PJN
ISSN 1680-5194

PAKISTAN JOURNAL OF NUTRITION

ANSInet
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan
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Pakistan Journal of Nutrition 15 (1): 58-65, 2016
ISSN 1680-5194
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Use of Lignin Formacell of Empty Bunch Palm Fiber as Feed Supplement and Prebiotics Candidate in Ruminant

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Abstract: A series of experiment had been done to evaluate the use of purified lignin formacell (PLF) and its derivative (MIL and MSL) as feed supplement and prebiotics candidate for ruminant. PLF was isolated from empty bunch palm fiber. First experiment was microbial test using PLF, MIL and MSL as substitute of glucose in medium. Six treatments were applied and growth of *Lactobacillus casei*, *Lactobacillus bulgaricus* and *Streptococcus thermophillus* were measured. Second experiment was also microbial test using PLF, MIL and MSL as substitute of yeast extract in medium. Six treatments were applied and growth of *Escherichia coli*, *Salmonella typhimurium* and *Staphylococcus aureus* were measured. Third experiment was *in vitro* test using fresh rumen liquid to study the following treatments: R_0 = basal diet; R_1 = R_0 + 1.25% of Inulin; R_2 = R_0 + 1.25% of PLF; R_3 = R_0 + 1.25% of MIL and R_4 = R_0 + 1.25% of MSL. Microbial test showed that PLF, MIL, or MSL could substitute the glucose in the medium as carbon sources for the growth of lactic acid bacteria; whereas PLF, MIL, or MSL could not be used as carbon sources in the medium for growth of the pathogenic bacteria. *In vitro* rumen fermentation revealed that the addition of PLF, MIL, or MSL has positive effects on rumen metabolism parameters. Higher ammonia was achieved by MIL and MSL; higher rate of microbial protein synthesis was achieved by PLF and MIL and higher crude fiber digestibility was achieved by MIL and MSL.

Key words: Lignin formacell, empty bunch palm fiber, prebiotic, *in vitro*, ruminant

INTRODUCTION

Improving animal production should be followed by improving the animal welfare and food safety of the animal products (Croney and Millman, 2007; Carenzi and Verga, 2009; Frank, 2011). In recent years, there has been a growing attention on the relevance of gastrointestinal microbes in animal in improving feed digestion, nutrients utilization, pathogen exclusion and immunity system of the host animals (Hill *et al*., 2011). Therefore, as critical organ system in mediating nutrients uptake and use as well as modulating immune system of the host animal, understanding factors that improve the performance of gastrointestinal microbes is critical to improve animal production and welfare.

Prebiotics are among material explored to be used to improve the performance of gastrointestinal microbes (Rai *et al*., 2013; Flickinger and Fahey, 2002). Related to that valuable benefit, considerable progress has been made in increasing the animal production using prebiotics as an alternative of using unsafe antibiotics product. The use of antibiotic to increase feed efficiency in animal production could lead to the emergence of resistant microorganisms and creation of antibiotic-resistant disease in animal (Houser *et al*., 2008).

Currently prebiotics are defined as selectively fermented ingredients that allow specific changes, both in the composition and activity, in the gastrointestinal microbes that confer benefits to the host animals (Gibson and Roberfroid, 1995; Roberfroid, 2007). Prebiotics promotes domination of beneficial bacteria and inhibits undesirable bacteria leading to improved feed utilization and animal productivity. Extensive studies were then focused on exploring the benefit of using prebiotics for modulating the gut ecosystem of ruminants. Prebiotics (Inulin) could reduce the rumen ammonia nitrogen, methane production, increase microbial protein synthesis and live weight gain in calves (Samanta *et al*., 2013). Cota and Whitefield (1998) and Samanta *et al*. (2012) also reported that a number of rumen bacteria, especially hemicellulolytic bacteria, could ferment prebiotics and then used it as source of energy. In most studies, lactobacilli and bifidobacteria are the common microbial target genera in prebiotics application. However, their responses to each prebiotic ingredient were unique, so that there is a need to explore the potential prebiotic ingredient for specific purposes, including in ruminant nutrition.

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Purified lignin was demonstrated to have prebiotic effects on ruminant nutrition (Baurhoo *et al*., 2008). *In vivo* experiment showed that the use of alcell lignin 12.5 g/kg of dry matter could improve the daily weight gain of beef cattle (Phillip *et al*., 2000). However, the mechanism of the process not yet explored intensively, especially its effects on rumen microbes and VFA profile of the rumen fluids. The hypotheses was that oligomer content of the lignin may provide beneficial effect to lactobacilli and bifidobacteria as well as decrease the pathogenic bacteria in gastrointestinal tract of ruminants. These actions most probably will alter and improve the rumen fermentation system. Since ruminant rely on thousands of bacteria in their digestive tract to break down their feed, a healthy rumen will lead to healthier animals and maximized ruminant production.

Oil palm (*Elaeis guineensis*) industry is one of the biggest agro-industries in South East Asia, therefore empty bunch palm fiber (EBPF) is one of the most abundant agro-waste fiber resources. EBPF could be used as a potential feed for ruminant, especially in crop livestock system of beef cattle palm oil integration. In this research purified lignin formacell (PLF) from empty bunch palm fiber (EBPF) and its derivative were studied to characterize its compound and also to explore the possibility of using it as a feed supplement and prebiotics candidate for ruminants.

MATERIALS AND METHODS

Preparation of purified lignin formacell and its oligomer: Empty bunch palm fiber (EBPF) was collected from local palm oil industry in Rejosari, Lampung Province, Indonesia. Lignin Formacell (LF) was isolated from ground EBPF (30-40 mesh) using pulping formacell method (Lehnen *et al*., 2005) and LF then further purified (Lin, 1992) to obtain Purified Lignin Formacell (PLF). PLF then extracted using methanol to obtain methanol soluble lignin and methanol insoluble lignin fraction. Fraction of methanol soluble lignin was fractionated from methanol extracted PLF using vacuum liquid chromatography (Sticher, 2008) with eluent 30% methanol: chloroform. Fractions of purified lignin that used in these experiments are fraction of methanol insoluble lignin (MIL) and fraction of methanol soluble lignin (MSL). To describe its chemical compounds, small part the PLF, MIL and MSL were further analyzed using GC-MS (Shimadzu GCMS-QP2010, MS detector, capillary column type Phase Rtx-5MS 60 m x 0.25 mm ID, column temperature 50°C and Helium as gas carrier).

Experiment 1: Lactic acid bacterial growth on PLF, MIL and MSL: This microbial test was designed to evaluate the possibility of using PLF, MIL and MSL as substitute of glucose in Man Rogosa Sharp (MRS) medium to culture the lactic acid bacteria as target microbes. Six treatments of culture medium with 3 replications each were applied in a completely randomized design and growth of *Lactobacillus casei*, *Lactobacillus bulgaricus and Streptococcus thermophillus* supplied from Food Microbiology Laboratory were counted. The treatments were culture medium of Normal MRS; Minimal MRS; Inulin MRS; PLF MRS; MIL MRS and MSL MRS (Table 1). Inulin (Sigma Product catalog # I 2255) was used as control positive, isolated from chicory root. The treatments were designed to make sure that the bacteria used lignin as a solely carbon source for growth. Colony of lactic acid bacteria carefully inoculated to medium and then incubated at 37°C for 24 h. The number of colony then measured using total plate count (TPC) at 0 and 24 h and the data were then expressed as increasing percentage of microorganism number.

Experiment 2: Pathogenic growth on PLF, MIL and MSL: This microbial test was designed to evaluate the possibility of using PLF, MIL and MSL as substitute of yeast extract in Luria Brooth (LB) medium to culture the pathogenic bacteria as non-target microbes. Six treatments of culture medium with 3 replications each were applied in a completely randomized design and growth of *Escherichia coli*, *S. typhimurium* and *S. aureus* supplied from Food Microbiology Laboratory were measured. The treatments were culture medium of Normal MRS; Minimal MRS; Inulin MRS; PLF MRS; MIL MRS and MSL MRS (Table 2). Colony of *E. coli*, *S. typhimurium* and *S. aureus* carefully inoculated to medium and then incubated at 37°C for 24 h. The number of colony was then counted using total plate count (TPC) at 0 and 24 h and the data were then expressed as decreasing percentage of microorganism number.

Experiment 3: Effect of PLF and its oligomer lignin to rumen metabolism: This *in vitro* experiment was conducted in a completely randomized block design with 5 treatments in 3 blocks, with rumen fluid from different cattle as a block. The treatments were R_0 = basal diet (contained 66.7% TDN and 14.7% crude protein); R_1 = R_0 + 1.25% of Inulin (commercial prebiotics); R_2 = R_0 + 1.25% of PLF; R_3 = R_0 + 1.25% of MIL and R_4 = R_0 + 1.25% of MSL. Ingredient composition of the basal diet was presented in Table 3.

Inoculum and medium: Rumen contents were collected just about 3 h after morning feeding from 400 kg ruminally fistulated Ongole crossbred beef cattle (in LIPI Cibinong), maintained on basal diet composed of 40% forage and 60% concentrate. The rumen fluid was filtered through a double layer cheese cloth. All animal procedures and protocols in this study were approved by Bogor Agricultural University animal care committee. The substrate for the fermentation is a basal diet composed

Table 1: Ingredient composition of MRS medium and treatments in experiment 1

	MRS Medium (in 100 ml)					
Ingredient	Normal	Minimal	Inulin	PLF	MIL	MSL
Pepton[c] (g)	1.8	0.18	0.18	0.18	0.18	0.18
Yeast[b] (g)	0.4	0.04	0.04	0.04	0.04	0.04
K_2HPO_4[a] (g)	0.2	0.2	0.2	0.2	0.2	0.2
Tween 80[a] (mL)	0.1	0.1	0.1	0.1	0.1	0.1
$MgSO_4.7H_2O$[a] (g)	0.02	0.02	0.02	0.02	0.02	0.02
$MnSO_4$[a] (g)	0.004	0.004	0.004	0.004	0.004	0.004
Diamonium hydrogen citrate[a] (g)	0.2	0.2	0.2	0.2	0.2	0.2
Glukosa[b] (g)	1.0	0.1	-	-	-	-
Inulin (g)	-	-	1.0	-	-	-
PLF (g)	-	-	-	1.0	-	-
MIL (g)	-	-	-	-	1.0	-
MSL (g)	-	-	-	-	-	1.0

[a]Merck products; [b]Oxoid products; [c]Difco lab products

Table 2: Ingredient composition of LB medium and treatment of the experiment 2

	LB Medium (in 100 ml)					
Ingredient	Normal	Minimal	Inulin	PLF	MIL	MSL
Tripton[c] (g)	1	0.1	0.1	0.1	0.1	0.1
Yeast[b] (g)	0.5	0.05	-	-	-	-
NaCl[a] (g)	1	1	1	1	1	1
Inulin (g)	-	-	0.5	-	-	-
PLF (g)	-	-	-	0.5	-	-
MIL (g)	-	-	-	-	0.5	-
MSL (g)	-	-	-	-	-	0.5

[a]Merck products; [b]Oxoid products; [c]Difco lab products

of 40% forage and 60% concentrate containing 66.5% TDN and 14.7% crude protein (CP).

Culture condition: *In vitro* rumen fermentation was conducted according to the method of Tilley and Terry (1963). An amount of 500 mg substrate and 40 ml of McDougal buffer solution were added to individual fermentation tube and then 10 ml of strained rumen fluid was inoculated to the medium. McDougal buffer solution contained $NaHCO_3$ 58.8 g, $Na_2HPO_4.7H_2O$ 42 g, KCl 3.42 g, NaCl 2.82 g, $MgSO_4.7H_2O$ 0.72 g, $CaCl_2$ 0.24 g and H_2O in 6 litres solution. The fermentation medium was stirred and flushed with carbon dioxide to establish anaerobic condition and the tubes were then sealed with a rubber cork fitted with the gas release valve. Fermentation tubes were then incubated in a shaker water-bath at 39°C for 24 h.

Sample analysis: Individual VFA of the rumen fluid aliquots were measured using gas chromatography (Chrompack CP9002, Netherlands, flame ionized detector, capillary column type and nitrogen as gas carrier). The pH of rumen fluid aliquots was adjusted to 3-4 with H_2SO_4 then 1.5 ml of it was mixed with 30 mg of sulfosalicylic acid ($C_7H_6O_6S.2H_2O$). The mixture was centrifuged at 12.000 rpm for 10 minutes (7°C) then 0.5 µl of supernatant was injected to the GC. Ammonia concentration of the rumen fluid aliquots was measured using the micro diffusion method (Conway, 1962). Rumen microbial protein synthesis was determined as acid precipitable nitrogen, described by Makkar *et al.* (1982) and Lowry's method (Lowry *et al.*, 1951).

Data analysis: All data collected were subjected to analysis of variance followed by least significant difference (LSD) test. Computation was performed using SPSS 13.0 for windows evaluation system and Microsoft Excel 2010.

RESULTS AND DISCUSSION

Lignin is unique in that is composed of up to three different phenyl propane monomer. An additional complexity of lignin is that there are many possible bonding patterns between individual units. It chemical compound and structure determine the properties of the lignin. Therefore, characterize the lignin is important step in exploring the beneficial effects of lignin in nutrition studies. The phenolic compounds of the purified lignin formacell (PLF) and its derivative (fraction of purified lignin formacell) used in these experiments were presented in Table 4 and 5, respectively. It appears from the Table 4 and 5 that Purified Lignin Formacell, fraction of Methanol Insoluble Lignin (MIL) and fraction of Methanol Soluble Lignin (MSL) are phenolic compounds, which were known to have antimicrobial effects. These potential antimicrobial substances could be used to reduce pathogenic bacteria in the digestive tract of animals.

Results of the first experiment on the lactic acid bacterial growth revealed that *Lactobacillus bulgaricus*, *Lactobacillus casei* and *Streptococcus thermophillus* could grow well in PLF, MIL, or MSL medium (Table 6). This evidence suggests that PLF and its derivative could substitute the glucose in MRS medium. Its means that PLF and its derivative could be used as energy sources for growth of microbes that confer benefits to the host animals. This result indicated that PLF, MIL, or MSL could be used as feed supplement and demonstrated prebiotic effects by supporting the growth of good bacteria (*L. bulgaricus*, *L. casei* and *S. thermophillus*). Result of this experiment is in line with the work of Cota

Table 3: Ingredients composition and nutritional content of the basal diet

		Nutrients content (% DM)			
Ingredient	Dry matter (%)	TDN	Crude protein	Ca	P
Forage					
Pennisetum purpureum	25.0	15.43	2.88	0.17	0.06
Gliricidia sepium	15.0	11.25	4.01	0.02	0.02
Concentrate					
Cassava waste	21.1	13.34	0.91	0.04	0.01
Rice bran	11.1	7.27	1.42	0.01	0.19
Corn meal	9.0	6.84	0.79	0.01	0.02
Tofu waste	9.3	6.88	2.48	0.02	0.00
Coconut oil meal	7.5	5.65	2.22	0.01	0.01
Bone meal	1.0	0.00	0.00	0.32	0.11
Vitamin and mineral mix	1.0	0.00	0.00	0.05	0.02
Total	100	66.65	14.71	0.63	0.45

Table 4: Phenolic compound of purified lignin formacell (PLF) isolated from empty bunch palm fiber

Phenolic compound*	Formula	Molecular weight
4H-1-Benzopyran-4-one, 2-[4-[(6-deoxyhexopyranosyl)oxy]phenyl]-8-(hexopyranosyloxy)-5,7-dihydroxy-	$C_{27}H_{30}O_{15}$	594.518
4,4'-Bis-(4-methoxy-phenoxy)-biphenyl	$C_{26}H_{22}O_4$	398.450
Benzene, 1-(1-phenylethoxy)-4-(phenylmethoxy)-	$C_{21}H_{20}O_2$	304.382
1,2-Benzenedicarboxylic acid diphenyl ester	$C_{20}H_{14}O_4$	318.228
Benzaldehyde, 4-[1-[4-(acetyloxy)-3,5-dimethoxyphenyl]ethoxy]-3-methoxy-	$C_{20}H_{22}O_7$	374.384
Benzaldehyde, 4-[[4-(acetyloxy)-3,5-dimethoxy-phenyl] methoxy]-3-methoxy	$C_{19}H_{20}O_7$	360.358
diconiferyl alcohol, dehydro-	$C_{20}H_{22}O_6$	358.385
Phenol, 4,4'-methylene bis[2,6-dimethoxy]-	$C_{17}H_{20}O_6$	320.337
2H-1-Benzopyran-7-ol, 3,4-dihydro-3-(3-hydroxy-2,4-dimethoxyphenyl)-	$C_{17}H_{18}O_5$	302.322
Benzoic acid, 2-hydroxy-4-[(3-methoxyphenyl)methoxy]-, methyl ester	$C_{16}H_{16}O_5$	288.2952
4H-1-Benzopyran-4-one, 5,7-dihydroxy-2-(2-methoxyphenyl)-	$C_{16}H_{12}O_5$	284.264
Dioxybenzone	$C_{14}H_{12}O_4$	244.243
2-Ethoxybenzoin	$C_{16}H_{16}O$	240.337
3,4,7-Trimethoxycoumarin	$C_{12}H_{12}O_5$	236.221
2-Hydroxyphenyl benzoate	$C_{13}H_{10}O_3$	214.217
3,3-Diphenyl-1-propanol	$C_{15}H_{16}O$	212.287
Sinapyl aldehyde	$C_{11}H_{12}O_4$	208.211
Acetosyringone	$C_{10}H_{12}O_4$	196.200
Methoxyeugenol	$C_{11}H_{14}O_3$	194.227
Ferulic acid	$C_{10}H_{10}O_4$	194.184
Syringaldehyde	$C_9H_{10}O_4$	182.173
Coniferyl alcohol	$C_{10}H_{12}O_3$	180.201
Vanillic acid	$C_8H_8O_4$	168,147
Acetovanillone	$C_9H_{10}O_3$	166.174
Eugenol	$C_{10}H_{12}O_2$	164.201
Syringol	$C_8H_{10}O_3$	154.163
Vanillin	$C_8H_8O_3$	152.147
3-Methoxyacetophenone	$C_9H_{10}O_2$	150.174
3-Methoxy-pyrocatechol	$C_7H_8O_3$	140.137
2-Methoxy-4-methylphenol	$C_8H_{10}O_2$	138.164
Guaiacol	$C_7H_8O_2$	124.137
Phenol	C_6H_6O	94.111

*Analyzed using Shimadzu GCMS-QP2010

and Whitefield (1998) and Samanta *et al.* (2012), who reported that a number of rumen bacteria could fermented prebiotics and then used it as source of energy for metabolism.

Results of the Second Experiment revealed that *E. coli*, *S. typhimurium* and *S. aureus* could not grow well in PLF, MIL, or MSL medium (Table 7). This result indicated that *E. coli*, *S. typhimurium* and *S. aureus* could not use PLF, MIL, or MSL in the medium as source of carbon nutrients for growth. This evidence suggests that fraction of PLF, MIL and MSL could not be used in the medium to grow the pathogenic bacteria. This result was in accordance with the work of Baurhoo *et al.* (2007), which revealed that purified lignin (Alcell lignin) could significantly reduce the cecal population of total *E. coli* in *E. coli* challenged broilers.

The exact mechanism of how PLF, MIL, or MSL reduced the growth of pathogenic bacteria is still need thoroughly investigate. However, two mechanisms could be proposed as most probably happened in the culture

Table 5: Phenolic compound of methanol insoluble lignin (MIL) isolated from empty bunch palm fiber

Phenolic compound*	Formula	Molecular weight
4H-1-Benzopyran-4-one, 2-[4-[(6-deoxyhexopyranosyl)oxy]phenyl]-8-(hexopyranosyloxy)-5,7-dihydroxy-	$C_{27}H_{30}O_{15}$	594.518
Quercetin 7,3',4'-trimethyl ether	$C_{18}H_{16}O_7$	344.3154
Phenol, 4,4'-methylene bis[2,6-dimethoxy]-	$C_{17}H_{20}O_6$	320.337
Dioxybenzone	$C_{14}H_{12}O_4$	244.243
3,3-Diphenyl-1-propanol	$C_{15}H_{16}O$	212.287
Vanillic acid	$C_8H_8O_4$	168.147
Syringol	$C_8H_{10}O_3$	154.163
Guaiacol	$C_7H_8O_2$	124.137

*Analyzed using Shimadzu GCMS-QP2010

Table 6: Phenolic compound of methanol soluble lignin (MSL) isolated from empty bunch palm fiber

Phenolic compound*	Formula	Molecular weight
1,4-Phenylenebis[(4-phenoxyphenyl)methanone]	$C_{32}H_{22}O_4$	470.515
4-biphenylyl 3,5-di-tert-butylbenzoate	$C_{27}H_{30}O_2$	386.526
Methanone, [1,1'-biphenyl]-3-ylphenyl-	$C_{19}H_{14}O$	258.314
Phenol, 4,4'-methylene bis[2,6-dimethoxy]-	$C_{17}H_{20}O_6$	320.337
Benzenemethanol, 3,4-dimethoxy-alpha-[1-(2-methoxyphenoxy)ethyl]-	$C_{18}H_{22}O_5$	318.364
Ethanone, 2-ethoxy-1,2-diphenyl-	$C_{16}H_{16}O$	240.297
3,3-Diphenyl-1-propanol	$C_{15}H_{16}O$	212.287
Acetosyringone	$C_{10}H_{12}O_4$	196.200
Methoxyeugenol	$C_{11}H_{14}O_3$	194.227
Ferulic acid	$C_{10}H_{10}O_4$	194.184
Syringaldehyde	$C_9H_{10}O_4$	182.173
Syringol	$C_8H_{10}O_3$	154.163
3-Methoxy-pyrocatechol	$C_7H_8O_3$	140.137
Guaiacol	$C_7H_8O_2$	124.137

*Analyzed using Shimadzu GCMS-QP2010

Table 7: Total plate count of *L. casei*, *L. bulgaricus* and *S. thermophilus* test

	------- Bacterial population (log cfu/ml) -------		
Bacteria	0 h	24 h	Increase (%)
Lactobacillus casei			
Minimal MRS	3.01	8.33	176.29±4.21^c
Normal MRS	3.03	8.71	187.83±4.95^b
Inulin MRS	3.04	8.40	176.29±0.85^c
PLF MRS	3.05	6.85	124.20±2.09^d
MIL MRS	2.01	7.10	252.71±3.76^a
MSL MRS	2.96	6.72	126.72±1.36^d
Lactobacillus bulgaricus			
Minimal MRS	3.16	7.62	140.52±4.56^b
Normal MRS	3.19	7.77	143.63±3.74^b
Inulin MRS	3.27	8.32	154.22±1.28^a
PLF MRS	3.19	6.93	117.43±0.75^c
MIL MRS	3.40	7.09	108.33±1.03^d
MSL MRS	2.69	6.80	153.29±7.98^a
Streptococcus thermophilus			
Minimal MRS	2.70	7.28	169.39±1.30^d
Normal MRS	2.74	8.29	202.04±1.44^c
Inulin MRS	2.75	7.04	155.77±1.67^e
PLF MRS	2.70	6.78	149.90±0.29^f
MIL MRS	1.97	7.05	258.65±1.73^a
MSL MRS	1.92	6.79	254.34±0.51^b

Values with different superscript at the same column (for each bacteria) differ significantly ($p<0.05$), tested by LSD test

Table 8: Total plate count of *E. coli*, *S. typhimurium* and *S. aureus* test

	------- Bacterial population (log cfu/ml) -------		
Bacteria	0 h	24 h	Decrease (%)
E. coli			
Minimal LB	3.01	8.75	190.28±2.10^b
Normal LB	3.00	9.05	201.78±3.92^b
Inulin LB	3.07	8.84	188.37±2.33^b
PLF LB	2.60	8.30	268.93±79.88^a
MIL LB	3.01	7.39	92.75±76.01^c
MSL LB	2.98	2.75	-7.78±1.11^d
S. typhimurium			
Minimal LB	2.72	8.54	258.81±73.32^a
Normal LB	3.11	9.07	191.09±1.87^b
Inulin LB	3.12	8.55	173.67±3.66^b
PLF LB	3.16	3.00	-4.94±0.52^d
MIL LB	3.15	7.10	55.90±71.68^c
MSL LB	3.18	3.11	-2.32±0.63^d
S. aureus			
Minimal LB	2.88	8.34	189.20±1.55^b
Normal LB	2.97	8.99	202.32±1.79^a
Inulin LB	2.92	8.59	194.10±1.44^b
PLF LB	2.93	0.00	-100.00±0.00^c
MIL LB	2.87	0.00	-100.00±0.00^c
MSL LB	2.29	3.03	11.21±48.67^d

Values bearing different superscript at the same column (for each bacteria) differ significantly ($p<0.05$), tested by LSD test

medium. First mechanism was because no energy source in medium for pathogenic bacteria as they could not use PLF, MIL, or MSL as carbon sources and therefore their population decreased. Another mechanism was the possibility of antimicrobial effect of phenolic compound in PLF, MIL, or MSL. Antimicrobial activity of plant phenolic has been intensively studied. Rauha *et al.* (2000) reported that Finnish plant extracts

Table 9: Effects of the treatments on rumen metabolism parameters

	Treatments				
Parameters	R_0 (basal diet)	R_1 (R_0+Inulin)	R_2 (R_0+PLF)	R_3 (R_0+MIL)	R_4 (R_0+MSL)
pH	6.3±0.21[a]	6.4±0.06[a]	6.5±0.15[a]	6.5±0.12[a]	6.2±0.06[a]
Total VFA, mM	124.31±23.71[a]	79.94±4.94[a]	78.56±12.92[a]	87.50±32.55[a]	122.17±41.52[a]
Acetic acid (C2), mM	85.60±17.96[a]	53.33±2.92[c]	49.77±9.34[c]	57.03±22.64[bc]	77.63±28.68[ab]
Propionic acid (C3), mM	27.72±4.89[ab]	18.65±1.59[c]	20.29±3.31[c]	21.17±7.69[bc]	31.46±9.72[a]
Iso-Butyric acid, mM	0.71±0.23[b]	0.49±0.11[c]	0.55±0.15[bc]	0.63±0.06[b]	1.03±0.40[a]
n-Butyric acid (C4), mM	9.11±0.59[ab]	6.74±0.53[c]	7.02±0.70[c]	7.49±2.27[bc]	10.66±2.75[a]
iso-Valeric, mM	0.54±0.15[b]	0.38±0.09[c]	0.39±0.08[c]	0.53±0.07[b]	0.68±0.24[a]
n-Valeric (C5), mM	0.63±0.09[ab]	0.35±0.02[c]	0.54±0.09[bc]	0.65±0.08[a]	0.71±0.10[a]
C2/C3 Ratio	3.08±0.12[a]	2.86±0.10[b]	2.69±0.16[c]	2.68±0.12[c]	2.43±0.16[d]
Non-Glucogenic Ratio (NGR)[a]	3.68±0.05[a]	3.54±0.11[b]	3.33±0.14[c]	3.32±0.07[c]	3.07±0.13[d]
Estimate of methane production[b]	34.55±6.94[a]	21.56±1.11[c]	21.78±3.49[c]	22.84±8.96[bc]	30.54±11.33[ab]
Efficiency of Hexosa conversion to VFA (%)[c]	79.96±1.20[c]	81.35±0.36[b]	81.96±1.24[ab]	82.20±1.04[a]	83.18±1.25[a]
Isoacid	1.88±0.40[b]	1.22±0.09[c]	1.48±0.16[c]	1.81±0.06[b]	2.41±0.68[a]
NH3, mM	9.65±2.00[b]	12.39±1.29[a]	11.12±2.39[ab]	12.24±0.92[a]	11.63±0.69[a]
Microbial Protein Synthesis, g protein/100 g digested organic matter	21.85±3.48[b]	24.20±1.58[ab]	24.49±2.81[a]	23.84±1.33[ab]	22.11±3.97[b]
Total gas, ml/mg digested organic matter	182.5±7.67[a]	176.1±4.81[a]	171.6±4.51[a]	174.0±8.69[a]	173.7±6.35[a]

Values with different superscript at the same row differ significantly ($p<0.05$), tested by LSD test

[a]NGR calculated as (C2+2C4+C5)/(C3+C5); (Abrahamse *et al*., 2008)

[b]CH_4 calculated as 0.45 C2-0.275C3+0.40 C4; (Moss *et al*., 2000)

[c]EHC calculated as ((0.622 x %C2)+(1.092 x %C3)+(1.56 x C4)/(%C2+C3+C4))*100; (Orskov and Ryle, 1980)

Table 10: Effects of the treatments on nutrients digestibility

	Treatments				
Nutrients digestibility	R_0 (Basal diet)	R_1 (R_0+Inulin)	R_2 (R_0+PLF)	R_3 (R_0+MIL)	R_4 (R_0+MSL)
Dry matter digestibility (%)	66.82±5.53[b]	72.98±6.28[a]	67.60±1.94[b]	72.91±4.72[a]	70.05±1.09[ab]
Organic matter digestibility (%)	65.85±6.43[b]	71.58±6.66[a]	65.90±1.95[b]	71.79±5.47[a]	68.45±1.29[ab]
Crude protein digestibility (%)	72.77±3.72[b]	72.87±3.02[b]	75.37±2.03[b]	79.00±4.86[a]	73.73±3.43[b]
Crude fiber digestibility (%)	38.34±4.69[b]	39.20±5.55[b]	42.12±4.26[ab]	47.85±5.38[a]	45.45±7.90[a]

Values with different superscript at the same row differ significantly ($p<0.05$), tested by LSD test

containing flavonoid and other phenolic compounds showed antimicrobial effect on some human pathogenic bacteria including *Escherichia coli* and *Staphylococcus aureus*.

Ruminal metabolism parameters of *in vitro* fermentation were presented in Table 8. It appears from the Table that the used of Inulin, PLF, MIL, or MSL did not change the rumen pH, which were stable in the range of 6.2-6.5 that is considered normal for rumen fermentation. This result indicated that the use of these additives in this experiment did not have adverse effects on rumen metabolism. The same result was reported by Santoso *et al*. (2003) that the rumen pH remained unchanged (6.7) when prebiotic is given to Holstein cows maintained on orchards grass silage or alfalfa silage.

It appears from Table 8 that ruminal ammonia concentration was significantly increased by addition of Inulin, MIL or MSL. Ammonia concentrations of MIL or MSL were comparable to that of inulin as a commercial prebiotic product. Improving in ammonia concentration in MIL was supported by higher crude protein digestibility (Table 9). Ammonia is well known as the preferred source of nitrogen for microbial protein synthesis in the rumen (Bryant and Robinson, 1963; Erfle *et al*., 1977). Rumen microbial protein synthesis data confirmed this theory and revealed that addition of PLF increased the rumen microbial protein synthesis. Although it was not significant, the use of Inulin, MIL and MSL also tended to increase the rumen microbial protein synthesis. This result indicated that the use of Inulin, PLF, MIL, or MSL could support rumen microbial growth.

In certain situation, increasing in rumen microbial protein synthesis was mostly followed by reducing in ruminal ammonia concentration (Mwenya *et al*., 2005a; Santoso *et al*., 2003). However, since nitrogen for microbial protein synthesis also supplied by amino acids and peptides (Cotta and Russell, 1982), in certain condition improving in rumen microbial protein synthesis not always followed by reducing in ammonia concentration. Study of Atasoglu *et al*. (2003) revealed that rumen microbial yield was increased by 42% when amino acids were included in the growth media. This result indicated that higher rumen microbial protein synthesis in Inulin, PLF, MIL or MSL treatment were possibly also supported by nitrogen from amino acids and peptides in media as main products of protein digestion.

Addition of inulin, PLF, MIL, or MSL did not significantly affect the total VFA concentration. However, the addition of these additives tended to change the individual VFA profile of the rumen fluid. It appears from Table 8 that the addition of inulin, PLF, MIL, or MSL significantly reduced the C2/C3 ratio and non-glucogenic ratio (NGR). Propionic acid (C3) is known as a main source for gluconeogenesis in ruminant (Leng *et al*., 1967). It means that addition of inulin, PLF, MIL, or MSL tended to

change the rumen metabolism in vapor of glucogenic precursors production. These phenomena will in turn support the process of gluconeogenesis in ruminant metabolism. With the diets of crop residues, little glucose is absorbed so that gluconeogenesis will expect to provide the more glucose to support needed energy for animal metabolism. This result suggesting that the use of PLF, O-MIL, or O-MSL may be nutritionally beneficial to fattening beef cattle with crop residues based diet.

Data on estimate of methane production revealed that the addition of additives were significantly reduced the methane production and improved the efficiency of hexose energy conversion to VFA, except for MSL. It means that the use of Inulin, PLF and MIL could alter the rumen metabolism in vapor of improving the energy metabolism and reducing the methane production. Work of Mwenya *et al.* (2005b) revealed that the use of galacto-oligosaccharides (GOS) could lower the methane production in steer. This result indicated that PLF and MIL could be used as good feed supplement and also demonstrated prebiotic effects by improving the microbial ecosystem in the rumen and efficiency of feed energy utilization.

Data on nutrients digestibility of the *in vitro* fermentation were presented in Table 9. It appears from the table that dry matter and organic matter digestibility were significantly improved by Inulin and MIL. Although not significant, addition of MSL also tended to improve dry matter and organic matter digestibility. Part of improvement in nutrients digestibility of the whole digestive tracts (Tilley and Terry method) were because of improving in some rumen metabolism parameters (Table 8). Since nutrients digestibility data measured by Tilley and Terry methods reflected the nutrients digestibility in the whole digestive tracts, this result suggesting that the addition of PLF, MIL and MSL were most probably have significant effects only in the rumen or foregut. However, this finding needs further investigation especially through microbial study in the intestine and colon (hindgut).

It was noteworthy that crude fiber digestibility was significantly improved by MIL and MSL compared with that of the basal diet. Although it was not significant, the use of PLF also tended to increase the crude fiber digestibility. This result was in accordance with Cota and Whitefield (1998) which reported that all the rumen hemicellulolytic bacteria are capable to utilize xylooligosaccharides as growth substrate. Improving in digestibility of crude protein and crude fiber will support the protein and energy metabolism of ruminant.

Conclusion: Purified lignin formacell from empty bunch palm fiber and its oligomers are phenolic compounds. It demonstrated prebiotic effects by stimulate the growth of lactic acids bacteria, therefore it could be promote as new feed supplements and prebiotic candidate. These materials also could not use as carbon sources for the growth of pathogenic bacteria and probably have antimicrobial effects by reduce the growth of pathogenic bacteria. The use of purified lignin formacell from empty bunch palm fiber and its oligomers in *in vitro* rumen fermentation could altered the rumen fermentation system by decrease the C2/C3 ratio of VFA, non-glucogenic ratio (NGR) and methane production. These materials also could improve nutrients digestibility and increased the ammonia concentration as well as rumen microbial protein synthesis. These evidences suggesting that purified lignin formacell from empty bunch palm fiber and its oligomers in the rumen were in vapor of glucogenic precursor production, which will in turn support the gluconeogenesis in ruminants. Therefore, these materials may be nutritionally beneficial to fattening beef cattle.

ACKNOWLEDGEMENTS

This study was partially supported by DP2M Directorate General of Higher Education, Republic of Indonesia and technically supported by Prof. Tati, Prof Yandri, Ulvi, Surya, Reska, Teta, Awan and Eka. Therefore it was a great pleasure of the authors to express their appreciation to all of them.

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