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2.3. Data collection and laboratory analyses

Throughout the whole experimental trial, the LW of each animal was recorded every Sunday and Thursday at 08:00 h before morning feeding using a digital platform weighing scale. Duplicate subsamples of feed offered were collected each day during the collection week. The feeds refused by each animal during the collection week were weighed and sampled (~200 g fresh matter, FM) stored at -20 ^{0}C temperature until the end of each collection week. Then, the daily refusals were pooled per animal, thoroughly homogenized, and sampled (~100 g FM) in duplicate. The samples offered and refused feed and daily fecal excretion were dried at 45 ^{0}C for 4 days and reweighed for determination of dry weight.

During seven consecutive days of the collection week, a urine feces separator was fitted below the floor of each individual pen for daily total urine and feces collection. The separator was equipped with a plastic mesh that directed the dropped feces to a 5-L bucket while urine passed through the mesh. Total urine excreted by each goat was collected into a closed 5-L bucket prefilled with 25% (v/v) sulfuric acid to preserve the nitrogen (N). Sample of urine (~200 mL) was taken daily after homogenizing and filtering with surgical gaze then stored at -20 ^{0}C for N analysis.

The daily urine and feces excretion was quantified at 8:00 h each day throughout the collection week. After homogenizing, the feces were sampled (~100 g FM) and dried at 45 ^{0}C for 4 days, and reweighed for dry weight determination. After drying, the feed and fecal samples were ground to pass a 1-mm mesh. At the end of each period, the samples were pooled per animal proportionally to the daily amount. The dried samples were stored in zipper plastic bags prior to laboratory analyses.

The samples of feed and feces were analyzed for concentrations of DM, ash (AOAC, 1990; Method 924.05), N (AOAC, 1990; Method 988.05), NDF, and acid detergent fiber (ADF) (using an Ankom200 fiber analyzer, Ankom Technology cooperation, Fairport, USA) with alpha-amylase and including residual ash (Van Soest et al., 1991). Organic matter (OM) concentrations were calculated by subtracting the ash concentration by 100 while CP content was calculated as N×6.25. The N concentrations of urine samples were determined by the micro Kjedhal method (AOAC, 1990; Method 988.05). The NO_3 was extracted by the method described by Liu et al. (2016) and the NO_3 content was

2.4.Data analysis

Nutrients intake was calculated by subtracting the daily amount of nutrients in the refused feed by the amount of nutrients in the offered feed. Metabolizable energy (ME, MJ/kg DM) was estimated as 0.0157 × digestible OM (g/kg DM) (AFRC, 1993). Intake composition was calculated by dividing the amount of a nutrient by total feed DM intake. Total tract apparent digestibility of DM, OM, NDF, and ADF were estimated by the difference between diets fed (accounting for differences in the composition of refused feed) and feces excreted over the 7-day collection week. Live weight gain was calculated by regression of LW over the time (day of weighing) by SLOPE function while feed conversion efficiency (FCE) was calculated by dividing the LW gain by DM intake during 4 experimental weeks. Absorbed N was calculated by subtracting the amount of fecal N excretion by the amount of N intake while N retention was calculated by subtracting the amount of urinary N excretion by Absorbed N.

Statistical analyses were performed using R (R Core Team, 2018). Analysis of variance was performed using the mixed procedure (*lme* function) after confirming the normality of residual data by the Kolmogorov–Smirnov test. A mixed model with dietary treatments and period as fixed effects and animal as a random effect was fitted on the data. Results are presented as arithmetic means and standard error of the mean and the Tukey post hoc test was applied to compare differences among means and the level of significance was determined at 0.05.

supplementation diet could not improve the nutrients intake and digestibility as the intake of the basal GG already contained 118 g CP/kg DM. Similar DM intakes of basal and supplemented diets were also reported by Ali et al. (2019) for heifers on mixed wheat straw and Rhodes grass hay (71 g CP and 730 g NDF/kg DM) supplemented with sweet potato vine silage and Pineiro-Vázquez et al. (2017) for heifers on *Pennisetum purpureum* (71 g CP and 660 g NDF/kg DM) supplemented with *Leucaena leucocephala*. However, a higher DM intake and digestibility was reported for goats fed *Ischaemum*

aristatum (68 g CP and 400 g NDF/kg DM) supplemented with fresh sweet potato vine (Aregheore,

Table 1

Dry matter (DM), organic matter (OM), crude protein (CP), neutral detergent fibre (NDF), acid detergent fibre (ADF), and nitrate concentrations of Guinea grass, Chinese violet weed and unpeeled

[Kutipan teks disembunyikan]

Effect of feeding *Asystasia gangetica* weed on intake, nutrient utilization, and gain in Kacang goat

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Abstract

A high concentration of nitrate and total nitrogen (N) in Chinese violet (CV) weed (*Asystasia gangetica*) might be a benefit for ruminant production in humid tropical regions; therefore, it was investigated in this study. Using a crossover design, twelve Kacang goats were allocated to four diets: Guinea grass (*Panicum maximum*) *ad libitum* (GG), GG *ad libitum* and CV (1% live weight, LW) (GG+CV), CV *ad libitum* (CV), and CV *ad libitum* plus cassava chip (1% LW) (CV+CC) (dry matter, DM basis). The goats on the CV diet had a higher intake of DM, metabolizable energy, and N, N retention, LW gain, and feed conversion efficiency than goats on the GG and GG+CV diets ($P < 0.001$) while DM digestibility was similar to the goats on the GG+CV diet but higher than the goats on the GG diet ($P < 0.001$). However, the goats on the CV diet had a higher urinary N loss compared to the GG and GG+CV diets ($P < 0.001$). The goats on the CV+CC had a higher digestibility of DM and organic matter (OM) and a lower urinary N loss compared to the goats on the CV diet ($P < 0.001$). Hence, the CV weed could be fed as a sole diet or as a supplement to the Kacang goat fed a low-quality forage for improvement of nutrients intake, digestibility, and gain while the urinary N loss could be lowered by cassava supplementation.

Keywords: Chinese violet, digestibility, growth, ruminant, urinary N loss

1. Introduction

A presence of weeds in a crop field has benefits such as lowering soil evaporation and erosion. However, weeds can negatively affect crop growth and yield due to competition for light, nutrients, and water (Zimdahl, 2004). In integrated crop-livestock farming, livestock serves a practical sustainable approach for controlling palatable weed species (MacLaren et al., 2020) which depends on the type of livestock and crops. Similar to the other tropical regions, the forage supply of small-scale farmers in the tropical humid regions is limited by resources of land, labors, and other inputs (Valbuena et al., 2012). Hence, ruminants were often supplied by the palatable weeds as the main resources of feed during a period of crop-growing season.

Asystasia gangetica (L.) T. Anderson is a perennial herb of the Acanthaceae family which widely distributed in tropical Africa and Asia (Mannetje and Jones, 1992) and currently threat Northern and Eastern part of Australia (Westaway et al., 2016). The plant is commonly known as Chinese violet (CV) (Mannetje and Jones, 1992). The weed is fast-growing species, tolerant to low soil fertility and shade, and widely grown in the ground of tree plantation (Adjorlolo et al., 2014; Chee and Faiz, 1990). The weed has been fed to livestock by the small-scale farmers in the humid regions of Asia and Africa (Bindelle et al., 2007; Bussmann et al., 2020; Mannetje and Jones, 1992).

Few studies have been conducted to evaluate the effects of CV feeding on ruminant performance and nutrient utilization (Adjorlolo et al., 2014; Bindelle et al., 2007; Dahlan et al., 1993; Merkel et al., 1999; Suarna et al., 2019). Comparing to legumes *Paraserianthes falcataria*, *Gliricidia sepium*, and *Calliandra calothyrsus*, CV had higher dry matter (DM), neutral detergent fiber (NDF), and crude protein (CP) digestibility while a higher nitrogen (N) urine of CV was related to NH_3 excess in the rumen due to a higher nitrate (NO_3) concentration in the weed (Merkel et al., 1999). The presence of NO_3 could be a benefit for lowering methane (CH_4) production and increasing N supply for microbial protein synthesis in the rumen. Nitrate is a hydrogen sink and has been reported to reduce CH_4 production in beef cattle fed low-quality forage (Callaghan et al., 2014; Hulshof et al., 2012). However, during the reduction of NO_3 to ammonia (NH_3), nitrite (NO_2) is formed. Because the reduction of NO_3 to NO_2 is generally faster than the reduction of NO_2 to NH_3, NO_2 can be accumulated in the rumen, and an excess of NO_2 results in methemoglobinemia (Callaghan et al., 2014; Ku-Vera et al., 2020).

The higher digestibility indicates that the CV weed could be supplemented to a low-quality forage (Ali et al., 2019b; Aregheore, 2004). Furthermore, an energy source of the readily fermented carbohydrates from cassava chips (CC) might improve the NO_3 and NH_3 utilization in the rumen (Hristov et al., 2005; Ku-Vera et al., 2020; Schwab et al., 2005) and so decrease urinary N loss when supplemented to a basal diet of the CV weed. Thus, an evaluation is needed to assess sustainable feeding strategies of the CV weed on the ruminant production of the smallholder farmers in the crop-livestock system. The objective of the study was to evaluate a range of locally available diets based on the CV weed to improve Kacang goat performances as well as reduce the associated CH_4 and N losses. Specifically, this study was to determine the effect of supplementing a basal Guinea grass diet (*Panicum maximum* Jacq.) with the CV weed and the basal CV weed diet with the CC.

2. Materials and methods

2.1. Animals and experimental design

The animals were cared for according to the Animal Welfare Guidelines of the Indonesian Institute of Sciences. Approval of the experiment was granted from the Faculty of Agriculture, Universitas Sriwijaya, Indonesia.

Twelve male local Kacang goats with live weight (LW) of 10 ± 1.4 kg and six to seven months old were used. The animals were housed in individual pens (1.5 m × 0.75 m) in an open-sided type of house. The animals were treated orally with Oxfendazole (Verm-O, 25 mg per 5 kg LW, PT Sanbe, Indonesia). The goats were gradually acclimated to the CV diet over 20 days for preventing the nitrate toxicity. Before starting the experiment, the goats stratified based on their LW and allocated to the four treatments. The experimental design was a crossover design which consisted of four diets tested in four periods (Appendix). Each experimental period lasted for four weeks with three weeks of adaptation and followed by one week of sample collection where feed intake along with feces and urine excretion were measured.

2.2. Experimental feeds

The experimental diets used in the present study consisted of Guinea grass (GG), Guinea grass plus Chinese violet weed (GG + CV), CV weed, and CV weed plus cassava root chip (CV + CC). The composition of the GG, CV, and CC is shown in Table 1. The Guinea grass was obtained from existing pastures near the experimental unit. The grass was harvest at 55 to 60 days intervals (after the blooming stage) about 25 cm from the ground every afternoon. The CV weed was obtained from an abandoned rubber plantation in the Universitas Sriwijaya campus. The weed was harvest in the post-blooming stage, at 45 days intervals about 5 cm from the ground every afternoon. The forages were chopped into 5.0 to 10.0 cm particle size the next morning before offering to the animals. The unpeeled cassava chips were made from cassava tubers by chopping the tubers to 0.5 to 1.0 cm pieces then sun-dried for 4 to 5 days depending on sunlight intensity. Cassava crop is a cash crop in sub-urban areas of Palembang city, the tubers for cassava chips were unmarketable size tubers (2 to 3 cm in diameter) which usually left on the field.

After removing and weighing the refusals from the previous day, the feeds were offered to the animals in separate buckets according to the experimental treatments, making it possible to give the

supplements at the same time as the basal feeds. The forages were offered 3 times at 9:00, 13:00, and 17:00 h while CC was offered at 9:00 h. The basal diet was offered *ad libitum* (allowing for 20% of refusal, fresh matter basis) while the CV and CC supplementations were offered at 1% of LW and were adjusted after each measurement of animal LW. Drinking water and salt-mineral lick were offered for *ad libitum* intake to all animals throughout the experiment.

2.3. Data collection and laboratory analyses

Throughout the whole experimental trial, the LW of each animal was recorded every Sunday and Thursday at 08:00 h before morning feeding using a digital platform weighing scale. Duplicate subsamples of feed offered were collected each day during the collection week. The feeds refused by each animal during the collection week were weighed and sampled (~200 g fresh matter, FM) stored at -20 ^{0}C temperature until the end of each collection week. Then, the daily refusals were pooled per animal, thoroughly homogenized, and sampled (~100 g FM) in duplicate. The samples offered and refused feed and daily fecal excretion were dried at 45 ^{0}C for 4 days and reweighed for determination of dry weight.

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2.4.Data analysis

Nutrients intake was calculated by subtracting the daily amount of nutrients in the refused feed by the amount of nutrients in the offered feed. Metabolizable energy (ME, MJ/kg DM) was estimated as 0.0157 × digestible OM (g/kg DM) (AFRC, 1993). Intake composition was calculated by dividing the amount of a nutrient by total feed DM intake. Total tract apparent digestibility of DM, OM, NDF, and ADF were estimated by the difference between diets fed (accounting for differences in the composition of refused feed) and feces excreted over the 7-day collection week. Live weight gain was calculated by regression of LW over the time (day of weighing) by SLOPE function while feed conversion efficiency (FCE) was calculated by dividing the LW gain by DM intake during 4 experimental weeks. Absorbed N was calculated by subtracting the amount of fecal N excretion by the amount of N intake while N retention was calculated by subtracting the amount of urinary N excretion by Absorbed N.

Statistical analyses were performed using R (R Core Team, 2018). Analysis of variance was performed using the mixed procedure (*lme* function) after confirming the normality of residual data by the Kolmogorov–Smirnov test. A mixed model with dietary treatments and period as fixed effects and animal as a random effect was fitted on the data. Results are presented as arithmetic means and standard error of the mean and the Tukey post hoc test was applied to compare differences among means and the level of significance was determined at 0.05.

3. Results

3.1. Feed intake and apparent digestibility of nutrients

The offered CV weed had lower NDF and ADF concentrations and higher CP and NO_3 content than GG whereas CC had the lowest NDF, ADF, and CP contents (Table 1). The effect of the CV weed and CC supplementations on nutrients intake and digestibility is shown in Table 2. Intake of GG was higher for goats on the basal GG diet than goats received supplements (GG+CV) whereas supplement intake was higher for goats offered GG+CV than those fed CV+CC ($P < 0.001$). The goats on CV and CV+CC had higher total DM and ME intake than those fed GG and GG+CV ($P < 0.001$). The goats on the CV diet had the highest CP concentration in their intake followed those observed on GG+CV, CV+CC, and GG diets. The highest NDF and ADF concentrations were observed in GG diet followed by GG+CV and CV diets and the lowest NDF and ADF concentration was found in CV+CC diet. The DM digestibility of the goats fed CV diet was similar to the goats on GG+CV diet but higher than those fed GG diet whereas the goats on CV+CC diet had the highest DM digestibility ($P < 0.001$). The goats fed CV+CC diet also had the highest OM digestibility ($P < 0.001$) with no difference among the other treatments. The goats offered GG had the highest NDF digestibility ($P < 0.001$) while the ADF digestibility was not significantly different among diets. The goats on CV+CC diets had similar NDF digestibility with the goats on GG+CV and CV alone diet.

3.2. Nitrogen balance and growth performance

Nitrogen intake was the highest for the goats fed CV alone ($P < 0.001$) whereas the goats fed GG had the lowest N intake (Table 3). The N intake of the goats on GG+CV diet was similar to the goats on GG diet. Daily fecal N excretion was the highest for the goats on CV diet ($P < 0.001$), with no difference among the other treatments. Absorbed N and urinary N were also the highest for goats fed CV diet. The goats on CV+CC diet had similar absorbed N but lower urinary N than the goats fed solely CV when the values were expressed as g/d. The goats on GG diet had the lowest N intake and total excreted N and the highest ratio of fecal and urinary N ($P < 0.001$). As the lowest for LW gain, the goats on GG diet had the lowest N retention which not significantly different from the goats on GG+CV diet whereas the higher N retention was found in goats receiving CV and CV+CC diets ($P < 0.001$). Live weight gain and feed conversion efficiency were higher for the goats on diets of CV and CV+CC than those fed GG and GG+CV ($P < 0.001$) (Table 4).

Eight goats on the GG and GG+CV diets experienced losses of LW during the adaptation and collection weeks (Figure 1). Regression analysis showed that LW gain significantly correlated ($P < 0.001$) with DM intake (R^2: 0.63) and ME intake (R^2: 0.54). The regression resulted in a daily ME requirement of 536 KJ/kg$^{0.75}$ LW for maintenance and 49.7 KJ/kg$^{0.75}$ LW for gain.

4. Discussion

4.1. Feed intake and apparent digestibility of nutrients

The NDF concentration of the CV weed in the present study was higher while CP concentration was within the range of the previous studies (Adjorlolo et al., 2014; Bindelle et al., 2007; Dahlan et al., 1993; Merkel et al., 1999; Suarna et al., 2019). The differences might depend on the plant age or the ratio of leaf and stem (Kumalasari et al., 2020). The NO_3 concentration in the present study was lower than the NO_3 concentration in the CV weed (22 - 98 g/kg) in Merkel et al. (1999) and have to offer less than 50% of a ration (DM basis) (Rasby et al., 2014). The high level of NO_3 in the weed would be expected to restrict the intake of the goats. However, the higher intake was found in the CV weed alone and cassava chip supplementation diets that could be related to the acclimation of the rumen microbes to the higher NO_3 in the weed.

The higher CP concentration and the lower NDF concentration of the intake in the CV weed supplementation diet could not improve the nutrients intake and digestibility as the intake of the basal GG already contained 118 g CP/kg DM. Similar DM intakes of basal and supplemented diets were also reported by Ali et al. (2019) for heifers on mixed wheat straw and Rhodes grass hay (71 g CP and 730 g NDF/kg DM) supplemented with sweet potato vine silage and Piñeiro-Vázquez et al. (2017) for heifers on *Pennisetum purpureum* (71 g CP and 660 g NDF/kg DM) supplemented with *Leucaena leucocephala*. However, a higher DM intake and digestibility was reported for goats fed *Ischaemum* *aristatum* (68 g CP and 400 g NDF/kg DM) supplemented with fresh sweet potato vine (Aregheore, 2004).

Though the DM digestibility was the lowest in the basal GG diet, the highest NDF digestibility on the basal GG diet might be attributed to the lowest DM intake of the diet. A lowered DM intake could be attributed to the escalated nutrient digestibility in the rumen as a result of a longer retention time in the rumen. A study of Ali et al. (2019a) on steers fed four levels of intake, observed a higher NDF and ADF digestibility at a lower intake level. An increase of NDF digestibility, as well as an increase of ruminating time per DM intake when the level of intake was lowered, was also reported in the study of Schulze et al. (2014) on heifers fed four levels of grass-clover silage. The lower NDF digestibility in the present study might be also related to the higher NO_3 concentration in the diets. Nitrite, as the result of the reduction of NO_3, was reported to reduce the cellulolytic microbial population in the rumen (Marais et al., 1988).

The higher DM and OM digestibility of the goats fed the CV weed and the cassava supplementation could be explained by the increased supply of both N and the readily available carbohydrates for the rumen fermentation. An improvement of DM and OM digestibility along with a higher N retention and LW gain of goats fed a mix of Gamba grass (*Andropogon gayanus*) and Cassava hay by cassava supplementation was reported (Phengvichith and Ledin, 2007).

4.2. Nitrogen balance and growth performance

Comparing to the basal GG diet, N intake and absorption were higher in the goats fed CV weed when the values were expressed as % of LW (Table 4). However, urinary N excretion was also elevated which might reflect an excess of NH_3 production in the rumen. A higher concentration of NO_3 in the CV weed was also reported (Merkel et al. 1999) which could be degraded to the NH_3 in the rumen. The excess of NH_3 is absorbed in the bloodstream, converted to urea in the liver, and then excreted in the urine (Schwab et al., 2005).

The additional fermentable carbohydrate of cassava chip would have utilized the excess of the rumen-degradable N of the CV weed by microbial uptake in the rumen. Though the present work did not estimate microbial protein supply by purine derivative in the urine, the lowered urinary N excretion by the cassava chips supplementation could be attributed to an improvement of the microbial protein supply to the host animal (Hristov et al., 2005; Ku-Vera et al., 2020; Schwab et al., 2005). However, in the present study, the improvement did not result in a significantly higher N retention and LW gain of the goats fed the cassava supplementation diet.

The present study demonstrated that feeding of the CV weed either as a supplement to the basal diet of low-quality tropical grass or as a sole diet could result in a higher urinary N excretion which potentially increases N_2O emission in the livestock sector. The supplementation of the locally available non-structural carbohydrate, which is affordable for the small-scale farmers, improved the nutrients utilization of the CV weed and thus ruminant growth as well as a reduction of N_2O and CH_4 emissions.

The averaged LW gain and FCE of the goats on the CV weed and cassava supplementation diets were in the range of gain and FCE (23.5 to 69.4 g/d and 0.04 to 0.11 for LW gain and FCE, respectively) for fifteen growing male Kacang goats fed three diets with three different CP and energy contents (Restitrisnani et al., 2013). The gain was also in the range of Adiwinarti et al. (2019) for growing male Kacang goats with soybean meal supplementations (41.5 to 79.9 g/d). The LW loss of the goats on the basal diet of GG and CV weed supplementation diets reflected an insufficiency of energy intake for their maintenance requirement which is estimated to be 536 KJ/kg$^{0.75}$LW. The value of ME requirement is lower than an averaged value for goat and sheep (542 KJ/kg$^{0.75}$LW) in the warm climate (Salah et al., 2014) but higher than a value obtained in the study of Luo et al. (2004) for growing indigenous goat (489 KJ/kg$^{0.75}$LW) while the value for gain is higher than values on their studies (24.3 and 19.8 KJ/kg$^{0.75}$LW).

5. Conclusion

In conclusion, the Chinese violet weed supplementation on the basal diet of Guinea grass enhanced CP intake and urinary N excretion. When the weed was offered alone, nutrients intake and digestibility were higher; the goats had higher N retention and LW gain compared with the goats with basal diet but urinary N loss was also higher. The inclusion of cassava chip supplementation improved DM and OM digestibility and lowered the urinary N excretion. Therefore the weed could be fed as a supplement to a poor-quality basal diet or fed solely without any adverse effects. Supplementation of cassava chip as a source of readily fermented carbohydrates to the basal diet of the weed has the potential to effectively improve nutrients utilization. A further study is needed to evaluate the effects of the weed on CH_4 production and rumen fermentation characteristic.

Conflict of interest

We state that we have no conflict of interest.

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Tables and figure

Table 1

Dry matter (DM), organic matter (OM), crude protein (CP), neutral detergent fibre (NDF), acid detergent fibre (ADF), and nitrate concentrations of Guinea grass, Chinese violet weed and unpeeled Cassava chip used in the study[1].

	DM	OM	CP	NDF	ADF	Nitrate
	(g/kg FM)	(g/kg DM)				
Guinea grass	213±15.6	901±3.2	107±3.9	755±26.0	371±29.3	0.3±0.02
Chinese violet	168±20.4	860±8.3	160±2.1	510±23.8	338±14.0	11.0±0.98
Cassava chip	863± 3.5	984±0.8	43±1.3	276±24.9	61± 8.4	n.a

[1]Values are means ± standard error of the mean

FM, fresh matter; n.a, not available

Table 2

Dry matter (DM) intake of basal diets and supplements, metabolizable energy (ME) intake, intake composition of crude protein (CP), neutral detergent fibre (NDF) and acid detergent fibre (ADF), digestibility of DM, OM, NDF and ADF of Kacang goats fed Guinea grass *ad libitum* (GG), GG *ad libitum* and Chinese violet weed (1% live weight, LW) (GG+CV), CV *ad libitum*, and CV *ad libitum* and unpeeled cassava chips (1% LW) (CV+CC).

Parameter	GG	GG+CV	CV	CV+CC	SEM	*P*-value
Basal diet intake						
g/d	313.0 ^{b}	220.2 ^{a}	533.6 ^{c}	398.8 ^{b}	23.64	<0.001
% LW	2.5 ^{b}	1.8 ^{a}	4.2 ^{d}	3.2 ^{c}	0.16	<0.001
Supplement intake						
g/d	n.a	148.6 ^{b}	n.a	119.7 ^{a}	5.85	<0.001
% LW	n.a	1.2 ^{b}	n.a	1.0 ^{a}	0.03	<0.001
Total DM intake						
g/d	313.0 ^{a}	368.8 ^{a}	533.6 ^{b}	518.6 ^{b}	22.89	<0.001
% LW	2.5 ^{a}	2.9 ^{a}	4.2 ^{b}	4.1 ^{b}	0.14	<0.001
ME intake						
MJ/d	3.4 ^{a}	4.1 ^{a}	6.0 ^{b}	6.3 ^{b}	0.31	<0.001
MJ/kg$^{0.75}$ LW	0.52 ^{a}	0.61 ^{a}	0.89 ^{b}	0.94 ^{b}	0.040	<0.001
Intake composition (g/kg DM)						
CP	118 ^{a}	136 ^{b}	164 ^{c}	135 ^{b}	2.7	<0.001
NDF	734 ^{d}	642 ^{c}	488 ^{b}	432 ^{a}	18.9	<0.001
ADF	347 ^{c}	339 ^{bc}	323 ^{b}	257 ^{a}	8.2	<0.001
Digestibility (g/kg)						
DM	675 ^{a}	694 ^{ab}	717 ^{b}	771 ^{c}	10.0	<0.001
OM	690 ^{a}	697 ^{a}	701 ^{a}	767 ^{b}	9.9	<0.001
NDF	643 ^{c}	615 ^{bc}	521 ^{a}	561 ^{ab}	19.5	<0.001
ADF	540	532	528	524	26.8	0.953

SEM, Standard error of the mean; n.a, not available

Values in the same row with different letters represent significant differences ($P < 0.05$).

Table 3

Nitrogen (N) balance in Kacang goats fed Guinea grass *ad libitum* (GG), GG *ad libitum* and Chinese violet weed (1% live weight, LW) (GG+CV), CV *ad libitum*, and CV *ad libitum* and unpeeled cassava chips (1% LW) (CV+CC).

Parameter	GG	GG+CV	CV	CV+CC	SEM	*P*-value
N intake						
g/d	6.0^{a}	8.1^{a}	14.0^{c}	11.3^{b}	0.64	<0.001
% LW	0.05^{a}	0.06^{b}	0.11^{d}	0.09^{c}	0.004	<0.001
Fecal N						
g/d	2.5^{a}	2.7^{a}	3.9^{b}	3.0^{a}	0.14	<0.001
% LW	0.21^{a}	0.22^{a}	0.31^{b}	0.23^{a}	0.009	<0.001
absorbed N						
g/d	3.5^{a}	5.3^{b}	10.1^{c}	8.3^{c}	0.54	<0.001
% LW	0.03^{a}	0.04^{b}	0.08^{d}	0.07^{c}	0.004	<0.001
Urinary N						
g/d	2.0^{a}	3.2^{b}	5.7^{c}	3.7^{b}	0.29	<0.001
% LW	0.016^{a}	0.026^{b}	0.045^{c}	0.030^{b}	0.0021	<0.001
Fecal N urinary N ratio	1.39^{b}	0.91^{a}	0.78^{a}	0.80^{a}	0.058	<0.001
N retention						
g/d	1.5^{a}	2.1^{a}	4.4^{b}	4.6^{b}	0.35	<0.001
% LW	0.011^{a}	0.017^{a}	0.035^{b}	0.036^{b}	0.0026	<0.001

absorbed N: N intake - Fecal N; N retention: absorbed N – urinary N

SEM, Standard error of the mean

Values in the same row with different letters represent significant differences ($P < 0.05$).

Table 4

Daily live weight (LW) gain, total gain and feed conversion efficiency (FCE) of Kacang goats fed Guinea grass *ad libitum* (GG), GG *ad libitum* and Chinese violet weed (1% live weight, LW) (GG+CV), CV *ad libitum*, and CV *ad libitum* and unpeeled cassava chips (1% LW) (CV+CC).

Parameter	GG	GG+CV	CV	CV+CC	SEM	*P*-value
LW gain (g/d)	2.0 ^{a}	13.7 ^{a}	47.2 ^{b}	48.2 ^{b}	4.04	<0.001
Total gain (g)	51.2 ^{a}	355.0 ^{a}	1227.5 ^{b}	1251.9 ^{b}	105.05	<0.001
FCE	-0.01 ^{a}	0.03 ^{a}	0.09 ^{b}	0.09 ^{b}	0.009	<0.001

FCE: g LW gain/g dry matter intake

Values in the same row with different letters represent significant differences ($P < 0.05$).

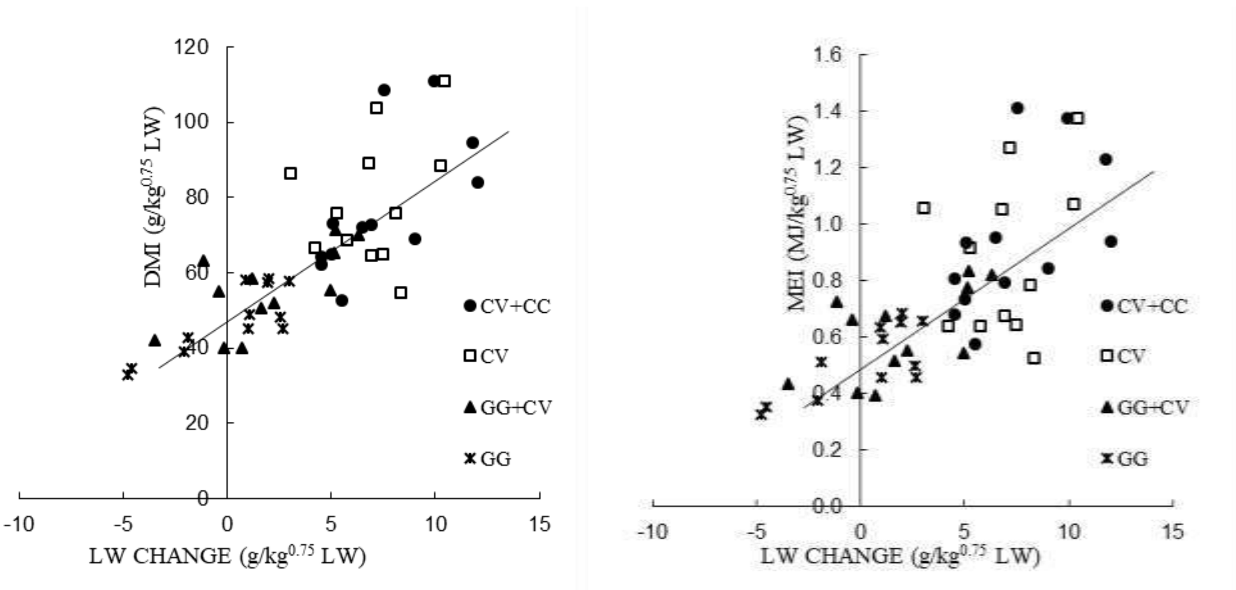

Fig. 1. Relationship between dry matter intake (DMI, left) and metabolizable energy intake (MEI, right) on live weight (LW) change in Kacang goats fed Guinea grass *ad libitum* (GG), GG *ad libitum* and Chinese violet weed (1% live weight, LW) (GG+CV), CV *ad libitum*, and CV *ad libitum* and unpeeled cassava chips (1% LW) (CV+CC). DMI= 3.81 LW change + 49.23 (n: 48, R^2: 0.63, P <0.001); MEI=0.049 LW change + 0.536 (n: 48, R^2: 0.54, $P < 0.001$).

Appendix

Allocation of the twelve Kacang goats (Group A (4, 8, 11), B (5, 10, 12), C (6, 7, 9) and D (1, 2, 3)) to Guinea grass (GG), GG and Chinese violet weed (GG+CV), CV, and CV plus unpeeled cassava chips (CV+CC) dietary treatments during 4 experimental periods.

Period	Animal group			
	A	B	C	D
1	GG	GG+CV	CV	CV+CC
2	CV+CC	GG	GG+CV	CV
3	GG+CV	CV	CV+CC	GG
4	CV	CV+CC	GG	GG+CV

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Utilization of invasive weed Asystasia gangetica to improve nutrient utilization and growth of Kacang goat

--Manuscript Draft--

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Abstract:	A high concentration of nitrate and total nitrogen (N) in Chinese violet (CV) weed (Asystasia gangetica) might be a benefit for ruminant production in humid tropical regions; therefore, it was investigated in this study. Using a crossover design, twelve Kacang goats were allocated to four diets: Guinea grass (Panicum maximum) ad libitum (GG), GG ad libitum and CV (1% live weight, LW) (GG+CV), CV ad libitum (CV), and CV ad libitum plus cassava chip (1% LW) (CV+CC) (dry matter, DM basis). The goats on the CV diet had a higher intake of DM, metabolizable energy, and N, N retention, LW gain, and feed conversion efficiency than goats on the GG and GG+CV diets (P < 0.001) while DM digestibility was similar to the goats on the GG+CV diet but higher than the goats on the GG diet (P < 0.001). However, the goats on the CV diet had a higher urinary N loss compared to the GG and GG+CV diets (P < 0.001). The goats on the CV+CC had a higher digestibility of DM and organic matter (OM) and a lower urinary N loss compared to the goats on the CV diet (P < 0.001). Hence, the CV weed could be fed as a sole diet or as a supplement to the Kacang goat fed a low-quality forage for improvement of nutrients intake, digestibility, and gain while the urinary N loss could be lowered by cassava supplementation.
Suggested Reviewers:	

Utilization of invasive weed *Asystasia gangetica* to improve nutrient utilization and growth of Kacang goat

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Conflict of interest

We state that we have no conflict of interest.

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June 24th, 2021

Dear Dr. Ali Abdelaziz Ali,
We wish to submit an original research article entitled "Utilization of invasive weed *Asystasia gangetica* to improve nutrient utilization and growth of Kacang goat" for consideration by Journal of Annals of Agricultural Sciences. We confirm that this work is original and has not been published elsewhere, nor is it currently under consideration for publication elsewhere.

We believe that this manuscript is appropriate for publication in the journal. It shows the improvement of nutrient utilization and growth of a local goat whilst reduce urinary N loss by feeding the weeds in an integrated crop-livestock system.

Asystasia gangetica is an invasive weed species that practically fed to livestock in tropical humid regions. However, the responses of the animals to the feeding of the N-rich weed are limited. This study shows options of diets based on the weed for boosting the ruminant performances.

We have no conflicts of interest to disclose.

Please address all correspondence concerning this manuscript to me at asep_ali@fp.unsri.ac.id.

Thank you for your consideration of this manuscript.

Sincerely,

Asep Ali

Abstract

A high concentration of nitrate and total nitrogen (N) in Chinese violet (CV) weed (*Asystasia gangetica*) might be a benefit for ruminant production in humid tropical regions; therefore, it was investigated in this study. Using a crossover design, twelve Kacang goats were allocated to four diets: Guinea grass (*Panicum maximum*) *ad libitum* (GG), GG *ad libitum* and CV (1% live weight, LW) (GG+CV), CV *ad libitum* (CV), and CV *ad libitum* plus cassava chip (1% LW) (CV+CC) (dry matter, DM basis). The goats on the CV diet had a higher intake of DM, metabolizable energy, and N, N retention, LW gain, and feed conversion efficiency than goats on the GG and GG+CV diets ($P < 0.001$) while DM digestibility was similar to the goats on the GG+CV diet but higher than the goats on the GG diet ($P < 0.001$). However, the goats on the CV diet had a higher urinary N loss compared to the GG and GG+CV diets ($P < 0.001$). The goats on the CV+CC had a higher digestibility of DM and organic matter (OM) and a lower urinary N loss compared to the goats on the CV diet ($P < 0.001$). Hence, the CV weed could be fed as a sole diet or as a supplement to the Kacang goat fed a low-quality forage for improvement of nutrients intake, digestibility, and gain while the urinary N loss could be lowered by cassava supplementation.

Keywords: invasive weed, digestibility, growth, ruminant, urinary N loss

1. Introduction

A presence of weeds in a crop field has benefits such as lowering soil evaporation and erosion. However, weeds can negatively affect crop growth and yield due to competition for light, nutrients, and water (Zimdahl, 2004). In integrated crop-livestock farming, livestock serves a practical sustainable approach for controlling palatable weed species (MacLaren et al., 2020) which depends on the type of livestock and crops. Similar to the other tropical regions, the forage supply of small-scale farmers in the tropical humid regions is limited by resources of land, labors, and other inputs (Valbuena et al., 2012). Hence, ruminants were often supplied by the palatable weeds as the main resources of feed during a period of crop-growing season.

Asystasia gangetica (L.) T. Anderson is a perennial herb of the Acanthaceae family which widely distributed in tropical Africa and Asia (Kiew and Vollesen, 1997) and currently threat Northern and Eastern part of Australia (Westaway et al., 2016). The weed is fast-growing species, tolerant to low soil fertility and shade, and widely grown in the ground of tree plantation (Adjorlolo et al., 2014; Kiew and Vollesen, 1997). The weed has been fed to livestock by the small-scale farmers in the humid regions of Asia and Africa (Bindelle et al., 2007; Bussmann et al., 2020).

Few studies have been conducted to evaluate the effects of CV feeding on ruminant performance and nutrient utilization (Adjorlolo et al., 2014; Bindelle et al., 2007; Dahlan et al., 1993; Merkel et al., 1999). Comparing to legumes *Paraserianthes falcataria*, *Gliricidia sepium*, and *Calliandra calothyrsus*, CV had higher DM, neutral detergent fiber (NDF), and crude protein (CP) digestibility while a higher N urine of CV was related to NH_3 excess in the rumen due to a higher nitrate (NO_3) concentration in the weed (Merkel et al., 1999). The presence of NO_3 could be a benefit for lowering methane (CH_4) production and increasing N supply for microbial protein synthesis in the rumen. Nitrate is a hydrogen sink and has been reported to reduce CH_4 production in beef cattle fed low-quality forage (Callaghan et al., 2014; Hulshof et al., 2012). However, during the reduction of NO_3 to ammonia (NH_3), nitrite (NO_2) is formed. Because the reduction of NO_3 to NO_2 is generally faster than the reduction of NO_2 to NH_3, NO_2 can be accumulated in the rumen, and an excess of NO_2 results in methemoglobinemia (Callaghan et al., 2014; Ku-Vera et al., 2020).

The higher digestibility indicates that the CV weed could be supplemented to a low-quality forage (Ali et al., 2019b; Aregheore, 2004). Furthermore, an energy source of the readily fermented carbohydrates from CC might improve the NO_3 and NH_3 utilization in the rumen (Hristov et al., 2005; Ku-Vera et al., 2020; Schwab et al., 2005) and so decrease urinary N loss when supplemented to a basal diet of the CV weed. Thus, an evaluation is needed to assess sustainable feeding strategies of the CV weed on the ruminant production of the smallholder farmers in the crop-livestock system. The objective of the study was to evaluate a range of locally available diets based on the CV weed to improve Kacang goat performances as well as reduce the associated CH_4 and N losses. Specifically, this study was to determine the effect of supplementing a basal GG diet with the CV weed and the basal CV weed diet with the CC supplementation.

2. Materials and methods

2.1. Animals and experimental design

The animals were cared for according to the Animal Welfare Guidelines of the Indonesian Institute of Sciences. Approval of the experiment was granted from the Faculty of Agriculture, Universitas Sriwijaya, Indonesia.

Twelve male local Kacang goats with live weight (LW) of 10 ± 1.4 kg and six to seven months old were used. The animals were housed in individual pens (1.5 m × 0.75 m) in an open-sided type of house. The animals were treated orally with Oxfendazole (Verm-O, 25 mg per 5 kg LW, Sanbe, Indonesia). The goats were gradually acclimated to the CV diet over 20 days for preventing the nitrate toxicity. Before starting the experiment, the goats stratified based on their LW and allocated to the four treatments. The experimental design was a crossover design which consisted of four diets tested in four periods (Appendix). Each experimental period lasted for four weeks with three weeks of adaptation and followed by one week of sample collection where feed intake along with feces and urine excretion were measured.

2.2. Experimental feeds

The experimental diets used in the present study consisted of GG, GG+CV, CV, and CV+CC. The composition of the GG, CV, and CC is shown in Table 1. The GG was obtained from existing pastures near the experimental unit. The grass was harvest at 55 to 60 days intervals (after the blooming stage) about 25 cm from the ground every afternoon. The CV weed was obtained from an abandoned rubber plantation in the Universitas Sriwijaya campus. The weed was harvest in the post-blooming stage, at 45 days intervals about 5 cm from the ground every afternoon. The forages were chopped into 5.0 to 10.0 cm particle size the next morning before offering to the animals. The unpeeled CC were made from cassava tubers by chopping the tubers to 0.5 to 1.0 cm pieces then sun-dried for 4 to 5 days depending on sunlight intensity. Cassava crop is a cash crop in sub-urban areas of Palembang city, the tubers for CC were unmarketable size tubers (2 to 3 cm in diameter) which usually left on the field.

After removing and weighing the refusals from the previous day, the feeds were offered to the animals in separate buckets according to the experimental treatments, making it possible to give the

supplements at the same time as the basal feeds. The forages were offered 3 times at 9:00, 13:00, and 17:00 h while CC was offered at 9:00 h. The basal diet was offered *ad libitum* (allowing for 20% of refusal, fresh matter basis) while the CV and CC supplementations were offered at 1% of LW and were adjusted after each measurement of animal LW. Drinking water and salt-mineral lick were offered for *ad libitum* intake to all animals throughout the experiment.

2.3. Data collection and laboratory analyses

Throughout the whole experimental trial, a digital platform weighing scale was used to record the LW every Sunday and Thursday before offering the feeds. Duplicate subsamples of feed offered were collected each day during the collection week. The daily refusal feeds during the collection week were weighed, sampled (~200 g fresh matter, FM) and then stored at -20 ^{0}C. Then, the daily orts were pooled per animal, and then sampled (~100 g FM) in duplicate. The samples offered and refused feed and daily fecal excretion were dried at 45 ^{0}C for 4 days and reweighed for determination of dry weight.

During seven consecutive days of the collection week, a urine feces separator was fitted below the floor of each individual pen for daily total urine and feces collection. The separator was equipped with a plastic mesh that directed the dropped feces to a 5-L bucket while urine passed through the mesh. Total daily urine excretion was collected using 250 ml/L sulfuric acid to preserve the N. For N analysis concentration, sample of urine (~200 mL) was taken daily after homogenizing and filtering with surgical gaze then kept at -20 ^{0}C.

The daily excretions were quantified in the morning during the collection week. Before sampling (~100 g FM), the individual fecal excretion was homogenized and dried at 45 ^{0}C for 4 days, and then reweighed for dry weight determination. After drying, the feed and fecal samples were ground to pass a 1-mm mesh. The samples were pooled per animal at the end of each period proportionally to the daily amount. The dried samples were stored in zipper polythene bags prior to analyses.

The samples of feed and feces were analyzed for concentrations of DM, ash, N (AOAC, 1990), NDF, and acid detergent fiber (ADF) (Ankom200 fiber analyzer, Ankom Technology cooperation, Fairport, USA) with alpha-amylase and including residual ash (Van Soest et al., 1991). Concentration of OM was calculated by subtracting the ash concentration by 100 while the content of CP was calculated as N×6.25. The micro Kjedhal method (AOAC, 1990) was used to determine the N concentrations of urine. The NO_3 was extracted by the method of Liu et al. (2016) and the NO_3 content was measured using a UV-Vis spectrophotometer (Shimadzu 1800).

2.4. Data analysis

Nutrients intake was calculated from the nutrients in the refusal and the nutrients in the offered feed. Supply of metabolizable energy (ME, MJ/kg DM) was estimated as 0.0157 × digestible OM (g/kg DM) (AFRC, 1993). Intake composition was calculated by dividing the amount of a nutrient by total feed DM intake. Apparent digestibility was estimated by subtracting the daily nutrients in the feses by the nutrients in the feed intake. Live weight gain was calculated by regression of LW over the time (day of weighing) by SLOPE function while feed conversion efficiency (FCE) was calculated by dividing the LW gain by DM intake during 4 experimental weeks. Absorbed N was estimated by subtracting the fecal N excretion by the N intake while N retention was calculated by subtracting the urinary N excretion by Absorbed N.

Analysis of variance was performed using the mixed procedure (*lme* function) after confirming the normality of residual data by the Kolmogorov–Smirnov test (R Core Team, 2018). A mixed model with diets and period as fixed effects and animal as a random effect was used. To compare differences among means the Tukey post hoc test was applied. The significance was determined at $P < 0.05$.

3. Results

3.1. Feed intake and apparent digestibility of nutrients

The offered CV weed had lower NDF and ADF concentrations and higher CP and NO_3 content than GG whereas CC had the lowest NDF, ADF, and CP contents (Table 1). The effect of the CV weed and CC supplementations on nutrients intake and digestibility is shown in Table 2. Intake of GG was higher for goats on the basal GG diet than goats received supplements (GG+CV) whereas supplement intake was higher for goats offered GG+CV than those fed CV+CC ($P < 0.001$). The goats on CV and CV+CC had higher total DM and ME intake than those fed GG and GG+CV ($P < 0.001$). The goats on the CV diet had the highest CP concentration in their intake followed those observed on GG+CV, CV+CC, and GG diets. The highest NDF and ADF concentrations were observed in GG diet followed by GG+CV and CV diets and the lowest NDF and ADF concentration was found in CV+CC diet. The DM digestibility of the goats fed CV diet was similar to the goats on GG+CV diet but higher than those fed GG diet whereas the goats on CV+CC diet had the highest DM digestibility ($P < 0.001$). The goats fed CV+CC diet also had the highest OM digestibility ($P < 0.001$) with no difference among the other treatments. The goats offered GG had the highest NDF digestibility ($P < 0.001$) while the ADF digestibility was not significantly different among diets. The goats on CV+CC diets had similar NDF digestibility with the goats on GG+CV and CV alone diet.

3.2. Nitrogen balance and growth performance

Nitrogen intake was the highest for the goats fed CV alone ($P < 0.001$) whereas the goats fed GG had the lowest N intake (Table 3). The N intake of the goats on GG+CV diet was similar to the goats on GG diet. Daily fecal N excretion was the highest for the goats on CV diet ($P < 0.001$), with no difference among the other treatments. Absorbed N and urinary N were also the highest for goats fed CV diet. The goats on CV+CC diet had similar absorbed N but lower urinary N than the goats fed solely CV when the values were expressed as g/d. The goats on GG diet had the lowest N intake and total excreted N and the highest ratio of fecal and urinary N ($P < 0.001$). As the lowest for LW gain, the goats on GG diet had the lowest N retention which not significantly different from the goats on GG+CV diet whereas the higher N retention was found in goats receiving CV and CV+CC diets ($P < 0.001$). Live weight gain and feed conversion efficiency were higher for the goats on diets of CV and CV+CC than those fed GG and GG+CV ($P < 0.001$) (Table 4).

Eight goats on the GG and GG+CV diets experienced losses of LW during the adaptation and collection weeks (Figure 1). Regression analysis showed that LW gain significantly correlated ($P <$ 0.001) with DM intake (R^2: 0.63) and ME intake (R^2: 0.54). The regression resulted in a daily ME requirement of 536 KJ/kg$^{0.75}$ LW for maintenance and 49.7 KJ/kg$^{0.75}$ LW for gain.

4. Discussion

4.1. Feed intake and apparent digestibility of nutrients

The NDF concentration of the CV weed in the present study was higher while CP concentration was within the range of the previous studies (Adjorlolo et al., 2014; Bindelle et al., 2007; Dahlan et al., 1993; Merkel et al., 1999). The differences might depend on the plant age or the ratio of leaf and stem (Kumalasari et al., 2020). The NO_3 concentration in the present study was lower than the NO_3 concentration in the CV weed (22 - 98 g/kg) in Merkel et al. (1999) and have to offer less than 50% of a ration (DM basis) (Rasby et al., 2014). The high level of NO_3 in the weed would be expected to restrict the intake of the goats. However, the higher intake was found in the CV weed alone and CV+CC diets that could be related to the acclimation of the rumen microbes to the higher NO_3 in the weed.

The higher CP concentration and the lower NDF concentration of the intake in the CV weed supplementation diet could not improve the nutrients intake and digestibility as the intake of the basal GG had 118 g CP/kg DM. Ali et al. (2019) reported similar basal and supplement intake for heifers fed mixed Rhodes grass hay and wheat straw (7.1% CP and 73% NDF) with sweet potato vine silage supplementation. Piñeiro-Vázquez et al. (2017) also found the similar evidence for heifers on *Pennisetum purpureum* (7.1% CP and 66% NDF) with *Leucaena leucocephala* supplementation. However, a higher DM intake and digestibility was reported for goats fed *Ischaemum aristatum* (68% CP and 40% NDF) with sweet potato vine supplementation (Aregheore, 2004).

Though the DM digestibility was the lowest in the basal GG diet, the highest NDF digestibility on the basal GG diet might be attributed to the lowest DM intake of the diet. A lowered DM intake could be attributed to the escalated nutrient digestibility in the rumen as a result of a longer retention time in the rumen. A study of Ali et al. (2019a) on steers fed four levels of intake, observed a higher NDF and ADF digestibility at a lower intake level. An increase of NDF digestibility, as well as an increase of ruminating time per DM intake when the level of intake was lowered, was also reported in the study of Schulze et al. (2014) on heifers fed four levels of grass-clover silage. The lower NDF digestibility in the present study might be also related to the higher NO_3 concentration in the diets. Nitrite, as the result of the reduction of NO_3, was reported to reduce the cellulolytic microbial population in the rumen (Marais et al., 1988).

The higher DM and OM digestibility of the goats fed the CV weed and the cassava supplementation could be explained by the increased supply of both N and the readily available carbohydrates for the rumen fermentation. An improvement of DM and OM digestibility along with a higher N retention and LW gain of goats fed a mix of Gamba grass (*Andropogon gayanus*) and Cassava hay by cassava supplementation was reported (Phengvichith and Ledin, 2007).

4.2. Nitrogen balance and growth performance

Comparing to the basal GG diet, N intake and absorption were higher in the goats fed CV weed when the values were expressed as % of LW (Table 4). However, urinary N excretion was also elevated which might reflect an excess of NH_3 production in the rumen. A higher concentration of NO_3 in the CV weed was also reported (Merkel et al. 1999) which could be degraded to the NH_3 in the rumen. The excess of NH_3 is absorbed in the bloodstream, converted to urea in the liver, and then excreted in the urine (Schwab et al., 2005).

The additional fermentable carbohydrate of CC would have utilized the excess of the rumen-degradable N of the CV weed by microbial uptake in the rumen. Though the present work did not estimate microbial protein supply by purine derivative in the urine, the lowered urinary N excretion by the CC supplementation could be attributed to an improvement of the microbial protein supply to the host animal (Hristov et al., 2005; Ku-Vera et al., 2020; Schwab et al., 2005). However, in the present study, the improvement did not result in a significantly higher N retention and LW gain of the goats fed the cassava supplementation diet.

The present study demonstrated that feeding of the CV weed either as a supplement to the basal diet of low-quality tropical grass or as a sole diet could result in a higher urinary N excretion which potentially increases N_2O emission in the livestock sector. The supplementation of the locally available non-structural carbohydrate, which is affordable for the small-scale farmers, improved the nutrients utilization of the CV weed and thus ruminant growth as well as a reduction of N_2O and CH_4 emissions.

The averaged LW gain and FCE of the goats on the CV weed and cassava supplementation diets were in the range of gain and FCE (23.5 to 69.4 g/d and 0.04 to 0.11 for LW gain and FCE, respectively) for fifteen growing male Kacang goats fed three diets with three different CP and energy contents (Restitrisnani et al., 2013). The gain was also in the range of Adiwinarti et al. (2019) for growing male Kacang goats with soybean meal supplementations (41.5 to 79.9 g/d). The LW loss of the goats on the basal diet of GG and CV weed supplementation diets reflected an insufficiency of energy intake for their maintenance requirement which is estimated to be 536 KJ/kg$^{0.75}$LW. The value of ME requirement is lower than an averaged value for goat and sheep (542 KJ/kg$^{0.75}$LW) in the warm climate (Salah et al., 2014) but higher than a value obtained in the study of Luo et al. (2004) for growing indigenous goat (489 KJ/kg$^{0.75}$LW) while the value for gain is higher than values on their studies (24.3 and 19.8 KJ/kg$^{0.75}$LW).

5. Conclusion

In conclusion, the CV weed supplementation on the basal diet of GG enhanced CP intake and urinary N excretion. When the weed was offered alone, nutrients intake and digestibility were higher; the goats had higher N retention and LW gain compared with the goats with basal diet but urinary N loss was also higher. The inclusion of CC supplementation improved DM and OM digestibility and lowered the urinary N excretion. Therefore the weed could be fed as a supplement to a poor-quality basal diet or fed solely without any adverse effects. Supplementation of CC as a source of readily fermented carbohydrates to the basal diet of the weed has the potential to effectively improve nutrients utilization. A further study is needed to evaluate the effects of the weed on CH_4 production and rumen fermentation characteristic.

Conflict of interest

We state that we have no conflict of interest.

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Tables and figure

Table 1

Chemical composition of Guinea grass, Chinese violet weed, and unpeeled Cassava chip ((g/kg dry matter) used in the study[1].

	Guinea grass	Chinese violet	Cassava chip
Dry matter (g/kg FM)	213±15.6	168±20.4	863± 3.5
Organic matter	901±3.2	860±8.3	984±0.8
Crude protein	107±3.9	160±2.1	43±1.3
Neutral detergent fiber	755±26.0	510±23.8	276±24.9
Acid detergent fiber	371±29.3	338±14.0	61± 8.4
Nitrate	0.3±0.02	11.0±0.98	n.a

[1]Values are means ± standard error of the mean

FM, fresh matter; n.a, not available

Table 2

Dry matter (DM) intake of basal diets and supplements, metabolizable energy (ME) intake, intake composition of crude protein (CP), neutral detergent fibre (NDF) and acid detergent fibre (ADF), digestibility of DM, OM, NDF and ADF of Kacang goats fed Guinea grass *ad libitum* (GG), GG *ad libitum* and Chinese violet weed (1% live weight, LW) (GG+CV), CV *ad libitum*, and CV *ad libitum* and unpeeled cassava chips (1% LW) (CV+CC).

Parameter	GG	GG+CV	CV	CV+CC	SEM	*P*-value
Basal diet intake						
g/d	313.0^{b}	220.2^{a}	533.6^{c}	398.8^{b}	23.64	<0.001
% LW	2.5^{b}	1.8^{a}	4.2^{d}	3.2^{c}	0.16	<0.001
Supplement intake						
g/d	n.a	148.6^{b}	n.a	119.7^{a}	5.85	<0.001
% LW	n.a	1.2^{b}	n.a	1.0^{a}	0.03	<0.001
Total DM intake						
g/d	313.0^{a}	368.8^{a}	533.6^{b}	518.6^{b}	22.89	<0.001
% LW	2.5^{a}	2.9^{a}	4.2^{b}	4.1^{b}	0.14	<0.001
ME intake						
MJ/d	3.4^{a}	4.1^{a}	6.0^{b}	6.3^{b}	0.31	<0.001
$MJ/kg^{0.75}$ LW	0.52^{a}	0.61^{a}	0.89^{b}	0.94^{b}	0.040	<0.001
Intake composition (g/kg DM)						
CP	118^{a}	136^{b}	164^{c}	135^{b}	2.7	<0.001
NDF	734^{d}	642^{c}	488^{b}	432^{a}	18.9	<0.001
ADF	347^{c}	339^{bc}	323^{b}	257^{a}	8.2	<0.001
Digestibility (g/kg)						
DM	675^{a}	694^{ab}	717^{b}	771^{c}	10.0	<0.001
OM	690^{a}	697^{a}	701^{a}	767^{b}	9.9	<0.001
NDF	643^{c}	615^{bc}	521^{a}	561^{ab}	19.5	<0.001
ADF	540	532	528	524	26.8	0.953

SEM, Standard error of the mean; n.a, not available

Values in the same row with different letters represent significant differences ($P < 0.05$).

Table 3

Nitrogen (N) balance in Kacang goats fed Guinea grass *ad libitum* (GG), GG *ad libitum* and Chinese violet weed (1% live weight, LW) (GG+CV), CV *ad libitum*, and CV *ad libitum* and unpeeled cassava chips (1% LW) (CV+CC).

Parameter	GG	GG+CV	CV	CV+CC	SEM	*P*-value
N intake						
g/d	6.0^{a}	8.1^{a}	14.0^{c}	11.3^{b}	0.64	<0.001
% LW	0.05^{a}	0.06^{b}	0.11^{d}	0.09^{c}	0.004	<0.001
Fecal N						
g/d	2.5^{a}	2.7^{a}	3.9^{b}	3.0^{a}	0.14	<0.001
% LW	0.21^{a}	0.22^{a}	0.31^{b}	0.23^{a}	0.009	<0.001
absorbed N						
g/d	3.5^{a}	5.3^{b}	10.1^{c}	8.3^{c}	0.54	<0.001
% LW	0.03^{a}	0.04^{b}	0.08^{d}	0.07^{c}	0.004	<0.001
Urinary N						
g/d	2.0^{a}	3.2^{b}	5.7^{c}	3.7^{b}	0.29	<0.001
% LW	0.016^{a}	0.026^{b}	0.045^{c}	0.030^{b}	0.0021	<0.001
Fecal N urinary N ratio	1.39^{b}	0.91^{a}	0.78^{a}	0.80^{a}	0.058	<0.001
N retention						
g/d	1.5^{a}	2.1^{a}	4.4^{b}	4.6^{b}	0.35	<0.001
% LW	0.011^{a}	0.017^{a}	0.035^{b}	0.036^{b}	0.0026	<0.001

absorbed N: N intake - Fecal N; N retention: absorbed N – urinary N

SEM, Standard error of the mean

Values in the same row with different letters represent significant differences ($P < 0.05$).

Table 4

Daily live weight (LW) gain, total gain and feed conversion efficiency (FCE) of Kacang goats fed Guinea grass *ad libitum* (GG), GG *ad libitum* and Chinese violet weed (1% live weight, LW) (GG+CV), CV *ad libitum*, and CV *ad libitum* and unpeeled cassava chips (1% LW) (CV+CC).

Parameter	GG		GG+CV		CV		CV+CC		SEM	*P*-value
LW gain (g/d)	2.0	a	13.7	a	47.2	b	48.2	b	4.04	<0.001
Total gain (g)	51.2	a	355.0	a	1227.5	b	1251.9	b	105.05	<0.001
FCE	-0.01	a	0.03	a	0.09	b	0.09	b	0.009	<0.001

FCE: g LW gain/g dry matter intake

Values in the same row with different letters represent significant differences ($P < 0.05$).

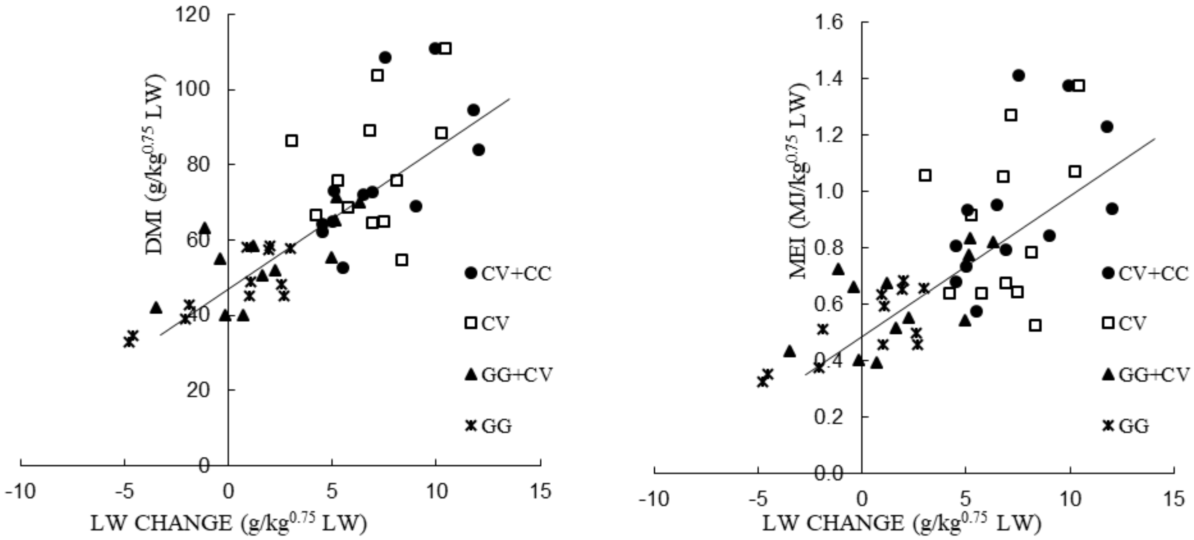

Fig. 1. Relationship between dry matter intake (DMI, left) and metabolizable energy intake (MEI, right) on live weight (LW) change in Kacang goats fed Guinea grass *ad libitum* (GG), GG *ad libitum* and Chinese violet weed (1% live weight, LW) (GG+CV), CV *ad libitum*, and CV *ad libitum* and unpeeled cassava chips (1% LW) (CV+CC). DMI= 3.81 LW change + 49.23 (n: 48, R^2: 0.63, P <0.001); MEI=0.049 LW change + 0.536 (n: 48, R^2: 0.54, P < 0.001).

Appendix

Allocation of the twelve Kacang goats (Group A (4, 8, 11), B (5, 10, 12), C (6, 7, 9) and D (1, 2, 3)) to Guinea grass (GG), GG and Chinese violet weed (GG+CV), CV, and CV plus unpeeled cassava chips (CV+CC) dietary treatments during 4 experimental periods.

Period	Animal group			
	A	B	C	D
1	GG	GG+CV	CV	CV+CC
2	CV+CC	GG	GG+CV	CV
3	GG+CV	CV	CV+CC	GG
4	CV	CV+CC	GG	GG+CV

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Utilization of invasive weed Asystasia gangetica to improve nutrient utilization and growth of Kacang goat

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Abstract:	A high concentration of nitrate and total nitrogen (N) in Chinese violet (CV) weed (Asystasia gangetica) might be a benefit for ruminant production in humid tropical regions; therefore, it was investigated in this study. Using a crossover design, twelve Kacang goats were allocated to four diets: Guinea grass (Panicum maximum) ad libitum (GG), GG ad libitum and CV (1% live weight, LW) (GG+CV), CV ad libitum (CV), and CV ad libitum plus cassava chip (1% LW) (CV+CC) (dry matter, DM basis). The goats on the CV diet had a higher intake of DM, metabolizable energy, and N, N retention, LW gain, and feed conversion efficiency than goats on the GG and GG+CV diets ($P < 0.001$) while DM digestibility was similar to the goats on the GG+CV diet but higher than the goats on the GG diet ($P < 0.001$). However, the goats on the CV diet had a higher urinary N loss compared to the GG and GG+CV diets ($P < 0.001$). The goats on the CV+CC had a higher digestibility of DM and organic matter (OM) and a lower urinary N loss compared to the goats on the CV diet ($P < 0.001$). Hence, the CV weed could be fed as a sole diet or as a supplement to the Kacang goat fed a low-quality forage for improvement of nutrients intake, digestibility, and gain while the urinary N loss could be lowered by cassava supplementation.

Utilization of invasive weed *Asystasia gangetica* to improve nutrient utilization and growth of Kacang goat

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Conflict of interest

We state that we have no conflict of interest.

Abstract

A high concentration of nitrate and total nitrogen (N) in Chinese violet (CV) weed (*Asystasia gangetica*) might be a benefit for ruminant production in humid tropical regions; therefore, it was investigated in this study. Using a crossover design, twelve Kacang goats were allocated to four diets: Guinea grass (*Panicum maximum*) *ad libitum* (GG), GG *ad libitum* and CV (1% live weight, LW) (GG+CV), CV *ad libitum* (CV), and CV *ad libitum* plus cassava chip (1% LW) (CV+CC) (dry matter, DM basis). The goats on the CV diet had a higher intake of DM, metabolizable energy, and N, N retention, LW gain, and feed conversion efficiency than goats on the GG and GG+CV diets (P < 0.001) while DM digestibility was similar to the goats on the GG+CV diet but higher than the goats on the GG diet (P < 0.001). However, the goats on the CV diet had a higher urinary N loss compared to the GG and GG+CV diets (P < 0.001). The goats on the CV+CC had a higher digestibility of DM and ~~organic matter (OM)~~ and a lower urinary N loss compared to the goats on the CV diet (P < 0.001). Hence, the CV weed could be fed as a sole diet or as a supplement to the Kacang goat fed a low-quality forage for improvement of nutrients intake, digestibility, and gain while the urinary N loss could be lowered by cassava supplementation.

Keywords: invasive weed, digestibility, growth, ruminant, urinary N loss

1. Introduction

A presence of weeds in a crop field has benefits such as lowering soil evaporation and erosion. However, weeds can negatively affect crop growth and yield due to competition for light, nutrients, and water (Zimdahl, 2004). In integrated crop-livestock farming, livestock serves a practical sustainable approach for controlling palatable weed species (MacLaren et al., 2020) which depends on the type of livestock and crops. Similar to the other tropical regions, the forage supply of small-scale farmers in the tropical humid regions is limited by resources of land, labors, and other inputs (Valbuena et al., 2012). Hence, ruminants were often supplied by the palatable weeds as the main resources of feed during a period of crop-growing season.

Asystasia gangetica (L.) T. Anderson is a perennial herb of the Acanthaceae family which widely distributed in tropical Africa and Asia (Kiew and Vollesen, 1997) and currently threat Northern and Eastern part of Australia (Westaway et al., 2016). The weed is fast-growing species, tolerant to low soil fertility and shade, and widely grown in the ground of tree plantation (Adjorlolo et al., 2014; Kiew and Vollesen, 1997). The weed has been fed to livestock by the small-scale farmers in the humid regions of Asia and Africa (Bindelle et al., 2007; Bussmann et al., 2020).

Few studies have been conducted to evaluate the effects of CV feeding on ruminant performance and nutrient utilization (Adjorlolo et al., 2014; Bindelle et al., 2007; Dahlan et al., 1993; Merkel et al., 1999). Comparing to legumes *Paraserianthes falcataria*, *Gliricidia sepium*, and *Calliandra calothyrsus*, CV had higher DM, neutral detergent fiber (NDF), and crude protein (CP) digestibility while a higher N urine of CV was related to NH_3 excess in the rumen due to a higher nitrate (NO_3) concentration in the weed (Merkel et al., 1999). The presence of NO_3 could be a benefit for lowering methane (CH_4) production and increasing N supply for microbial protein synthesis in the rumen. Nitrate is a hydrogen sink and has been reported to reduce CH_4 production in beef cattle fed low-quality forage (Callaghan et al., 2014; Hulshof et al., 2012). However, during the reduction of NO_3 to ammonia (NH_3), nitrite (NO_2) is formed. Because the reduction of NO_3 to NO_2 is generally faster than the reduction of NO_2 to NH_3, NO_2 can be accumulated in the rumen, and an excess of NO_2 results in methemoglobinemia (Callaghan et al., 2014; Ku-Vera et al., 2020).

The higher digestibility indicates that the CV weed could be supplemented to a low-quality forage (Ali et al., 2019b; Aregheore, 2004). Furthermore, an energy source of the readily fermented carbohydrates from CC might improve the NO_3 and NH_3 utilization in the rumen (Hristov et al., 2005; Ku-Vera et al., 2020; Schwab et al., 2005) and so decrease urinary N loss when supplemented to a basal diet of the CV weed. Thus, an evaluation is needed to assess sustainable feeding strategies of the CV weed on the ruminant production of the smallholder farmers in the crop-livestock system. The objective of the study was to evaluate a range of locally available diets based on the CV weed to improve Kacang goat performances as well as reduce the associated CH_4 and N losses. Specifically, this study was to determine the effect of supplementing a basal GG diet with the CV weed and the basal CV weed diet with the CC supplementation.

2. Materials and methods

2.1. Animals and experimental design

The animals were cared for according to the Animal Welfare Guidelines of the Indonesian Institute of Sciences. Approval of the experiment was granted from the Faculty of Agriculture, Universitas Sriwijaya, Indonesia.

Twelve male local Kacang goats with live weight (LW) of 10 ± 1.4 kg and six to seven months old were used. The animals were housed in individual pens (1.5 m × 0.75 m) in an open-sided type of house. The animals were treated orally with Oxfendazole (Verm-O, 25 mg per 5 kg LW, Sanbe, Indonesia). The goats were gradually acclimated to the CV diet over 20 days for preventing the nitrate toxicity. Before starting the experiment, the goats stratified based on their LW and allocated to the four treatments. The experimental design was a crossover design which consisted of four diets tested in four periods (Appendix). Each experimental period lasted for four weeks with three weeks of adaptation and followed by one week of sample collection where feed intake along with feces and urine excretion were measured.

2.2. Experimental feeds

The experimental diets used in the present study consisted of GG, GG+CV, CV, and CV+CC. The composition of the GG, CV, and CC is shown in Table 1. The GG was obtained from existing pastures near the experimental unit. The grass was harvest at 55 to 60 days intervals (after the blooming stage) about 25 cm from the ground every afternoon. The CV weed was obtained from an abandoned rubber plantation in the Universitas Sriwijaya campus. The weed was harvest in the post-blooming stage, at 45 days intervals about 5 cm from the ground every afternoon. The forages were chopped into 5.0 to 10.0 cm particle size the next morning before offering to the animals. The unpeeled CC were made from cassava tubers by chopping the tubers to 0.5 to 1.0 cm pieces then sun-dried for 4 to 5 days depending on sunlight intensity. Cassava crop is a cash crop in sub-urban areas of Palembang city, the tubers for CC were unmarketable size tubers (2 to 3 cm in diameter) which usually left on the field.

After removing and weighing the refusals from the previous day, the feeds were offered to the animals in separate buckets according to the experimental treatments, making it possible to give the

supplements at the same time as the basal feeds. The forages were offered 3 times at 9:00, 13:00, and 17:00 h while CC was offered at 9:00 h. The basal diet was offered *ad libitum* (allowing for 20% of refusal, fresh matter basis) while the CV and CC supplementations were offered at 1% of LW and were adjusted after each measurement of animal LW. Drinking water and salt-mineral lick were offered for *ad libitum* intake to all animals throughout the experiment.

2.3. Data collection and laboratory analyses

Throughout the whole experimental trial, a digital platform weighing scale was used to record the LW every Sunday and Thursday before offering the feeds. Duplicate subsamples of feed offered were collected each day during the collection week. The daily refusal feeds during the collection week were weighed, sampled (~200 g fresh matter, FM) and then stored at -20 ^{0}C. Then, the daily orts were pooled per animal, and then sampled (~100 g FM) in duplicate. The samples offered and refused feed and daily fecal excretion were dried at 45 ^{0}C for 4 days and reweighed for determination of dry weight.

During seven consecutive days of the collection week, a urine feces separator was fitted below the floor of each individual pen for daily total urine and feces collection. The separator was equipped with a plastic mesh that directed the dropped feces to a 5-L bucket while urine passed through the mesh. Total daily urine excretion was collected using 250 ml/L sulfuric acid to preserve the N. For N analysis concentration, sample of urine (~200 mL) was taken daily after homogenizing and filtering with surgical gaze then kept at -20 ^{0}C.

The daily excretions were quantified in the morning during the collection week. Before sampling (~100 g FM), the individual fecal excretion was homogenized and dried at 45 ^{0}C for 4 days, and then reweighed for dry weight determination. After drying, the feed and fecal samples were ground to pass a 1-mm mesh. The samples were pooled per animal at the end of each period proportionally to the daily amount. The dried samples were stored in zipper polythene bags prior to analyses.

The samples of feed and feces were analyzed for concentrations of DM, ash, N (AOAC, 1990), NDF, and acid detergent fiber (ADF) (Ankom200 fiber analyzer, Ankom Technology cooperation, Fairport, USA) with alpha-amylase and including residual ash (Van Soest et al., 1991). Concentration of OM was calculated by subtracting the ash concentration by 100 while the content of CP was calculated as N×6.25. The micro Kjedhal method (AOAC, 1990) was used to determine the N concentrations of urine. The NO_3 was extracted by the method of Liu et al. (2016) and the NO_3 content was measured using a UV-Vis spectrophotometer (Shimadzu 1800).

2.4. Data analysis

Nutrients intake was calculated from the nutrients in the refusal and the nutrients in the offered feed. Supply of metabolizable energy (ME, MJ/kg DM) was estimated as 0.0157 × digestible OM (g/kg DM) (AFRC, 1993). Intake composition was calculated by dividing the amount of a nutrient by total feed DM intake. Apparent digestibility was estimated by subtracting the daily nutrients in the feses by the nutrients in the feed intake. Live weight gain was calculated by regression of LW over the time (day of weighing) by SLOPE function while feed conversion efficiency (FCE) was calculated by dividing the LW gain by DM intake during 4 experimental weeks. Absorbed N was estimated by subtracting the fecal N excretion by the N intake while N retention was calculated by subtracting the urinary N excretion by Absorbed N.

Analysis of variance was performed using the mixed procedure (*lme* function) after confirming the normality of residual data by the Kolmogorov–Smirnov test (R Core Team, 2018). A mixed model with diets and period as fixed effects and animal as a random effect was used. To compare differences among means the Tukey post hoc test was applied. The significance was determined at $P < 0.05$.

3. Results

3.1. Feed intake and apparent digestibility of nutrients

The offered CV weed had lower NDF and ADF concentrations and higher CP and NO_3 content than GG whereas CC had the lowest NDF, ADF, and CP contents (Table 1). The effect of the CV weed and CC supplementations on nutrients intake and digestibility is shown in Table 2. Intake of GG was higher for goats on the basal GG diet than goats received supplements (GG+CV) whereas supplement intake was higher for goats offered GG+CV than those fed CV+CC ($P < 0.001$). The goats on CV and CV+CC had higher total DM and ME intake than those fed GG and GG+CV ($P < 0.001$). The goats on the CV diet had the highest CP concentration in their intake followed those observed on GG+CV, CV+CC, and GG diets. The highest NDF and ADF concentrations were observed in GG diet followed by GG+CV and CV diets and the lowest NDF and ADF concentration was found in CV+CC diet. The DM digestibility of the goats fed CV diet was similar to the goats on GG+CV diet but higher than those fed GG diet whereas the goats on CV+CC diet had the highest DM digestibility ($P < 0.001$). The goats fed CV+CC diet also had the highest OM digestibility ($P < 0.001$) with no difference among the other treatments. The goats offered GG had the highest NDF digestibility ($P < 0.001$) while the ADF digestibility was not significantly different among diets. The goats on CV+CC diets had similar NDF digestibility with the goats on GG+CV and CV alone diet.

3.2. Nitrogen balance and growth performance

Nitrogen intake was the highest for the goats fed CV alone ($P < 0.001$) whereas the goats fed GG had the lowest N intake (Table 3). The N intake of the goats on GG+CV diet was similar to the goats on GG diet. Daily fecal N excretion was the highest for the goats on CV diet ($P < 0.001$), with no difference among the other treatments. Absorbed N and urinary N were also the highest for goats fed CV diet. The goats on CV+CC diet had similar absorbed N but lower urinary N than the goats fed solely CV when the values were expressed as g/d. The goats on GG diet had the lowest N intake and total excreted N and the highest ratio of fecal and urinary N ($P < 0.001$). ~~As the lowest for LW gain,~~ the goats on GG diet had the lowest N retention which not significantly different from the goats on GG+CV diet whereas the higher N retention was found in goats receiving CV and CV+CC diets ($P < 0.001$). Live weight gain and feed conversion efficiency were higher for the goats on diets of CV and CV+CC than those fed GG and GG+CV ($P < 0.001$) (Table 4).

Eight goats on the GG and GG+CV diets experienced losses of LW during the adaptation and collection weeks (Figure 1). Regression analysis showed that LW gain significantly correlated ($P < 0.001$) with DM intake (R^2: 0.63) and ME intake (R^2: 0.54). The regression resulted in a daily ME requirement of 536 ~~K~~J/kg$^{0.75}$ LW for maintenance and 49.7 KJ/kg$^{0.75}$ LW for gain.

4. Discussion

4.1. Feed intake and apparent digestibility of nutrients

The NDF concentration of the CV weed in the present study was higher while CP concentration was within the range of the previous studies (Adjorlolo et al., 2014; Bindelle et al., 2007; Dahlan et al., 1993; Merkel et al., 1999). The differences might depend on the plant age or the ratio of leaf and stem (Kumalasari et al., 2020). The NO_3 concentration in the present study was lower than the NO_3 concentration in the CV weed (22 - 98 g/kg) in Merkel et al. (1999) and have to offer less than 50% of a ration (DM basis) (Rasby et al., 2014). The high level of NO_3 in the weed would be expected to restrict the intake of the goats. However, the higher intake was found in the CV weed alone and CV+CC diets that could be related to the acclimation of the rumen microbes to the higher NO_3 in the weed.

The higher CP ~~concentration~~ and the lower NDF ~~concentration of the~~ intake in the CV weed ~~supplementation~~ diet could not improve the nutrients intake and digestibility as the intake of the basal GG had 118 g CP/kg DM. Ali et al. (2019) reported similar basal and supplement intake for heifers fed mixed Rhodes grass hay and wheat straw (7.1% CP and 73% NDF) with sweet potato vine silage supplementation. Piñeiro-Vázquez et al. (2017) also found the similar evidence for heifers on *Pennisetum purpureum* (7.1% CP and 66% NDF) with *Leucaena leucocephala* supplementation. However, a higher DM intake and digestibility was reported for goats fed *Ischaemum aristatum* (68% CP and 40% NDF) with sweet potato vine supplementation (Aregheore, 2004).

Though the DM digestibility was the lowest in the basal GG diet, the highest NDF digestibility on the basal GG diet might be attributed to the lowest DM intake of the diet. A lowered DM intake could be attributed to the escalated nutrient digestibility in the rumen as a result of a longer retention time in the rumen. A study of Ali et al. (2019a) on steers fed four levels of intake, observed a higher NDF and ADF digestibility at a lower intake level. An increase of NDF digestibility, as well as an increase of ruminating time per DM intake when the level of intake was lowered, was also reported in the study of Schulze et al. (2014) on heifers fed four levels of grass-clover silage. The lower NDF digestibility in the present study might be also related to the higher NO_3 concentration in the diets. Nitrite, as the result of the reduction of NO_3, was reported to reduce the cellulolytic microbial population in the rumen (Marais et al., 1988).

The higher DM and OM digestibility ~~of the~~ goats fed the CV weed and the cassava supplementation could be explained by the increased supply of both N and the readily available carbohydrates for the rumen fermentation. An improvement of DM and OM digestibility along with a higher N retention and LW gain of goats fed a mix of Gamba grass (*Andropogon gayanus*) and Cassava hay by cassava supplementation was reported (Phengvichith and Ledin, 2007).

4.2. Nitrogen balance and growth performance

Comparing to the basal GG diet, N intake and absorption were higher in the goats fed CV weed when the values were expressed as % of LW (Table 4). However, urinary N excretion was also elevated which might reflect an excess of NH_3 production in the rumen. A higher concentration of NO_3 in the CV weed was also reported (Merkel et al. 1999) which could be degraded to the NH_3 in the rumen. The excess of NH_3 is absorbed in the bloodstream, converted to urea in the liver, and then excreted in the urine (Schwab et al., 2005).

The additional fermentable carbohydrate of CC would have utilized the excess of the rumen-degradable N of the CV weed by microbial uptake in the rumen. Though the present work did not estimate microbial protein supply ~~by purine derivative in the urine~~, the lowered urinary N excretion by the CC supplementation could be attributed to an improvement of the microbial protein supply to the host animal (Hristov et al., 2005; Ku-Vera et al., 2020; Schwab et al., 2005). However, in the present study, the improvement did not result in a significantly higher N retention and LW gain of the goats fed the cassava supplementation diet.

The present study demonstrated that feeding of the CV weed either as a supplement to the basal diet of low-quality tropical grass or as a sole diet could result in a higher urinary N excretion which potentially increases N_2O emission in the livestock sector. The supplementation of the locally available non-structural carbohydrate, which is affordable for the small-scale farmers, improved the nutrients utilization of the CV weed and thus ruminant growth as well as a reduction of N_2O and CH_4 emissions.

The averaged LW gain and FCE of the goats on the CV weed and cassava supplementation diets were in the range of gain and FCE (23.5 to 69.4 g/d and 0.04 to 0.11 for LW gain and FCE, respectively) for fifteen growing male Kacang goats fed three diets with three different CP and energy contents (Restitrisnani et al., 2013). The gain was also in the range of Adiwinarti et al. (2019) for growing male Kacang goats with soybean meal supplementations (41.5 to 79.9 g/d). The LW loss of the goats on the basal diet of GG and CV weed supplementation diets reflected an insufficiency of energy intake for their maintenance requirement which is estimated to be 536 KJ/kg$^{0.75}$LW. The value of ME requirement is lower than an averaged value for goat and sheep (542 KJ/kg$^{0.75}$LW) in the warm climate (Salah et al., 2014) but higher than a value obtained in the study of Luo et al. (2004) for growing indigenous goat (489 KJ/kg$^{0.75}$LW) while the value for gain is higher than values on their studies (24.3 and 19.8 KJ/kg$^{0.75}$LW).

5. Conclusion

In conclusion, the CV weed supplementation on the basal diet of GG enhanced CP intake and urinary N excretion. When the weed was offered alone, nutrients intake and digestibility were higher; the goats had higher N retention and LW gain compared with the goats with basal diet but urinary N loss was also higher. The inclusion of CC supplementation improved DM and OM digestibility and lowered the urinary N excretion. Therefore the weed could be fed as a supplement to a poor-quality basal diet or fed solely without any adverse effects. Supplementation of CC as a source of readily fermented carbohydrates to the basal diet of the weed has the potential to effectively improve nutrients utilization. A further study is needed to evaluate the effects of the weed on CH_4 production and rumen fermentation characteristic.

Conflict of interest

We state that we have no conflict of interest.

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Tables and figure

Table 1

Chemical composition of Guinea grass, Chinese violet weed, and unpeeled Cassava chip ((g/kg dry matter) used in the study[1].

	Guinea grass	Chinese violet	Cassava chip
Dry matter (g/kg FM)	213±15.6	168±20.4	863± 3.5
Organic matter	901±3.2	860±8.3	984±0.8
Crude protein	107±3.9	160±2.1	43±1.3
Neutral detergent fiber	755±26.0	510±23.8	276±24.9
Acid detergent fiber	371±29.3	338±14.0	61± 8.4
Nitrate	0.3±0.02	11.0±0.98	n.a

[1]Values are means ± standard error of the mean

FM, fresh matter; n.a, not available

Table 2

Dry matter (DM) intake of basal diets and supplements, metabolizable energy (ME) intake, intake composition of crude protein (CP), neutral detergent fibre (NDF) and acid detergent fibre (ADF), digestibility of DM, OM, NDF and ADF of Kacang goats fed Guinea grass *ad libitum* (GG), GG *ad libitum* and Chinese violet weed (1% live weight, LW) (GG+CV), CV *ad libitum*, and CV *ad libitum* and unpeeled cassava chips (1% LW) (CV+CC).

Parameter	GG	GG+CV	CV	CV+CC	SEM	*P*-value
Basal diet intake						
g/d	313.0^{b}	220.2^{a}	533.6^{c}	398.8^{b}	23.64	<0.001
% LW	2.5^{b}	1.8^{a}	4.2^{d}	3.2^{c}	0.16	<0.001
Supplement intake						
g/d	n.a	148.6^{b}	n.a	119.7^{a}	5.85	<0.001
% LW	n.a	1.2^{b}	n.a	1.0^{a}	0.03	<0.001
Total DM intake						
g/d	313.0^{a}	368.8^{a}	533.6^{b}	518.6^{b}	22.89	<0.001
% LW	2.5^{a}	2.9^{a}	4.2^{b}	4.1^{b}	0.14	<0.001
ME intake						
MJ/d	3.4^{a}	4.1^{a}	6.0^{b}	6.3^{b}	0.31	<0.001
MJ/kg$^{0.75}$ LW	0.52^{a}	0.61^{a}	0.89^{b}	0.94^{b}	0.040	<0.001
Intake composition (g/kg DM)						
CP	118^{a}	136^{b}	164^{c}	135^{b}	2.7	<0.001
NDF	734^{d}	642^{c}	488^{b}	432^{a}	18.9	<0.001
ADF	347^{c}	339^{bc}	323^{b}	257^{a}	8.2	<0.001
Digestibility (g/kg)						
DM	675^{a}	694^{ab}	717^{b}	771^{c}	10.0	<0.001
OM	690^{a}	697^{a}	701^{a}	767^{b}	9.9	<0.001
NDF	643^{c}	615^{bc}	521^{a}	561^{ab}	19.5	<0.001
ADF	540	532	528	524	26.8	0.953

SEM, Standard error of the mean; n.a, not available

Values in the same row with different letters represent significant differences ($P < 0.05$).

Table 3

Nitrogen (N) balance in Kacang goats fed Guinea grass *ad libitum* (GG), GG *ad libitum* and Chinese violet weed (1% live weight, LW) (GG+CV), CV *ad libitum*, and CV *ad libitum* and unpeeled cassava chips (1% LW) (CV+CC).

Parameter	GG	GG+CV	CV	CV+CC	SEM	*P*-value
N intake						
g/d	6.0^{a}	8.1^{a}	14.0^{c}	11.3^{b}	0.64	<0.001
% LW	0.05^{a}	0.06^{b}	0.11^{d}	0.09^{c}	0.004	<0.001
Fecal N						
g/d	2.5^{a}	2.7^{a}	3.9^{b}	3.0^{a}	0.14	<0.001
% LW	0.21^{a}	0.22^{a}	0.31^{b}	0.23^{a}	0.009	<0.001
absorbed N						
g/d	3.5^{a}	5.3^{b}	10.1^{c}	8.3^{c}	0.54	<0.001
% LW	0.03^{a}	0.04^{b}	0.08^{d}	0.07^{c}	0.004	<0.001
Urinary N						
g/d	2.0^{a}	3.2^{b}	5.7^{c}	3.7^{b}	0.29	<0.001
% LW	0.016^{a}	0.026^{b}	0.045^{c}	0.030^{b}	0.0021	<0.001
Fecal N urinary N ratio	1.39^{b}	0.91^{a}	0.78^{a}	0.80^{a}	0.058	<0.001
N retention						
g/d	1.5^{a}	2.1^{a}	4.4^{b}	4.6^{b}	0.35	<0.001
% LW	0.011^{a}	0.017^{a}	0.035^{b}	0.036^{b}	0.0026	<0.001

absorbed N: N intake - Fecal N; N retention: absorbed N – urinary N

SEM, Standard error of the mean

Values in the same row with different letters represent significant differences ($P < 0.05$).

Table 4

Daily live weight (LW) gain, total gain and feed conversion efficiency (FCE) of Kacang goats fed Guinea grass *ad libitum* (GG), GG *ad libitum* and Chinese violet weed (1% live weight, LW) (GG+CV), CV *ad libitum*, and CV *ad libitum* and unpeeled cassava chips (1% LW) (CV+CC).

Parameter	GG	GG+CV	CV	CV+CC	SEM	*P*-value
LW gain (g/d)	2.0 [a]	13.7 [a]	47.2 [b]	48.2 [b]	4.04	<0.001
Total gain (g)	51.2 [a]	355.0 [a]	1227.5 [b]	1251.9 [b]	105.05	<0.001
FCE	-0.01 [a]	0.03 [a]	0.09 [b]	0.09 [b]	0.009	<0.001

FCE: g LW gain/g dry matter intake

Values in the same row with different letters represent significant differences ($P < 0.05$).

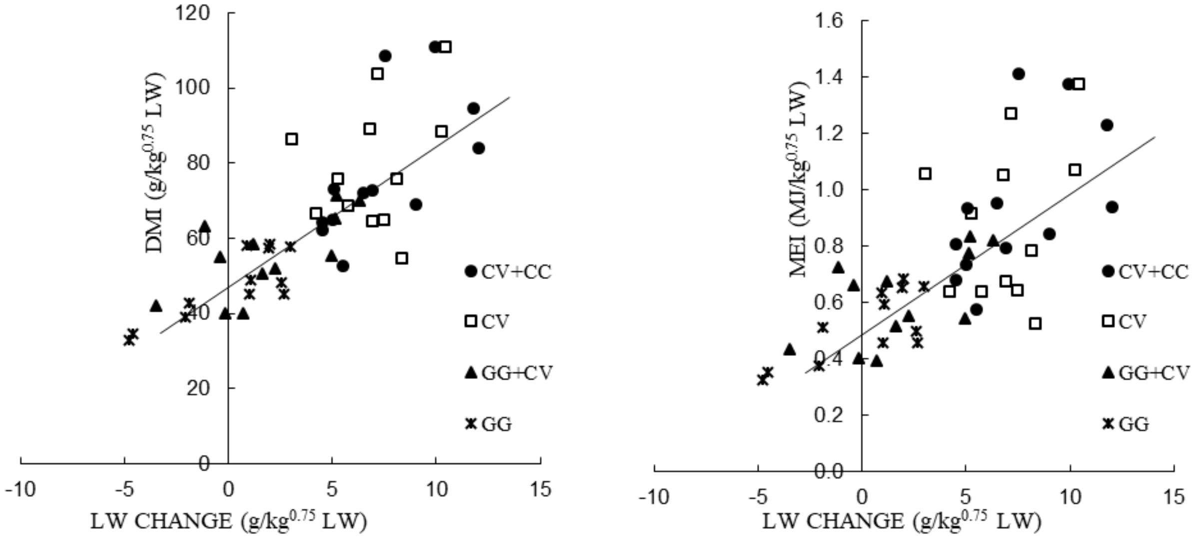

Fig. 1. Relationship between dry matter intake (DMI, left) and metabolizable energy intake (MEI, right) on live weight (LW) change in Kacang goats fed Guinea grass *ad libitum* (GG), GG *ad libitum* and Chinese violet weed (1% live weight, LW) (GG+CV), CV *ad libitum*, and CV *ad libitum* and unpeeled cassava chips (1% LW) (CV+CC). DMI= 3.81 LW change + 49.23 (n: 48, R^2: 0.63, P <0.001); MEI=0.049 LW change + 0.536 (n: 48, R^2: 0.54, P < 0.001).

Appendix

Allocation of the twelve Kacang goats (Group A (4, 8, 11), B (5, 10, 12), C (6, 7, 9) and D (1, 2, 3)) to Guinea grass (GG), GG and Chinese violet weed (GG+CV), CV, and CV plus unpeeled cassava chips (CV+CC) dietary treatments during 4 experimental periods.

Period	Animal group			
	A	B	C	D
1	GG	GG+CV	CV	CV+CC
2	CV+CC	GG	GG+CV	CV
3	GG+CV	CV	CV+CC	GG
4	CV	CV+CC	GG	GG+CV

Asep Indra Munawar Ali fp <asep_ali@fp.unsri.ac.id>

Your Submission

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Ms. Ref. No.: AOAS-D-21-00216
Title: Utilization of invasive weed Asystasia gangetica to improve nutrient utilization and growth of Kacang goat
Annals of Agricultural Sciences

Dear asep,

The reviewers have commented on your above paper. They indicated that it is not acceptable for publication in its present form.

However, if you feel that you can suitably address the reviewers' comments (included below), I invite you to revise and resubmit your manuscript.

Please carefully address the issues raised in the comments.

If you are submitting a revised manuscript, please also:

a) outline each change made (point by point) as raised in the reviewer comments

AND/OR

b) provide a suitable rebuttal to each reviewer comment not addressed

To submit your revision, please do the following:

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Yours sincerely,

Ali Ali, Ph.D.
Editor in Chief
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Reviewers' comments:

Reviewer #1: Utilization of invasive weed Asystasia gangetica to improve nutrient utilization and growth of Kacang goat
Comments
Title
Title may be corrected as suggested on the body of the text?
Abstract
If CV diet as sole feed showed low digestibility and higher N losses? How can you have concluded that this may be used as sole feed? Pl. see your results?
Few corrections have been suggested on the body of the text.
Introduction

Pl. define the objective of the study precisely?
Materials and methods
L 89: Pl. describe the dietary treatments properly? How did you estimate the ME content in feeds?
Besides these some corrections have been suggested on the body of the text.
Discussion
L 177: 11.8 % CP of diet is good enough to take care of goats?
Authors earlier claimed that intake was increased with CV diets?
What was the CP, DCP and ME intake of goats?
L 189: The theory is applicable for DM digestibility also but you observed lower DM digestibility?
L-192: This statement is not clear, pl. revise it?
L-198: The cassava is a very good source of condensed tannins, that improved the N utilization in ruminants. Therefore, results may be discussed this point of view with suitable recent references?
L-208: The lowered urinary N excretion by the CC supplementation could be attributed to improved N bio-availability/ protection of N in rumen due to the presence of phenolic compound in cassava?
Pl. discuss the results accordingly?
L-214: The higher urinary N excretion indicated that the N was poorly utilized in this group, therefore, results may be discussed accordingly?
L-220: Pl. discuss the results on the basis of higher/lower or better FCE among the treatment groups?
L-225: What was the DCP and ME intake of different groups? That can be compared with the requirements?
Besides these some corrections have been suggested on the body of the text.
Conclusion
Pl. give your conclusion on the basis of results obtained?
Do not give results here?
Table 1: title may be modified as Intake and digestibility of nutrients in goats?
How did you estimate the ME content in feeds?
Table 3: Merge table 4 with table 3? Pl. use superscript properly?
Some corrections have been suggested on the body of the text.

Reviewer #2:

English in the paper must be thoroughly revised and edited.

Reviewer #3: The authors presented an important and valuable manuscript about the utilizition of Chinese violet in feeding of goats. This study is based on original experiments. Goals of the study are clearly defined. Materials and methods of the experiment are well-presented and based on animal welfare statements and regulations. The results are presented in tables and figures, the discussion is based on a compairison with the relevant references. Conclusions are clear and not far-reaching.

Recommendations of the reviewer:

- Title of the manuscript should be reconsidered (very long and the word "Utilization" is repeated).
- Very old references (from 1988, 1991 etc.) should be avoided if possible.
- Recommended reference with similar topic: DOI: 10.15835/nbha49112197
- Recommended to use detailed footnotes at the tables, according to the different diets (GG, GG+CV, CV, CV+CC).
- Due to the allocation and rotation of the twelve goats, it is very difficult to follow the individual growth performance of each animal. For example, if the reviewer is focusing on Table 4, this can be very confusing and it can make feels as if some individuals are dying by the end of the experiment due to weight loss. According to this, it is recommended to give an explanation about the LWs and ADGs of the individuals, with special regards on the animal welfare regulations.

Reviewer #4: Dear Author,

1. This study is important as it shows the use of a natural resource as goat feed.
2. I consider that the document is well written.
3. Restate the objective, since no results are presented where CH_4 losses are analyzed, then I suggest take off the losses by CH_4.

****

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Ms. Ref. No.: AOAS-D-21-00216R1
Title: Effect of feeding Asystasia gangetica weed on intake, nutrient utilization, and gain in Kacang goat
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Effect of feeding Asystasia gangetica weed on intake, nutrient utilization, and gain in Kacang goat

--Manuscript Draft--

Manuscript Number:	AOAS-D-21-00216R1
Article Type:	Original Article
Keywords:	invasive weed; digestibility; growth; ruminant; urinary N loss
Corresponding Author:	asep indra munawar ali, Ph.D Universitas Sriwijaya Ogan Ilir, Sumatera Selatan INDONESIA
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Abstract:	A high concentration of nitrate and total nitrogen (N) in Chinese violet (CV) weed (Asystasia gangetica) has a potential benefit as a digestible protein source for ruminant production in the humid tropical regions. We conducted a study using twelve Kacang goats in a crossover design to investigate the effect of CV weed on intake, nutrient utilization, and growth. Four dietary treatments were tested: Guinea grass (Panicum maximum) ad libitum (GG), GG ad libitum and CV (1% live weight, LW) (GG+CV), CV ad libitum (CV), and CV ad libitum plus cassava chip (1% LW) (CV+CC) (dry matter, DM basis). No sign of nitrite toxicity was observed when the goat fed CV diet while intake, N retention, and LW gain was higher than goats on the GG and GG+CV diets ($P < 0.001$). Digestibility of DM was similar to the goats on the GG+CV diet but higher than the goats on the GG diet ($P < 0.001$). However, the goats on the CV diet had a higher urinary N loss compared to the goats on GG and GG+CV diets ($P < 0.001$). The cassava supplementation (CV+CC) increased DM digestibility and lowered urinary N loss ($P < 0.001$). Hence, the CV weed could be fed as a sole diet or as a supplement to the Kacang goat fed a low-quality forage for improvement of nutrients intake, digestibility, and gain while the urinary N loss could be lowered by cassava supplementation

Conflict of interest

We state that we have no conflict of interest.

Asep Ali
Department of Animal Science, Faculty of Agriculture, Universitas Sriwijaya
Jalan Palembang-Prabumulih KM32 Ogan Ilir 30862, Indonesia

June 24th, 2021

Dear Dr. Ali Abdelaziz Ali,
We wish to submit an original research article entitled "Utilization of invasive weed *Asystasia gangetica* to improve nutrient utilization and growth of Kacang goat" for consideration by Journal of Annals of Agricultural Sciences. We confirm that this work is original and has not been published elsewhere, nor is it currently under consideration for publication elsewhere.

We believe that this manuscript is appropriate for publication in the journal. It shows the improvement of nutrient utilization and growth of a local goat whilst reduce urinary N loss by feeding the weeds in an integrated crop-livestock system.

Asystasia gangetica is an invasive weed species that practically fed to livestock in tropical humid regions. However, the responses of the animals to the feeding of the N-rich weed are limited. This study shows options of diets based on the weed for boosting the ruminant performances.

We have no conflicts of interest to disclose.

Please address all correspondence concerning this manuscript to me at asep_ali@fp.unsri.ac.id.

Thank you for your consideration of this manuscript.

Sincerely,

Asep Ali

Effect of feeding *Asystasia gangetica* weed on intake, nutrient utilization, and gain in Kacang goat

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Reviewer Comments		Authors Responses	
Comments	**Line**	**Comments/Correction**	**New line**
Reviewer 1			
Title Title may be corrected as suggested on the body of the text? Effect of *Asystasia gangetica* weed supplementation on intake, nutrient utilization, and gain in Kacang goat		The title has been revised. Since the weed was not only as a supplement, the "supplementation" has been substituted with "feeding": Effect of feeding *Asystasia gangetica* weed on intake, nutrient utilization, and gain in Kacang goat	
Abstract Pl. rephrased the statement precisely?	2-4	The statement has been revised	2-4
Pl. rewrite it clearly?	4-7	The statement has been revised	3-7
Pl. rephrased for more clarity?	10-11	The sentence has been revised	11-12
If CV diet as sole feed showed low digestibility and higher N losses? How can you have concluded that this may be used as sole feed? Pl. see your results? Few corrections have been suggested on the body of the text.	13-15	The digestibility of the CV diet was higher than the digestibility of GG diet but lower than CV+CC diet.	10 12-13
		CV has a high concentration of Nitrate that converted to nitrite (a potential toxic for the ruminants) in the rumen. We did not observe a sign of toxicity or any negative effect when the goats consumed the CV diet alone. Despite the higher N loss, this indicates that CV could be fed as a sole diet without the negative effect on the animals. It was explained that This could be related to the acclimation of the population of rumen microbes to the higher NO_3 in the weed.	Introduction 44-46 8 166-168 169 - 170
Introduction Pl. define the objective of the study precisely?	52-55	The objective of the study has been revised	53-55
Materials and methods L 89: Pl. describe the dietary treatments properly?	72-73	The diets have been clearly explained	72-75
How did you estimate the ME content in feeds?		ME was estimated from digestible OM (AFRC, 1993)	116-117
Besides these some corrections have been suggested on the body of the text.		The corrections have been followed, one by one	
Discussion 11.8 % CP of diet is good enough to take care of goats?	177	The improvement of intake & digestibility depend on CP content. When CP of basal diet was less than 7%, supplementation of CP source could increase intake of basal diet which means that the additional CP intake could enhance microbial degradation since CP was very limited for microbial requirement. However, when the CP was higher than 7%, the supplementation only improved DM and N intake (Schwab, et al 2005, page 30) which was evidenced by the present study and the earlier authors. The sentences have been revised	171-174 176-180 174-176
Authors earlier claimed that intake was increased with CV diets?			
What was the CP, DCP and ME intake of goats?		CP intake and digestible CP have been added in Table 2	Table 2

		The ME intake is shown in Table 2 as well	
The theory is applicable for DM digestibility also but you observed lower DM digestibility?	189	DM digestibility was the lowest but NDF digestibility was the highest on GG diet.	185-187
This statement is not clear, pl. revise it?	192	The statement has been revised	189-190
The cassava is a very good source of condensed tannins, that improved the N utilization in ruminants. Therefore, results may be discussed this point of view with suitable recent references?	198	Some literatures showed that the tannin content in the cassava leaves is high (200 – 800 mg/kg) while the tannins content in the cassava tubers is only 10-57 mg/g. The improved N utilization in the ruminant is mainly due to the high content of starch (readily fermentable carbohydrate) as the main energy source to convert NO_3, NO_2 and NH_3 to microbial protein. The lower urinary N excretion by the CC supplementation (CV+CC) than sole CV diet could be attributed to the utilization of (excess) NO_2 and NH_3	
The lowered urinary N excretion by the CC supplementation could be attributed to improved N bio-availability/ protection of N in rumen due to the presence of phenolic compound in cassava? Pl. discuss the results accordingly?	208		
The higher urinary N excretion indicated that the N was poorly utilized in this group, therefore, results may be discussed accordingly?	214	Compared to CV diet, urinary N in the CV+CC was higher when the values are shown as g/d, %LW, and % N intake The result has been discussed	197-209
Pl. discuss the results on the basis of higher/lower or better FCE among the treatment groups?	220	The additional discussion has been added	215-221
What was the DCP and ME intake of different groups? That can be compared with the requirements?	225	The ME intake has been discuused. For the DCP requirement, I am not confidence to discuss the requirement based on the values of LW gain since the metabolizable protein could not be estimated in the present study	226-232
Besides these some corrections have been suggested on the body of the text.		The corrections have been followed, one by one. Thank you	
Conclusion			
Pl. give your conclusion on the basis of results obtained? Do not give results here?		The conclusion has been revised	
Table 2: title may be modified as Intake and digestibility of nutrients in goats?		The title has been modified as suggested	Table 2
How did you estimate the ME content in feeds?		Estimation had been described in the MM and the footnote of Table 2.	116-117, Table 2
Table 3: Merge table 4 with table 3? Pl. use superscript properly? Some corrections have been suggested on the body of the text.		Table 4 and Table 3 have been merged and superscript letters in all tables have been revised	Table 3
Reviewer 2			
English in the paper must be thoroughly revised and edited.		The English has been rechecked and edited	
Reviewer 3:			
The authors presented an important and valuable manuscript about the utilizition of Chinese violet in feeding of goats. This study is based on original experiments. Goals of the study are clearly defined. Materials and methods of the experiment are well-presented and based on animal welfare statements and regulations. The results are presented in tables and figures, the discussion is based on a			

compairison with the relevant references. Conclusions are clear and not far-reaching			
Title of the manuscript should be reconsidered (very long and the word "Utilization" is repeated).		The title has been modified as suggested by the reviewers	
Very old references (from 1988, 1991 etc.) should be avoided if possible		Dahlan et al. (1993) has been deleted. Marais (1988) has been replaced by more-relevant references (Latham et al., 2016; Zhou et al., 2011). Van Soest et al. (1991) & AFRC (1993) could not be avoided	190
Recommended reference with similar topic: DOI: 10.15835/nbha49112197		We tried to relate the recommended reference but had difficulties finding a link or supported idea. A suggestion is needed	
Recommended to use detailed footnotes at the tables, according to the different diets (GG, GG+CV, CV, CV+CC)		The detailed footnotes has been added in the tables	Table 2 Table 3
Due to the allocation and rotation of the twelve goats, it is very difficult to follow the individual growth performance of each animal. For example, if the reviewer is focusing on Table 4, this can be very confusing and it can make feels as if some individuals are dying by the end of the experiment due to weight loss. According to this, it is recommended to give an explanation about the LWs and ADGs of the individuals, with special regards on the animal welfare regulations	Table 4	The value is averaged values during 4 weeks. The negative value was from GG diet. In the next period, we changed the dietary treatments (appendix) so the weight loss would not continue and the animal were not dying.	Table 3
Reviewer 4			
This study is important as it shows the use of a natural resource as goat feed			
I consider that the document is well written		The English has been checked and edited again	
Restate the objective, since no results are presented where CH4 losses are analyzed, then I suggest take off the losses by CH4		The “CH4” was deleted in the objectives of the study	
		Thank you so much for your correction and suggestions	

Abstract

A high concentration of nitrate and total nitrogen (N) in Chinese violet (CV) weed (*Asystasia gangetica*) has a potential benefit as a digestible protein source for ruminant production in the humid tropical regions. We conducted a study using twelve Kacang goats in a crossover design to investigate the effect of CV weed on intake, nutrient utilization, and growth. Four dietary treatments were tested: Guinea grass (*Panicum maximum*) *ad libitum* (GG), GG *ad libitum* and CV (1% live weight, LW) (GG+CV), CV *ad libitum* (CV), and CV *ad libitum* plus cassava chip (1% LW) (CV+CC) (dry matter, DM basis). No sign of nitrite toxicity was observed when the goat fed CV diet while intake, N retention, and LW gain was higher than goats on the GG and GG+CV diets (P < 0.001). Digestibility of DM was similar to the goats on the GG+CV diet but higher than the goats on the GG diet (P < 0.001). However, the goats on the CV diet had a higher urinary N loss compared to the goats on GG and GG+CV diets (P < 0.001). The cassava supplementation (CV+CC) increased DM digestibility and lowered urinary N loss (P < 0.001). Hence, the CV weed could be fed as a sole diet or as a supplement to the Kacang goat fed a low-quality forage for improvement of nutrients intake, digestibility, and gain while the urinary N loss could be lowered by cassava supplementation.

Keywords: invasive weed, digestibility, growth, ruminant, urinary N loss

1. Introduction

A presence of weeds in a crop field has benefits such as lowering soil evaporation and erosion. However, weeds can negatively affect crop growth and yield due to competition for light, nutrients, and water (Zimdahl, 2004). In integrated crop-livestock farming, livestock serves a practical sustainable approach for controlling palatable weed species (MacLaren et al., 2020) which depends on the type of livestock and crops. Similar to the other tropical regions, the forage supply of small-scale farmers in the tropical humid regions is limited by resources of land, labors, and other inputs (Valbuena et al., 2012). Hence, ruminants were often supplied by the palatable weeds as the main resources of feed during a period of crop-growing season.

Asystasia gangetica (L.) T. Anderson is a perennial herb of the Acanthaceae family which widely distributed in tropical Africa and Asia (Kiew and Vollesen, 1997). The weed is commonly known as the Chinese violet (CV) and currently threat Northern and Eastern part of Australia (Westaway et al., 2016). The weed is fast-growing species, tolerant to low soil fertility and shade, and widely grown in the ground of tree plantation (Adjorlolo et al., 2014; Kiew and Vollesen, 1997). The weed has been fed to livestock by the small-scale farmers in the humid regions of Asia and Africa (Bindelle et al., 2007; Bussmann et al., 2020).

Few studies have been conducted to evaluate the effects of CV feeding on ruminant performance and nutrient utilization (Adjorlolo et al., 2014; Bindelle et al., 2007; Merkel et al., 1999). Comparing to legumes *Paraserianthes falcataria*, *Gliricidia sepium*, and *Calliandra calothyrsus*, CV had higher dry matter (DM), neutral detergent fiber (NDF), and crude protein (CP) digestibility while a higher N urine of CV was related to NH_3 excess in the rumen due to a higher nitrate (NO_3) concentration in the weed (Merkel et al., 1999). The presence of NO_3 could be a benefit for lowering methane (CH_4) production and increasing N supply for microbial protein synthesis in the rumen. Nitrate is a hydrogen sink and has been reported to reduce CH_4 production in beef cattle fed low-quality forage (Callaghan et al., 2014; Hulshof et al., 2012). However, during the reduction of NO_3 to ammonia (NH_3), nitrite (NO_2) is formed. Because the reduction of NO_3 to NO_2 is generally faster than the reduction of NO_2 to NH_3, NO_2 can be accumulated in the rumen, and an excess of NO_2 results in methemoglobinemia (Callaghan et al., 2014; Ku-Vera et al., 2020).

The higher digestibility indicates that the CV weed could be supplemented to a low-quality forage (Ali et al., 2019b; Aregheore, 2004). Furthermore, an energy source of the readily fermented carbohydrates from CC might improve the NO_3 and NH_3 utilization in the rumen (Hristov et al., 2005; Ku-Vera et al., 2020; Schwab et al., 2005) and so decrease urinary N loss when supplemented to a basal diet of the CV weed. Thus, an evaluation is needed to assess sustainable feeding strategies of the CV weed on the ruminant production of the smallholder farmers in the crop-livestock system. The objective of the study was to evaluate the effects of supplementing a low-quality forage with the CV weed and the basal CV weed diet with the CC supplementation on intake, nutrient utilization, and gain of Kacang goat.

2. Materials and methods

2.1. Animals and experimental design

The animals were cared for according to the Animal Welfare Guidelines of the Indonesian Institute of Sciences. Approval of the experiment was granted from the Faculty of Agriculture, Universitas Sriwijaya, Indonesia.

Twelve male local Kacang goats with live weight (LW) of 10 ± 1.4 kg and six to seven months old were used. The animals were housed in individual pens (1.5 m × 0.75 m) in an open-sided type of house. The animals were treated orally with Oxfendazole (Verm-O, 25 mg per 5 kg LW, Sanbe, Indonesia). The goats were gradually acclimated to the CV diet over 20 days for preventing the nitrite toxicity. Before starting the experiment, the goats stratified based on their LW and allocated to the four treatments. The experimental design was a crossover design which consisted of four diets tested in four periods (Appendix). Each experimental period lasted for four weeks with three weeks of adaptation and followed by one week of sample collection where feed intake along with feces and urine excretion were measured.

2.2. Experimental feeds

The experimental diets used in the present study consisted of Guinea grass (*Panicum maximum*) *ad libitum* (GG), GG *ad libitum* and CV (1% live weight, LW) (GG+CV), CV *ad libitum* (CV), and CV *ad libitum* plus cassava chip (1% LW) (CV+CC) (DM basis). The composition of the GG, CV, and CC is shown in Table 1. The GG was obtained from existing pastures near the experimental unit. The grass was harvest at 55 to 60 days intervals (after the blooming stage) about 25 cm from the ground every afternoon. The CV weed was obtained from an abandoned rubber plantation in the Universitas Sriwijaya campus. The weed was harvest in the post-blooming stage, at 45 days intervals about 5 cm from the ground every afternoon. The forages were chopped into 5.0 to 10.0 cm particle size the next morning before offering to the animals. The unpeeled CC were made from cassava tubers by chopping the tubers to 0.5 to 1.0 cm pieces then sun-dried for 4 to 5 days depending on sunlight intensity. Cassava crop is a cash crop in sub-urban areas of Palembang city, the tubers for CC were unmarketable size tubers (2 to 3 cm in diameter) which usually left on the field.

After removing and weighing the refusals from the previous day, the feeds were offered to the animals in separate buckets according to the experimental treatments, making it possible to give the supplements at the same time as the basal feeds. The forages were offered 3 times at 9:00, 13:00, and 17:00 h while CC was offered at 9:00 h. The basal diet was offered *ad libitum* (allowing for 20% of refusal, fresh matter basis) while the CV and CC supplementations were offered at 1% of LW and were adjusted after each measurement of animal LW. Drinking water and salt-mineral lick were offered for *ad libitum* intake to all animals throughout the experiment.

2.3. Data collection and laboratory analyses

Throughout the whole experimental trial, a digital platform weighing scale was used to record the LW every Sunday and Thursday before offering the feeds. Duplicate subsamples of feed offered were collected each day during the collection week. The daily refusal feeds during the collection week were weighed, sampled (~200 g fresh matter, FM) and then stored at -20 ^{0}C. Then, the daily orts were pooled per animal, and then sampled (~100 g FM) in duplicate. The samples offered and refused feed and daily fecal excretion were dried at 45 ^{0}C for 4 days and reweighed for determination of dry weight.

During seven consecutive days of the collection week, a urine feces separator was fitted below the floor of each individual pen for daily total urine and feces collection. The separator was equipped with a plastic mesh that directed the dropped feces to a 5-L bucket while urine passed through the mesh. Total daily urine excretion was collected using 250 ml/L sulfuric acid to preserve the N. For N analysis concentration, sample of urine (~200 mL) was taken daily after homogenizing and filtering with surgical gaze then kept at -20 ^{0}C.

The daily excretions were quantified in the morning during the collection week. Before sampling (~100 g FM), the individual fecal excretion was homogenized and dried at 45 ^{0}C for 4 days, and then reweighed for dry weight determination. After drying, the feed and fecal samples were ground to pass a 1-mm mesh. The samples were pooled per animal at the end of each period proportionally to the daily amount. The dried samples were stored in zipper polythene bags prior to analyses.

The samples of feed and feces were analyzed for concentrations of DM, ash, N (AOAC, 1990), NDF, and ADF (Ankom200 fiber analyzer, Ankom Technology cooperation, Fairport, USA) with alpha-amylase and including residual ash (Van Soest et al., 1991). Concentration of OM was calculated by subtracting the ash concentration by 100 while the content of CP was calculated as N×6.25. The micro Kjedhal method (AOAC, 1990) was used to determine the N concentrations of urine. The NO_3 was extracted by the method of Liu et al. (2016) and the NO_3 content was measured using a UV-Vis spectrophotometer (Shimadzu 1800). Supply of metabolizable energy (ME, MJ/kg DM) was estimated as 0.0157 × digestible OM (g/kg DM) (AFRC, 1993).

2.4. Statistical analysis

Analysis of variance was performed using the mixed procedure (*lme* function) after confirming the normality of residual data by the Kolmogorov–Smirnov test (R Core Team, 2018). A mixed model with diets and period as fixed effects and animal as a random effect was used. To compare differences among means the Tukey post hoc test was applied. The significance was determined at $P < 0.05$.

3. Results

3.1. Feed intake and apparent digestibility of nutrients

The offered CV weed had lower NDF and ADF concentrations and higher CP and NO_3 content than GG whereas CC had the lowest NDF, ADF, and CP contents (Table 1). Intake of GG was higher for goats on the basal GG diet than goats received supplements (GG+CV) whereas supplement intake was higher for goats offered GG+CV than those fed CV+CC ($P < 0.001$). The goats on CV and CV+CC had higher total DM and ME intake than those fed GG and GG+CV ($P < 0.001$). The goats on the CV diet had the highest CP intake ($P < 0.001$) followed those observed on CV+CC, GG+CV, and GG diets. The highest ADF intake was observed in the CC diet and followed by CC+CV, GG+CC, and GG diets while NDF intake was not significantly different among the dietary treatments. The DM digestibility in the goats fed CV diet was similar to the goats on GG+CV diet but higher than those fed GG diet whereas the goats on CV+CC diet had the highest DM digestibility ($P < 0.001$). The goats fed CV+CC diet also had the highest OM digestibility ($P < 0.001$) with no difference among the other treatments. Digestibility of CP was the highest in the goats on the CV+CC diet ($P < 0.001$) that was similar to the value on the CV diet. The goats offered GG had the highest NDF digestibility ($P < 0.001$) while the ADF digestibility was not significantly different among diets. The goats on CV+CC diets had similar NDF digestibility with the goats on GG+CV and CV alone diet.

3.2. Nitrogen balance and growth performance

Nitrogen intake was the highest for the goats fed CV alone ($P < 0.001$) whereas the goats fed GG had the lowest N intake (Table 3). The N intake in the goats on GG+CV diet was similar to the goats on GG diet. Daily fecal N excretion was the highest for the goats on CV diet ($P < 0.001$), with no difference among the other treatments. Absorbed N and urinary N were also the highest for goats fed CV diet. The goats on CV+CC diet had similar absorbed N but lower urinary N than the goats fed solely CV diet. The goats on GG diet had the lowest N intake and total excreted N and the highest ratio of fecal and urinary N ($P < 0.001$). The goats on GG diet had the lowest N retention which was not significantly different from the goats on GG+CV diet whereas the higher N retention was found in goats receiving CV and CV+CC diets ($P < 0.001$). Live weight gain and feed conversion efficiency were higher for the goats on diets of CV and CV+CC than those fed GG and GG+CV ($P < 0.001$).

Eight goats on the GG and GG+CV diets experienced losses of LW during the adaptation and collection weeks (Figure 1). Regression analysis showed that LW gain significantly correlated ($P < 0.001$) with DM intake (R^2: 0.63) and ME intake (R^2: 0.54). The regression resulted in a daily ME requirement of 536 KJ/kg$^{0.75}$ LW for maintenance and 49.7 KJ/kg$^{0.75}$ LW for gain.

4. Discussion

4.1. Feed intake and apparent digestibility of nutrients

The NDF concentration of the CV weed in the present study was higher while CP concentration was within the range of the previous studies (Adjorlolo et al., 2014; Bindelle et al., 2007; Merkel et al., 1999). The differences might depend on the plant age or the ratio of leaf and stem (Kumalasari et al., 2020). The NO_3 concentration in the present study was lower than the range of NO_3 concentrations in the CV weed (22 - 98 g/kg) in Merkel et al. (1999) and have to offer less than 50% of a ration (DM basis) (Rasby et al., 2014). The high level of NO_3 in the weed would be expected to restrict the intake of the goats. However, the higher intake was found in the CV weed alone and CV+CC diets without the negative effect of the NO_2 toxicity. This could be related to the acclimation of the population of rumen microbes to the higher NO_3 in the weed (Latham et al., 2016; Zhou et al., 2011).

The higher CP and the lower NDF concentration in the CV weed supplemented diet could not improve the nutrients intake and digestibility as the intake of the basal GG had 118 g CP/kg DM. Ali et al. (2019b) reported similar basal and supplement intake for heifers fed mixed Rhodes grass hay and wheat straw (7.1% CP and 73% NDF) with sweet potato vine silage supplementation. Piñeiro-Vázquez et al. (2017) also found the similar evidence for heifers on *Pennisetum purpureum* (7.1% CP and 66% NDF) with *Leucaena leucocephala* supplementation. However, when the basal diet had a lower CP concentration, a higher DM intake and digestibility was reported in goats fed *Ischaemum aristatum* (6.8% CP and 40% NDF) with sweet potato vine supplementation (Aregheore, 2004) as well as in steer fed wheat straw (2% CP and 81% NDF) with *Calliandra calothyrsus* supplementation (Korir et al., 2016).

Though the DM digestibility was the lowest in the basal GG diet, the highest NDF digestibility on the basal GG diet might be attributed to the lowest DM intake of the diet. A lowered DM intake could be attributed to the escalated nutrient digestibility in the rumen as a result of a longer retention time in the rumen. A study of Ali et al. (2019a) on steers fed four levels of intake, observed a higher NDF and ADF digestibility at a lower intake level. An increase of NDF digestibility, as well as an increase of ruminating time per DM intake when the level of intake was lowered, was also reported in the study of Schulze et al. (2014) on heifers fed four levels of grass-clover silage. The lower NDF digestibility when the goat consumed CV weed might be also related to the higher NO_3 concentration in the weed. The higher NO_3 concentration in the feed was reported to reduce the cellulolytic microbial population in the rumen (Latham et al., 2016; Zhou et al., 2011).

The higher DM and OM digestibility in goats fed the CV weed and the cassava supplementation could be explained by the increased supply of both N and the readily available carbohydrates for the rumen fermentation. An improvement of DM and OM digestibility along with a higher N retention and LW gain of goats fed a mix of Gamba grass (*Andropogon gayanus*) and Cassava hay by cassava supplementation was also reported by a previous study (Phengvichith and Ledin, 2007).

4.2. Nitrogen balance and growth performance

Comparing to the basal GG diet, N intake and absorption were higher in the goats fed CV weed (Table 3). Urinary N excretion was also elevated which might reflect an excess of NH_3 production in the rumen. A higher concentration of NO_3 in the CV weed was also reported (Merkel et al. 1999) which could be degraded to the NH_3 in the rumen. The excess of NH_3 is absorbed in the bloodstream, converted to urea in the liver, and then excreted in the urine (Schwab et al., 2005).

The additional fermentable carbohydrate of CC would have utilized the excess of the rumen-degradable N of the CV weed by microbial uptake in the rumen. This is suggested by the similar value of the absorbed N but the lower N intake in the CV+CC diet (Table 3). Though the present work did not estimate microbial protein supply, the similar absorbed N and lowered urinary N excretion by the CC supplementation could be attributed to an improvement of the microbial protein supply to the host animal (Hristov et al., 2005; Ku-Vera et al., 2020; Schwab et al., 2005). However, in the present study, the improvement did not result in a significantly higher N retention and LW gain of the goats fed the cassava supplementation diet.

The present study demonstrated that feeding of the CV weed either as a supplement to the basal diet of low-quality tropical grass or as a sole diet could result in a higher urinary N excretion which potentially increases N_2O emission in the livestock sector. The supplementation of the locally available non-structural carbohydrate, which is affordable for the small-scale farmers, improved the nutrients utilization of the CV weed as well as a reduction of N_2O and CH_4 emissions.

Improved growth performance has been well known when ruminants were fed a degradable protein source in the form of forage legume (Korir et al., 2016; Piñeiro-Vázquez et al., 2017) and leafy crop residue (Aregheore, 2004; Phengvichith and Ledin, 2007). In the present study, feeding of CV weed resulted in an improved gain of Kacang goats. The supplementation with the energy source of CC, however, could not result in a significantly higher LW gain which is related to the similar daily ME intake (Table 2) and digestible CP of the diets (Table 3). Moreover, the goats had the capacity to grow more than 48 g/day when they were fed CV+CC diets.

The averaged LW gain and FCE of the goats on the CV weed and cassava supplementation diets were in the range of gain and FCE (23.5 to 69.4 g/d and 0.04 to 0.11 for LW gain and FCE, respectively) for fifteen growing male Kacang goats fed three diets with three different CP and energy contents (Restitrisnani et al., 2013). The gain was also in the range of Adiwinarti et al. (2019) for growing male Kacang goats with soybean meal supplementations (41.5 to 79.9 g/d). The LW loss of the goats on the basal diet of GG and GG+CV diets reflected an insufficiency of energy intake for their maintenance requirement which is estimated to be 536 KJ/kg$^{0.75}$LW. The value of ME requirement is lower than an averaged value for goat and sheep (542 KJ/kg$^{0.75}$LW) in the warm climate (Salah et al., 2014) but higher than a value obtained in the study of Luo et al. (2004) for growing indigenous goat (489 KJ/kg$^{0.75}$LW) while the value for gain is higher than values on their studies (24.3 and 19.8 KJ/kg$^{0.75}$LW).

5. Conclusions

The CV weed could be fed as a supplement to a poor-quality forage or fed solely without any negative effects. Feed intake, nutrient utilization, and gain can be improved when the weed is offered. The supplementation of cassava chips as a source of readily fermented carbohydrates to the weed has the potential to effectively improve nutrients utilization and benefits for lowering urinary N loss. A further study is needed to evaluate the effects of the weed on CH_4 production and rumen fermentation characteristic.

Conflict of interest

We state that we have no conflict of interest.

Acknowledgements

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Tables and figure

Table 1

Chemical composition of Guinea grass, Chinese violet weed, and unpeeled Cassava chip (g/kg dry matter) used in the study.

	Guinea grass	Chinese violet	Cassava chip
Dry matter (g/kg FM)	213±15.6	168±20.4	863± 3.5
Organic matter	901±3.2	860±8.3	984±0.8
Crude protein	107±3.9	160±2.1	43±1.3
Neutral detergent fiber	755±26.0	510±23.8	276±24.9
Acid detergent fiber	371±29.3	338±14.0	61± 8.4
Nitrate	0.3±0.02	11.0±0.98	n.a

FM: fresh matter, n.a: not available

Table 2

Intake and digestibility of nutrients in Kacang goats.

Parameter	GG	GG+CV	CV	CV+CC	SEM	*P*-value
Basal diet intake						
g/d	313.0^{b}	220.2^{a}	533.6^{c}	398.8^{b}	23.64	<0.001
% LW	2.5^{b}	1.8^{a}	4.2^{d}	3.2^{c}	0.16	<0.001
Supplement intake						
g/d	n.a	148.6^{b}	n.a	119.7^{a}	5.85	<0.001
% LW	n.a	1.2^{b}	n.a	1.0^{a}	0.03	<0.001
Nutrients intake (g/d)						
DM	313.0^{a}	368.8^{a}	533.6^{b}	518.6^{b}	22.89	<0.001
OM	282.0^{a}	326.7^{a}	459.6^{b}	460.0^{b}	20.04	<0.001
CP	37.4^{a}	50.4^{a}	87.6^{c}	70.7^{b}	3.97	<0.001
NDF	233.8	240.2	263.7	229.1	12.40	0.4483
ADF	113.2^{a}	128.0^{a}	173.9^{b}	137.6^{ab}	8.23	0.0022
ME (MJ/d)	3.4^{a}	4.1^{a}	6.0^{b}	6.3^{b}	0.31	<0.001
ME (MJ/kg$^{0.75}$ LW)	0.52^{a}	0.61^{a}	0.89^{b}	0.94^{b}	0.040	<0.001
Digestibility (g/kg)						
DM	675^{a}	694^{ab}	717^{b}	771^{c}	10.0	<0.001
OM	690^{a}	697^{a}	701^{a}	767^{b}	9.9	<0.001
CP	556^{a}	652^{b}	715^{c}	726^{c}	15.4	<0.001
NDF	643^{c}	615^{bc}	521^{a}	561^{ab}	19.5	<0.001
ADF	540	532	528	524	26.8	0.953

GG: Guinea grass ad libitum, CV: Chinese violet weed ad libitum, GG+CV: GG ad libitum and CV weed (1% live weight, LW), CV+CC: CV ad libitum and unpeeled cassava chips (1% LW).

n.a: not available, DM: dry matter, OM: organic matter, CP: crude protein, NDF: neutral detergent fiber, ADF: acid detergent fiber, ME: metabolizable energy was estimated as 0.0157 × digestible OM (g/kg DM) (AFRC, 1993).

Values in the same row with different letters represent significant differences ($P < 0.05$).

Table 3

Nitrogen (N) balance, total gain, and feed conversion efficiency (FCE) in Kacang goats.

Parameter	GG	GG+CV	CV	CV+CC	SEM	*P*-value
N balance (g/d)						
N intake	6.0^{a}	8.1^{a}	14.0^{c}	11.3^{b}	0.64	<0.001
Fecal N	2.5^{a}	2.7^{a}	3.9^{b}	3.0^{a}	0.14	<0.001
Absorbed N	3.5^{a}	5.3^{b}	10.1^{c}	8.3^{c}	0.54	<0.001
Urinary N	2.0^{a}	3.2^{b}	5.7^{c}	3.7^{b}	0.29	<0.001
Fecal N urinary N ratio	1.39^{b}	0.91^{a}	0.78^{a}	0.80^{a}	0.058	<0.001
N retention	1.5^{a}	2.1^{a}	4.4^{b}	4.6^{b}	0.35	<0.001
Total gain (g)	51.2^{a}	355.0^{a}	1227.5^{b}	1251.9^{b}	105.05	<0.001
FCE	-0.01^{a}	0.03^{a}	0.09^{b}	0.09^{b}	0.009	<0.001

GG: Guinea grass *ad libitum*, CV: Chinese violet weed *ad libitum*, GG+CV: GG *ad libitum* and CV weed (1% live weight, LW), CV+CC: CV *ad libitum* and unpeeled cassava chips (1% LW).

FCE: g LW gain/g dry matter intake.

Values in the same row with different letters represent significant differences ($P < 0.05$).

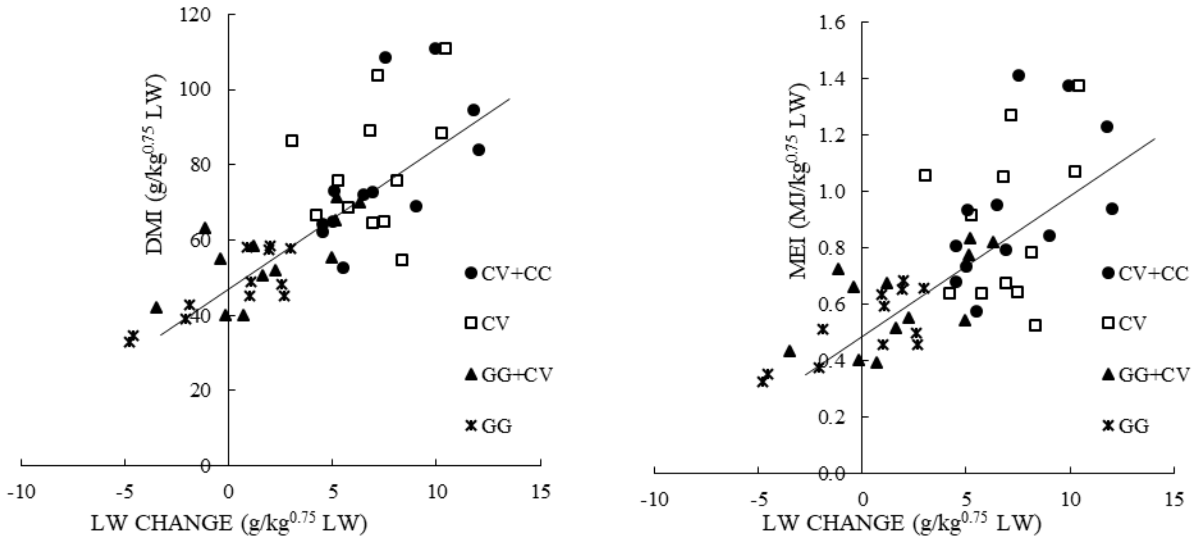

Fig. 1. Relationship between dry matter intake (DMI, left) and metabolizable energy intake (MEI, right) on live weight (LW) change in Kacang goats fed Guinea grass *ad libitum* (GG), GG *ad libitum* and Chinese violet weed (1% live weight, LW) (GG+CV), CV *ad libitum*, and CV *ad libitum* and unpeeled cassava chips (1% LW) (CV+CC). DMI= 3.81 LW change + 49.23 (n: 48, R^2: 0.63, P <0.001); MEI=0.049 LW change + 0.536 (n: 48, R^2: 0.54, $P < 0.001$).

Appendix

Allocation of the twelve Kacang goats (Group A (4, 8, 11), B (5, 10, 12), C (6, 7, 9) and D (1, 2, 3)) to Guinea grass (GG), GG and Chinese violet weed (GG+CV), CV, and CV plus unpeeled cassava chips (CV+CC) dietary treatments during 4 experimental periods.

Period	Animal group			
	A	B	C	D
1	GG	GG+CV	CV	CV+CC
2	CV+CC	GG	GG+CV	CV
3	GG+CV	CV	CV+CC	GG
4	CV	CV+CC	GG	GG+CV

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Your Submission

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Effect of feeding *Asystasia gangetica* weed on intake, nutrient utilization, and gain in Kacang goat

Q1

The corrections made in this section will be reviewed and approved by a journal production editor.

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Q2

Abstract

A high concentration of nitrate and total nitrogen (N) in Chinese violet (CV) weed (*Asystasia gangetica*) has a potential benefit as a digestible protein source for ruminant production in humid tropical regions. We conducted a study using twelve Kacang goats in a crossover design to investigate the effect of CV weed on intake, nutrient utilization, and growth. Four dietary treatments were tested: Guinea grass (*Panicum maximum*) *ad libitum* (GG), GG *ad libitum* and CV (1% live weight, LW) (GG + CV), CV *ad libitum* (CV), and CV *ad libitum* plus cassava chip (1% LW) (CV + CC) (dry matter, DM basis). No sign of nitrite toxicity was observed when the goat fed CV diet while intake, N retention, and LW gain was higher than goats on the GG and GG + CV diets ($P < 0.001$). Digestibility of DM was similar to the goats on the GG + CV diet but higher than the goats on the GG diet ($P < 0.001$). However, the goats on the CV diet had a higher urinary N loss compared to the goats on GG and GG + CV diets ($P < 0.001$). The cassava supplementation (CV + CC) increased DM digestibility and lowered urinary N loss ($P < 0.001$). Hence, the CV weed could be fed as a sole diet or as a supplement to the Kacang goat fed a low-quality forage for improvement of nutrients intake, digestibility, and gain, while the urinary N loss could be lowered by cassava supplementation.

Keywords:

Invasive weed, Digestibility, Growth, Ruminant, Urinary N loss

~~1.~~1 Introduction

A presence of weeds in a crop field has benefits such as lowering soil evaporation and erosion. However, weeds can negatively affect crop growth and yield due to competition for light, nutrients, and water (Zimdahl, 2004). In integrated crop-livestock farming, livestock serves a practical sustainable approach for controlling palatable weed species (MacLaren et al., 2020), which depends on the type of livestock and crops. Similar to the other tropical regions, the forage supply of small-scale farmers in the tropical humid regions is limited by resources of land, labors, and other inputs (Valbuena et al., 2012). Hence, ruminants were often supplied by the palatable weeds as the main resources of feed during a period of crop-growing season.

Asystasia gangetica (L.) T. Anderson is a perennial herb of the Acanthaceae family, which is widely distributed in tropical Africa and Asia (Kiew and Vollesen, 1997). The weed is commonly known as the Chinese violet (CV) and currently threat Northern and Eastern part of Australia (Westaway et al., 2016). The weed is fast-growing species, tolerant to low soil fertility and shade, and widely grown in the ground of tree plantation (Adjorlolo et al., 2014; Kiew and Vollesen, 1997). The weed has been fed to livestock by the small-scale farmers in the humid regions of Asia and Africa (Bindelle et al., 2007; Bussmann et al., 2020).

Few studies have been conducted to evaluate the effects of CV feeding on ruminant performance and nutrient utilization (Adjorlolo et al., 2014; Bindelle et al., 2007; Merkel et al., 1999). Comparing to legumes *Paraserianthes falcataria*, *Gliricidia sepium*, and *Calliandra calothyrsus*, CV had higher dry matter (DM), neutral detergent fiber (NDF), and crude protein (CP) digestibility while a higher N urine of CV was related to NH_3 excess in the rumen due to a higher nitrate (NO_3) concentration in the weed (Merkel et al., 1999). The presence of NO_3 could be a benefit for lowering methane (CH_4) production and increasing N supply for microbial protein synthesis in the rumen. Nitrate is a hydrogen sink and has been reported to reduce CH_4 production in beef cattle fed low-quality forage (Callaghan et al., 2014; Hulshof et al., 2012). However, during the reduction of NO_3 to ammonia (NH_3), nitrite (NO_2) is formed. Because the reduction of NO_3 to NO_2 is generally faster than the reduction of NO_2 to NH_3, NO_2 can be accumulated in the rumen, and an excess of NO_2 results in methemoglobinemia (Callaghan et al., 2014; Ku-Vera et al., 2020).

The higher digestibility indicates that the CV weed could be supplemented to a low-quality forage (Ali et al., 2019b; Aregheore, 2004). Furthermore, an energy source of the readily fermented carbohydrates from CC might improve the NO_3 and NH_3 utilization in the rumen (Hristov et al., 2005; Ku-Vera et al., 2020; Schwab et al., 2005) and so decrease urinary N loss when supplemented to a basal diet of the CV weed. Thus, an evaluation is needed to assess sustainable feeding strategies of the CV weed on the ruminant production of the smallholder farmers in the crop-livestock system. The objective of the study was to evaluate the effects of supplementing a low-quality forage with the CV weed and the basal CV weed diet with the CC supplementation on intake, nutrient utilization, and gain of Kacang goat.

~~2.~~2 Materials and methods

~~2.1.~~2.1 Animals and experimental design

The animals were cared for according to the Animal Welfare Guidelines of the Indonesian Institute of Sciences. Approval of the experiment was granted from the Faculty of Agriculture, Universitas Sriwijaya, Indonesia.

Twelve male local Kacang goats with live weight (LW) of 10 ± 1.4 kg and six to seven months old were used. The animals were housed in individual pens (1.5 m × 0.75 m) in an open-sided type of house. The animals were treated orally with Oxfendazole (Verm-O, 25 mg per 5 kg LW, Sanbe, Indonesia). The goats were gradually acclimated to the CV diet over 20 days to prevent nitrite toxicity. Before starting the experiment, the goats were stratified based on their LW and allocated to the four treatments. The experimental design was a crossover design which consisted of four diets tested in four periods (Appendix A). Each experimental period lasted for four weeks with three weeks of adaptation and followed by one week of sample collection where feed intake along with feces and urine excretion were measured.

~~2.2.~~2.2 Experimental feeds

The experimental diets used in the present study consisted of Guinea grass (*Panicum maximum*) *ad libitum* (GG), GG *ad libitum* and CV (1% live weight, LW) (GG + CV), CV *ad libitum* (CV), and CV *ad libitum* plus cassava chip (1% LW) (CV + CC) (DM basis). The composition of the GG, CV, and CC is shown in Table 1. The GG was obtained from existing pastures near the experimental unit. The grass was harvest at 55 to 60 days intervals (after the blooming stage) about 25 cm from the ground every afternoon. The CV weed was obtained from an abandoned rubber plantation in the Universitas Sriwijaya campus. The weed was harvest in the post-blooming stage, at 45 days intervals about 5 cm from the ground every afternoon. The forages were chopped into 5.0 to 10.0 cm particle size the next morning before offering to the animals. The unpeeled CC were made from cassava tubers by chopping the tubers to 0.5 to 1.0 cm pieces then sun-dried for 4 to 5 days depending on sunlight intensity. Cassava crop is a cash crop in sub-urban areas of Palembang city, the tubers for CC were unmarketable size tubers (2 to 3 cm in diameter) which are usually left on the field.

alt-text: Table 1

Table 1

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Chemical composition of Guinea grass, Chinese violet weed, and unpeeled Cassava chip (g/kg dry matter) used in the study.

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Organic matter	901 ± 3.2	860 ± 8.3	984 ± 0.8
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Neutral detergent fiber	755 ± 26.0	510 ± 23.8	276 ± 24.9
Acid detergent fiber	371 ± 29.3	338 ± 14.0	61 ± 8.4
Nitrate	0.3 ± 0.02	11.0 ± 0.98	n.a

FM: fresh matter, n.a: not available.

After removing and weighing the refusals from the previous day, the feeds were offered to the animals in separate buckets according to the experimental treatments, making it possible to give the supplements at the same time as the basal feeds. The forages were offered 3 times at 9:00, 13:00, and 17:00 h, while CC was offered at 9:00 h. The basal diet was offered *ad libitum* (allowing for 20% of refusal, fresh matter basis), while the CV and CC supplementations were offered at 1% of LW and were adjusted after each measurement of animal LW. Drinking water and salt-mineral lick were offered for *ad libitum* intake to all animals throughout the experiment.

~~2.3.~~2.3 Data collection and laboratory analyses

Throughout the whole experimental trial, a digital platform weighing scale was used to record the LW every Sunday and Thursday before offering the feeds. Duplicate subsamples of feed offered were collected each day during the collection week. The daily refusal feeds during the collection week were weighed, sampled (~200 g fresh matter, FM) and then stored at –20 °C. Then, the daily orts were pooled per animal and then sampled (~100 g FM) in duplicate. The samples offered and refused feed and daily fecal excretion were dried at 45 °C for 4 days and reweighed for determination of dry weight.

During seven consecutive days of the collection week, a urine feces separator was fitted below the floor of each individual pen for daily total urine and feces collection. The separator was equipped with a plastic mesh that directed the dropped feces to a 5-L bucket while urine passed through the mesh. Total daily urine excretion was collected using Q3 250 ~~ml~~/L sulfuric acid to preserve the N. For N analysis concentration, sample of urine (~200 mL) was taken daily after homogenizing and filtering with surgical gaze then kept at –20 ~~0~~°C.

The daily excretions were quantified in the morning during the collection week. Before sampling (~100 g FM), the individual fecal excretion was homogenized and dried at 45 °C for 4 days, and then reweighed for dry weight determination. After drying, the feed and fecal samples were ground to pass a 1-mm mesh. The samples were pooled per animal at the end of each period proportionally to the daily amount. The dried samples were stored in zipper polythene bags prior to analyses.

The samples of feed and feces were analyzed for concentrations of DM, ash, N (AOAC, 1990), NDF, and ADF (Ankom200 fiber analyzer, Ankom Technology cooperation, Fairport, USA) with alpha-amylase and including residual ash (Van Soest et al., 1991). The concentration of OM was calculated by subtracting the ash concentration by 100 while the content of CP was calculated as N × 6.25. The micro Kjeldahl method (AOAC, 1990) was used to determine the N concentrations of urine. The NO_3 was extracted by the method of Liu et al. (2016) and the NO_3 content was measured using a UV–Vis spectrophotometer (Shimadzu 1800). Supply of metabolizable energy (ME, MJ/kg DM) was estimated as 0.0157 × digestible OM (g/kg DM) (AFRC, 1993).

~~2.4.~~2.4 Statistical analysis

Analysis of variance was performed using the mixed procedure (*lme* function) after confirming the normality of residual data by the Kolmogorov–Smirnov test (R Core Team, 2018). A mixed model with diets and period as fixed effects and animal as a random effect was used. To compare differences among means the Tukey *post hoc* test was applied. The significance was determined at $P < 0.05$.

~~3.~~3 Results

~~3.1.~~3.1 ~~-~~Feed intake and apparent digestibility of nutrients

The offered CV weed had lower NDF and ADF concentrations and higher CP and NO_3 content than GG, whereas CC had the lowest NDF, ADF, and CP contents (Table 1). Intake of GG was higher for goats on the basal GG diet than goats who received supplements (GG + CV), whereas supplement intake was higher for goats offered GG + CV than those fed CV + CC ($P < 0.001$). The goats on CV and CV + CC had higher total DM and ME intake than those fed GG and GG + CV ($P < 0.001$). The goats on the CV diet had the highest CP intake ($P < 0.001$) followed by those observed on CV + CC, GG + CV, and GG diets. The highest ADF intake was observed in the CC diet and followed by CC + CV, GG + CC, and GG diets, while NDF intake was not significantly different among the dietary treatments. The DM digestibility in the goats fed CV diet was similar to the goats on GG + CV diet but higher than those fed GG diet, whereas the goats on CV + CC diet had the highest DM digestibility ($P < 0.001$). The goats fed CV + CC diet also had the highest OM digestibility ($P < 0.001$) with no difference among the other treatments. Digestibility of CP was the highest in the goats on the CV + CC diet ($P < 0.001$), which was similar to the value on the CV diet. The goats offered GG had the highest NDF digestibility ($P < 0.001$), while the ADF digestibility was not significantly different among diets. The goats on CV + CC diets had similar NDF digestibility with the goats on GG + CV and CV alone diet.

~~3.2.~~3.2 ~~-~~Nitrogen balance and growth performance

Nitrogen intake was the highest for the goats fed CV alone ($P < 0.001$) whereas the goats fed GG had the lowest N intake (Table 3). The N intake in the goats on GG + CV diet was similar to the goats on GG diet. Daily fecal N excretion was the highest for the goats on CV diet ($P < 0.001$), with no difference among the other treatments. Absorbed N and urinary N were also the highest for goats fed CV diet. The goats on CV + CC diet had similar absorbed N but lower urinary N than the goats fed CV diet solely. The goats on GG diet had the lowest N intake and total excreted N and the highest ratio of fecal and urinary N ($P < 0.001$). The goats on GG diet had the lowest N retention, which was not significantly different from the goats on GG + CV diet whereas the higher N retention was found in goats receiving CV and CV + CC diets ($P < 0.001$). Live weight gain and feed conversion efficiency were higher for the goats on diets of CV and CV + CC than those fed GG and GG + CV ($P < 0.001$).

Eight goats on the GG and GG + CV diets experienced losses of LW during the adaptation and collection weeks (Fig. 1). Regression analysis showed that LW gain significantly correlated ($P < 0.001$) with DM intake (R^2: 0.63) and ME intake (R^2: 0.54). The regression resulted in a daily ME requirement of 536 $KJ/kg^{0.75}$ LW for maintenance and 49.7 $KJ/kg^{0.75}$ LW for gain.

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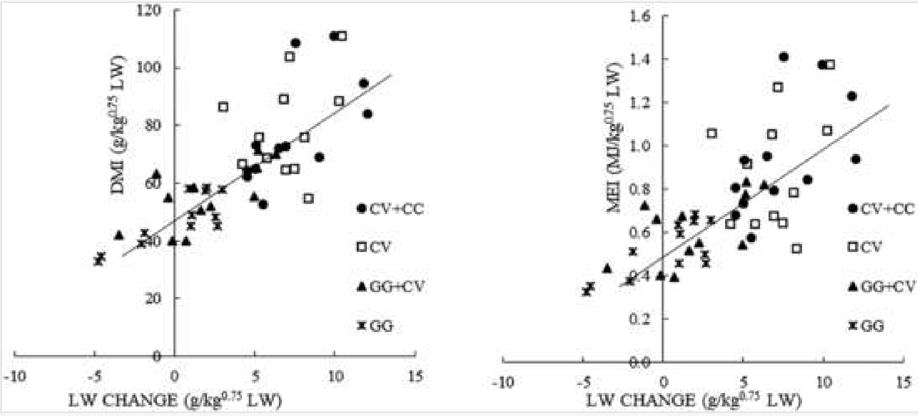
alt-text: Fig. 1

~~Fig. 1.~~Fig. 1



Relationship between dry matter intake (DMI, left) and metabolizable energy intake (MEI, right) on live weight (LW) change in Kacang goats fed Guinea grass *ad libitum* (GG), GG *ad libitum* and Chinese violet weed (1% live weight, LW) (GG + CV), CV *ad libitum*, and CV *ad libitum* and unpeeled cassava chips (1% LW) (CV + CC). DMI = 3.81 LW change + 49.23 (n: 48, R^2: 0.63, $P < 0.001$); MEI = 0.049 LW change + 0.536 (n: 48, R^2: 0.54, $P < 0.001$).

~~4.~~4 Discussion

~~4.1.~~4.1 Feed intake and apparent digestibility of nutrients

The NDF concentration of the CV weed in the present study was higher, while CP concentration was within the range of the previous studies (Adjorlolo et al., 2014; Bindelle et al., 2007; Merkel et al., 1999). The differences might depend on the plant age or the ratio of leaf and stem (Kumalasari et al., 2020). The NO_3 concentration in the present study was lower than the range of NO_3 concentrations in the CV weed (22~~-~~–98 g/kg) in Merkel et al. (1999) and have to offer less than 50% of a ration (DM basis) (Rasby et al., 2014). The high level of NO_3 in the weed would be expected to restrict the intake of the goats. However, a higher intake was found in the CV weed alone and CV + CC diets without the negative effect of the NO_2 toxicity. This could be related to the acclimation of the population of rumen microbes to the higher NO_3 in the weed (Latham et al., 2016; Zhou et al., 2011).

The higher CP and the lower NDF concentration in the CV weed supplemented diet could not improve the nutrients intake and digestibility as the intake of the basal GG had 118 g CP/kg DM. Ali et al. (2019b) reported similar basal and supplement intake for heifers fed mixed Rhodes grass hay and wheat straw (7.1% CP and 73% NDF) with sweet potato vine silage supplementation. Piñeiro-Vázquez et al. (2017) also found the similar evidence for heifers on *Pennisetum purpureum* (7.1% CP and 66% NDF) with *Leucaena leucocephala* supplementation. However, when the basal diet had a lower CP concentration, a higher DM intake and digestibility was reported in goats fed *Ischaemum aristatum* (6.8% CP and 40% NDF) with sweet potato vine supplementation (Aregheore, 2004) as well as in steer fed wheat straw (2% CP and 81% NDF) with *Calliandra calothyrsus* supplementation (Korir et al., 2016).

Though the DM digestibility was the lowest in the basal GG diet, the highest NDF digestibility on the basal GG diet might be attributed to the lowest DM intake of the diet. A lowered DM intake could be attributed to the escalated nutrient digestibility in the rumen as a result of a longer retention time in the rumen. A study of Ali et al. (2019a) on steers fed four levels of intake observed a higher NDF and ADF digestibility at a lower intake level. An increase of NDF digestibility, as well as an increase of ruminating time per DM intake when the level of intake was lowered, was also reported in the study of Schulze et al. (2014) on heifers fed four levels of grass-clover silage. The lower NDF digestibility when the goat consumed CV weed might be also related to the higher NO_3 concentration in the weed. The higher NO_3 concentration in the feed was reported to reduce the cellulolytic microbial population in the rumen (Latham et al., 2016; Zhou et al., 2011).

The higher DM and OM digestibility in goats fed the CV weed and the cassava supplementation could be explained by the increased supply of both N and the readily available carbohydrates for the rumen fermentation. An improvement of DM and OM digestibility along with a higher N retention and LW gain of goats fed a mix of Gamba grass (*Andropogon gayanus*) and Cassava hay by cassava supplementation was also reported by a previous study (Phengvichith and Ledin, 2007).

~~4.2.~~4.2 Nitrogen balance and growth performance

Comparing to the basal GG diet, N intake and absorption were higher in the goats fed CV weed (Table 3). Urinary N excretion was also elevated, which might reflect an excess of NH_3 production in the rumen. A higher concentration of NO_3 in the CV weed was also reported (Merkel et al., 1999), which could be degraded to the NH_3 in the rumen. The excess of NH_3 is absorbed in the bloodstream, converted to urea in the liver, and then excreted in the urine (Schwab et al., 2005).

The additional fermentable carbohydrate of CC would have utilized the excess of the rumen-degradable N of the CV weed by microbial uptake in the rumen. This is suggested by the similar value of the absorbed N but the lower N intake in the CV + CC diet (Table 3). Though the present work did not estimate microbial protein supply, the similar absorbed N and lowered urinary N excretion by the CC supplementation could be attributed to an improvement of the microbial protein supply to the host animal (Hristov et al., 2005; Ku-Vera et al., 2020; Schwab et al., 2005). However, in the present study, the improvement did not result in a significantly higher N retention and LW gain of the goats fed the cassava supplementation diet.

The present study demonstrated that feeding the CV weed either as a supplement to the basal diet of low-quality tropical grass or as a sole diet could result in a higher urinary N excretion which potentially increases N_2O emission in the livestock sector. The supplementation of the locally available non-structural carbohydrate, which is affordable for small-scale farmers, improved the nutrients utilization of the CV weed as well as a reduction of N_2O and CH_4 emissions.

Improved growth performance has been well known when ruminants were fed a degradable protein source in the form of forage legumes (Korir et al., 2016; Piñeiro-Vázquez et al., 2017) and leafy crop residue (Aregheore, 2004; Phengvichith and Ledin, 2007). In the present study, feeding of CV weed resulted in an improved gain of Kacang goats. The supplementation with the energy source of CC, however, could not result in a significantly higher LW gain which is related to the similar daily ME intake (Table 2) and digestible CP of the diets (Table 3). Moreover, the goats had the capacity to grow more than 48 g/day when they were fed CV + CC diets.

alt-text: Table 2

Table 2

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Intake and digestibility of nutrients in Kacang goats.

Parameter	GG	GG + CV	CV	CV + CC	SEM	*P*-value
Basal diet intake						
g/d	313.0^{b}	220.2^{a}	533.6^{c}	398.8^{b}	23.64	<0.001
% LW	2.5^{b}	1.8^{a}	4.2^{d}	3.2^{c}	0.16	<0.001
Supplement intake						
g/d	n.a	148.6^{b}	n.a	119.7^{a}	5.85	<0.001
% LW	n.a	1.2^{b}	n.a	1.0^{a}	0.03	<0.001
Nutrients intake (g/d)						
DM	313.0^{a}	368.8^{a}	533.6^{b}	518.6^{b}	22.89	<0.001
OM	282.0^{a}	326.7^{a}	459.6^{b}	460.0^{b}	20.04	<0.001
CP	37.4^{a}	50.4^{a}	87.6^{c}	70.7^{b}	3.97	<0.001
NDF	233.8	240.2	263.7	229.1	12.40	0.4483
ADF	113.2^{a}	128.0^{a}	173.9^{b}	137.6^{ab}	8.23	0.0022
ME (MJ/d)	3.4^{a}	4.1^{a}	6.0^{b}	6.3^{b}	0.31	<0.001

ME (MJ/kg$^{0.75}$ LW)	0.52^{a}	0.61^{a}	0.89^{b}	0.94^{b}	0.040	<0.001
Digestibility (g/kg)						
DM	675^{a}	694^{ab}	717^{b}	771^{c}	10.0	<0.001
OM	690^{a}	697^{a}	701^{a}	767^{b}	9.9	<0.001
CP	556^{a}	652^{b}	715^{c}	726^{c}	15.4	<0.001
NDF	643^{c}	615^{bc}	521^{a}	561^{ab}	19.5	<0.001
ADF	540	532	528	524	26.8	0.953

GG: Guinea grass *ad libitum*, CV: Chinese violet weed *ad libitum*, GG + CV: GG *ad libitum* and CV weed (1% live weight, LW), CV + CC: CV *ad libitum* and unpeeled cassava chips (1% LW).
n.a: not available, DM: dry matter, OM: organic matter, CP: crude protein, NDF: neutral detergent fiber, ADF: acid detergent fiber, ME: metabolizable energy was estimated as 0.0157 × digestible OM (g/kg DM) (AFRC, 1993).
Values in the same row with different letters represent significant differences ($P < 0.05$).

alt-text: Table 3

Table 3

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Nitrogen (N) balance, total gain, and feed conversion efficiency (FCE) in Kacang goats.

Parameter	**GG**	**GG + CV**	**CV**	**CV + CC**	**SEM**	***P*-value**
N balance (g/d)						
N intake	6.0^{a}	8.1^{a}	14.0^{c}	11.3^{b}	0.64	<0.001
Fecal N	2.5^{a}	2.7^{a}	3.9^{b}	3.0^{a}	0.14	<0.001
Absorbed N	3.5^{a}	5.3^{b}	10.1^{c}	8.3^{c}	0.54	<0.001
Urinary N	2.0^{a}	3.2^{b}	5.7^{c}	3.7^{b}	0.29	<0.001
Fecal N urinary N ratio	1.39^{b}	0.91^{a}	0.78^{a}	0.80^{a}	0.058	<0.001
N retention	1.5^{a}	2.1^{a}	4.4^{b}	4.6^{b}	0.35	<0.001
Total gain (g)	51.2^{a}	355.0^{a}	1227.5^{b}	1251.9^{b}	105.05	<0.001
FCE	−0.01^{a}	0.03^{a}	0.09^{b}	0.09^{b}	0.009	<0.001

GG: Guinea grass *ad libitum*, CV: Chinese violet weed *ad libitum*, GG + CV: GG *ad libitum* and CV weed (1% live weight, LW), CV + CC: CV *ad libitum* and unpeeled cassava chips (1% LW).
FCE: g LW gain/g dry matter intake.
Values in the same row with different letters represent significant differences ($P < 0.05$).

The averaged LW gain and FCE of the goats on the CV weed and cassava supplementation diets were in the range of gain and FCE (23.5 to 69.4 g/d and 0.04 to 0.11 for LW gain and FCE, respectively) for fifteen growing male Kacang goats fed three diets with three different CP and energy contents (Restitrisnani et al., 2013). The gain was also in the range of Adiwinarti et al. (2019) for growing male Kacang goats with soybean meal supplementations (41.5 to 79.9 g/d). The LW loss of the goats on the basal diet of GG and GG + CV diets reflected an insufficiency of energy intake for their maintenance requirement, which is estimated to be 536 KJ/kg$^{0.75}$LW. The value of ME requirement is lower than an averaged value for goat and sheep (542 KJ/kg$^{0.75}$LW) in the warm climate (Salah et al., 2014) but higher than a value obtained in the study of Luo et al. (2004) for growing indigenous goat (489 KJ/kg$^{0.75}$LW) while the value for gain is higher than values on their studies (24.3 and 19.8 KJ/kg$^{0.75}$LW).

5.5 Conclusions

The CV weed could be fed as a supplement to a poor-quality forage or fed solely without any negative effects. Feed intake, nutrient utilization, and gain can be improved when the weed is offered. The supplementation of cassava chips as a source of readily fermented carbohydrates to the weed has the potential to effectively improve nutrients utilization and benefits for lowering urinary N loss. A further study is needed to evaluate the effects of the weed on CH_4 production and rumen fermentation characteristic.

Declaration of competing interest

All the authors have no conflict of interest to declare.

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Appendix A

Allocation of the twelve Kacang goats (Group A (4, 8, 11), B (5, 10, 12), C (6, 7, 9) and D (1, 2, 3)) to Guinea grass (GG), GG and Chinese violet weed (GG + CV), CV, and CV plus unpeeled cassava chips (CV + CC) dietary treatments during 4 experimental periods.

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Period	Animal group			
	A	B	C	D
1	GG	GG + CV	CV	CV + CC
2	CV + CC	GG	GG + CV	CV
3	GG + CV	CV	CV + CC	GG
Q4 4	CV	CV + CC	GG	GG + CV

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The corrections made in this section will be reviewed and approved by a journal production editor. The newly added/removed references and its citations will be reordered and rearranged by the production team.

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