

Productivity, bioeconomy and physiological responses of *Leptobarbus hoevenii* seed in a recirculation system with high density

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Abstract. The decline in Hoven's carp, *Leptobarbus hoevenii*, production in Jambi Province is due to the low production of ready-to-stock seeds in the rearing phase. Production intensification needs to be carried out to enhance seed production by increasing stocking density. This study aimed to analyze the production performance, bioeconomics and physiological responses of *L. hoevenii* seeds maintained in a recirculation system with high stocking density. This study was conducted for 2 months at the Telanaipura Ornamental Fish Installation, Jambi Province Marine and Fisheries Service. The study design was a completely randomized design with five treatments with three replications, namely five stocking densities: 3 ind L⁻¹ (A), 5 ind L⁻¹ (B), 10 ind L⁻¹ (C), 15 ind L⁻¹ (D) and 20 ind L⁻¹ (E). The results of the study showed that there was no significant difference between low and high densities, in all parameters tested, with a survival rate (SR) from 96.69±0.25% to 99.56±0.44%, a specific growth rate (SGR) from 2.50±0.12% day⁻¹ to 2.77±0.02% day⁻¹, an absolute weight growth (AWR) from 1.79±0.17 g to 2.18±0.04 g, an absolute length growth (ALG) from 1.95±0.08 cm to 2.63±0.05 cm, a feed conversion ratio (FCR) from 1.70±0.02 to 1.85±0.11, a final weight diversity coefficient (fWDC) from 14.11±2.05% to 15.27±2.93% and a final length diversity coefficient (fLDC) ranging from 4.56±0.76% to 6.93±0.65%. The stocking density treatment significantly affected the blood glucose (BG) and hematocrit (Ht) parameters and did not significantly affect the total cholesterol (TC), Ketone and hemoglobin (Hb) parameters. All economic analysis indicators produced the highest values in treatment E. The results of the analysis of the rearing media showed that the water quality was suitable for maintaining *L. hoevenii*. An important finding of the study was that 96% of *L. hoevenii* seeds were able to survive at a stocking density of 20 ind L⁻¹.

Key Words: fish seed nursery, Hoven's carp, Jambi Province, stocking density, stress response.

Introduction. Aquaculture consists of cultivating fish, shellfish, algae and other aquatic organisms in marine, brackish and fresh waters equipped with a water recirculation system (EU 2023). Aquaculture has become the fastest growing industrial activity producing food in the world (Santurtun et al 2018). The high demand for aquaculture products requires this industry to keep providing continuous production results. In Indonesia, freshwater aquaculture has quite high prospects. In 2020, the potential for freshwater aquaculture was 2,830,540 hectares with a utilization of 287,521 hectares or 10.16% so that there is still opportunity to develop of 2,543,019 hectares (KKP 2022). One of the aquaculture segments is nursery. Nursery activities aim to produce fish seeds that are ready to be released or spread in the next phase, namely enlargement (Harifuzzumar et al 2018). One type of freshwater fish that has potential and high economic value in Indonesia is the Hoven's carp, known as the jelawat fish (*Leptobarbus hoevenii*). *L. hoevenii* is a cyprinid fish that can be found in several river and lake areas in Malaysia, Cambodia, Indonesia, Laos, Thailand, and Vietnam (Mohsin & Ambak 1983; Vidthayanon et al 1997; Kottelat 2001). In Indonesia, *L. hoevenii* are found in the waters of the Batanghari River, Jambi Province (Sutisna et al 2020).

There has been a decline in the production of *L. hoevenii* farming in Jambi Province from 2019 to 2021, the total seed production of 16,108 fish and consumption size of 7,538 tons (Sutisna et al 2020). The decline in *L. hoevenii* production in Jambi Province was due to the low production of ready-to-scatter seeds in the growing phase. The nursery phase seed production technology is still carried out at a low density of 2-5 ind L⁻¹ (Sunarno & Syamsunarno 2017; Putri et al 2021; Harianto et al 2023a; Harianto et al 2024). Production intensification must be carried out in order to increase the amount of seed production. One way to increase production is intensification by increasing stocking density (Crab et al 2012; El-Dahhar et al 2021; Stanivuk et al 2024). Stocking density is the number or mass of fish in a certain amount of water (Jones & Sloman 2024) and is a relevant technological parameter in aquaculture (Enache et al 2011; Nuwansi et al 2021; Stickney 1993). Expanding stocking density is a way to increase production, space utilization in maintenance media and cultivation land (Biswas et al 2006; Adineh et al 2019).

Several recent research report that the stocking density of *L. hoevenii* in the nursery segmentation reared in aquarium containers ranges from 2-5 ind L⁻¹ (Sunarno & Syamsunarno 2017; Putri et al 2021; Harianto et al 2023b; Harianto et al 2024). The use of aquarium containers is the best for the nursery phase, in accordance with the opinion of Harianto et al (2024). The stocking density in the rearing of *L. hoevenii* that has been carried out still has the potential to be escalated. Increasing the stocking density will be followed by an increase in the amount of feed, metabolic waste, oxygen consumption and can reduce water quality (Latifah et al 2022). In addition, it can interfere with the physiological processes and behavior of fish towards movement space which can ultimately reduce the health and physiological conditions of the fish (Huisman 1987). Increasing *L. hoevenii* production by increasing the stocking density must be followed by improving water quality. One method that can be used to improve water quality is the use of a recirculation system.

The recirculation system is a fish production system that reuses rearing water by treating it to depurify the water (Bregnballe 2015; Takeuchi 2017; Goddek et al 2019). Water management is carried out using filters to reduce fish farming waste and uneaten feed (Chen et al 1994; Couturier et al 2009). The recirculation system is equipped with a filter unit, either a mechanical (FAO 2015) or biological filter (Gutierrez-Wing 2006). Research on *L. hoevenii* using a recirculation system has been conducted by (Putri et al 2021; Damayanti et al 2018; Rusliadi et al 2015). The latest research show that until now the stocking density of *L. hoevenii* in aquarium containers ranges from 2-5 ind L⁻¹ (Sunarno & Syamsunarno 2017; Putri et al 2021; Harianto et al 2023a; Harianto et al 2024), but the results of this research are suboptimal, without physiological and bioeconomic response information. This study aimed to analyze the production performance, bioeconomics and physiological responses of *L. hoevenii* seeds reared in a recirculation system with high stocking density.

Material and Method

Description of the study sites. This research was conducted for 2 months, from September to October 2024 at the Telanaipura Ornamental Fish Installation, Jambi Province Marine and Fisheries Service. Blood biochemical analysis was carried out at the Veterinary Teaching Hospital, Faculty of Veterinary Medicine, IPB University. Hematology and water quality analysis were carried out at the Basic Laboratory of Batanghari University.

Research design. This study used a completely randomized design with five treatments and three replications. The treatments applied were the differences in stocking density including stocking density of 3 ind L⁻¹ (treatment A), stocking density of 5 ind L⁻¹ (treatment B), stocking density of 10 ind L⁻¹ (treatment C), stocking density of 15 ind L⁻¹ (treatment D), stocking density of 20 ind L⁻¹ (treatment E).

Preparation of research containers. The research container in this study was an aquarium measuring 70x40x30 cm³, comprising 12 units with a water volume of 56 L.

Before use, the rearing container was washed with soap, rinsed with clean water and dried in the sun. The clean and dry container was filled with water according to the specified volume. Each research container was equipped with an aeration unit in the form of 2 aeration stones in each aquarium, a filtration unit (multilevel filtration) consisting of a 20 watt water pump machine, physical filter materials (synthetic cotton, gravel, shells) chemical filters (zeolite, activated charcoal, activated carbon) and biological filters (bio ball, kaldness) (Figure 1).

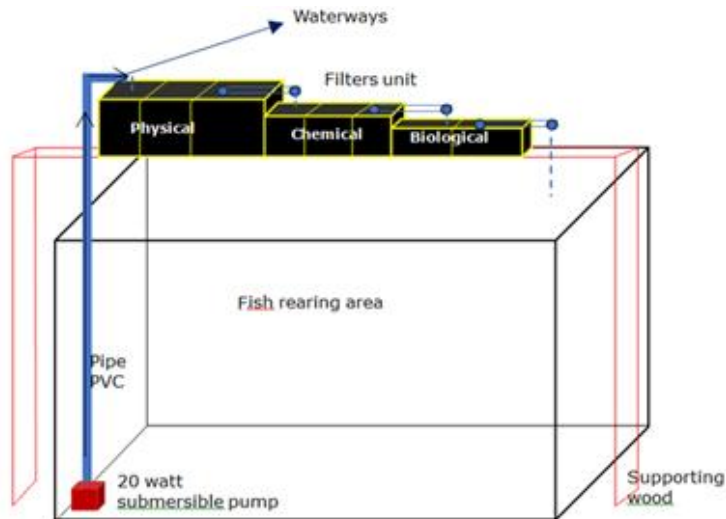


Figure 1. Filtration unit in the recirculation system.

Fish preparation and cultivation. The fish used in this study were 1 ± 0.2 inch. *L. hoevenii* were produced from artificial spawning by the Tenaipura Ornamental Fish Installation of the Jambi Province Marine and Fisheries Service. The number of fish used in this study was 8,904 fish. Fish rearing was carried out for 60 days. During the cultivation period, the fish were given commercial feed in the form of pellets with a protein content of 41%. The feeding method was an ad-restricted feeding rate given in the range of 10-5%. Feeding was delivered in the morning, afternoon and evening. The amount of feed was evaluated at each sampling period. The number of samples used in this study was 30% of the total population. The samples taken included test fish, blood, and water. During maintenance, water changes were carried out once every 3 days, by replacing as much as 20% of the total volume of rearing water.

Data collection. The data in this study include production performance, bioeconomics, physiological responses and water quality data. Data were collected by sampling every 10 days for measuring the production performance and once every 20 days for determining the physiological responses. Production performance data include weight and length, number of live and dead fish and amount of feed given during the rearing. The weight of the test fish samples was measured using a 0.001 g digital scale and the length was measured using a digital caliper (mm). The number of live and dead fish was observed and recorded during the cultivation, while the amount of feed was calculated for each feeding period. Bioeconomic data were collected by compiling cost components including investment costs, fixed costs and variable costs. Physiological response data were collected at the beginning, middle and end of the study by taking blood samples with a 0.5 mL syringe at the top between the anus and the tip of the anal fin. Then the blood samples were collected in a 1 mL Eppendorf tube and analyzed in the laboratory. Water samples collected at the beginning, middle and end of the study were taken with sample bottles and analyzed in the laboratory.

Observed parameters. The parameters observed in this study include the survival rate (SR), specific growth rate (SGR), absolute weight growth (AWG), absolute length growth (ALG), feed conversion ratio (FCR), final weight diversity coefficient (fWDC) and final length

diversity coefficient (fLDC) (Steel & Torrie 1981; Huisman 1987; Goddard 1996; NRC 2011). The bioeconomic analysis calculated is a feasibility analysis including revenue cost ratio C (RC^{-1}), benefit cost ratio (BC^{-1}), total cost (TC) (Ibrahim 2009), break even point (BEP) payback period (PP) and cost of goods manufactured (COGM) (Martin et al 1991; Rahardi et al 1998; Ibrahim 2009). Physiological response analysis includes blood biochemistry and hematology analysis. The blood biochemistry analysis measured includes blood glucose (BG), total cholesterol (TC) and ketones. The analysis was conducted using an ARKRAY blood chemical analyzer (SPOTCHEM-EZ sp 4430) equipped with a paper test indicator for each parameter. Hematology analysis includes hemoglobin (Hb) (Wedemeyer & Yasutake 1977) and hematocrit (Ht) (Anderson & Siwicki 1993). Water quality parameters consisted of temperature, pH and dissolved oxygen (DO) which were measured directly every day using a digital thermometer, Hanna HI98107 pH meter and Lutron-5510 DO meter. Measurements of ammonia, nitrite, nitrate and alkalinity were carried out at the beginning and end of the study. The analysis procedure used a spectrophotometer, referring to APHA (2006).

Data analysis. The data obtained were tabulated using Ms Office Excel 2016 and analyzed using SPSS version 22.0. This analysis was applied to determine the effect of treatment on each parameter tested at a 95% confidence level. In case of significant differences, the Duncan test was performed.

Results. Production performance parameters showed varying results in each treatment. Length and weight are important indicators in observing fish growth. In general, there was an increase in the average weight and length of *L. hoevenii* during the study. The average weight of fish stocked at the beginning of the study was 0.52 g increasing at the end of the study ranging from 2.32 ± 0.17 to 2.70 ± 0.04 g (Figure 2), while the average length of fish stocked at the beginning of the study was 4.12 cm increasing at the end of the study ranging from 6.06 ± 0.08 to $6.75 \pm 0.06 \text{ cm}$ (Figure 3). Production performance parameters are indicators of the fish farming techniques and are often used for evaluation. In this study, the production performance of *L. hoevenii* seeds reared for 60 days with different stocking densities in a recirculation system showed a good performance.

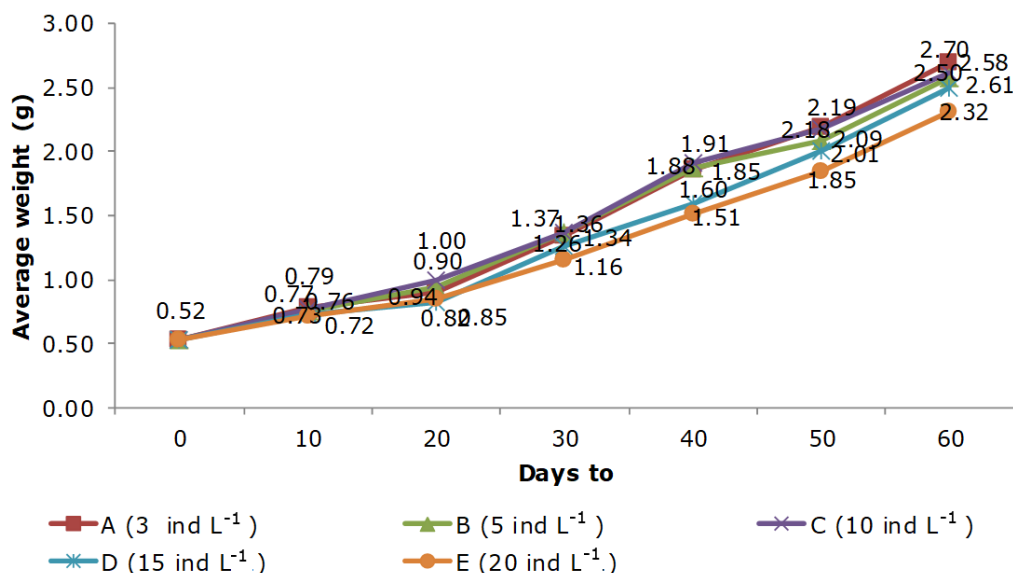


Figure 2. Average weights of *Leptobarbus hoevenii* seed with different stocking densities during 60 days of rearing.

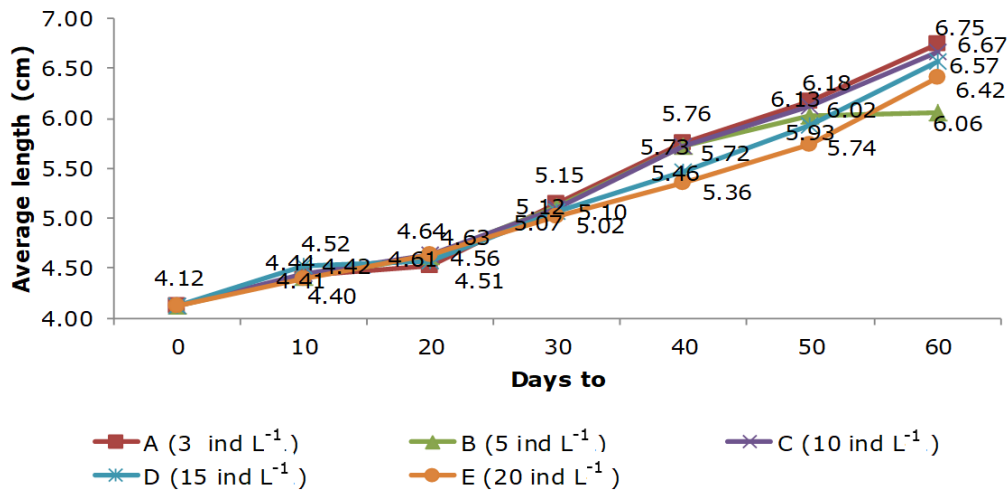


Figure 3. Average length of *Leptobarbus hoevenii* seed with different stocking densities during 60 days of rearing.

In this study, the production performance of *L. hoevenii* seeds cultivated for 60 days with different stocking densities in a recirculation system showed good performance. SR for all treatments ranged from $96.69 \pm 0.25\%$ to $99.56 \pm 0.44\%$, SGR ranged from $2.50 \pm 0.12\%$ day⁻¹ to $2.77 \pm 0.02\%$ day⁻¹, AWG for all treatments ranged from 1.79 ± 0.17 g to 2.18 ± 0.04 g, ALG ranged from 1.95 ± 0.08 cm to 2.63 ± 0.05 cm, FCR ranged from 1.70 ± 0.02 to 1.85 ± 0.11 , fWDC ranged from $14.11 \pm 2.05\%$ to $15.27 \pm 2.93\%$, and fLDC ranged from $4.56 \pm 0.76\%$ to $6.93 \pm 0.65\%$ (Table 1).

Table 1
Production performance of *Leptobarbus hoevenii* seeds with different stocking densities during the 60 days of rearing

Parameters	Treatment (Stocking density, ind L ⁻¹)				
	A (3)	B (5)	C (10)	D (15)	E (20)
SR (%)	99.56 ± 0.44^d	98.49 ± 0.41^c	98.67 ± 0.27^c	97.72 ± 0.51^b	96.69 ± 0.25^a
SGR (% day ⁻¹)	2.77 ± 0.02^c	2.69 ± 0.04^{bc}	2.71 ± 0.01^{bc}	2.64 ± 0.02^b	2.50 ± 0.12^a
AWG (g)	2.18 ± 0.04^c	2.06 ± 0.05^{bc}	2.09 ± 0.01^{bc}	1.98 ± 0.02^b	1.79 ± 0.17^a
ALG (cm)	2.63 ± 0.06^c	1.95 ± 0.08^a	2.56 ± 0.16^c	2.45 ± 0.18^{bc}	2.30 ± 0.06^b
FCR	1.70 ± 0.02^a	1.80 ± 0.06^a	1.79 ± 0.05^a	1.72 ± 0.03^a	1.85 ± 0.11^a
fWDC (%)	14.54 ± 2.40^a	14.11 ± 2.05^a	15.27 ± 2.93^a	15.02 ± 2.87^a	15.03 ± 1.08^a
fLDC (%)	6.93 ± 0.65^a	7.38 ± 2.13^a	4.56 ± 0.76^a	6.45 ± 2.49^a	5.03 ± 0.60^a

Data are presented as mean \pm standard error of mean. The values with same letters in the same line indicate non-significant differences ($P > 0.05$) in 5% significance level. One-way analysis of variance (ANOVA) followed by Duncan's multiple-range test was used to test significant differences among groups; SR-survival rate, SGR-specific growth rate, AWG-absolute weight growth, ALG-absolute length growth, FCR-feed conversion ratio, fWDC-final weight diversity coefficient, fLDC-final length diversity coefficient.

Based on the results of the analysis of variance, the stocking density treatment has a significant effect on the parameters of SR, SGR, AWG and ALG and has no significant effect on the parameters of FCR, fWDC and fLDC. The highest SR value was in treatment A of $99.56 \pm 0.44\%$, significantly different from other treatments. The highest SGR values were found in treatment A ($2.77 \pm 0.02\%$ day⁻¹) and treatment B ($2.69 \pm 0.04\%$ day⁻¹) and C ($2.71 \pm 0.01\%$ day⁻¹). The lowest SGR values were found in treatments D and E. The highest AWG values were identified in treatments A (2.18 ± 0.04 g), and in treatments B (2.06 ± 0.05 g) and C (2.09 ± 0.01 g). The lowest AWG values were observed in treatments D and E. The highest ALG values were found in treatment A (2.63 ± 0.06 cm), and in treatments C (2.56 ± 0.16 cm) and D (2.45 ± 0.18 cm). The lowest PPM values were found in treatments B and E.

Physiological response. Physiological response parameters in this study consisted of blood biochemical and hematological analysis. The blood biochemical analysis measured blood glucose (BG), total cholesterol (TC) and ketones while hematological analysis consisted of hemoglobin (Hb) and hematocrit (Ht) (Table 2). The BG value at the beginning of rearing period was 71.75 ± 25.87 mg dL⁻¹, at the end of the study it showed varying values in all treatments. There was a decrease in BG level in treatment A and B to 61.33 ± 7.51 and 68.00 ± 7.21 mg dL⁻¹ and an upscale in treatments C, D, and E to 77.00 ± 14.00 mg dL⁻¹, 95.33 ± 11.68 mg dL⁻¹, and 84.63 ± 32.84 mg dL⁻¹, respectively. The TC value at the beginning of cultivation was 314.67 ± 17.21 mg dL⁻¹. At the end of rearing, there was a decrease in TC value in treatment E to 299.67 ± 13.58 mg dL⁻¹ and an increase in treatments A (342.67 ± 46.14 mg dL⁻¹), B (328.33 ± 42.57 mg dL⁻¹), C (334.33 ± 28.22 mg dL⁻¹) and D (320.00 ± 9.17 mg dL⁻¹). The ketone value at the beginning of rearing was 9.78 ± 1.65 mg dL⁻¹. At the end of the study, the values fluctuated in all treatments. There was a decrease in ketone levels in treatments A, B, C and E to 8.80 ± 0.56 mg dL⁻¹, 8.23 ± 3.00 mg dL⁻¹, 9.10 ± 0.70 mg dL⁻¹ and 9.27 ± 2.72 mg dL⁻¹ and an increase in treatment D to 10.27 ± 1.14 mg dL⁻¹. The Hb value at the beginning of rearing was 15.00 ± 1.28 g dL⁻¹. At the end of maintenance there was a decrease in all treatments and ranged from 8.80 ± 0.56 g dL⁻¹ to 10.27 ± 1.14 g dL⁻¹. The Ht value at the beginning of rearing was $43.25 \pm 5.19\%$ and there was a decline at the end of the maintenance period in treatments A ($35.00 \pm 4.00\%$), B ($37.33 \pm 4.16\%$), D ($40.67 \pm 4.73\%$) and E ($43.00 \pm 1.00\%$), and a rise in treatment C ($43.67 \pm 4.04\%$).

Table 2
Analysis of physiological responses of *Leptobarbus hoevenii* seeds with different stocking densities during 60 days of rearing

Parameters	Before treatment	Treatment (Stocking density, ind L ⁻¹)				
		A (3)	B (5)	C (10)	D (15)	E (20)
BG (mg dL ⁻¹)	71.75 ± 25.87	61.33 ± 7.51^a	68.00 ± 7.21^a	77.00 ± 14.00^a	95.33 ± 11.68^b	84.63 ± 32.84^{ab}
	314.67 ± 17.21	342.67 ± 46.14^a	328.33 ± 42.57^a	334.33 ± 28.22^a	320.00 ± 9.17^a	299.67 ± 13.58^a
TC (mg dL ⁻¹)	9.78 ± 1.65	8.80 ± 0.56^a	8.23 ± 3.00^a	9.10 ± 0.70^a	10.27 ± 1.14^a	9.27 ± 2.72^a
	15.00 ± 1.28	11.53 ± 0.35^a	12.47 ± 1.50^a	14.40 ± 1.06^a	13.57 ± 2.42^a	13.80 ± 2.23^a
Keton (mg dL ⁻¹)	43.25 ± 5.19	35.00 ± 4.00^a	37.33 ± 4.16^{ab}	43.67 ± 4.04^b	40.67 ± 4.73^{ab}	43.00 ± 1.00^b
Hb (g dL ⁻¹)						
Ht (%)						

Data are presented as mean \pm standard error of mean. The values with same letters in the same line indicate non-significant differences ($P > 0.05$) in 5% significance level. One-way analysis of variance (ANOVA) followed by Duncan's multiple-range test was used to test significant differences among groups; BG-blood glucose, TC-total cholesterol, Hb-hemoglobin, Ht-hematocrit.

Based on the results of the analysis of variance, it shows that the stocking density treatment has a significant effect on the BG and Ht parameters and has no significant effect on the TC, ketone and Hb parameters. The highest BG value was found in treatment D, at 95.33 ± 11.68 mg dL⁻¹ and the lowest was found in treatment A, at 61.33 ± 7.51 mg dL⁻¹. The highest Ht value was found in treatment C, at $43.67 \pm 4.04\%$ and the lowest was found in treatment A, at $35.00 \pm 4.00\%$.

Bioeconomy analysis. The bioeconomic analysis calculated is a feasibility analysis including RC⁻¹ ratio, BC⁻¹ ratio, total cost (TC), break-even point (BEP), payback period (PP) and cost of goods manufactured (COGM) (Table 3). Business analysis in this study was calculated based on research data to determine the amount of production. The price components used in calculating the costs incurred were assumed and standardized with current year prices. There are several assumptions used, including 1) the bioeconomic analysis is carried out for a period of one year, 2) The fish farming cycle adjusted the research period, which is 6 cycles for 1 year, 3) The use of production units is the same

for all treatments. The differences lie in the use of seed inputs and adjusting the treatments applied, 4) the investment cost components for buildings are not calculated because this research is at a laboratory scale, 5) Factor prices were considered constant throughout the production cycle, 6) Production data adjusted to research results, and 7) The selling price of the product followed the selling price of *L. hoevenii* seeds at the time of the study. Based on the assumptions and calculation results used, it can be seen that the bioeconomic analysis of *L. hoevenii* seed nursery shows different results between treatments. In general, the data shows that the higher the stocking density, the more efficient and profitable the nursery business.

Table 3

Bioeconomy analysis rearing of *Leptobarbus hoevenii* seeds with different stocking densities during 60 days of rearing

Component	Treatment (Stocking density, ind L ⁻¹)				
	A (3)	B (5)	C (10)	D (15)	E (20)
Investment (USD)	203.38	203.38	203.38	203.38	203.38
Fixed costs (USD year ⁻¹)	170.64	170.64	170.64	170.64	170.64
Variable costs (USD year ⁻¹)	52.93	77.94	192.66	281.72	370.77
Total costs (USD year ⁻¹)	223.57	248.58	363.30	452.36	541.42
Fish production volume (ind year ⁻¹)	3.154	4.680	13.320	19.788	26.106
Revenue (USD year ⁻¹)	286.63	425.32	1210.51	1798.28	2372.44
Income (USD year ⁻¹)	63.06	176.74	847.21	1345.92	1831.02
RC ⁻¹ ratio	1.28	1.71	3.33	3.98	4.38
BC ⁻¹ ratio	0.28	0.71	2.33	2.98	3.38
BEP (individual)	1877.99	1877.99	1877.96	1877.96	1877.96
BEP (USD)	64.91	95.42	229.12	334.05	439.45
PP (year)	3.23	1.15	0.24	0.15	0.11
COGM (USD)	0.071	0.053	0.027	0.023	0.021

RC⁻¹ ratio-revenue cost ratio, BC⁻¹ ratio-benefit cost ratio, BEP-break even point, PP-payback period, COGM-cost of goods manufactured.

Water quality analysis. Water quality parameters consist of temperature, pH and dissolved oxygen (DO), ammonia, nitrite, nitrate and alkalinity (Table 4).

Table 4

Water quality measurement results rearing media of *Leptobarbus hoevenii* seeds with different stocking densities during 60 days of rearing

Treatment	Temp (°C)	pH	DO (mg L ⁻¹)	Ammonia (mg L ⁻¹)	Nitrite (mg L ⁻¹)	Nitrate (mg L ⁻¹)	Alkalinity (mg L ⁻¹)
A (3 ind L ⁻¹)	27.73-	7.76-	5.61-	<0.15-	0.1-	25-50	13.4-
	29.86	7.81	5.77	<0.15	0.25		13.10
B (5 ind L ⁻¹)	27.81-	7.75-	5.52-	<0.15-	0.1-	25-50	13.4-
	29.91	7.78	5.55	0.25	0.25		14.10
C (10 ind L ⁻¹)	27.76-	7.68-	5.06-	<0.15-	0.1-	25-50	13.4-
	29.99	7.69	5.08	0.5	0.25		12.50
D (15 ind L ⁻¹)	27.71-	7.60-	5.01-	<0.15-	0.1-	25-50	13.4-
	29.76	7.62	5.09	0.5	0.25		12.10
E (20 ind L ⁻¹)	27.71-	7.71-	5.33-	<0.15-	0.1-	25-50	13.44-
	29.94	7.81	5.39	0.5	0.50		11.80

Temperature values ranged from 27.71 to 29.99°C. pH values ranged from 7.60 to 7.81, DO values ranged from 5.01 to 5.77 mg L⁻¹. The initial nitrite value was 0.1 mg L⁻¹ and at the end of the study it ranged from 0.1 to 0.50 mg L⁻¹, the initial nitrate value was 25 mg L⁻¹ and at the end of the study ranged from 25 to 50 mg L⁻¹, the initial ammonia value was 25 mg L⁻¹ and at the end of the study it was less than 0.15 mg L⁻¹ and the initial alkalinity

value was $<15 \text{ mg L}^{-1}$ and there was no change at the end of the study. In general, water quality is still within the feasible range for maintaining *L. hoevenii* seeds in all treatments.

Discussion. Several recent research report that the stocking density of *L. hoevenii* in nursery segmentation maintained in aquarium containers ranges from 2 to 5 ind L^{-1} (Sunarno & Syamsunarno 2017; Putri et al 2021; Harianto et al 2023a; Harianto et al 2024). These studies evaluate the potential of the stocking density to be increased. Increasing the stocking density will be followed by an increase in the amount of feed, body metabolic waste, oxygen consumption and can reduce water quality (Latifah et al 2022). In addition, it can interfere with the physiological processes and behavior of fish. Less movement space can ultimately reduce the health and physiological conditions of the fish (Huisman 1987). This study brings data support on the physiological and bioeconomic response.

In this study, four main indicators of the *L. hoevenii* nursery process have been analyzed, namely the production performance, physiological response, bioeconomic analysis and water quality. In the production performance parameters, it can be seen that differences in stocking density levels have a significant effect on the SR, SGR, AWG and ALG and have no significant effect on the FCR, fWDC and fLDC. SR is the main parameter in aquaculture; high SR indicates that the cultivation activities carried out have been successful. The SR value for all treatments ranged from $96.69 \pm 0.25\%$ to $99.56 \pm 0.44\%$. The highest SR value was in treatment A at $99.56 \pm 0.44\%$, significantly different from other treatments. The results of this study found that increasing stocking density to 20 ind L^{-1} did not reduce the SR value. Treatment C gave the best SR value compared to treatments D and E. Previous studies with lower stocking densities produced smaller SR values than in this study (Hasan et al 2014; Rusliadi et al 2015; Harianto et al 2023b). The high SR value in this study was supported by good water quality conditions. Water quality during the rearing period was in optimal condition for maintaining *L. hoevenii* seeds. In addition, during the rearing period, water changes were carried out in each cultivation container as much as 20% of the total water volume. Aquaculture activities with good water management and quality will produce more and healthier fish and vice versa (Boyd & Tucker 2014; Rana & Jain 2017; Olanubi et al 2024). Stocking density is one of the most important parameters that affects growth performance and productivity in fish farming activities. Growth is usually seen from the increase in volume and length of body cells in wet weight and dry weight in a certain time unit (Effendie 1979). Previous studies reported that increasing fish stocking density had adverse effects on growth performance, for example in tambaqui (*Colossoma macropomum*), ayu (*Plecoglossus altivelis*), and tiger puffer (*Takifugu rubripes*) (Costa et al 2019; Iguchi et al 2003; Kikuchi et al 2006).

There are three main growth indicators analyzed including SGR, AWG and ALG. The results of the study showed that the treatment of different types of containers had a significant effect on the three parameters. All growth indicators analyzed showed that the highest stocking density that still provided the best growth value was treatment C (10 ind L^{-1}) with SGR values ($2.71 \pm 0.01\% \text{ day}^{-1}$), AWG ($2.09 \pm 0.01 \text{ g}$) and ALG ($2.56 \pm 0.16 \text{ cm}$). In this treatment, the growth value was the highest and was not significantly different from the low stocking density. The three growth indicators in this study were also higher than in previous studies, with SGR, AWG and ALG values ranging from $1.64\% \text{ day}^{-1}$ to $1.99\% \text{ day}^{-1}$, 0.0438 to 1.08 g and 1.53 to 1.84 cm, respectively (Kamarudin et al 2013; Rusliadi et al 2015; Sunarno & Syamsunarno 2017; Harianto et al 2023). The high values of weight and length growth in this study were supported by an optimal water quality performance, due to the use of a recirculation system (Table 4). Good water quality at high stocking densities does not interfere with the process of food intake and metabolism. There is very little competition for feed so that growth variations are very small, the test fish get the same portion of food with high growth values. Optimal growth occurs up to a stocking density of 10 ind L^{-1} . Optimal density plays an important role in increasing the growth of farmed fish (Aliabad et al 2022). The feed given in the study was commercial feed with an average protein content of 41%. This protein content is in accordance with the recommendations of previous researchers where *L. hoevenii* seeds measuring 0.065-1.6 g should be given feed with a protein content of 36-40% (Sunarno & Syamsunarno 2017).

High protein content is needed by *L. hoevenii* seeds, considering that the fish are still in a high growth phase. Growing fish seeds require feed that is rich in nutrients and energy for stable growth (Yúfera 2011). FCR is the amount of feed given to produce 1 kg of fish body weight (NRC 1977). Based on the results of the analysis of variance, the treatment of different levels of stocking density has no significant effect on the FCR parameter. The FCR value ranged from 1.70 ± 0.02 to 1.85 ± 0.11 , fWDC $14.11 \pm 2.05\%$ to $15.27 \pm 2.93\%$ and fLDC from $4.56 \pm 0.76\%$ to $6.93 \pm 0.65\%$ (Table 1). The FCR value in this study is quite low, showing a very high efficiency level of feed transformation into biomass. This condition also shows that the feed given to the fish is evenly distributed to each individual fish. The level of feed competition is very low so that the level of size uniformity is high. Radona et al (2017) and Fry et al (2018) reported that lower FCR values indicate a more efficient use of the feed. The FCR results in this study were more efficient than in previous studies, where *L. hoevenii* seeds cultivated using aquariums with different feeding levels produced FCR values ranging from 1.72 to 3.57 (Sonavel et al 2020; Harianto et al 2023). The final weight coefficient of variation (fWDC) and the final length coefficient of variation (fLDC) describe the level of diversity of fish weight and length at the end of maintenance; the higher the coefficient of variation, the lower the level of uniformity. Based on the results of the analysis of variance, different stocking density treatments have no significant effect on the fWDC and fLDC values. The fWDC value ranged from $14.11 \pm 2.05\%$ to $15.27 \pm 2.93\%$ and fLDC ranged from 4.56 ± 0.76 to $6.93 \pm 0.65\%$ (Table 1). The fWDC and fLDC values in this study were quite good because they were still below 25%. This is in accordance with the findings of Baras et al (2011), namely that the fWDC and fLDC values below 25% mean that the uniformity of the fish at the end of the study was high. In addition, the fWDC results in this study were better than in previous studies, where the fWDC values produced ranged from 20.69 to 24.84% (Harianto et al 2023b).

Stocking density in aquaculture is an important factor in reflecting and evaluating physiological stress levels (Barton 2002). Stocking density usually results in physiological stress and impaired fish immunity (Lisen et al 2021). Stress is a behavioral and physiological response shown by fish due to the influence of the environment and other factors on homeostasis or allostasis (Braithwaite & Ebbesson 2014). The metabolic rate of fish kept at high density will be faster as a consequence of hyperglycemia, because plasma glucose levels are positively correlated with metabolic rates as observed in *Micropterus salmoides*. As a result, fish require greater energy sources to effectively manage stressors, which potentially inhibit optimal growth performance (Wang et al 2019). Hematological and biochemical parameters are often used as indicators of the physiological status of fish (Minahal et al 2024). The physiological responses analyzed in this study included blood glucose (BG), total cholesterol (TC), ketones, hemoglobin (Hb) and hematocrit (Ht). Indicators of fish stress are glucose and cortisol levels (Ahmad 2000; Martinez-Porchas 2009).

BG is a secondary stress response in fish, BG levels usually increase due to increased carbohydrate metabolism in response to environmental stress (Kim et al 2018). Increased glucose levels indicate the mobilization of gluconeogenesis and glycogenolysis pathways to meet increased energy needs in stress caused by high density (Minahal et al 2024). A rapid increase in blood glucose and its stabilization at high levels will increase energy use. The resulting energy deficiency will be followed by death (Jentoft et al 2005). BG levels at the beginning of the maintenance period were 71.75 ± 25.87 mg dL⁻¹ and at the end of the maintenance period ranged from 61.33 ± 7.5 to 95.33 ± 11.6 mg dL⁻¹ (Table 2). There was a decrease in BG in treatments A, B and an increase in treatments C, D and E. Based on the results of the analysis of variance, the stocking density treatment had a significant effect on the BG parameter. Other research results reported that *L. hoevenii* kept in aquarium containers showed an increase in BG values at the end of rearing ranging from 51.17 to 95.67 mg dL⁻¹ (Putri et al 2021; Harianto et al 2023a). The decline in BG at the end of rearing that occurred in treatment A and B indicated that there was no stress in *L. hoevenii*, while the increase in BG that occurred in treatment C, D and E was still within the normal range of blood glucose of *L. hoevenii*. The normal blood glucose range for jealwat fish is 40.00-90.00 mg dL⁻¹ (Patriche 2009; Rahardjo et al 2011; Rizki et al 2020).

TC is a blood biochemical parameter used to determine the health, nutritional, physiological and reproductive status of fish (Javed 2017). TC is closely related to fish

stress through the hypothalamic-pituitary-interrenal axis (HPI axis) (Mormède et al 2007). Increased TC levels are an indicator that fish are experiencing stress due to total lipids being synthesized maximally to be converted into cholesterol and triglycerides so that they can be used as an energy source. Increased TC is part of liver dysfunction and lipid metabolism disorders (Javed et al 2017). The TC value at the beginning of rearing was 314.67 ± 17.2 mg dL⁻¹ and fluctuated at the end of the rearing period. There was an increase in TC in treatments A, B, C and D and a decrease in treatment E. Based on the results of the analysis of variance, it showed that the stocking density treatment had no significant effect on the TC parameter. The TC value at the end of rearing ranged from 299.67 ± 13.58 to 342.67 ± 46.1 mg dL⁻¹. An interesting finding in this study showed that the decline in TC occurred at the highest stocking density (treatment E). The decline indicates that the fish are not stressed, the energy from fat is not fully used by the fish as the main energy source to overcome stress conditions. The TC value produced in this study is still within the normal range for freshwater fish in general. TC levels in the species *Labeo rohita*, *Catla catla*, *Cirrhana mrigala* and *Labeo fimbriatus* were reported at 202.16, 272.16, 206.5 and 211.83 mg dL⁻¹, respectively (Kulkarni & Bedjargi 2016). In purple moray eels (*Gymnothorax vicinus*) TC values ranged from 233 to 433 mg dL⁻¹ (Erlacher-Reid et al 2011). TC levels of *Dicentrarchus labrax* were reported to be 160.5-314.6 mg dL⁻¹ (Chatzifotis et al 2011). High blood cholesterol concentrations may indicate a lipid imbalance in the diet (Wedemeyer et al 1990).

Ketone bodies are produced as a by-product of the fat metabolism process. When the liver metabolizes circulating free fatty acids, these acids are converted to acetyl-coenzyme A (acetyl-CoA), a molecule used in energy production (Prabhakar et al 2014). In fish, ketones are commonly observed during migration processes that cause fish to starve (Ramos & Smith 1978). During this process, there is an increase in fat mobilization from the depot and a decrease in carbohydrate utilization, this condition supports ketogenesis (Wakil & Bressler 1962). The blood ketone value at the beginning of rearing was 9.78 ± 1.65 mg dL⁻¹ and fluctuated at the end of the rearing period. There was an increase in blood ketones in treatment D and a decrease in treatments A, B, C and E. Based on the results of the analysis of variance, the stocking density treatment had no significant effect on blood ketone parameters. Blood ketone values at the end of maintenance ranged from 8.23 ± 3.00 to 10.27 ± 1.14 mg dL⁻¹. Hemoglobin is a protein in erythrocytes which is composed of colorless globin protein and heme pigment produced in erythrocytes (Nabi et al 2022). An increase in hemoglobin levels indicates that the fish are under stress. Hb levels are related to the number of erythrocytes, so that stress conditions also affect Hb levels in the blood. The ability of blood to transport oxygen depends on the Hb levels in the blood (Lagler et al 1977). Wells et al (2005) stated that 1 g of hemoglobin can bind approximately 1.34 ml of oxygen. The Hb value at the beginning of rearing was 15.00 ± 1.28 g dL⁻¹ and at the end of the maintenance period it ranged from 11.53 ± 0.35 to 14.40 ± 1.06 g dL⁻¹. Based on the results of the analysis of variance, it showed that the stocking density treatment had no significant effect on Hb. The Hb content in this study was still within the normal range for eelfish. The Hb content in healthy fish ranges from 12 to 14 g dL⁻¹ (Bastiawan et al 1995; Fazio et al 2019). The Hb value in fish is related to the metabolism, through its influence on the formation of energy due to the oxygen transported through Hb. Hemoglobin binds oxygen, which is used for the catabolism process to produce energy (Lagler et al 1977). In this study, there were no significant problems with growth and FCR, all treatments showed positive growth and low FCR values. This indicates that Hb is normally binding oxygen in the blood so that the metabolic process is not disturbed. Other research results show Hb values in several freshwater fish species, including hemoglobin levels of 4.72 g dL⁻¹ in semah fish, of 6.12 g dL⁻¹ in biawan fish, of 5.50 g dL⁻¹ in botia fish and of 5.40 g dL⁻¹ in *L. hoevenii* (Yuni et al 2018). Hematocrit (Ht) is the ratio between the volume of human blood and the total volume of blood (Affandi & Tang 2002). Based on the results of the analysis of variance, it shows that the stocking density treatment has a significant effect on Ht. The highest Ht value was found in treatment C at $43.67 \pm 4.04\%$; this value is the same as in treatments B, D and E but different from treatment A. The lowest Ht value was found in treatment A at $35.00 \pm 4.00\%$. In general, the Ht value decreased when compared to the Ht value before treatment at $43.25 \pm 5.19\%$. The normal hematocrit value in teleost fish,

especially freshwater fish, ranges from 22 to 60% (Nabib & Pasaribu 1989). The Ht value in this study was still within the normal range of *L. hoevenii*, better than in previous studies. Yuni et al (2018) reported that the average hematocrit value in five types of freshwater fish, namely semah fish, *L. hoevenii*, tengadak fish, biawan fish, and botia fish ranged from 17.38%±0.10 to 28.50%±0.71.

All economic analysis indicators produced the highest value in treatment E where the RC⁻¹ ratio is 4.38 and >1. The condition shows that every USD 1 of costs incurred will generate revenue of USD. 4.38. The RC⁻¹ ratio value in this study shows the economic performance of the *L. hoevenii* seed nursery, because the revenue value is greater than the total costs incurred (Nugroho & Mas'ud 2021). The RC⁻¹ ratio measures the ratio between total revenue and total costs, thus focusing on gross income rather than expenses. The RC⁻¹ ratio is used to assess the profitability of short-term operations or per production cycle (Astari et al 2024). The BC⁻¹ is the ratio or ratio between absolute income or profit and total production costs (Nasruddin et al 2024). The BC⁻¹ ratio compares total benefits to total costs, with benefits including income and other added value. The BC⁻¹ ratio is used to assess long-term economic feasibility (Poudel et al 2024). Based on the results of the analysis, treatment E produced a BC⁻¹ ratio value of 3.38, meaning that every USD. 1 of costs incurred for *L. hoevenii* seed nursery will generate revenue of USD 3.38. According to Rahardi & Hartono (2003), if the BC⁻¹ ratio value is greater than zero (BC⁻¹>0), then a business can be said to be feasible.

BEP is an analysis tool used to determine the minimum of the production value or production volume of a business to reach profitability (Rahardi et al 1998; Nasruddin et al 2024). The BEP (USD) and BEP (units) values in treatment E obtained the highest values compared to other treatments. This is because the high value of revenue and variable costs causes a high BEP value. The payback period (PP) is the time required to cover the return on an investment expenditure (Damayanti 2018). The lowest PP value in this study was 0.11 years (E), this value shows that the time needed for capital returns in treatment E was 1.32 months, other treatments required 38.76 months (A), 13.8 months (B), 2.88 months (C) and 1.8 months (D). The cost of production, assigned to the inventory of goods before the goods are sold, is the cost spent to produce 1 unit of product (Nasruddin et al 2024; Amir et al 2023). Treatment A generated the highest COGM, of USD 0.071 unit⁻¹ compared to other treatments. Treatment E generated the lowest COGM value, of USD 0.021 unit⁻¹. The high value of COGM is due to the low production volume, being determined by the number of fish stocked in the cultivation container. A higher stocking density and survival will reduce the production cost per unit of product produced.

The water quality of the rearing media for *L. hoevenii* seeds maintained at different stocking densities includes: temperature, pH, DO, ammonia, nitrite, nitrate and alkalinity. The results of the analysis of the water quality of the maintenance media show that the water quality is still in the range suitable for maintaining *L. hoevenii*. The temperature value ranges from 27.71 to 29.99°C, the optimal temperature for maintaining *L. hoevenii* seeds ranges from 25 to 30°C (Utami et al 2018; Putri et al 2021; Harianto et al 2023a). The pH value ranges from 7.60 to 7.81, optimal for maintaining *L. hoevenii* seeds; the optimal range in the literature is 6-8.4 (Putri et al 2021; Harianto et al 2023). The DO value ranges from 5.01 to 5.77 mg L⁻¹, in the range suitable for maintaining eel fish. The optimal DO for the maintenance of eel fish fry is >3 mg L⁻¹ (Rusliadi et al 2015; Putri et al 2021; Harianto et al 2023). The ammonia value ranges between 0.15 and 0.50 mg L⁻¹, the optimal ammonia value for the maintenance of eel fish fry being not more than 0.5 mg L⁻¹ (Rusliadi et al 2015; Putri et al 2021; Harianto et al 2023b). The nitrite value ranges from 0.15 to 0.50 mg L⁻¹. In general, the nitrite value in fish maintenance with a recirculation system ranges from 0.2-5 mg L⁻¹ (Masser et al 1999). The nitrate value ranges from 25-50 mg L⁻¹ and the alkalinity value ranges from 13.44 to 14.10 mg L⁻¹. This value is quite low, compared to the optimal conditions for fish maintenance in general. Alkalinity above 20 mg L⁻¹ provides a good rearing medium for fish life. If alkalinity is less than 20 mg L⁻¹, nitrifying bacteria will not function (Francis-Floyd et al 1996). Good water quality in this study was supported by the applied water quality management. During the rearing period, water changes were carried out as much as 20% of the total water volume. In addition, each maintenance container was given aeration for oxygen supply.

Conclusions. A stocking density of 10 ind L⁻¹ (treatment C) is optimal for rearing *L. hoevenii* seed in the recirculation system. This stocking density achieves the best survival rate, specific growth rate, absolute weight growth, absolute length growth, and blood biochemical conditions suggesting that fish are not in a state of stress. The bioeconomic analysis study showed that a high stocking density resulted in the best economic performance. In general, water quality is still within the feasible range for maintaining *L. hoevenii* seeds. This study recommends that the rearing density of catfish fry should not exceed 10 ind L⁻¹ for technical and economic considerations.

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