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Application of Microbubble Technology to Increase Oxygen Content in The Aquaculture of Tambaqui (*Colossoma macropomum*)

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Abstract

Oxygen plays an important role in aquaculture by serving as an essential component for oxidizing food substances to produce energy, thereby influencing metabolic rates often quantified by oxygen consumption per unit of time. Particularly in intensive aquaculture systems characterized by high stocking densities and maximal feeding rates, oxygen content assumes paramount importance. This study aimed to evaluate the growth performance of tambaqui (*Colossoma macropomum*) treated with increased oxygen in two different systems. The study was conducted on a laboratory scale by applying T-test data analysis. The difference in treatment level applied when comparing aeration and microbubble consists of two treatments and three replications. This research used commercial floating pellets PF0 with 25% protein content, the feeding rate was 5% of the biomass. The feed was given daily during the culture for two times a day. Data from the study were analyzed using a T-test on the Statistical Product and Service Solutions (SPSS) software Version 17.0. Results of the study showed that aeration system using microbubble resulted in dissolved oxygen (DO) of 6.5 ± 0.17, 100% pomfret fish survival rate (SR), Survival Growth Rate (SGR) of 1.83 ± 0.24, Feed Conversion Ratio (FCR) of 1.37 ± 0.17, Protein Efficiency Ratio (PER) of 2.79 ± 0.37, Feed Utilization Efficiency (FUE) of 0.67 ± 0.09, Absolute Weight Growth of 52.02 ± 1.60; it was better than using aeration. This study has developed a model of tambaqui aquaculture to provide growth value.

INTRODUCTION

Oxygen is one of the most important water quality parameters in aquaculture (Zang *et al.*, 2001; Petranich *et al.*, 2018). Oxygen plays an important role in aquaculture by serving as an essential component for oxidizing food substances to produce energy, thereby influencing metabolic rates often quantified by oxygen

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consumption per unit of time (Pardamean *et al*., 2021). In intensive aquaculture systems characterized by high stocking densities and maximal feeding rates (Pangestika and Putra, 2020; Jefri *et al*., 2020), oxygen content is paramount. The heightened stocking density frequently leads to oxygen deficiencies, posing a threat to aquatic life and necessitating a keen consideration of oxygen as a limiting factor (Stickney and Van Liere, 1953). They revealed that lack of oxygen endangers aquatic animals since it causes stress, is susceptible to disease, and can even cause death. The need for oxygen varies depending on the species, size, activity, sex, rate of feed consumption, temperature, and dissolved oxygen concentration (Salvanes *et al*., 2018).

Dissolved oxygen, a critical aspect of water quality, is intricately linked to respiratory processes crucial for fish survival and well-being (Hasan and Islam, 2020; Hasan and Widodo, 2020; Hasan *et al*., 2021). Insufficient levels of dissolved oxygen may adversely affect aquaculture water quality, resulting in growth abnormalities and heightened susceptibility to diseases among fish populations (Nafisyah *et al*., 2018; Tavares-Dias, 2021; Amin *et al*., 2021; Amin *et al*., 2022). Mitigating oxygen insufficiency typically involves water changes and aeration; however, the latter often proves inefficient due to challenges in accurately determining the minimum required dissolved oxygen concentration for fish. Consequently, aeration often persists for prolonged periods until oxygen saturation levels are achieved, leading to significant energy wastage, particularly in electrical energy consumption.

A potential solution to this inefficiency lies in the utilization of Microbubble Systems, a cutting-edge aquaculture technology designed to optimize fish cultivation productivity by refining and modifying aquaculture systems. Microbubble technology has garnered substantial interest in recent years owing to its diverse applications across scientific and technological domains. A study by Mahasri *et al*. (2018) demonstrated the efficacy of microbubble technology in augmenting oxygen content in tilapia culture, achieving a concentration of 0.61 pp/minute. Notably, the microbubble model proposed by Luo exhibited superior oxygen distribution compared to conventional high bubble distribution trends (Ren *et al*., 2019).

Furthermore, microbubble technology has been shown to enhance growth outcomes in seawater fish compared to freshwater species (Duangjai and Punroob, 2017). Microbubble technology is pivotal in enhancing fish survival and growth rates by optimizing water quality through increased dissolved oxygen concentrations. Additionally, microbubbles contribute to waste treatment in culture containers by supplying dissolved oxygen for microbial decomposition of organic and inorganic matter. Due to the lack of studies concerning the application of microbubble technology in Tambaqui cultivation, this study aims to evaluate the comparative effectiveness of aeration versus microbubble technology in enhancing dissolved oxygen content and growth performance of tambaqui.

METHODOLOGY
Ethical Approval

All research procedures are carried out according to standards without damaging or polluting the environment and testing laboratory.

Place and Time

The study was conducted from January - December 2020. Comparative biological testing of the use of microbubbles and aeration on the oxygen content and growth of pomfret was conducted at the Fisheries and Marine Sciences Faculty Research Laboratory, Jenderal Soedirman University. This study compared two cultivation system methods aeration and microbubble. Biological tests were conducted by pomfret samples on the growth performance, FCR, PER, and survival of the fish. Maintenance activities were conducted for 30 days with sampling every seven days.

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Research Materials

The tools used in this study were aquarium 40 x 30 x 30, an aerator pump (LP 100), a microbubble pump, a DO meter (Lutron DO-5510), a thermometer, a pH meter digital (00-14.00 pH), an aerator hose, a digital scale, a ruler, and a timer socket. Furthermore, this experiment used pomfret fish (size of 6-7 g) derived from Rempoa Area, Banyumas, Central Java.

Research Design

This research was carried out in a laboratory setting utilizing T-test data analysis. The comparison between aeration and microbubble was conducted using two treatments and three replications. The treatments given were as follows: P1 (The use of aeration) and P2 (The use of microbubble).

Work Procedure

Container Preparation

The process of preparing the containers commenced with the cleaning of six units of aquariums with the size of 60 cm × 35 cm × 30 cm each. Subsequently, 10L of water was added to each container. The aquariums were thoroughly cleaned, and water filling was carried out over three days to stabilize the water conditions in each aquarium to provide the natural habitat of pomfret fish. The temperature of the research room was maintained within the range of 25-27°C. After the fish exhibited signs of health and activity, they were allowed a three-day period to adapt to their new environment. This adaptation period was crucial to ensure an accurate assessment of the effects of aeration and microbubble cultivation systems.

Preparation and Feeding

Pomfret fish were fed with commercial floating pellets PF0 containing 25% protein, at a feeding rate equivalent to 3% of the biomass. The fish were fed twice daily during the rearing period. The amount of feed provided was determined based on the weekly weight gain of the sample fish, with adjustments made every seven days (Hossain *et al.*, 2010).

Water Quality Observation

The parameters of the water quality observed in this experiment were dissolved oxygen (DO), temperature, and pH. Dissolved oxygen and temperature were measured using a Lutron DO meter, while pH levels were assessed with a pH meter. Water quality observations were conducted twice daily, in the morning and afternoon, throughout the study.

Data Collection

Collected data included dissolved oxygen (DO) levels, survival rate (SR), Specific Growth Rate (SGR), Feed Conversion Ratio (FCR), Protein Efficiency Ratio (PER), Feed Utilization Efficiency (FUE), and Absolute Weight Growth. (Hossain *et al.*, 2010).

Data Analysis

Data analysis in this study performed the T-test method. The research design compared the average growth achieved using an aeration system versus a microbubble system. Statistical analysis of the data was conducted using T-tests within the Statistical Product and Service Solutions (SPSS) software Version 17.0, enabling conclusions to be drawn regarding the research design.

RESULTS AND DISCUSSIONS

According to the growth performance outcomes and observations concerning the effects of aeration and microbubble treatment on pomfret fish growth, the findings are outlined in Table 1 as follows:

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Table 1. Observation results of the study parameters.

Parameter	P1	P2
Dissolved oxygen (mg/L)	5.38 ± 0.57[b]	6.5 ± 0.17[a]
Survival Rate (SR) (%)	100[a]	100[a]
Survival Growth Rate/SGR (%/day)	1.16 ± 0.03[b]	1.83 ± 0.24[a]
Feed Conversion Ratio (FCR)	2.27 ± 0.14[b]	1.37 ± 0.17[a]
Protein Efficiency Ratio (PER)	1.66 ± 0.11[b]	2.79 ± 0.37[a]
Feed Utilization Efficiency (FUE)	0.40 ± 0.02[b]	0.67 ± 0.09[a]
Absolute Weight Growth (W)	27.15 ± 3.67[b]	52.02 ± 1.60[a]

Note: All data listed are mean values (n = 3; $\bar{x}$ ± SD). The numbers followed by different superscript lowercase letters indicate significant differences (P <0.05).

Dissolved Oxygen

The statistical analysis revealed a significant difference ($P<0.05$) in dissolved oxygen content between the aeration and microbubble treatment methods. The average dissolved oxygen content in the microbubble treatment was notably higher compared to the aeration treatment, with respective averages of 6.5 ± 0.17 mg/L and 5.38 ± 0.57 mg/L. This study establishes that the microbubble technique offers a more substantial supply of dissolved oxygen than the aeration technique, aligning with findings by Ushikubo *et al*. (2010) indicating that microbubble systems maintain stable oxygen levels in water, thereby extending the availability of oxygen and increasing dissolved oxygen content. The solubility of oxygen in water is influenced by factors such as water temperature, salinity, agitation, and air pressure.

Additionally, Boyd (1990) noted that oxygen solubility in water decreases with rising temperatures, while gas solubility decreases as salinity levels increase up to 40%, with a salinity value of 6.56 mg/L. When dissolved oxygen concentration falls below saturation levels, oxygen from the atmosphere dissolves into the water, and the process of oxygen dissolution or release is accelerated by the oxygen concentration in air and water (Hepher and Pruginin, 1981). The oxygen dissolves into water through atmospheric diffusion and photosynthesis (Stickney, 1979), primarily through direct diffusion between air and water surfaces (Spotte, 1979). The rate of diffusion is largely contingent on three key factors: oxygen deficiency in water, water surface area exposed to air, and the degree of turbulence (Boyd, 1982).

In addition to its application in fish cultivation, microbubble technology has proven successful in microbiology and plant cultivation. Ago *et al*. (2005) conducted a microbubble design study focusing on bacterial and plant growth, while Ikeura *et al*. (2017) explored its impact on plants. Microbubble technology has also been utilized in waste treatment, as demonstrated by Wen *et al*. (2011). The development of microbubble venturi design methods has enhanced Nile tilapia cultivation and emerged as a viable technology in intensive aquaculture. Microbubbles are engineered with small exhaust air, resulting in highly pressurized air bubbles. This design feature enables microbubbles to expedite the decomposition of organic matter in aquaculture waters, as indicated by Budhijanto *et al*. (2017).

While microbubble technology has advanced to address oxygen quality in aquaculture waters, its drawback lies in the considerable costs associated with pumps used for oxygen diffusion. Attention must be paid to the appropriate duration of microbubble use to optimize their effectiveness in maintaining scheduled oxygen levels, as suggested by Rofik *et al*. (2020), who reported an increase in oxygen content of up to 8.8 mg/L within an hour. The oxygen enhancement achieved is contingent upon factors such as water depth, microbubble placement, and resulting water pressure, as detailed by Endo *et al*. (2008), who observed an increase in oxygen content from 0.52 mg/L to 0.87 mg/L and a decrease in temperature from 0.08-0.12 °C

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through microbubble application in freshwater fish culture.

Survival Rate (SR)

Statistical analyses revealed no significant differences in fish SR between aeration and microbubble treatments ($P > 0.05$). Both methods exhibited an average SR of 100% for pomfret fish, indicating their robust resistance and adaptability to consistent water quality conditions. Contrary to the assertions of Duangjai and Punroob (2017) regarding microbubble technology's role in enhancing fish survival and growth through water quality maintenance, the findings suggest that the similar SR in both treatments may stem from meeting the optimal dissolved oxygen standards for pomfret fish cultivation.

This aligns with Retnani and Abdulgani's (2013) observations that pomfret fish can thrive even at low salinity levels around 4 ppt, with a survival rate of 100%. In a study by Ayuningrum *et al.* (2020), microbubble technology applied in intensive vannamei shrimp *(Litopenaues vannamei)* farming significantly improved SR to approximately 60-100%, whereas non-microbubble treatments exhibited considerably lower SR of around 39-78%. Similarly, catfish farming recorded a high SR of 97.22% (Hidayat *et al.*, 2016). Microbubbles, characterized by small sizes ranging from 50-100 m, facilitate enhanced oxygen diffusion, which accelerates organic matter decomposition and reduces ammonia content in the water, as noted by Deendarlianto *et al.* (2015) and Jainontee *et al.* (2019).

Specific Growth Rate (SGR)

The statistical analysis results revealed significant differences in the impact of treatment methods, specifically between aeration and microbubble techniques, on the SGR ($P < 0.05$). The SGR mean value under the microbubble technique was notably higher compared to that of the aeration method; specifically, the SGR for the microbubble technique stood at 1.83 ± 0.24% per day, while the aeration technique registered 1.16 ± 0.03% per day. This finding aligns with previous research by Duangjai and Punroob (2017), which highlighted the enhanced growth of pomfret fish through microbubble utilization, particularly in seawater environments.

The variance in pomfret growth rates between these two techniques is attributed to several factors, as noted by Hasan *et al.* (2015), who mentioned that fish tend to reduce food consumption in low-dissolved oxygen conditions, thereby affecting growth inversely. Optimal dissolved oxygen concentrations stimulate fish appetite, consequently bolstering growth rates. This elucidates the observed growth rate disparity in pomfret fish between aeration and microbubble techniques. Heriyati *et al.* (2020) further elaborated that microbubble technology facilitated individual fish weight gains and increased fish biomass by 268%. Although aeration improves dissolved oxygen (DO) levels, incorporating microbubble aeration enhances DO levels, growth rates, and fish biomass.

The decline in oxygen availability within the aeration system is considered the primary factor influencing food intake determinants. Reduced oxygen levels result in diminished appetite and growth performance in pomfret fish during feeding sessions, highlighting the importance of adequate oxygen content. Similar findings have been reported in other studies, such as those on *Oncorhynchus mykiss* by Pedersen (1987); Glencross (2009); D. labrax L (Thetmeyer *et al.*, 1999), *Scophthalmus maximus* (Pichavant *et al.*, 2000), *Anarhichas minor* Olafsen (Foss *et al.*, 2003), *Ictalurus punctatus* (Buentello *et al.*, 2000), *O. niloticus* (Tran-Duy *et al.*, 2008), and *Morone saxatilis* (Brandt *et al.*, 2009).

Feed Conversion Ratio (FCR)

Statistical analyses revealed significant differences in treatment methods, specifically between aeration and microbubble techniques, regarding the Feed Conversion Ratio (FCR) ($P <0.05$). The FCR average calculated for the microbubble treatment

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was 1.37 ± 0.17, whereas that of the aeration treatment was 2.27 ± 0.14. This suggests that pomfret fish cultured using the microbubble method exhibited improved feed intake compared to those cultured using aeration. These findings align with a study by Heriyati *et al*. (2020), which compared conventional aeration and microbubble techniques in red tilapia aquaculture. In that study, the FCR for the microbubble method was 1.33, while that of the conventional aeration method was 1.18; notably, cultures without aeration or microbubble had an FCR of 4.42.

The microbubble treatment demonstrated a low FCR value, indicating efficient use of feed by the fish for growth, as evidenced by the high biomass of fish observed at the study's conclusion. The relatively stable Dissolved Oxygen (DO) concentration in the microbubble treatment, ranging from 6.2 to 6.7 mg/L until the study's end, influenced the fish's appetite, metabolism, and growth. This adherence to standard DO values for tilapia culture, set at >3 ppm according to SNI (2009), supports continuous growth. Several studies have reported that microbubbles may enhance fish growth (Onari *et al*., 2002; Saputra *et al*., 2018) and impact survival rates (SR). Notably, the SR of the aeration treatment was higher than that of the control ($P < 0.05$).

The oxygen content in water significantly affects feed conversion; an oxygen content level between 80-120% tends to result in a favorable feed conversion effect. This underscores the importance of adequate oxygen content for nutrient absorption in fish, thereby influencing feed conversion. Oxygen also plays a crucial role as a growth-limiting factor for fish (Welker *et al*., 2019). Insufficient oxygen levels hinder the protein synthesis of pomfret, as demonstrated by Smith *et al*. (1996), who observed over a 56% reduction in protein synthesis rates in the liver, red muscle, and white muscle of crucian carp exposed to 48 hours of anoxia.

Feed Utilization Efficiency (FUE)

The statistical analysis results indicated a significant difference ($P<0.05$) in feed efficiency between the aeration and microbubble treatment methods. The mean feed efficiency in the aeration group was 0.40 ± 0.02, whereas in the microbubble group, it was 0.67 ± 0.09. This finding suggests that pomfret fish subjected to microbubble treatment exhibited greater feed utilization efficiency compared to those under aeration treatment.

The feed efficiency achieved in the microbubble treatment reached an optimal level, consistent with Febrianti *et al*. (2016), who noted that a fine feed efficiency value should exceed 50% or 0.5. This contrasts with the aeration treatment, which did not attain the desired level of feed efficiency, as indicated by Maskur (2005), who linked reduced dissolved oxygen levels in water to decreased feed efficiency. Adequate oxygen levels are crucial for supporting fish growth as they positively influence feed efficiency. Recommended oxygen levels typically range between 60-80%, and sometimes up to 100%, offering various benefits to fish farmers. Higher oxygen content in the culture environment stimulates feed consumption in fish, ultimately promoting favorable growth outcomes, as emphasized by Buentello *et al*. (2000).

Protein Efficiency Ratio (PER)

Statistical analysis revealed significant differences ($P<0.05$) in the Protein Efficiency Ratio (PER) between aeration and microbubble treatments for pomfret fish. The average PER value in the aeration group was 1.66 ± 0.11, whereas in the microbubble group, it was 2.79 ± 0.37. This discrepancy could be attributed to the dissolved oxygen levels in the water, which impact the activity of protease enzymes. As a result, tambaqui were able to efficiently utilize protein-rich food intakes, leading to enhanced PER values. This phenomenon aligns with findings by Mulyani *et al*. (2014), indicating that increased enzyme activity likely corresponds to fish

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intensifying their efforts to digest nutrients, particularly proteins, to optimize feed protein utilization for growth.

The lower PER value observed in the aeration treatment is attributed to the diminished activity of protease enzymes compared to the microbubble treatment, thereby resulting in Treatment 1 exhibiting a lower PER value than Treatment 2. Additionally, oxygen levels significantly influence antioxidant enzyme activity and malondialdehyde levels in both liver and serum. As noted in previous observations, optimal dissolved oxygen (DO) levels contribute to improved growth performance, feed utilization, and antioxidant response in fish, as highlighted by Yang *et al*. (2015). Moreover, Abdel-Tawwab *et al*. (2015) elaborated that low oxygen content in fish culture compromises fish immunity, rendering them more susceptible to diseases. It's noteworthy that providing fish with an oxygen content of 3.5 mg/L leads to decreased growth, while an oxygen content of 6 mg/L positively impacts weight gain, protein retention, amino acids, and feed efficiency, as discussed by Gan *et al*. (2013). Thus, inadequate oxygen levels pose risks to fish health and may even cause organ damage.

Absolute Weight Growth (W)

The statistical analysis revealed a notable and significant difference ($P<0.05$) in pomfret biomass between the aeration and microbubble treatments. The average weight gain in the aeration group was 27.15 ± 3.67 grams, while in the microbubble group, it was 52.02 ± 1.60 grams. This data indicates that the pomfret biomass was higher in the microbubble treatment compared to aeration. This finding aligns with Heriyati *et al*. (2020), who reported a significant difference ($p<0.05$) in red tilapia biomass at the end of their study, with the microbubble treatment showing a 32.5% increase (6.15 kg) compared to conventional aeration treatment (4.64 kg). The consistent oxygen supply from the microbubble aerator contributes to enhanced fish growth as dissolved oxygen is vital for respiration, metabolism, and substance exchange, thereby fueling growth (Boyd, 1982).

This suggests that microbubble treatment can lead to increased fish growth compared to conventional aeration. Budhijanto *et al*. (2017) similarly applied microbubble aeration in tilapia culture, resulting in accelerated organic matter degradation and subsequent fish growth. Fish intake and growth are notably affected by high oxygen levels compared to low levels. The oxygen needs of fish are also influenced by their size, with smaller fish exhibiting higher feed intake and growth rates than larger fish, especially under low oxygen conditions where hematological adjustments are limited due to a restricted gill surface area (Tran-Duy *et al*., 2008).

CONCLUSION

Aeration system using microbubble resulted in dissolved oxygen (DO) of 6.5 ± 0.17, 100%pomfret fish survival rate (SR), Survival Growth Rate (SGR) of 1.83 ± 0.24, Feed Conversion Ratio (FCR) of 1.37 ± 0.17, Protein Efficiency Ratio (PER) of 2.79 ± 0.37, Feed Utilization Efficiency (FUE) of 0.67 ± 0.09, Absolute Weight Growth of 52.02 ± 1.60; it was better than using aeration. This study has developed a model of tambaqui fish aquaculture to provide growth value.

CONFLICT OF INTEREST

There is no conflict of interest in this manuscript between all authors upon writing and publishing this manuscript.

AUTHOR CONTRIBUTION

The contribution of each author is as follows: Ren Fitriadi, Mustika Palupi, and Sesillia Rani Sanudra collected and analyzed data, drafting, and manuscript preparation. Joni Johandra Putra and Muh. Azril participated in the conception and experimental design.

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