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by Ahmad G. Mustofa, Ardiansyah, Sri Wahidah Mulyati, Hasniar, Indrayani

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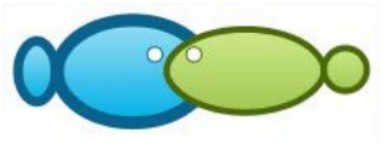

Use of duckweed (*Lemna minor*) harvested from IRAS as a partial replacement for fishmeal proteins in barramundi (*Lates calcarifer*) diets

[1]Ahmad G. Mustofa, [1]Ardiansyah, [2]Sri Wahidah, [2]Mulyati, [1]Hasniar, [3]Indrayani

[1] Fish Nursery Technology Study Program, Department of Aquaculture, Pangkep State Polytechnic of Agriculture, Pangkep, South Sulawesi, Indonesia; [2] Aquaculture Technology Study Program, Department of Aquaculture, Pangkep State Polytechnic of Agriculture, Pangkep, South Sulawesi, Indonesia; [3] Agricultural Technology Education Study Program, Makassar State University, Makassar, South Sulawesi, Indonesia. Corresponding author: Ardiansyah, ardi_kimsan@yahoo.com

Abstract. The optimum inclusion level of fermented duckweed (*Lemna minor*) in formulating feed for the juvenile barramundi (*Lates calcarifer*) was evaluated using growth performance, feed utilization, and physiological parameters. The juveniles were randomly stocked at a density of 15.86 kg m^{-3} in 15 independent integrated recirculating aquaculture systems (IRAS) and fed diets containing 0, 15, 25, 35, and 45% fermented duckweed (diets CD, F1, F2, F3, and F4, respectively). The average weight gain (WG) and protein efficiency ratio (PER) of fish fed-diet F4 were lower ($p < 0.05$) than those fed diets F1, F2, F3, and CD. Average WG of barramundi juveniles increased in all the dietary treatments when compared to the CD except diet F4. The highest WG and specific growth rate (SGR) were recorded for F2 fish. Fish-fed diet F3 also showed good performance in terms of WG and SGR. PER was highest in fish-fed F3 ($p < 0.05$). PER was lowest in CD-fed fish. Feed conversion ratio (FCR) was lowest for fish-fed F3 and highest for F4 ($p < 0.05$). With increasing dietary fermented duckweed meal inclusion, glucose and high-density lipoprotein cholesterol (HDL-C) concentrations increased, whereas triglyceride and low-density lipoprotein cholesterol (LDL-C) levels decreased. Activities of plasma alanine aminotransferase (ALT) and aspartate aminotransferase (AST) in juvenile barramundi fed the CD and F1 diets were higher ($p < 0.05$) than in fish fed other experimental diets. Plasma alkaline phosphatase (ALP) activities were lower ($p < 0.05$) in all duckweed supplemented diets. The highest carcass protein deposition was observed in fish fed F2 diet. These results indicated that F3 diet did not adversely affect the growth or metabolism of juvenile barramundi.

Key Words: barramundi, duckweed, fermentation, integrated recirculating aquaculture systems, recycling.

Introduction. Sustainable growth and intensification of aquaculture worldwide demand large quantities of high-quality protein feed. Fishmeal is considered to be an ideal source of dietary protein due to its high protein content, essential amino acid profile, and presence of phospholipids and essential fatty acids with high digestibility and palatability (Gasco et al 2018). The limiting factors in applying fishmeal to the production of aquafeed are high prices, limited resources, and variable microbiological quality (Gasco et al 2018). In the long-term, fishmeal availability may pose a potential threat to the sustainability of targeting cultured species that require high quantities of fishmeal in their feed. Furthermore, due to the increasing demand for fishmeal concerns have been raised over the reliance and sustainability of certain capture fisheries as sources of raw materials (Kok et al 2020).

The time-series data show a downward trend in the catch of fish for feed since the 1980s (FAO 2016). The accelerated usage of fishmeal, coupled with decreasing catches in world fisheries, has resulted in significant increases in the use of various alternative

protein ingredients for aquafeeds (Liland et al 2021). Due to the importance of low-cost and nutritionally balanced diets for fish, there are increasing research efforts to evaluate the nutritional value of different, unconventional feed resources, including the utilization of aquatic macrophytes or recycling of organic waste or plant by-products (Velásquez et al 2015).

In integrated recirculating aquaculture systems (IRAS), organic by-products may be reclaimed, recycled, and re-used. This farming system has been proposed as an environmentally sustainable way of recycling unused by-products, especially those produced through the culture of high trophic level fish species that require the supply of exogenous energy (FAO 2018). Reclamation, recycling, and re-use help to solve the problems of by-product disposal while relieving the scarcity of resource materials. In this experimental study, duckweed (*Lemna minor*) was used as a producer and biofilter media, the target species, barramundi (*Lates calcarifer*) as a consumer, and aquatic or substrate attached microorganisms as decomposers which form a complete ecosystem (Gupta et al 2017). Barramundi is a carnivorous species and accepts most forms of frozen and live feed (Palma et al 2020). Barramundi is a highly desired marine fish species for commercial culture due to its high market demand, fast growth, euryhaline nature, and its adaptability to various growing environments (FAO 2009).

Plant proteins represent a good option for replacing fishmeal in formulated diets. Previous work has shown that the substitution of fishmeal with plant-based protein is feasible without compromising the growth and performance of cultured barramundi (Nandakumar et al 2017). Duckweed has been successfully used as a complete feed for fish and shrimp (Flores-Miranda et al 2015). Duckweed responds with linear increases in biomass yield and crude protein content when fertilized with biodigester effluent (Dang et al 2011). Furthermore, a reduction of 30% in tilapia feeding costs was obtained by substituting a duckweed meal for fishmeal for Nile tilapia (*Oreochromis niloticus*) (Mohedano et al 2005). However, apart from previous reports (Flores-Miranda et al 2015), there appears to be no information on the potential value of duckweed as a fish feed supplement when co-produced in an IRAS.

Duckweed contains 28 to 43% crude protein, 5% fiber in dry weight, a high concentration of trace minerals, such as phosphorus and potassium, as well as xanthophylls and carotenes (Chaturvedi et al 2003). Duckweed also contains a higher amount of lysine (7.5%) and methionine (2.6%) than other plant feedstuffs (Chantiratikul et al 2010; Herawati et al 2020). The composition of duckweeds is highly variable and influenced by the nutrient status of their growth media (Ansal et al 2010). Duckweeds grown in nutrient-poor media have lower protein content associated with high fiber, ash, and carbohydrate content than that grown in nutrient-rich media (Ansal et al 2010). However, the presence of fiber and anti-nutritional factors, such as tannins and phytic acid in duckweed negatively affects their nutritional value and, consequently, the growth of the cultured species (Kumaraguru-Vasagam et al 2007; Flores-Miranda et al 2015). In this regard, processing duckweed materials through a simple and cheap method of fermentation may considerably enhance flavor and texture and decrease the anti-nutritional factors and crude fiber content thereby increasing the duckweeds' nutritional values and digestibility (Iji et al 2017).

The origin of duckweed may be varied, but, in this study, it was derived as a by-product from IRAS culturing barramundi. The use of fermented duckweed as one of the ingredients in barramundi diets would reduce the cost of fish production. However, no published data exist for the effects of fermented duckweed on the growth performance and feed utilization of juvenile barramundi. Additionally, the relationship between dietary duckweed protein inclusion and fish physiology remains neglected (Herawati et al 2020). Here, duckweed was fermented by a cellulose-degrading fish gut bacterium, *Bacillus* sp., and the optimum inclusion level was determined based on growth performance, feed utilization, and physiological parameters of juvenile barramundi.

Material and Method

Animals. Barramundi juveniles (n = 1200; mean weight 7.53±0.21 g) were used in 63-day long feeding trials in IRAS at Curtin Aquatic Research Laboratory, Perth, Western Australia from April to June 2017. Handling and rearing of the experimental animals were carried out according to ethical and welfare standards (approval number of AEC-2013-16).

Fermentation of duckweed. Fermentation of duckweed was performed according to the method of Bairagi et al (2002) with some modifications. *Bacillus* spp., used for fermentation of duckweed was isolated from the intestine of marron (*Cherax canii*). Duckweeds harvested from previous studies in IRAS were spread on trays and sun-dried for 48 h until they reached a constant weight. The dried duckweed was finely ground and passed through a fine-meshed sieve to ensure homogeneity. A portion of sieved duckweed was moistened with 50% w/v liquid basal medium containing (g L^{-1}): KH_2PO_4, 4; Na_2HPO_4, 4; $MgSO_4$. $7H_2O$, 0.2; $CaCl_2$, 0.001; $FeSO_4$. $7H_2O$, 0.004, and autoclaved for sterilization. *Bacillus* spp. were inoculated at 1 x 10^8 CFU g^{-1} of the dried duckweed for 16 days at 37°C in an incubator.

Diet preparation. Experimental diets were formulated using fermented (F1 to F4) duckweed meals at 15%, 25%, 35%, and 45% inclusion levels. A diet containing fishmeal as the main protein source was used as the control diet (CD). To each of the formulated diets, 1% chromic oxide was added as a digestibility marker. All the diets were prepared in the pelleted form using 0.5% carboxy-methyl-cellulose as a binder. The pellets were sun-dried for a few days and crumbled (1.5 mm particle sizes) before feeding.

Experimental design and rearing conditions. The experiment was conducted in 15 independent IRAS. Barramundi juveniles were transported to Curtin Aquatic Research Laboratory and acclimatized to the laboratory conditions for 21 days and fed with a commercial barramundi diet. Barramundi juveniles were then randomly distributed at an optimum stocking density of 15.86 kg m^{-3} (Ardi & Fotedar 2016) with three replicates for each treatment. All the fish were fed twice daily at 10.00 and 17.00 h at a fixed feeding rate of 2.5% body weight per day for 63 days. The feeding rate was adjusted every 10 days after weighing the fish. To determine feed consumption, any leftover diet was collected 6 h after each feeding and weighed after oven drying. Fecal samples were regularly collected in the morning by siphoning, and following the "immediate pipetting" method described by Vandenberg & De La Noüe (2001) from every tank. Water quality parameters from each tank, measured following the standard methods for the examination of water and wastewater (APHA 2005), were monitored each week throughout the experimental period.

Chemical analyses and data collection. Proximate analysis of the experimental diets and fecal samples was performed following AOAC (2000) procedures as follows: moisture was determined after drying the samples in an oven at 105°C for 24 h. Crude protein was determined by micro-Kjeldahl digestion (N x 6.25) and distillation after acid digestion using a Kjeltec 1026 Distilling Unit together with a Tecator Digestion System (Tecator, Sweden). Ash was determined by incineration at 550°C for 12 h in a muffle furnace to constant weight. Lipid was determined by Soxhlet extraction with diethyl ether at 40-60°C for 7-8 h while crude fiber content was determined as a loss on ignition of dried lipid-free residues after digestion with 1.25% H_2SO_4 and 1.25% NaOH. Nitrogen-free extract (NFE) was calculated by subtracting the sum values of crude protein, crude lipid, crude fiber, and moisture from 100 (Ndidi et al 2014). Chromic oxide in the diets and fecal samples was estimated following the method of Divakaran et al (2002).

At the end of the 63-day experiment, five individual fish from each tank were euthanized with 60 mg L^{-1} Aqui-S containing 50% isoeugenol (New Zealand Ltd., Lower Hutt, New Zealand), then oven-dried at 105°C for 24 h, ground and stored at -20°C for subsequent analysis. The whole body was analyzed for moisture, crude protein, crude lipid, and ash following the aforementioned methods. Tannin concentration in both dried

and fermented duckweed was determined using Folin–Denis reagent (Polshettiwar et al 2007). The phytic acid concentration was determined using a spectrophotometric procedure following the method of Gao et al (2015). The absorbance was measured at 830 nm against a blank. The result was calculated as mg phytic acid/100 g dry sample using standard phytic acid, whereas estimation of total free amino acids was conducted according to Friedman (2004) using ninhydrin reagent dissolved in methyl cellosolve.

The juveniles were weighed at the beginning and the end of the experiment. Weight gained (WG, g), specific growth rate (SGR, % day^{-1}), feed conversion ratio (FCR), and protein efficiency ratio (PER) were calculated according to Amaya et al (2007), whereas apparent protein digestibility (APD, %) and apparent net protein utilization (ANPU, %) were calculated using standard methods (Noor et al 2000).

Physiological parameters. At the end of the 63-day feeding trial, five fish were randomly taken from each tank, anesthetized with 60 mg L^{-1} Aqui-S, containing 50% isoeugenol (New Zealand Ltd., Lower Hutt, New Zealand). The blood samples were collected by a heparinized syringe from the caudal vein. The collected blood was immediately centrifuged at 3,000 x *g* for 10 min at 4°C. Plasma samples were pooled and stored at -80°C for subsequent analysis of physiological parameters. Plasma glucose, triglyceride, cholesterol, high-density lipoprotein (HDL), low-density lipoprotein (LDL), and activities of aminotransferase, including alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP) were measured by using commercially available kits (BioSino Biotechnology and Science, Inc., China). All the physiological parameters were determined by using an automatic biochemical analyzer (Hitachi 7020, Japan).

Data analysis. A one-way variance (ANOVA) analysis was used to examine the differences in growth performances, feed utilization, and biochemical parameters among dietary treatments. All data obtained were expressed as means±standard error (SE). All computations were performed with IBM SPSS Statistics 22.0. Statistical significance was measured at $p \leq 0.05$ in all cases.

Results. The proximate compositions of feed ingredients and experimental diets are presented in Tables 1 and 2, respectively. Fermentation of duckweed resulted in a significant increase in crude protein and a significant decrease in the levels of crude fiber and antinutritional factors, phytic acid, and tannin. A comparison of the proximate composition of the CD, and duckweed incorporated diets indicated that the crude fiber level in the CD was 11.82%, whereas, it ranged from 3.92 to 5.36% in diets F1-F4. In diets containing fermented duckweed, the tannin, and phytic acid contents were below the detection limit.

The growth performance and feed utilization of barramundi juveniles in terms of WG, SGR, FCR, PER, ANPU, and APD are presented in Table 3. When compared to CD the average weight of juvenile barramundi increased in all except F4 diets. No significant differences in the WG and SGR of barramundi fed diets F1, F2, and F3, whereas fish fed diets F4 and control showed poor performance in terms of WG and SGR. PER was highest in F3 fish ($p < 0.05$). PER value was lowest with CD. FCR was lowest for F3 fish and highest for the F4 diet. ANPU was highest in juvenile barramundi reared on diet F2 ($p < 0.05$) and lowest with diet F4 ($p < 0.05$). Apparent protein digestibility (APD) for all diets was, however, high, ranging from 80.85 to 86.17%. Juvenile barramundi-fed diet F3 showed the highest APD value (86.17%). Lower protein digestibility was recorded on the fish-fed CD.

Table 1

Proximate composition of dried and fermented duckweed (% dry weight)

Recycled duckweed	*Moisture (%)*	*Crude protein (%)*	*Crude lipid (%)*	*Crude fiber (%)*	*Ash (%)*	*NFE (%)*	*Gross energy (K cal.g^{-1})*
Dried	3.2±0.12^{a}	29.86±0.07^{a}	3.80±0.14^{a}	13.22±0.21^{b}	14.00±0.15^{b}	41.24±0.12^{a}	3.32±0.04^{a}
Fermented	3.4±0.14^{b}	35.43±0.04^{b}	3.92±0.12^{b}	11.85±0.17^{a}	11.86±0.10^{a}	52.23±0.16^{b}	4.06±0.07^{b}

Values are expressed as mean±SEM. Different letters in the same row indicate a significant difference at $p < 0.05$.

Table 2

Ingredient composition (% dry weight) and proximate analyses of fermented duckweed incorporated feeds (on dry matter basis)

Ingredients	*Experimental diets*				
	Control diet	*F1 (15%)*	*F2 (25%)*	*F3 (35%)*	*F4 (45%)*
Fishmeal	40	37	35	33	31
Duckweed meal	-	15	25	35	45
Soybean meal	22	25	20	15	10
Corn flake	34	19	16	13	10
Fish oil	2	2	2	2	2
Vitamin mineral mix	1	1	1	1	1
Chromic oxide	1	1	1	1	1
Total	100	100	100	100	100
Proximate composition					
Crude protein	35.45	34.63	33.74	32.92	32.17
Crude lipid	8.4	5.10	5.36	5.87	5.88
Crude fibre	11.82	3.92	4.24	4.85	5.36
Ash	10	10	12	12	14
Dry matter	98	96	98	96	95
NFE	32.50	47.30	46.90	40.50	38.70
Gross energy (K cal g^{-1})	4.5	4.2	4.1	3.9	3.8
Phyctic acid	ND	ND	ND	ND	ND
Tannins	ND	ND	ND	ND	ND
Chromic oxide	0.97	1.01	1.01	1.02	1.02

ND = not detectable; NFE = nitrogen free extract.

Table 3

Growth and feed utilization efficiencies in juvenile barramundi fed experimental diets for 63 days

Parameters	*CD*	*Fermented duckweed meal*			
		F1 (15%)	*F2 (25%)*	*F3 (35%)*	*F4 (45%)*
Initial weight (g)	7.53 ± 0.02^{a}	7.53 ± 0.02^{a}	7.53 ± 0.01^{a}	7.53 ± 0.01^{a}	7.53 ± 0.02^{a}
Final weight (g)	18.24 ± 0.06^{b}	18.68 ± 0.08^{b}	18.84 ± 0.03^{c}	18.82 ± 0.04^{c}	18.01 ± 0.28^{a}
WG (g)	10.71 ± 0.06^{a}	11.15 ± 0.09^{b}	11.31 ± 0.03^{b}	11.28 ± 0.05^{b}	10.58 ± 0.30^{a}
SGR (% day^{-1})	1.40 ± 0.01^{a}	1.44 ± 0.01^{b}	1.46 ± 0.01^{b}	1.45 ± 0.01^{b}	1.39 ± 0.03^{a}
FCR	2.45 ± 0.01^{bc}	2.43 ± 0.01^{b}	2.41 ± 0.01^{a}	2.40 ± 0.01^{a}	2.48 ± 0.03^{c}
PER	1.17 ± 0.01^{a}	1.19 ± 0.01^{a}	1.23 ± 0.01^{b}	1.26 ± 0.01^{c}	1.24 ± 0.01^{b}
APD (%)	80.85 ± 0.01^{a}	83.01 ± 0.02^{b}	85.27 ± 0.01^{c}	86.17 ± 0.02^{c}	85.52 ± 0.01^{c}
ANPU (%)	33.82 ± 0.05^{bc}	25.71 ± 0.07^{b}	44.63 ± 0.04^{c}	40.19 ± 0.02^{c}	12.48 ± 0.02^{a}

Values are expressed as mean±SEM. Different letters in the same row indicate a significant difference at $p < 0.05$.

The carcass composition of juvenile barramundi before and after the experiment is presented in Table 4. Juvenile barramundi-fed diet F4 had a slightly higher percentage of moisture than fish-fed other experimental diets. The deposition of protein in the carcass of juvenile barramundi increased over the initial value in all the dietary treatments. Although all the fish were fed on isonitrogenous diets, the greatest accumulation of carcass protein as recorded in the group of fish reared on diet F2. The carcass lipid content varied significantly ($p < 0.05$) among different dietary treatments, in which the highest tissue lipid accumulation occurred in fish fed the CD. The ash content of fish carcass was highest ($p < 0.05$) in fish diet F3, while the moisture content of fish carcass was low ($p < 0.05$) in fish diet CD and F1.

Table 4

Proximate carcass compositions (% wet weight) of juvenile barramundi at the start and end of the 63-day feeding trial

Carcass composition	*Initial*	*CD*	*F1*	*F2*	*F3*	*F4*
Crude protein	13.21^{a}	16.45±0.15^{c}	15.62±0.04^{b}	17.32±0.03^{d}	16.83±0.12^{cd}	14.25±0.07^{a}
Crude lipid	2.43^{a}	2.76±0.11^{c}	2.34±0.04^{b}	2.60±0.04^{bc}	2.28±0.02^{a}	2.18±0.05^{a}
Moisture	76.19^{a}	75.31±0.04^{c}	75.29±0.02^{a}	75.87±0.03^{ab}	75.62±0.04^{b}	76.12±0.03^{a}
Ash	4.87^{a}	3.59±0.02^{a}	4.17±0.04^{ab}	4.59±0.03^{b}	5.01±0.03^{c}	4.87±0.05^{bc}

Values are expressed as mean±SEM. Different letters in the same row indicate a significant difference at $p < 0.05$.

Glucose, cholesterol, LDL-C, and HDL-C levels were significantly influenced by the dietary treatments (Table 5). The glucose concentration increased ($p < 0.05$) with dietary fermented duckweed meal inclusion above 15%. The cholesterol levels of juvenile barramundi fed the control and F1 diets were higher ($p < 0.05$) than barramundi fed other diets. Triglyceride levels decreased ($p < 0.05$) with the increasing fermented duckweed meals. The LDL-C concentration decreased, but the HDL-C concentration gradually increased with increasing fermented duckweed meal inclusion. The HDL-C concentration was higher ($p < 0.05$), while the LDL-C concentration was lower ($p < 0.05$) in fish-fed with F3 and F4 diets. Plasma ALT and AST activities in fish-fed CD and F1 diets were greater ($p < 0.05$) than in other groups while plasma ALP of F1, F2, F3, and F4 fish were lower ($p < 0.05$) than CD barramundi.

Table 5

Glucose, cholesterol, LDL-C, HDL-C, ALT, AST, and ALP changes of juvenile barramundi in response to dietary fermented duckweed meal inclusion

Parameters	*Control diet*	*Fermented duckweed inclusion level*			
		F1 (15%)	*F2 (25%)*	*F3 (35%)*	*F4 (45%)*
Glucose (mmol L^{-1})	5.40±0.03^{a}	5.52±0.01^{a}	5.93±0.02^{b}	5.97±0.01^{b}	6.21±0.02^{c}
Cholesterol (mmol L^{-1})	5.89±0.02^{b}	6.04±0.03^{b}	4.71±0.01^{a}	4.60±0.03^{a}	4.65±0.01^{a}
Triglyceride (mmol L^{-1})	5.38±0.03^{c}	5.01±0.02^{bc}	4.65±0.03^{b}	4.23±0.04^{ab}	4.07±0.03^{a}
LDL-C (mmol L^{-1})	4.35±0.02^{c}	4.33±0.03^{c}	3.91±0.02^{b}	3.72±0.05^{a}	3.79±0.04^{a}
HDL-C (mmol L^{-1})	1.31±0.10^{a}	1.26±0.05^{a}	1.37±0.07^{a}	1.59±0.10^{b}	1.59±0.11^{b}
ALT (U L^{-1})	6.07±0.05^{c}	6.12±0.03^{c}	5.89±0.02^{b}	5.67±0.02^{a}	5.84±0.03^{b}
AST (U L^{-1})	7.98±0.03^{c}	7.91±0.05^{c}	7.35±0.10^{a}	7.39±0.07^{a}	7.71±0.12^{b}
ALP (U L^{-1})	172.43±0.03^{c}	161.52±0.05^{b}	160.82±0.02^{b}	150.24±0.02^{a}	149.57±0.02^{a}

Values are expressed as mean±SEM. Different letters in the same row indicate a significant difference at $p < 0.05$.

Discussion. Fermented duckweed can be utilized as a feed ingredient in formulating diets for juvenile barramundi effectively up to 35% inclusion without compromising growth performance. WG, SGR, and FCR of barramundi juveniles were better with 25% and 35% fermented duckweed than other diets, while APD and ANPU of barramundi juveniles fed with F2 and F3 were better than the CD. Goswami et al (2020) demonstrated the possibility of incorporating dried *Lemna polyrhiza* as a feed ingredient for the juvenile Indian major carp (*Labeo rohita*), and observed higher carbohydrate digestibility concerning that of the control diet, although the SGR and FCR in the fish fed 25% and 35% fermented duckweed did not differ to controls. Aslam & Zuberi (2017) considered duckweed (*Lemna* sp.) as a highly nutritious ingredient for herbivorous fish such as grass carp (*Ctenopharyngodon idella*) due to its tenderness and high protein

content compared to other aquatic plants. In the present study, the protein content of dried and fermented duckweed was estimated to be 29.86 and 35.43%, respectively (Table 1). However, the presence of antinutritional factors in dried duckweed limits the direct use of this floating plant as a dietary ingredient.

Dietary tannin as low as 0.5% causes growth depression in chicke41 (Choi & Kim 2020) and there are also reports on the toxicity of tannin to fish (Omnes et al 2017). In the present study, tannin and phytic acid could not be detected in fermented duckweed incorporated diets. Apart from the presence of some antinutritional factors, fiber contents of plant ingredients could also be responsible for their poor digestibility (Zhou & Yue 2012). High levels of fiber in the diet are known to reduce feed intake, which can lead to growth retardation (Choi et al 2021). A possible reason for the poor performance of the juvenile barramundi on the control diet and 45% fermented duckweed meal diet may have been its relatively higher level of crude fiber compared to other fermented duckweed incorporated diets. Fiber is cellulose, which has less value in the nutrition of carnivorous fish. Fiber content should be restricted to less than 7% of fish diets (Hilton et al 2011). In comparison to other vertebrates in the evolutionary process, carnivorous fish have a rather simple, little-developed digestion system, and therefore, they have a reduc5d ability to digest carbohydrates as an energy source (Gatlin 2010). As dietary fiber is part of the carbohydrate component of plant ingredients, most teleost fish cannot utilize it (Eusebio et al 2004). Previous studies revealed that increasing the level of dietary lipids above 8% negatively affects growth performance and nutrient utilization, resulting in decreased protein accretion and slower growth rate (Borges et al 2009), while the other species such as red-spotted grouper (*Epinephelus akaara*), the increase of dietary lipid level above 9.11%, did not provide a beneficial effect on growth (Jiang et al 2015).

During fermentation, nutrient losses may occur as a result of leaching, destruction by light, heat, or oxygen, or as a result of microbial activity (Nkhata et al 2018). Nevertheless, loss of nutrients during fermentation is commonly negligible, and there may be an increase in the nutrient level through microbial synthesis (Nkhata et al 2018). The protein utilization efficiency of fish-fed 35% fermented duckweed meal incorporated diets was significantly better than those fed other diets. Hontiveros et al (2021) reported better growth of tilapia, *Oreochromis mossambicus*, fed diets containing composted water hyacinth (*Eichhornia crassipes*) than with conventional tilapia diet, although the latter contained more protein. Hui et al (2008) also indicated the possibility of incorporating composted *Salvinia cucullata* in supplementary diets for the Indian major carp substituting the conventional diet up to 20% level.

The better performance of juvenile barramundi in terms of percentage WG, SGR, FCR, APD, and ANPU has been observed in fish fed the 25% fermented duckweed meal diet followed by the 35% fermented duckweed meal diet. Fish fed the diet with 45% fermented duckweed gave the poorest performance in terms of WG and SGR. The reduced growth of the fish fed appeared to be due to increased fiber contents in the diets. Fish do not possess cellulase enzymes to hydrolyze cellulose, which is the main ingredient of plant cell walls (Ray et al 2012). Thus, increased dietary fiber in diet F4 may negatively affect fish growth by increasing FCR. The result suggests that high fermented duckweed inclusion might inhibit fish growth by decreasing feed efficiency.

Biochemical variables of plasma blood can be used as indicators to evaluate the health condition of the fish (Çelik et al 2012; Yılmaz & Ergün 2012). The present study demonstrated that glucose levels increased with an increased dietary level of fermented duckweed meal above 15%. A similar result was observed in cobia (*Rachycentron canadum*) (Ren et al 2011). Although, there has been considerable debate about the limited ability of carnivorous teleost fish to utilize dietary carbohydrates efficiently. Altered dietary carbohydrate contents lead to prominent alterations in metabolic enzyme activities in the fish liver (Leung & Woo 2012). Dietary carbohydrates impaired the control of plasma glucose levels, leading to glucose intolerance in such species (Polakof et al 2008). Nutritional status is a factor that can affect the glucose response. The intake of diets with different lipid and protein content resulted in different responses of the blood glucose of the fish (Martínez-Porchas et al 2009).

Variations in physiological parameters are an indicator of fish responses to their diet (Satheeshkumar et al 2012). Thus, changes in cholesterol, triglyceride, LDL-C, and HDL-C are related to feeding composition and what is used as energy for daily activities. Cholesterol is a precursor of steroid hormones, which has an essential role in the function of nerve fibers, the formation of bile salts, and the maintenance of cell membrane structure. Meanwhile, triglycerides may act as a reserve source of energy for body metabolism (Hoseini & Ghelichpour 2012). Cholesterol and triglyceride levels can be used as the main indicator of liver function, particularly lipid metabolism (Arvind et al 2019). In the present study, plasma cholesterol and triglycerides were lower in the fish-fed diets containing 35 and 45% fermented duckweed meal than in fish-fed CD. Similarly, Romarheim et al (2006) reported that the plasma cholesterol and triglyceride levels of rainbow trout (*Oncorhynchus mykiss*) were significantly reduced when 50% of the fishmeal was replaced by soybean meal. The result indicates that a high proportion of fat in the chemical composition of the feed resulted in high cholesterol levels of *L. calcarifer* (Satheeshkumar et al 2012).

The use of alternate plant proteins in fish diets has been associated with disturbances in lipid and cholesterol metabolism (Romano et al 2020). This may reflect the negative impacts of various compounds on bile salt dynamics and lipid absorption (Romarheim et al 2006). Flavonoid and phenolic compounds in duckweed may, therefore, have been responsible for the differences observed in cholesterol, triglyceride, and LDL levels.

Observations relating to results ALT and AST were similar to those reported for tilapia (*O. niloticus*) (Lin & Luo 2011) and Atlantic cod (*Gadus morhua*) (Hansen et al 2007). In humans, elevated plasma ALT and AST enzymes have been associated with increased cell damage, tissue necrosis, the risk of cardiovascular diseases, and myocardial infarction (Alvarez & Mukherjee 2011). Plasma AST can give information on the damage to organs and in particular to liver cells (Kumar et al 2010), which leak aminotransferases, including ALT and AST, into the blood (McGill 2016). If this was the case with fish, then the results herein indicate that juvenile barramundi-fed fermented duckweed meal was healthier than controls. ALP is also considered indicative of enterocyte activity and a marker for the intensity of nutrient absorption in fish (Minghetti et al 2017). In this study, activities of ALP decreased with increasing dietary fermented duckweed meal. This is similar to the findings of Sangavi et al (2020) who reported that the activities of ALP in fish-fed soybean meal-based diets were likely lower due to the decreased content of phosphoproteins generally found in vegetable meals (Silva et al 2010). All experimental diets tested increased carcass protein of the juvenile barramundi, while the carcass lipid content was reduced in F1, F3, and F4 diets.

Conclusions. The results suggest that fishmeal can be replaced with up to 35% fermented duckweed meal without adversely affecting the growth, survival, or physiological parameters of the juvenile barramundi. Decreased plasma ALP observed in the fish fed the fermented duckweed meal diets have been attributed to the healthier state of the plasma membranes of these fish compared to those in the control group. Therefore, duckweed besides acting as an efficient biological filter in IRAS can be recycled to reduce dependency on fishmeal-based diets.

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Authors:
Ahmad G. Mustofa, Fish Nursery Technology Study Program, Department of Aquaculture, Pangkep State of Polytechnic of Agriculture, Pangkep 90655, South Sulawesi, Indonesia, e-mail: aghufronm@gmail.com
Ardiansyah, Fish Nursery Technology Study Program, Department of Aquaculture, Pangkep State Polytechnic of Agriculture, Pangkep 90655, South Sulawesi, Indonesia, e-mail: ardi_kimsan@yahoo.com
Sri Wahidah, Aquaculture Technology Study Program, Aquaculture Department, Pangkep State of Agriculture, Pangkep 90655, South Sulawesi, Indonesia, e-mail: sriwahidah@gmail.com
Mulyati, Aquaculture Technology Study Program, Department of Aquaculture, Pangkep State of Agriculture, Pangkep 90655, South Sulawesi, Indonesia, e-mail: mulyatimakmur@gmail.com
Hasniar, Fish Nursery Technology Study Program, Department of Aquaculture, Pangkep State Polytechnic of Agriculture, Pangkep 90655, South Sulawesi, Indonesia, e-mail: niar6691@yahoo.com
Indrayani, Agricultural Technology Education Study Program, Makassar State University, Makassar 90222, South Sulawesi, Indonesia, e-mail: indrayani_tajudin@yahoo.com.au

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