J. Appl. Environ. Biol. Sci., 3(9)166-171, 2013

ISSN: 2090-4274
Journal of Applied Environmental and Biological Sciences
www.textroad.com

Addition of Lignocellulolytic Enzymes Into Rice Straw Improves In Vitro Rumen Fermentation Products

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ABSTRACT

This study aimed to assess lignocellulolytic enzymes in rice straw with an attempt to improve in vitro rumenfermentationproducts. The products were volatile fatty acid, acetate, propionate, butyrate, and ammonia. The experimental methods used completely randomized design with five treatments and five replicates. Five treatments consisted of various levels of lignocellulolytic enzymes and bacterial lignocellulolytic in addition to control, which was no enzymes addition. The results revealed there were significant difference of all the fermentation products. The new finding was an amount of 5% lignocellulolytic enzymes resulted in optimal products of volatile fatty acid and ammonia.

KEYWORDS: lignocellulolytic enzymes, rice straw, in vitro, rumen fermentation, volatile fatty acid, ammonia.

INTRODUCTION

Production potential of agricultural waste in the form of rice straw was quite high in Indonesia and could be used as alternative feed forage. However, the nutritional values were low, characterized by a high content of crude fiber such as cellulose and hemicellulose, and low crude protein. Molecules of cellulose and hemicellulose were polysaccharides with ß-bond 1-4glycosidic, which were poorly digested by microbial ruminants, and therefore the agricultural waste was characterized by low digestibility. Polysaccharides were the main energy source for ruminant livestock feed derived from fibrous waste such as rice straw. Polysaccharide utilization was limited due to the high content of lignin in lignocellulosic residues, polymerization of the cellulose and hemicellulose, and therefore could not be used as a carbon source. One of the goals of biotechnology development was the use of enzymes in the bioconversion lignocellulosic waste of rice straw[1].

Lignocellulolytic enzymes formed an enzyme complex consisting of the enzyme cellulase and hemicellulase. Cellulase enzymes consisted of three components of the enzymes that were Cx component (endo-β-1,4-glucanase), C1 component (β-1,4-glucan cellobiohydrolaseorexo-β-1,4-glucanase) and components cellobiase (β-glucocidase)[2,3]. An essential enzymes complex for the degradation of the hemicellulose component of the enzymes xylanase consisted of five component of the enzyme endoxylanase-β-1.4, β-xylosidase,α-L-arabinofuranosidase, α-D-glukuronidase, andacetylxylanesterase [4]. Lignocellulolytic enzymes group could ferment cellulose and hemicellulose into glucose and xylose by enzymatic process.

Biodegradation of cellulose using cellulase enzymes produced by microbes were very important in the processing of agricultural waste[5]. Animal feed supplementation with cellulase enzymes to improve feed efficiency and animal performance through increased fiber digestibility in vitro[6,7], in-situ [8,9] or in-vivo [10].

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Ruminant animal feed usually contained 60-70% carbohydrates. In general, the end product of carbohydrate fermentation in the rumen was volatile fatty acid (VFA) which was a source of energy for ruminants. Agro-industrial waste feed material contained cellulose (45-50%), hemicellulose (35-40%), which could be utilized by ruminants as a source of energy. Cellulose and hemicellulose in ruminants rumen fermentation process that produced VFA could meet 50-60% of energy needs. The end product of fermented feed in the form of VFA and ammonia NH_3 used as measure of microbial activity in the rumen microbial protein synthesis and energy [11,12].

Therefore, the purposeof the current research was to assess the addition of lignocellulolytic enzymes into rice straw for in vitro rumen fermentation with an objective to produce optimum products of VFA and NH_3.

MATERIALS AND METHODS

Inoculum was prepared by inoculating one loop lignocellulolytic bacterial culture into 25mL of inoculum medium. Cultures were inoculated at a temperature of 39^0C with a speed of 170rpm. As much as 1% inoculum put into 200mL of media production. Cultures were incubated under the same conditions as above. CMC substrate added to the medium used for cell harvesting. Cells were harvested after 14hours of growth by centrifugation for 15minutes at 6000rpm. Supernatant was a crude extract lignocellulolytic enzymes to be used in testing the potential of rice straw.

Treatment of rice straw fermentation enzymes lignocellulolytic consisted of JPF1=control, JPF2=JP+5% EL, JPF3=JP+10% EL, JPF4=JP+5%EL+5%BL, JPF5 =JP+5%EL+10%BL (Notes: JP stands for rice straw, JPF stands for fermented rice straw, EL stands for enzymes lignocellulolytic; BL stands for bacterial lignocellulolytic). The fermented rice straw(JPF) was obtained through the following steps: Straw was dried and chopped to the size of 2.5-3cm. Enzymes treatment used lignocellulolytic then sprinkle on dried rice straw and stir until evenly across the surface of wet straw. Straw then incubated for seven days according to treatment incubation time. Nutrient content of fermented rice straw used for experiments in vitro were presented in Table1.

Table1**.**Nutrient contentof rice strawfermentedbyenzymes lignocellulolytic

Nutrition (% DM)	JPF1	JPF2	JPF3	JPF4	JPF5
DM	94.26	93.61	94.32	94.86	95.47
CP	5.14	6.19	6.74	8.05	8.18
CF	39.10	38.01	37.70	35.84	35.10
OM	80.40	83.43	84.23	86.25	86.05
Cellulose	34.44	31.72	30.57	29.90	29.86

In vitro method[13] used McDougall's buffer solution that included in the storage stirer flask and placed in a heater and escaping CO_2 gas. Rumen fluid was filtered and placed in a flask and transferred into storage flasks which already contain a buffer solution. Composition of buffer solution with rumen fluid was 4 : 1. Conditions of buffer and rumen fluid mixture was maintained at pH of 6.9 to 7.0 with a temperature of 38-39^{o}C. Feed samples to be tested were ±0.5grams that inserted into the fermenter tube and 38-39°C incubation. Taken 50mL of a mixture of rumen fluid and buffer solution, put in the fermenter tube containing the sample and the tube was empty fermenter (blank) using dispencer and immediately closed with rubber coated stoppers with a Bunsen valve. Before closing, first the ferementer fed with CO_2 gas. After 48hours of incubation, rumen fermentation was stopped by soaking in cold water. Then the samples were filtered with a filter paper. Supernatant was taken for measurement of NH_3 according to the method[14] and measurement of VFA by gas chromatography according to the method[15]. And the molar proportion of acetic acid, propionate and butyrate production determined molfermentation [16].

The experimental design used in this study was completely randomized design. Data of rumen fermentation parameters in vitro (production of VFA and NH_3) were analyzed using One-way Analysis of Varians (ANOVA) ($P<0.05$) on Statistical Package For Social Science (SPSS) program. To find the differences between the treatments were analyzed byDuncan's Multiple Range Test[17].

RESULTS AND DISCUSSION

The average concentration of NH_3, total VFA, acetate acid levels, propionic acid and butyric acid of rumen fluid to the different treatment of feed were presented in Table 2.

Table 2. Rumen fermentationcharacteristicsin vitroof lignocellulolytic rice straw

Variable	Treatments				
	JPF1	**JPF2**	**JPF3**	**JPF4**	**JPF5**
N-NH_3(mg/100 mL)	11.70 ^{d}	13.92 ^{c}	14.71 ^{bc}	15.46 ^{ab}	16.79 ^{a}
Total VFA (mM/L)	53.79 ^{a}	57.11 ^{bc}	59.86 ^{b}	65.45 ^{a}	63.67 ^{a}
Acetate(mM/L)	38.03^{c}	39.47 ^{bc}	41.12 ^{b}	44.74 ^{a}	43.70 ^{a}
Propionate(mM/L)	10.80^{c}	12.56 ^{b}	13.35 ^{b}	14.90 ^{a}	14.34 ^{a}
Butyrate(mM/L)	4.96^{c}	5.08 ^{bc}	5.39 ^{c}	5.75 ^{a}	5.63 ^{a}

The results obtained by the highest concentrations of NH_3 to JPF5 not significantly different ($P>0.05$) with JPF4 but significantly different ($P<0.05$) with JPF3, JPF2, JPF1(Table 2). The high concentrations of NH_3 treatment compared with JPF5, JPF4 because enzymes activity could degrade lignocellulose and lignohemicellulose bonds and the release of bound lignin content of soluble N so that increases N, also lignocellulolytic proliferation of bacteria that contributes to increasing concentrations of NH_3.

According to Kamalak et al [18], rumen fluid NH_3 concentration varied depending on the amount of feed protein, protein degradation rate and feeding time. Protein feed into the rumen was fermented by microorganisms proteolytic that produce enzymes protease, peptidase and deaminase to degrade the protein into amino acids, peptides and finally to ammonia. Ammonia was the main product of the deamination of amino acids in the rumen and their adequacy to supply most of N for microbial growth and protein synthesis. Other researchers [19,20] who used a feed protein purified protein and NPN, further reported that the degradation of feed protein in the rumen depends on the solubility of proteins and soluble protein(NPN), which will be faster in the rumen acid hydrolyzed and deaminated will affect rumen fluid concentrations of NH_3. The combination of bacteria and enzymes lignocellulolytic in feed JPF4 and JPF5 contribute to improving the nutritional value of feed thus increasing the solubility of the protein degradation mainly increased rumen NH_3. The high concentration of NH_3 for JPF5 and JPF4 could be assumed to increase the ability of rumen microbes in feeds high fiber, and this was due to JPF5 and JPF4 treatment other than support high levels JPF5 and JPF4 feed as a source of N was also available carbon and energy units sufficient for synthesis of microbial feed.

The average concentration of NH_3 obtained from this study of 11.70 to 16.79mgN/100mL (Table 2), still within the normal range of rumen microbial growth. According to Wallace [21] who indicated optimal NH_3 concentrations between 2 and 20 mg NH_3 /l00 mL rumen fluid. The results were lower than those obtained by Eun et al [22] who reported the use of exogenous enzymes(cellulases and hemicellulases) on rice straw to a 24 hour incubation ammoniation produced NH_3 production of 49.1mg/L.

The results obtained the highest total VFA obtained feed JPF5 and JPF4 were significantly different ($P<0.05$) with the JPF3, JPF2 and JPF1(Table 2). Volatile Fatty Acid was a product of carbohydrate fermentation by rumen microbes in addition to other products, namely CH_4 and CO_2. Volatile Fatty Acid compositions of rumen fluid in the most were: acetic acid, propionic and butyric, while small amounts were

among others: formic acid, isobutirat, valerate, and isovalerat. Volatile Fatty Acid was the main energy source for the energy needs of ruminants. It was appropriate according to[11,12], VFA was an important source of metabolic energy for ruminants and the source of the carbon chain for microbial synthesis, because VFA was capable of supplying 50-60% of the energyneeds of livestock.

One effort that could be done to improve the nutritional value and degrade plant cellwall constituent compounds in the form of agro-industry waste was to utilize the help of enzymes. Lignocellulolytic enzymes consisting of ellulase and hemicellulase in particular could act as biokatalysator in elaborating cellulose and hemicellulose into simple compounds and decrease the value of crude fiber in the agro-industry waste. Lignocellulolytic enzymes group could ferment cellulose and hemicellulose to glucose and xylose by enzymatic processes, which then undergo fermentationin the rumen produce VFA. The high concentration of total VFA treatment on JPF5, JPF4, JPF3, JPF2 influenced by the high organic matter(OM) treatment that was higher than the JPF1. This means that the potential degradation of feed in the JPF5, JPF4 indicated that microbial activity in the fermentation process more digestibility in the rumen, resulting in a higher concentration of total VFA. Increased concentrations of total VFA was closely related to organic matter digestibility of feed. Total VFA produced from this study of 63.67-65.45mM/L was still higher than the results obtained[23] that was byfeeding rye grass and oat hay, producing total VFA of 59.3mM/L.

Proportion of acetate produced from this study of 38.03-44.74 mM/L was still lower than results obtained[24] with supplemental feeding of grains1% in the proportion of dairy cows, producing acetate 73mM/L. Proportion of propionic acid produced from this study of 10.80-14.90 mM/L were lower than the results obtained by Hervas et al [25] who reported feeding barley and maize in sheep produces propionic acid proportion amounted to24.2 and 24.3mM/L.

CONCLUSION

Lignocellulolytic enzyme utilization in rice straw brought about the decomposition of nutrients, affecting the rumen fermentation products in vitro. Addition of enzymes lignocellulolytic into rice straw improved significantly the production of volatile fatty acid and ammonia. An optimal products was achieved by addition 5% of enzyme level.

ACKNOWLEDGEMENT

This research was financed by DIPA BOPTN (Project Number: 001/UN3.6/KU/PPK/2013, May 2nd, 2013 through the foundation for Penelitian Unggulan Perguruan Tinggi (UPT), Universitas Airlangga.

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