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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)**

4
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16
17 **ABSTRACT**

18
19 *Ficus racemosa* has considerable potential as a plant-based alternative to partially replace
20 soybean meal in giant gourami diets. Fermentation of fig flour using Effective Microorganisms
21 4 (EM-4) is expected to improve feed protein quality and nutrient utilization. This study
22 evaluated the effects of substituting artificial feed with fermented fig flour on protein and lipid
23 retention in giant gourami (*Osphronemus goramy*) cultured under aquaculture conditions. The
24 experiment employed a completely randomized design with five dietary treatments containing
25 0%, 10%, 20%, 30%, and 40% fermented fig flour, each with five replicates. All diets were
26 formulated to be isonitrogenous, with a crude protein level of 32%. Fig flour was aerobically
27 fermented for 72 h using EM-4 prior to feed formulation. A total of 250 juvenile giant gourami
28 (15–20 g initial body weight) were reared for 70 days and fed twice daily at a feeding rate of
29 3% of total biomass. Fish length and weight were recorded at 14-day intervals. Protein and
30 lipid retention were determined based on proximate analyses conducted at the beginning and
31 end of the experiment. The results showed that dietary inclusion of fermented fig flour
32 significantly affected protein and lipid retention ($p < 0.05$). The highest protein retention (18.41
33 $\pm 0.86\%$) and lipid retention ($36.03 \pm 1.60\%$) were observed in fish fed the diet containing 30%
34 fermented fig flour. These findings indicate that partial substitution of artificial feed with 30%
35 fermented *F. racemosa* flour can enhance nutrient retention efficiency and represents a
36 promising strategy for developing cost-effective and sustainable feeds for giant gourami
37 aquaculture.

38
39 **KEYWORDS:** *Ficus racemosa*; retention; protein; lipid; fermentation; giant gourami

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

44
45 *Ficus racemosa* memiliki potensi yang besar sebagai bahan nabati alternatif untuk
46 menggantikan sebagian tepung kedelai dalam pakan ikan gurami. Fermentasi tepung buah ara
47 menggunakan Effective Microorganisms 4 (EM-4) diharapkan dapat meningkatkan kualitas
48 protein pakan dan pemanfaatan nutrisi. Penelitian ini bertujuan untuk mengevaluasi
49 pengaruh substitusi pakan buatan dengan tepung buah ara terfermentasi terhadap retensi
50 protein dan lemak pada ikan gurami (*Osphronemus goramy*) yang dibudidayakan dalam sistem
51 akuakultur. Penelitian ini menggunakan rancangan acak lengkap dengan lima perlakuan
52 pakan yang mengandung 0%, 10%, 20%, 30%, dan 40% tepung buah ara terfermentasi,

53 masing-masing dengan lima ulangan. Seluruh pakan diformulasikan secara isonitrogen
54 dengan kadar protein kasar sebesar 32%. Tepung buah ara difermentasi secara aerob selama
55 72 jam menggunakan EM-4 sebelum proses formulasi pakan. Sebanyak 250 ekor ikan gurami
56 juvenil dengan bobot awal 15–20 g dipelihara selama 70 hari dan diberi pakan dua kali sehari
57 dengan dosis 3% dari total biomassa. Pengukuran panjang dan bobot ikan dilakukan setiap
58 14 hari. Retensi protein dan lemak ditentukan berdasarkan analisis proksimat yang dilakukan
59 pada awal dan akhir penelitian. Hasil penelitian menunjukkan bahwa penambahan tepung
60 buah ara terfermentasi dalam pakan berpengaruh nyata terhadap retensi protein dan lemak (p
61 $< 0,05$). Nilai retensi protein tertinggi ($18,41 \pm 0,86\%$) dan retensi lemak tertinggi ($36,03 \pm$
62 $1,60\%$) diperoleh pada perlakuan pakan dengan penambahan 30% tepung buah ara
63 terfermentasi. Temuan ini menunjukkan bahwa substitusi parsial pakan buatan dengan 30%
64 tepung *F. racemosa* terfermentasi mampu meningkatkan efisiensi retensi nutrisi dan
65 berpotensi dikembangkan sebagai strategi pakan yang efektif, ekonomis, dan berkelanjutan
66 dalam budidaya ikan gurami.

67
68 **KATA KUNCI:** *Ficus racemosa*; retensi; protein; lemak; fermentasi; ikan gurami

69 70 71 INTRODUCTION

72 Aquaculture production in Indonesia continues to grow in line with increasing demand
73 for fish protein as an animal protein source. The high cost of formulated feeds remains a
74 significant constraint on the economic sustainability of commercial fish farming, which
75 account for the most significant portion of total production costs (50–70%) (Fitra and Zakaria,
76 2022). Therefore, efforts to reduce feed costs by substituting conventional feed ingredients
77 (e.g., fish meal or soybean meal) with low-cost local ingredients are crucial to improving the
78 economic efficiency of aquaculture businesses (Chen *et al.*, 2019; Kim & Cho, 2024).
79 Therefore, strategies to reduce feed costs without compromising fish performance are a key
80 focus in modern aquaculture nutrition research.

81 Giant gourami (*Osphronemus goramy*) is a high value commodity in Indonesia, but
82 often exhibits relatively slow growth and specific feeding requirements. Optimizing feed
83 composition is necessary to improve feed conversion efficiency, growth rate, and protein and
84 lipid retention in fish. Feed efficiency continues to be a limiting factor affecting the productivity
85 of giant gourami farming, particularly in improving protein and lipid retention, which directly
86 impacts fish growth and meat quality (Afriyanti *et al.*, 2020). The nutritional quality of dietary

87 protein can be evaluated through protein retention in the fish body, which is strongly ¹influenced
88 by the source and quality of protein in the diet (Chen *et al.*, 2019).

89 Giant gourami farming generally uses commercial feed, which can account for up to
90 60% of production costs, thereby reducing giant gourami production. Feed is a major factor
91 affecting fish growth and survival (Sonavel *et al.*, 2020). One effort made to increase giant
92 gourami is by using high-quality feed (Suwarsito & Susylowati, 2024). One of the nutritional
93 factors required for fish growth is protein content and amino acid components (Fitra & Zakaria,
94 2022). Therefore, feed containing plant-based protein derived from fermented fig flour (*Ficus*
95 *racemosa*) is applied as a dietary ingredient to enhance the growth performance of giant
96 gourami

97 The use of fig flour has been proven in alternative feeding as a substitute for commercial
98 feed for giant gourami to improve feed quality and fish growth (Zakaria *et al.*, 2022).
99 Substituting a portion of the feed with fig flour is hypothesized to improve digestive
100 performance and increase feed utilization efficiency, thereby positively impacting protein
101 retention and lipid retention in fish (Yonarta *et al.*, 2023).

102 Conventional feed used in fish farming is generally based on imported raw materials
103 such as fish meal and soybeans, which are expensive and limited in availability. The use of
104 alternative raw materials in fish feed formulation is an increasingly interesting topic, especially
105 for reducing dependence on conventional feed ingredients such as fish meal and soybeans.
106 Therefore, economical local alternatives with high nutritional value are needed. One potential
107 local ingredient is fig fruit (*F. racemosa*), which is the material contains high concentrations of
108 bioactive constituents such as flavonoids, tannins, and saponins, along with adequate protein
109 and fiber contents, supporting its use as an alternative feed ingredient. The nutrient profile of
110 figs shows relatively high protein (28.125%) and carbohydrate (15.84%) contents, along with

111 essential minerals (2%), calcium (30.5%), carotene (20%), ascorbic acid (5.3%), and notable
112 amounts of phosphorus and iron (Kannan *et al.*, 2024; Rasyid *et al.*, 2017; Sharma *et al.*, 2020).

113 Previous research indicates that the antioxidant compounds in figs play a role in
114 strengthening fish immunity and enhancing feed utilization efficiency (Prasad *et al.*, 2014).
115 Replacing part of the artificial feed with fig flour results in significant improvements in feed
116 quality and growth performance of giant gourami (Zakaria *et al.*, 2022). Accordingly, the partial
117 replacement of dietary ingredients with fig flour may improve protein and lipid retention
118 efficiency in giant gourami.

119 Research on the effects of feed substitution with fermented fig flour (*F. racemosa*) on
120 protein and lipid retention. The objective of this study was to assess the impact of fig flour
121 substitution in diets on protein and lipid retention in giant gourami. The results are anticipated
122 to provide scientific insights into the use of local feed resources and to support the development
123 of efficient and sustainable fish farming.

124

125 **MATERIALS AND METHODS**

126 **Time and Location**

127 The research was conducted during the period of August–December 2021 at the Fish
128 Seed Center in Bungus Timur Village, Bungus Teluk Kabung District, Padang, Indonesia, and
129 data analysis was carried out at the Animal Ecology Research Laboratory, Department of
130 Biology, Faculty of Mathematics and Natural Sciences, Andalas University, Padang, West
131 Sumatra.

132 **Experimental Design and Formulated Feed**

133 This study employed an experimental approach using a completely randomized design
134 comprising 5 treatments with 5 replicates each. The experimental treatments were as follows:

135 (F1) Feed with 0% fermented fig flour

136 (F2) Feed with 10% fermented fig flour

137 (F3) Feed with 20% fermented fig flour

138 (F4) Feed with 30% fermented fig flour

139 (F5) Feed with 40% fermented fig flour

140 The feed to be used in this study was first analyzed proximately to determine its
141 nutritional content. The feed formulation used was based on the study (Fitra, 2021) with fig
142 fermentation. The feed formulation was prepared using the isonitrogenous method with a
143 protein content of 32%. The raw materials used in the feed mixture were soybean meal, fish
144 meal, tapioca flour, corn flour, fine bran, fish oil, vitamins and minerals (Premix Aquavita),
145 and water, as well as fermentation materials, namely Effective Microorganism-4 (EM-4) and
146 distilled water. Fermentation of fig flour was conducted under aerobic conditions using
147 Effective Microorganisms 4 (EM-4) with an incubation period of 72 h. Initially, 2.000 g of fig
148 flour was weighed and gradually mixed with 1.200 mL of distilled water until a homogeneous
149 slurry was obtained. The mixture was transferred into a heat-resistant plastic container and
150 steamed for 30 min to reduce microbial contamination. After steaming, the fig flour was
151 allowed to cool at room temperature for approximately 30 min. Subsequently, 2.000 g of the
152 cooled fig flour was mixed thoroughly with a 5% EM-4 solution until uniform. The fermented
153 mixture was then placed into a plastic bag measuring 14 × 30 cm for further incubation
154 (Listiwati & Pramono, 2014).

155 The test fish used were 250 giant gourami weighing 15 - 20 g, obtained directly from
156 the Padang City Fish Seed Center. The experiment was carried out in a 12 × 8 m earthen pond
157 fitted with hapa nets to separate experimental units and maintain uniform rearing conditions.
158 Water depth was consistently maintained between 60 and 75 cm throughout the experimental
159 period. Feeding was carried out at 07:00 and 17:00 WIB with a dose of 3% of the fish biomass.
160 The experimental fish were reared for 70 days, and growth parameters (length and weight)

161 were measured at 14 day intervals (Arifiina *et al.*, 2020). Fish weight and length were observed
162 every 14 days by weighing all fish from each treatment container (Fitra, 2021).

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

172 Three fish samples for each treatment were taken at the beginning and end of the study
173 and analyzed for moisture, protein, lipid, crude fiber, and ash content to determine protein and
174 lipid retention in giant gourami. Chemical analyses to determine protein and lipid contents of
175 fish bodies at the start and end of the experiment were carried out at the Chemistry Laboratory,
176 Bung Hatta University, Padang City. Protein and lipid levels in fish and feed samples were
177 analyzed using standard Kjeldahl and Soxhlet methods based on AOAC (2019).

178 Crude protein levels in fish and feed samples were quantified using the Kjeldahl
179 procedure following AOAC Official Methods 978.04 and 984.13. This method involved
180 determination of total nitrogen through acid digestion, distillation, and titration, after which
181 nitrogen values were converted to crude protein using a conversion factor of 6.25. Crude lipid
182 content was determined by Soxhlet extraction in accordance with AOAC Official Method
183 920.39, where oven-dried samples were subjected to continuous extraction with a non-polar
184 solvent and lipid content was subsequently measured gravimetrically.

185 **Parameters Assessed**

186 This study assessed protein retention, lipid retention, length and body weight of giant
187 gourami.

188 Fish absolute weight gain was computed using the formula of Effendie (1997), as
189 presented in Equation (1):

$$190 W_m = W_t - W_o \dots\dots\dots (1)$$

191 Notes:

192 W_m : Average absolute weight gain of fish (g)

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

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

195 Fish absolute length increment was computed using the formula of Effendie (1997), as
196 presented in Equation (2):

$$197 L_m = L_t - L_o \dots\dots\dots (2)$$

198 Notes:

199 L_m : Average absolute length gain of fish (mm)

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

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

202 Protein retention is defined as the proportion of dietary protein that is deposited in the
203 fish body relative to the total protein intake during the rearing period. The increase in fish body
204 protein is calculated by multiplying the fish's dry weight at the end of the study by its final
205 body protein content, minus the dry weight at the start of the study multiplied by its initial body
206 protein content. The protein retention value was obtained using the formula provided in
207 Equation (3) (Willer *et al.*, 2024).

$$208 RP (\%) = \left(\frac{P_t - P_o}{P_p} \right) \times 100 \dots\dots\dots (3)$$

209 Notes:

210 RP = Protein retention (%)

211 Pt = Total protein in fish at the end of the study (%)

212 Po = Total protein in fish at the beginning of the study (%)

213 Pp = Total protein in feed consumed by fish (%)

214 Lipid retention in fish is the percentage of lipid from feed that is stored as body tissue
215 during the maintenance period. The increase in fish body lipid was calculated by multiplying
216 the fish's dry weight at the end of the study by its final body lipid content, minus the dry weight
217 at the start of the study multiplied by its initial body lipid content. The calculation is generally
218 performed using the formula presented in Equation (4) (Van Nguyen *et al.*, 2024).

219 $RL (\%) = \left(\frac{L_t - L_o}{L_p} \right) \times 100 \dots\dots\dots (4)$

220 Notes:

221 RL = Lipid retention (%)

222 Lt = Total body lipid of fish at the end of the study (%)

223 Lo = Total body lipid of fish at the beginning of the study (%)

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

225

226 **Data Statistic**

227 Protein and lipid retention data were subjected to one-way analysis of variance
228 (ANOVA) at a 5% significance level ($\alpha = 0.05$). When significant differences among treatments
229 were detected, Duncan's New Multiple Range Test (DNMRT) was applied using SPSS
230 software version 26. Prior to analysis, data normality and homogeneity of variances were
231 assessed using the Shapiro–Wilk and Levene's tests, respectively (Zakaria *et al.*, 2022).

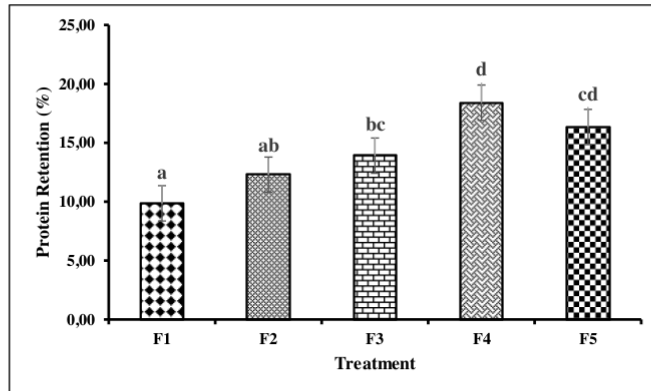
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233 **RESULTS AND DISCUSSION**

234 **Protein Retention**

235 Based on one-way ANOVA, variation in the proportion of fermented fig flour included
236 in the artificial diet had a significant effect on protein retention in giant gourami ($p < 0.05$).
237 Further analysis using Duncan's New Multiple Range Test revealed significant differences
238 between treatment F4 and the control (F1), as well as between treatments F2 and F3 (Figure
239 1). However, treatment F3 did not differ significantly from treatment F1. Treatment F2 had the
240 same effect on protein retention as treatment F1 or the control in giant gourami.

241



242

243

244 Figure 1. Giant gourami fish protein retention in each treatment: (F1) Diet containing 0%
245 fermented fig flour; (F2) diet containing 10% fermented fig flour; (F3) diet containing
246 20% fermented fig flour; (F4) diet containing 30% fermented fig flour; and (F5) diet
247 containing 40% fermented fig flour. Different superscript letters above the bars
248 indicate statistically significant differences among treatments at the 95% confidence
249 level ($p < 0.05$).

250

251 Protein retention represents the proportion of dietary protein that is deposited in the fish
252 body relative to the total protein intake. Based on the experimental results, the highest mean
253 protein retention in giant gourami was recorded in treatment F4, corresponding to a diet
254 containing 30% fermented fig flour, with a value of $18.41 \pm 0.86\%$ at a dietary protein level of
255 35.46%. Meanwhile, treatment F1 or the control, had the lowest average protein retention value
256 with artificial feed without fermented fig flour, at $9.86 \pm 0.10\%$ (feed protein content 31.65%).

257 Treatment F4 is the best feed for achieving effective, efficient retention in giant gourami.
258 The protein content of the F4 diet (32%) indicates that the inclusion of 30% fermented fig flour
259 was appropriate for meeting the nutritional requirements of giant gourami and promoting
260 efficient protein utilization. This indicates that feed containing fermented fig flour is better for
261 giant gourami than feed without it, which served as the control.

262 According to Langi *et al.* (2024), the protein requirement range for carnivorous fish is
263 35–45%, whereas herbivorous fish generally require only 25–35%, depending on growth phase
264 and environmental conditions. This is in line with the results of research that giant gourami fish
265 as a herbivorous fish is able to produce optimal protein retention. Serra *et al.* (2024), stated that
266 protein utilization efficiency is also greatly influenced by alternative protein ingredients,
267 including single cell protein, insect meal, and fermented plant-based materials, have been
268 reported to enhance digestibility and achieve higher protein retention than conventional protein
269 sources when properly formulated.

270 Appropriate essential amino acid balance and protein to energy ratios significantly
271 influence protein retention and growth performance in fish and crustaceans (Xing *et al.*, 2024).
272 Dietary protein quality is not solely defined by its concentration but is strongly affected by
273 amino acid bioavailability and digestibility, which ultimately determine protein retention in
274 fish (Buttle *et al.*, 2024). Thus, protein retention can be used as a key indicator of feed protein
275 quality, as high retention values reflect the ability of fish to utilize protein for tissue synthesis
276 and growth (Serra *et al.*, 2024).

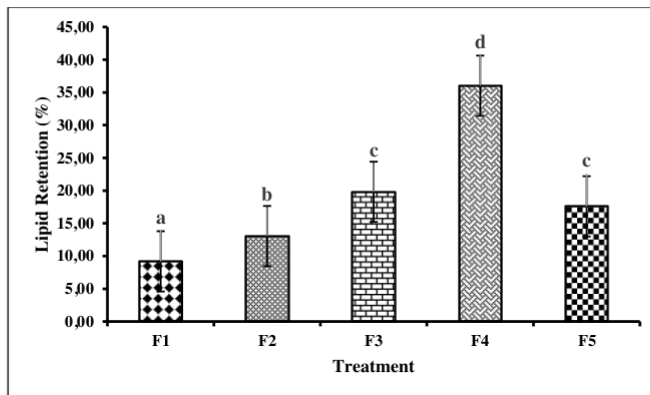
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278 **Lipid Retention**

279 One-way ANOVA indicated that variations in the proportion of fermented fig flour
280 incorporated into the formulated diet significantly affected lipid retention in giant gourami (p
281 < 0.05). Subsequent analysis using Duncan's New Multiple Range Test revealed that treatment

282 F4 differed significantly from the control (F1) as well as from treatments F2, F3, and F5 (Figure
283 2). However, treatments F3 and F5 had the same effect on lipid retention in giant gourami.

284 The highest lipid retention value for giant gourami during the study was observed in
285 treatment F4, at 36.30%. The findings demonstrated that treatment F4, incorporating 30%
286 fermented fig flour into the diet, resulted in higher lipid retention in giant gourami compared
287 with the control group, which received a diet without fermented fig flour.



288
289

290 Figure 2. Giant gourami fish lipid retention in each treatment: (F1) Diet containing 0%
291 fermented fig flour; (F2) diet containing 10% fermented fig flour; (F3) diet containing
292 20% fermented fig flour; (F4) diet containing 30% fermented fig flour; and (F5) diet
293 containing 40% fermented fig flour. Different superscript letters above the bars
294 indicate statistically significant differences among treatments at the 95% confidence
295 level ($p < 0.05$).
296

297 Lipid retention is an indicator of the efficiency of energy utilization from feed by fish,
298 defined as the ratio of lipid stored in the fish's body to the lipid provided during maintenance.
299 This parameter is closely related to feed nutrient composition, protein-to-lipid ratio, and lipid
300 metabolism processes (Li *et al.*, 2023; Všeticková *et al.*, 2020). The inclusion of fermented fig
301 in artificial feed can increase lipid retention in giant gourami. Based on the research data
302 treatment F4 had the highest average lipid retention of $36.03 \pm 1.60\%$ (feed lipid content:

303 5.71%). Meanwhile, the lowest average lipid retention value was observed in treatment F1
304 (control), at $9.19 \pm 0.09\%$ (feed lipid content 6.35%).

305 The higher the lipid retention value, the more efficiently fish utilize energy from feed
306 for tissue growth, rather than just as a metabolic energy reserve (Phan *et al.*, 2021; Tacon &
307 Metian, 2015). According to Li *et al.* (2023), increasing the lipid content of feed to an optimal
308 level can improve the protein-to-energy (P:E) ratio and shift the main energy source from
309 protein oxidation, thereby simultaneously increasing protein and lipid retention efficiency.
310 However, excessively high lipid levels (>15%) can lead to visceral fat accumulation and
311 reduced retention efficiency due to an imbalance in energy metabolism.

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

319

320 **CONCLUSIONS**

321 Variations in the inclusion level of fermented fig flour (*Ficus racemosa*) in the
322 formulated diets significantly affected protein and lipid retention in giant gourami ($p < 0.05$).
323 Among the treatments, F4, containing 30% fermented fig flour, proved to be the most effective
324 formulation for achieving optimal protein and lipid retention in giant gourami.

325

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328 research.

329

330 **AUTHOR CONTRIBUTION**

331 All authors have contributed to the completion of this research. The contributions of each
332 author were as follows: RF; conceptualization, data curation, formal analysis, investigation,
333 methodology, resources, software, visualization, writing original draft, review, and editing. IJZ;
334 conceptualization, data curation, funding acquisition, project administration, supervision, and
335 validation. FAF; conceptualization, data curation, funding acquisition, project administration,
336 supervision, and validation. WW; investigation and methodology. MF; investigation and
337 methodology. All authors participated in the discussion of the results and contributed to the
338 preparation of the final manuscript.

339

340 **DECLARATION OF COMPETING INTEREST AND USE GENERATIVE AI**

341 The authors declare no competing interests. Artificial intelligence-based tools were
342 used to assist in literature retrieval and language refinement. All content was reviewed and
343 approved by the authors, who assume full responsibility for the final manuscript.

344

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