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Nutrient Intake, Digestibility, Rumen Parameters and Blood Metabolites of Kacang Goats Fed Silage of Forage Mixture Produced from Intercropping of Sorghum Differing in Planting Space with Butterfly Pea (*Clitoria ternatea*)

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ABSTRACT

An experiment was conducted to investigate the effect of feeding silage of sorghum-butterfly pea mixture (which was made of forage harvested from sorghum sown at differing planting space and intercropped with *Clitoria ternatea*) on intake, digestibility, rumen environment and blood metabolites of Kacang goats. The experiment was designed following a replicated latin square with three treatments and three periods. In each period, two goats were randomly fed one of the three silage mixture which was made of clitoria and sorghum mixture harvested from sorghum planting space of 20×20 cm^2 (S_{20}), 40×40 cm^2 (S_{40}) and 60×60 cm^2 (S_{60}). Data on feed intake, digestibility, ruminal pH, ammonia (NH_3) and volatile fatty acid (VFA) concentrations as well as blood glucose and total plasma protein were collected during the last week of each period which lasted for three weeks. Results showed that crude protein (CP) content were increased with increasing sorghum planting space as the proportion of clitoria in the forage mixture increased. Meanwhile, crude fiber (CF) were comparable among different silage. Dry matter intake (DMI) and digestibility were not different (P>0.05) in Kacang goats fed different silage. However, rumen NH_3 and VFA concentrations were significantly (P<0.05) lower in S_{20} compared to S_{40} and S_{60} groups. It is concluded that in order to produce good quality silage for Kacang goats there is no benefit of increasing sorghum planting space beyond 40×40 cm^2 when intercropped with *Clitoria ternatea*.

Key words: *Clitoria ternatea,* Intercropping, Kacang Goats, Silage, Sorghum

INTRODUCTION

There has been a growing interest of farmer in The Province of Nusa Tenggara Timur, Indonesia to utilize Sorghum (*Sorghum bicolor* L) fodder as ruminant feed to cope with feed scarcity during dry season. Compared to maize, sorghum is more drought tolerant capability and fodder production is also higher due to forage addition from second and third cut (Nullik, 2009). It is also palatable for ruminants when fed as fresh, hay or silage (Gupta *et al.,* 2000). Sorghum is commonly harvested at early generative stage and given to animals either fresh or in ensiled form. The quality of sorghum silage, however, is only sufficient for moderate level of daily gain. CP content of sorghum silage varies from 5.2% to 7.4% (Mahanta and Pachauri, 2005) with digestibility of 55-62% when harvested at milk stage (Owen, 1967). For higher level of animal production, it is therefore necessary to improve silage CP content by mixing with high-protein forages such as a variety of forage legumes during the ensiling process. Alternatively, sorghum can be sown inter-cropped with forage legumes, hence, it will produce a certain mixture of forage with higher quality of silage. In addition, forage legumes may provide additional soil nitrogen which improves forage production (Willey, 1990) and often nutritive quality of the companion plants (Mureithi and Thorpe, 1993). One of those forage legumes which have been reported to successfully thrive and producing appreciable amount of forage in intercropping system with grass, maize and sorghum in semi-arid area is butterfly pea (*Clitoria ternatea).* Mureithi and Thorpe (1993) reported that *C. ternatea* was the better legume to Napier grass inter-croppping compared to *Macroptilium atropupureus* and *Stylosanthes guyanensin* in coastal lowland of Kenya. This legume forage is also found to be well adapted to

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the local environment (Nullik, 2009; Jelantik *et al.,* 2015).

To obtain the expected silage quality from a clitoria-sorghum inter-cropping system, it is required to know the appropriate plant density for at least one of the species in the plant mixture. Different plant density obtained by planting certain forage species at certain planting space will determine the ratio of clitoria and sorghum biomass produced as it involves competition among the system component for light as well as for water and nutrients (Ali *et al.,* 2013). This further affects silage nutritive value which consequently influences nutrient intake, digestibility and the silage utilisation by goats. Scientific evidence on the effect of feeding silage made from forage mixture harvested from sorghum-clitoria intercropping system to meat type goats in the tropics is limited. Therefore, this study was designed to investigate the intake and nutrient utilization by Kacang goats fed silage made from sorghum clitoria inter-cropping system where sorghum was planted at different space.

MATERIALS AND METHODS

Eighteen 2×2 m^2 plots were prepared in vertisol soil during March 2018 and 1 kg cows manure compost were applied during land preparation. Clitoria seeds (*Clitoria ternatea* cv Millagra) were sown at planting space of 20×20 cm^2 in April 2018. Seeds were directly broadcasted and immediately watered. Two weeks after clitoria was sown and already germinating, sorghum was planted at different planting space, *i.e.* 20×20 cm^2, 40×40 cm^2 and 60×60 cm^2, respectively at six plots for each planting space randomly. Nitrogen fertilizer was applied at 120 g/plot or 300 kg/ha 20 days after planting. All plots were watered every 2 days and weeds were removed every two weeks.

Forages were harvested at early flowering stage *i.e.* at 60 days after planting. The harvested forage mixture was then wilted for 6 hours and thereafter chopped at 2-4 cm length before put in a plastic mini silo (50 kg each) and ensiled for four weeks. No additive was added during the ensiling process.

Six mature Kacang goats weighing 11.54 ± 1.15 kg were used in this experiment following a replicated 3×3 latin square design with three treatments and three periods of 21 days each. The animals were randomly allotted to one of the three experimental feeds, *i.e.* silage of forage produced form clitoria-sorghum inter-crops with different sorghum planting space: 20×20 cm (S_{20}), 40×40 cm (S_{40}) and 60×60 cm (S_{60}). All animals were also offered concentrate mixture containing ground maize, rice bran, coconut cake, coconut oil and premix. The nutrient content of concentrate mixture was 11.19% CP, 19.95% CF, 5.4% ether extract (EE), 57.74% nitrogen free extract (NFE) and 17.58 MJ gross energy (GE)/kg DM. Experimental goats had *ad libitum* access to clean water.

As much as 50 g of silage were sampled from every silo and about 10 g was sub-sampled for DM determination. The rest were sun-dried and pooled for each silage type every period. Silage was offered *ad libitum* to the animals twice a day at 0800 and 1600 h as soon as the concentrate mixture was completely consumed. Residues were collected before morning feeding, weighed and sampled for DM determination.

A digestion trial of five-day total fecal collection was conducted for determination of apparent digestibility of DM and nutrients at the last week of each period. Feces was collected before morning feeding. The collected feces were weighed and about 10% was sampled. Each fecal sample was sub-sampled for DM determination and the rest was sun-dried. The sun-dried fecal samples were then pooled at the end of the collection period for each animal and stored till further chemical analyses. Apparent dry matter digestibility was determined by the difference between dry matter or nutrients offered and dry matter or nutrients recovered in feces. Urine was also collected at the same period. The collected urine was weighed and sample was taken and frozen thereafter.

Rumen liquid was taken 2-4 hours after morning feeding by using a stomach tube from each animals at the last day of each period. The collected rumen liquid was strained with layers of nylon cloth followed by pH determination and thereafter quickly acidified using concentrated sulfuric acid to a pH below 3.0. The samples were then stored frozen. At the end of the experiment, the frozen rumen liquids were thawed before analyses for ammonia and VFA concentration.

Blood samples were taken from jugular vein of each animals quickly after rumen fluid collection. Blood was collected using a venojeck connected to a vacuum glass bottle containing disodium EDTA as anticoagulant. Blood samples were then centrifuged at 3000 rpm for 15 minutes. Blood plasma was taken and stored frozen until analyses for glucose and total plasma protein.

Dry matter determination was conducted by drying in the ventilated oven at 105°C until constant weight. Proximate analyses were conducted following methodology of AOAC (2005). Ash content was determined by ashing feeds and fecal matter in a furnace at 550°C. CP content was determined by using Kjeldahl method and CF was determined after acid and alkali extractions. NH_3-N concentration was analyzed by micro-diffusion technique (Conway, 1957). Total VFA concentration was determined by steam distillation technique.

All data were analyzed using Proc. GLM (SPSS version 21). Significant difference between means was assigned at P<0.05.

RESULTS AND DISCUSSION

Biomass proportion of sorghum and clitoria as well as nutrient content of silage made from forage which was produced from a clitoria-sorghum inter-crops with different sorghum planting space is presented in Table 1. As expected, the biomass proportion of *C. ternatea* in the total fresh forage mix increased with higher sorghum planting space while sorghum biomass was reduced. Clitoria is known as a fast growing climbing forage legume (Gomez and Kalamani, 2003) that has been intercropped with maize (Jelantik *et al.,* 2016), grass (Mureithi and Thorpe, 1993; Ali *et al.,* 2013) and sorghum (Ayoub, 1985) with varying success. The biomass production of clitoria declines with narrower planting space of the companion plant due to the strong negative effect of shade on plant growth (Minson and Wilson, 1994). Ali *et al.* (2013) found that clitoria biomass production was suppressed when *C. ternatea* was grown intercropped with *Setaria splendida*. Similarly, Ayoub (1985) reported that biomass production of clitoria was reduced by 42% when grown inter-cropped with sorghum compared to monoculture. Some results, however, indicates that *C. ternatea* is one of shade-tolerant forage legumes (Congdon and Addison, 2003; Nicodemo *et al.,* 2015) with negligible effect on its biomass production when intercropped with dense planting space. Jelantik *et al.* (2016) reported that when harvested at 60 days the total biomass production was not reduced when intercropped with maize at different planting space, i.e 40×40 cm compared to 180×40 cm.

Silage CP content in the present experiment increased with increasing sorghum planting space due to increasing proportion of clitoria in the silage. Clitoria contains higher CP compared to sorghum. Ratnawati *et*

Table 1. Forage production and nutrient content of silage used in the experiment

Item	Sorghum planting space		
	S_{20}	S_{40}	S_{60}
Forage biomass proportion before ensiled, kg DM/kg			
Sorghum	0.652	0.590	0.462
Clitoria	0.348	0.410	0.538
Nutrient content (% DM basis)			
OM	80.76	80,06	79,73
CP	16.83	19,22	18,36
CF	31.51	30,54	31,21
EE	3.44	2,70	5,75
Nitrogen NFE	28.98	27,80	24,41
GE (kcal)	2.75	2,71	2,84

al. (2013) reported CP content varying 17.85-18.73% in Clitoria harvested at 120 days after planting. Hartutik *et al.* (2012) reported that CP content of *C. ternatea* grown monoculture and harvested at 90 days after planting was 18.38%. Sorghum harvested at 50 days contained 9.3% CP and declined to 5.1 % at 100 day after sowing (Saini, 2012). Ayub *et al.* (2004) also reported that CP content of green sorghum fodder was 9.29%. Depending on the ratio of the mixture, CP content of the mixture varied from 13.23% to 16.16 % in rice bean dominating forage mixture (*Ayub et al.,* 2004).

Unlike CP content, the silage CF content in the present experiment was comparable among different type of silage despite higher proportion of sorghum in the forage mixture with increasing planting space. Similar result was reported by Ayub *et al.* (2004) who found that CF content of sorghum rice-bean mixture varied between 30 to 34%, even when a larger variation in CF content was noted for rice-bean and sorghum grown alone, *i.e.* 29.5-36.5%, respectively. This was possibly due to the increasing sorghum fiber content with increasing plant density while that of clitoria remain unchanged. Aydemir and Kizilsimsek (2018) found that NDF and ADF of sorghum were increased in lower plant density but this did not occur with soybean when they were grown as intercrops.

Nutrient intake and digestibility of silage of forage mixture produced from sorghum-clitoria inter-crops with different sorghum planting space in Kacang goats is presented in Table 2. DMI varied narrowly between 35.3 to 38.2 g $LW^{0.75}$ day^{-1} or about 1.9-2.0% LW of Kacang goats weighing 10.06 to 13.45 kg. This level of DMI was lower than that reported earlier in goats consuming sorghum silage. Barros *et al.* (1986) reported DMI of goats consuming sorghum and clitoria silage were 65.9 and 77.2 g/kg $LW^{0.75}$, respectively. This low level of intake was unexpected

Table 2. Nutrient intake and digestibility in goats consuming sorghum-*C. ternatea* silage

Parameter	Treatments			SEM	P-value
	S_{20}	S_{40}	S_{60}		
Dry matter intake (g/day)					
DMI (% of BW)	2.068	1.915	2.018	0.112	0.634
DMI (g/kg $BW^{0.75}$)	38.12	35.30	37.18	2.132	0.651
Nutrient intake (g/day)					
OM	219.77	202.99	214.85	13.541	0.680
CP	40.98	40.95	41.82	2.34	0.981
CF	47.26	44.29	45.73	3.229	0.814
EE	13.70	10.27	14.75	1.091	0.046
NFE	106.74	96.44	101.63	6.65	0.572
GE (MJ/day)	37.40	33.81	36.83	2.357	0.277
Digestibility (%)					
DM	66.06	68.90	62.98	2.528	0.307
OM	68.42	70.69	64.79	2.360	0.262
CP	71.82	76.46	72.02	1.78	0.180
CF	35.73	46.60	35.53	4.596	0.211
EE	79.03a	70.89b	69.82b	2.127	0.025
NFE	75.84	74.14	69.58	2.741	0.156
Energy	73.87	74.84	70.27	1.960	0.277

Means within the same rows followed by a different alphabet differ significantly (P<0.05) considering the diets offered to the animals were relatively of good quality. All silage used in the present experiment contained high crude protein, *i.e.* much higher than 6.2% at which the intake of ruminant animals started to fall (Minson, 1990). Potchoiba (1990) observed DMI between 3.6 to 3.9% BW in goats fed good quality feeds high in CP content.

There are a number of factors that may cause the low silage intake by Kacang goats in the present experiment. High fiber content in sorghum-based silage is often reported to limit intake. Ngongoni *et al.* (2008) explained the observed lower intake of sorghum based silage compared to maize-based silage as sorghum silage contained higher NDF as compared to maize silage. Nichols *et al.* (1998) found that DMI of dairy cows were reduced with increasing NDF content of sorghum silage. Cooke *et al.* (2008) also found high correlation between DMI and forage NDF content. Legumes often do not ensile well due to moisture content and high buffering capacity which can cause high effluent loss and unstable silage of high pH and such silage has low palatability (Titterton, 1997).

Nevertheless, the increasing DMI with increasing sorghum planting space observed in this experiment was expected due to the increasing proportion of clitoria biomass in the silage. As discussed before, the increase of clitoria in the silage improved protein content of the silage by 14.2%. With this increase, it would be expected that DMI is increased. Negesse *et al.* (2001) reported a 36% increase of DMI when dietary CP level was increased from 8% to 15.5%. Similarly, Jia *et al.* (1995) found increasing DMI with increasing dietary CP level. In addition, *C. ternatea* was reported to have higher intake compared to forage sorghum at any stage of maturity (Kawas *et al.,* 1985). The results of the present experiment, however, showed that DMI and nutrient intakes were not different (P>0.05) between goats offered different types of silage. This finding is contrary to other research finding where legumes were included during sorghum ensiling. Ngongoni *et al.* (2008) reported that the inclusion of 40% lablab (*Lab lab purpureus*) in sorghum silage increased DMI in sheep. One explanation is that dietary CP content of the control diet was optimum that provided NH_3 and/or amino acid or peptida for microbial population and activities, thus further increase in CP content of the diet did not result in any further improvement in intake. This phenomenon was for example reported by Negesse *et al.* (2001) who observed a significant increase of intake of Saanen kids when dietary CP was improved from 80 to 105 g/kg but no further increase was noted at higher dietary CP content. In our experiment, dietary CP content was above that level. Previously, Lu and Potchoiba (1990) observed a sharp 35.0% increase in DMI when dietary CP was increased from 11.2 to 12.7% compared to only 9.0% increase when dietary CP was increased from 12.7 to 15.1%.

Since the level of intake in this experiment was low in all treatments, the difference of CP content of silage used in the present experiment was expected to have more profound effect on the DM digestibility.

Table 3. Rumen and blood attributes in goats consuming Sorgum-*C. ternatea* silage

Parameters	Treatment			SEM	P-value
	S_{20}	S_{40}	S_{60}		
Rumen parameters					
pH	6.97	6.72	7.02	0.18	0.467
VFA (mmol/l)	104.18[a]	114.58[b]	121.81[b]	2.15	0.001
NH_3 (mmol/l)	10.13[a]	12.21[b]	11.07[ab]	0.39	0.017
Blood metabolites					
Total plasma protein (g/dl)	6.02	6.38	6.32	0.28	0.63
Glucose (mmol/dl)	115.29	113.18	112.86	3.789	0.835

Means within the same rows followed by a different alphabet differ significantly (P<0.05)

Results showed that the digestibility of nutrients did not differ ($P>0.05$) among treatments. This finding appears to corroborate well with some previous reports which indicate that *C. ternatea* had reasonably high digestibility due to its high CP content (Nasrullah *et al.,* 2003). Hartutik *et al.* (2012) reported *in vitro* DM digestibility (IVDMD) and *in vitro* organic matter digestibility (IVOMD) of 65.95 and 66.28% for *C. ternatea* harvested at early flowering stage. Other researchers reported that IVDMD of *C. ternatea* varied between 59.9% (Ratnawati *et al.,* 2013) to 74.15% (Nasrullah *et al.,* 2003). Those recorded level of IVDMD was higher than that of sorghum. Hence, when there was an increase of biomass proportion of clitoria in the silage with higher sorghum planting space, it was rather expected that DM digestibility would be higher in S_{40} and S_{60} compared to S_{20}. The absence of increased digestibility with increasing clitoria proportion in the silage mixture may be caused by many different factors. One of these factors is the fermentative profile of the silage. Although CP content of silage with higher clitoria proportion due to higher sorghum planting space is higher, but most of the protein is degraded with improper ensiling process which ends up with high NH_3-containing silage which has lower digestibility (Nussio *et al.,* 2002). The other factor is that the content of water-soluble carbohydrates in sorghum was higher than in clitoria.

Rumen pH in this experiment varied between 6.72 and 7.02. This level is considered as favourable pH range which provides suitable environment for optimum ruminal fibre degradation (Van Soest, 1994). Similarly, NH_3 concentration in this experiment were well above the threshold level of 50 to 80 mg/l for optimal microbial growth and activities as suggested by Satter and Slyter (1974). Rumen pH did not differ ($P>0.05$) between goats consuming different type of silage, however, rumen liquid concentration of NH_3 and VFA were significantly lower ($P<0.05$) in S_{20}, *i.e.* in goats receiving silage made of forage with narrower sorghum planting space. Silage made from higher sorghum planting space (S_{40}) and (S_{60}) were comparable. Rumen pH is the balance between the acid production rate and the buffering capacity from saliva and to a lesser extent from weak base NH_3 produced in the rumen. It was therefore expected that when there was an increase VFA concentration in S_{40} and S_{60}, rumen pH would decline. However, since at the same time NH_3 concentration was also increased, it neutralized the effect.

Ruminal NH_3 concentration depends on CP content and its rate of degradation in the rumen. In this experiment, CP content of S_{60} was higher than S_{20}. However, the more determinant factors for ruminal NH_3 concentration is the extent of protein degradation in the rumen. In poor ensiling conditions, much of the protein is degraded into peptide, amino acids, NH_3 and amines. When consumed by the animals, these end products of ensilage will be quickly converted to NH_3 in the rumen which therefore gives rise of rumen NH_3 concentration. This probably explains for the increase of NH_3 concentration in goats consuming S_{60}.

Rumen VFA concentration was lower ($P<0.05$) in goats consuming silage made of a mixture of forage produced from clitoria-sorghum inter-cropping with narrow sorghum planting space compared to sparser planting space. But rumen VFA concentration was comparable between S_{40} and S_{60}. The increase indicated that there was a higher extent and rate of fermentation of OM in the rumen in S_{40} and S_{60} compared to S_{20}.

Nevertheless, rumen concentrations of both NH_3 and VFA were within the normal range reported earlier. Mc Donald *et al.* (2010) reported that rumen VFA concentration in ruminant typically varying between 70-150 mmol/l. Suryani *et al.* (2014) reported that goats consuming various forage and concentrate had VFA concentration of 40.45 mmol/l. Similarly, Naswantara (2006) noted a level of 71.81 mmol/l in goats consuming rice straw supplemented with different protein and energy sources. This indicates that silage of sorghum-clitoria mix in this experiment has reasonably high fermentation rate.

Blood glucose and total plasma protein were not different ($P>0.05$) in goats consuming different type of silage. However, the level of blood glucose was under normal level which is stated to be 50-80 mg/dl (Kaneko, 1997). Manu *et al.* (2007) reported blood glucose concentration varying between 51.00 to 94.80 mg/dl for Kacang goats in Timor savanas, Indonesia. The increase of glucose concentration in blood plasma in this experiment did not occur despite the increase of VFA concentration in the rumen fluid. In ruminant, glucose absorbed from carbohydrate digestion in the small intestine is generally low, only 3-8% of glucose synthesized in sheep (Preston and Leng, 1986). Hence, ruminants rely on the gluconeogenesis mechanism from propionate and amino acids to maintain blood glucose level. Major part (50-60%) of precursor for gluconeogenesis comes from propionate (Preston and Leng, 1986).

Total plasma protein in the present experiment. varied narrowly between 6.02 to 6.38 g/dl and no treatment effect (P>0.05) was found. This level was within the normal range reported by Mitruka and Rawnsley (1981), between 4.5-7.2 g/dl. Thus, it is apparent that the animal tends to maintain plasma protein concentration in a normal homeostatic level (Swenson and Reece, 1993).

CONCLUSIONS

It is concluded that CP content of the silage increased with increasing sorghum planting space as the proportion of clitoria in the mixture was increasing. Nutrient intake and digestibility, however, are not improved with increasing planting space although rumen NH_3 and VFA concentration was improved. It is therefore inferred that there is no benefit of increasing sorghum planting space beyond 40×40 cm^2 when sown together with *C. ternatea* to produce good quality silage for Kacang goats.

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