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1 **EFFECT OF FERMENTED FIG FLOUR (*Ficus racemosa* L.) SUBSTITUTION ON**  
2 **PROTEIN AND LIPID RETENTION IN GIANT GOURAMI (*Osphronemus goramy***  
3 **Lacepede, 1801)**

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16  
17 **ABSTRACT**

18  
19 *Ficus racemosa* is one plant species that can be used as a substitute for soybean meal in giant  
20 gourami feed. Substitution of fermented fig flour with Effective Microorganisms 4 (EM-4) can  
21 improve protein quality in feed and the growth of giant gourami. The objective of this study  
22 was to analyze the effect of feeding giant gourami with fig flour-based substitute feed on  
23 protein and lipid retention values in aquaculture systems. The method used was an experiment  
24 in a completely randomized design with five treatments (0%, 10%, 20%, 30%, 40% fermented  
25 fig flour) and five replicates. The feed formulation was prepared using the five isonitrogenous  
26 method with a protein content of 32%. The fermentation of fig flour was carried out aerobically,  
27 incubated for 72 hours using EM-4. The test fish used were 250 giant gourami weighing  
28 between 15 and 20 g. Feeding was carried out at 07:00 and 17:00 WIB with a dose of 3% of  
29 the fish biomass. Giant gourami fish were kept for 70 days, with length and weight  
30 measurements taken every 14 days. The results showed that treatment F4, with 30% fermented  
31 fig flour in the feed, had the highest protein retention ( $18.41 \pm 0.86\%$ ) and lipid retention ( $36.03$   
32  $\pm 1.60\%$ ) in giant gourami. Treatment F4, with 30% fermented fig flour in the artificial feed,  
33 was the best treatment for producing effective and efficient protein and lipid retention values  
34 in giant gourami.

35  
36 **KEYWORDS:** *Ficus racemosa*; retention; protein; lipid; fermentation; giant gourami

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38 **ABSTRAK:** *Pengaruh Substitusi Tepung Buah Ara (*Ficus racemosa* L.) Fermentasi*  
39 *terhadap Retensi Protein dan Lemak Ikan Gurami (*Osphronemus goramy**  
40 *Lacepede, 1801)*

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42 *Ficus racemosa* adalah salah satu jenis tumbuhan yang dapat digunakan sebagai pengganti  
43 tepung kedelai dalam pakan ikan gurami. Substitusi tepung buah ara yang difermentasi dengan  
44 EM-4 dapat meningkatkan kualitas protein dalam pakan dan pertumbuhan ikan gurami.  
45 Tujuan penelitian ini adalah menganalisis pengaruh pemberian pakan pengganti berbasis  
46 tepung buah ara pada nilai retensi protein dan lemak dalam sistem budidaya perikanan.  
47 Metode yang digunakan adalah eksperimen dengan desain acak lengkap yang terdiri dari lima  
48 perlakuan (0%, 10%, 20%, 30%, 40% fermentasi tepung buah) dan lima ulangan. Formulasi  
49 pakan disiapkan menggunakan metode isonitrogenus dengan kandungan protein 32%.  
50 Fermentasi tepung buah ara dilakukan secara aerobik, diinkubasi selama 72 jam  
51 menggunakan EM-4. Ikan uji yang digunakan adalah 250 ekor ikan gurami dengan bobot  
52 berkisar 15-20 g. Pemberian pakan dilakukan pada pukul 07:00 dan 17:00 WIB dengan dosis

53 3% dari biomassa ikan. Ikan gurami dipelihara selama 70 hari, pengukuran panjang dan bobot  
54 ikan diukur setiap 14 hari. Hasil menunjukkan bahwa perlakuan F4, dengan 30% tepung buah  
55 ara fermentasi dalam pakan, memiliki retensi protein ( $18,41 \pm 0,86\%$ ) dan retensi lemak ( $36,03$   
56  $\pm 1,60\%$ ) tertinggi pada ikan gurami. Perlakuan F4 dengan 30% tepung buah ara fermentasi  
57 dalam pakan buatan merupakan perlakuan terbaik untuk menghasilkan nilai retensi protein  
58 dan lemak yang efektif dan efisien pada ikan gurami.

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60 **KATA KUNCI:** *Ficus racemosa*; retensi; protein; lemak; fermentasi; ikan gurami

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## 63 INTRODUCTION

64 Aquaculture production in Indonesia continues to grow in line with increasing demand  
65 for fish protein as an animal protein source. One of the main constraints in commercial fish  
66 farming is feed costs, which account for the most significant portion of total production costs  
67 (50–70%) (Fitra and Zakaria, 2022). Therefore, efforts to reduce feed costs by substituting  
68 conventional feed ingredients (e.g., fish meal or soybean meal) with low-cost local ingredients  
69 are crucial to improving the economic efficiency of aquaculture businesses (Chen *et al.*, 2019;  
70 Kim & Cho, 2024). Therefore, strategies to reduce feed costs without compromising fish  
71 performance are a key focus in modern aquaculture nutrition research.

72 Giant gourami (*Osphronemus goramy*) is a high value commodity in Indonesia, but  
73 often exhibits relatively slow growth and specific feeding requirements. Optimizing feed  
74 composition is necessary to improve feed conversion efficiency, growth rate, and protein and  
75 lipid retention in fish. One of the main challenges in gurami fish farming is feed efficiency,  
76 particularly in improving protein and lipid retention, which directly impacts fish growth and  
77 meat quality (Afriyanti *et al.*, 2020). The quality of fish feed protein can also be seen from the  
78 protein retention value in the fish body. The source and quality of protein influences protein  
79 retention value in the feed (Chen *et al.*, 2019).

80 Giant gourami farming generally uses commercial feed, which can account for up to  
81 60% of production costs, thereby reducing giant gourami production. Feed is a major factor  
82 affecting fish growth and survival (Sonavel *et al.*, 2020). One effort made to increase giant

83 gourami is by using high-quality feed (Suwarsito & SusyLOWATI, 2024). One of the nutritional  
84 factors required for fish growth is protein content and amino acid components (Fitra & Zakaria,  
85 2022). Therefore, feed containing plant-based protein derived from fermented fig flour (*Ficus*  
86 *racemosa*) is used to improve the growth performance of giant gourami.

87         The use of fig flour has been proven in alternative feeding as a substitute for commercial  
88 feed for giant gourami to improve feed quality and fish growth (Zakaria *et al.*, 2022). Partial  
89 substitution of feed with fig flour is expected to improve the digestive profile and increase feed  
90 utilization efficiency, thereby positively impacting protein retention and lipid retention in fish  
91 (Yonarta *et al.*, 2023).

92         Conventional feed used in fish farming is generally based on imported raw materials  
93 such as fish meal and soybeans, which are expensive and limited in availability. The use of  
94 alternative raw materials in fish feed formulation is an increasingly interesting topic, especially  
95 for reducing dependence on conventional feed ingredients such as fish meal and soybeans.  
96 Therefore, economical local alternatives with high nutritional value are needed. One potential  
97 local ingredient is fig fruit (*F. racemosa*), which is rich in bioactive compounds such as  
98 flavonoids, tannins, and saponins, and contains adequate levels of protein and fiber to serve as  
99 a feed substitute. The nutrients contained in figs are 28.125% protein, 2% minerals, 30.5%  
100 calcium, 15.84% carbohydrates, 20% carotene, 5.3% ascorbic acid, and are rich in phosphorus  
101 and iron (Kannan *et al.*, 2024; Rasyid *et al.*, 2017; Sharma *et al.*, 2020).

102         Several studies have shown that the antioxidant content in figs can increase immunity  
103 and feed efficiency in fish (Prasad *et al.*, 2014). Substituting fig flour into artificial feed has a  
104 significant effect on feed quality and the growth of the giant gourami (Zakaria *et al.*, 2022).  
105 Therefore, partially substituting feed with fig flour has the potential to improve protein and  
106 lipid retention efficiency in giant gourami.

107            Research on the effects of feed substitution with fermented fig flour (*F. racemosa*) on  
108 protein and lipid retention. This study aims to analyze the effects of feeding giant gourami with  
109 fig flour feed substitutes on protein and lipid retention in aquaculture systems. The results of  
110 this study are expected to provide scientific information on the use of local resources in fish  
111 feed formulation and support the development of more efficient and sustainable fish farming.

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## 113 **MATERIALS AND METHODS**

### 114 **Time and Location**

115            This research was conducted from August to December 2021 at the Fish Seed Center,  
116 Bungus Timur Village, Bungus Teluk Kabung District, Padang. Data analysis was carried out  
117 at the Animal Ecology Research Laboratory, Department of Biology, Faculty of Mathematics  
118 and Natural Sciences, Andalas University, Padang, West Sumatra.

### 119 **Experimental Design and Formulated Feed**

120            The method used in this study was an experimental design with a completely randomized  
121 design consisting of 5 treatments and 5 replicates. The treatments given in this study were as  
122 follows:

123 (F1) Feed with 0% fermented fig flour

124 (F2) Feed with 10% fermented fig flour

125 (F3) Feed with 20% fermented fig flour

126 (F4) Feed with 30% fermented fig flour

127 (F5) Feed with 40% fermented fig flour

128            The feed to be used in this study was first analyzed proximately to determine its  
129 nutritional content. The feed formulation used was based on the study (Fitra, 2021) with fig  
130 fermentation. The feed formulation was prepared using the isonitrogenous method with a  
131 protein content of 32%. The raw materials used in the feed mixture were soybean meal, fish

132 meal, tapioca flour, corn flour, fine bran, fish oil, vitamins and minerals (Premix Aquavita),  
133 and water, as well as fermentation materials, namely Effective Microorganism-4 (EM-4) and  
134 distilled water. The fermentation of fig flour was carried out aerobically, incubated for 72 hours  
135 using EM-4. Weigh 2.000 g of fig flour, then gradually pour in 1.200 ml of distilled water until  
136 a uniform mixture is obtained. The mixture is placed in a heat-resistant plastic container and  
137 steamed for 30 minutes. Allow the fig flour to cool for 30 minutes. Next, 2.000 g of fig flour  
138 is weighed and mixed a 5% EM-4 solution until homogeneous. The mixture of fig flour and  
139 EM-4 is placed in a 14×30 cm plastic bag (Listiwati & Pramono, 2014).

140         The test fish used were 250 giant gourami weighing 15 - 20 g, obtained directly from  
141 the Padang City Fish Seed Center. The experiment was carried out in a 12 × 8 m earthen pond  
142 fitted with hapa nets to separate experimental units and maintain uniform rearing conditions.  
143 Water depth was consistently maintained between 60 and 75 cm throughout the experimental  
144 period. Feeding was carried out at 07:00 and 17:00 WIB with a dose of 3% of the fish biomass.  
145 Giant gourami were reared for 70 days, and their length and weight were measured every 14  
146 days (Arifiina *et al.*, 2020). Fish weight and length were observed every 14 days by weighing  
147 all fish from each treatment container (Fitra, 2021).

148         Total length was measured from the anterior tip of the snout to the posterior end of the  
149 caudal fin using a measuring board or digital caliper with a precision of 0.1 cm. All  
150 measurements were conducted under consistent handling procedures to reduce stress and  
151 measurement bias. After measurement, the fish were immediately returned to their respective  
152 rearing containers. The collected data were used to calculate growth performance parameters  
153 during the experimental period. Prior to measurement, all fish from each treatment container  
154 were carefully collected to ensure accurate biomass estimation. Excess water on the fish body  
155 surface was gently removed using a soft cloth to minimize weighing error. Individual fish were  
156 weighed using a digital balance with a precision of 0.01 g to obtain accurate body weight data.

157 Three fish samples for each treatment were taken at the beginning and end of the study  
158 and analyzed for moisture, protein, lipid, crude fiber, and ash content to determine protein and  
159 lipid retention in giant gourami. To determine the protein and lipid content in fish bodies at the  
160 beginning and end of the study, tests were conducted at the Chemistry Laboratory at Bung  
161 Hatta University in Padang City. The protein and lipid content of fish and feed were analyzed  
162 using the Kjeldahl and Soxhlet methods based on AOAC (2019).

163 The crude protein content of fish and feed samples was determined using the Kjeldahl  
164 method in accordance with AOAC Official Methods 978.04 and 984.13, based on total nitrogen  
165 determination following acid digestion, distillation, and titration, with nitrogen values  
166 converted to crude protein using a factor of 6.25. Crude lipid content was analyzed by Soxhlet  
167 extraction following AOAC Official Method 920.39, in which dried samples were continuously  
168 extracted with a non-polar solvent and the lipid residue was quantified gravimetrically.

169 **Parameters Assessed**

170 The parameters observed in this study were protein retention, lipid retention, and the  
171 relationship between feed quality and the length and weight of fish.

172 The absolute weight gain of fish can be calculated using the formula (1) of Effendie  
173 (1997):

174  $W_m = W_t - W_o$ ..... (1)

175 Notes:

176  $W_m$  : Average absolute weight gain of fish (g)

177  $W_t$  : Average individual weight of fish at the end of the study (g)

178  $W_o$  : Average individual weight of fish at the start of the study (g)

179 The absolute length increment of fish can be calculated using the formula (2) of  
180 Effendie (1997):

181  $L_m = L_t - L_o$ ..... (2)

182 Notes:

183  $L_m$  : Average absolute length gain of fish (mm)

184  $L_t$  : Average individual length of fish at the end of the study (mm)

185  $L_o$  : Average individual length of fish at the start of the study (mm)

186 Protein retention is the ratio of the amount of protein stored in the fish's body to the  
187 amount of protein provided during maintenance. The increase in fish body protein is calculated  
188 by multiplying the fish's dry weight at the end of the study by its final body protein content,  
189 minus the dry weight at the start of the study multiplied by its initial body protein content.  
190 Protein retention is calculated using the formula (3) (Willer *et al.*, 2024).

191  $RP (\%) = \left( \frac{P_t - P_o}{P_p} \right) \times 100 \dots\dots\dots (3)$

192 Notes:

193  $RP$  = Protein retention (%)

194  $P_t$  = Total protein in fish at the end of the study (%)

195  $P_o$  = Total protein in fish at the beginning of the study (%)

196  $P_p$  = Total protein in feed consumed by fish (%)

197 Lipid retention in fish is the percentage of lipid from feed that is stored as body tissue  
198 during the maintenance period. The increase in fish body lipid was calculated by multiplying  
199 the fish's dry weight at the end of the study by its final body lipid content, minus the dry weight  
200 at the start of the study multiplied by its initial body lipid content. It is generally calculated  
201 using the formula (4) (Van Nguyen *et al.*, 2024).

202  $RL (\%) = \left( \frac{L_t - L_o}{L_p} \right) \times 100 \dots\dots\dots (4)$

203 Notes:

204  $RL$  = Lipid retention (%)

205  $L_t$  = Total body lipid of fish at the end of the study (%)

206  $L_o$  = Total body lipid of fish at the beginning of the study (%)

207 Lp = Total feed lipid consumed by fish (%)

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#### 209 **Data Statistic**

210 The protein and lipid retention data were analyzed using one-way ANOVA at the 5%  
211 significance level. If there were significant differences among treatments, DNMRT was  
212 performed in SPSS version 26. Normality and homogeneity were validated using the Shapiro-  
213 Wilk and Levene's tests (Zakaria *et al.*, 2022).

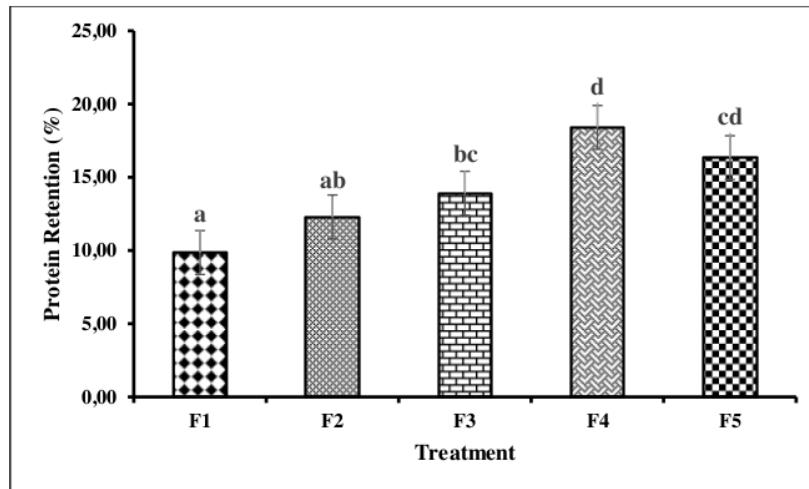
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### 215 **RESULTS AND DISCUSSION**

#### 216 **Protein Retention**

217 Based on the results of the one-way ANOVA statistical test, it was found that differences  
218 in the percentage of fermented fig flour in artificial feed had a significant effect ( $p < 0.05$ ) on  
219 the protein retention of giant gourami. The results of Duncan's New Multiple Range Test  
220 showed that treatments F4 and F1 (control) were significantly different, and that treatments F2  
221 and F3 were significantly different (Figure 1). However, treatment F3 did not differ  
222 significantly from treatment F1. Treatment F2 had the same effect on protein retention as  
223 treatment F1 or the control in giant gourami.

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Figure 1. Giant gourami fish protein retention in each treatment: (F1) Feed with 0% fermented fig flour, (F2) Feed with 10% fermented fig flour, (F3) Feed with 20% fermented fig flour, (F4) Feed with 30% fermented fig flour, (F5) Feed with 40% fermented fig flour. Different letters above the bar in each treatment indicate significant differences at the 95% confidence level ( $p < 0.05$ ).

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Protein retention is the ratio between the amount of protein in the fish's body and the amount of protein consumed in the feed. Based on the research data the highest average protein retention value for giant gourami was observed in treatment F4, with the addition of 30% fermented fig flour to the artificial feed, at  $18.41 \pm 0.86\%$  (feed protein content 35.46%). Meanwhile, treatment F1 or the control, had the lowest average protein retention value with artificial feed without fermented fig flour, at  $9.86 \pm 0.10\%$  (feed protein content 31.65%).

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Treatment F4 is the best feed for achieving effective, efficient retention in giant gourami. The protein content in treatment F4 feed is 32%, indicating that the addition of 30% fermented fig flour is suitable for the needs of giant gourami, enabling optimal absorption by the giant gourami's body. This indicates that feed containing fermented fig flour is better for giant gourami than feed without it, which served as the control.

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According to Langi *et al.* (2024), the protein requirement range for carnivorous fish is 35–45%, whereas herbivorous fish generally require only 25–35%, depending on growth phase

246 and environmental conditions. This is in line with the results of research that giant gourami fish  
247 as a herbivorous fish is able to produce optimal protein retention. Serra *et al.* (2024), stated that  
248 protein utilization efficiency is also greatly influenced by alternative protein sources such as  
249 single-cell protein, insect meal, and fermented plant materials, which can improve digestibility  
250 and support higher protein retention values than conventional sources when formulated  
251 appropriately.

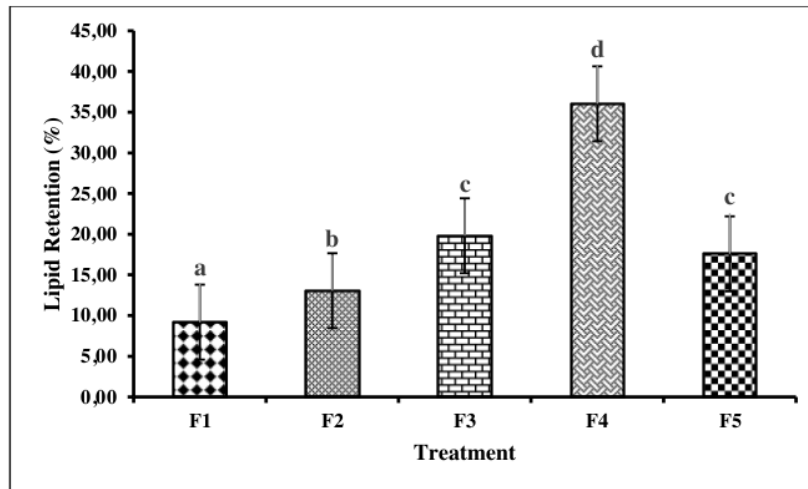
252 The balance of essential amino acids and the protein-to-energy ratio has a significant  
253 effect on protein retention and growth efficiency in various species of fish and crustaceans  
254 (Xing *et al.*, 2024). Feed protein quality is not only determined by total protein content, but  
255 also by amino acid bioavailability and crude protein digestibility, which are the main factors  
256 determining the level of protein retention in fish (Buttle *et al.*, 2024). Thus, protein retention  
257 can be used as a key indicator of feed protein quality, as high retention values reflect the ability  
258 of fish to utilize protein for tissue synthesis and growth (Serra *et al.*, 2024).

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## 260 **Lipid Retention**

261 Based on the results of the one-way ANOVA statistical test, it was found that differences  
262 in the percentage of fermented fig flour formulated in artificial feed had a significant effect  
263 ( $p < 0.05$ ) on the lipid retention value of giant gourami. The results of DNMRT showed  
264 significant differences between treatments F4 and F1 (control), F2, F3 and F5 (Figure 2).  
265 However, treatments F3 and F5 had the same effect on lipid retention in giant gourami.

266 The highest lipid retention value for giant gourami during the study was observed in  
267 treatment F4, at 36.30%. The results showed that treatment F4, with 30% fermented fig flour  
268 in the feed, was able to increase the lipid retention value in giant gourami compared to the  
269 control treatment, which was given feed without fermented fig flour.



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Figure 2. Giant gourami fish lipid retention in each treatment: (F1) Feed with 0% fermented fig flour, (F2) Feed with 10% fermented fig flour, (F3) Feed with 20% fermented fig flour, (F4) Feed with 30% fermented fig flour, (F5) Feed with 40% fermented fig flour. Different letters above the bar in each treatment indicate significant differences at the 95% confidence level ( $p < 0.05$ ).

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Lipid retention is an indicator of the efficiency of energy utilization from feed by fish, defined as the ratio of lipid stored in the fish's body to the lipid provided during maintenance. This parameter is closely related to feed nutrient composition, protein-to-lipid ratio, and lipid metabolism processes (Li *et al.*, 2023; Všetická *et al.*, 2020). The inclusion of fermented fig in artificial feed can increase lipid retention in giant gourami. Based on the research data treatment F4 had the highest average lipid retention of  $36.03 \pm 1.60\%$  (feed lipid content: 5.71%). Meanwhile, the lowest average lipid retention value was observed in treatment F1 (control), at  $9.19 \pm 0.09\%$  (feed lipid content 6.35%).

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The higher the lipid retention value, the more efficiently fish utilize energy from feed for tissue growth, rather than just as a metabolic energy reserve (Phan *et al.*, 2021; Tacon & Metian, 2015). According to Li *et al.* (2023), increasing the lipid content of feed to an optimal level can improve the protein-to-energy (P:E) ratio and shift the main energy source from protein oxidation, thereby simultaneously increasing protein and lipid retention efficiency.

291 However, excessively high lipid levels (>15%) can lead to visceral fat accumulation and  
292 reduced retention efficiency due to an imbalance in energy metabolism.

293 In addition to fatty acid composition, feed quality and fermentation processes also  
294 significantly affect lipid retention. According to Sartipiyarahmadi *et al.* (2023), fermentation  
295 of plant materials can reduce crude fiber content, increase lipid digestibility, and improve lipid  
296 retention efficiency without causing excessive Lipid accumulation in fish tissue. A similar  
297 finding was reported by Mao *et al.* (2024), who discovered that supplementing feed with  
298 naturally fermented ingredients can improve Lipid digestibility and increase the lipid retention  
299 ratio in freshwater fish.

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## 301 **CONCLUSIONS**

302 The difference in the percentage of fermented fig flour (*Ficus racemosa*) in the artificial  
303 feed had a significant effect ( $p<0.05$ ) on protein and lipid retention in giant gourami. Treatment  
304 F4, with 30% fermented fig flour in the artificial feed, was the best treatment for producing  
305 effective and efficient protein and lipid retention values in giant gourami.

306

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308 The authors declare that this research was conducted without any external financial  
309 support.

310

## 311 **AUTHOR CONTRIBUTION**

312 All authors have contributed to the completion of this research. The contributions of each  
313 author were as follows: RF; conceptualization, data curation, formal analysis, investigation,  
314 methodology, resources, software, visualization, writing original draft, review, and editing. IJZ;  
315 conceptualization, data curation, funding acquisition, project administration, supervision, and

316 validation. FAF; conceptualization, data curation, funding acquisition, project administration,  
317 supervision, and validation. WW; investigation and methodology. MF; investigation and  
318 methodology. All authors discussed the results and contributed to the final manuscript.

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#### 320 **DECLARATION OF COMPETING INTEREST AND USE GENERATIVE AI**

321 The authors declare no competing interests. "During the preparation of this work, the  
322 authors used Consensus, Chat GPT and Quillbot in order to enrich the literature and do  
323 paraphrasing. The authors reviewed and edited the content of the created material as needed  
324 and take full responsibility for the content of the publication".

325

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