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Feeding diet containing concentrate with fermented *Arenga pinnata* by-product on nutrient intakes, digestibility, and milk quality of lactating dairy cows

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Abstract Utilizing fermented Arenga pinnata, a product produced by *Pleurotus ostreatus* that was transferred for 5 weeks until the full hyphae were grown, as an alternative feedstuff. This biomass was ready to be used as one of the ingredients in substitution with rice bran in the concentrate. They were concentrated with fermented Arenga (CFA) by-products, specifically CFA0 (0/35), CFA10 (10/25), CFA15 (15/20), and CFA20 (20/15). In comparison, other ingredients were ground corn, soybean meal, palm oil, mineral mix, *Curcuma xanthorrhiza,* yeast, NaCl, CaCO3, and TSP (Triple Superphosphate). Those were all in the same amount, totalling 65%. The results showed that the crude protein content of fermented *A. pinnata* was improved by 11.62%, compared to 1.55% in non-fermented *A. pinnata*. Its crude fibre content decreased from 30.18% to 24.31%, respectively, in non-fermented and fermented *A. pinnata.* Meanwhile, the contents of NDF, ADF, hemicellulose, and cellulose were reduced with increased levels of fermented A. pinnata by-product in rice bran. On the other hand, Ca, P, and Ca/P (2.04) were found to be high in CFA20. Feces production (19.50 kg/d on average) was not different (P>0.05) among the three diets containing fermented *A. pinnata* by-product concentrates in lactating dairy cows. The nutrient contents in feces were not differed (P>0.05) among these diets, except for ether extract (P<0.05), which was shown to be higher in CFA15 and CFA20. All nutrient intakes were very significantly (P<0.01) decreased with increased the fermented *A. pinnata* by-product in concentrates. Digestibility of nutrients was not differed substantially (P > 0.05), while ether extract was significantly decreased (P < 0.05) in CFA15 (86.57%) and CFA20 (87.51%), compared to a diet without fermentation. *A. pinnata* in the concentrate. Milk yielded (8.52 kg/d in FCA0 and 8.31 kg/d in CFA20 diets) and milk composition were not significantly affected (P > 0.05). These results indicated that incorporating up to 20% fermented *A. pinnata* into the concentrate, replacing rice bran found to be a suitable option for lactating dairy cows' diets.

Keywords: *A. pinnata*, Concentrate, Dairy, Digestibility, Fermented

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Introduction

The palm tree (*Arenga pinnata* Merr.), also known by its local name, Enau, is a perennial plant. The palm sugar tree is classified as a multi-purpose plant due to its various benefits that can be taken from its leaves, fruit, and stems, namely palm sugar, palm fruit, palm broom stuff, and palm flour. Potential agricultural waste as an alternative feed biomass is the Arenga by-product of the Aren tree. Aren tree (*Arenga pinnata*) is a tropical fruit that thrives in various regions in Indonesia, including Bengkulu (Sulistyowati *et al.,* 2023). Its By-product is waste from making palm flour. To extract the starch (flour), the palm tree must be around 20 years old. By-products have not been utilised optimally as feed ingredients. Kusmiyati and Agustini (2007) reported that by-products contain dry matter at 26.47%, organic matter at 89.67%, crude protein at 3.19%, ether extract at 0.13%, and crude fibre at 31.90%. Judging from its nutritional content, especially low crude protein, this is an obstacle to using sugar by-products as a feed ingredient. The high crude fibre content reduces the digestibility value when used as feed. Therefore, efforts are needed to enrich its nutritional value. One effort to increase the nutritional content of sugar by-products is by fermentation. The application of biotechnology, utilising bioprocesses with fungi that exhibit good ligninolytic capabilities, is an alternative that warrants further study. The *Pleurotus ostreatus* mushroom, commonly known as the white oyster mushroom, may be an option for Miles and Chang (2004). The fungus *P. ostreatus* is classified as a white-rot fungus, which is capable of degrading lignin because it produces extracellular ligninolytic enzymes, such as laccase, lignin peroxidase, and Mn peroxidase (Periasamy and Natarajan, 2004). The ability of *Pleurotus ostreatus* to degrade lignocellulosic materials has been widely utilised in bioderegulation and biobleaching processes (Herliyana *et al*., 2008), as well as in the bioconversion of lignocellulosic waste for animal feed (Badarina *et al*., 2013; Sulistyowati *et al*., 2016). This research aimed to evaluate the nutritional value, milk quality, and nutrient digestibility of by-products fermented using the *Pleurotus ostreatus* fungus, with the goal of enhancing their nutritional quality for use as ruminant feed.

Materials and methods

Fermentation of Arenga pinnata by-product

After collecting a certain amount of the Arenga by-product or dreg, it was dried in the sun until it was ready to be ground, resulting in a coarse powder. Together with 13% rice bran, 85% Arenga dreg powder, and 2% CaCO3, they were watered (60-70% v/w) and composted for 24 hours. These substrates were then placed into bags of 600g each and sterilized at 120 °C for 4 hours. These baglogs were cooled down to about 400 °C and then inoculated with a 3.5% *Pleurotus ostreatus* starter. This procedure was modified from the previous experiment conducted by Sulistyowati *et al.* (2020a). The fermented Arenga by-product can be harvested in approximately 4-5 weeks when the full hyphae are grown. This fermented Arenga by-product was removed from the baglogs, air-dried, and then ready to be incorporated into the concentrate according to each treatment in the formula.

Preparation of feed additives

The yeast additive was prepared according to a procedure reported by Sulistyowati *et al.* (2015). There were several ingredients (fresh cassava tuber, rice flour, garlic, lime, sugar, *Alpinia galanga*, local commercial yeast, and water) with a certain ratio. *Curcuma xanthorrhiza* powder can be prepared by slicing it thinly, drying it, and then grinding it into a powder. Ground corn-soybean flour and other ingredients were weighed as calculated in the formula, including fermented Arenga by-product, which was used to supplement the diet of lactating dairy cows and contain 14% crude protein (NRC, 1989). The modification of the formula found to be the most reasonable in previous research (Sulistyowati *et al*., 2015, 2020a, 2020b) is presented in Table 2. Those were CFA0 (0/35), CFA10 (10/25), CFA15 (15/20), and CFA20 (20/15).

Chemical analysis

Before and after fermentation of Arenga – a by-product, the four fermented Arenga – by-product concentrates, forage, tofu product, and feces were analysed in PAU – IPB Laboratory for proximate analyses (DM- dry matter, OM- organic matter, CP- crude protein, EE- ether extract, CF-crude fibre contents) according to AOAC (2005). Fractions of crude fibre were analysed according to the method of Goering and Van Soest (1970) in the Nutrition and Feed Technology Laboratory at IPB. Minerals of Ca and P were assayed using an atomic absorption spectrophotometer (AA 7000, Shimadzu Co., AAS).

Experimental design and data collection

The essential diet contains 65% forage (corn stover, King grass, and paddy hay), 19% tofu by-product, and 16% concentrate with fermented Arenga by-product. A 4 x 4 Latin square experimental design was used, according to Lentner and Bishop (1986), with four lactating dairy cows (445.00 ± 23.5 kg). The cows were housed individually, with feed and water baskets provided for each cow. The procedure was conducted by animal ethics approval from the Research Ethics Committee of the Institute for Research and Community Service at the University of Bengkulu, as evidenced by Certificate of Ethical Approval No. 26/KERLPPM/EC/2023. The four treatments were allocated to four cows over four 10-day periods, with a 10-day preliminary period. The total duration was 50 days. The treatments were CFA0was the concentration of 0% fermented Arenga -by-product/35% rice bran in an elemental diet, CFA10 was the concentration of 10% fermented Arenga- by-product/25% rice bran in an elemental diet, CFA15 was the concentration of 15% fermented Arenga - by-product/20% rice bran in an elemental diet, and CFA20 was the concentration of 20% fermented Arenga - by-product/15% rice bran in the essential diet.

The concentrate was administered at a rate of 2.5 kg/head/day, consisting of 1 kg in the mornings and 1.5 kg in the afternoons. Other feedstuffs total approximately 25 kg/head/day; forage and tofu by-products were provided in half in the mornings and the other half in the afternoons. Any leftovers were weighed the following morning. Milk yields were recorded from each milking in the mornings and afternoons after the milk had been weighed and measured. Milk samples from each experimental unit were collected on the last day of treatment in each period and then stored frozen until analysis of milk composition (ether extract, protein, and lactose) using Lactoscan, as well as SCC (somatic cell count). It was conducted at the Kesmavet laboratory, FKH-IPB University.

Feces collections were conducted over the last four days of each period; samples were taken, air-dried, and ground for proximate analyses. Apparent digestibility (%) was calculated as the intake of nutrients minus the nutrients in feces, divided by the intake of nutrients. The diets were applied at a dairy farm in the upland (900-1000m ASL) of Kabawetan, Kepahiang, Bengkulu, Indonesia. All data were collected and tabulated, then analysed using ANOVA (analysis of variance). Any significant differences were further analysed using Duncan's Multiple Range Test (DMRT) according to Lentner and Bishop (1986).

Results

Nutrient contents and fibre fractions of fermented Arenga- by-product

The nutritional contents (DM, OM, EE, CP, and CF) of non-fermented and fermented Arenga are presented in Table 1. The results showed that fermenting the Arenga by-product with P. ostreatus improved the nutritional value of the by-product, specifically increasing crude protein levels by approximately 740% and reducing crude fibre levels by approximately 19%.

Formula and nutrient contents of concentration **concentrates**

The formula and nutrient concentration of concentrates containing fermented Arenga by-products are presented in Table 2. The dry matter, organic matter, and ether extract contents in the concentrate with 20% fermented Arenga by-product decreased the most compared to the control.

Table 1. Nutrient contents of non and fermented Arenga- by-product, forage, and tofu by-product

Nutrient (%)	Non fermented Arenga	Fermented Arenga	Forage	Tofu by-product
Moisture	84.09	65.16	34.50	58.86
Dry matter	15.92	34.84	65.51	41.15
Ash	1.58	7.47	9.89	2.18
Organic matter	14.34	27.38	55.62	38.97
Ether extract	1.73	0.85	1.03	3.64
Crude protein	1.55	11.62	11.52	15.86
Crude fiber	30.18	24.31	17.70	9.24

Table 2. Formula and nutrient contents of concentration concentrates containing fermented Arenga-by-product for dairy cows

Feedstuffs (%)	CFA0	CFA10	CFA15	CFA20
Fermented Arenga (FA)	0	10	15	20
Rice bran	30	20	15	10
Ground corn	30	30	30	30
Soybean meal	32	32	32	32
Palm oil	3.5	3.5	3.5	3.5
Mineral mix	0.5	0.5	0.5	0.5
C. xanthorriza	1.5	1.5	1.5	1.5
Yeast	1	1	1	1
NaCl	0.5	0.5	0.5	0.5
CaCO3	0.5	0.5	0.5	0.5
TSP	0.5	0.5	0.5	0.5
Nutrient contents (%)				
Dry matter	88.71	83.28	81.92	79.36
Organic matter	81.33	75.75	75.28	73.29
Ether extract	12.05	10.18	10.62	10.94
Crude protein	13.48	16.22	16.24	16.38
Crude fiber	7.42	9.25	6.01	7.01
NDF	26.91	26.15	26.87	24.15
Hemicellulose	12.17	11.80	12.15	10.27
ADF	14.74	14.35	14.72	13.88
Cellulose	9.15	8.11	8.67	7.02
Ash	7.11	6.83	6.99	8.05
Ca	0.71	0.63	0.64	0.98
P	0.47	0.36	0.41	0.48
Ca/P	1.51	1.75	1.56	2.04

Nutrient digestibility

Fecal production and nutrient contents are presented in Table 3. There were no significant differences ($P > 0.05$) in most nutrients across all treatments, except for a significantly higher value in ether extract ($P < 0.05$) in CFA15.

Table 3. Feces production and its nutrient contents of the dairy cow-fed diet with concentration concentrate containing fermented *Arenga pinnata* by-product

Nutrients (kg/d)	CFA0	CFA10	CFA15	CFA20	SEM	P
Feces production	19.33	19.41	19.28	19.80	0.235	>0.05
Dry matter	3.49	3.67	3.63	3.10	0.195	>0.05
Organic matter	2.34	2.58	2.53	2.13	0.105	>0.05
Ether extract	0.06[b]	0.06[b]	0.08[a]	0.07[a]	0.005	<0.05
Crude protein	0.32	0.32	0.34	0.33	0.005	>0.05
Crude fiber	0.74	0.79	0.83	0.69	0.025	>0.05

Note: CFA0: diet with no fermented Arenga in concentrate; CFA10: diet with 10% fermented Arenga in concentrate; CFA15: diet with 15% fermented Arenga in concentrate; CFA20: diet with 20% fermented Arenga in concentrate. A significant effect was observed in the ether extract content ($P < 0.05$).

Nutrient intakes and digestibility of a dairy cow-fed diet with a concentrate containing fermented Arenga by-product are presented in Table 4. All nutrient intakes were significantly different ($P<0.05$) among treatments. They were mainly found in diets with 15 and 20% of fermented Arenga in concentrates. They contained lower amounts of dry matter, organic matter, ether extracts, crude protein, and crude fibre than the control and 10% fermented Arenga in concentrates. On the other hand, in terms of digestibility, only the ether extract was found to be significantly different. Again, diets with 15% and 20% fermented Arenga in concentrates were lower than those in the control and the 10% fermented Arenga in concentrates.

Table 4. Nutrient intakes and digestibility of dairy cow-fed diet with a concentrate containing fermented Arenga by-product

Intakes (kg/d)	CFA0	CFA10	CFA15	CFA20	SEM	P
Dry matter	12.12[a]	12.01[b]	11.93[c]	11.89[c]	0.115	<0.01
Organic matter	6.73[a]	6.52[b]	6.45[c]	6.38[d]	0.175	<0.01
Ether extract	0.64[a]	0.60[b]	0.59[c]	0.59[c]	0.025	<0.01
Crude protein	1.59[c]	1.63[a]	1.62[b]	1.62[b]	0.015	<0.01
Crude fiber	1.62[b]	1.65[a]	1.57[d]	1.59[c]	0.015	<0.01
Digestibility (%)						
Dry matter	71.51	69.50	69.61	73.98	1.235	>0.05
Organic matter	65.60	60.46	60.86	66.72	0.560	>0.05
Ether extract	90.66[a]	89.83[a]	86.57[b]	87.51[b]	1.575	<0.05
Crude protein	79.90	80.25	79.37	79.47	0.215	>0.05
Crude fiber	54.98	52.48	47.59	56.85	0.935	>0.05
NFE	48.64	50.41	48.19	46.86	1.27	>0.05
TDN	77.10	78.63	74.84	74.85	1.60	>0.05

Note: CFA0: diet with no fermented Arenga in concentrate; CFA10: diet with 10% fermented Arenga in concentrate; CFA15: diet with 15% fermented Arenga in concentrate; CFA20: diet with 20% fermented Arenga in concentrate. Significant effects were found in nutrient intakes ($P < 0.01$) and digestibility ($P < 0.05$).

Milk quality

Milk yield and milk components of a dairy cow-fed diet with concentration concentrate containing fermented *Arenga pinnata* by-product are presented in Table 5. No significant differences were observed in milk yield and milk composition of dairy cows fed at all levels of fermented Arenga in concentrates. Based on the averages, the diet with 20% fermented Arenga in concentrate exhibited a low-fat percentage and a low SCC (Somatic Cell Count) in milk. The milk yield was relatively high on average at this level of fermentation with Arenga. The milk density of all four diets was found to be above the standard, which was 1.028.

Table 5. Milk yield and milk components of the dairy cow-fed diet with a concentrate containing fermented *Arenga pinnata* by-product

Items	CFA0	CFA10	CFA15	CFA20	SEM	P
Milk yield, kg/d	8.52	7.97	7.75	8.31	0.105	>0.05
Fat, %	3.91	3.93	3.41	2.38	0.765	>0.05
Fat yield, kg/d	0.28	0.35	0.35	0.25	0.015	>0.05
Protein, %	3.30	3.24	3.47	3.36	0.030	>0.05
Protein yield, kg/d	0.28	0.26	0.22	0.32	0.020	>0.05
Solid non-fat, %	9.04	8.86	9.49	9.21	0.085	>0.05
Solid non-fat, kg/d	0.77	0.71	0.74	0.77	0.030	>0.05
Lactose, %	4.96	4.86	5.20	5.05	0.045	>0.05
Lactose, kg/d	0.42	0.39	0.40	0.42	0.010	>0.05
Density	1.032	1.031	1.033	1.034	1.030	>0.05
SCC, x cfu 10^3	293	130	107	80	106.5	>0.05

Note: CFA0: diet with no fermented Arenga in concentrate; CFA10: diet with 10% fermented Arenga in concentrate; CFA15: diet with 15% fermented Arenga in concentrate; CFA20: diet with 20% fermented Arenga in concentrate. No significant effects were found in any variable across all treatments ($P > 0.05$).

Discussion

There are differences in nutrient contents between non-fermented and fermented Arenga- by-products. The increase in crude protein content of by-products after fermentation using *P. ostreatus* is in line with the results of fermenting sago dregs with this yeast, as reported by Sangadji (2019). Hatta and Sugiarto (2015) reported a similar result: an increase in crude protein content and a decrease in crude fibre content in corn cobs after fermentation using *P. ostreatus*. An increase in crude protein in fermented by-products can occur due to the growth and reproduction of mould during the fermentation process.

There is an increase in nutritional quality, particularly in the crude protein content of the Arenga by-product after fermentation, reaching 11.62% CP. The increase in crude protein (10.7%, equivalent to a 7.5-fold increase) enables fermented Arenga by-product to serve as a protein-rich feed equivalent to rice bran. This is because the protein value of rice bran is recommended to be at least 8% (SNI, 1997). Thus, fermented Arenga by-products can be used as a concentrate component for dairy cows as a substitute for rice bran. The substitution of fermented Arenga by-product for rice bran in the concentrate ration of dairy cows shows that the nutritional value (fibre fraction) decreased by as much as 5.87%, equivalent to 19.5%. In this study, fermenting Arenga by-products using *P. ostreatus* can enhance their nutritional value by increasing the crude protein content, reducing the crude fibre, and improving the mineral content, particularly Ca/P.

The substitution of fermented by-products for rice bran in the fermented Arenga concentrate (CFA), especially in CFA20, apparently did not increase the value of the fibre fraction component of the concentrate ration. This indicates that the fermentation process of fibre dregs using P. ostreatus can reduce the fibre fraction components of the by-product. This concentrate was also low in fibre fractions, including NDF, hemicellulose, ADF, and cellulose. This condition aligns with the research results of Azzahra *et al.* (2022), who reported a decrease in the levels of NDF, ADF, and hemicellulose in oil palm plantation waste after fermentation by *P. ostreatus*.

Neutral Detergent Fiber (NDF) comprises cellulose, hemicellulose, and lignin. Rice bran replacement of more than 100% (CFA20) showed the lowest NDF value. The ADF and cellulose values of rice bran are 21.88 ± 0.13 and 12.21 ± 0.28, respectively, as reported by Maesaroh *et al*. (2023). At the same time, non-fermented by-products (original) contain 57.78% ADF and 38.80% cellulose (Febrianti *et al*., 2020). The content of the fibre fraction (NDF) component of non-fermented by-product is higher than that of rice bran.

A research result of concentrate with fermented Durio rind (30%) also showed a decrease in NDF and hemicellulose (Sulistyowati *et al.,* 2020b). The substitution of fermented by-products in the CFA20 treatment increased the ash, mineral Ca, and P content of the fermented Arenga concentrate. This indicates an increase in mineral content in the degraded substrate (by-product) since *P. ostreatus* immediately utilised the lignocellulosic constituents after inoculation on the substrate. In this way, fermented by-products become a feed ingredient rich in minerals. Çağlarırmak (2007) stated that the *Pleurotus* species is a rich food source in proteins, minerals (Ca, P, Fe, K, and Na), vitamin C, and the vitamin B complex (thiamine, riboflavin, folic acid, and niacin).

All nutrient intakes are found to be significant differences ($P < 0.01$) in all treatments. Intakes of dry matter, organic matter, and ether extract decreased with increasing levels of fermented Arenga by-product in the concentrate. The average dry matter intake with these diets (11.89-12.01 kg/d) slightly met the required dry matter intake (DMI) for small breed dairy cows (454 kg) with 10-20 kg milk production, and a milk ether extract content of 4% was between 12.4-16.0 kg/d (NRC, 1989). The intakes of dry matter, organic matter, crude protein, and crude fibre in this present study were higher than those of diets with alfalfa hay, corn stover, and rice straw, as reported by (Wang *et al*., 2014). These current results were higher than those reported by Damborg *et al.* (2019) for grass-clover silage.

The digestibility of all nutrients was not significantly different ($P > 0.05$), except for ether extract, which decreased significantly ($P < 0.05$) with increasing levels of fermented Arenga by-product in the concentrate. On average, the digestibility of dry matter and organic matter increased with the addition of this feedstuff. It was also found in feces that the ether extract content was significantly different ($P < 0.05$). All nutrient digestibility in this study was higher in averages than those with the forages and alfalfa hay, as reported by (Wang *et al*., 2014) and those with 100% alfalfa silage inclusion (Hassanath *et al*., 2014), as well as substitution with millet silage (Brunette *et al*., 2014) and those with low and high energy diets as reported by Zhou *et al*. (2015). Positive effects were reported on nutrient digestibility and rumen fermentation with yeast or mannan oligosaccharide (Bagheri *et al*., 2009). A diet with a concentrate containing 20% fermented Durio rind and 10% rice bran showed the highest digestibility of crude protein and crude fibre (Sulistyowati *et al*., 2020b). This present study on the diet containing a concentrate with 10% fermented Arenga pinnata by-product and 20% rice bran appeared to be optimal for the intake and digestibility of nutrients in dairy cows. This diet also yielded the highest milk production, fat, and protein weights, along with optimal levels of milk fatty acids (Sulistyowati *et al*., 2020b).

However, this present study, which examined a diet containing 10-20% fermented Arenga pinnata by-product, found no significant effects ($P > 0.05$) on milk yield, milk components (protein, SNF, and lactose), and SCC (Somatic Cell Count). There were decreasing SCC numbers with increasing levels of this fermented Arenga in the diet. On average, the diet resulted in slightly higher milk production, particularly with a higher level of this feedstuff. This could be a positive sign of utilising the fermented Arenga pinnata by-product as more than 20% substitute for rice bran in terms of milk yield and mastitis indicators in dairy cows' milk. In conclusion, based on the general performance of nutrient quality, nutrient digestibility, milk yield, and milk quality, including 20% fermented Arenga pinnata by-products in the diet concentrate, a diet containing rice bran could provide a potential feed for lactating dairy cows.

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