The effect of *Ketepeng Cina* leaf (*Cassia alata L*), as a source of anthraquinone, methanogenesis inhibitor agent on rumen microbial protein synthesis for beef cattle in Sedyo Rukun Farmer Group

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ABSTRACT: *Ketepeng Cina* leaf meal (***Cassia alata* L)**, a source of anthraquinone has been tested in the previous experiment, as an agent with ability to reduce methane production in cellulolytic fermentation by rumen microbes. The present experiments were conducted in the farm belong to the farmer group of Sedyo Rukun, Berbah, Sleman,Yogyakarta. Ten male Ongole crossed cattle were used in this experiment to evaluate the effect of *Ketepeng Cina* leaf on the rumen microbial protein synthesis. The experiment contained 4 feeding periods. In the first period, cattle were fed as usual, without *Ketepeng Cina* leaf addition as a control treatment. In the second period, cattle were fed with addition of *Ketepeng Cina* leaf equal to 5 ppm antraquinone, based on rumen volume during 10 days for feeding trial adaptation and continued for 7 days feeding trial. The experiment was continued by Period III and Period IV which began two weeks and four weeks after the end of period when the animals were treated by *Ketepeng Cina* leaf addition. In the third and fourth period, the animals received ordinary diet as in the first period. In each period, urine was collected by spot sampling methods for 7 days. Urine samples were analysed for purine derivatives content including allantoin and uric acid. Samples of feedstuff, refusal feed and feces were collected daily, for dry matter and organic matter analysis. The result showed that microbial protein synthesis, which was estimated based on the purine derivatives excretion tended to increase as an effect of *Ketepeng Cina* leaf (from 18,24 to 21,93 g/day). After 4 weeks of *Ketepeng Cina* leaf addition, microbial protein synthesis return to the initial condition. It could be concluded that *Ketepeng Cina* leaf which has a high potency for methanogenesis inhibitor, were recommended as a feed additive to increase feed efficiency.

Key words: purin derivatives, *cassia alata* l. leaf, anthraquinone, methan**.**

INTRODUCTION

About 70% of methane production arises from anthropogenic sources, and two third of those sources was considered come from agricultural sources including flooded rice paddies, enteric fermentation and animal waste (Moss *et al.,* 2000). The stability of global methane concentration at current levels would require reductions in methane emission or the increase of sink for methane at the same level. Livestock is one of the large sources of methane emission with 80-115 tons produced per year. The global cattle population is responsible for 73% methane emission of all livestock, and methane produced during rumen fermentation represents a loss of 2-15% of gross energy intake and may contribute to global warming (Johnson and Johnson, 1995). Many researches had been carried out to find ways to decrease methane production especially from ruminants.

The compound with the ability to capture the electron such as unsaturated fatty acid was tested to reduce methane production (Minami et al., 1992). Coconut oil have been used to decrease methane production (Machmuller *et al.,* 2000; Machmuller *et al.,* 2003). Sardine fish oil which contains unsaturated fatty acids was proved for its ability in decreasing methane production on *in vitro* rumen fermentation (Yusiati *et al*., 2008). The addition of 9,10 anthraquinone, a known inhibitor of methanogenesis had no effect on blood metabolite, apparent digestion of nutrients in the total gastrointestinal tract (Kung et al., 2003). *Cassia alata L* leaf which contains anthraquinone (Syamsuhidayat dan Hutapea, 1991), has been tested its ability in decreasing methane production as well as its effect on rumen fermentation pattern (Yusiati *et al*, 2006). It was reported that the addition of *Ketepeng Cina* leaf in the *in vitro* fermentation reduced methane production significantly, as much as 47% of methane production. It was stated that the use of *Ketepeng Cina* leaf as a source of anthraquinone did not has any negative effect on rumen microbes metabolism. Cellulolytic enzyme activity, the concentration of acetic acid and butyric acid, as well as microbial protein synthesis did not change while propionic acid concentration increased (Yusiati *et al.,* 2006).

Although *Ketepeng Cina* leaf was proved as an excellent source of methane inhibitor agent during *in vitro* rumen fermentation, it was still needed *in vivo* trial to examine its potencies. Since *Ketepeng Cina* is easily cultivated, its application in the cattle diet should be considered. One of the indicators that application of *Ketepeng Cina* leaf in the ruminants diet does not give any negative effect on the rumen fermentation, was by measuring of rumen microbes biomass synthesized, which was supplied to the host animal.

The estimation of microbial protein supply based on the purine derivatives (PD) including allantoin, uric acid, xanthine and hypoxanthine excretion in the urine of ruminants was considered to be an interesting method. The method is simple and non invasive, therefore the use of urinary PD excretion as an indicator of rumen microbial protein synthesis has been extensively applied to ruminant nutrition research such as studies dealing with the effect of defaunation on animal performance (Eugène *et al*. ,2004), with rumen methanogenesis (Hess, *et al*., 2004) and with the effect of tannin on rumen fermentation (Koenig, *et al*. 2000; Yáñez Ruiz *et al*., 2004 and Abia1 *et al*., 2006). Yusiati (2005) applied that method for Ongole Crossed cattle, Friesian Holstein Crossed cattle, Bali Cattle and buffalo. The model to estimate rumen microbial protein synthesis has been postulated for those Indonesian ruminants. The model based on her finding that there was correlation between microbial purine absorbed (MPA=X) and purine derivatives excreted in the urine (Y). The prediction model for Ongole Crossed cattle was showed by equation $Y= 132\ W^{0,75} + 0,85\ X$ and Estimated Microbial Nitrogen Supply (g/day) = (MPA x 70)/ (0,83 x 0,200 x1000).

Although the estimation of rumen microbial protein supply became popular among nutritionists, it is still difficult to be applied in the field condition, as it needs 24 hours urine collection for at least 6 days. Chen *et al*. (1995) suggest collection of spot urine sample to overcome that problem. Since the pattern of urine output might be influenced by climatic factors (Nsahlai *et al.,* 2000), the prediction of PD excretion based on the PD:C in the spot urine samples was validated (Yusiati, 2005). Spot urine sample could be used to assess the total PD excretion, especially for the morning samples. Purine derivatives excretion (Y) could be estimated by PD:C in the spot urine samples (X) by these equation $Y = -5.48 + 1.46\ X$ ($r^2= 0.99$, n=6, sampling time 4.00-7.00 Am.) for Bali cattle, and $Y =17.64 + 0.87\ X$ ($r^2= 0.61$, n=6, sampling time 04.00-07.00 h) for Ongole cattle.

MATERIALS AND METHODS

Ketepeng Cina (Cassia alata L) Leaf Preparation

Ketepeng Cina leaf as a source of anthraquinone a known inhibitor of methanogenesis were collected from the plantation growing in the Gadjah Mada University campus. The leaves were sun dried, ground them by hand into meal and used as feed *additive*.

Biological Treatment

This experiment was conducted in the farmers group namely Sedyo Rukun, Berbah, Sleman, Yogyakarta. Ten male Ongole Crossed cattle were selected from the farmers who kept cattle, which were receiving similar feeding and management conditions. All animals received regular diet, namely farm diet which were served at the first period and treatment diet were given at the second period. Each period consisted of 18 days adaptation and three days collection. At the beginning and the end of each period, the animals were weighed (before adaptation and after collection period). The diets were served twice a day, at 8.00 h and 15.00 h. Feedstuff samples and refusal feed samples were taken every day and were bulked weekly for each animal.

Spot samples of the urine were taken on the last three days of periods. Samples were before feeding time in the morning at 4.00-7.00. About 20 ml of urine were collected and added by amounts of 10% H_2SO4 to adjust the pH become less than 3. The urine samples were stored in the freezer (-20 C), before PD (allantoin and uric acid) and creatinine analysis.

Samples Analysis

Feed and faeces samples were analyzed for dry matter (DM) and organic matter (OM) according to standard procedure (AOAC). Urine samples were analyzed for allantoin and uric acid according to procedure described in the laboratory manual (*Chen et al.,* 1997), while urinary creatinine analysis was carried out according to procedure described by Hawk *et al.* (1980).

Data Analysis

The ratio of purine derivatives (allantoin and uric acid) and creatinine concentration in the *spot sampling* urine, were used as an index of total PD excretion in the urine to assess the prediction of rumen microbial protein synthesis. The data obtained were analyzed by analysis of variance using completely randomized design 4X2 factorial. The differences between the mean values were tested by Duncant's New Multiple Range Test (DMRT).

RESULTS AND DISCUSSION

The concentration of purine derivatives (allantoin and uric acid) and creatinine in the *spot sampling* urine is shown in Table 1.

Allantoin content in the urine of Ongole Crossed cattle was significantly affected by addition of *Ketepeng Cina* leaf. *Ketepeng Cina* leaf addition tended to increase allantoin and purine derivatives concentration in the urine about 14% and 14%. Two and four weeks after received diet with *Ketepeng Cina* leaf addition, purine derivatives concentration in the urine decreased significantly, but the concentration were not lower compared to before addition of *Ketepeng Cina* leaf. The effect of *Ketepeng Cina* leaf addition on urinary uric acid concentration was nearly the same as allantoin. In this experiment, xanthine and hypoxanthine were not measured because their concentration in urine cattle are too low due to the high activities of xanthine oxidase in the intestinal mucous and liver of the cattle (Chen, 1990).

Table 1. The concentration of purine derivatives in the urine of Ongole Crossed cattle fed by *Ketepeng Cina* leaf containing diet

	Ketepeng Cina Leaf addition			
Concentration (mmol/l)	I	II	III	IV
Allantoin	6.78^{ab}	7.73^{a}	5.71^{bc}	4.69^{d}
Uric acid	0.27^{ab}	0.31^{ab}	0.35^{a}	0.25^{b}
Purine Derivatives	7.05^{ab}	8.04^{a}	6.06^{bc}	4.95^{d}
Creatinine ^{ns}	8.45	9.44	7.49	7.15

^{a,b,c,d} Different superscript in the same row showed different effect of treatment (P< 0.05)
^{ns}Not significant.

Allantoin and uric acid concentration in all of the cattle urine were higher compared to allantoin (3.86 mmol/l) and uric acid (0.25 mmol/l) concentration in the urine of Ongole Crossed cattle reported by Yusiati *et al. (*1999), due to the differences of diet. Creatinine concentration were not affected by *Ketepeng Cina* leaf addition, and the values were also higher compared to creatinine concentration (5,01 mmol/l) in the urine of Ongole Crossed cattle reported by Yusiati *et al.* (1999).

The ratio of purine derivatives : creatinine (PD:C index) were used to asses PD excretion in the cattle urine (Yusiati, 2005). The result of calculation is presented in Table 2. Purine derivatives excretion in the urine of Ongole Crossed cattle was significantly affected by addition of *Ketepeng Cina* leaf. The addition tended to increase purine derivatives excretion in the urine about 16%. Two and four weeks after received diet with *Ketepeng Cina* leaf addition, purine derivatives excretion in the urine decreased significantly, but the concentration were not lower compared to before addition of *Ketepeng Cina* leaf. Purine derivatives excretion in the urine of all the cattle were also higher compared to PD excretion of Ongole Crossed cattle (554 μmol/$W^{0.75}$/day) reported by Yusiati *et al.* (1999).

Table 2. Purine derivatives excretion in the urine, digested dry matter intake and digested organic matter intake of Ongole Crossed cattle fed by *Ketepeng Cina* leaf containing diet (mmol/day)

	Ketepeng Cina Leaf addition			
	I	II	III	IV
Purine derivatives (μmol/$W^{0.75}$/day)	714.38[ab]	827.40 [a]	692.71 [ab]	562.66 [b]
Absorbsi purin (μmol/$W^{0.75}$/day)	43.25 [ab]	51.99 [a]	43.67 [ab]	32.47 [b]
Estimated N-Protein Supply (g/d)	18.24 [ab]	21.93 [a]	18.41 [ab]	13.69 [b]
Digested dry matter intake (kg/h/d)[ns]	1.84	1.79	2.80	2.61
Digested organic matter intake (kg/h/d) [ns]	1.69	1.74	2.70	2.18

[a,b]Different superscript in the same row showed different effect of treatment ($P<0.05$)
[ns]Not significant.

Based on the data of PD excretion in the urine, purine absorbed and rumen microbial protein supply could be predicted (Yusiati *et al*., 1999). The result showed that the effect of *Ketepeng Cina* leaf addition on purine absorbed and rumen microbial protein supply were similar to their effect on PD concentration in the urine. Nsahlai *et al.* (2000) reported microbial N-supply for cattle fed by straw was 18.14 while for cattle fed by Napier grass was 19.58 g/day. The result in this experiment showed that estimated N-protein supply ranged from 13.69 to 21.93 g/day. Spot urine sampling which was used in urine collection, appeared to produce satisfactory estimated of PD excretion, therefore the method seems to be potentially used under field condition to asses the extent of microbial protein supply.

Ketepeng Cina leaf as a source of antraquinone in *in vitro* rumen closed fermentation did not increased microbial protein synthesis (Yusiati *et al.,* 2003). This recent experiment proved that the addition of *Ketepeng Cina* leaf in the diet of Ongole Crossed cattle tended to increase rumen microbial protein synthesis or supply, although digested organic matter intake did not change. Rumen continues fermentation which was carried out in *in vivo* trial seems to give the microbes a chance to grow better without any effect of feedback inhibition during their metabolism.

CONCLUSIONS

It could be concluded that *Ketepeng Cina* leaf which has a high potency for methanogenesis inhibitor, were recommended as a feed additive to increase rumen microbial protein supply.

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