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Sustainable production of bullfrogs (*Lithobates catesbeianus*) with reused water from a Biofloc system

Producción sostenible de rana toro (*Lithobates catesbeianus*) con agua de reúso de un sistema Biofloc

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ABSTRACT

In Mexico, bullfrog (*Lithobates catesbeianus*) production is carried out in only 35 animal production units, and improvements in its commercialization are necessary to increase demand. Faced with these challenges, innovative technological options such as Biofloc are emerging, which improve production efficiency in favor of environmental, economic and social sustainability. Thus, this study measured the effect of using reused water from a Biofloc system for tilapia (*Oreochromis niloticus)* culture in the intensive production of bullfrogs (*Lithobates catesbeianus*). With a biological material of 4,000 organisms (*Lithobates catesbeianus*), treatments were evaluated: T1 (drinking water), T2 (30% Biofloc reuse water), T3 (60% Biofloc reuse water) and T4 (90% Biofloc reuse water); the variables Weight Gain (WG), Specific Growth Rate (SGR), Survival Percentage (%S), Survival Rate (SR) and Feed Conversion (FC) were measured. The results were analyzed in SPSS Statistic version 27.0.0 with an ANOVA and Mauchly's test of sphericity. The results showed that intensive production of bullfrogs (*Lithobates catesbeianus*) with reused water from a Biofloc system of tilapia (*Oreochromis niloticus*) culture is feasible.

Keywords: production, sustainability, Biofloc, *Lithobates catesbeianus.*

RESUMEN

En México, la producción de rana toro (*Lithobates catesbeianus*) se realiza en apenas 35 unidades de producción animal, siendo necesario realizar mejoras en su comercialización para aumentar la demanda. Frente a estos retos, surgen opciones tecnológicas innovadoras como Biofloc, que mejoran la eficiencia de la producción en pro de la sostenibilidad ambiental, económica y social. Siendo así que este estudio midió el efecto de la utilización de agua de reúso de un sistema de Biofloc de cultivo de tilapia (*Oreochromis niloticus*) en la producción intensiva de rana toro (*Lithobates catesbeianus*). Con un material biológico de 4,000 organismos (*Lithobates catesbeianus*), se evaluaron los tratamientos: T1 (agua potable), T2 (30% de agua de reúso Biofloc), T3(60% de agua de reúso Biofloc) y T4 (90% de agua de reúso Biofloc); se midieron las variables Ganancia de Peso (GP), Tasa Específica de Crecimiento (TEC), Porcentaje de Sobrevivencia (%S), Tasa de Sobrevivencia (TS) y Conversión Alimenticia (CA). Los resultados fueron analizados en SPSS Statistic versión 27.0.0 con un ANOVA y prueba de esfericidad de Mauchly. Observando que es factible la producción intensiva de rana toro (*Lithobates catesbeianus*) con agua de reúso de un sistema Biofloc del cultivo de tilapia (*Oreochromis niloticus*).

Palabras claves: producción, sostenibilidad, Biofloc, *Lithobates catesbeianus.*

INTRODUCTION

Global fish production has increased steadily over the past five decades and the supply of edible fish has increased at an average annual rate of 3.2%, thus outpacing the global population growth rate of 1.6%. Per capita consumption internationally increased from an average of 9.9 kg in 1960 to 19.2 kg in 2012 (FAO, 2018). Specifically in Mexico, during the last decade the consumption of aquaculture and fishery species has been increasing. Currently, the main aquaculture species in the country are shrimp (150 thousand 76 tons), mojarra tilapia (149 thousand 54 tons), oyster (45 thousand 148 tons), carp (30 thousand 300 tons) and trout (7 thousand tons) (CONAPESCA, 2018).

Regarding the production and market of bullfrog (*Lithobates catesbeianus*), statistics are scarce. Even so, the Food and Agriculture Organization of the United Nations (FAO, 2018) reports that in the year 1980, it was estimated that 3% of the global frog market (all species) was supplied by aquaculture; while the contribution for the year 2002 was estimated at 15% (taking into account the calculated growth rate of the industry). Taiwan, Brazil and Mexico are the main producers of live frogs (capture and aquaculture). Some documented statistics place the United States of America as the largest consumer of frogs, followed by France and Canada; with three main market niches: frog legs, live frogs and frogs for educational and scientific needs (FAO, 2009)

In Mexico, bullfrog (*Lithobates catesbeianus*) production is led by the Mexico State of, followed by Sinaloa, Nayarit and Jalisco with 35 animal production units, with an average of 60 hectares of surface area used (INAPESCA, 2018). The main aquaculture production systems used in the country are extensive (farming in reservoirs with minimal human intervention after planting and low yields), semi-intensive (farming in ponds, pens and bodies of water) and intensive (farming in controlled systems, ponds, cages, fast-flow channels or water recirculation and reconditioning systems) (INAPESCA, 2018).

While, the demand for live frogs for food has increased, it is expected that conducting research on nutrition, pathology and reproduction will lead to significant improvements that will boost their production. As well as an increase in market prices, because as the trade and capture of wild frogs is restricted, their cultivation increases; however, improvements must be made in marketing, since frog meat and its qualities are far from being widely known (FAO, 2009).

To face these challenges, innovative technological options are emerging that improve production efficiency, in favor of environmental, economic and social sustainability. For although we know that freshwater is a fundamental requirement for aquaculture, it is necessary to recognize that reserves are finite in arid regions and water is scarce, generating competition between productive sectors for the distribution of water resources (Neto & Ostrensky, 2015). Thus, the use of non-conventional water resources in aquaculture is identified as a potential mechanism to improve food production yields while preserving non-renewable and renewable freshwater resources (Corner *et al.,* 2020).

In response to this problem, Biofloc technology emerges as an alternative for water reuse at industrial levels, with a positive impact on the environment (Mancipe *et al.,* 2019) as its application can be carried out in integrated production systems (Bossier & Ekasari, 2017). In the production of aquaculture alternative species, the implementation of a Biofloc system means a reduction of more than 50% of the water footprint involved in production; in addition to creating a positive effect on animal health (Bossier & Ekasari, 2017).

The implementation of such technology in the aquaculture area is based on the creation of a microbiome that reuses organic fish waste and unused feed; creating flocs of bacterial aggregates large enough to be detected by fish and feed them; these microbiota aggregates usually contain protein percentages of up to 27.5% and 7.5% lipids (Ekasari *et al*., 2014). Being so, these protein and energy levels can even be compared to the quality of commercial feed for production fish.

Considering that microorganisms are an essential part of aquifer ecosystems, their role in nutrient recycling is essential in the trophic chain of systems. For decades they have been used as prebiotics and immunostimulants, for disease control, as well as water quality improvers in aquaculture production ponds (Martínez *et al*., 2017). Microbial-based systems represent one of the most viable strategies to achieve sustainable aquaculture, as these systems are based on the promotion of microbial proliferation; expecting these to use, recycle and transform excess nutrients from feces, dead organisms, uneaten food and various metabolites into biomass; in addition to displacing pathogenic organisms in production systems (Martínez *et al*., 2015; Huerta *et al*., 2019).

Besides taking into account that Biofloc system application in aquaculture production achieves a nitrification process, this happens through the carbohydrate source that is added to ponds, since it allows bacteria and microorganisms to convert organic waste from feces and wasted feed; decreasing the amount of ammonium, improving water quality and allowing it to be practically eternal in ponds (Wei *et al*., 2016).

Therefore, the aim of this study was to measure the effect of using reuse water from a Biofloc system for tilapia (*Oreochromis niloticus*) culture in the intensive production of bullfrogs (*Lithobates catesbeianus*), as an alternative use for arid and semi-arid zones from Mexico.

MATERIAL AND METHODS

Study area: the study was conducted at the Bullfrog Reproduction, Research and Technology Transfer Center "El Chaveño" in agreement to the Autonomous University of Aguascalientes; located in Jesús María, Aguascalientes, Mexico; with an average annual temperature of 17 ºC, average annual rainfall of 531 mm and located at 1,880 m a.s.l. (INEGI, 2021).

Biological material: a total of 4,000 bullfrogs (*Lithobates catesbeiana*), with an average initial weight of 49.8 grams/organism, distributed in 40 pens with a semi-flooded system with an effective volume of 400 L, were used.

Experimental design: the experiment was established under a completely randomized design of 4 treatments with 10 replications, obtaining a total of 40 experimental units. Each experimental unit consisted of 100 bullfrogs (*Lithobates catesbeiana*).

Treatments evaluated: the treatments evaluated were as follows: T1: culture system with weekly 100% fresh water replacement and bottom cleaning. T2: culture system with 30% reuse water from a tilapia (*Oreochromis niloticus*) Biofloc system and 70% potable water, without water replacement and with the addition of unrefined sugar as a carbon source in a C:N ratio of 15:1. T3: culture system with 60% reuse water from a tilapia (*Oreochromis niloticus*) Biofloc system and 40% potable water, without water exchange and with the addition of unrefined sugar as a carbon source at a C:N ratio of 15:1. T4: culture system with 90% reuse water from a tilapia (*Oreochromis niloticus*) Biofloc system and 10% potable water, without water replacement and with the addition of unrefined sugar as a carbon source at a C:N ratio of 15:1.

Production system: the production system used in the study was of the semi-flooded type with uniform confinement surfaces of 8 m^2, the floodable capacity of each pond was 0.4 m^3, with a dry area of 0.4 m^2 with feeding in the dry area of the floor. With light lamps distributed in the aquaculture production unit to maintain a photoperiod of 14 hours of light

(10 hours of darkness), with a temperature between 28 - 42 °C in a 24 hours cycle and a constant water temperature between 26-28 °C. Ambient humidity was maintained at 95-98% using water sprinklers. The study period was 15 weeks, August - November 2020.

Diets and feeding: an isoproteic and isocaloric diet was used (Rincón *et al.,* 2012), based on commercial feed for trout (*Salmo trutta*) and catfish (*Ictalurus punctatus*) Nutripec Purina® brand with 40% crude protein and 9% fat for the development stage. The amount of ration was supplied once a day (SENASICA, 2016) and it was calculated based on the biomass at a feeding rate of 6% maintained during the experimental period and adjusted 20 days after the experiment starting to 3% of the biomass. For the determination of weight gain, the total weight of bullfrogs was recorded at the beginning of the experiment and weekly with a digital scale with a sensitivity of 0.1g (303D, DESEGO, Mexico).

Zootechnical parameters evaluated: the variables evaluated were: Weight Gain (WG) with the formula WG= FWIW, where FW is Final Weight and IW is Initial Weight. The Specific Growth Rate (SGR) was calculated with the formula SGR (%) = (Ln) (Fw)-Ln (Iw)/tx100; where: Fw and Iw are Final weight and Initial weight, t is time1 and Ln is the natural logarithm of weights. The percentage of Survival (%S) at the period end was calculated with the formula %S= final organism No/initial organism No x 100. Survival Rate (SR) and Feed Conversion (FC) obtained from the ratio between consumed feed and biomass at the end of the experimental period (Gutiérrez *et al*., 2016).

Water quality: during the study, water quality remained within parameters established for bullfrogs (*Lithobates catesbeiana*) in intensive production (SENASICA, 2016). The physicochemical parameters evaluated weekly were: temperature (T °C), conductivity (µs), pH and ammonium (mg/l); being taken with multiprobe (556 MPS, YSI, USA). While hardness (mg/l $CaCO_3$) and alkalinity (mg/l $CaCO_3$) were taken with a test kit (FF-1A, HACH, Germany), as described by Plazas & Paz, (2019).

Bacterial flocs: for the establishment of bacterial flocs in tilapia (*Oreochromis niloticus*) culture, it was inoculated with leachate from Californian red worm (*Eisenia foetida*) litter; for which 3 L of leachate was used for every 10m^3 of water in tanks 1L/10m^3 of nitrifying bacteria for fish of the PondPerfect 4in brand. For Biofloc establishment, unrefined sugar was used at a rate of 0.02 g/L to ensure a C source and 5 mg/L ammonium chloride (NH_4Cl) as an N source; in addition to 2 g/L sea salt and 50 g/L sodium bicarbonate ($NaHCO_3$) to ensure an initial source of alkalinity for the bacteria according to the methodology of Luo *et al.,* (2014). Unrefined sugar continued to be added every two days according to the Biofloc volume measured in the Imhoff cones.

Statistical analysis: IBM SPSS Statistic version 27.0.0 was used. In each experience the hypothesis of "the reuse of Biofloc water affects the productive parameters of bullfrog (*Lithobates catesbeiana*)" was assessed by means of variance analysis (ANOVA) with a confidence level of 95% (Ducoing, 2019), applying a Mauchly's test of sphericity Mauchly, (1940). When this null hypothesis was rejected, a Greenhouse-Geisser or Huynh-Feldt test of adjustment was used (Bardera, 2019). When significant overall effects were found, simple effects tests were performed followed by post hoc tests. Post hoc analyses were performed using Tukey's test to analyze differences between treatments with different percentage of reused water from a Biofloc system and drinking water, using Bonferroni pairwise comparisons to test for differences between the behaviors analyzed (Bardera, 2019).

RESULTS AND DISCUSSION

The mean of the average water quality parameters recorded during the study were: temperature 20°C, conductivity 0.4µs, pH 7.2, ammonium 1.19 mg/l, hardness 46 mg/l $CaCO_3$ and alkalinity 40 mg/l $CaCO_3$.

Regarding the study variables, results show statistical difference of means between treatments; however, Mauchly's test of sphericity is rejected as it does not show significance. Due to the violation of sphericity, the Greenhouse-Geisser or Huynh-Feldt corrections were applied; which shows significance among treatments in the variables biomass weight, average weight and feed conversion. In turn, in the post hoc test, the difference between treatments under the Bonferroni test showed significant differences for the use of Biofloc 30, 60, and 90% in the variables biomass weight, feed intake and feed conversion, respectively.

Weight gain (WG) behavior (Table 1) showed to be statistically better in the wastewater-based treatments of a Biofloc system; however, this may be related to a higher mortality observed in T1 (drinking water). The estimation of the average weight of marginal means shows no significant differences, suggesting that the average weight of the bullfrog (*Lithobates catesbeiana*) is not altered with the use of different percentages of Biofloc in the water, as growth rates and weight gain are estimated to be similar in treatments.

The effect on feed consumption (Table 2) is significantly higher in the Biofloc treatments, compared to organisms that received only drinking water; these results correlate with those obtained on feed conversion (Table 3), where a better effect is observed in the Biofloc wastewater-based treatments. The estimates of marginal means for feed consumption show significant differences in T3 (60% Biofloc) and T4 (90% Biofloc), showing a relationship with the higher weight gain observed.

Table 1. Effect of the different treatments on weight gain (WG).

Variable			Differences between means (I-J)	Standard Error	Confidence Interval 95%	
					Inferior Limit	Superior Limit
Weight Gain	T1 (drinking water)	30% Biofloc	**-2.2900***	.08809	-2.5965	-1.9836
		60% Biofloc	**-2.6011***	.08809	-2.9076	-2.2947
		90% Biofloc	**-2.3919***	.08809	-2.6984	-2.0855
	T2 (30% Biofloc)	Drinking water	**2.2900***	.08809	1.9836	2.5965
		60% Biofloc	**-.3111***	.08809	-.6176	-.0047
		90% Biofloc	-.1019	.08809	-.4084	.2046
	T3 (60% Biofloc)	Drinking water	**2.6011***	.08809	2.2947	2.9076
		30% Biofloc	**.3111***	.08809	.0047	.6176
		90% Biofloc	.2092	.08809	-.0973	.5157
	T4 (90% Biofloc)	Drinking water	**2.3919***	.08809	2.0855	2.6984
		30% Biofloc	.1019	.08809	-.2046	.4084
		60% Biofloc	-.2092	.08809	-.5157	.0973

According to the observed means, Mean Squared Error (Error) = .002.
* Differences between means show a significance level of 0.05.

Table 2. Effect of treatments on feed intake

Variable			Differences between means (I-J)	Standard Error	Confidence Interval 95%	
					Inferior Limit	Superior Limit
Food consumption	T1 (drinking water)	30% Biofloc	**-524.6222***	44.71786	-680.1903	-369.0542
		60% Biofloc	**-642.3333***	44.71786	-797.9014	-486.7653
		90% Biofloc	**-627.7778***	44.71786	-783.3458	-472.2097
	T2 (30% Biofloc)	Drinking water	**524.6222***	44.71786	369.0542	680.1903
		60% Biofloc	-117.7111	44.71786	-273.2792	37.8569
		90% Biofloc	-103.1556	44.71786	-258.7236	52.4125
	T3 (60% Biofloc)	Drinking water	**642.3333***	44.71786	486.7653	797.9014
		30% Biofloc	117.7111	44.71786	-37.8569	273.2792
		90% Biofloc	14.5556	44.71786	-141.0125	170.1236
	T4 (90% Biofloc)	Drinking water	**627.7778***	44.71786	472.2097	783.3458
		30% Biofloc	103.1556	44.71786	-52.4125	258.7236
		60% Biofloc	-14.5556	44.71786	-170.1236	141.0125

According to the observed means, Mean Squared Error (Error) = .002.
* Differences between means show a significance level of 0.05.

Regarding feed conversion, it is observed (Table 3 and Figure 1) that the organisms of T1 (drinking water) show lower feed conversion than those of T4 (90% Biofloc) during the first weeks of the study; however, after week 8 the conversions are equalized, ending without significant differences by week 15 of the study. It is important to highlight that from week 4 onwards, feed consumption begins to be higher in T2 (30% Biofloc), T3 (60% Biofloc) and T4 (90% Biofloc); this is the effect of the lower mortality and the greater number of surviving individuals and better assimilation of nutrients.

Table 3. Effect of treatments on feed conversion

Variable			Differences between Means (I-J)	Standard Error	Confidence Interval 95%	
					Inferior Limit	Superior Limit
Feed conversion	T1 (Drinking water)	30% Biofloc	**.2369***	.03918	.1006	.3732
		60% Biofloc	**.2462***	.03918	.1099	.3825
		90% Biofloc	**.2208***	.03918	.0845	.3571
	T2 (30% Biofloc)	Drinking water	**-.2369***	.03918	-.3732	-.1006
		60% Biofloc	.0093	.03918	-.1270	.1456
		90% Biofloc	-.0161	.03918	-.1524	.1202
	T3 (60% Biofloc)	Drinking water	**-.2462***	.03918	-.3825	-.1099
		30% Biofloc	-.0093	.03918	-.1456	.1270
		90% Biofloc	-.0253	.03918	-.1616	.1110
	T4 (90% Biofloc)	Drinking water	**-.2208***	.03918	-.3571	-.0845
		30% Biofloc	.0161	.03918	-.1202	.1524
		60% Biofloc	.0253	.03918	-.1110	.1616

According to the observed means, Mean Squared Error (Error) = .002.
* Differences between means show a significance level of 0.05.

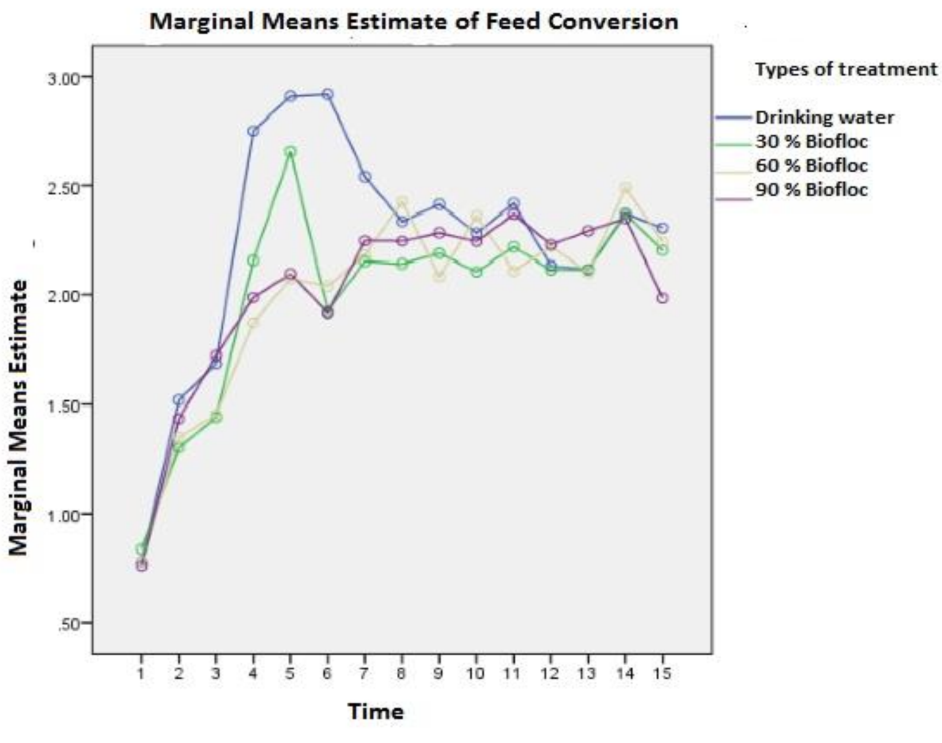

Figure 1. Feed conversion performance by treatment

Several studies have demonstrated more efficient diet and nutrient assimilation in systems where Biofloc is used (Figure. 1). Da Silva *et al.* (2013), found that the application of Biofloc technology in the intensive culture of Pacific white shrimp (*Litopenaeus vannamei*) significantly improves the improved efficiency of N and P utilization by up to 70 and 66%, respectively, in relation to conventional intensive culture systems with regular water exchange. Authors such as Mercante *et al*., (2014) have described that high levels of phosphorus and nitrogen in the water of intensive bullfrog (*Lithobates catesbeiana*) production ponds decrease water quality parameters and interfere with productivity; these same effects have been found in the use of Biofloc in tilapia (*Oreochromis niloticus*) culture (Schveitzer *et al*., 2013; Widanarni *et al.,* 2012).

While Table 4 shows a significant difference between means in the specific growth rate in T2 (30% Biofloc) with respect to T1 (drinking water); in the same way this effect is observed in T3 (60% Biofloc). With respect to the effect of treatments on the survival rate (Table 5), the difference between means of the variables T1 (drinking water), compared to organisms of the other treatments, is observed.

Table 4. Effect of treatments on specific growth rate

Variable			Differences between Means (I-J))	Standard Error	Confidence Interval 95%	
					Inferior Limit	Superior Limit
Specific growth rate per treatment	T1 (drinking water)	30% Biofloc	-.1850*	.02100	-.2581	-.1120
		60% Biofloc	-.2048*	.02100	-.2778	-.1318
		90% Biofloc	-.1940*	.02100	-.2670	-.1210
	T2 (30% Biofloc)	Drinking water	.1850*	.02100	.1120	.2581
		60% Biofloc	-.0198	.02100	-.0928	.0533
		90% Biofloc	-.0090	.02100	-.0820	.0641
	T3 (60% Biofloc)	Drinking water	.2048*	.02100	.1318	.2778
		30% Biofloc	.0198	.02100	-.0533	.0928
		90% Biofloc	.0108	.02100	-.0622	.0838
	T4 (90% Biofloc)	Drinking water	.1940*	.02100	.1210	.2670
		30% Biofloc	.0090	.02100	-.0641	.0820
		60% Biofloc	-.0108	.02100	-.0838	.0622

According to the observed means, Mean Squared Error (Error) = .002.
* Differences between means show a significance level of 0.05.

The specific growth rate of the organisms of T2 (30% Biofloc), T3 (60% Biofloc) and T4 (90% Biofloc) is higher than in the organisms that received drinking water, which suggests that the microbial diversity in the water has a beneficial effect on the growth and development of this species under intensive production conditions; this effect coincides with the results observed in the survival rate of this study. These results suggest that it is feasible to use water from intensive tilapia (*Oreochromis niloticus*) farming for reuse in the intensive production of bullfrogs (*Lithobates catesbeianus*), since the microbial quality existing in the medium benefits interactions with pathogenic microorganisms, decreasing mortality in frogs that receive reuse water in different proportions as happens in other aquaculture species (Vinatea *et al*., 2018).

Table 5. Effect of treatments on survival rate

Variable			Differences between Means (I-J)	Standard error	Confidence Interval 95%	
					Inferior Limit	Superior Limit
Survival rate	T1 (drinking water)	30% Biofloc	-11.3333	3.80058	-24.5551	1.8884
		60% Biofloc	-13.6667*	3.80058	-26.8884	-.4449
		90% Biofloc	-12.0000	3.80058	-25.2218	1.2218
	T2 (30% Biofloc)	Drinking water	11.3333	3.80058	-1.8884	24.5551
		60% Biofloc	-2.3333	3.80058	-15.5551	10.8884
		90% Biofloc	-.6667	3.80058	-13.8884	12.5551
	T3 (60% Biofloc)	Drinking water	13.6667*	3.80058	.4449	26.8884
		30% Biofloc	2.3333	3.80058	-10.8884	15.5551
		90% Biofloc	1.6667	3.80058	-11.5551	14.8884
	T4 (90% Biofloc)	Drinking water	12.0000	3.80058	-1.2218	25.2218
		30% Biofloc	.6667	3.80058	-12.5551	13.8884
		60% Biofloc	-1.6667	3.80058	-14.8884	11.5551

According to the observed means, Mean Squared Error (Error) = .002.
* Differences between means show a significance level of 0.05.

It was observed that survival was similar among treatments evaluated, highlighting that in T3 (60% Biofloc) the organisms showed a better survival rate. Results suggest that the great diversity of organisms present in the reused water of a Bioflc system, exert a competition with potential pathogenic microorganisms that attack frogs; this effect has been observed in aquaculture cultures using a Biofloc system (Martinez *et al*., 2016; Ekasari *et al*., 2014). Suggesting that this effect creates a competition of potential pathogenic organisms, reducing their proliferation in the experimental ponds as well as in the digestive tract of the fish (Manduca *et al*., 2021).

Published studies show that the autochthonous microbiota of the skin and gastrointestinal tract could be affected by many factors, such as microbial interactions, water flows, husbandry, techniques and disinfection; which could alter the balance of microbial ecosystems. These aspects, together with the stress produced by overcrowding, can overcome immune barriers, causing microbial microorganisms to attack, leading to outbreaks of infectious diseases (Mauel *et al*., 2002); providing bullfrogs (*Lithobates catesbeiana*) with a microbial environment rich in beneficial microorganisms improves performance in intensive production systems. It has been shown that different strains of Gram (+) as well as Gram (-) lactic acid bacteria isolated from fish cultures have been used for the control of disease-causing bacteria in frogs, such as *Proteus vulgaris*, *Pseudomonas aeruginosa* and *Staphylococcus epidermidis* (Pasteris *et al*., 2009).

On the other hand, Mayorga *et al*., (2015), found that Biofloc was the main food source consumed preferentially by tilapia (*Oreochromis niloticus*) versus balanced feed. Therefore, it is important to highlight that in Mexico, given the availability of feed (Fattening extruded, at 20 and 25% crude protein El Pedregal and Los Belenes), they can be used in Biofloc culture to minimize the impact of feed cost and take advantage of the preference of tilapia (*Oreochromis niloticus*), for biofloc; and thus reduce production costs remain preponderant.

There is a scientific reality that indicates the high nutritional content of bioflocs (Ekasari & Maryam, 2012), an aspect that does not seem to apply in Mexico, since most of them use balanced feed with high levels of protein 45/32/25 respectively. When the feed could be eliminated at 32% and used at 25% to favor the consumption of microbial flocs that are preferred by tilapia. Finally, Martínez *et al.,* (2017), argue that global evidence supports the hypothesis that the use of microorganisms as a direct feed source in aquaculture will revolutionize the industry, closing the gap towards sustainability.

CONCLUSION

The intensive production of bullfrogs (*Lithobates catesbeianus*) with reuse water from a Biofloc system for tilapia (*Oreochromis niloticus*) culture is feasible, since variables evaluated, weight gain, specific growth rate and survival; as well as feed conversion in bullfrogs (Lithobates catesbeianus), showed a positive statistical difference in relation to aquaculture production with potable water reuse, being an option for the efficient use of water resources in arid and semi-arid zones of Mexico.

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