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308 Lasani Town, Sargodha Road, Faisalabad - Pakistan
Mob: +92 300 3008585, Fax: +92 41 8815544
E-mail: editorpjn@gmail.com

Supplementation of Fermented Palm Press Fibre on Digestibility of Rice Straw and Rumen Bacteria Profile

A. Fariani¹, A. Abrar¹, G. Muslim¹ and Lili Wary²

¹Department of Animal Science, Faculty of Agriculture, Sriwijaya University, South Sumatera, Indonesia

²Faculty of Animal Science, Andalas University, West Sumatera, Indonesia

Abstract: The objectives of the present study were to evaluate the potential of fermented palm press fibre (FPPF) as supplement feed to increase *in vitro* rice straw digestibility. Complete randomized design experiment was used to determine digestibility (*in vitro*) of rice straw supplemented with palm pressed fiber. Three treatments were composed as control (P0), rice straw supplemented with 5% FPPF (P1) and rice straw supplemented with 10% FPPF (P2) with 6 replication for each treatment. Observed parameters were digestibility of dry matter (%), organic matter (%), crude protein (%), crude fiber (%) ammonia-N (mM) and Total VFA (mM) concentration. Rumen bacteria population post 24 h incubation were also determined. The result showed that digestibility of dry matter and crude protein were not altered by treatment ($p>0.05$) while organic matter and crude fiber were significantly ($p<0.05$) affected by PPF supplementation. Rumen bacteria population post incubation were not significantly ($p>0.05$) altered by PPF supplementation but the number tends to increase.

Key words: *In vitro*, palm press fiber, rumen bacteria

INTRODUCTION

Rice straw has been widely used as animal feed especially ruminants because it has a carbohydrate rich material which can be utilized as a potential source of dietary energy for the ruminants. However rice straw characterized by poor palatability, low digestibility and protein. Some studies have shown that high silica and lignin contents of the rice straw impede digestion by rumen microbes. Silica reduces palatability and the degradability of rice straw in the rumen due to its direct action in preventing colonization by ruminal microorganisms (Agbagla-Dohnani *et al.*, 2003; Van Soest, 2006).

Several possible strategies to improve utilization of rice straw by ruminants were physical treatment, chemical treatment and biological method. Chemical treatments to improve the utilization of rice straw may be alkaline, acidic or oxidative agents. The most commonly used alkaline agents are sodium hydroxide (NaOH), ammonia (NH₃) and urea (Elseed *et al.*, 2003; Yulistiani *et al.*, 2003; Vadiveloo, 2000) or combine a physical and chemical method that has been done by Zia-ur-Rehman *et al.* (2000) on rice straw that treated with alkalies and pressure steam. Biological methods use fungi and their enzyme that metabolizes lignocelluloses is a potential biological treatment to improve the nutritional value of straw by selective delignification, as mentioned in the review by Jalç (2002).

Fermented palm pressed fiber (FPPF) is a product from the fermentation process on Palm pressed fiber by

using white rot fungi (*Phanaerochaeta chrysosporium*) as inoculant. As fresh fermentation product PPF was subjected to carry over metabolites exerted from white rot fungi, while also has contain single cell protein substance, therefore in the present study, FPPF were evaluate *in vitro* to enhance digestibility of rice straw and its effect on rumen bacteria profile post 24 h incubation.

MATERIALS AND METHODS

Palm press fibre fermentation: Palm press fibre (500 g) was fine grinding then soaked in mineral solution (0.6 g MgSO₄; 0.5 g KCl; 5 g NH₄NO₃; 0.001 g CuSO₄; 0.01 g FeSO₄ and distilled water in to 1000 mL solution) for 3 h, then air-dried for 6 h before inoculated with white rot fungi solution. White rot fungi (*Phanaerochaeta chrysosporium*) solution was prepared from our collection and re-suspension (1% v/v) in nutrient broth medium which later incubated for 24 h in room temperature. This suspension then sprayed evenly in prepared palm press fiber then incubated in room temperature for 7 days. After 7 days all palm press fiber was covered by white rot fungi then samples were taken for proximate analysis and fiber fraction determination using Van Soests method as described elsewhere by Santoso *et al.* (2009). Data on proximate analysis of control PPF and fermented PPD are shown in Table 1.

***In vitro* digestibility assay:** *In vitro* digestibility analysis as describe by Tilley and Terry (1963) were performed in the present study. Rumen fluid from cattle was diluted

Table 1: Proximate analysis of control and fermented palm press fiber (PPF)

Items (%)	Control PPF	Fermented PPF
Dry matter	89.90±1.20	86.20±3.10
Crude protein	5.20±1.10	9.80±1.70
Crude fiber	28.30±2.60	20.70±2.50
Ether extract	1.20±0.20	1.10±0.10
Ash	9.10±2.10	10.50±3.10
Neutral detergent fiber	43.40±2.70	42.30±2.10
Acid detergent fiber	36.3±3.70	33.40±1.70
Lignin	9.20±1.90	7.10±2.10
Cellulose	22.50±2.70	19.10±1.80
Hemicellulose	7.10±1.30	8.90±2.20

Values are mean±SD deviation, n = 5

with McDougal buffer (1:2) and dispensed into 1 g substrate-prepared incubation tube while also purged by CO₂ to maintain anaerobic condition. Incubation was applied in 39°C water bath for 24 h. Substrate composition for present experiment and its chemical composition are shown in Table 2. After 24 h incubation, incubation tubes were mix vigorously and 1 mL liquid were collected for determination of rumen bacterial population quantification. Determination of proteolytic, amylolytic and cellulolytic bacterial populations were used the roll tube techniques of Hungate based on colony forming unit. Media for each population were described as in Ogimoto and Imai (1980). Subsequently, 2 drops of HgCl₂ was added to each incubation tubes then the tubes were centrifuged at 4000 rpm for 10 min. Supernatants were collected for NH₃-N and Total Volatile Acid (Total VFA) assay. NH₃-N concentration was determined by micro diffusion Conway method and TVFA concentration was measured with steam distillation method. The residue was added with 20 mL pepsin HCl 0.2%, mixed and incubated with previous condition for another 24 h. The samples of previous incubation were vacuum-filtered with Whatman no.41 and dried at 60°C in oven. The dried samples were used for *in vitro* digestibility assay of dry matter, organic matter, crude fiber and crude protein.

Experimental design and statistical analysis: The study of rice straw digestibility supplemented with fermented palm press fiber were assigned to completely randomized designed experiment with three treatment as follows; control, 5% FPPF and 10% FPPF on dry matter basis substrate. All treatment has 6 replications and data were analyzed using one-way ANOVA and the mean values were tested using Duncan Multi Range Test (Steel and Torrie, 1960)

RESULTS AND DISCUSSION

***In vitro* digestibility:** Digestibility of supplemented rice straw with FPPF is shown in Table 3. *In vitro* dry matter digestibility (IVDMD) and crude protein digestibility results was not differ significantly ($p>0.05$), however organic matter digestibility (IVOMD) of PPF supplementation showed significant result ($p<0.05$) than

Table 2: Substrate composition and its chemical analysis

Chemical composition	Control	5% FPPF	10% FPPF
Dry matter	89.8±1.2	91.3±2.1	90.2±2.5
Organic matter	84.2±2.7	85.1±2.5	85.5±2.9
Crude protein	5.2±1.1	6.8±1.2	7.2±1.3
Crude fiber	28.3±2.6	29.7±1.8	29.5±1.6
Extract ether	1.2±0.2	1.5±0.1	1.4±0.2
Treatments	Rice straw (g)	Fermented PPF (g)	
Control	1	-	
5% FPPF	0.95	0.05	
10% FPPF	0.90	0.10	

Values are mean±SD deviation, n = 3

control. Rice straw were known has low digestibility due to high content of silica (Van Soest, 2006) and high fiber (Toharmat *et al.*, 2006) therefore it was required to supplemented with N source to improve its digestibility. The treatment of the present study was using fermented palm press fiber on substrate which seems contained higher crude protein than control (Table 1).

Supplementation of FPPF on the present study was aimed not only as diet substrate but also provide N source from fermentation substances. Sharma and Arora (2013) reported that white rot fungi enhance digestibility of lignocellulosic residues including rice straw. Reynal and Broderick (2005) reported that low concentration of protein required by rumen microbes may alter microbial growth, production of microbial protein and rumen digestion. The protein content of substrate is very important and limiting factor for microorganism growth. Blummel *et al.* (2003) and Parissi *et al.* (2005) found positive correlation between crude protein, metabolic energy and organic matter digestibility. However, due to result of IVDMD in the present study protein content of treated substrate was not enough to improve substrate dry matter digestibility. Protein in the rumen will be utilized by rumen microbes to increase its population which later on impacted on substrate digestibility. Regarding to the rumen microbes population data on Table 4, it seems that FPPF treatments level was not enough to improve the digestibility.

Crude protein digestibility were not altered by FPPF supplementation ($p>0.05$) but crude fiber digestibility showed significant result ($p<0.05$). The FPPF was treated with *Phanaerochaeta chrysosporium* which known has the ability to degraded fiber and increase crude protein content (Syahrir *et al.*, 2013; Islamiyati *et al.*, 2013). Fresh PPF may carry over metabolites or substance which contained protein, thus the organic substance of FPPF may improve the digestibility of crude fiber.

NH₃-N and total vfa concentration: Data on ammonia-N and Total VFA of the present study are shown in Table 3, FPPF supplementation were not significantly ($p>0.05$) alter ammonia-N (NH₃-N) concentration of rice straw and Total VFA. NH₃-N concentration values depends on

Table 3: Digestibility of rice straw supplemented with PFPF

Measured parameters	Treatments			Sig.
	Control	5% FPPF	10% FPPF	
Dry MD (%)	66.21±3.22	65.03±1.1	64.2±3.6	ns
Organic MD (%)	43.8±2.2 ^a	54.8±8.1 ^b	54.9±2.7 ^b	*
Crude PD (%)	6.2±0.2	6.7±1.1	7.1±0.7	ns
Crude FD (%)	11.2±1.4 ^a	14.6±1.7 ^a	17.8±2.1 ^b	*
Ammonia-N (mM)	0.8±0.3	1.1±0.1	0.8±0.1	ns
Total VFA (mM)	43.2±3.1 ^a	44.6±2.1 ^a	49.1±2.1 ^a	*

*Values are mean±standard deviation, n = 6

Means on the same row with different superscript differ significantly (p<0.05)

MD: Matter digestibility, PD: Protein digestibility, FD: Fiber digestibility, Sig: Significance

Table 4: Rumen bacteria population of rice straw supplemented with palm press fibre prebiotic

Bacteria population (cfu/mL)	Treatments			Sig.
	P0	P1	P2	
cellulolytic, cellx10 ⁷	3.30±0.4 ^a	5.15±1.7 ^a	5.85±1.9 ^a	ns
Amylolytic, cellx10 ⁶	7.35±1.1 ^a	11.25±2.4 ^a	10.30±3.2 ^a	ns
Proteolytic, cellx10 ⁶	5.75±2.3 ^a	14.45±3.6 ^a	12.55±3.2 ^a	ns

Values are mean±standard deviation, n = 6. Sig: Significance means within same row with different superscript differ significantly (p<0.05)

protein intake. Higher protein intake will stimulant the higher microbial activities then causing higher of ammonia N concentration. Low rumen NH₃-N could be a limiting factor on rumen fermentation thus concentration of ammonia-N in the present study might regarding to such situation.

Total VFA concentration in the present study was altered by FPPF supplementation (p<0.05). Nevertheless, total VFA production for each treatment was considerably low in extent to improve rumen ecosystem. Waldron *et al.* (2002) reported that total VFA concentration on rumen was 60-120 mM while in the present study was 43-49 mM. Total VFA was the end product of rumen metabolism therefore higher level of FPPF was needed to increase the total VFA concentration. Since FPPF was considered feedstuff itself, then addition of higher level FPPF was considerably applicable.

Microbial (cellulolytic, amylolytic and proteolytic) Populations:

Ruminal bacteria are classified as cellulolytic and amylolytic based on their preferential use of energy. Microbial (cellulolytic, amylolytic and proteolytic) population on post incubation of rice straw supplemented with FPPF were not significantly different (p>0.05). Nevertheless, number of each population increased along with treatment. This may indicate that population of rumen microbial may altered by FPPF treatment, since total VFA of FPPFP treatment considerably higher than control. Total VFA was source of carbon for growth of rumen bacteria but these conditions require optimum NH₃-N concentration.

Conclusion: The fermented palm press fibre was potential feedstuff and it supplementation showed

potential result not only as feedstuff but also enhancing agent of rice straw digestibility. Nevertheless, higher level of ratio on diet should be increased to obtain higher impact on rice straw digestibility.

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