer from high financial losses due to surra affecting their livestock.

ACKNOWLEDGEMENT

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   Elucidating clearly about materials used and method carried out. If the material using animals in the experiment, please indicate that the animals are performed according to animal ethics and welfare. See ethical statement in the attachment.

6. **Results and Discussion:**
   It presents and discusses clearly and completely achieved research results based on the purpose. Result and discussion may be presented separately or united. Result description may be completed by consise tables and clear illustrations (black and white graphics, figures or photos) on separated page. Tabel description (on top) and illustrations (in bottom) should be clear and independent, so readers may easily understand the table without read the text. Discussion description consists of description of result and research mean and benefit associated with issue which will be solved. Measurement units both in table or illustrations use metric system.

7. **Conclusion:**
   It is a manuscript final summary.

8. **Acknowledgement:**
   It can be written if needed.

9. **References:**
   The author is recommended to use Mendeley Program (http://www.mendeley.com) and citation style of Taylor & Francis - Council of Science Editors (author-date). Mendeley program utilization is aimed to avoid mistakes in citations and references writting. Cited references (preferably, 80% is primary article and the last 10 years publication). and should not from unpublished articles such as practical guidance and research report, except thesis and dissertation. Download is allowed if it is from electronic magazine, genom database or patent.

   Citation in the references:
   Literatures in reference are written alphabetically based on the author's name. Same author is written sequentially starting from earlier order.

   Example of reference writing

   Primary paper:
   Bhanja SK, Anjali DC, Panda AK, Sunder GS. 2009. Effect of post hatch feed deprivation on yolk-sac utilization and young broiler chickens. Asian-Aust J Anim Sci. 22:1174-1179.

   Book:
   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.
   c. Paloheimo M, Piironen J, Vehmaanpera J. 2010. Xylanases and cellulases as feed additives. In: Bedford MR, Partridge GG, editors. Enzymes in farm animal nutrition. 2nd ed. New York (USA): CABI Publishing. p. 12-53.

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:
a. [NRC] National Research Council. 1985. Nutrient requirements of sheep. 6th revised. Washington DC (USA): National Academic Press.
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.

10. **Citation in text:**
Citation consists author's last name and publication year.

Example:

a. One author: ..... grow slower than lamb fed cattle's milk (Supriyati 2012). Supriyati (2012) formulates.....
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