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Research Article

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Dietary Thymol Supplementation to Improve Growth in Common Carp (*Cyprinus carpio*) and Tambaqui (*Colossoma macropomum*)

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Abstract Common carp (*Cyprinus carpio*) and tambaqui (*Colossoma macropomum*) are the fish that are commonly farmed in Indonesia. The advancement of improved feeding practices is crucial for the sustainable production of both common carp and tambaqui, leading to better growth performance. This research was conducted to enhance growth performance and improve feed utilization efficiency by using thymol as a feed additive. For common carp, thymol was supplemented at a dose of 0, 0.1, 0.2, 0.4, or 0.8 g/kg commercial feed. While thymol was added to the tambaqui feed formulation at doses of 0.0, 0.2, 0.4, or 0.6 g/kg. Common carp with an initial weight of 3.23±0.00 g were reared in net enclosures for 60 days, while tambaqui weighing 8.22±0.01 g were kept in aquariums for 70 days. The study found that supplementing carp feed with 0.4 g/kg thymol led to the highest growth performance. Meanwhile, feeding tambaqui with thymol at 0.6 g/kg resulted in significant improvements in growth-related parameters compared to other treatments, including final total biomass, final average weight, protein efficiency ratio, specific growth rate, and antioxidant status. In conclusion, the optimal thymol supplementation levels were found to be 0.4 g/kg for carp and 0.6 g/kg for tambaqui.

Keywords Thymol • Feed additive • *Cyprinus carpio* • *Colossoma macropomum* • Growth Performance

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INTRODUCTION

Aquaculture has become one of the fastest—growing sectors in animal protein production in recent decades. Common carp (*Cyprinus carpio*) and tambaqui (*Colossoma macropomum*) are among the most commonly farmed fish in Indonesia. These species are highly valuable due to their desirable traits, such as fast growth rates, efficient feed conversion ratio for both natural and supplementary feed, and relative resistance to adverse environmental conditions and diseases (Ljubojević et al., 2013). Common carp, belonging to the Cyprinidae family, the largest family of freshwater fish, is widely farmed in Indonesia. This species is an excellent source of valuable nutrients and plays a significant role in promoting healthy human nutrition. Common carp typically inhabit freshwater environments such as ponds, lakes and rivers (Barus et al., 2001; Rahman, 2015). Tambaqui, originally from the Amazon River, is another important aquaculture commodity. Advancing feeding practices is essential for the sustainable production of both common carp and tambaqui, as it enhances growth performance. Continuous efforts to optimize feed utilization efficiency are crucial for reducing operational costs and enhancing overall aquaculture productivity. Enhancing feed efficiency directly contributes to better growth performance, including increased growth rates and improved feed conversion ratios.

Using common carp and tambaqui as models for fish growth can provide valuable insights into growth rates and feed efficiency. Studying these species helps in understanding how different feeds affect growth performance, feed conversion ratios, and overall health. Additionally, both species exhibit strong adaptability to various farming conditions, making them ideal for evaluating the performance of different feed formulations across diverse environments. This can contribute to the development of more universally applicable feeds or enhance understanding of species-specific nutritional requirements. The relevance of common carp and tambaqui in growth studies, along with the differences in their natural diets and feeding behaviors, highlights the importance of considering these factors when formulating effective fish feeds.

Feeds formulated with both animal- and plant-based ingredients have demonstrated numerous benefits for fish health, overall condition, production performance, and meat quality (Ljubojević et al., 2013 & Ljubojević et al., 2014). Thymol, a natural essential oil, has the ability to stimulate fish growth (Amer et al., 2018). Thymol, also known as 2-isopropyl-5-methylphenol, is a natural monoterpene present in specific essential oils derived from plants (Alagawany et al., 2020). The primary compounds in thymol are the terpenoid thymol itself and the phenol isomer, carvacrol (Putra, 2016). Thymol content is found in thyme (*Thymus vulgaris*), oregano, and *Carum copticum* plants. The thyme plant is an aromatic compound with an essential oil chemical compound of 0.32–4.9%. In comparison, the essential oil component has several contents, such as thymol 61.6%, carvacrol 3.33%, pinene, eugenol, and others. Thymol essential oil has properties as an antioxidant, antibacterial, immunomodulator, expectorant, and carminative and functions as an antifungal, antiseptic, carminative, expectorant, sedative, antioxidant, and antimicrobial (Queiroz et al., 2012; Rahardjo et al., 2014). Thymol is a feed additive that improves animal performance and feed digestibility by influencing the structure and function of the digestive system, improving nutrient absorption, stimulating metabolic activity, exerting antimicrobial effects within the intestines, and reducing oxidative stress. By promoting digestive enzyme activity, thymol is believed to facilitate greater nutrient uptake in fish (Platel and Srinivasan, 2004; Jang et al., 2007). Thymol in feed can activate the structure and the role of the digestive system and increase absorption and metabolism in the bodies of animals, fish, and poultry. Thymol has been reported to exhibit several beneficial physiological effects, including the stimulation of digestive enzyme secretion, enhancement of salivary amylase activity in humans, and the promotion of bile acid, gastric, and pancreatic enzyme production (El-Hack et al., 2016).

Earlier studies have shown that supplementing tilapia (*Oreochromis niloticus*) feed with 0.1 g/kg of thymol can enhance growth performance, improve the growth performance of catfish (*Clarias sp.*) at a dose of 0.2 g/kg and with a dose of 0.3 g/kg can improve the growth performance of catfish (*Pangasius hypopthalmus*) (Amer et al., 2018; Casandra, 2021; Octaviana, 2021). Therefore, research is needed on the addition of thymol to common carp and tambaqui feed using different feed processing methods, such as coating and additives in feed formulations, with varying doses of thymol in each type of feed method as a way to increase the efficiency of feed utilization and growth performance.

MATERIAL AND METHODS

Experimental diet

Thymol supplementation in common carp and tambaqui feed followed a Completely Randomized Design (CRD). The common carp experiment included five treatments (0, 0.1, 0.2, 0.4, and 0.8 g/kg) with three replications, while the tambaqui experiment had four treatments (0.0, 0.2, 0.4, and 0.6 g/kg) with three replications.

In this study, the diet for common carp consisted of a commercial feed supplemented with thymol through a coating technique. For 1 kg of feed, 100 mL of water, one chicken egg, and thymol were added to each feed treatment in amounts of 0 g, 0.1 g, and 0.2 g. 0.4 g and 0.8 g. All coating materials were stirred to form a homogeneous suspension using a hand mixer and mixed with commercial feed using a mixer. The homogenized feed is dried in an oven at 40°C for 4 hours, after which it undergoes proximate analysis using the method outlined by the Association of Official Analytical Chemistry (AOAC, 2012).

On the other hand, the experimental diet for tambaqui was prepared using carefully selected raw materials. These ingredients were ground into fine flour using a grinder and then sieved to ensure uniform particle size. The sieved materials were subsequently weighed according to Table 2 and thoroughly mixed using a mechanical mixer to achieve a consistent blend. During the final stage of the feed preparation, liquid raw materials were gradually incorporated at a ratio of 250 mL per kilogram of the dry mixture. The ingredients were continuously stirred until a homogeneous consistency was obtained. The thoroughly mixed feed was shaped into floating pellets using an extruder machine, producing pellets with a uniform diameter of 2 mm. Following pellet formation, the feed was dried in an oven at a controlled temperature of 50°C to reduce moisture content and enhance shelf stability. Once adequately dried, the feed was allowed to cool to room temperature before being transferred into an airtight container to prevent moisture absorption and nutrient degradation. The container was kept at room temperature in a dry, well-ventilated space to ensure proper preservation. The composition of the formulated diet is provided in Table 2, along with the proximate composition of each feed formulation, including moisture, protein, lipid, fiber, ash, and nitrogen-free extract contents, which were determined and are presented in Table 3.

Table 1.
Proximate Composition (%) of Common Carp Feed with Thymol Addition in Different Dose

Composition	Thymol Dose (g/kg)				
	0.0	0.1	0.2	0.4	0.8
Moisture (%)	9.25	9.31	9.46	9.74	9.35
Fat (%)	5.22	5.60	5.45	5.90	5.40
Protein (%)	31.07	30.51	31.14	31.20	30.77
Fiber (%)	3.68	3.58	3.74	3.45	3.80
Ash (%)	9.29	9.25	9.45	9.48	9.25
NFE* (%)	41.29	41.75	40.75	40.22	41.43

*NFE = nitrogen free extract

Table 2.
Feed Formulation for the Tambaqui Experiment

Raw Material	Thymol Dose (g/kg)			
	0.0	0.2	0.4	0.6
Fish Meal	40.0	40.0	40.0	40.0
Poultry Meal	50.0	50.0	50.0	50.0
Meat Bone Meal	97.0	97.0	97.0	97.0
Soybean Meal	130	130	130	130
Corn Gluten Meal	50.0	50.0	50.0	50.0
Cassava Chips	50.0	50.0	50.0	50.0
Wheat Pollard	328	328	328	328
Wheat Flour	200	199	199	199
Fish Oil	12.7	12.7	12.7	12.7
Cruide Palm Oil	24.4	24.4	24.4	24.4
Lysine	4.0	4.0	4.0	4.0
Vitamin C	0.5	0.5	0.5	0.5
NaCl	5.0	5.0	5.0	5.0
Methionine	2.0	2.0	2.0	2.0
Vitamin Mix	1.5	1.5	1.5	1.5
Mineral Mix	1.5	1.5	1.5	1.5
Polymethylolcarbamide	2.0	2.0	2.0	2.0
Anti-mold	0.15	0.15	0.15	0.15
Thymol	0.0	0.2	0.4	0.6
Total	1000.0	1000.0	1000.0	1000.0

Table 3.
Tambaqui's Feed Proximate Analysis with Thymol Additive in Different Dose

Composition	Thymol dose (g/kg)			
	0.0	0.2	0.4	0.6
Moisture (%)	7.75	7.50	7.75	7.50
Protein (%)	28.67	29.15	29.01	29.42
Fat (%)	3.50	3.50	3.83	4.00
Ash (%)	7.00	7.25	7.00	7.25
Fiber (%)	2.87	2.67	2.38	2.11
NFE* (%)	50.21	49.23	50.03	49.72
GE** (kcal kg^{-1})	4122	4129	4144	4160

*Nitrogen Free Extract = 100% - (moisture + protein + fat + ash + fiber)
**Gross Energy Composition (GE) was calculated based on protein = 5.64 kcal/g protein, fat = 9.44 kcal/g fat, and carbohydrate/NTEC = 4.11 kcal/g carbohydrate (Watanabe, 1998).

Fish maintenance

The research on common carp and tambaqui was conducted using two different schemes. In common carp, research was conducted in the experimental ponds of the Department of Aquaculture, Faculty of Fisheries and Marine Sciences, IPB University. The maintenance container used in this study was a pond lined with HDPE (high-density polyethylene) and equipped with 15 nets measuring (2×1×1) m with a water height of 70 cm. The nets used were brushed and then rinsed with water until clean, after which they were dried for 24 hours and then installed in the pond.

Common carp obtained from cultivators in Parung (Bogor District, Indonesia) with an average weight of 3.23 ± 0.00 g, were distributed into each hapa at a density of 50 fish per cubic meter, resulting in a total of 750 fish for this study. The fish were maintained for 60 days. Throughout the maintenance period, the common carp were fed based on their respective treatments, with feeding occurring three times a day at 8:00, 12:00, and 16:00 at satiation. The amount of feed provided and the number of fish that died were recorded to calculate the individual feed consumption for each carp. The final sampling was carried out at the end of maintenance by calculating the total weight of the entire fish population.

In tambaqui, the study used tambaqui from Rancabungur breeders (Bogor, Indonesia). The fish used were divided into 4 group of treatments with an initial weight of 8.22 ± 0.01 g, which were kept in 16 aquariums measuring 90×45×45 cm with a water height of 30 cm. Each aquarium contained 15 fish, with a net placed above to prevent them from jumping out. The water used as a maintenance medium was collected in a reservoir that had been deposited. One-point aeration was added to all aquariums, and they were equipped with a thermostat to maintain a water temperature of 29.3–29.8°C. Water changes of 60–70% were performed daily to replace the old water with new. The fish were maintained for 70 days and were fed three times a day at satiation throughout the period.

Water quality measurement

The water quality parameters measured during maintenance include temperature, dissolved oxygen (DO), pH, and total ammonia nitrogen (TAN). Temperature measurements were carried out three times a day, namely morning, afternoon, and evening, with values of 26.2-29.1ºC. Dissolved oxygen values are between 6.2 and 8.5 mg/L, pH ranges from 7.4 to 9, and TAN ranges from 0.07 mg/L to 0.21 mg/L. DO, pH, and TAN were measured weekly to meet quality standards (SNI 7550:2009). Water quality parameters were measured at the Environmental Laboratory, Department of Aquaculture, Faculty of Fisheries and Marine Sciences, also IPB University.

Parameters and data analysis

Based on Stickney and Gatlin (2022) growth performance in aquatic organisms is often evaluated using parameters that reflect changes over the life cycle, which supports the focus on early growth phases when assessing feed efficiency and overall development. This foundational understanding guides the selection of growth metrics in the present study. The parameters used in the study were Survival Rate (SR), Specific Growth Rate (SGR), Feed Intake (FI), Feed Conversion Ratio (FCR), Protein Efficiency Ratio (PER), malondialdehyde (MDA) in tambaqui and Hepatosomatic Index (HSI) in common carp (Huisman, 1987; Goddard, 1996; Tacon, 1987; Fan et al. 2019; Tsaknis, 1999). Data were analyzed using variance analysis in the Independent Sample t-Test SPPS with a 95% confidence interval using the SPSS Statistics 26.0 application (IBM, USA).

RESULT AND DISCUSSION

Growth performance

Adding thymol to the feed with different doses affected the growth performance of common carp consuming the feed. The higher the dose of thymol added to the common carp's feed, the higher the growth performance, including fish weight, SGR, PER, and FCR, but at a dose of thymol 0.8 g/kg feed experienced a decrease in growth performance compared to the thymol treatment of 0.4 g/kg.

The growth performance of common carp fed with thymol showed a positive effect (Table 4), with the addition of thymol to the feed resulting in improved growth performance values when compared to the treatment without thymol; this is characterized by better individual weight values, individual FCR, SGR, and PER. As is well known, the SGR value shows the ability of fish to store feed nutrients in the body, which are converted into energy and growth because it is determined by the nutrient content in the feed that can be digested and absorbed and provides an increase in the length and weight of the fish (Putra, 2016). The SGR of common carp in the 0.8 g/kg treatment was lower compared to the 0.4 g/kg treatment. This is considered an adverse effect of excess thymol doses, where thymol is also antimicrobial, so the microbes in the digestive tract are thought to be reduced or killed. The statement highlights that thymol exhibits antibacterial effects against *Escherichia coli* and *Salmonella typhimurium* (Gianneanas et al., 2012). One of the contents of thymol is phenol, which destroys bacterial membranes and causes the release of membranes from cells to the external media. Essential oils containing thymol function as antimicrobials, antifungals, and antioxidants and contain molecules that have intrinsic bioactivity in fish metabolism and physiology (Ahmadifar et al., 2011). However, it should be noted that the present study did not directly measure the intestinal microbial load; therefore, the proposed link between thymol's antimicrobial effects and changes in SGR remains speculative. Further studies are needed to investigate the intestinal microbiota to confirm these potential effects.

Table 4.

Growth Performance of Common Carp with the Addition of Thymol to Feed

Test Parameters	Thymol Dose (g/kg)				
	0.0	0.1	0.2	0.4	0.8
W0 (g)	3.23±0.00^{ab}	3.23±0.00^{b}	3.23±0.00^{ab}	3.24±0.00^{a}	3.23±0.00^{ab}
Wt (g)	14.70±0.55^{c}	16.22±0.37^{b}	17.43±0.32^{b}	19.30±0.68^{a}	17.43±0.32^{b}
Bt (g)	602.3±8.0^{d}	665.0±9.5^{c}	738.7±12.9^{b}	843.3±52.9^{a}	807.3±11.7^{a}
FI (g)	1097±34^{b}	1133±2^{b}	1223±53^{ab}	1293±21^{a}	1303±44^{ab}
SGR (%)	2.52±0.06^{c}	2.69±0.04^{b}	2.77±0.11^{b}	2.98±0.06^{a}	2.81±0.03^{b}
FCR	2.34±0.05^{d}	2.13±0.06^{c}	2.04±0.08^{bc}	1.85±0.06^{a}	1.98±0.03^{b}
SR (%)	82.0±2.0^{b}	82.0±2.0^{b}	86.7±4.2^{b}	87.3±3.1^{ab}	92.7±3.1^{a}
HSI	0.41±0.03^{a}	0.36±0.14^{a}	0.37±0.07^{a}	0.31±0.05^{a}	0.32±0.04^{a}
PER	1.38±0.03^{d}	1.54±0.04^{c}	1.58±0.06^{bc}	1.74±0.05^{a}	1.64±0.03^{b}

The values shown are the average ± standard deviation. Different letters in the same row indicate significant differences ($p<0.05$). The values shown are the average and standard deviation, W0 = initial weight, Wt = final weight, Bt = final biomass, FI = Feed Intake, SGR = Specific Growth Rate, FCR = Feed Conversion Ratio, SR = Survival Rate, HSI = Hepatosomatic Index, PER = Protein Efficiency Ratio.

Meanwhile, the FI of common carp treated with thymol increased. SGR and FI are related to the fish's response to the feed given, commonly referred to as palatability. Al-Souti et al. (2019) stated that fish have palatability properties, which means the level of preference for feed to be accepted by the body based on aroma, which is chemical feed factors. The level of palatability can affect FI; if the FI is high, it indicates a decent level of palatability, and vice versa. If the FI is low, the level of fish palatability is unacceptable. Moreover, adding thymol to the feed of tilapia enhanced its palatability. They stated that feed additives will affect the palatability of fish in receiving feed (Aanyu et al., 2019); Eriegha et al., 2017; Platel & Srinivasan, 2004).

The FI of common carp in the 0.2, 0.4, and 0.8 g/kg treatments did not differ significantly, but the feed conversion ratio (FCR) in the 0.4 g/kg treatment was better than in the other treatments. Low FCR values indicate more efficient feed utilization. Amer et al. (2018) demonstrated that the addition of thymol at doses of 1 ml/kg and 2 ml/kg in tilapia feed can reduce FCR values. This improvement is suggested to be linked to enhanced nutrient absorption also some nutrients are consumed by microbes, while some microbes produce secondary metabolites, potentially due to the reduction of anaerobic bacteria, making nutrients more available and decreasing bacterial metabolites, which are toxic during digestion (Giannenas et al., 2012). In the present study, the 0.4 g/kg thymol treatment for common carp resulted in better nutrient utilization compared to other treatments, as indicated by the highest SGR and lowest FCR. However, since digestive enzyme activity was not measured, this mechanism remains speculative and warrants further investigation.

Table 5 presents the growth performance of tambaqui maintained for 70 days, with different doses of thymol added to the feed. The results of the analysis indicated that supplementing the feed with thymol had a significant impact ($p<0.05$) on the growth performance of tambaqui. Increasing the thymol dose treatment increased individual weight and final biomass, with the highest weight in the thymol treatment of 0.6 g/kg feed. The 0.6 g/kg thymol treatment also had the highest SGR value. Adding thymol 0.6 g/kg feed increased the efficiency of feed utilization; the PER value increased, and the FCR value was low. Thymol also acts as an antioxidant because the administration of thymol reduces the malondialdehyde value. The treatment of adding thymol at various doses resulted in the same fish survival ($p>0.05$).

Table 5.

Growth Performance of Tambaqui with the Addition of Thymol to Feed

Test Parameters	Thymol Dose (g/kg)			
	0.0	0.2	0.4	0.6
W0 (g)	8.22±0.01	8.23±0.01	8.23±0.01	8.22±0.00
Wt (g)	39.84±2.80^{b}	41.18±1.17^{b}	42.29±0.90^{ab}	44.67±1.11^{a}
B0 (g)	123.3±0.2	123.5±0.2	123.5±0.2	123.3±0.1
Bt (g)	597.2±42.0^{b}	617.3±17.5^{b}	634.4±13.5^{ab}	670.1±16.6^{a}
FI (g)	581±11.4^{a}	587±4.8^{a}	561±4.2^{a}	585±13.4^{a}
SR (%)	96.8±6.7^{a}	98.3±3.3^{a}	100.0±0.0^{a}	100.0±0.0^{a}
SGR (%)	2.25±0.10^{b}	2.30±0.04^{b}	2.34±0.03^{ab}	2.41±0.04^{a}
FCR	1.23±0.11^{c}	1.18±0.05^{bc}	1.09±0.02^{ab}	1.07±0.04a
PER	2.84±0.24^{b}	2.88±0.12^{b}	3.13±0.06^{a}	3.17±0.11^{a}
MDA (μM)	0.80±0.04^{b}	0.74±0.01^{a}	0.73±0.01^{a}	0.72±0.01^{a}

W0 = initial weight, Wt = final weight, B0 = Initial biomass, Bt = final biomass, FI = Feed Intake, SGR = Specific Growth Rate, FCR = Feed Conversion Ratio, SR = Survival Rate, PER = Protein Efficiency Ratio, MDA = Malondialdehyde. Different letters in the same row indicate significant differences in each treatment ($p<0.05$)

Adding thymol to tambaqui feed shows better growth performance values than the feed treatment without thymol, which can be seen from the final biomass value, PER, FCR, and SGR. The final weight and biomass values in feed with the addition of 0.6 g/kg have more optimal results, which were 44.67±1.11 g and 670.1±16.6 g (Table 5). This follows the statement that thymol can increase growth in rainbow trout, on grass carp and tilapia (Ahmadifar et al., 2011; Amer et al., 2018; Morselli et al., 2019).

In tambaqui, the most optimal SGR was found in the treatment of adding thymol 0.6 g/kg of 2.41±0.04 %; this treatment was significantly different from the addition of thymol 0.0 g/kg and 0.2 g/kg of 2.25±0.10 g and 2.30±0.04 g. This is supported by the PER and FCR value of 0.6 g/kg thymol addition, which was better than the others. The addition of 0.6 g/kg of thymol to tambaqui feed showed a low FCR value of 1.07±0.04, while PER gave more optimal results at this dose, namely 3.17±0.11. The high protein efficiency ratio (PER) and low feed conversion ratio (FCR) indicate that tambaqui can utilize the feed given optimally, leading to optimal absorption and conversion into muscle mass. A lower FCR indicates greater feeding efficiency, as the fish are able to convert the feed they consume into growth, resulting in increased body weight (Shofura et al., 2017). Research conducted by Jang et al. (2007) reported that supplementing broiler chicken feed with 0.05 g/kg of thymol can enhance the activity of digestive enzymes, namely trypsin enzymes. In addition, it can also affect the feed conversion ratio value. The trypsin enzyme can convert feed protein into amino acids more easily absorbed by the body.

Hepatosomatic Index (HSI) in common carp and Malondialdehyde (MDA) in tambaqui

Further testing on common carp is the HSI value. Adding thymol to the feed showed results that did not significantly affect the HSI value of common carp. Based on the statement of the previous study, the increasing amount of nutrients absorbed by the body will increase the HSI value of gourami seeds (Yandes et al., 2003). In this study, it is suspected that there is no accumulation of fat in the liver, and it is suspected that fat is stored in the form of glycogen in the body tissue of the fish. These results were also obtained in the malondialdehyde (MDA) value of tambaqui, where the treatment of adding thymol to the feed of 0.2 g/kg, 0.4 g/kg and 0.6 g/kg of feed showed results that were not significantly different. The efficient value was found in the treatment of adding thymol 0.6 g/kg of feed of 0.72±0.01 μM and the treatment of adding thymol 0.0 g/kg of feed of 0.80±0.04 μM. These values indicate that the antioxidant system contained in thymol works well so that the fish are not easily stressed. This follows the study's results above, which indicate that the SR in the treatment with thymol at a dose of 0.6 g/kg of feed was higher than in the other treatments. The SR value for the 0.6 g/kg thymol treatment reached 100%, whereas the SR in the treatment with 0.0 g/kg thymol was only 96.75±6.67% (Alagawany et al., 2020). Adding thymol to feed can increase antioxidant status, immune function, and meat quality and reduce animal mortality rates.

CONCLUSION

The findings of this study demonstrate that supplementing common carp feed with thymol at a concentration of 0.4 g/kg significantly enhances their growth performance. Similarly, incorporating 0.6 g/kg of thymol into tambaqui feed promotes better growth and improves the fish's antioxidant status, potentially strengthening their overall health and resilience against oxidative stress. These results suggest that thymol supplementation may have beneficial effects on certain growth and health parameters in fish, including improvements in survival rate, growth performance, and some indicators of metabolic and oxidative status. However, further comprehensive analyses of oxidative stress markers are needed to fully understand thymol's potential as a natural feed additive in aquaculture.

Ethical Approval: This study did not require ethical clearance as all procedures adhered to standard aquaculture practices and were conducted in accordance with applicable guidelines for the care and use of fish in research.
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