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## Increasing nutrient quality and in vitro digestibility of mixed feed oil palm waste for ruminant feed by solid-state fermentation technology

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# Increasing nutrient quality and in vitro digestibility of mixed feed oil palm waste for ruminant feed by solid-state fermentation technology

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**Abstract.** Palm oil cake (BS) and Solid ex decanter (SD) are feed ingredients produced from palm oil processing which is available in large quantities. BS and SD have high protein content could be used as ruminant feed but have high fat content could be decreased rumen metabolism. Therefore, solid-state fermentation (SSF) technology is one way to overcome this problem. This study aims to improve the nutritional quality and digestibility of mixed feed palm oil cake and solid ex decanter (BS/SD) which is fermented by *Rhizopus oligosporus* (RO) mushroom. Application SSF technology for different combination of mixed feed BS/SD (70/30, 50/50 and 30/70) was carried out for 6 days which was then analyzed on the quality of the proximate, fiber fractions and in vitro true dry matter digestibility (IVTDMD) value (daisy incubator ANKOM Technology). The result showed that the mixed feed BS/SD 50/50 was the best combination with *Rhizopus oligosporus* inoculum with doses of 0.2, 0.4 and 0.8%. Hence, this SSF technology was effective process for increasing protein content and in vitro true dry matter digestibility (IVTDMD) 60% - 67.26% with combination BS/SD 50/50 and RO inoculum with doses 0.2 and 0.4 percent

## 1. Introduction

The production of waste or by-products of food and agriculture processing industry has a fairly large proportion of processing with an average 30% of all raw material before processing. They consisting of materials containing lignocellulose, fruit and vegetable waste and sugarcane waste [1]. They also generally containing many bioactive plants (polyphenolic, carotenoid and fiber) which can be used as a source of animal feed ingredients. Thus, it could be a solution to the problem of adequate nutritional needs of feed as energy and protein ratio. Technology of material processing could enhance nutrient quality of the feed. Currently, some of processing technology are found in industrial such as solid-state fermentation (SSF), silage (ensiling) and processing of materials containing high solid or water content. These technologies have various objectives including the production of certain enzymes [2,3], increasing protein content [4] and protection certain nutritional content which can be developed related to material specification and economic value as feed ingredients material [1].

Other agricultural waste by-product of agriculture industry is palm oil processing industrial. They are categorized as non-conventional food resources for feed ingredient. Palm oil cake (BS) is a by-product of processing palm kernels into palm kernel oil, which accounts for 3% of the total fresh fruits of oil palm [5]. Other, Solid ex decanter (SD) also oil palm waste by-product which is produced from the palm oil clarification process using decanter machine [6]. Solid ex decanter was calculated obtained 4.2% of fresh fruit bunches [7]. Solid brownish black color with a fat content of 7.12% [8].



Some of limitation to the use of palm oil cake and solid decanter waste feed ingredient are high fat contain that's is difficult in feed formulation related with the nutritional requirement of fat for livestock. Other presence of by-product that are difficult to digest such as fragments of kernel shell in palm kernel cake. The waste by-product of palm oil processing containing was depend on the method and condition of the palm fruit processing machine in to crude palm oil (CPO) and palm kernel oil (PKO). Therefore, the study of physical and chemical properties of palm oil cake and solid ex decanter by-product can complement the data for suitable processing purposes. Based on physical chemical properties of this ingredient, would be carried out with the aim of improving nutritional quality. Solid-state fermentation technology could be an alternative processing of palm oil cake (BS) and solid ex decanter as feed raw material.

Solid-state fermentation (SSF) processing technology is determined in general as the growth of microorganisms on a solid substrate. SSF technology has some advantages resulting in low water content, large amounts of fermentation, does not require large energy, uses simple fermentation media/substrate, reduces bacterial contamination and uses only a small amount of water [10]. The SSF technology is also proven to be able to improve nutrient quality of agriculture waste by product feed ingredients which could be used as a model for solving problems in the supply of feed ingredients in developing countries [4]. Oil palm waste by-product such as palm oil cake (BS) and solid ex decanter (SD) could be fermented utilizes fungi, yeast and bacteria as fermentation agent microorganisms for the purposes of increasing protein nutrition and production of feed additives in the form of enzymes, vitamins, and others.

The object of this study was to determine the effect of fermented mixed feed palm oil cake (BS) and solid ex decanter (SD) with different doses of *Rhizopus oligosporus* on rumen in vitro true DM disappearance (IVTDM) and neutral detergent fiber disappearance (IVNDFD) using ANKOM DAISY<sup>II</sup> incubator

## 2. Materials and methods

### 2.1. Palm oil cake (BS) and solid ex decanter (SD)

Palm oil cake (BS) and Solid ex Decanter (SD) are feed ingredients from palm oil processing in Industry. They were obtained from Oil Palm Processing Company Sari Lembah Subur Palm Oil mill and Makmur Andalans Sawit Company (MAS) in Riau. The Palm oil cake was grinded to small size particle for reduce nut shell crackers. Stored solid ex decanter (SD) material will look rotten for a few days, therefore SD was always taken from the inside of stored SD pile. They were mixed as fed ingredients to be mixed feed oil palm waste.

### 2.2. Solid-state fermentation technology (SSF)

Solid-state fermentation Technology (SSF) utilizes fungi *Rhizopus oligosporus* which is used as traditional food fermentation processing in Indonesia, was called Tempeh. *Rhizopus oligosporus* was used as a commercial food fermentation inoculum from PT. Aneka Fermentasi Raprima in Bandung. The fungi widely and easily were found at every market in Indonesia.

Mixed fermented feed BS and SD (BS/SD) was fermented by *Rhizopus oligosporus* with some different doses, it was fermented with 0.2%, 0.4% and 0.8% doses of *Rhizopus oligosporus* for 6 days. They also were mixed with mineral vitamins as 0.1% of the total mixing. Fermentation is stored in room temperature conditions with a humidity of 97.5%

### 2.3. Proximate and fiber fractionation

Proximate fractionation of mixed feed BS/SD was analyzed by Methodology AOAC 2012 [21] while fiber fractionation was analyzed by modified van Soest methods using paper bag technique ANKOM Fiber analyze [22]. Modified van Soest method for fiber fractionation is different with other methods because the method was added with amylase enzyme for starch correction in NDF. Thus, the NDF values was corrected from starch carbohydrate (NDFa).

#### 2.4. *In vitro* digestibility methods

*In vitro* digestibility methods is using ANKOM DAISY<sup>II</sup> incubator [23]. The method has several steps: 0.25 g substrat of fermented mixed Feed BS/SD preparing in filter bag (F57); buffer solutions and rumen inoculum mixing; 30 hours incubation and NDF analysis. The final bag weight after NDF analysis was recorded as final weight (W3) which its values of samples was used for energy and digestibility estimation.

*In vitro* digestibility value was calculated as *in vitro* dry matter disappearance (IVTDMD) and *in vitro* NDF disappearance (IVNDFD). They were calculated with below equation (1 and 2).

$$- \text{IVNDFD (\% DM)} = 100 \times [(W2 \times \% \text{NDF}_{\text{Feed}}) - (W3 - (W1 \times C1))]/(W2 \times \% \text{DM}_{\text{Feed}}) \quad (1)$$

$$- \text{IVTDMD (\%DM)} = 100 - [(W3 - (W1 \times C1)) \times 100] / (W2 \times \% \text{DM}_{\text{Feed}}) \quad (2)$$

where W1 is weight of filter bag. W2 is weight of sample. W3 is final weight (Filter bag + sample).  $\text{NDF}_{\text{Feed}}$  is % of NDF contain in Feed (%DM).  $\text{DM}_{\text{Feed}}$  is % of dry matter contain in feed and C1 is correction of factor (blank filter bag NDF value).

Cannulated cows for rumen inoculum were used and individually penned indoors at Research and Application Farm. LIPI Cibinong. Ruminal fluids were collected from different sites within the rumen before morning feeding at 08.00 am.

#### 2.5. *Statistical analysis*

The effect of different combination of mixed feed BS/SD (70/30; 50/50 and 30/70) and different doses of *Rhizopus* inoculum (0%, 0.2%, 0.4% and 0.8%) on nutrient quality and *in vitro* digestibility values was assigned and in two by two factorial arrangement in completely randomized design (2x3x4). Resulted data was analyzed using GLM procedure of SAS 9.1.3 for windows statistical packaged analyzed [25].

### 3. Results and discussion

#### 3.1. *Nutrient quality (Proximate and fiber fractionation)*

Implementation of SSF technology at mixed feed palm oil cake and solid ex decanter (BS/SD) at different doses *Rhizopus oligosporus* was affected on nutrient quality for proximate and fiber fractionation (Table 1, Table 2). Proportion of mixed feed BS/SD has good growth of *Rhizopus oligosporus* at BS/SD 50/50 and BS/SD 70/30 while mixed feed 30/70 had no or small growth of *Rhizopus oligosporus* for 6 days fermentation. It was caused by high content of crude fat. Protein content was highest at mixed feed BS/SD 70/30 but there is increasing protein after 6 days fermentation at mixed feed BS/SD 50/50, while mixed feed BS/SD 30/70 experienced a decrease in protein content.

Fiber fraction using paper bag technic ANKOM technology of mixed feed BS/SD also affected by SSF technology processing. Highest lignin values at mixed feed BS/SD 70/30 was caused by high content of palm oil cake (BS) containing nut shell crackers. The palm oil cake was grinded to small particle size but still has a lot of small particle of left nut shell of palm kernel after processing. However, the Acid detergent fiber (ADF) content was similar between proportion of mixed feed BS/SD. It showed that the ADF with low content of lignin and DIA has nutrient potential to be used by ruminant animal using feed technology processing.

Mixed feed BS/SD 30/70 has high nutrient quality values as proximate and fiber fraction. but has low protein content. High value of organic matter in NDF ( $\text{NDF}_{\text{om}}$ ) at mixed feed BS/SD 50/50 is more than 60% with high protein content. It could be as optimize proportion for mixed feed palm oil waste by-product which could fermented by *Rhizopus oligosporus* until 0.8%, the proportion of mixed feed BS/SD 50/50 also has low content of crude fat contain with low digestible insoluble ash (DIA).

**Table 1.** Proximate fractionation of fermented mixed feed palm oil cake and solid decanter (BS/SD) with different doses of *Rhizopus oligosporus* (RO).

Proximate	DM (%)	OM	CF	CP	EE	ASH	NFE	
		% DM						
BS/SD 70/30	RO-0%	40.28	89.93	23.44	21.60	2.73	10.07	42.16
	RO-0.2%	40.30	88.01	20.67	23.03	2.78	11.99	41.53
	RO-0.4%	39.89	88.33	17.26	21.67	2.90	11.67	46.50
	RO-0.8%	39.89	88.91	23.25	21.55	2.85	11.09	41.26
BS/SD 50/50	RO-0%	52.66	92.09	21.51	18.40	2.69	7.91	49.49
	RO-0.2%	61.16	91.18	20.32	20.30	2.88	8.82	47.68
	RO-0.4%	54.59	90.21	23.90	20.33	2.71	9.79	43.27
	RO-0.8%	54.59	91.56	20.27	20.00	2.84	8.44	48.45
BS/SD 30/70	RO-0%	65.04	94.19	18.61	14.07	5.26	5.81	56.25
	RO-0.2%	74.65	94.62	19.22	13.42	4.24	5.38	57.74
	RO-0.4%	76.40	94.41	23.26	13.39	5.30	5.59	52.46
	RO-0.8%	79.42	94.32	17.23	12.96	7.74	5.68	56.39

Where : DM = Dry Matter, OM = Organic Matter, CF = Crue Fiber, CP = Crude Protein, EE = Ether Extract, NFE = Nitrogen Free Extract

Nutrient quality of mixed feed BS/SD should be determined also by in vitro rumen digestibility after 30 hours incubation at Daisy incubator. Different containing of nutrient quality at mixed feed BS/SD effect to rumen fermentation because of microbial fermentation. In vitro rumen fermentation uses daisy incubator technique for NDF digestibility (NDFD) and Digestible NDF (dNDF) analysis. Hence, it could calculate the In vitro true digestibility for in vitro rumen fermentation models (IVTDM). The value of Fiber fraction of Mixed feed BS/SD would have high correlation with the in vitro digestibility. NDF digestibility (NDFD) is a coefficient digestible of dry matter content.

**Table 2.** Fiber fractionation of fermented mixed feed palm oil cake and solid decanter (BS/SD) with different doses of *Rhizopus oligosporus* (RO)

Fiber Fraction	DM (%)	NDF	NDFom	DIA	HEMISEL	ADF	CELLULOSE	LIGNIN	
		% DM							
BS/SD 70/30	RO-0%	40.28	59.71	55.69	4.02	13.71	46.00	31.05	14.96
	RO-0.2%	40.3	58.89	54.53	4.35	11.24	47.65	26.94	20.71
	RO-0.4%	39.89	64.21	60.28	3.93	14.44	49.77	33.07	16.70
	RO-0.8%	39.89	64.82	58.75	6.07	15.54	49.28	34.38	14.91
BS/SD 50/50	RO-0%	52.66	69.18	66.17	3.01	22.71	46.47	40.71	5.75
	RO-0.2%	61.16	67.39	64.22	3.17	17.54	49.85	43.40	6.45
	RO-0.4%	54.59	69.09	68.11	0.97	27.00	42.09	36.71	5.38
	RO-0.8%	54.59	62.26	59.25	3.01	16.88	45.37	40.28	5.10
BS/SD 30/70	RO-0%	65.04	74.40	71.00	3.40	27.36	47.04	44.68	2.36
	RO-0.2%	74.65	73.38	70.06	3.32	25.67	47.71	45.12	2.59
	RO-0.4%	76.40	78.04	72.32	5.72	29.78	48.26	46.02	2.24
	RO-0.8%	79.42	67.40	64.48	2.92	26.16	41.23	38.78	2.45

Where : DM = Dry Matter, NDF = Neutral Detergent Fiber, ADF = Acid Detergent Fiber

### 3.2. *In vitro* rumen fermentation digestibility values

Mixed feed palm oil cake and solid ex decanter (BS/SD) was affected by their proportion significantly ( $P < 0.01$ ) on *in vitro* rumen digestibility values while doses of *Rhizopus oligosporus* (RO) was not significant (Table 3). Mixed feed BS/SD 30/70 has highest values of *in vitro* rumen digestibility without affected by doses of *Rhizopus oligosporus*. The combination effect also significant at NDF digestibility and digestible NDF values.

The increase in the value of *in vitro* digestibility with the incubation method for 30 hours in all fermented mixed feed with 0.4% *Rhizopus oligosporus* was caused by lower fraction that was difficult to digest in feed using palm oil waste by-product. Meanwhile, the other treatments experienced decrease in the value of *in vitro* true dry matter disappearance (IVTDMD) with 0.8% doses of *Rhizopus oligosporus*. Digestion coefficient of NDF (NDFD) was highest at mixed feed BS/SD 30/70 (58.85% NDF) was caused by lower proportion of palm oil cake (BS) containing high difficult digestible nutrient (lignin, ADF). Thus, the proportion of mixed feed BS/SD 30/70 also has highest proportion of dry matter that is digestible NDF (dNDF).

Solid-state fermentation technology for mixed feed BS/SD has an effect on increasing crude protein content in Mixed feed BS/SD 50/50, while the other nutritional values relatively do not change in each treatment. Highest values of undigestible fractionation (ADF, Lignin and DIA) also contribute to decide the optimum SSF-technology method for mixed feed palm oil waste by-product. Increasing protein content at mixed feed BS/SD 50/50 while the *in vitro* digestibility also higher than 60% could be optimum combination proportion of mixed feed BS/SD. The value of dNDF always lower than NDF which is showed that the proportion of dry matter that is digestible NDF. *In vitro* true dry matter disappearance (IVTDMD) of mixed feed BS/SD is more than 60% for Mixed feed BS/SD 50/50 and 30/70. However, the mixed feed BS/SD 50/50 is the optimum proportion for mixed feed from palm oil waste by-product.

**Table 3.** *In vitro* digestibility values of fermented mixed feed palm oil cake and solid decanter (BS/SD) with different doses of *Rhizopus oligosporus* (RO)

In vitro digestibility parameter		NDFD		dNDF		IVTDMD	
		(%NDF)	(%DM)	(g/Kg DM)	(%DM)	(g/Kg DM)	
BS/SD 70/30	RO-0%	16.26	9.71	97.12	50.00	500.00	
	RO-0.2%	16.70	9.83	98.33	50.94	509.43	
	RO-0.4%	16.03	10.29	102.91	46.08	460.78	
	RO-0.8%	32.58	21.12	211.15	56.30	562.97	
BS/SD 50/50	RO-0%	39.29	27.18	271.80	58.00	580.00	
	RO-0.2%	40.65	27.39	273.92	60.00	600.00	
	RO-0.4%	48.35	33.40	334.03	64.31	643.14	
	RO-0.8%	47.41	29.52	295.16	67.26	672.59	
BS/SD 30/70	RO-0%	55.64	41.40	413.97	67.00	670.00	
	RO-0.2%	58.85	43.18	431.81	69.80	698.01	
	RO-0.4%	55.63	43.41	434.11	65.37	653.72	
	RO-0.8%	45.62	30.75	307.49	63.35	633.53	
SE		4.50		30.52		4.79	
P Value BS/SD		0.00		0.00		0.00	
P value RO		0.62		0.67		0.41	
P value BS/SD * RO		0.07		0.02		0.18	

Where : NDFD = Neutral Detergent Fiber digestibility. dNDF = digestible NDF. IVTDMD = *in vitro* dry matter disappearance

#### 4. Conclusion

Solid-state fermentation technology in Mixed feed palm oil cake and solid ex decanter (B/S) with combination 50/50 and *Rhizopus oligosporus* 0.4% increase nutrient quality and in vitro rumen digestibility values of mixed feed BS/SD as ruminant animal feed.

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