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Research Article Influence of Different Stocking Densities on Growth, Feed Efficiency and Carcass Composition of Bonylip Barb (*Osteochilus vittatus* Cyprinidae) Fingerlings

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Abstract

Background and Objective: Bonylip barb (*Osteochilus vittatus*) is an herbivorous freshwater fin-fish species native in Indonesia. These species has high demand and price in the market. A 90 days study was conducted to evaluate the effects of different stocking densities on growth, carcass composition, survival and water quality of *Osteochilus vittatus* (*O. vittatus*) in a synthetic sheet pond. **Materials and Methods:** Fingerlings with an average weight of 2.45 ± 0.071 g (Mean \pm SD) were randomly stocked in 12 synthetic sheet ponds ($2 \times 2 \times 1$ m) at densities of 50 fish m⁻³ (T₅₀), 75 fish m⁻³ (T₇₅), 100 fish m⁻³ (T₁₀₀) and 125 fish m⁻³ (T₁₂₅) in triplicate groups. One-way ANOVA followed by Duncan's new multiple range test was used to analyze obtained data a significance level of p<0.05. **Results:** After 90 days of the trial, the growth in terms of weight gain (WG) and specific growth rate (SGR) of fish from T₅₀ were significantly higher than those from T₇₅, T₁₀₀ and T₁₂₅. The feed conversion ratio (FCR) was significantly lower in T₅₀ followed by T₇₅, T₁₀₀ and T₁₂₅ consecutively. Protein efficiency ratio (PER) was higher in T₅₀ followed by T₇₅, T₁₀₀ and T₁₂₅. Significantly lower amounts of carcass lipid and carbohydrate contents were found in T₁₂₅ compared to the contents of the T₅₀, T₇₅ and T₁₀₀ fish. **Conclusions:** Based on the growth performance and feed efficiency, the T₅₀ fish m⁻³ of *O. vittatus* in a synthetic sheet pond may be technically feasible.

Key words: Aquaculture, synthetic sheet pond, bonylip barb, growth, body composition

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The success of fish farming activity depends not only on the fish species, feeding rate, feeding frequency and water quality management¹⁻⁴, but also on the fish stocking density⁵⁻⁸. Stocking density is one of the most important variables in aquaculture because it directly affects the growth performance, survival, feed conversion ratio, water quality and production efficiency⁹⁻¹¹ of the cultured fish.

High stocking density is a potential source of stress for fish^{5,12,13} and has a negative effect on the specific growth rate¹⁴⁻¹⁶, survival rate, feed conversion ratio and body composition¹⁷⁻¹⁹. Therefore, the optimum stocking densities must be determined for each species and cultivation phase to allow for maximum profitability.

Osteochilus vittatus, Cyprinidae, is an herbivorous freshwater finfish and a which the native species in Indonesia^{20,21}. These species live in Singkarak lake, the Antokan river, the Koto Panjang reservoir²² and Kampar Kanan river²³ in Indonesia. The over-exploitation of these natural fishes, fish stocks have occurred due to increased market demand and a height retail fish²³. A study performed by Uslichah and Syandri²¹, Azrita *et al.*²⁴, Syandri *et al.*^{25,26}, Aryani *et al.*²⁷ describe information relevant to the aquaculture activity. *Osteochilus vittatus* has been identified as one the best prospect species for middle-scale commercial aquaculture in Indonesia.

The aim of this study was to evaluate the effects of different fish stocking densities on the growth performance, feed efficiency, survival and carcass composition of synthetic sheet pond-cultured *O. vittatus*. The results of this study increase the scientific understanding of *O. vittatus*.

MATERIALS AND METHODS

Fish culture technique: *Osteochilus vittatus* fingerlings were produced from broodstock, which are kept in a concrete tank in the hatchery unit of the Faculty Fisheries and Marine Sciences, Bung Hatta University Padang, West Sumatra, Indonesia. This study was performed in March-June, 2017. A total of 3,000 individual fingerlings were used in the study. The larvae were nursed in a pond fertilized with poultry manure prior to the initiation of the experiment and the larvae were gradually weaned to a commercial prawn diet (MS Pengli Zero, produced by Tunas Prima Indonesia). The approximate composition of the feed was 11% moisture content, 40% crude protein, 6% crude lipid, 3% crude fiber and 11% crude

ash. After initial nursing, the fingerlings were stocked in synthetic sheet ponds ($6 \times 2 \times 1$ m) for an adaptation period of 3 weeks, during which they were fed a commercial feed. The synthetic sheet ponds had supplemental aeration with a continuous flow of underground water at a velocity of 5 L min⁻¹. Twelve synthetic sheet pond units were used measuring with the size of 2 m × 1 m × 1 m (L×W×H, 2 m³). The water depth of the synthetic sheet ponds was 0.5 m.

Experimental design: The average randomly distributed initial weight of the *O. vittatus* fingerlings was 8.55 ± 0.57 g (Mean \pm SD). The initial length (cm) and weight (g) of the fishes were determined using a measuring scale and a digital electronic balance (OHAUS, Model CT 1200-S, USA) and these values were recorded. The stocking densities (1 synthetic sheet ponds/density of 50 fish m⁻³, 75 fish m⁻³, 100 fish m⁻³ and 125 fish m⁻³) was designated T_{50} , T_{75} , T_{100} and T_{125} , respectively, in 3 replicates for each treatment group. The experiment was carried out for a duration of 90 days. The fishes were fed daily at rate of 5% from their biomass. During the experiments the fingerlings were fed twice/day (at 9.00 am and 17.00 pm) with a sinking and extruded diet containing 3,340.50 kcal kg⁻¹ of cross energy, 30% crude protein, 6.85% crude lipid, 7.24% crude fiber, 4.10% crude ash and 12% moisture contents. The composition of the feed was prepared from fish meal, soybean cake, coconut cake, fine rice brain, wheat starch, tapioca starch, vitamin premix and salt. The formulation was ground thoroughly and passed through a 0.5 mm mesh-size sieve. All the ingredients were mixed well according to the formula composition of the pelleted feed (Table 1). The feed was then put into a manually operated pellet machine to produce 1 mm sized pellets.

Fishes weight was evaluated every 30 days and the fishes were fasted 24 h prior to analysis. For sampling, 10% of fishes in each synthetic sheet pond were captured with a gillnet that formed a net bag of the appropriate mesh size. Subsequently, the fishes were anesthetized with 0.2 mL L^{-1} clove oil and their weights and lengths were measured. After each sampling period, the amount of feed was adjusted depending on the mean weight and biomass in each synthetic sheet pond. The biomass of the fish was calculated and the amount of feed was adjusted. The feed intake was recorded daily. Following evaluation, the fishes were returned to their synthetic sheet ponds. No mortality was noted. To determine the mortality of the fish, a daily collection was performed using a gillnet net bag and the dead fish were counted both on the surface and background of each synthetic sheat pond.

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Ingredients	Gross energy (Kcal)	Protein (%)	Lipid (%)	Crude fiber (%)	Amount (g kg ⁻¹)	
Fish meal	2,7292.52	43.92	2.50	6.45	460.52	
Soybean cake	3,956.70	35.90	1.32	5.80	155.10	
Coconut cake	4,250.90	12.60	15.20	3.50	126.50	
Fine rice bran	4,101.25	15.03	12.51	12.43	235.88	
Wheat starch	3,828.40	1.50	1.30	-	10.00	
Tapioca starch	3,560.17	0.95	0.76	-	9.00	
Mineral premix ¹					1.00	
Vitamin premix ²	-	0.10	-	-	1.00	
Salt	-	0.20	-	-	1.00	

Table 1: Composition of diets for feeding Q *vittatus* reared in synthetic sheet ponds

*Analysis results was obtained from Animal Science Laboratory, Bung Hatta University, Indonesia. 1Mineral mixture (g kg⁻¹ diet) KH₂PO₄ 4.2, CaHPO₄ 3.3, NaH₂PO₄ 6.25, KCl 2, KI 1.6, MgSO₄. 7H₂O 2, ZnSO₄ 0.1, MnSO₄ 0.1, ²Vitamin mixture (mg kg⁻¹ diet): Thiamine hydrochloride 25, vitamin B₁₂ 0.005, inositol 2,000, biotin 6, folic acid 6, choline chloride 1,000, ascorbyl-polyphosphate 250, vitamin E 200, vitamin K₃ 12, vitamin A 12,000 IU, vitamin D₃ 2400 IU

Measurements parameters: To determine the growth performance of the fish, the following parameters were calculated, weight gain (WG), specific growth rate (SGR %/day), feed conversion ratio (FCR), protein efficiency ratio (PER) and survival rate (SR). The parameters were analyzed according to Aryani et al.28 and Igbal et al.29 with the following equation:



Specific growth rate (SGR %/day):
$$\left[\frac{(\ln Wt-\ln Wi)}{T}\right] \times 100$$

Where:

Wt = Mean final weight

= Mean initial weight Wi

Т = Total experimental days

FCR = Total feed fed (g)/total wet weight gain (g)

PER = Fish weight again/feed intake

SR% = 100 (number of fish survived/number of fish stocked)

Proximate composition analyses: Proximate composition analyses of the experimental diet and whole fish body were performed by the standard methods of the Association of Official Analytical Chemists³⁰. After the completion of the experiment, three fishes from each treatment group were collected for further analysis of body carcass composition. For determining the moisture content, samples of the diets and wet fish were dried at 135°C for 2 h. The ash content was determined by using a muffle furnace (600°C for 4 h). The crude lipid content was determined using a Soxhlet apparatus with the Soxtec system 1046 (Foss, Hoganas, Sweden). The crude protein content was determined by the Kjeldahl method (N×6.25) after acid digestion, distillation and titration of samples. The total carbohydrates were determined by subtracting the sum of % crude protein, % crude lipid, % crude ash and % moisture contents from 100³¹. The samples were analyzed in the Animal

Science Lab, Department of Aquaculture, Faculty of Fisheries and Marine Science Bung Hatta University, Padang, Indonesia.

Water quality: The water samples were collected at a depth of 10 cm from each synthetic sheet pond for the determination of the dissolved oxygen (DO) contents. An oxygen meter (YSI model 52, Yellow Spring Instrument Co., Yellow Springs, OH, USA) was used in situ and pH values were determined with a pH meter (Digital Mini-pH Meter, 0-14PH, IQ Scientific, Chemo-science [Thailand]) Co., Ltd., Thailand). The levels of phosphorus, ammonia-nitrogen, alkalinity and hardness of the water in each replication were measured according to standard procedures³².

Statistical analysis: The mean values for the water quality parameters, growth, survival and production of different treatments were subjected to one-way ANOVA followed by Duncan's new multiple range test³³. All statistical analyses were performed using SPSS software (version 16.0 for Windows, SPSS Inc., Chicago, IL). The standard deviation of each parameter and treatment was determined and expressed as the Mean \pm SD. The treatment effects were considered to be significant at p<0.05.

RESULTS

Determination of weight growth responses: During the experimental period the growth performance of O. vittatus at 30, 60 and 90 days showed significant differences (p<0.05). The lowest final weight was observed in T_{125} , followed by T_{100} , T_{75} and T_{50} (Fig. 1). The influence of stocking densities on weight gain (WG), specific growth rate (SGR), feed conversion ratio (FCR), protein efficiency ratio (PER) and survival rate (SR) were affected significantly (p<0.05) by stocking density. Mean final weight increases in T_{50} followed by in T_{75} , T_{100} and T_{125} , respectively (Fig. 2). Mean specific growth rate, feed conversion ratio and feed efficiency were presented in Fig. 3-5.

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	bie 2. Summary of cares compositions of <i>O. Mitatus</i> current at dimenent stocking defisities over 90 days					
Stocking	Final carcass composi	Final carcass compositions (%)				
density						
(fish m ⁻³)	Moisture	Protein	Lipid	Ash	Carbohydrate	
T ₅₀	78.29±0.12ª	14.28±1.17ª	1.83±0.01ª	1.45±0.02ª	4.15±0.01ª	
T ₇₅	78.24±0.07ª	14.25±1.10 ^a	1.97±0.01 ^b	1.51±0.03ª	4.03±0.02 ^b	
T ₁₀₀	77.80±0.04ª	14.92±1.04ª	1.03±0.02°	1.90±0.02ª	3.81±0.09°	
T ₁₂₅	78.94±0.17ª	14.48±1.02ª	1.27±0.02 ^d	1.69±0.02ª	3.62±0.02 ^d	

Table 2: Summary of carcass cor	npositions of <i>O. vittatus</i>	cultured at different stockin	g densities over 90 da	ys
,				

Values are $\%\pm$ SD, *Values in the same row having different superscript are significantly different (p<0.05)

Table 3: Water quality parameters at different stocking densities

	Stocking densities (iisn m ⁻²)				
Parameters	T ₅₀	T ₇₅	T ₁₀₀	T ₁₂₅	
Temperature (°C)	27.250±1.22ª	28.060±1.15ª	28.220±1.11ª	28.720±1.19ª	
рН	7.310±0.07ª	7.430±0.15ª	7.760±0.15ª	7.800±0.16ª	
DO (mg L ⁻¹)	6.890±0.06ª	6.730±0.07ª	6.660±0.05ª	6.620±0.05ª	
Ammonia (mg L^{-1})	0.160±0.01ª	0.190±0.01 ^b	0.230±0.72°	0.280 ± 0.01^{d}	
Nitrite-N (mg L ⁻¹)	0.031±0.01ª	0.043±0.01 ^b	0.056±0.03°	0.068 ± 0.02^{d}	
Nitrate-N (mg L^{-1})	0.290±0.01ª	0.300±0.01ª	0.310±0.07 ^b	0.310±0.02 ^b	
Phosphorus (mg L ⁻¹)	0.240±0.05ª	0.250±0.01 ^b	0.270±0.01°	0.300 ± 0.01^{d}	
Alkalinity (mg L ⁻¹)	85.330±0.57ª	86.660±1.52ª	86.830±0.35ª	86.450±0.80ª	
Hardness (mg L ⁻¹)	27.190±0.65ª	27.940±0.06°	28.060±0.37ª	28.030±0.15ª	

Mean values (Mean±SD), *Values in the same row having a different superscript are significantly different (p<0.05)



Fig. 1: Mean weight \pm SD (g) of fingerlings *O. vittatus* at four different stocking densities



Fig. 2: Mean weight gain (mean weight increases in $grams \pm SD$) growth response for the fingerlings during the experiment Bars represent mean standard deviation weight increases





Mean body carcass: In the present study, author did not find any significant differences in body carcass moisture, protein and ash content in T_{50} , T_{75} , T_{100} and T_{125} (p>0.05) (Table 2). However, amounts of carcass lipid and carbohydrate contents in T_{125} were significantly lower than those of the other treatments (p<0.05) (Table 2).

Mean pond water quality parameters: Mean values $(Mean \pm SD)$ and ranges of water guality parameters measured in the synthetic sheet ponds over the experimental period are summarized in Table 3. The average temperature, pH, DO, total hardness and total alkalinity did not change significantly among the treatments (p>0.05). Meanwhile, the ammonia, nitrite-N and phosphorus were significantly among the treatments (p<0.05).







Bars represent mean standard deviation feed conversion ratio

Fig. 5: Mean protein efficiency ratio (mean protein efficiency ratio in grams±SD)

Bars represent mean standard deviation protein efficiency ratio

DISCUSSION

In the present study the WG, SGR, FCR and PER of O. vittatus were significantly higher at the stocking density of $T_{\rm 50}$ compared to those at $T_{\rm 757}, T_{\rm 100}$ and $T_{\rm 125}.$ When the same feed was applied in an equal ratio, the growth performance of O. vittatus was significantly different in all the stocking densities. This may be due to increased space and decreased competition for feed in lower density treatments of fish. This finding agrees with the findings reported by Moniruzzaman et al.¹⁹ and Gibtan et al.³⁴ for mono sex Oreochromis niloticus in a cage culture system. Bag et al.⁶ for Catla catla, Labeo rohita and Cirrhinus mrigala fingerlings in a pond. Kareem and Olanrewaju³⁵ for fry *Clarias gariepinus* in tanks and Rahman et al.³⁶ for Barbonymus gonionotus fingerlings in a pond. In contrast, De Oliveira et al.¹¹ described an increase in specific growth rate, feed conversion ratio and production of Arapaima gigas in cages.

In this study, the density of stocking stocks T_{50} , T_{75} , T_{100} and T_{125} had an effect on the growth performance of *O. vittatus* (Fig 3). Rowland *et al.*³⁷ stated that stocking densities of 12, 25, 50, 100 or 200 fish m⁻³ of silver perch (*Bidyanus bidyanus*) in cages did not affect the final weight or specific growth rate. However, feed conversion ratios (FCR) of fish stocked at 25 or 50 fish m⁻³ were significantly higher than FCRs at other densities. According to Gibtan *et al.*³⁴, *Oreochromis niloticus* cultured in cages with stocking densities of 50, 100, 150 and 200 fish m⁻³ obtained the best results at a stocking density of 50 fish m⁻³. No study has been performed evaluating a stocking density higher than 250 fish m⁻³. Meanwhile, a study on koi carps in concrete tanks showed that a stocking density of 300 fish m⁻³ was recommended³⁸.

The *O. vittatus* cultured in T_{125} stocking densities may develop stress. A high stocking density is a potential source of stress for fish^{12,17,39}, with a negative effect on the growth^{6,40}, survival, specific growth rate and feed conversion ratio^{8,41}. Furthermore, Liu *et al.*¹³ stated that an overly high stocking density of turbot (*Scophthalmus maximus*) might block the activities of metabolic and antioxidant enzymes, cause physiological stress and immunosuppression. In addition, higher stocking densities of *Argyrosomus regius* can result in increased values of muscle triglycerides, amino acids and lactate¹⁷.

In this study, the higher stocking densities $(T_{50}-T_{125})$ resulted in higher FCR values (Fig. 4). This is likely due to the stress condition of the fish, which can reduce the appetite of *O. vittatus*. Therefore, excess feed will result in a build-up of sediment in the bottom of synthetic sheet ponds. Alternatively, the lower FCR value obtained in this study indicates better food utilization efficiency. Research by Osofero *et al.*¹⁰, found that *Oreochromis niloticus* cultured in bamboo net cages during 90 days at stocking density of 50, 100, 150 and 200 fish m⁻³ had FCR values of 1.92, 1.56, 2.15 and 2.21, respectively. An FCR value that is less than 2.0 or very close to 2.0 is considered "good" in aquaculture industry⁶.

Protein efficiency ratio (PER) always decreases with increasing stocking density, when feeding rates are below the level of fish maintenance and however, increases as the amount of stock density increases as the density stock falls above the level of fish maintenance. However, increases as the amount of stock density increases as the density stock falls above the level of fish maintenance⁹. In this study, protein efficiency ratio decreased linearly with increasing stocking density from T₅₀ to T₁₂₅ (Fig. 5). One possible explanation for this finding from a report by Calabrese *et al.*⁴² on Atlantic salmon (*Salmo salar* L.) and from a study by Naderi *et al.*⁸ for rainbow trout (*Oncorhynchus mykiss*).

Stocking density has a significant effect (p<0.05) on the survival rate of *O. vittatus*. The mean of survival rate ranged from 84.40 ± 1.44 to $95.33 \pm 0.66\%$. The pathological results of *O. vittatus* did not reveal any association between stocking density and mortality. Fish mortality occurs as a result of poor water quality parameters and poor reception of food. Fish emaciation is a clear sign of fish mortality. Thus, mortality rates are related to stocking density. Stocking density can affect the survival rate and increases the number of emerging diseases in fish culture^{15,19,43} and influence growth and activities of metabolic and antioxidant enzymes^{5,7,13}. Similar results have been confirmed in freshwater fish^{9,34,44} and it is well-accepted that high stocking density can cause reductions in growth rate⁴⁵ and increases in mortality^{7,16}.

At stocking densities of T_{125} and T_{100} the approximate composition of O. vittatus have a lower amount of crude protein, crude lipid and carbohydrate content. This may be due to over expenditure of body energy to maintain the normal metabolic activity of the fish during the experimental period. At the end of the experimental period, O. vittatus with stocking densities of $T_{\scriptscriptstyle 50}$ and $T_{\scriptscriptstyle 75}$ were more active and consumed more feed compared with fish stocking densities of T_{125} and T_{100} . High stocking density (T_{125} and T_{100}) cause fish to consume less feed and the excess of feed will result in a build-up of sediment in the bottom of synthetic sheet ponds. According to Hussain et al.46, the herbivorous fish (Catla catla, Cirrhinus mrigala and Labeo rohita) have carcass compositions with a crude protein of 14.86 ± 2.24 , 15.15 ± 1.85 and 14.44±1.93%, respectively. In this study, O. vittatus (herbivorous) with stocking densities of $T_{\rm 50},\,T_{\rm 75},\,T_{\rm 100}$ and $T_{\rm 125}$ have crude proteins of 14.28±1.17, 14.25±1.10, 14.92±1.04 and 14.48±1.02%, respectively. There are many factors that influence of carcass composition. In this study, carcass composition appeared to be influenced by stocking density. Alternatively, carcass composition may be a reflection of diet 47 , feeding rate^{48,49}, protein and lipid levels of the diet⁵⁰⁻⁵², type of diet⁵³ and supplementation of tannic acid (TA) the diet⁵⁴.

Physico-chemical parameters play a significant role in the growth of *O. vittatus*. These parameters are required for the growth performance of *O. vittatus*²⁷. The average water temperature ranged from 27.25 ± 1.22 to 28.72 ± 1.19 °C which is below the tolerance limit of water quality parameters for fish culture (25-30°C). The effects of pH on fish ponds illustrated by Boyd⁵⁵ and indicated that culturing fish in more acidic environments at a pH of 6.5 or a more alkaline pH of 9-9.5 for an extended period of time resulted in diminished fish growth. However, water pH that ranged from

 7.31 ± 0.07 to 7.80 ± 0.16 had no effect on the growth of O. vittatus. The DO level recorded during the experiment ranged from 6.32 ± 0.05 to 6.89 ± 0.06 mg L⁻¹. Certain fish species such as Cyprinidae have DO dependents growth performance. Syandri et al.56 suggested that a DO concentration that ranged from 5.08 ppm to saturated condition is ideal for fish growth in all species that are culture in Maninjau lake. Alternatively, Bag et al.⁶, found that a DO level above 4.02 mg L^{-1} is ideal for Indian major carp growth. In this study, the values of nitrite-nitrogen ranged from 0.031 ± 0.01 mg L⁻³ to 0.068 ± 0.02 mg L⁻³ and nitrate-nitrogen ranged from 0.29 ± 0.01 to 0.31 ± 0.02 mg L⁻¹. These reported results fell within the ranges reported to be safe for aquaculture activity ranged from found by Boyd⁵⁵. According to Boyd⁵⁵, the phosphorus contents which are safe for aquaculture activity ranged from 0.005-0.2 mg L^{-1} . In this study, the phosphorus contents ranged from 0.24±0.05 to 0.28 ± 0.01 mg L⁻¹. These phosphorus results are likely due to waste feed and fish feces.

The alkalinity levels during the experiment ranged from 85.33 ± 0.57 to 86.45 ± 0.80 mg L⁻¹ CaCO₃. Similar results were reported by Boyd et al.57, the total alkalinity for fish culture ranged from 75-200 mg L⁻¹ CaCO₃. The water hardness of *O. vittatus* culture ranged from 27.19 \pm 0.65 mg L⁻¹ to 28.06 ± 0.37 mg L⁻¹. Water hardness is an important factor for fish culture and is a commonly reported aspect of water quality. Water hardness can be a mixture of divalent salts, however, calcium and magnesium are the most common sources of water hardness. During the experiment of O. vittatus cultured in synthetic sheet ponds, water quality was an important parameters that reflected the ideal environmental conditions. All water quality parameters that measure within the optimal conditions for the growth of O. vittatus in synthetic sheet ponds are acceptable and are recommended for tropical fish aquaculture. Further research is warranted regarding the fish stocking size and culturing activities in other habitats.

CONCLUSION

Based on growth performance, feed efficiency and carcass composition, 50 fish m^{-3} exhibited the highest performance out of all the stocking densities. Therefore, a stocking density of 50 fish m^{-3} may be recommended for successful synthetic sheet ponds. The findings of this are relevant for sustainable and effective synthetic sheet pond practices.

SIGNIFICANCE STATEMENT

This study analyzed the difference stocking density of Bonylip barb (*Osteochilus vittatus*) in the synthetic sheet pond on the growth performance, feed efficiency and carcass composition. The stocking density T_{50} fish m⁻³ was the better results in terms of growth performance, feed efficiency and carcass composition compared than T_{75} , T_{100} and T_{125} fish m⁻³. The stocking density of T_{50} fish m⁻³ can be considered for the cultured of *O. vittatus* for the development of new species in the future.

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