

## Substitution of Concentrate with Mulberry Leaves in Ongole Grade Cattle Fed Rice Straw Based Diet

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### ABSTRAK

Penelitian dilakukan untuk mengkaji substitusi konsentrat dengan tepung daun murbei (*Morus spp.*) yang dikombinasikan dengan jerami padi sebagai pakan sapi potong. Penelitian menggunakan 12 ekor sapi peranakan ongole (PO) jantan dengan bobot badan  $217 \pm 10,53$  kg. Ransum terdiri atas jerami padi, tepung daun murbei dan konsentrat. Penelitian menggunakan rancangan acak lengkap dengan 3 perlakuan dan 4 kali ulangan, dengan susunan perlakuan sebagai berikut: P1= 50% jerami padi + 50% konsentrat; P2= 50% jerami padi + 25% konsentrat + 25% murbei; P3= 50% jerami + 50% murbei. Hasil penelitian menunjukkan bahwa fermentasi pakan dalam rumen sapi yang mendapat ransum P2 lebih efektif dibandingkan dengan P1 dan P3. Hal tersebut ditandai dengan konsentrasi VFA total dan ekskresi allantoin yang lebih tinggi pada P2. Kehadiran senyawa 1-deoxynojirimycin (DNJ) daun murbei dalam sistem rumen pada perlakuan P2 dan P3 meningkatkan daya cerna fraksi serat ransum berbasis jerami padi. Daun murbei dalam ransum juga menyumbang *isoacids* dalam sistem rumen, yang mendukung perkembangan bakteri selulolitik. Disimpulkan bahwa daun murbei berpotensi digunakan untuk mensubstitusi konsentrat pakan yang digunakan bersama dengan sumber pakan serat seperti jerami padi.

*Kata kunci: murbei, konsentrat, jerami padi, peranakan ongole, rumen, pencernaan*

### ABSTRACT

An experiment was conducted to assess the substitution of concentrate with mulberry (*Morus spp.*) leaves combined with rice straw offered to beef cattle. The study used 12 male Ongole grade cattle weighing  $217 \pm 10.53$  kg. Rations consisted of rice straw, mulberry leaves and concentrate. Research was carried out according to completely randomized design with 3 treatments and 4 replications for each treatment. The treatments were: P1= 50% rice straw + 50% concentrate; P2= 50% rice straw + 25% concentrate + 25% mulberry; P3= 50% rice straw + 50% mulberry. The results of the study indicated that rumen fermentation in cattle fed on P2 ration was more effective than those receiving P1 and P3 rations. The fermentation was characterized by a higher production of total VFA and allantoin excretion in the urine. The presence of 1-deoxynojirimycin (DNJ) compound of mulberry leaves in the rumen system in treatment P2 and P3 improved digestibility of fiber fractions of rice straw-based rations. Mulberry leaves in rations also accounted for isoacids in the rumen system, which supported the development of cellulolytic bacteria. In conclusion, mulberry leaves containing concentrate had a potency to substitute concentrate when used together with fiber source feed such as rice straw.

*Key words: mulberry, concentrates, rice straw, Ongole cattle, rumen, digestibility*

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## INTRODUCTION

Mulberry (*Morus spp.*) plants have potential as good quality feed ingredient due to its potential production, nutrient content, and good adaptability of growth. Doran *et al.* (2007) reported that biomass production of mulberry could reach 25 tons DM/ha/year. That potential yield is higher than that of *Gliricidia sepium* with is 7-9 tons DM/ha/year.

High nutrient content and leaves production make the mulberry leaves are possible to be utilized as a feed ingredient to replace concentrate especially for ruminants (Doran *et al.*, 2007). In Indonesia, mulberry leaves are used only as feed for silkworms. There is no information available yet on its use as feed for ruminants.

One of the active compounds contained in the mulberry leaves is 1-deoxynojirimycin (DNJ) (Oku *et al.*, 2006; Kimura *et al.*, 2004.). DNJ compounds have the potential to inhibit the ( $\alpha$ ,  $\beta$ ) glucosidase specifically (Chapel *et al.*, 2006). Furthermore, the DNJ in a form of D-glucose could inhibit intestinal  $\alpha$ -glucosidase and pancreatic  $\alpha$ -glucosidase, and therefore DNJ compounds inhibit the formation of oligosaccharides (Romaniouk *et al.*, 2004; Yatsunami *et al.*, 2003).

These compounds could be expected to inhibit the hydrolysis of non-structural carbohydrates in the rumen system. The existence of mulberry leaves containing the active compound in the ration are expected to provide a balance and sustained non-structural carbohydrates in the rumen system resulting in a better fermentability of high fiber diet.

In addition to continuous provision of readily available carbohydrates (RAC), slow-release mechanism of the RAC in the rumen system will also improve the energy efficiency of feed. The site change of non-structural carbohydrate digestion in the ruminant's digestive tract could affect the energy efficiency of the diet. Feed energy efficiency in ruminants increases when non-structural carbohydrates are digested in the small intestine.

Carbohydrates are hydrolyzed in the rumen in different patterns. Addition of mulberry leaves extract containing 1-deoxynojirimycin compound improves the fermentability of RAC in rumen system (Syahrir *et al.*, 2009a). An *in vitro* study on the substitution of concentrates by mulberry leaves in a rice straw based ration indicated that the optimum level of concentrates substitution by mulberry leaves was 50% (Syahrir *et al.*, 2009b). The aim of this study was to assess the potential use of mulberry leaves to substitute concentrates when used together with rice straw in Ongole grade cattle.

## MATERIALS AND METHODS

An *in vivo* experiment was conducted using 12 Ongole grade cattle weighing  $217 \pm 10.53$  kg. Ration offered to the cattle consisted of rice straw, mulberry leaf meal and concentrate. Concentrate composed of yellow corn, soybean meal, coconut meal, pollard, cassava waste (onggok), molasses, Ca (urea)  $4Cl_2$ , sodium salt and dicalcium phosphate (DCP). Composition of each dietary treatment is shown in Table 1. Rice straw used in

the experiment was prepared by drying and chopping into 3-5 cm long.

Research was done according to a completely randomized design with 3 treatments and 4 replications. The dietary treatments were: P1= 50% rice straw + 50% concentrates; P2= 50% rice straw + 25% concentrates + 25% mulberry; and P3= 50% straw + 50% mulberry.

The experimental animals were maintained in an individual stall. Feed as much as 2.5%-3.0% of body weight was offered to each animal at 6:00 a.m. to 07:00 and 16:00 to 17:00 pm. The animals had free access to drinking water.

Experimental animals were maintained for 10 weeks (2 weeks for adaptation period, 8 weeks for observation). Total collection period was carried out in the middle of the observation period (week 5) for one week. Variables measured were rumen physiological conditions, urine allantoin concentration, digestibility of fiber fractions and quality of feed protein. The rumen physiological conditions variables measured were pH, ammonia analyzed based on *phenol hypochlorite assay*, total and partial VFA were analyzed using *Gas Chromatography* (Cruwys *et al.*, 2002). Nitrogen retention, net protein utilization (NPU) and biological value (BV) of ration were determined (Puastuti, 2005). Urine and rumen fluid samples were collected at the end of the experimental period. The data were analyzed by analysis of variance using a completely randomized design (CRD) according procedure of Steel & Torrie (2003).

Table 1. Feed components and nutrient composition of ration offered to Ongole Grade cattle (%)

	Treatments		
	P1	P2	P3
Feed ingredients:			
Rice straw	50.00	50.00	50.00
Corn	10.72	5.35	0.00
Soybean meal	8.93	4.47	0.00
Coconut meal	7.50	3.75	0.00
Pollard	15.10	7.55	0.00
Onggok	3.00	1.50	0.00
Molasses	3.50	1.75	0.00
Mulberry leaf meal	0.00	25.00	50.00
Ca(Urea) $_4Cl_2$	1.00	0.50	0.00
DCP	0.25	0.125	0.00
Nutrients content:			
Crude protein	13.70	13.70	13.70
Crude fat	3.35	2.73	2.11
Crude fiber	15.48	16.95	18.41
TDN	60.60	59.40	58.20

Note: DCP= Dicalcium Phosphate; TDN= Total Digestible Nutrient. P1= 50% rice straw + 50% concentrate; P2= 50% rice straw + 25% concentrate + 25% mulberry leaf meal; P3= 50% rice straw + 50% mulberry leaf meal.

## RESULTS AND DISCUSSION

### Rumen Physiological Conditions, Urine Allantoin, and Fiber Digestibility

Data on rumen physiological conditions and urine allantoin concentration are presented in Table 2. The dynamics of the pH described the hydrolysis rate of feed. A faster hydrolysis rate of non-structural carbohydrate produced higher total acid, and the decreased ruminal pH. The pH of cattle rumen fluid was 6.66-7.18, which were in the range of optimal pH for rumen microbial activity.

Cattle receiving different rations indicated the different ruminal fluid acidity ( $P < 0.05$ ). Acidity of rumen of cattle fed 50% concentrate (P1) was significantly lower compared to that fed mulberry replacing partly of the concentrates (P2) or all of the concentrate (P3). The results of this study illustrated that the hydrolysis rate of the non-structural carbohydrates in the concentrate was more rapidly in P1 compared to the rations containing mulberry (P2 and P3).

Reduction of hydrolysis rate of non-structural carbohydrate in P2 and P3 was likely related to the DNJ contained in the mulberry leaves. The process created a more stable rumen system, resulting in the stability of the microbial population producing the fiber-digesting enzymes. Overall effect of the reduction rate of non-structural carbohydrate hydrolysis increased the digestibility of rations containing rice straw.

Rumen ammonia concentration depends upon the level of feed protein degradation and rumen microbial growth conditions. Ammonia concentration increased in the period of 2-4 h post feeding (Nolan & Dobos, 2005). Cattle receiving different treatments did not indicate differences in the ammonia concentration of rumen

fluid. The mean of ammonia concentration in rumen fluid was high (19.22-19.66 mM). High concentration of ammonia is due to the high protein content of experimental rations. Concentrate and mulberry leaves used in this study contained crude protein of 20.8%. There was no difference in ammonia concentration among the treatments. The results indicated that the use of ammonia in the rumen was relatively similar, although the type of bacteria that used ammonia might differ among treatments.

The concentration of VFA in the fermentation medium indicates the level of effectiveness of the fermentation process. In general, the higher concentration of VFA, the more effective the fermentation process. Data of VFA concentration in the rumen fluid are presented in Table 2.

Cattle rumen system given mulberry diet replacing part of concentrate (P2) produced the highest VFA concentration in their rumen fluid, compared to that of treatments P1 and P3. High VFA production reflected the high overall degradation of carbohydrate in P2 ration. High degradation of dietary components might take place when nutrients as precursors for fermentation are sufficient and continuously available in the rumen system. P2 ration allowed non-structural carbohydrates continuously available in sufficient quantity, since DNJ compounds inhibited the hydrolysis of non-structural carbohydrates. The inhibition of non-structural carbohydrate hydrolysis resulted in the continuous supply of RAC in the rumen system and increased the degradation rate of feed.

The use of rice straw up to 50% in the ration had no significant effects on the proportion of C2:C3:C4 due to the high availability of fiber in all treatments. The ratio of acetate: propionate: butyrate in the rumen fluid of cattle that have high fiber feed sources was 70:20:10, compared to 50:40:10 when the cattle received high concentrate diet (Nagaraja *et al.*, 1997). The highest acetate proportion was observed in cattle fed P3, namely 79% of total VFA (Table 2). High proportion of acetic acid reflected the dominance of high fiber-digesting microbes in the rumen of cattle receiving P3 ration. Fiber-digesting bacteria species grow in the rumen of cattle given high fiber ration and produced high acetic acid content of rumen liquor. The use of mulberry leaves in the rations (P2 and P3) increased the concentration of isoacids metabolites (IC4 + IC5) in the rumen by 31%, from 2:25-2.95 mM. Isoacids are essential metabolites as source of branched chain fatty acids needed for the development of cellulolytic bacteria. Increased isoacids metabolites in the rumen of cattle received P2 and P3 rations indicated the dominance of cellulolytic bacteria in the rumen fluid which was associated with the high acetate content in P3 compared with P1. The presence of isovaleric and isobutyric acids in the rumen could improve the fiber digestibility of the diet, which was represented by an increased in the population of cellulolytic microbial groups.

The development of rumen bacteria is reflected in the excretion of allantoin in the urine. The excretion of allantoin in the urine is an indicator of the microbial protein supply from rumen. In this study, treatment P2 yielded the highest excretion of allantoin in the urine.

Table 2. Characteristics of rumen liquor and urinary allantoin excretion of Ongole Grade cattle offered rations composed of concentrate with or without mulberry leaves meal and rice straw

Variables	Treatments		
	P1	P2	P3
Rumen pH	6.66±0.35 <sup>b</sup>	7.18±0.19 <sup>a</sup>	7.02±0.14 <sup>ab</sup>
NH <sub>3</sub> -N (mM)	19.22±1.08	19.66±0.30	19.37±2.06
Total VFA (mM)	75.33±7.54 <sup>b</sup>	108.75±20.32 <sup>a</sup>	89.13±3.12 <sup>ab</sup>
Acetic acid (mM)	55.27±6.17 <sup>b</sup>	81.69±15.44 <sup>a</sup>	69.86±3.50 <sup>ab</sup>
Propionic acid (mM)	11.30±1.11 <sup>b</sup>	15.54±3.13 <sup>a</sup>	11.80±0.74 <sup>b</sup>
n-Butyric acid (mM)	6.19±0.82 <sup>b</sup>	8.35±1.11 <sup>a</sup>	4.49±0.68 <sup>c</sup>
i-Butyric acid (mM)	1.29±0.58	1.57±0.36	1.85±0.29
i-Valeric acid (mM)	0.95±0.17	1.38±0.25	1.12±0.39
C2:C3:C4	75:15:10	76:15:9	79:13:7
iC4 + iC5 (mM)	2.245	2.950	2.973
Urine allantoin (g/d)	1.73±0.08 <sup>b</sup>	2.23±0.18 <sup>a</sup>	1.29±0.14 <sup>c</sup>

Note: Mean in the same row with different superscript differ significantly ( $P < 0.05$ ). P1= 50% rice straw + 50% concentrate; P2= 50% rice straw + 25% concentrate + 25% mulberry leaves meal; P3= 50% rice straw + 50% mulberry leaves meal.

High growth of rumen bacteria in the treatment P2 would have an impact on the ability of cattle to digest feed better. Indication of a better level of digestibility in treatment P2 was also supported by high total VFA production.

Treatment P1 supports the development of rumen microbes which was good for the fermentation indicated by the higher level of allantoin excreted in the urine compared to that of cattle in treatment P3. However, fiber degradation rate of P1 was lower compared to that of treatments P2 and P3. There was lower rumen pH and total VFA concentration in P1 than those of P2 and P3 rations. Proportion of concentrate in P1 ration supported the better development of amylolytic bacteria which was then producing more lactic acid. Bacteria producing fibers digestive enzymes such as *Butyrivibrio spp* and *Bacteroides rimunicola* are more dominant in the rumen of animal receiving high fiber diet while *Bacteroides amylophilus* dominant in those having high concentrate diet. Contrary to P1, P3, the low concentration of allantoin excreted in the urine indicating the low development of rumen bacteria which was in turn had an impact on low degradation rate of ration compared to that of treatment P1 and P2. However, fiber digestibility in cattle offered P3 was better which was marked by higher acetic acid proportion and higher ruminal pH, total VFA production as well as greater isoacids production than those of P1.

Coefficient digestibility for fiber fractions in cattle given P3 treatment was higher than that for P1 and P2 treatments (Table 3). Mulberry leaves in the P2 and P3 rations contributed to the achievement of stable conditions of rumen supporting the development of the rumen cellulolytic bacteria, which was characterized by a higher digestibility of fiber components, especially ADF and cellulose, than that of the ration without mulberry leaf (P1). The high digestibility of fiber fraction of ration P3 was also characterized by a higher proportion of acetate compared to that of P1 and P2 and also higher value in pH, total VFA and isoacids than that of P1 treatment (Table 2).

### Nitrogen Utilization

The higher the nitrogen retention value and the less nitrogen excreted into the urine, the better quality of protein ration, because most of the undigested protein ration can be deposited into the body. NPU value as the ratio of nitrogen retention to nitrogen consumed and the value of BV as the ratio of nitrogen retention and nitrogen absorbed also will be higher if the quality of protein of feed is better.

Nitrogen retention value, NPU and BV of experimental cattle are presented in Table 4. Diatrayment of P3 produced higher ( $P<0.05$ ) nitrogen retention value, NPU and BV compared to those of P1 and P2 treatments. High quality protein of dietary treatment P3 was likely due to the complete amino acid composition in mulberry leaves.

About 95% of crude protein in mulberry leaves is true protein providing all types of amino acids (Machii *et al.*, 2002). Although cellulolytic microbes are capable of

Table 3. Digestibility rate of fiber components in Ongole Grade cattle offered rations composed of concentrate with or without mulberry leaves meal and rice straw (%)

Variables	Treatments		
	P1	P2	P3
NDF	57.73±0.91	58.91± 4.83	61.10± 9.61
ADF	48.27±2.70 <sup>a</sup>	50.06± 5.72 <sup>ab</sup>	60.04± 9.89 <sup>b</sup>
Cellulose	58.14±2.30 <sup>a</sup>	68.23± 3.55 <sup>b</sup>	72.94± 6.69 <sup>b</sup>
Hemicellulose	72.49±2.42	65.45±12.27	66.25±11.47

Note: Mean in the same row with different superscript differ significantly ( $P<0.05$ ). P1= 50% rice straw + 50% concentrate; P2= 50% rice straw + 25% concentrate + 25% mulberry leaves meal; P3= 50% rice straw + 50% mulberry leaves meal.

Table 4. Nitrogen retention, net protein utility (NPU) and biological value (bv) in Ongole Grade cattle offered rations composed of concentrate with or without mulberry leaves meal and rice straw

Variables	Treatments		
	P1	P2	P3
Nitrogen retention (g/d)	40.27±3.40 <sup>b</sup>	46.01±5.44 <sup>a</sup>	47.36± 6.53 <sup>a</sup>
NPU (%)	28.39±0.85 <sup>b</sup>	29.93±3.47 <sup>b</sup>	35.22± 4.17 <sup>a</sup>
BV (%)	54.41±4.69 <sup>b</sup>	64.84±6.42 <sup>b</sup>	83.90±10.60 <sup>a</sup>

Note: Mean in the same row with different superscript differ significantly ( $P<0.05$ ). P1= 50% rice straw + 50% concentrate; P2= 50% rice straw + 25% concentrate + 25% mulberry leaves meal; P3= 50% rice straw + 50% mulberry leaves meal.

using ammonia from amino acids as the main nitrogen source, the true protein in the ration is always superior to urea in improving digestibility of fiber fractions of the diet. The results of this study revealed the potential of mulberry leaves to be used to improve the digestibility of fiber feed sources such as rice straw.

Fermentation of feed in the rumen of cattle receiving ration P2 was more effective than that in cattle receiving P1 and P3 rations. The effective ruminal fermentation was characterized by a higher total VFA concentration and urinary allantoin excretion. The presence of DNJ compounds in the rumen of cattle in the treatments P2 and P3 improved the digestibility of fiber fractions of rice straw-based rations. Mulberry leaf in the rations also accounted isoacids in the rumen system, which supported the development of ruminal cellulolytic bacteria.

### CONCLUSION

Mulberry leaves had potency to substitute concentrate for feeding of beef cattle. Substitution of concentrates by mulberry leaves increases digestibility of fiber fractions of ration containing rice straw as a fiber source resulting in a better nutritive value for rice straw. Substitution of 50% concentrate by mulberry leaves in the diet composed of rice straw as a fiber source might increase the productivity of ruminant.

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