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EFFECT OF DIETARY PAPAYA PEEL AND BAKER'S YEAST SUPPLEMENTATION ON GROWTH PERFORMANCE, HEMATOLOGICAL VALUE OF SILVER BARB (*Barbodes gonionotus*) AND ITS RESISTANCE TO *Aeromonas hydrophila*

PHINYO MAHATTANEE*[1,2], THAIKOSA AREEYA[1] AND WANGKAHART EAKAPOL[3]

[1]Department of Agricultural Science, Faculty of Agriculture, Natural Resources and Environment, [2]Center of Excellence in Research for Agricultural Biotechnology, Naresuan University, Phitsanulok 65000, Thailand. [3]Division of Fisheries, Department of Agricultural Technology, Faculty of Technology, Mahasarakham University, Mahasarakham, Thailand.

**Corresponding author: mahattaneep@nu.ac.th*
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Abstract: The aim of this study is to investigate the effects of dietary papaya peel and baker's yeast (*Saccharomyces cerevisiae*) as growth supplements for the silver barb (*Barbodes gonionotus*), their influence on the fish's hematological values and disease resistance against *Aeromonas hydrophila*. The study comprised four experimental diets, namely a control (C); 4 % (w/w) papaya peel (P); 2 % (w/w) baker's yeast (Y); and, 4 % (w/w) papaya peel plus 2% (w/w) baker's yeast (PY). After eight weeks of feeding, the silver barb fed with PY diet had significantly higher values of final body weight (14.53±0.29 g/fish) and specific growth rate (SGR, 1.87±0.05 %/day) compared with the control (P<0.05). They also recorded an improved feed conversion ratio (FCR) (P<0.05). However, the C and P diets did not exhibit significant differences in FCR and SGR. During the feeding trial, no mortality was observed. Silver barbs on PY diet had significantly higher red blood cells (RBC) and white blood cells (WBCs) than the other diet groups (P<0.05). Intraperitoneal injection of 0.1 ml (0.5×10^7 CFU mL^{-1}) *A. hydrophila* resulted in increased mortality, which was reduced with the intake of Y and PY diets. In conclusion, the P diet alone showed no differences in growth performance and haematological parameters when compared with the control. However, the dietary inclusion of baker's yeasts may improve growth performance, as well as resistance to *A. hydrophila*. These results indicate that papaya peel and baker's yeast may be used as feed additives for silver barb to produce better quality fish with protection from pathogenic infection.

Keywords: Baker's yeast, silver barb, papaya peels, growth, disease resistance.

Introduction

The silver barb (*Barbonymus gonionotus*) is a herbivorous freshwater fish belonging to the family Cyprinidae, and its habitat is mostly distributed in Southeast Asia, including Indonesia, Vietnam, Cambodia, Lao PDR and Thailand (Tantong *et al*., 1980). This fish is one of the most important species in the Thai aquaculture industry due to its rich protein content, good taste and relative ease to rear, with a culture period ranging from three to four months. Moreover, the fish is fermented and consumed as a traditional food in northeast Thailand (Tesana *et al*., 2014; Vuthiphandchai *et al*., 2015).

Aeromonas hydrophila, a Gram-negative bacterium, is a pathogen that causes hemorrhagic septicaemia in many kinds of fish and amphibians. Once infection occurs, this bacterium may wreak havoc and inflict enormous economic losses in the aquaculture industry. Fish infected with *A. hydrophila* will develop hemorrhagic spots on the body and tail, and there is increased mortality in embryonic fish (İlhan *et al*., 2006; Vijayakumar *et al*., 2017). The bacteria is usually transmitted after insemination of the fish eggs with tainted cryopreserved sperm, which leads to reduced fertilization capacity as the embryos die and egg production gets disrupted. (Boonthai *et al*., 2016; Boonthai *et al.,* 2018).

Saccharomyces cerevisiae, or baker's yeast, is a single-cell eukaryotic fungus that is widely used in fermentation. The major components of the yeast cell wall include β-glucan, nucleic acids, mannan oligosaccharides and *N*-acetylglucosamine. Baker's yeast is also a probiotic that has been used as a feed additive in the diet of the Nile (*Oreochromis niloticus*) and Galilee tilapias (*Sarotherodon galilaeus*). It has been shown to improve growth, boost the fishes' immune response and enhance resistance against pathogenic bacteria (Abdel-Tawwab *et al*., 2010; Pandiyan *et al*., 2013).

β-Glucans are polysaccharides that, besides yeast, are also found in mushrooms, bacteria and algae. The polysaccharides comprise of D-glucose monomers linked by β-glycosidic bonds. They have been observed to play a role in anti-tumor, anti-inflammatory and immuno-stimulating reactions in aquatic animals like the black tiger shrimp (*Penaeus monodon*). Dietary beta-1, 3 glucan has been found to enhance innate immunity of the large yellow croaker (*Umbrina roncador*) and is used as a feed additive for various fish species, including the Nile tilapia and common carp (*Cyprinus carpio*) (Sahoo & Mukherjee, 2001; Suphantharika *et al.*, 2003; Ai *et al*., 2007; El-Boshy *et al*., 2010; Gopalakannan & Arul, 2010; Przybylska-Diaz *et al*., 2013; Petit & Wiegertjes, 2016; Zhu *et al*., 2016).

Meanwhile, papaya is a tropical fruit that is used to make the famous Thai green salad, or "som tam", usually before the fruit is ripe. Unripe papaya extract may also help treat an enlarged spleen as it contains anti-inflammatory and antibacterial properties (Krishna *et al.*, 2008; Boshra & Tajul, 2013). However, papaya may contain active substances (e.g. carpine and papain) that are known to be toxic to aquatic life. Carpine, which is present in papaya seeds, may cause acute toxicity in catfish (*Clarias gariepinus*) and the Nile tilapia (Ayotunde and Ofem, 2008; Eyo *et al*., 2013).

Additionally, papaya peel is an agricultural waste (Pathak *et al.*, 2019) that has been observed to reduce fertility. A previous study showed that the peel extract may affect the testes of male Wistar rats (Ovie *et al*., 2019). However, no toxicity has been reported in fish. Its proximate and mineral constituents include phenolic compounds, proteins, minerals (major minerals like potassium, phosphorus, and trace minerals like magnesium, calcium, sodium and iron), ferulic acid, caffeic acid and proteases (papain, chymopapain, cyclortransferase, glycyl, endopeptidase and caricain) (Chaiwut *et al.,* 2010; Ikram *et al*., 2015: Martial-Didier *et al*., 2017). Moreover, the peel also contains pectin, which is a polysaccharide found abundantly in plant cell walls. Pectin is complex and contains linear chains of (1, 4)-linked α-D-galacturonic acid (Gal*p*A) and carboxyl groups (Ridley *et al.,* 2001; Koubala *et al.,* 2014).

The current study aims to determine the dietary effects of papaya peel and baker's yeast (*S. cerevisiae*) on growth performance, hematological values and pathogenic resistance against *A. hydrophila* in silver barbs. This may contribute in environmental sustainability by providing a solution for this agricultural waste as an alternative ingredient in fish feed.

Materials and Methods

Production of Yeast on Yeast Extract-Peptone-Dextrose (YPD) Medium with Papaya Peel

Unripe papaya peel discarded as kitchen waste were collected from Thai restaurants and dried in an oven at 60 ^{o}C for 24 hours. Proximate analysis was carried out based on the 1990 protocols of the Association of Official Agricultural Chemists (AOAC) International (Table 2). *In vitro* tests were performed using the yeast on yeast extract-peptone-dextrose (YPD) culture medium. The YPD liquid medium (3 ml) was inoculated with a single colony of yeast for 24 h and a hundred micro-litre of yeast cultured was then transferred into 50 ml of the YPD medium and grown at 30^{o}C in an incubator shaker at 200 rpm, this served as a YPD. 2% (w/v) of oven-dried papaya peels (YPD/papaya peels) was added and collected of yeast cells were examined at 0, 4, 12, 24, 48, 60, 84, and 108 hours using a Neubauer hemocytometer.

Diet Preparation

The experimental diets contained 30% protein and 9.5% lipid. The diet supplements included 2% (w/w) of baker's yeast and/or 4% (w/w) of papaya peel as a replacement of the gradient broken rice. Baker's yeast was purchased from a local store. To prepare the fish pellets, each ingredient in Table 1 was filtered through a 30-mesh sieve (0.6 mm) before being mixed in a 3 mm grinder. The pellets were dried overnight at 45°C until the moisture content was less than 10%. The pelleted diets were analyzed according to AOAC International protocols and kept in plastic bags at 4°C.

Fish Culture

Silver barbs (*Barbonymus gonionotus*) with an average weight of 5 g/fish were randomly divided into 10-fish triplicates (40*90*46 cm) in aquaculture tanks. They were fed with 30% crude protein and 9.5% crude lipid (control diet) twice a day (8 am and 4 pm) for one week. Silver barb was fed twice a day at a rate of 5% (w/w) of their body weight for 8 weeks. They were weighed every two weeks. Before taking the weights, they were starved for 24 hours. The fish were exposed to a 12-h light and dark cycle. The water quality were maintained with temperatures between 26 ^{o}C and 28 ^{o}C, pH 6.5 to 8, dissolved oxygen at >6 mg L^{-1}, and total ammonia within 0.05 and 0.5 mg L^{-1}). Growth performance, feed conversion ratio (FCR) and survival rate were determined using Equations one, two and three, respectively (E1, E2 and E3).

Table 1: Formulation of the different experimental diets for silver barb

Ingredients	**Experimental Diets**			
	Control (C)	**4% papaya peels (P)**	**2% yeast (Y)**	**4% papaya peel and 2% yeast (PY)**
Fish meal	21.5	21.5	21.5	21.5
Soybean meal	25	25	25	25
Corn meal	8.5	8.5	8.5	8.5
Rice bran	28	28	28	28
Broken rice	15.5	11.5	13.5	9.5
Baker's yeast	0	0	2	2
Papaya peels	0	4	0	4
Fish oil	0.5	0.5	0.5	0.5
Vitamin premix[1]	0.5	0.5	0.5	0.5
Mineral premix[2]	0.5	0.5	0.5	0.5
Proximate analysis (%)				
Moisture	5.86±0.05	5.46±0.03	5.23±0.24	5.63±0.08
Crude protein	30.04±0.08	30.14±0.11	30.32±0.30	31.09±0.40
Crude lipid	9.47±0.25	9.50±0.12	9.69±0.25	9.54±0.07
Total fiber	3.60±0.07	3.94±0.04	3.42±0.09	3.87±0.04
Crude ash	7.01±0.10	7.30±0.03	6.90±0.06	7.31±0.03

[1] kg of vitamin premix contains: A, 20,000 IU; D3, 6,000 IU; E, 5,500 IU; K, 8 g; C, 21 g; B1, 2.5 g; B2, 10 g; B6, 4.5 g; B12, 2.5 g, pantothenic acid, 7.5 g; nicotinic acid, 47.5 g; folic acid, 1 g.

[2] One kilogram of mineral premix contains: calcium, 100 g; phosphorous, 80 g; magnesium, 2.16 g; iron, 1.24 g; zinc, 1.6 g; copper, 1.2 g; manganese, 1.2 g; potassium, 0.23 g; iodine, 0.76 g; selenium, 0.01 g; cobalt, 0.2 g.

Specific growth rate (SGR) = 100 (ln W2-ln W1) / T (E1)

where W2 = Final fish weight, W1 = Initial fish weight, and T = Number of days in feed period (days).

Feed conversion ratio (FCR) = Feed intake (g) / Weight gain (g) (E2)

Survival rate = (Number of final fish / Number of Initial fish) x100 (E3)

Hematological Parameters

After feeding with experimental diets for eight weeks, five fish were randomly selected from each triplicate. Blood samples were collected from the caudal veins and centrifuged for five min to determine hematocrit or packed cell volume (% PCV). The red blood cells (RBC) and white blood cells (WBC) were diluted using Grower's Solution and 3% (v/v) acetic acid, respectively. The cells were counted using the improved Neubauer hemocytometer (Voigt, 2000).

Disease Resistance

Finally, the fish (*N*=5 per replicate) were randomly selected from each aquarium and tested for resistance against *A. hydrophila*. They were intraperitoneally injected (IP) with 0.1 ml ($0.5x10^7$ CFU mL^{-1}) of *A. hydrophila* and the controls (*N*=2) were injected with 0.85% NaCl. After the IP injection, the fish were observed for mortality for 14 days.

Statistical Analysis

The effects of dietary baker's yeast and/or papaya peels were statistically tested using one-way analysis of variance (ANOVA) and Duncan's multiple range test was used to compare the differences among the experimental diets. Results were considered significant when *P*<0.05.

Results

The composition of raw papaya peel is shown in Table 2.

Table 2: Proximate composition of oven-dried unripe papaya peels

Nutrients compositions	Percentage composition
Moisture	8.94±0.11
Crude protein	18.93±0.07
Crude lipid	2.36±0.24
Total fiber	15.50±0.19
Crude ash	9.53±0.14

Results are showed as mean ± standard deviation of 3 replicates

The growth of yeast started at 12 h and then remained consistent until 108 hours post culture, while the growth rate of yeast mixed with papaya peel reached its highest peak at 48 hours, and then declined (Figure 1).

Growth performance of the fish was estimated based on final body weight (FBW), specific growth rate (SGR), and feed conversion ratio (FCR) as shown in Table 3. Silver barbs fed with a 4% papaya peel/2% baker's yeast (PY) diet showed increased FBW, SGR and improved FCR (*P*<0.05) compared with the control (C), 4% papaya peel (P), and 2% baker's yeast (Y) diets. No mortality was observed in any of the experimental dietary groups.

Hematological parameters are shown in Table 4. Silver barbs fed with PY had significantly higher RBC (*P*<0.05) than those in other diet groups and WBC of fish fed with Y and PY diets were higher than those in the C and P diet groups (*P*<0.05). However, there was no significant difference in the hematocrit level and mean corpuscular volume (MCV) of the silver barbs.

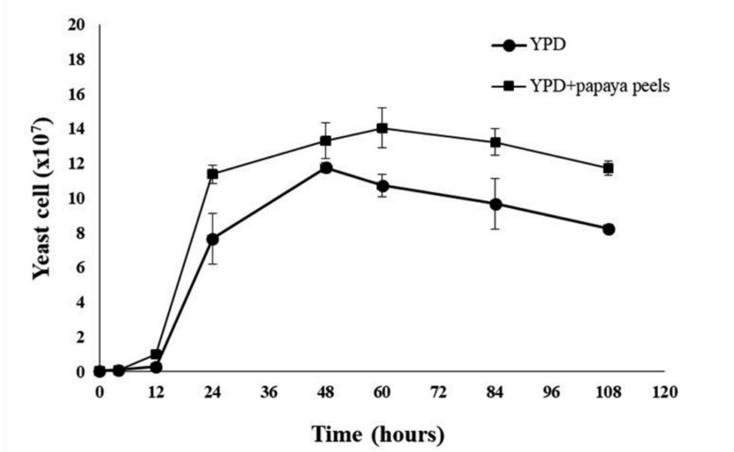

Figure 1: Growth curve of baker's yeast grown in YPD medium and YPD medium containing 2% (w/v) oven-dried papaya peel for 0-108 hours

Table 3: Growth performance of silver barb fed with yeast and/or papaya peels

Parameters	Experimental Diets			
	C	P	Y	PY
Initial weight (IW; g/fish)	5.03±0.06	5.07±0.06	5.07±0.12	5.10±0.10
Final body weight (FBW; g/fish)	12.83±0.46[a]	12.97±0.51[a]	13.43±0.23[a]	14.53±0.29[b]
Weight gain (g/fish)	7.80±0.44[a]	7.90±0.53[a]	8.37±0.31[a]	9.43±0.31[b]
Specific growth rate (SGR; %/day)	1.67±0.06[a]	1.68±0.08 [a]	1.74±0.06[a]	1.87±0.05[b]
Feed conversion ratio (FCR)	2.76±0.16[b]	2.74±0.07[b]	2.70±0.07[b]	2.51±0.03[a]
Survival (%)	100	100	100	100

All variables are presented as mean ±SD. Means (along column or inter-group) with different superscript letter(s) are significantly different ($P < 0.05$).

Table 4: Hematological and biochemical changes of silver barb fed with yeast and or papaya peel diets (N=5)

Parameter	C	P	Y	PY
Hematocrit (% PCV)	33.67±0.92[a]	33.33±4.70[a]	32.4 ±3.17[a]	35.87±1.15[a]
RBC (10^6 /mm^3)	1.75±0.18[a]	1.76±0.24[a]	1.81±0.04[a]	2.19±0.21[b]
WBC (10^4 /mm^3)	2.08±0.11[a]	2.06 ±0.27[a]	2.39±0.10[b]	2.49±0.07[b]
MCV (fL)	193.63±20.15	190.10±32.50	178.74±13.12	163.57±9.27

All variables are presented as mean ±SD, RBC = Red blood cell, WBC = White blood cell, MCV=mean corpuscular volume (hematocrit (% PCV) x10)/ RBC (10^6/mm^3). Means (along column or inter-group) with different superscript letter(s) are significantly different (P<0.05). To investigate disease-resistance in fish post-feeding, the silver barbs were injected with viable *A. hydrophila*. Results showed that the fish mortality had steadily increased until seven days following bacterial infection. Moreover, mortality was lower in silver barbs fed with supplementary diets of yeast (Y and PY group) compared to all other groups. The PY group had increased mortality compared with the Y group (Figure 2). However, no mortality was recorded in the control group after injecting 0.85% of sodium chloride.

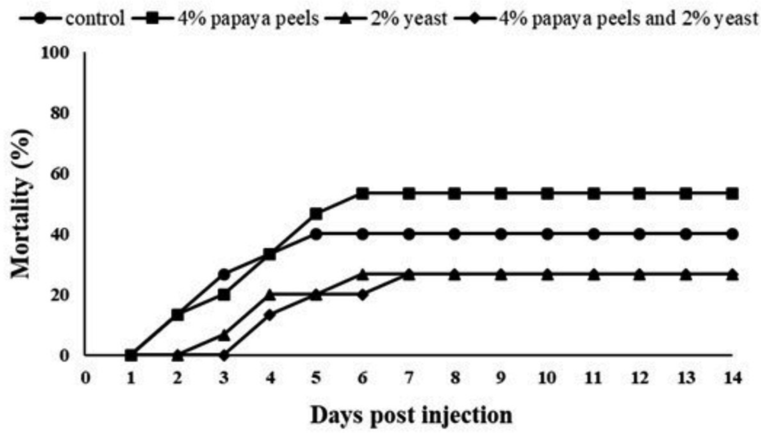

Figure 2: Mortality of Silver barb after intraperitoneal injection with 0.1 ml (0.5×10^{7} CFU mL^{-1}) of *A. hydrophila*

Discussion

There was a rapid expansion in fisheries and aquaculture in Thailand, which serve as important sources of food for millions around the world (FAO, 2012). Tropical countries are affected by greater loss in aquaculture during disease outbreaks (Leung & Bates, 2013). *Aeromonas* are pathogenic organisms in freshwater fish. Many studies have desired to investigate the role of a feed additive on growth and disease resistance in culturing silver barbs (Mondal *et al*., 2019). Moreover, fish feed represented about 50% of the farmer's production cost. Therefore, supplementation of a diet with papaya peel would help reduce feed cost in the aquaculture industry, besides putting an agricultural waste to good use.

The experimental diets contained crude protein and crude lipid not less than 30% and 9.5%, respectively. Crude fiber was less than 4%. The crude ash in P and PY diets was hardly different than each other because they contained equivalent amount of papaya peel, but the values were higher than other diets. Crude ash refers to inorganic matter such as mineral content. Papaya peel contains nutrients, vitamins and main minerals, such as potassium (516.3 mg/100 g of dry weight product) and phosphorus (221.5 mg/100 g of dry weight product), besides trace minerals like magnesium, calcium, sodium and iron (Martial-Didier *et al*., 2017; Vora *et al*., 2018). The experimental diet contained less than 7.4% crude ash. Commercially-produced diets also did not contain more than 8.5% crude ash (Craig *et al*., 2017).

The important component of papaya peel was pectin, which is rich in glucose, L-arabinose and D-galacturonate, and could also function as a prebiotic (Koubala *et al*., 2014; Paull *et al*., 1999). Several studies had demonstrated that prebiotics could stimulate the growth and activity of beneficial microorganisms in the host gut (Srivastava & Malviya, 2011; Minzanova *et al*., 2018). In addition, papaya peel contained protease. Previous studies showed that papaya peel boiled in water at a temperature of 55 °C and pH 7.0 could maintain its protease-specific activity of approximately 133.8 U/mg in breaking down proteins (Chaiwut *et al*., 2010; Morais *et al*., 2017).

Papaya peel also contained polyphenols, which were bioactive compounds acting as antioxidants, and could inhibit the growth pathogenic bacteria such as *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli* and *Klebsiella pneumonia* (Faller & Fialho, 2010; Machado *et al.*, 2015; Maran & Prakash, 2015). However, it is worth stating that the growth rate of yeast in the YPD medium containing oven-dried papaya peel was higher than in YPD medium alone. The present study indicated that papaya peel did not inhibit the growth of yeast cells *in vitro.* Moreover, supplementation of 4% papaya peel (P) in the diet showed no differences in growth performance, survival rate and hematological parameters of the silver barb when compared with the control (C) group. This suggested that the supplementation with papaya peel and baker's yeast in fish feed could be used in aquaculture and might yield great economic benefits.

Silver barb fed with the Y and PY diets had increased body weight, specific growth rate and hematological parameters than those fed with the C and P diets. Supplementation of baker's yeast may improve growth, besides modulating gut morphology an intestinal microbiota. It was also observed to increase the expression of Heat Shock Protein 70 genes in Nile tilapia (Abdel-Tawwab *et al.*, 2008; Lara-Flores *et al.*, 2003; Ran *et al*., 2016). Moreover, *S. cerevisiae* could also affect various fish species, such as groupers (*Epinephelus coioides*), rainbow trouts (*Oncorhynchus mykiss*), hybrid striped bass, beluga (*Huso huso*), Galilee tilapia (*Sarotherodon galilaeus*) and the giant tiger shrimp (*Penaeus monodon*) (Abdel-Tawwab *et al*., 2008; Chiu *et al*., 2010; Hoseinifar *et al*., 2011; Li & Gatlin, 2005; Thanardkit *et al*., 2002).

Supplementation of Y and PY diets also elevated the RBC and WBC ($P<0.05$) of the fish. A previous study had demonstrated that supplementation of yeast in Nile tilapia fingerlings (*Oreochromis niloticus*) at 1, 2 and 5 g yeast kg^{-1} diets could increase both hematological (red blood cell, hematocrit, and haemoglobin) and biochemical parameters (glucose, albumin, and glubulin) in Beluga juveniles (*Huso huso*). Other studies showed that yeast could improve fish health (Abdel-Tawwab *et al*., 2008; Hoseinifar, *et al*., 2011). Moreover, dietary supplementation of 1% yeast and 0.1% β-glucan (*Channa striata*) improved RBC and WBC of cultured snakeheads after eight weeks of feeding when compared with control diets (Talpur *et al*., 2014).

Baker's yeast supplemented diet significantly improved the growth and resistance of the silver barb against *A. hydrophila.* Based on a previous report, the yeast might act as a probiotic, which led to growth improvement in the Nile tilapia (Goda *et al.*, 2012). It may improve gut microvilli (length and density), prevent against *A. hydrophila* infection, and increase protein digestibility by enhancing trypsin activity (Ran *et al*., 2016; Ran *et al*., 2015).

Nile tilapia and snakehead fed with a 1 g/kg diet and 1% yeast supplement showed increased activity of lysozyme and disease resistance against *A. hydrophila* (Abdel-Tawwab *et al*., 2008). This could be because the yeast was a good source of β-1, 3 glucan that could improve the fish's immune response (Vetvicka *et al.*, 2013). In a recent study, a diet containing 0-0.18% β-glucan could enhance disease resistance in large yellow croakers against *Vibrio harveyi* infection. Fish fed with 0.09% of β-glucan for eight weeks had increased growth and serum lysozyme activities (Ai *et al*., 2007). In another study, β-1, 3 glucan could increase immunity against pathogenic *Edwardsiella tarda* and confer specific immunity to aflatoxin in rohu fish (*Labeo rohita* Hamilton) (Sahoo & Mukherjee, 2001).

Conclusion

The present study showed that the dietary supplementation of baker's yeast and papaya peel could improve growth of the silver barb, and also confer disease resistance against *A. hydrophila.* However, the diet containing 4% papaya peel alone had no adverse effects on the growth and blood variables of the silver barb.

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