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AN *IN VITRO* EVALUATION OF THREE CLONES OF KATUK LEAF (*Sauropus Androgynus* L. Merr) AS PROTEIN SUPPLEMENTS FOR RUMINANTS

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ABSTRACT

An *in vitro* evaluation is conducted to study the potential use of three clones of katuk leaf (Bastar, Paris and Zanzibar) as protein supplements for ruminants. A factorial randomized completed block experiment (3x3) was carried out for studying degradability and fermentability of katuk leaf clones, and its effects on total bacterial and protozoal populations. Factor A was the three clones of katuk leaf (Bastar, Paris and Zanzibar), and factor B was the *in vitro* incubation period (1, 2 and 3 h); rumen fluid from three cattle was used as block. A randomized complete block design was also used in an *in vitro* digestibility experiment. Data was analysed with analysis of variance to evaluate treatment effects, and a contrast or polynomial orthogonal test was used to determine differences among treatments. The results indicate that katuk leaf clones affect ammonia concentration ($P<0.01$), protozoal population ($P<0.05$) and dry matter digestibility ($P<0.10$), but the effect is not significant on VFA concentration, total bacterial population and organic matter digestibility. VFA concentration ($P<0.01$) and protozoal population ($P<0.01$) are affected by the incubation period, but its effects are not significant on ammonia concentration and total bacterial population. Interaction between katuk leaf clones and incubation period does not produce significant effects on all variables measured. It is concluded that Bastar clone is the best clone of katuk leaf as a protein supplement for ruminants, and the highest ammonia and VFA concentrations producing the largest microbial populations is achieved at 3 h incubation period.

INTRODUCTION

Katuk (*Sauropus androgynus* L. Merr) is a shrub plant which belongs to family Euphorbiaceae (Becker *et al.*, 1963; Prajogo and Santa, 1997). This plant grows well in humic tropical area with heavy rains at the altitude of 3-1300 m above sea level (Sumantera, 1997). In Indonesia, katuk is commonly grown in West Java and other parts such as Madura, Bali, Sumatra and Kalimantan as it is known by its local name (Sudiarto *et al.*, 1997; Sastrapradja *et al.*, 1977; Sumantera, 1997; Setyowati, 1997).

Katuk leaf is used for human consumption as vegetable, traditional medicine, lactogogum – food that can stimulate milk production, and other purposes such as life fences (Sumantera, 1997). For these purposes, there are three clones of katuk leaf (Bastar, Paris and Zanzibar) that are usually planted, especially in Bogor area (Sudiarto *et al.*, 1997). Its use for stimulating milk production has also been studied in dairy goat

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(Suprayogi, 2000). Katuk leaf contains 33.68% crude protein, 52.11% carbohydrate and 7.89% crude fibre (Directorate of Nutrition, Ministry of Health of Republic Indonesia, 1979; Directorate of Nutrition, Ministry of Health of Republic Indonesia, 1992), 24 mg Ca, 83 mg P, 2.7 mg Fe, 3111 g vitamin D, 0.10 mg vitamin B6 and 200 mg vitamin C per 100 g fresh leaf (Oei, 1987; Padmavati and Rao, 1990). This nutrient composition indicates that katuk leaf can be used as a protein supplement. However, its nutrient composition may vary among plant varieties which can be affected by factors such as plant varieties, soil fertility and other factors (Prajogo and Santa, 1997). Its potential use as protein supplements for ruminants depends on its metabolism in the rumen, and this process may also differ among its clones. Therefore, an *in vitro* evaluation is conducted to study the potential use of three clones of katuk leaf as protein supplements for ruminants.

MATERIALS AND METHODS

Plant leaves

Katuk (*Sauropus androgynus* L. Merr) leaf was obtained from the area of Cinangneng – Ciampea, Bogor. There are clones of katuk leaf used in this experiment : Bastar, Paris and Zanzibar. Fresh leaves were harvested from each clones; the leaves were dried under the sun for 4-5 days which were then dried in the over at 60 °C for 24 h. Dried leaves from each clones were ground for further analysis. Each clone was also analysed its DM, ash and crude protein contents using proximate analysis.

in vitro fermentability experiment

The first step of digestion study of Tilley and Terry method (1963) was used in *in vitro* fermentability experiment. One gram of each dried sample was mixed with 12 mL of McDougall buffer solution and 8 mL of beef rumen fluid obtained from a slaughtered house. These mixtures were then incubated anaerobically in a shaker bath at 39 °C for 1, 2 and 3 h. At the end of each incubation period, samples were taken from each mixtures for counting bacterial and protozoal populations. Fermentations were stopped completely by adding 0.2 mL saturated $HgCl_2$ solution. The mixtures were centrifuged at 10,000 rpm for 10 min; the supernatants were collected for ammonia (NH_3) and volatile fatty acid (VFA) analysis, and the residues were discarded. Micro diffusion Conway was used for analyzing NH_3 concentration, and VFA concentration was determined using steam-distillation method (General Laboratory Method of Department of Dairy Science – University of Wisconsin, 1966 in Sutardi *et al.*, 1983).

Counting microbial populations

A serial dilution method described by Ogimoto and Imai (1981) was applied for counting total bacterial population. A dilution solution was used to dilute sample (0.5 mL), and the diluted sample was inoculated into sterile solid medium in a Hungate tube. The tubes were then incubated anaerobically at 39 °C. After 12-24 h incubation, colonies grown in the medium were counted, and bacterial populations were calculated on the basis of sample volume and dilution factor.

Each samples from incubation mixtures was mixed with MFS (methyl formal saline) solution with a ratio of 1:1. Using a Pasteur-pipette, the mixture sample was

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inserted into a counting chamber. Protozoal population was counted after placing the counting chamber under a microscope.

***in vitro* digestibility experiment**

Dry matter (DM) and organic matter (OM) digestibilities were determined following a two-stage digestion method of Tilley and Terry (1963) which was described in Sutardi *et al.* (1983). The first stage of digestion was conducted by following the same procedure of *in vitro* fermentability experiment as described above; however, the incubation period was conducted for 24 h. After stopping the fermentation by adding 0.2 mL saturated $HgCl_2$ solution and centrifuging the mixture at 10,000 rpm for 10 min, the residues were mixed with 20 mL of pepsin-HCl solution. The second stage of digestion was carried out by incubating the mixtures aerobically at 39 °C in a shaker water bath. After 24 h incubation, the mixtures were filtered using a vacuum pump, and dried in oven (105 °C) to determine DM content of residues. The ash content was analysed by placing the samples in a furnished oven (600 °C), OM content was calculated by subtracting the DM content with the as content.

Variables measured

The concentrations of NH_3 and VFA, and populations of total bacteria and protozoa were measured as variables for studying fermentation of katuk leaf in the rumen. Other variables were DM and OM digestibilities which were measured as digestibility coefficients of DM and OM.

Experimental design

A factorial 3x3 randomised block design was used to study effects of treatments on variables for studying fermentation of katuk leaf in the rumen. The first factor was katuk leaf clones (Bastar, Paris and Zanzibar), and the second factor was the incubation period (1, 2, and 3 h). A randomized block design was applied in studying DM and OM digestibilities. In both experiments, rumen fluids obtained from three different cattle were used as blocks. The data were examined with analysis of variance to determine effects of treatment on each variables, and differences between each treatment were determined with contrast and polynomial orthogonals (Steel and Torrie, 1980).

RESULTS AND DISCUSSION

Nutrient composition and clone characteristics

Table 1 indicates DM, ash and crude protein contents of each clone of katuk leaf after the leaf samples were dried at 60 °C. The data indicate that there are no very much different in DM, ash and crude protein contents among the clones. A slightly higher ash content is present in Zanzibar leaf indicating a slightly lower of OM content in this clone compared to that of Bastar and Paris leaves.

Table 1.Nutrient composition of Bastar, Paris and Zanzibar katuk leaves

Clones	Dry matter (%)	Ash content (% DM basis)	Crude protein content (% DM basis)
Bastar	19.67	7.98	30.05
Paris	18.29	7.63	29.36
Zanzibar	19.80	8.63	31.27

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Table 2. Katuk leaf characteristics

Part of leaf	Katuk clones		
	Bastar	Paris	Zanzibar
Colour	Green	Light green-green	Green-dark green
Form	Oval	Oval	Oval-long
Tip and bottom	Sharp	Sharp	Sharp
Top	White spot-scattered	None	White spot-centered
Length (cm)	3.4-7.5	2.9-5.4	3.9-7.5
Width (cm)	2.2-3.2	2.0-2.3	2.3-2.5
Leaf number per stalk	6-13	7-15	9-15
Stalk number	6-16	7-15	8-18
Stalk number per branch	0-4	0-5	0-5
Branch number per plant	1-6	1-4	1-6

Source : Sudiarto *et al.* (1998)

These two clones contain slightly lower crude protein contents than Zanzibar clone. Differences in nutrient contents among katuk leaf clones could be due to differences in plant varieties, planting/growing plant method, soil fertility in a location where the plants are grown, and age when the plants are harvested (Prajogo and Santa, 1997).

However, data in crude protein content indicate that all the three clones can be used as protein supplements.

Each clone has specific characteristics, and their leaf characteristics (colour, form, presence of white spots, size and number of leaf per plants) can be used to differentiate the three clones of katuk leaf (Table 2).

Leaf of all clones has green colour, but the degree of this colour varied among the clones. Zanzibar clones has darker green colour than the others, and this may indicate high concentrations of chlorophylls. High concentrations of chlorophylls may associate with high concentration of protein as chlorophylls in katuk leaf was found in a complexed with protein (Yuliani *et al.*, 1997). This possibility is also supported by crude protein content of Zanzibar clone (Table 3). High concentrations of chlorophylls and proteins may also demonstrate the presence of tannin which is supported by strong astringent taste of Zanzibar leaf. Tannin is able to bind protein forming a complex between protein-tannin which is unavailable for digestion. Katuk leaf has been identified to contain organic acids, volatile fatty acids, saponin, flavonoid, alkaloids and tannins (Agusta *et al.*, 1997; Malik, 1997; Suprayogi, 2000). The presence of scattered white spots on the top of Bastar leaf can differentiate this clone from Zanzibar clone. These two clones have comparable leaf size; Paris clone can be identified by its small size of leaf. Based on the total leaf numbers per plant, it can be estimated that the smallest, intermediate, and highest leaf yield can be obtained, respectively, from Paris, Bastar and Zanzibar.

Katuk leaf fermentability and digestibility

Table 3 demonstrates the results of fermentability study of katuk leaf clones, the effect of incubation period is shown in Figure 1.

The effect of katuk leaf clones is highly significant on ammonia concentration ($P<0.01$) with Zanzibar leaf produced the smallest concentration of ammonia. There is no significantly different in ammonia concentration between Bastar clone and Paris

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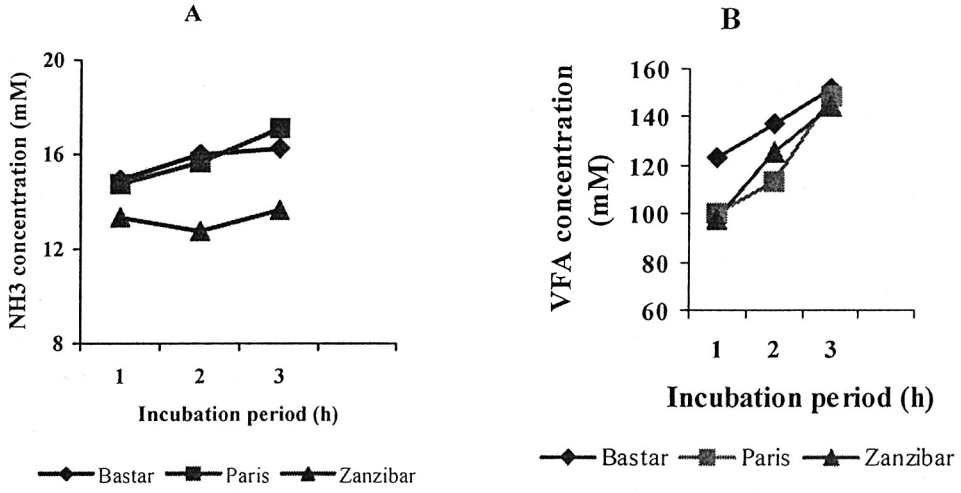
clone. This result indicates that protein of Zanzibar leaf is less degraded than the other clones; this may be due to the content of tannin in Zanzibar leaf which may be higher than in the other clones. Katuk leaf contains tannin (Suprayogi, 2000); however, tannin concentration may vary among plant varieties/accessions as this has been observed in *Acacia* spp., *Leucaena* spp., *Calliandra* spp., and *Lotus* spp. (Norton, 2000; Wina *et al.*, 2000; McNabb *et al.*, 2000). The effect of incubation period and interaction between katuk leaf clones and incubation period are not significant on ammonia concentration (Figure 1A). Ammonia concentration in all clones increase with a longer period of incubation; the highest ammonia concentration is reached at 3 h incubation.

VFA concentration is not affected by katuk leaf clones and by interaction between katuk leaf clones and incubation period. Incubation period produced effect on VFA concentration ($P<0.01$) with a linier effect is obtained ($P<0.01$); the result can be seen in Figure 1B. An increase in VFA concentration is obtained as incubation period is extended. This result also demonstrates that OM of all clones are readily fermentable which could be due to its low content of crude fibre (Arora, 1989; Directorate of Nutrition, Ministry of Health of Republic Indonesia, 1992). The regression equation for Bastar clone : $Y=14.925X + 108.72$ ($R^2=0.9985$), Paris clone : $Y=24.495X + 71.693$ ($R^2=0.9381$), and Zanzibar clone : $Y=23.0925X + 76.503$ ($R^2=0.9877$).

Table 3. Concentrations of NH_3 and VFA, total bacterial and protozoal populations of three clones of katuk leaf

Variables	Clones		
	Bastar	Paris	Zanzibar
NH_3 concentration (mM)[1]	15.73 ± 0.99^{A}	15.84 ± 0.86^{A}	13.25 ± 1.33^{B}
VFA concentration (mM)	137.31 ± 9.06	120.68 ± 8.21	122.69 ± 9.39
Total bacterial population (log CFU/mL)	9.25 ± 0.48	9.99 ± 0.39	9.59 ± 0.42
Protozoal population (log CFU/mL)[1,2]	4.56 ± 0.05^{A}	4.39 ± 0.05^{Bb}	4.48 ± 0.05^{Ba}

1) Means within row with capital letter were highly significantly different ($P<0.01$)
2) Means within row with small letter were significantly different ($P<0.05$)



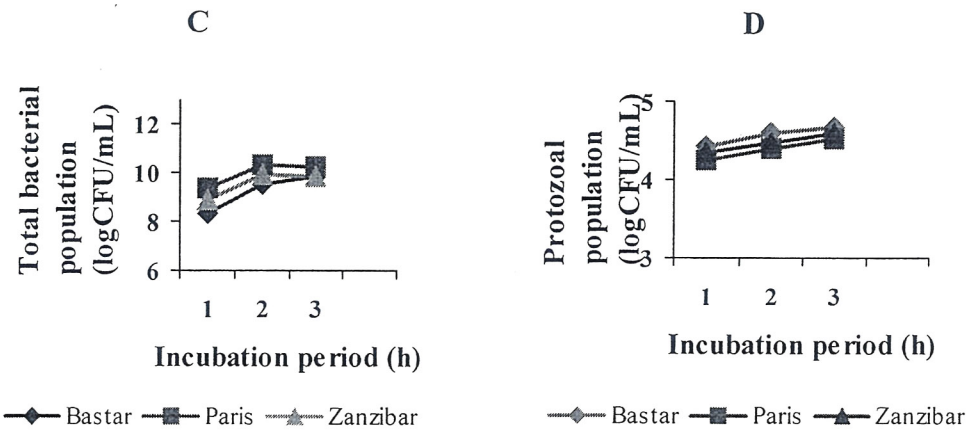


Figure 1. Effect of incubation period on ammonia concentration (A), VFA concentration (B), total bacterial population (C) and protozoal population (D)

Table 4. Digestibility coefficients of DM and OM of katuk leaf clones

Variables	Katuk leaf clones		
	Bastar	Paris	Zanzibar
DM digestibility coefficient (%)	76.35 ± 1.64^{A}	72.79 ± 0.57^{A}	68.99 ± 1.71^{B}
OM digestibility coefficient (%)	76.06 ± 1.71	71.63 ± 1.04	69.35 ± 3.31

1) Means within row with capital letter were highly significantly different (P<0.01)
2) Means within row with small letter were significantly different (P<0.05)

Effects of katuk clones (Table 3), incubation period and its interaction (Figure 1C) do not cause any significant effects on total bacterial population. On the other hand, protozoal population is affected by differences in katuk clones (P<0.05), and by incubation period (P<0.05); however, there is no effect of interaction between the two factors on protozoal population. Although the effect of katuk clones on protozoal populations is statistically significant, the result may not produce significant effect biologically. The effect of incubation period indicates that protozoal numbers increase with an increase in incubation period (Figure 1D). The highest protozoal population occurred at 3 h incubation.

Differences in katuk leaf clones produced significant effects on digestibility coefficients of DM (Table 4). DM digestibility coefficient (%) of Bastar and Paris leaves are greater than that of Zanzibar leaf. This indicates that DM of Bastar and Paris leaves are more digested and its nutrients can be more useful for animals (Preston and Leng, 1987). This is also supported by data in fermentability study that proteins and carbohydrates of those clones are more degradable and fermentable (Table 3; Figure 1). A low digestibility of Zanzibar leaf could be due to its low OM content and its low protein degradability which could be related to high concentration of tannin content (Table 3). The effect of katuk leaf clones on OM digestibility coefficient are similar to that of DM digestibility coefficient, but the effect of katuk leaf clones is not significant on OM digestibility.

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CONCLUSION

The best clone of katuk leaf as a protein supplement for ruminants is Bastar clone, and the highest ammonia and VFA concentrations producing the largest microbial populations is achieved at 3 h incubation period.

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