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The Effect Fermentation of Local Agroindustry Waste Using Cellulolytic Bacteria Cellulomonas on Nutrient Content as Feed Stuff

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Abstract

This study aimed to evaluate fermentation of local agro-industry waste to produce CSCF as alternative feedstuff nutritious and palatable for rabbit. Experimental research method that uses a completely randomized factorial design 3x4 is cellulolytic bacteria colonies concentration and duration of incubation. Data were analyzed for variance and Duncan's Multiple Range Test (DMRT) to determine differences between treatments. The results showed that increasing the use of cellulolytic bacteria concentrations and the incubation time in the fermentation can improve CSCF quality with CP rose and lowering the content of CF, NDF, ADF and cellulose. The optimum fermentation CSC are using a treatment concentration of 10^8 cfu /g DM and incubation 8 days, the nutrient content of OM 92.64 % ; CP 11.55 % ; EE 31.29 % ; CF 17.34 % ; NDF 28.23 % ; ADF 18.33 % ; cellulose 8.89 % and lignin 7.13 %.

Keywords: Agro-industry waste, cellulolytic bacteria, nutrient content

1. Introduction

An alternative to reduce the cost of feed in the rabbit breeding intensification is the use of unconventional feedstuffs which is available enough in the roomy and the price is cheap from local agro-industry wastes such as coconut meat skin (*Cocos nucifera*), soybean seed coat (*Glycine max*) and cassava waste (*Manihot esculenta*) as pollard substitution and coconut oilcake so it can benefit farmers.

In the area of Batu and Malang Indonesia there are many agro-industry businesses like coconut milk, tempe and tapioca flour industries. The DM production of organic waste is abundant reached 5230.07 kg/day and there is still thrown away so it can reduce the aesthetic environment, but potentially as a constituent of a rabbit complete feed. A combination of 60 % SCM, 20 % SSC and 20 % CW called CSC is suitable as a feed of energy source, but all three waste so high CF content varies 14.7 % - 26.5 % (Ali, 2011).

In order to make the agro-industrial wastes powerful, not rancid and more palatable for rabbits it is necessary to be fermented and stored dry condition according to Purwadaria and Sari (2004). The fermentation of CFC use bacteria cellulolytic *Cellulomonas* produces enzymes that work synergistically to decompose CF and anti nutrients into metabolites materials, nitrogen cell addition, various digestive enzymes and palatable feed products. This fermentation process is expected to replace the feed fermentation activity in the rabbit caecum thus improving feed efficiency and nutrient retention. This study aims to determine the level of concentration of colonies cellulolytic bacteria *Cellulomonas* and the incubation time in fermentation local agro-industry waste to produce CSCF high protein and low CF, NDF and cellulose.

2. Methods

2.1 Procurement of Materials

SCM collection is taken from coconut industry, SSC from *Tempe* industry and CW from manufacture of tapioca flour. This organic waste separately sun dried for 3 - 4 days to moisture content 14 %, all materials are milled to get the same particle size. Then took CSC as a sample of the material to be analyzed nutrient content and formulated from 60 % SCM, 20 % SSC and 20 % CW. Starter bacteria cellulolytic using *Cellulomonas* from isolates organic compost with containing colonies 2.56 x 10^9 cfu/ ml.

2.2 Experiment methods

This study use an experiment with Completely Randomized Design (CRD) of factorial 3x5 repeated 3 times. The first factor, the concentration (C) cellulolytic bacterial colonies: C_1=10^7 cfu/g DM; C_2=10^8 cfu/g DM and C_3=10^9 cfu/g DM, factors II, incubation periods (I) at room temperature: I_2= 2 days, I_4= 4 days, I_6= 6 days and I_8= 8 days and I_{10}=10 days we're according to Muwakhid et al (2007).

Variables measured are the CSCF nutrient content includes of organic material (OM), crude protein (CP), ether extract (EE), crude fiber (CF) according to AOAC (1990), whereas neutral detergent fiber (NDF), acid detergent fiber (ADF), cellulose and lignin (Van Soest and Robertson, 1985).

2.3 Statistical analysis

The data were statistically analysed using the analysis of variance (ANOVA). The significant differences between treatment means were separated using DMRT according to Steel and Torrie (1992).

3. Results and Discussion

3.1 Nutrien content of OM, CP, EE and CF

The results show that the concentration of cellulolytic bacteria colonies and incubation period in fermentation are highly significant (P<0.01) to the content of OM, CP, EE and CF.

Table 1. Content Rate of OM, CP, EE and CF in SSCF (DM)

Treatment	% OM	% CP	% EE	% CF
Concentration				
$C_1(10^7$cfu/g DM)	94,37 ± 1,23 ^{b}	10,29 ± 0,30^a	31,42 ± 0,13^b	19,04 ± 1,88^b
$C_2(10^8$cfu/g DM)	93,57 ± 1,27 ^{a}	10,81 ± 0,83^b	31,33 ± 0,19^a	18,56 ± 1,72^a
$C_3(10^9$cfu/g DM)	92,96 ± 1,38 ^{a}	11,38 ± 1,25^c	31,27 ± 0,25^a	18,39 ± 1,77^a
Incubation				
I_2 (2 days)	95,21 ± 0,95 ^{c}	10,41 ± 1,21^a	31,44 ± 0,06^c	20,14 ± 0,77^d
I_4 (4 days)	94,74 ± 1,45 ^{c}	10,58 ± 0,91^{ab}	31,39 ± 0,09^{bc}	19,47 ± 0,85^c
I_6 (6 days)	93,34 ± 1,39 ^{b}	10,78 ± 0,78^b	31,33 ± 0,12^b	18,73 ± 0,91^b
I_8 (8 days)	92,62 ± 1,32 ^{a}	11,16 ± 0,99^c	31,31 ± 0,15^{ab}	17,57 ± 1,68^a
I_{10}(10 days)	92,27 ± 1,32 ^{a}	11,18 ± 1,08^c	31,23 ± 0,21^a	17,39 ± 1,07^a

Description: The different superscript in the same column indicates the difference among treatments

The increasing concentration of bacteria *Cellulomonas* and the longer incubation in fermentation CSC reduce the content of OM, EE and CF, but the content of CP raises. The lowest content of OM, EE, CF is at C_3, sequentially 92.96 %, 31.27 %, 18.39 % and at the long incubation of 10 days 92.27 %, 31.23 % and 17.39 %, while the value of OM, EE and CF highest at C_1 consecutive 94.37 %, 31.42 %, 19.04 % and the incubation period for 2 days are consecutive 95.21 %, 31.44 % and 20.14 %. The nutrient decrease is caused by hydrolytic activity of bacteria during fermentation to decompose complex compounds into simpler forms. Cellulolytic bacteria secrete cellulase enzymes breaks down cellulose into glucose and cellubiose which can provide nutrients for livestock (Cai et al, 1999). Cellulase enzymes degrade cellulose which is a component of CF, the C_3 and the longer incubation results decreasing of CF 21.0 % to 18.39 %. The content of OM decreases with changes in the value of EE, CF and CP because the three components belong to OM other than NFE.

The increasing content of CP on CSCF products in line with the increasing concentrations of bacterial colonies and incubation period caused by the addition of nitrogen from biomass cells bacterial during the incubation that grows and thrive. On the concentration of C_3 and incubation period is longer, so increasing bacterial biomass can lead to increase nitrogen and CP content. Sutrisno et al (2005) reported that the fermentation of tea waste increases the content of CP caused by biomass increasing of microbial cell which is rich in protein. Generally, microbial cell contains of CP 31 - 78 % which can be used as an alternative protein source for livestock (Triwiyono, 1996).

The lowest content CP Pat C_1 10.29 % and C_3 is the greatest 11.38 % and on I_{10} 11.18%, it means that the fermentation treatment can improve the content of CP to 12.58 % (CP 10.11 % before fermentation). The increasing of CP on CSCF is still smaller compared than Rokhmani research results (2005) reported that fermentation cassava waste using *Aspergillus niger* fermentation product yield of CP 15 %. This is due to fermentation using additional media inorganic nitrogen such as urea and ammonium sulfate minerals, besides that it can get additional nitrogen fixation N and biomass of microbial cells that grow during fermentation.

The results of DMRT shows that between C_3 and C_2 influence equal on the content of OM, EE and CF of CSC except the CP and C_1, are different and incubation period I_{10} and I_8 influence equal on the content of OM, CP, EE and CF and different I_2, I_4 and I_6. Furthermore the treatment combination is the most effective and economical is

C_2I_8 the interaction between concentration 10^8 cfu/g DM materials and 8 days of incubation, therefore it is suggested to get the best CSC fermentation using cellulolytic bacteria concentration of 10^8 cfu/g DM materials were incubated at room temperature for 8 days, in addition to the anaerobic fermentation can prevent spoilage microbial contamination. The interaction between concentration and duration of incubation was highly significant ($P<0.01$) on the content of CP but on the nutrient content of OM, EE and CF are not significant ($P>0.05$).

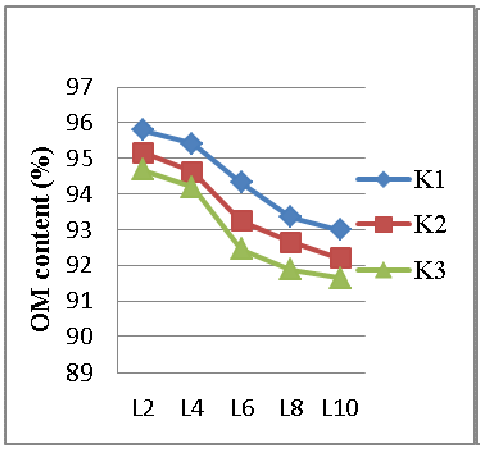

Figure1. The content of OM

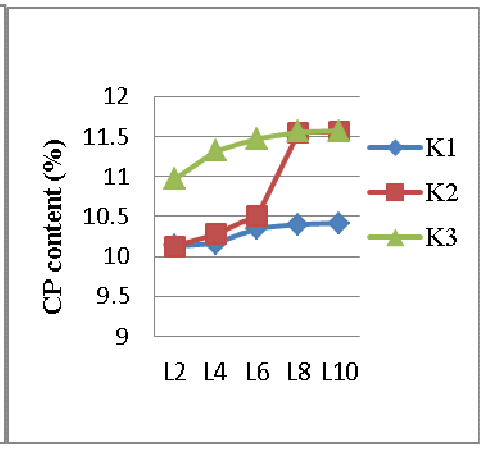

Figure 2. The content of CP

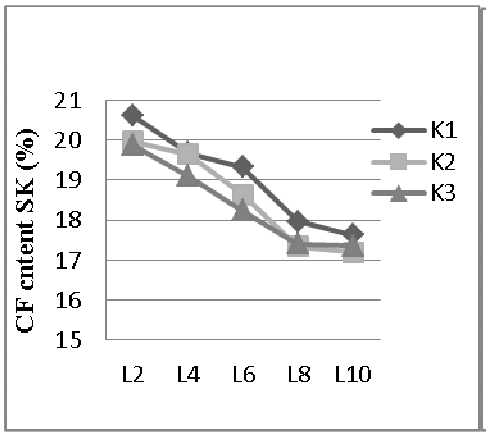

Figure 3. CF contant

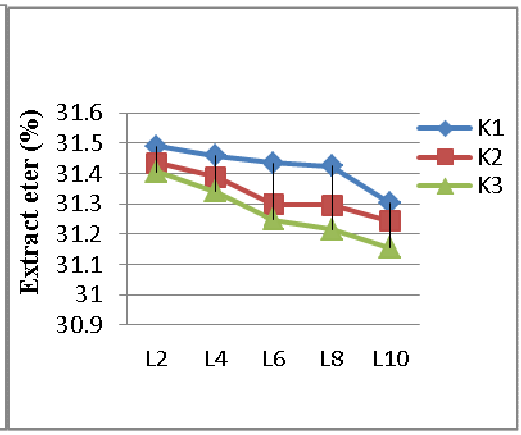

Figure 4. EE contant

Based on the picture above shows that there is no interaction between treatment concentration and duration of incubation on the content of OM, EE and CF, but CP being in the womb is significant interaction. The increasing concentrations of bacterial colonies (10^7-10^9 cfu /g DM materials) and long incubation 2-10 days reduce the content of OM, EE and CF, but the CP increases. Increasing concentrations of bacterial colonies and the use of long incubation, the enzyme excreted growing so it will increase the concentration of the enzyme. Increasing the concentration of the enzyme activity is increasing, so the faster and more degradated substrate can be simpler components according to Badriyah and Ali (2010).

3.2 The content of NDF. ADF, Cellulose and Lignin in CSCF

The results showed that the concentration of cellulolytic bacteria and long incubation significant effect on the content of NDF, ADF and cellulose, but not in lignin content. Concentration combination treatment of bacterial colonies and long incubation non significant effect ($P>0.05$) on the content of NDF, ADF, cellulose and lignin. The average content of NDF, ADF, cellulose and lignin in CSCF are presented in Table 2.

Table 2. Content rate of NDF, ADF, Cellulose and lignin in CSCF

Treanment	NDF (%)	ADF (%)	Cellulose (%)	Lignin (%)
Concentration				
C_1(10^7cfu/g DM)	30,18 ± 1,57^b	19,59 ± 1,48^b	10,10 ± 0,97 ^{b}	7,14 ± 0,04
C_2(10^8 cfu/g DM)	29,35 ± 1,29^a	19,33 ± 1,40^{ab}	09,81 ± 1,15^{ab}	7,13 ± 0,03
C_3 (10^9 cfu/g DM)	29,32 ± 1,03^a	19,16 ± 1,47^a	09,64 ± 1,13^a	7,13 ± 0,02
Incubation				
I_2 (2 days)	31,45± 0,01^d	20,61 ± 0,29 ^{d}	11,23 ± 0,09 ^{d}	7,14 ± 0,03
I_4 (4 days)	30,50 ± 0,67^c	20,13 ± 0,36^c	10,66 ± 0,46 ^{c}	7,14 ± 0,02
I_6 (6 days)	29,27 ± 0,82^b	19,47 ± 0,53^b	09,81 ± 0,43 ^{b}	7,13 ± 0,03
I_8 (8 days)	28,56 ± 0,52^a	18,39 ± 0,34^a	09,02 ± 0,54^a	7,13 ± 0,03
I_{10} (10 days)	28,29 ± 0,25^a	18,20 ± 0,19^a	08,53 ± 0,30^a	7,13 ± 0,02

Description: Superscript different in the same column indicate difference among treatments

The concentrations and long incubation cellulolytic bacteria fermentation CSC increasing can decrease the content of NDF, ADF, cellulose and lignin CSCF. The content of NDF, ADF and cellulose lowest at C_3, sequentially 30.18 %, 19.59 % and 10.29 % and the greatest value in C_1 respectively 29.32 %, 19.16 % and 11.38 %. Factor of the long incubation medium content of NDF, ADF and cellulose long incubation lowest in 10 days consecutive 28.29 %, 18.20 % and 10.41 %, while the biggest in a long time 2 days incubation respectively 31.45 %, 20.61 % and 11.19 %.

It shows that low bacterial concentrations and short incubation time so the activity of complex fiber substrate degradation have not been up it means that the quality products fermentation CSCF unfavorable. According to Cai et al (1999) microbial activity of cellulolytic secrete enzymes endoglucanase or carboxy methyl cellulose (CMC-ase), exoglucanase and β-glucosidase. The three types of this enzyme synergistically degrade cellulose to glucose. CMC-ase enzymes break down the hydrogen bonds in the crystal structure of cellulose to form individual cellulose chains, exoglucanase cuts the edge individual cellulose chains to produce disaccharide and tetra saccharide such as cellobiose and β-glucosidase hydrolyze disaccharide and tetra saccharide into glucose.

The treatment effect at C_3 and C_2 and C_1 to different levels of NDF, ADF and cellulose. Substrate NDF, ADF, cellulose and lignin as a constituent of plant cell wall hemicelluloses and silica addition. The presence of cellulose and hemicelluloses in feed stuffs can inhibit the enzymatic digestion of the body, but it can be degraded by microbial activity microbial *cellulomonas* as cellulolytic bacteria that secrete the enzyme cellulose. Lowering the content of NDF, ADF and cellulose with increasing concentrations of bacterial colonies and long incubation, it shows that the activity of cellulolytic bacteria decomposing fiber happens continuously during fermentation can remodel extracellular bacteria cellulose, hemicelluloses and lignin, because the fraction of water insoluble fiber according to Blanco et al (1999). Peres et al (2002) reported that two types of microbes working system hydrolytic extracellular enzymes that produce hydrolase system that responds to revamp cellulose and hemicellulose, both systems unique extracellular oxidative and lignolitic for lignin polymerization. Hemicellulose as a component of the NDF is a heterogeneous polysaccharide linked cellulose and lignin in the cell walls of plants and some types of microbes able to remodel into sugars and acetic acid. Xylan is the major constituent of hemicelluloses carbohydrates (Maillet et al, 2004). Overhaul of hemicelluloses requires various hydrolytic enzymes are endo-1,4-β-xylanase (endoxylanase), acetylesterase, α-glucuronidase and β-xylosidase, the enzyme will randomly attack the glycosidic bond.

Treatment of non significant effect on lignin content, this is due to lignin has a glycosidic bond that is difficult to degrade by microbial enzymes. Flagel and Meetivison (1988) stated that lignin is a polymer of phenilprophyl form complex connective tissue is water resistant, amorphous and bind strongly to polysaccharides, cellulose and hemicelluloses in plant cell walls and is the perfect protection materials. Lignin is the most important factor in limiting the availability of food for herbivorous and anaerobic digestion systems. Lignin polymer forma three dimensional structure of phenyl propane units are structure coumeryl alcohol, conyferyl alcohol and sinaphyl alcohol. Lignin is found in many woody stems and grasses that streng then the stem.

4. Conclusion

a. Concentration increasing of cellulolytic bacteria colonies and incubation time of 2 to10 days in CSC fermentation can improve the feed quality with content of CP and enzyme activity rose, lowering the content of CF, NDF and cellulose
b. Fermentation which is effective and economical use colony concentration 10^8 cfu /g DM materials and long incubation with 8 days of CSCF containing of CP 11.55 % ; CF 17.34 % ; NDF 678 %; 54.98 % ADF and cellulose 25.67 %.

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