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GROWTH OF CARP (*Cyprinus carpio* L.) FED WITH RICE BRAN-COCONUT BAGASSE MIXED SUBSTRATE FERMENTED USING *Rhizopus oryzae*

Pertumbuhan Ikan Mas (*Cyprinus carpio*. L) yang Diberi Pakan Fermentasi Substrat Campuran Dedak Padi dan Ampas Kelapa Menggunakan *Rhizopus oryzae*

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ABSTRAK
Dedak padi dan ampas kelapa dicampur dengan perbandingan tertentu dan kemudian difermentasi menggunakan Rhizopus oryzae *untuk pakan ikan. Uji pemberian pakan lalu dilakukan untuk mengetahui pengaruh pakan terhadap pertumbuhan ikan mas (*Cyprinus carpio *L.). Dalam penelitian ini digunakan 5 perlakuan: satu perlakuan pakan tanpa fermentasi (pakan komersial 100%), dan empat perlakuan pakan fermentasi substrat campuran bekatul dan ampas kelapa dengan empat perbandingan yang berbeda, yakni 75%:25%, 50%:50%, 25%:75%, dan 0%:100%. Hasil penelitian menunjukkan bahwa pemberian pakan komersial 100% (protein sejati 15,25% dan serat kasar 6,27%) memperlihatkan hasil terbaik terhadap pertumbuhan ikan mas dengan pertambahan bobot badan 2,56 g dan rasio konversi pakan 1,95. Sementara itu pemberian pakan fermentasi (protein sejati berkisar 4,89-9,97% dan serat kasar 22,87-25,70%) hanya menghasilkan pertambahan bobot badan ikan pada kisaran 0,47-0,64 g dengan rasio konversi pakan 2,50-2,64. Dengan demikian pakan fermentasi tersebut mampu mendorong pertumbuhan ikan mas meskipun masih kurang optimal dibandingkan dengan pakan komersial.*

Kata Kunci: Rhizopus oryzae, Cyprinus carpio*, rice bran, coconut bagasse, fermentation*

ABSTRACT
Rice bran and coconut bagasse were mixed and then fermented using *Rhizopus oryzae* for preparing aquafeed. Subsequent feeding test was carried out to determine the effect on the growth of carps (*Cyprinus carpio* L). Five feeding treatments were employed, one unfermented feed (commercial feed 100%), and the other four feeds produced by fermentation using substrate mixture of rice bran and coconut pulp in four different ratios, namely 75%:25%, 50%:50%, 25%:75%, and 0%:100%. The results showed that feeding 100% commercial feed (true protein 15.25% and crude fibre 6.27%) showed the best results on the fish growth with body weight gain of 2.56 g and feed conversion ratio of 1.95. Meanwhile, feeding fermented feeds (true protein 4.89-9.97% and crude fiber 22.87-25.70%) only resulted in body weight gain in the range of 0.47 to 0.64 g with feed conversion ratio of 2.50 to 2.64. Thus, the fermented feeds promoted growth in tested carps albeit less optimally than commercial feed.

Keywords*:* *Rhizopus oryzae, Cyprinus carpio,* dedak, ampas kelapa, fermentasi

INTRODUCTION

Up to 80% of animal feed raw materials in Indonesia are supplied from abroad (IFT Online 2013), an irony as Indonesia is an agrarian country that could have been self sufficient in supplying national demand for animal- and plant-based animal feedstuffs. The locally produced raw materials have been so far underutilized and poorly managed, causing discontinuous supply and scarcity (Kementrian Pertanian Indonesia 2014). Thus, the so called national "self-produced feeds" program has been launched to encourage fish farmers to produce aquafeed on their own.

Constituting about 60-70% of the total fish production cost, the use of feed (Sidik 2014) or feed raw materials must be as efficient as possible so as to maximise the profit. To achieve this, technology that can improve the nutritional quality of such cheap, locally available, feed raw materials is of highly important, aimed at increasing their inclusion rate in fish diet. Such technology would be widely adopted when proven to be highly applicable, low cost, and profitable to the farmers (Animal Feed Directorate 2014).

Solid fermentation of agroindustrial byproducts using the edible tempe mould *Rhizopus* spp. could provide solution to this problem owing to the fungus ability to confer on the fermented substrates better nutritional qualities such as higher contents of protein, vitamins (Sriherwanto 2010), as well as lower antinutrient like mycotoxins (Varga et al. 2005). Moreover, being the origin country of tempe, Indonesia provides an ample collection of edible *Rhizopus* strains as well as the practical solid fermentation technology which could be applicable for fermentation of animal feed. Initially used in soybean tempe production, *Rhizopus* spp. has been studied in the fermentation of other various plant materials intended for fish feed ingredients such as palm kernel cake (Amri 2007), leaves of *Alocasia macrorrhiza* (L) Schott (Bakhtiar 2002), cassava bagasse (Antika et al. 2014), *Sesbania grandiflora* leaf powder (Utami et al. 2012), and okara (Rijal 2014). However, all these fermented feedstuffs were used partially, not wholly, in limited proportion in the diets of the test fishes.

In this study, solid fermentation of rice bran-coconut bagasse mixed substrate using *Rhizopus oryzae* was carried out to prepare floating aquafeeds. The fermented feeds were subsequently fed wholly on carps (*Cyprinus carpio* L.) with the aim of finding out the effect on the fish growth relative to those fed with commercial feeds.

MATERIALS AND METHODS

Fermented feed preparation

Fermentation was carried out at the Feed Biotechnology Laboratory-BPPT, Serpong, Banten and the feed test in Cibinong, Gunung Sindur, Bogor, West Java. Substrates consisting of rice bran (RB) and coconut bagasse (CB) in various ratios (Table 1) were mixed well before being sterilized at 121°C for 15 minutes. Having cooled down to room temperature, the substrates were mixed with starter powder of *R. oryzae* (3.3×10^7 cfu/g) at the concentration of 1-1.5 g/100 g substrate, and moisturized to 75% using CIS™ mineral solution. Incubation was then carried out at 28-30°C for 96-120 hours after which the substrate physically transformed into cottony white cake with soft cheesy texture (Figure 1). The cake was then diced into small cubes, approximately 5 mm per side, and dried at 50°C for 24 hours. The dried fermented feeds were subjected to analyses of true protein content (Sriherwanto 2010), and crude fibre (Badan Standardisasi Nasional 1992).

Feeding test

Feeding test on carps were undertaken in a completely randomized design with three replications per treatments. Five feeding treatments were carried out, namely: commercial feed 100% (unfermented), 75%

Table 1. Ratio of rice bran (RB) and coconut bagasse (CB) in the mixed substrate to be fermented

Fermentation treatment	Ratio percentage of mixed substrate
1	75% RB + 25% CB
2	50% RB + 50% CB
3	25% RB + 75% CB
4	0% RB + 100% CB

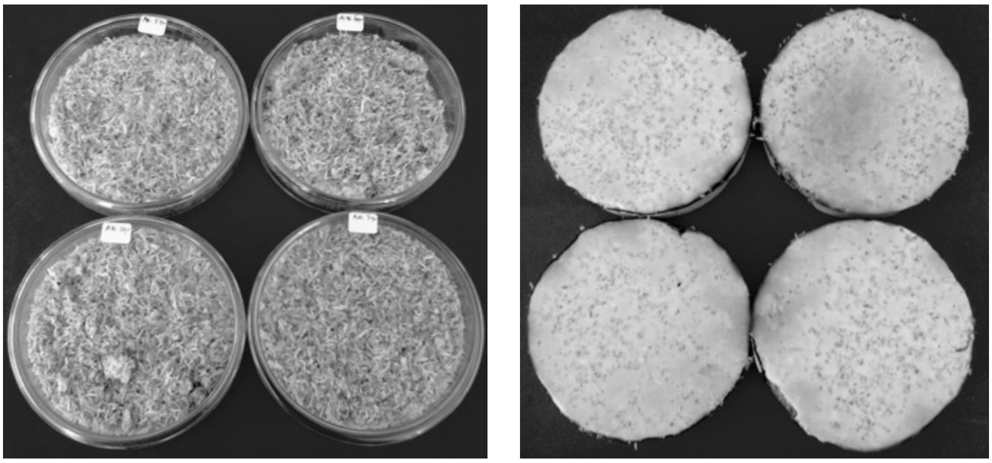

Figure 1. RB-CB mixed substrate before (left) and after fermentation (right) using *R. oryzae*

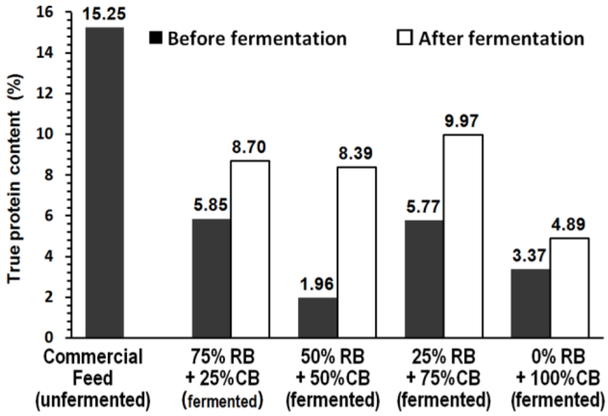

Figure 2. True protein content of RB-CB mixed substrate before and after fermentation using *R. oryzae*

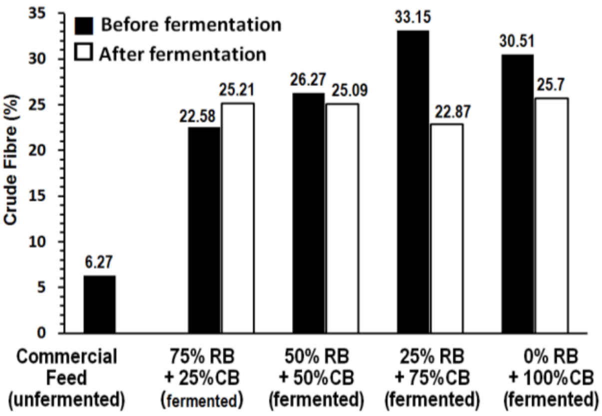

Figure 3. Crude fibre content of RB-CB mixed substrate before and after fermentation using *R. oryzae*

RB + 25% CB (fermented), 50% RB + 50% CB (fermented), 25% RB + 75% CB (fermented), and 100% CB (fermented).

Carp fingerlings were obtained from Parung fish market, Bogor, and acclimatized for two weeks to feeding on the fermented feed inside aquarium environment in Gunung Sindur, Bogor. Post acclimatization, the 3-5 g weighed fingerlings were fasted for 24 hours, weighed out and transferred into 40 $\times$ 30 $\times$ 30 cm glass aquaria placed randomly and in apparently similar positions in a shaded house. Six fishes were reared in each of the 15 aquaria for 60 days, aerated for 24 hour.

To maintain the quality of aqueous environment, at least 30% of aquarium water was replaced every two days, regularly removing all dirt attached on the aquarium glass sides. Water temperature, dissolved oxygen (DO), and pH values were taken three times monthly, at the beginning, middle, and end of the month. Feed was given *at satiation* twice daily in the morning and afternoon with a daily dosage of 4% of the fish weigh. Fish performance was evaluated by measuring the fish weight, survivability, daily growth rate, feed conversion ratio (FCR), as well as protein efficiency ratio (PER).

RESULTS AND DISCUSSION

The substrate consisting RB and CB supported good growth of *R. oryzae* as seen from the fungal white dense mycelial mass overwhelming the whole substrate post fermentation (Figure 1). When thrown into water, these fresh fermented feeds showed floating ability, which was enhanced further through overnight drying in an oven.

The fermented feeds were subjected to true protein content and crude fibre analyses. The true protein concentration was measured based on the total nitrogen

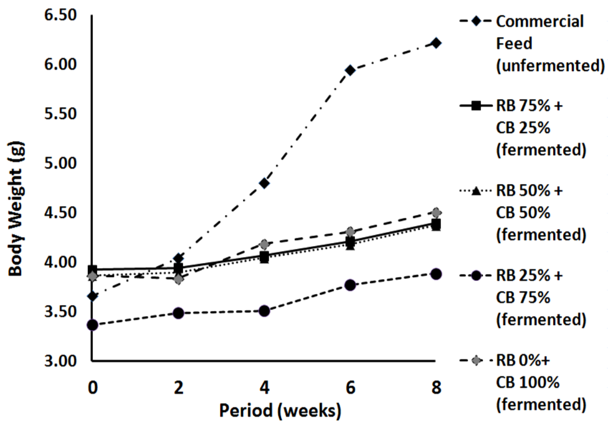

Figure 4. Increase of body weight of carps (*C. carpio* L.) fed with commercial feed or *R. oryzae*-fermented feeds

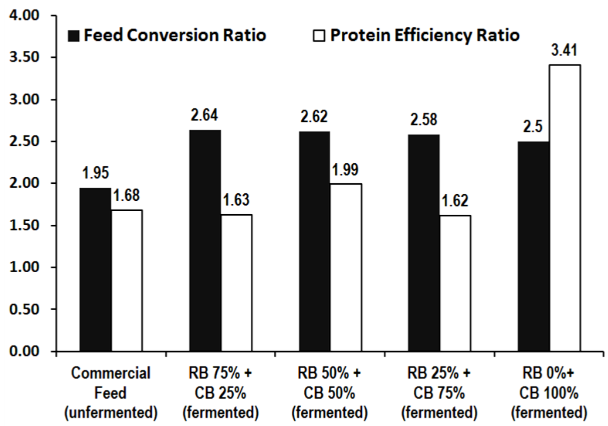

Figure 5. FCR and PER of the 60-day reared carps (*C. carpio* L.) fed with unfermented commercial or *R. oryzae*-fermented feeds

content of trichloroaetic acid-precipitated sample using Kjeldahl method. The resulted protein values differed from available literatures which commonly provide information on the crude protein contents. The later inaccurately took into account all non-protein nitrogenous compounds contained in samples. Figure 2 showed that the *R. oryzae* fermentation increased the true protein content of the RB-CB mixed substrates from 1.96-5.85% to 4.89-9.97%, which is still below that of the commercial feed (15.25%). Highest increase (from 1,96% to 8,39%) in the protein content was achieved with 50% RB-50% CB substrate, whereas the lowest was that of 100% CB (from 5.77% to 9.97%). Variation in the true protein content might be due to the different initial substrate composition.

Changes in protein content through *Rhizopus* fermentation have been previously reported. Sago flour fermented using *Rhizopus* sp. contained higher protein content (3.4%) than the unfermented one (1.6%) (Ab Jalil et al. 2015). CB fermented with *Rhizopus* sp. resulted in 531% increase in true protein content (from 1.45% to 9.15%) (Sriherwanto 2010).

The fermentation also modified the crude fibre content of the RB-CB mixed substrates from 22.58-33.15% to 22.87-25.70% (Figure 3). These values were much higher than that of the commercial aquafeed (6.27%). In general, the fermentation caused the crude fibre content to decrease in all substrates, except when 75% RB-25% CB combination was used. This increase might be due to the fibre being hardly degraded cellulolytically while at the same time significant dry matter loss occurred. Thus, the seemingly percentage increase in the crude fibre might actually be the decrease of dry matter. Lower crude fibre content post fermentation in the other substrate combinations might be attributed to better fungal cellulolytic activity, whereby the highest decline (by 31%) in crude fibre was obtained by 25% RB-75% CB substrate combination. This value is higher than previous report, in which 10 day-fermentation of rapeseed (*Brassica napus*) using *Rhizopus oligosporus* reduced the crude fibre content by 25.5% (Vig and Walia 2001).

The fermented feed prepared in this study contained 22.87-25.70% crude fibre, which is much higher than the recommended value of maximum 5% (FAO 2015). This might be the reason behind the poor growth of all the fishes fed with the fermented feeds as compared to those fed with the commercial feed (Figure 4). It is well known that crude fibre is poorly digested by fish due to the lack of cellulolytic activity inside fish digestive tract. Thus, not only nutritionally poor, crude fibre might also act as antinutrient since cellulose, one of the main components of crude fibre, is classified as *Non-Starch Polysaccharide* (NSP). As stated previousY (Sinha et al. 2011), NSPs were shown to negatively effect the lipid digestion as well as absorption in Atlanticsalmon. Nutrient availability was demonstrated to be impaired when salmonids were fed with diets containing NSP.

Table 2. Feed conversion ratio from week six to eight

Fish Diet	FCR week 6 to 8
Commercial feed (unfermented)	0.73
75% RB + 25% CB (fermented)	0.61
75% RB + 25% CB (fermented)	0.61
75% RB + 25% CB (fermented)	0.62
75% RB + 25% CB (fermented)	0.60

FCR varies depending on nutrition and physical quality of aquafeed, as well as environmental conditions such as temperature, production intensity, availability of natural feed, and other factors such as genetics (New & Wijkström 2002). In this study, varying the diets resulted in different FCRs (Figure 5). Feeding the fermented feeds gave FCR values ranging from 2.50 to 2.64, which are still much higher than that of commercial feed (1.95) (Figure 5). This indicates that the commercial feed promoted better growth than the fermented feeds. Thus, feeding carps exclusively *R. oryzae*- fermented feed based on CB, alone or in combination with RB, resulted in slower growth relative to commercial feed. However, other studies demonstrated that when fermented feeds were not given exclusively but used as feed constituent, better growth performance was possible. It was reported that carp fish fed with diets containing 12% and 18% fermented palm kernel gave FCR values of 2.40 and 2.14, respectively (Amri 2007).

PER is used in feed industry to determine protein quality in feed and as effectivity reference for protein sources. PER is calculated based on the increase in body weight of the test animal divided by the amount of the feed protein consumed during the test period (Bhilave 2011). As seen in Figure 5, fermented feeds resulted in PERs in the range of 1.62-3.41, whereas that of commercial feed gave 1.68. The later value is close to that of 100% CB fermented feed (1.62) which is the lowest amongst all the fermented feeds. Taken together, the average PER value of all four fermented feeds is 2.16, which is much better than control. This result might indicate that, even though containing lower levels of true protein content than the commercial feed, the fermented feeds might contain better quality amino acid composition than commercial feed did. Indeed, essential amino acid profile of *R. oryzae* had been demonstrated to be closely similar to that of FAO standard (Hamdy 2013).

Fermented organic materials added as ingredients in fish diets at certain levels have been reported by others to improve PER values. Fermented *lamtoro* (white leadtree, *Leucaena leucocephala*) leaf powder was used in red tilapia (*Oreochromis niloticus*) diets at the inclusion rates of 5%, 10% and 15%. The PERs achieved were 1.39, 2.03, and 1.43, respectively. These three values were higher than control diet without the fermented leaf, which was 1.27 (Restiningtyas 2015). All these suggest that PER can be improved by using fermented organic materials at appropriate inclusion levels.

The difference in inclusion levels of the fermented feeds as mentioned above was not so much, but could cause significant difference in PER values. This suggests that the quality of amino acid composition in the fermented samples might be the reason. It was reported that adding amino acid DL-methionine into methionine-poor diets increased protein digestibility, improving body weight gain and feed efficiency of carps (Nwanna et al. 2012).

Feeding carps with commercial feed resulted in very steep and almost linear growth rate within the first six weeks, outperforming all those fermented-diet fed carps (Figure 4). However, all the four graphs representing the growth of the fishes fed with fermented feeds were of the same trends, being linear with slight slopes from the beginning to the end of the rearing period. This could be explained by the fact that the four fermented feeds contained lower protein levels (Figure 2) and higher fibre contents (Figure 3) than the commercial feed did. Feed rich in fibre might have antinutritional effect and cause the diet hardly digestible by carps (Sinha et al. 2011). Previous study demonstrated that supplementing fish diet with alpha-cellulose at the level of 25% and 40% decreased growth and feed conversion efficiency of tilapia (*Oreochormis niloticus*) (Anderson et al. 1984).

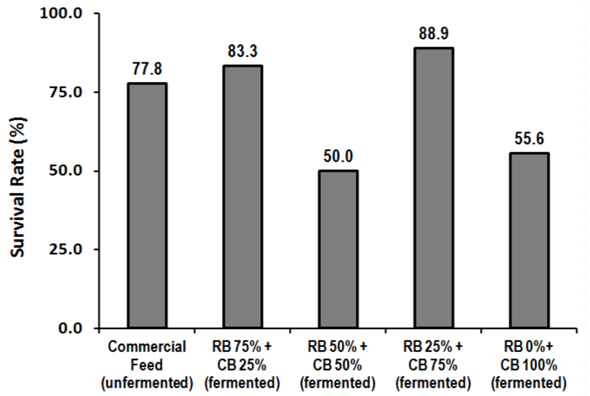

Figure 6. Survival rate of carp (*C. carpio* L.) fed with commercial feed and *R. oryzae*-fermented RB-CB mixed substrate

It is worth to note that although the fermented feed treated carps overall showed poorer growth than the control fishes, their growth curves from week six to week eight showed very similar gradients to the control (Figure 4), with very close FCR values (Table 2) indicating the fermented feeds possible ability to wholly substitute commercial feed in the later phase of the rearing period (from week six to week eight).

Variation in survival rates was observed for the fermented feed treated carps (Figure 6), ranging from 50.0% to 88.9% with the average value of 69.5%. This is lower than that of commercial feed treated carps. In general, fermented feeds resulted in poor survival rate, considering the standard values of 80-90% (SNI 2015). Nutrient quality of the commercial feed which is much better than fermented feeds might be responsible for this discrepancy.

Table 3 shows temperature and dissolved oxygen (DO) values which were within the recommended values, except pH (SNI 2015). Low pH, which is below the recommended level of 6.5, might account for the poor survival rates of the carps (Figure 6).

CONCLUSION

Solid fermentation using *R. oryzae* did increase the true protein content in all substrate combination and decrease crude fibre content in all but one substrate mixture (75% RB-25% CB). Used wholly as aquafeed, the fermented feeds promoted growth of carps but with much slower rates than those fed with the commercial feed.

Table 3. Water quality measured during carp fish feeding experiment using the fermented feed

Parameter	Measured Value	SNI Reference (2015)
pH	4.9-7.3	6.5-8.5
Temperature (^{0}C)	24-27	25-30
DO (ppm)	5.0-7.6	at least 1.7

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