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The Effect of Combination Indigenous Microbes Isolated from Waste Market to Improve Quality of Coconut Dreg as Animal Feeding

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Abstract : The purposed of the research was to find the combination of indigenous microbes and fermentation time that can be increase of quality such as crude protein, crude fiber and crude fat of coconut dreg. The design used is Completely Randomized Design (RAL) factorial pattern. The treatment of this research includes two factors namely factor A consists of 4 combinations indigenous microbes (A1: 80% *Rhizopus* sp + 10% *Lactobacillus* sp + 10 *Yeast*; A2: 70% *Rhizopus* sp + 20% *Lactobacillus* sp + 10 *Yeast* sp; A3: 60% *Rhizopus* sp + 30% *Lactobacillus* sp + 10 *Yeast* sp; A4: 50% *Rhizopus* sp + 40% *Lactobacillus* sp + 10 *Yeast* sp) sp and B factor consists of six of time fermentation (0, 2, 4, 6, 8 and 10 days) The results of the research showed that the combination of indigenous microbes and fermentation time give significant effect to crude protein, crude fiber and crude fat ($P<0.05$). The best treatment that produce of highest protein and lowest fiber and fat was A3B6 (60% *Rhizopus* sp + 30% *Lactobacillus* sp + 10 *Yeast* sp and fermentation time for 6 day). The research can be conluded that the best treatment was A3B6 which was 9.41% of crude protein, 9.88 % of crude fiber and 7.28% crude fat of coconut dreg.

Introduction

Indigenous Microorganisms (IMO) is called beneficial microbes because it inhabits the soil and the surfaces of all living things, inside and out. IMO is involved indifferent processes such as fermentation, decomposition, nitrogen fixation, and nutrient fixation. It aids in the assimilation of the plants. It enables better nutrient absorption and hence healthier plant growth. IMO is said to be cheap because of the availability of the materials in cultivating it. It can be collected in rice or in fermented vegetables like mustard. A large diversity of IMO can be found in forests, bamboo groves and areas with thick accumulation of plant residues. The introduction of IMO as substitute to chemical fertilizer in the production of tomato would lessen the use of these hazardous chemicals.

Indigenous Micro Organisms (IMO) are naturally occurring microbes that has adapted to the environmental condition where they are found and as such, are capable of accelerating rapid decomposition of organic materials found in the same location. Singh and Sharma (2003) [1], inoculated various kinds of wastes (mixed solid waste, municipal solid waste and horticultural waste) with different micro-flora. Acceleration of decomposition of crop residues high in lignin with the application of IMO has been reported [2]. Microbial inoculation in relation to waste decomposition for agricultural production offers the advantage

of releasing essential compounds stored in plants and animal waste to a stable state that can be used again for plant growth [3]. As reported by [4], microbial inoculants are vital component in the agro-ecosystems as they play an important role in reducing indiscriminate use of chemical fertilizers and offers farmers an attractive economically acceptably substitute for improving soil properties. Microbial inoculant produces metabolites that facilitate decomposition of organic waste and increase nutrient quality [5]. The nutrient status of sorghum stalk and wheat straw compost was improved after inoculation with *Aspergillus niger* and *Penicillium* spp. [6].

Indonesia is the world's second largest producer of copra. Indonesian coconut dreg production were predicted to be increased 520.000 tons (3.2%) from 1.56 to 1.58 million tons in 2013/2014 (USDA 2013). Coconut dreg is coconut residual cake that discharges as a byproduct in the process of oil extraction which is abundantly available and has a quite competitive price (Sundu & Dingle)[7] . Coconut dreg has considerable potential as a source of protein and carbohydrates, but cannot be fully utilized as feed ingredients for monogastric animals (Mendoza *et al.*) [8]. Limited use of coconut dreg is due to high level of non-starch polysaccharides (Purwadaria *et al.*) [9]. Therefore, it is important to manage coconut dreg as feedstuffs for animal feed formulas. Such us the increase of the coconut dreg quality to be fully utilized as feed ingredients. In this research we will try to see effect of combination of indigenous mirobes isolated from waste market to improve coconut dreg quality.

Materials And Methods

Coconut Dreg and Physico-Chemical Analysis.

The coconut residual cakes, usually called coconut dreg, were collected from Pasar Alai, Padang, West Sumatera. Coconut dreg as a carbon source for medium fermentation. Coconut dreg was milled and dried by sun drying for 12 h. The particle size of coconut dreg was 100 mesh. Coconut dreg physico-chemical properties including: moisture, protein, lipid, crude fibre and ash contents of the isolated samples were determined using approved methods (AOAC 1995)[10].

Isolation of Indigenous Microbes.

Indigenous Microbes was isolated from waste market carried out using Nutrient broth, Potato Dextrose Agar and MRS agar medium approved methods by (Yetti *et al.*, 2016)[11]

Combination of Indigenous Microbes

The selected microorganisms are developed into a liquid medium of coconut water. To make a 250 ml medium of coconut water in a glass of erlemeyer is added 5% palm sugar and 1% salt. Then sterilized in autoclav for 2 hours at 120 ^{0}C, and cooled to 37 ^{0}C. Each microbe-starter was taken as many as 3 needles from the test tube tilted and inserted into elemeyer glass and closed tightly. Fermentation mediums each of which have been inoculated (*Rhizopus* sp, *Lactobacillus* sp, *Yeast*) were incubated for 3 days. Incubation is carried out at 30 ° C with shaker at 250 rpm. If it already looks cloudy water means the microbial-starter is growing, is for mold will look.

Research Method

The treatment of this research includes two factors namely factor A consists of 4 combinations indigenous microbes (A1: 80% *Rhizopus* sp + 10% *Lactobacillus* sp + 10 *Yeast*; A2: 70% *Rhizopus* sp + 20% *Lactobacillus* sp + 10 *Yeast* sp; A3: 60% *Rhizopus* sp + 30% *Lactobacillus* sp + 10 *Yeast* sp; A4: 50% *Rhizopus* sp + 40% *Lactobacillus* sp + 10 *Yeast* sp) sp and B factor consists of six of time fermentation (0, 2, 4, 6, 8 and 10 days)

Results and Discussions

Effect of Treatment to Crude Protein

In Table 1, it can be seen that the crude protein content of fermented coconut dreg using various combinations of indiginous microbes with different fermentation times can be seen that the crude protein

content of fermented coconut dreg ranged between 4.81 to 9.14%. The highest combination was found in combination of A3B6 microbial-starter treatment mixture (Rhizopus sp 60%, Lactobacillus sp 30%, Yeast 10%) with a 6 day fermentation times of 9.41%, and the lowest was from combination of A3B0 treatment microbial mixer Rhizopus sp 60%, Lactobacillus sp 30%, Yeast 10%) without fermentation.

The analysis of result showed that the combination of starter microbe did not give significant different effect (P> 0,05), the fermentation time had significantly different effect (P <0,05) while interaction between fermentation length and combination of starter microbe gave significant different effect (P < 0.05) to the crude protein content of the fermented coconut dreg (Appendix 6). Further Tukey-HSD test showed that the combination treatment of A3B6 (Rhizopus sp 60%, Lactobacillus sp 30%, Yeast 10%) with 6-day fermentation time was not significantly different (P> 0.05) on crude coconut protein content compared to combination Treatment of A3B8 (Rhizopus sp 60%, Lactobacillus sp 30%, Yeast 10%) with fermentation time 8 days, A4B6 (Rhizopus sp 50%, Lactobacillus sp 40%, Yeast 10%) with 6 days of fermentation, A2B6 (Rhizopus sp 70 %, Lactobacillus sp 20%, Yeast 10%) with 6 days of fermentation, A2B8 (Rhizopus sp 70%, Lactobacillus sp 20%, Yeast 10%) with 8 days of fermentation, A3B10 (Rhizopus sp 60%, Lactobacillus sp 30% , Yeast 10%) with 10 days fermentation time, and A4B8 (Rhizopus sp 50%, Lactobacillus sp 40%, Yeast10%) with fermentation time 8 days. Conversely, it gives a tangible effect (P <0.05) higher than with other treatment combinations.

Table 1. The Effect of Microbial-starter Combinations (*Rhizopus* sp, *Lactobacillus* sp, *Yeast*) with Different Fermentation times to Crude Protein (%)

Microbes combination	Times (Day)						Average
	B0	B2	B4	B6	B8	B10	
A1	4,99^{H}	7,32^{DEFG}	7,62^{CDEFG}	7,91^{BCDEFG}	7,89^{BCDEFG}	6,98^{FG}	7,12
A2	4,90^{H}	7,32^{DEFG}	7,88^{BCDEFG}	8,47^{ABCD}	8,24^{ABCDE}	7,04^{FG}	7,31
A3	4,81^{H}	5,37^{H}	7,27^{EFG}	9,14^{A}	8,81^{AB}	8,11^{ABCDEF}	7,25
A4	4,88^{H}	6,76^{G}	7,24^{EFG}	8,68^{ABC}	7,98^{ABCDEF}	7,23^{EFG}	7,13
Average	4,89^{d}	6,69^{c}	7,50^{b}	8,55^{a}	8,23^{a}	7,34^{b}	

Note: Superscripts with different letters between treatments showed significantly different effects (P <0.05)

In this study, it was seen that the combination with reduced percentage of *Rhizopus* sp from 80% to 60%, or vice versa, increased percentage of *Lactobacillus* bacteria 10% to 40% with increasing fermentation time up to 6 days, increased the crude protein content of coconut dregs to 9, 14%. The results of this combination were higher than single-microbial inoculum-starter using only *Rhizopus* sp alone 8.89%, *Lactobacillus* sp 7.29% and *Yeast* 6.86%, but with the increase of fermentation time to 10 days, the crude protein content of coconut dreg decreased.

Increased protein content crude coconut dregs, this happens because the factors that affect the increase in crude protein in the fermentation of a substrate is the number of micro-organisms of body proteins that are formed. The more bacteria that grow, the more protein produced. In line with the opinion of Suriani *et al* [12] which said the increased number of bio-mass cells will increase the substrate protein level. The enzyme produced by micro-organisms during the 6-day fermentation period also causes an increase in the crude protein content produced by the fermented coconut pulp. This is supported by the opinion of Ratledge (1994)[13] said that the increase in protein content of fermentation products caused by microbes and enzymes, because microbes are a source of protein ie single cell protein. Furthermore (Buckle *et al*., 1987)[14] added the process of fermentation of foodstuffs produce some advantages such as improving the quality of food from both nutritional aspects digestibility, but it also increases the shelf life. Fermented foods usually have higher nutritional value than their original ingredients. This is because microbes are catabolic or break down complex components become simpler and more easily digested. (Winarno *et al*., 1997)[15]. The content of amino acids, fats, carbohydrates, vitamins and mineral materials undergoes changes after fermentation. This is due to the activity and development of microorganisms during fermentation (Al-Ahmad)[16].

The results also showed that, the mixture of microbial-starter mixtures in terms of protein content is higher than single microbial starter as inoculum in coconut dreg fermentation. This suggests that there is a synergistic and mutually beneficial work mechanism between molds, bacteria and yeasts in the product. Where molds, bacteria and yeast make a reshuffle to the compounds contained in the coconut pulp, and the end product produced is a source of energy and nitrogen source for mold growth, bacteria and other yeast. So there is no mutual contradiction between microbes with each other.

Effect of Treatment to Crude Fiber

The results of the research on the effect of combining the mixture of microbial starter with different fermentation time on the crude fiber content of coconut dreg can be seen in Table 2. The crude fiber of the lowest fermented coconut dreg 9.88% was found in combination of A3B6 treatment (*Rhizopus* sp 60%, *Lactobacillus* sp 30%, *Yeast* 10%) with 6 days of fermentation, and the highest in combination of A4B0 treatment (*Rhizopus* sp 50%, *Lactobacillus* sp 40%, *Yeast* 10%) without fermentation.

The analysis of result showed that the combination of starter microbe gave significant different effect (P <0,05), the fermentation time had significantly different effect (P <0,05) while interaction between fermentation length and combination of starter microbe gave significant different effect (P <0 , 05) to the crude fiber content of the fermented coconut dreg.

Table 2. The Effect of Microbial-starter Combinations (*Rhizopus* sp, *Lactobacillus* sp, *Yeast*) with Different Fermentation times to Crude Fiber (%)

Microbes combination	Times (Day)						Average
	B0	B2	B4	B6	B8	B10	
A1	21,67^{A}	16,04^{BC}	15,39^{BCD}	9,82H	10,85^{FGH}	11,88^{DEFGH}	14,28^{ab}
A2	21,88^{A}	15,44^{BCD}	14,39^{BCDEF}	11,32^{EFGH}	11,94^{DEFGH}	10,05^{H}	14,17^{ab}
A3	20,74^{A}	14,88^{BCDE}	11,57^{EFGH}	9,88^{H}	10,61^{GH}	12,17^{DEFGH}	13,31^{b}
A4	22,53^{A}	16,85^{B}	13,98^{BCDEFG}	10,99^{FGH}	10,99^{FGH}	13,19^{CDEFGH}	14,76^{a}
Average	21,71^{a}	15,80^{b}	13,83^{c}	10,50^{d}	11,10^{d}	11,82^{d}	

Note: Superscripts with different letters between treatments showed significantly different effects (P <0.05)

Further Tukey HSD Test Results between treatments, combination of A3B6 factor treatment (*Rhizopus* sp 60%, *Lactobacillus* sp 30%, *Yeast* 10%) with a significantly different 6-day fermentation length (P <0.05) with combination of A1B0 factor treatment (*Rhizopus* Sp 80%, *Lactobacillus* sp 10%, *Yeast* 10%) with no fermentation, A2B0 (*Rhizopus* sp 70%, *Lactobacillus* sp 20%, *Yeast* 10%) with no fermentation time, A3B0 (*Rhizopus* sp 60%, *Lactobacillus* sp 30% *Yeast* 10%) with no fermentation time, A4B0 (*Rhizopus* sp 50%, *Lactobacillus* sp 40%, *Yeast* 10%) with no fermentation time, A1B2 (*Rhizopus* sp 80%, *Lactobacillus* sp 10%, *Yeast* 10%) with fermentation length 6 Day, A2B2 (*Rhizopus* spv70%, *Lactobacillus* sp 20%, *Yeast* 10%) with 2 days of fermentation, A3B2 (*Rhizopus* sp 60%, *Lactobacillus* sp 30%, *Yeast* 10%) with 2 days of fermentation, A4B2 (*Rhizopus* sp 50% *Lactobacillus* sp 40%, *Yeast* 10%) with 2 days of fermentation, A1B6 (*Rhizopus* sp 80%, *Lactobacillus* sp 10%, *Yeast* 10%) with fermentation time 6 days, A2B6 (*Rhizopus* sp 70%, *Lactobacillus* sp 20%, *Yeast* 10%) with 6 days fermentation time, and A4B6 (*Rhizopus* sp 50%, *Lactobacillus* sp 40%, *Yeast* 10%), but different not significant with combination other treatment (P <0.05) on crude fiber of coconut dreg.

The 6-day fermentation period is the best time to reduce the content of the crude protein of coconut dreg. Compared to the same time using a single microbial-starter inoculum, the coarse fiber content of coconut dreg is higher in content. The coarse fiber content of coconut dreg with single microbial-starter inoculum with 6 day fermentation time is *Rhizopus* sp 9.96%, *Lactobacillus* sp 12.97% and *Yeast* 9.72%.

The reduction in the percentage of Rhizopus sp in mixed combinations (Rhizopus sp, Lactobacillus sp and Yeast), the crude fiber content of the fermented coconut pulp also decreased to the lowest crude fiber

content of 9.88%. The decrease of crude fiber content to 6 days fermentation time is also characterized by the most optimal enzyme activity which digested the crude fiber of coconut pulp, ie Manannase 4.42 U / ml, cellulose 2,48 U / ml and Xylannase 3,39U / ml. The result showed that Mannanase activity was higher than the activity of cellulose enzyme and xylanase. According Kansoh (2004)[17] states that the coconut dreg contains galactomannan of 61% which also can lower blood cholesterol levels.

Effect of Treatment to Crude Fat

The crude fat of coconut dreg fermented can be seen in Table 3. Table 3 showed that the highest crude fat content was found in combination of A4B0 treatments (Rhizopus sp 50%, Lactobacillus sp 20%, Yeast 10%) without fermentation was 33.63%. While the lowest on the combination of treatment A1B8 (Rhizopus sp 80%, Lactobacillus sp 10%, Yeast 10%) with 8 days fermentation time yielded a crude fat value of 7.28%. The result of analysis showed that the combination of starter microbe gave significant different effect (P <0.05), the fermentation time gave no significant effect (P> 0.05) while the interaction between fermentation length and the combination of starter microbe gave significant different effect (P < 0.05) to the crude fat content of the fermented coconut dreg.

Further Tukey-HSD test results between treatments resulted in no significant differences (P> 0.05) between a combination of A2B0 factor treatment (Rhizopus sp 70%, Lactobacillus sp 20%, Yeast 10%) with no fermentation time, compared with A1B0 (Rhizopus Sp 80%, Lactobacillus sp 10%, Yeast1 0%) with no fermentation time, A3B0 (Rhizopus sp 60%, Lactobacillus sp 30%, Yeast 10%) with time without fermentation and A4B0 (Rhizopus sp 50%, Lactobacillus sp 40% , Yeast 10%) with no fermentation time. In contrast, the combination of other treatments was significantly different (P <0.05) against the coarse fat of coconut dreg.

The results also showed that the combination of treatment with the longer fermentation time and the increasing percentage of Lactobacillus sp with the percentage of Yeast is fixed, the crude fat content decreases. Enzyme lipases produced by microbial-starter especially *Yeast* can break down fat to fatty acid and glycerol, then fatty acid and glycerol used by microbial starter as energy source for its growth process. Purwadaria *et al*. (1995)[9] reported that fermentation of steamed coconut cake by using *A. niger* for 4 days can decrease crude fat content by 63.89%. Added by Kurniawan *et al*. (2016)[18], which states that *Aspergillus niger* produces the highest lipase enzyme in the fourth day incubation either on coconut substrate (0.85 U / ml) and coconut husk (1.81 U / ml) Reducing coconut fat content 29.20%.

Table 3. The Effect of Microbial-starter Combinations (*Rhizopus* sp, *Lactobacillus* sp, *Yeast*) with Different Fermentation times to Crude Fat (%)

Microbes combination	Day (hari)						Average
	B0	B2	B4	B6	B8	B10	
A1	$34,97^{A}$	$33,32^{BCD}$	15.01^{EFG}	$11,01^{BCDEFG}$	$7,28^{G}$	$8,65^{DEFG}$	15,04
A2	$35,51^{A}$	$12,24^{BCDEF}$	$13,79^{BC}$	$9,35^{CDEFG}$	$7,80^{FG}$	$8,66^{DEFG}$	14,56
A3	$34,65^{A}$	$11,92^{BCDEFG}$	$11,99^{BCDEFG}$	$11,09^{BCDEFG}$	$9,19^{CDEFG}$	$8,23^{EFG}$	14,51
A4	$33,63^{A}$	$12,79^{BCDE}$	$12,69^{BCDEF}$	$8,94^{CDEFG}$	$8,32^{EFG}$	$8,02^{EFG}$	14,07
Average	$34,69^{a}$	$12,57^{b}$	$13,37^{b}$	$10,10^{c}$	$8,15^{d}$	$8,39^{cd}$	

Note: Superscripts with different letters between treatments showed significantly different effects (P <0.05)

Conclussions

The best treatment that produce of highest protein and lowest fiber and fat was A3B6 (60% *Rhizopus* sp + 30% *Lactobacillus* sp + 10 *Yeast* sp and fermentation time for 6 day). The research can be conluded that the best treatment was A3B6 which was 9.41% of crude protein, 9.88 % of crude fiber and 7.28% crude fat of coconut dreg.

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References

1. Singh A., Sharma S. 2003. Effect of microbial inocula on mixed solid waste composting, vermi composting and plant response. *Compost Sci. Util*., 11: 190-199.
2. Khadem, S.A., Galavi, M., Ramrodi, M., Mousavi, S.R., Rousta, M.J., Rezvani-Moghadam, P. 2010. Effect of animal manure and super absorbent polymer on corn leaf relative water content, cell membranestability and leaf chlorophyll content under dry condition. *Aus. J. Crop Sci*. 4,8, 642-647.
3. Barker, A.V., Bryson, G.M. 2006. Comparisons of composts with low or high nutrient status for growth of plants in containers. *Comm. Soil Sci. Plant Ana*. 37, 1303-1319.
4. Berc J, Muñoz O, Calero B. 2004. Vermiculture offers a new agricultural paradigm. *Biocycle*. 45(6):56-7.
5. Barker, A.V., Bryson, G.M. 2006. Comparisons of composts with low or high nutrient status for growth of plants in containers. *Comm. Soil Sci. Plant Ana*. 37, 1303-1319,
6. Gaur A.C., Sadasivam K.V. 1993. Theory and practical considerations of composting organic wastes. In: Thampan P.K., Ed., Organics in Soil Health and Crop Production, Peekay Tree Crops Development Foundation, Cochin, pp. 1-22.
7. Sundu B, Dingle J. 2003. Use of enzyme to improve the nutritional value of palm kernel meal and copra meal. *Proc Queensland Poult Sci Symp Australia* 11:1-15.
8. Mendoza NS, Arai M, Kawaguchi T, Yoshida T, Joson LM. 1994. Purification and properties of mannanase from *Bacillus subtilis*. *World J Microbiol Biotechnol* 10:551-555. http://dx.doi.org/10.1007/BF00367665
9. Purwadaria T, Haryati T, Darma J, Munazat OI. 1995. In vitro digestibility evaluation of fermented coconut meal using *Aspergilius Niger* NRRL 337. *Bull Anim Sci (Special Edition)*: 375-381.
10. Association of Official Analytical Chemists. 1995. *Official Methods of Analysis, 16th edn*. Association of Official Analytical Chemists. Arlington, Virginia, USA.
11. Yetti.M. Harnentis and Nurmiati. 2016. Isolation and Screening of Lactic Acid Bacteria from Dadih for Glutamic Acid Production as Precursor of γ-Amino Butyric Acid (GABA) Induced Heat Stress in Broiler. *International Journal of ChemTech Research* 9 (12) 534-540.
12. Suriani N.W. Purnomo.H. Estiasih T and Suteja I.K. 2014. Physicochemical Properties, Fatty Acids Profile and Cholesterol Content of Indigenous Manado Chicken, Broiler and Spent Hen Meat *International Journal of ChemTech Research* 6 (7) 3896-3902.
13. Ratledge C (1994) Yeasts, moulds, algae and bacteria as sources of lipids. In: BS Kamel Y. Kakuda. Technological advances in improved and alternative sources of lipids. London: Blackie Academic and Professional. pp. 235-291
14. Buckle, K. A, R. A. Edwards, G. H. Fleet, dan M. Wootton. 1989. Ilmu Panga Terjemahan Hadi Purnomo dan Adiono. UI Press, Jakarta.
15. Winarno, F. G. 1997. Kimia Pangan dan Gizi. Gramedia Pustaka, Jakarta. *Winarno*, F. G. 2002. Kimia Pangan dan Gizi. Gramedia Pustaka Utama. Jakarta.
16. Al-ahmad S. 2014. The Effect of Starter Cultures on the Physicochemical,Microbiological and Sensory Characteristics of Semi-dried Sausages. *International Journal of ChemTech Research* Vol.7, No.4, pp 2020-2028.
17. Kansoh AL, Nagieb ZA. 2004. Xylanase and mananase enzymes from *Streptomyces galbus* NR and their use in biobleaching of softwood kraf pulp. *Antonie van Leeuwenhoek* 85:103-114
18. Kurniawan H, Utomo R, dan Yusiati L.M. 2016. Nutritional quality of fermented coconut dregs using *Aspergillus niger* . Buletin Peternakan Vol. 40 (1): 26-33 Februari 2016 ISSN-0126-4400 E-ISSN-2407-876X

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