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Research paper

The Effect of Supplementing Lignolitic Probiotic in Rice Straw on in Vitro Gas Production,Concentration of Ammonia (NH3),and Volatile Fatty Acid (VFA)

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Abstract

The aims of this research were to know the effect of supplementing lignolitic probiotic in rice straw on in vitro gas production, concentration of ammonia (NH3), and VFA and to know the optimal use of lignolitic probiotic in rice straw. The materials used were rice straw and lignolitic probiotic. The methods used was Randomized Block Design (RBD) with 4 treatments and 3 groups. The treatments were P0: rice straw; P1: rice straw + 0.5% probiotic; P2 : rice straw + 1.0% probiotic; and P3: rice straw + 1.5% probiotic. The materials was incubated for 96 hours. The result showed that treatments significantly affected on in vitro gas production ($P<0.01$). Treatment of P3 had the highest gas production (73.86 ml/500mg DM) and VFA production (51.08 Mmol/l). While treatment of P0 had the lowest gas production (67.38 ml/500 mg DM) and VFA production (42.48 Mmol/l). Concentration of ammonia observed in all treatments were adequately support the ability of rumen microbes to digest fiber. Based on the result of this reserach, it could be concluded that supplementing 1.5% lignolitic probiotic (P3) achieved the highest gas production at 96 hour incubation (73.86 ml/500 mg DM).

Keywords: *gas production, concentration of ammonia (NH3), volatile fatty acid (VFA)*

1. Introduction

Forage is source of especial feed which is common to be given for the livestock of ruminant. At dry season, amount of available forage experience lowering so that need feed source of other fiber was rice straw. Principal constraint in rice straw is nutrient content and lower digestibility, because cellulose fibre rate height (33%), hemicellulose (26%), and lignin (23,4%) so difficult rice straw digested by rumen microbe. Low digestibility because existence lignin and silica bind cellulose and hemicellulose in form of bunch double, so difficult to digested by enzyme from rumen microbe.

Lignolitic probiotic is result bacteria isolate of lignolitic microbe select capable to lignin degradation. Result of Prihartini research (2007) obtained 3 lignolitic isolate have high growth potency, production, enzyme activity, and lignin degradation. Third of specific isolate only lignin degradation, good grow at natural carrier media is rice straw. The three isolate were TPG (isolate from Tumpang), BAS2 (isolate from Bumiasri), and Combination of TPG and BAS2. Third of isolate is included negative gram bacteria group, type of Bacillus, and aerob optionally character. Every isolate have different character, TPG have high ability in lignin degradation while BAS2 have high ability to organochlorin degradation.

Probiotic is biotechnology product of content lignolitic bacteria and cellulotic selected. Lignolitic yield ecto enzyme lignase which consist of phenol oksidase, lacasse, and peroksidase. Character of ecto enzyme lignase is reorganize methoxyl double bunch at lignin chain structure so hydro group its structure lignin derivate become more simple and had high cation exchange rate, so assist to permeate nutrient included NPN for microbe growth in rumen (Jasmal, 2009). The research aim to know the effect of supplementing lignolitic probiotic in rice Straw on gas production, concentration of ammonia (NH3), and VFA, and to know supplementing lignolitic probiotic optimal level in rice straw evaluated from gas production, NH3 concentration, and VFA on in vitro.

Result of this research is expected can be used as information about supplementing lignolitic probiotic to increase rice straw quality for ruminant livestock.

Therefore, require performed furthermore research about the effect of supplementing lignolitic probiotic in rice straw on in vitro gas production, NH3 concentration, and VFA.

2. Methods

2.1. Location and Time Research

The research was carried out at the Nutrition Laboratory of Animal Husbandry Faculty, University of Brawijaya in Malang and PAU Food and Nutrition Laboratory, University of Gadjah Mada in Yogyakarta from November until December 2009.

2.2. Materials Research

The materials used in this research is IR64 rice straw which is obtained from karang ploso village, rumen liquid take from fistulation cattle in Sumber Sekar wide laboratory which given elephant grass feed (pennisetum purpureum) and concentrate, lignolitiv probiotic representating combination of mineral M1, pollard, rice straw extract and lignochlorin isolate, and chemical materials and equipments used for gas production, NH3 concentration, and VFA.

2.3. Methods Research

The materials used were rice straw and lignolitic probiotic. The methods used was Randomized Block Design (RBD) with 4 treatments and 3 groups. The treatments were P0: rice straw; P1: rice straw + 0.5% probiotic; P2 : rice straw + 1.0% probiotic; and P3: rice straw + 1.5% probiotic. The materials was incubated for 96 hours.

3. Result and Discussion

3.1. In vitro Gas Production

Gas production is result of fermentation process that happened in rumen and can use to the amount of organic materials which is fermentation or digestible organic materials (DOM). Result of gas production measurement for use feed material can be seen at Table 1.

Table 1: Gas Production at Each Treatment

Treatment	Gas Production (ml/500mg DM) at each incubation period (hour)								
	2	4	8	16	24	36	48	72	96
P_0	0.75^{a}	2.23^{a}	3.73^{a}	9.05^{a}	19.09^{a}	33.78^{a}	44.68^{a}	57.14^{a}	67.38^{a}
P_1	1.08^{a}	2.41^{a}	4.32^{a}	10.19^{a}	20.97^{a}	34.92^{a}	45.96^{a}	59.70^{a}	69.48^{a}
P_2	1.33^{b}	2.99^{a}	5.23^{b}	10.96^{b}	21.74^{b}	36.71^{b}	47.33^{b}	62.33^{b}	71.67^{b}
P_3	1.58^{b}	3.32^{a}	5.73^{b}	12.05^{b}	23.38^{b}	38.05^{b}	48.93^{b}	62.92^{b}	73.86^{b}

Statement : $^{a-b}$ different superskrip in same column show significantly effect ($p<0.05$; 2,16,72 hour) and very significantlyeffect ($p<0.01$; 8, 24-48,96 hour).

From Table 1. incubation period 2-8 hour speed of gas production still slow. This matter because of happened phase lag in fermentation tube (syringe). After 8 hour (8-96 hour), probiotik already experience of adaptation so can reed degradation. With the mentioned, more fermentation BO so its gas production swift more fast.

Result of Table 1. indicating more and more supplementing probiotic and longer incubation period so higher gas production. This matter because the time of rice straw enter to rumen, feed materials which easy digestible will advance dissolve. Then feed materials which is cannot digestible but gas production potency (lignin, cellulose, and hemicellulose) requiring time to able degradation and can digested in rumen. With existence of probiotic supplementing so lignin, cellulose, and hemicellulose able degradation in rumen.

Table 1. indicating that longer time of gas production incubation more progress, this matter because rumen microbe activity in feed degradation more progress and lignolitic probiotic not have antagonist characteristic to rumen microbe so gas production more progress. Lignolitic probioticcan digest other materials in addition to digest by rumen microbe characteristic was lignin, so with supplementing lignolitic probiotic can cellulose, hemicellulose, and lignin digest in rice straw. The mention expected nutrien in feed materials become better.

Gas production in P3 treatment more high than other treatment because organic materials content at P3 many used by rumen microbe to yield VFA can see at Table 6. this matter according with sugoro (2005) declare that yield gas represent feed fermentation especially organic materials become VFA that rumen microbe do. This research result was better if compared with Prihartini result of research (2007) declare that highest gas production happen at control treatment 96 hour incubation was 63.167 ml/500mg DM.

The lowest gas production at control treatment (P0), low rate at P0 was compared other treatment because low lignin digestible process on rice straw in rumen effect lost of supplementing probiotic which can lignin digest characteristic (lignolitic), cellulose, and hemicellulose. Suparjo (2008) declare lignin hard to degradable because complex structure bunch with celluolose and hemicellulose in plant tissue.

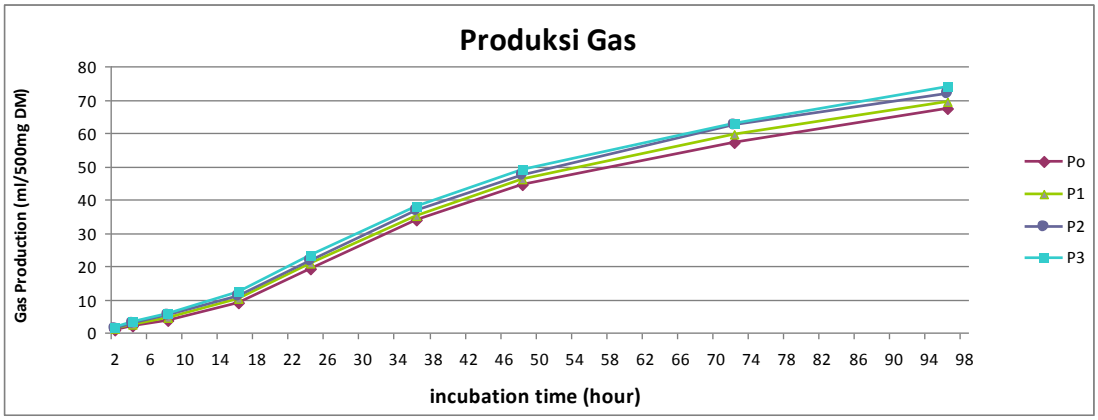

Fig 1: Relation between incubation time with feed tratment gas production graphic.

From the graphic longer time incubation time so gas production more increase because microbe activity in rumen to degradation high feed. This matter according with Ella (1997) explaning that high gas production show degradable increase at rice straw by microbe activity in rumen.

3.2. Concentration of Ammonia (NH3)

Concentration of Ammonia in rumen enough to rumen microbe growth was one of guidance in evaluating protein feed to ruminant livestock. NH3 concentration can see at table 2.

Table 2: Concentration of Ammonia (NH3) at each treatment

treatment	NH_3 Concentration (mg N-NH_3/liter rumen liquid)
P_0	247.65[a]
P_1	256.59[a]
P_2	269.08[a]
P_3	264.82[a]

Statement : a different superskrip in same column show different unsignificantly (P>0.05)

The statistic analysis result show that unsignificantly treatment effect (P>0.05) at concentration of ammonia (NH3). Table 2.indication that NH3 concentration enough to optimum degradable fibre feed process were ≥ 200 mg/liter rumen liquid (Ørskov, 1982). The more supplementing probiotic so more high NH3¬ concentration, this matter because probiotic have protein and protein mention was changed become ammonia (NH3).

Material organic specially protein, will experience hydrolysis by proteolitic enzyme become amino acid and oligopeptida in rumen. Furthermore amino acids experience further catabolism produce ammonia, VFA, and CO2. Ammonia become especial nitrogen source to de novo synthesis amino acids for rumen microbe. Metabolism process was showed that protein nutrition ruminant livestock very depend on rumen microbial protein synthesis process. Protein hydrolysis product a considerablepart will experience further catabolism (deaminasi), so produce ammonia (NH3). With existence of the mentioned, NH3 and degradable organic materials have relevance where more high supplementing probiotic and more high gas production, so more high NH3. This matter because research concurrently, degradable organic materials more increase were 156.92 g (P0), 166.17 g (P1), 187.43 g (P2), and 202.53 g (P3) (Julfilia, 2009).

Result from the research are showing NH3 concentration have done used by microbe rumen to protein synthesis. if NH3 concentration too high without made balance with energy source so will excretion in form urea by urine. According to Mc Donald and Edward (1988) NH3 absorption speed up can sause no poisoning livestock because NH3 pass blood stream will be brought to liver for modify be urea which later will enter in saliva and urine loosing upon through kidney. But in this research unhappened NH3 absorption by rumen wall because using syringe, so happen accumulation on in vitro within syringe so NH3 concentration become high.

3.3. VFA (Volatile Fatty Acid)

VFA (Volatile Fatty Acid) is one of carbohydrate fermentation product bu microbe rumen beside other product were CO2 and CH4. VFA was produced especially acetate acid (C2), propionate acid (C3), and butyrate acid (C4). This VFA is prime energy source for body ruminant livestock requirement (Preston and Leng, 1987).

Data of average actate acid, propionate acid, butyrate acid on the research can seen at table 3.

Table 3: Average acetate acid, propionate acid, and butrate acid total.

Treatment	asetat (C2) Mmol/l	propionat (C3) Mmol/l	butirat (C4) Mmol/l	total Mmol/l
P_0	27.78[a]	9.99[a]	4.71[a]	42.48
P_1	32.25[a]	11.09[a]	5.03[a]	48.38
P_2	29.02[a]	10.38[a]	4.62[a]	44.02
P_3	33.41[a]	12.33[a]	5.34[a]	51.08

Statement : a different superskrip in same column show different unsignificantly (P>0.05)

From statistic analysis result was got that treatment give unsignificantly effect (P>0.05) acetate acid, propionate acid, and butrate acid concentration.

Acetate acid proportion P3 was 33.41 Mmol/l higher if compare with P1 was 32.25 Mmol/l, P2 was 29.02 Mmol/l, and P0 was 27.78 Mmol/l. the matter cause probiotic in lignin degradation also make use of organic materials were VFA as energy source, but can lignin degradation quicker was compared VFA substantion degradable or organic materials.

From table 3 can be seen, more high supplementing probiotic so VFA total more high, this matter caused digestible crude fibre pocess increase and microbe activity in rumen also increase.

By virtue of the research result from gas production, CH4, and CO2 was presented in table 4.

Table 4: Methan gas percentage average (CH4) dan CO2

Treatment	C2 : C3 : C4 (%)	CH4 (%)	CO2 (%)
P_0	65.43 : 23.51 : 11.06	32.38	55.18
P_1	66.68 : 22.91 : 10.41	32.82	54.69
P_2	66.37 : 23.41 : 10.22	32.44	54.37
P_3	65.27 : 24.18 : 10.55	31.87	54.5
	65.94 : 23.50 : 10.56		

Source :

Result of Stoichiometry Calculation (Van Soest (1994)) were :

$Y = 0,5\ Ma + 0,25\ Mp + 1,5\ Mb$

$Z = Ma + 2Mb - Y$

Statement :

Y : Molar CO2

Z : Molar CH4

Ma : acetat acid proportion

Mp : propionate acid proportion

Mb : butyrate acid proportion

From the table 4. are indicating that compare of acetate acid (C2), propionate acid (C3), and butrate acid (C4) agree with Mc Donald and Edward opinion (1988) that C2:C3:C4 compared were 70% : 20%: 10%. The mentioned because microbe activity have worked well.

The methan and CO2 product were indicator which often used to energy usefull eficeincy determine. From Stoichiometry calculation table 4. able know that methan gas and CO2 was formed inclined increaseenergy usefull efficiency. Energy usefull efficiency very related with NH3 total able to incorporation in microbial protein synthesis.

From result of this research, more increase gas production so NH3 concetration and VFA also more increase. This matter is caused supplementing probiotic can increase rumen microbe population as reported by Julfilia (2009) was more high supplementing probiotic from P0 until P4, microbe protein synthesis efficiency also increase in succession P0, P1, P2, and P3 were 10.95; 19.03; 27.48; and 34.26 (g N/kg DOM). This matter accord with priyono (2008), concentration of ammonia in rumen follow determine microbial protein synthesis efficiency in turn will influence result of feed organic materials fermentation. Fermentation result can be seen as VFA concentration in rumen liquid. Concentration of ammonia was mentioned a given by feed protein level consumed, degradability degree, duration feed be rumen and pH rumen.

This fact are giving indication that balance protein and energy which better with more increase probiotic level.

4. Conclusion

The result conclusion that :

1. The supplementing lignolitic probiotic improve gas production and VFA production. Gas production and VFA total were highest at P3 treatment with added 1.5% probiotic from rice straw feed. Gas production has 73.86 ml/500 mg DM and VFA production has 51.08 Mmol/l at 96 hour incubation.

2. NH3 concentration at all treatment with supplementing lignolitic probiotic have been enough to support microbial growth in rumen so that able crude fibre degradation.

3. Thereby of the best research was rice straw added 1.5% probiotic DM (treatment of P3).

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