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Nutritive Value Evaluation on Rumen Content and Sludge Fermented with *Cellulomonas* sp. as Rabbit Feed

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Abstract: Livestock feed availability is affected by more green field conversion to residential, industrial, and transportation areas. To anticipate the limited land for animal feed plants, and to reduce the environmental pollution. Waste utilization for animal feed has important and meaning for efficient farm development. Rumen content and sludge are wastes available in high number and even can contaminate the environment. Both animal wastes are rich in essential amino acid and potential as animal feed. The use of cellulolytic bacteria (*Cellulomonas* sp.) starter in the fermentation is expected to be able to degrade crude fibers and to increase protein content. This study aims to evaluate the nutritive value of rumen content and sludge fermented using *Cellulomonas* sp. that the best level of microbe addition and incubation to make feed matter mixture for rabbits. The study applied factorial experiments in completely randomized design with 4 replications. First factor was cellulolytic bacteria colony concentration (K), K_1 = 10^7 cfu/g dry matter, K_2 = 10^8 cfu/g dry matter, and K_3 = 10^9 cfu/g dry matter, respectively, and second factor was incubation period (L) at room temperature, L_1 = 6 days, L_2 = 8 days, and L_3 = 10 days, respectively. Results revealed that rumen content and sludge mixture fermented at the bacteria concentration of 10^7 cfu/g dry matter and 8-day incubation gave an optimal outcome based upon its nutrient content for rabbit feed. In addition, the use of these wastes could also solve quality and sustainable feed availability problems and reduce environmental pollution impacts.
Keywords: Rumen content, sludge, fermentation, *Cellulomonas* sp.

Introduction

As one of the most wastes produced in the slaughterhouse is rumen content, but it has not much been used yet and often considered as one of the matters that can disturb the environmental equilibrium since this waste often results in pollution due to unpleasant smell for the surrounding area. Rumen content is a digested food but unused by the livestock. Its nutrient composition is dry matter of 80.5%, crude protein of 8.1%, crude fibers of 38.02%, calcium of 0.37%, phosphorus of 0.26%, and 2.361 ccal/kg of metabolic energy[1]. Soepranianondo and Koesnoto found that cow's rumen content was feed matters in the rumen before turning to feces and released from the rumen after the animal was slaughtered. The nutrient of the rumen content is high enough because the food contained in the rumen are not absorbed yet so that the food is not quite different from that of the raw material[2].

Sludge is a by-product of biogas production in form of odorless black mud with a composition of 64.73% dry matter, 10.84% crude protein, 34.02% crude fiber, 2.00% crude fat, 16.84% ash, 3,305.84 ccal/kg

of gross energy, 52.54% ADF, and 74.12% NDF (product analysis of Animal Nutrition and Feed Laboratory, UB Malang, 2012). The composition of the dry solid waste and liquid waste from oxidation pond and the juice have pH above 7 [3]. This biogas waste still has good nutritive content and inexpensive price, but it has so far been merely used as plant fertilizer.

Rumen content and sludge are a useful nutrient-containing cattle wastes as livestock feed. The former has passed mechanically or biologically digestive process in the rumen and possesses protein of rumen microbial cell biomass. The latter comes from cow's feces that also contains endogenous N, proteins from rumen degradation, and undigested matters of the abomasum that are released as feces**.** The use of rumen content and sludge has a limiting factor as livestock feed, one of which has sufficiently high lignocellulose content causing low digestibility and in fresh condition, the rumen content and sludge still contain pathogenic microbes.

Fermentation is a hereditary method applied in food processing in order to raise the storability, improve the palatability and the digestibility, and increase the nutritive value[4]. *Cellulomonas* sp., as other bacteria, possesses various biochemical activities, such as growth and multiplication utilizing the nutrients from its surroundings, has also biological catalyst called enzyme. Enzyme held by *Cellulomonas* sp. is capable of degrading the cellulose. Yustianti stated that *Cellulomonas* sp. is significantly influential in degrading much cellulose-containing bagasse[5].

This study was aimed to obtain an optimum concentration of *Cellulomonas* sp. and incubation period in the fermentation process in order to increase the quality of rumen content and sludge mixture.

Materials and Methods

Materials

This study used cow's rumen content waste collected from the slaughterhouse Gadang, Malang, East Java, and sludge taken from biogas processing unit of dairy cow owner in Bocek, Karangploso District, Malang Regency. Rumen content and sludge were mixed in 50 : 50 ratio. Equipment used were *O''haus* balance to weigh feed sample matters analyzed, sack, scoop, tarpaulin, bucket, proximate and fiber analyzer kits.

Methods

The study used factorial experiments in completely randomized design with 4 replications. First factor was colony concentration (K) of cellulolytic bacteria, $K_1 = 10^7$ cfu/g dry matter, $K_2 = 10^8$ cfu/g dry matter, and $K_3 = 10^9$ cfu/g dry matter, respectively, and second factor was incubation period (L) at room temperature, $L_1 = 6$ days, $L_2 = 8$ days, and $L_3 = 10$ days, respectively.

Procedure

The study was done as follows: cattle wastes, rumen content, and sludge, were cloth-pressed for water removal and sun-dried to reach a water content of 10 – 15 %. After the wastes had dried, they were milled to minimize their size and be evenly mixed, then added with cellulolytic bacteria starter at the concentration of 10^7 cfu/g dry matter, 10^8 cfu/g dry matter, and 10^9 cfu/g dry matter, respectively, as treatment. Bacteria, *Cellulomonas* sp., culture contained a colony of 2.56×10^9 cfu/ml.

One kilogram of rumen content and sludge mixture (dry matter of 85.5%) contains dry matter of 85.5 / 100 x 1000 g = 855 g, so that each treatment requires bacteria starter of 855×10^7 cfu, 855×10^8 cfu, and 855×10^9 cfu, and thus, total milliliter of stock bacteria starter of each treatment was 3.33 ml (855×10^7 cfu divided by 2.56×10^9 cfu/ml), 33.3 ml (855×10^8 cfu divided by 2.56×10^9 cfu/ml), and 333 ml (855×10^9 cfu divided by 2.56×10^9 cfu/ml). For fermentation optimation effort, the mixture of rumen content and sludge (dry matter of 85.5 %) was conditioned in water content of 60 % (dry matter = 40 %) by adding water following the formula of $M_1C_1 = M_2C_2$ as follows: 1000 g of matter x 85.5% = M_2 x 40%, so that the amount of readily fermented rumen content and sludge mixture (M_2) was 2,136 g and the amount of water (ml) added in each 1 kg of air-dried rumen content and sludge mixture was 2,136 ml – 1,000 ml = 1,136 ml. The mixture was added

with ml of starter as each treatment by spraying and stirred up to be homogenous then put into a black plastic polybag, tightly strapped to be anaerobically conditioned, and incubated at room temperature for 6, 8, and 10 days, respectively[6].

Data Analysis

Data were tabulated and analyzed using a linear model of factorial Complete Randomized Design. Mathematical model was used to analyze the effect of 2 treatment factors as follows:

$$Yijk = \mu + \alpha i + \beta j + (\alpha\beta)ij + \epsilon ijk$$

If there is any effect, Duncan's Multiple Range Test was applied to see inter-treatment difference following Sudjana[7].

Results and Discussion

Effect of *Cellulomonas* sp. concentration on the nutritive content of rumen content and sludge mixture

The result showed that concentration treatment of the bacteria, *Cellulomonas* sp., colony demonstrated highly significantly different effect ($P<0.01$) on the content of protein, crude fiber, NDF, ADF, hemicellulose, and cellulose. The mean values of Duncan's Multiple Range Test are presented in Table 1.

Table 1. Effect of *Cellulomonas* sp. concentration on the nutritive content of the rumen content and sludge mixture.

Variable	Treatment (Concentration)		
	K_1	K_2	K_3
Protein (%)	12.18±0.30[a]	11.69±0.16[c]	11.94±0.36[b]
Crude fiber (%)	26.15±0.80[c]	29.13±1.59[b]	30.04±0.46[a]
NDF (%)	79.88±5.09[b]	80.98±7.95[a]	75.24±1.36[c]
ADF (%)	68.35±4.34[a]	68.35±6.58[a]	67.43±0.94[b]
Hemicellulose (%)	11.53±1.17[b]	12.39±1.37[a]	7.89±0.80[c]
Cellulose (%)	24.77±1.54[c]	26.69±2.71[a]	25.33±0.54[b]

Note: Different alphabet on the same row indicates highly significant difference ($P<0.01$)

Protein content of the rumen content and sludge mixture ranged from 11.69±0.16 to 12.18±0.30, in which the highest protein content during the incubation process was found in K_1 treatment, *Cellulomonas* sp. of 10^7 cfu/g, 12.18%, followed by K_3, *Cellulomonas* sp. of 10^9 cfu/g, 11.94%, and the lowest at K_2, *Cellulomonas* sp. of 10^8 cfu/g, 11.69%.

Crude fiber of the substrate ranged from 26.15±0.80 to 30.04±0.46 with the lowest in the substrate of *Cellulomonas* sp. of 10^7 cfu/g (K_1), 26.15 %. The low crude fiber content of K_1 reflects that the microorganisms work optimally in degrading the complex compound of the crude fiber to be simpler compound.

According to Russell, decrease in feed fiber content from fermentation of cellulolytic bacteria results from a number of cellulolytic bacteria in line with number of nutrient sources available so that no inter-microbial competition occurs, and then the microbes can grow optimally to better degrade the cellulose in food or otherwise, the cellulolytic bacteria could yield the cellulolytic enzyme capable of degrading the cellulose[8].

Mean NDF content was 80.98±7.95 in K_2, followed with 79.88±5.09 in K_1 and then 75.24±1.36 in K_3. This result reflects that NDF content increases from K_1 to K_2 as much as 1.37 % and decreases from K_2 to K_3 as much as 7.08 %. ADF content ranged from 67.43±0.94 to 68.35±4.34 in which the lowest was recorded in K_3, 67.43 %, while that of K_1 and K_2 had the same value, 68.35 %. Mean hemicellulose ranged from 7.89±0.80 to 12.39±1.37 in which the lowest occurred at *Cellulomonas* sp. concentration of 10^9 cfu/g (K_3).

Bacteria, *Cellulomonas* sp., concentration also revealed highly significantly different effect (P<0.01) on the cellulose content in which mean cellulose content was 24.77±1.54, 25.33±0.54, and 26.69±2.71, respectively. This fact indicates that the lowest cellulose content occurs in K_1, 24.77 %.

Effect of incubation duration on the nutritive content of rumen content and sludge mixture.

ANOVA showed that incubation period gave significant effect (P<0.01) on protein content, crude fiber, NDF, ADF, hemicellulose and cellulose content of the rumen content and sludge mixture. The mean value of Duncan's Multiple Range Test is shown in Table 2.

Table 2. Effect of incubation duration on the nutritive content of rumen content and sludge mixture.

Variable	Treatment (Incubation Time)		
	L_1	L_2	L_3
Protein (%)	12.03±0.30[a]	11,84±0,47[b]	11.94±0.20[ab]
Crude fiber (%)	28.50±1.27[b]	29,06±2,27[a]	27.75±2.17[b]
NDF (%)	81.43±7.72[a]	73.91±1.33[b]	80.76±4.43[a]
ADF (%)	70.77±4.56[a]	64.10±2.61[c]	69.41±3.06[b]
Hemicellulose (%)	10.66±2.75[b]	9.81±2.03[c]	11.34±1.83[a]
Cellulose (%)	26.70±2.63[a]	24.65±1.35[c]	25.45±1.04[b]

Note: Different alphabet on the same row indicates highly significant difference (P<0.01)

Protein content in 6-day incubation (L_1) shows a higher value than that in 8-day (L_2) or 10-day (L_3) incubation. It means that 6 days of fermentation is an optimal duration for the proteolytic bacteria to degrade the protein. Utama claimed that in the incubation process, the microorganisms will utilize the substrate nutrient to synthesize their body protein so that the proteolytic bacteria will eventually multiply and increase the crude protein of the feed matters[9]. Hamdiyah also found that cell growth will unlimitedly grow, but since the growth occurred through nutrient consumption and releasing metabolic product simultaneously, after a certain period of time, the growth will decline and finally stop[10]. The growth termination could result from a reduction of several essential nutrients in the medium and formation of metabolic products inhibiting the growth. Laesari and Purwadia who studied palm cake substrate found that protein content increment in the fermentation substrate could result from protein addition obtained from inorganic nitrogen conversion to cell protein during the microbial growth.

The optimum incubation period for cellulolytic microbes to degrade crude fibers during this incubation process was 10 days (L_3) in which mean crude fiber content was 29.06, 28.50, and 27.75, respectively. The lowest was recorded in 10-day incubation treatment. This decline reflects that *Cellulomonas* sp. can grow well in this substrate so that it can secrete the cellulose enzyme acting as a biocatalyst to break the glycoside bond of the crude fiber to be the simpler form[11].

There were several factors causing the decline of the crude fiber content: 1) during their life, the microbes will take this crude fiber as carbon source to meet their energy need; 2) during their life, the microbes will secrete the cellulose enzyme capable of breaking the crude fiber to be simpler compounds, such as cellulose-containing 2 molecules of glucose then hydrolyzed by beta glycosidase[12].

The lowest NDF content was recorded in 8-day incubation period (L2). It indicates that 8-day fermentation treatment is an optimum duration for the microbes to degrade this substrate fiber component.

ANOVA showed that incubation period had a highly significant effect (P<0.01) on ADF content. It reflects that incubation period has significant different effect in decomposing ADF in the substrate. Mean ADF content was 70.77, 64.1, and 69.41, respectively, in which the lowest was found at 8-day incubation treatment (L_2), then it increased to 69.41 at day-10 (L_3). It could result from that in early fermentation, the microbes actively used the nutrient content in the substrate for growth and enzymatic activity increment at day-8, but the substrate nutrient content was decreasing with time or ending so that it could not use for growth and enzymatic activity increment of the microbes.

Incubation period has a highly significant effect (P<0,01) on hemicellulose content. It reflects that incubation period possesses significant different effect in breaking the hemicellulose in the substrate. Mean hemicellulose was 11.34, 10.66, and 9.81 %, respectively, in which the lowest was found in 8-day incubation treatment (L_2), 9.81 %. Low hemicellulose content at 8-day incubation indicates that the activity and the number of bacteria exist at the optimum point where fast multiplication of hemicellulose fraction-degrading microorganisms occurs.

Incubation period treatment has a significant effect (P<0.05) on cellulose content. The highest mean cellulose content occurred at 6-day incubation treatment (L_1), 26.7, followed by 10-day incubation (L_3), 25.45, and the lowest at 8-day incubation (L_2), 24.65, respectively. It reflects that the microbes degrading the cellulose in the substrate were at the optimum point in 8-day incubation period.

Interaction effect of incubation period and *Cellulomonas* sp. concentration on nutrition content and fiber component

ANOVA showed that incubation period and *Cellulomonas* sp. concentration interaction gave highly significant effect (P<0.01) on protein content, crude fiber, NDF, ADF, hemicellulose and cellulose of the rumen content and sludge mixture.

Table 3. Interaction effect of *Cellulomonas* sp. concentration and incubation time on the nutrient content of rumen content and sludge mixture

Incubation Time	Concentration		
	K1	K2	K3
	Protein (%)		
L_1	12.26±0.16^{ab}	11.67±0.13^{cd}	12.16±0.13^{b}
L_2	12.45±0.14^{a}	11.56±0.12^{d}	11.51±0.17^{d}
L_3	11.83±0.07^{c}	11.85±0.07^{c}	12.14±0.23^{b}
	Crude fiber (%)		
L_1	27.06±0.36^{e}	28.48±0.31^{c}	29.95±0.32^{b}
L_2	26.12±0.29^{f}	31.20±0.33^{a}	29.86±0.45^{b}
L_3	25.27±0.15^{g}	27.69±0.16^{d}	30.30±0.58^{b}
	NDF (%)		
L_1	78.49±1.03^{c}	90.91±1.00^{a}	74.89±0.79^{e}
L_2	74.79±0.83^{e}	72.51±0.76^{f}	74.42±1.12^{e}
L_3	86.36±0.53^{b}	79.52±0.45^{c}	76.39±1.46^{d}
	ADF (%)		
L_1	68.28±0.90^{c}	76.83±0.85^{a}	67.19±0.71^{c}
L_2	63.34±0.70^{d}	61.62±0.65^{e}	67.34±0.01^{c}
L_3	73.42±0.45^{b}	67.32±0.39^{c}	67.49±1.29^{c}
	Hemicellulose (%)		
L_1	10.21±0.13^{f}	14.08±0.16^{a}	7.69±0.08^{h}
L_2	11.45±0.13^{d}	10.89±0.11^{e}	7.08±0.11^{i}
L_3	12.93±0.08^{b}	12.19±0.07^{c}	8.91±0.17^{g}
	Cellulose (%)		
L_1	25.03±0.33^{de}	30.24±0.33^{a}	24.83±0.26^{e}
L_2	22.87±0.25^{g}	25.69±0.27^{c}	25.38±0.38^{cd}
L_3	26.42±0.16^{b}	24.14±0.14^{c}	25.78±0.49^{c}

Note: Different alphabet on the same row and column indicates highly significant difference (P<0.01)

Mean protein content varied from 11.51% to 12.45 %. Duncan's Multiple Range Test indicated that the highest protein content was recorded at the interaction of 8-day incubation (L_2) and *Cellulomonas* sp.

concentration of 10^7 cfu/g (K_1), while the lowest crude protein, 11.51 %, was found at the interaction of 8-day incubation (L_2) and *Cellulomonas* sp. concentration of 10^9 cfu/g (K_3).

The result above could claim that the growth period of *Cellulomonas* sp. occurs at the stationary phase in 8 days and leads to mortality phase in 10 days. It could also result from that at early incubation, the microbes are actively utilizing the substrate nutrient content for growth and enzymatic activity increment, but the substrate nutrient content is decreasing with time.

Widjastuti et al., found that palm kernel cake substrate fermented using fungus, *Marasmius* sp. at a concentration of 5%, 7.5% and 10% under 2, 3, and 4-week incubation resulted in protein content decline even though the inoculum dose and incubation period increased, in which the highest protein content was recorded at the dose of 7.5% in 2-week fermentation[13]. Palupi and Imsya, also claimed that high percent of cellulolytic bacteria unbalanced with appropriate nutrient content could make the cellulolytic bacterial activity during the fermentation work slowly. Without complete nutrient content, the decomposition could not work optimally since the bacteria will not live and well develop[14].

Mean crude fiber content varied from 25.27 to 31.20 %, in which the lowest crude fiber was recorded in the substrate incubated for 10 days (L_3) at *Cellulomonas* sp. concentration of 10^7 cfu/g (K_1). According to Hernawati *et al.*, Decline in feed fiber content as an output of cellulolytic bacterial fermentation could be caused by the presence of number of cellulolytic bacteria appropriate to number of nutrient sources available that did not yield intermicrobial competition, and the microbes could optimally grow to degrade the cellulose better in the feed matter or otherwise, the cellulolytic bacteria are capable of yielding cellulose enzyme to degrade the cellulose[8].

Incubation period and waste type interaction gave highly significant effect ($P<0.01$) on NDF content, in which the NDF varied from 72.51 – 90.91 %. Duncan's Multiple Range Test indicates that the lowest NDF was found at the interaction of *Cellulomonas* sp. concentration of 10^8 cfu/g (K_2) and 8-day incubation (L_2), 72.51 %. The highest, 90.91 %, occurred at the interaction of *Cellulomonas* sp. concentration of 10^8 (K_2) and 6-day incubation (K_1). This study revealed that NDF content of each *Cellulomonas* sp. concentration treatment showed declining trend at day-8 and then rose. It could result from that at day-8 of the incubation, the microbes were actively using the substrate nutrient content for growth and raising the enzymatic activity, but the nutrient content of the substrate was decreasing with time.

ADF content varied from 61.62±0.65 to 76.83±0.85 with the lowest recorded at *Celulomonas* sp. concentration of 10^8 cfu/g (K_2) in 8-day incubation (L_2), 61.62%. ADF degradation is dealt with both NDF and hemicellulose content because ADF is the difference between NDF and hemicellulose.

Mean hemicellulose content ranged from 7.08±0.11 to 14.08±0.16. Duncan's Multiple Range Test indicates that the interaction of 8-day incubation (L_2) and *Cellulomonas* sp. concentration of 10^9 cfu/g (K_3) has the lowest hemicellulose content, 7.08 %. The decline of the hemicellulose content in this treatment could result from *Cellulomonas* sp. fermentation degrading the hemicellulose to simpler polymers, such as glucose, fructose, mannose, galactose, and arabinose.

The highest cellulose content was recorded in 6-day incubation (L_1) treatment and a *Cellulomonas* sp. concentration of 10^8 cfu/g (K_2), 30.24±0.33, and the lowest in 8-day incubation (L_2) and *Cellulomonas* sp. concentration of 10^7 cfu/g (K_1), 22.87±0.25. Eight-day incubation treatment and *Cellulomonas* sp. concentration of 10^7 cfu/g exhibited the lowest cellulose content in which crude fiber decomposition occurs that causes crude fiber fraction reduce and the cellulolytic enzyme produced during the incubation will break the cellulose to glucose.

Fermentation of rumen content and sludge mixture at *Cellulomonas* sp. concentration of 10^7 cfu/g dry matter and 8-day incubation gave a optimum product through improvement of nutrient content or fiber component, i.e. increase in crude protein to 12.45%, reduction of crude fiber to 26,12%, NDF of 74.79%. ADF of 63.34%, hemicellulose of 11.45%, cellulose of 22.87%,respectively.

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