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Effects of the addition of oak (*Quercus robur* L.) and yucca (*Yucca schidigera*) on the water quality and growth performance of pacific white shrimp (*Litopenaeus vannamei*) cultured intensively in concrete tanks

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Abstract

The application of mixed natural extracts from oak (*Quercus robur* L.) and yucca (*Yucca Schidigera*) (OY) in an intensive culture system for Pacific white shrimp (*Litopenaeus vannamei*) was able to improve the growth performance of shrimp and the quality of its rearing media. Twelve tanks sized $8 \times 8 \times 1.3$ m were stocked with 500 post-larvae m^{-2} per tank and treated with 2, 2.5, and 3 Kg Ha^{-1} of OY. Physical and chemical water parameters, including pH, dissolved oxygen, temperature, and salinity, were measured 4 times day^{-1} during the trial. Measurements of other chemical parameters, including Ammonia (NH_3-N), Nitrite (NO_2-N), and Nitrate (NO_3-N), were performed the second and sixth day after the addition of OY. The same measurements were conducted in the control treatment every seventh-day post water exchange during the 90 days of the culture period. The growth performance parameters (biomass, final body weight, feed conversion ratio, survival rate, and percentage weight gain) and nutrient retention rate were evaluated after 90 days of shrimp culture. The utilization of OY significantly increased the final body weight

(FBW), percentage weight gain (PWG), feed conversion ratio (FCR), survival rate (%), and the final biomass of shrimp. Different dose applications of OY significantly reduced the accumulation of ammonia (NH_3-N), nitrite (NO_2-N), and nitrate (NO_3) in the rearing media compared to the control. However, there were no significant differences in protein, fat, ash, dry fiber, and water content among the shrimp treated with different addition levels of OY. This research concludes that the application of OY could maintain a safe level of ammonia and other nitrogen metabolites in the rearing media and enhance the growth performance of shrimp cultured in intensive production systems using commercial concrete tanks.

KEYWORDS
growth, intensive, *Litopenaeus vannamei*, oak, water quality, yucca

1 | INTRODUCTION

The toxicity of concentrated nitrogen compounds, especially ammonia, has become one of the major limiting factors of successful shrimp aquaculture production systems (Frias-Espericueta, Harfush-Melendez, & Páez-Osuna, 2000; Rostami, Davoodi, Nafisi Bahabadi, Salehi, & Nooryazdan, 2019). Ammonia in shrimp culture media could come from the ammonification of organic matter (Chen, Liu, & Lei, 1990), the excretion from aquatic organisms as the end product of protein metabolism (Walsh & Wright, 1995), the decomposition of organic nitrogen in feces, and uneaten feed (Avnimelech & Ritvo, 2003). In intensive recirculating systems, a biofilter or bioreactor as well as the application of probiotics become the common way to biologically remove ammonia (Ebeling, Timmons, & Bisogni, 2006; Farizky, Satyantini, & Nindarwi, 2020; Rajasekar et al., 2020). In addition, when using concrete tanks, graded daily water exchange adjusted to the amount of feed given has been successfully applied to maintain the ammonia level within the acceptable range for shrimp *Litopenaeus vannamei* (Novriadi et al., 2021). However, the complexity of the biological application and high operational costs of water exchange and bioreactor systems are only economically feasible for small and highly controlled shrimp production systems (Santacruz-Reyes & Chien, 2012). Therefore, the use of natural substances to control the level of ammonia in large intensive shrimp production systems could be an alternative approach.

Saponins can bind ammonia (Makkar, Francis, & Becker, 2007). They are commonly found in plants and contain either a steroid or a triterpenoid aglycone, to which one or more sugar chains are attached (Oda et al., 2000). In aquaculture, the most commonly used sources of saponins are yucca (*Yucca schidigera)* and quillaja (*Quillaja Saponaria*). However, the majority of studies on the application of saponins in aquaculture have focused on their use as natural growth promoters when included in feeds for fish and shrimp (Francis, Makkar, & Becker, 2002; Kelly & Kohler, 2003; Paray, Hoseini, Hoseinifar, & Van Doan, 2020; Serrano, Focken, Francis, Makkar, & Becker, 2000; Yang, Tan, Dong, Chi, & Liu, 2015). Only a few authors have assessed their potential benefits when applied directly in the tanks or ponds, in order to reduce the concentration of ammonia within the culture environment (Castillo-Vargasmachuca et al., 2015; Fayed et al., 2019; Khalil, Saad, Ragab, & Mohammed, 2015; Santacruz-Reyes & Chien, 2010, 2012). Oak (*Quercus robur*) is known to have a high content of bioactive compounds (Burlacu, Nisca, &

Tanase, 2020). Its bark is rich in polyphenols, and secondary metabolites such as triterpenoids and their derivatives can also be extracted from it (Morales, 2021; Perez et al., 2017).

Studies on the use of oak in animal production are limited (Focant, Froidmont, Archambeau, Van, & Larondelle, 2019; Morales, 2021; Paray et al., 2020). To the best of our knowledge, the study by Paray et al. (2020) was the only example of the application of oak in aquaculture. The authors reported that the inclusion of an oak (*Quercus castaneifolia*) leaf extract in diets for common carp (*Cyprinus carpio*) had antioxidant, antibacterial, and immunostimulant effects. There are no studies on the assessment of the effects of oak as an ammonia binder in aquaculture. Yucca (*Yucca schidigera)* has been used in livestock production systems to control the accumulation of ammonia (Cheeke & Otero, 2005). The plant extract, rich in sarsaponin, has shown beneficial effects, such as an antiprotozoal activity and the ability to trap ammonia and improve the feeding value of low-quality roughage (Cheok, Salman, & Sulaiman, 2014). The extract can also reduce ammonia levels in aquatic environments (Santacruz-Reyes & Chien, 2012). Wallace, Arthaud, and Newbold (1994), in a study in ruminants, reported that the binding capacity of yucca at an ammonia level of 0.4 mM was about 2 μmol of ammonia per ml of yucca extract, and led to a 6% decrease in the ammonia concentration. Furthermore, a study from Castillo-Vargasmachuca et al. (2015) demonstrated that 0.75 mg L^{-1} of yucca extract was recommended to reduce the ammonia concentration in marine water during the acclimatization of red snapper (*Lutjanus peru*). These results imply that the application of yucca offers a promising sustainable solution to reduce ammonia pollution from intensive culture systems.

Studies regarding the supplementation of yucca in aquafeed formulations unanimously reported improvements in the growth performance of several fish species. Fayed et al. (2019) suggested that the yucca-supplemented feed significantly increased the final weight, weight gain, and specific growth rate of European seabass (*Dicentrarchus labrax*) fingerlings fed with 0.5 and 1 g Kg^{-1} of yucca, compared to the control. Moreover, Nile tilapia (*Oreochromis niloticus*, L) fingerlings fed with feed supplemented with 0.075% yucca extract showed excellent growth rates (Gaber, 2006). In Pacific white shrimp (*Litopenaeus vannamei*), Yang et al. (2015) reported beneficial effects from the supplementation of 0.2% yucca extract on the growth and nonspecific immunity of the animals, as well as improved the rearing water quality condition. The inclusion of yucca in combination with quillaja extracts showed potential benefits as a feed additive for shrimp cultured at low-salinity (Hernández-Acosta et al., 2016). Despite their rigorous results, most of these researches were done under a controlled shrimp rearing environment. There is limited information available regarding the application of yucca within commercial intensive culture systems.

Therefore, the present study was conducted to determine the effects of combining oak and yucca on the reduction of ammonia levels in the Pacific white shrimp culture environment. The growth performance and protein retention of the shrimp cultured in intensive concrete tank systems were also determined.

2 | MATERIALS AND METHODS

2.1 | Ammonia binding product

Sapotan Powder™ (OY, Tanin Sevnica, Slovenia) is a commercial mixture of oak and yucca in powder form, containing polyphenols, triterpenoid and steroidal saponins, and crude fibers. This product is a commercial additive used in aquaculture.

2.2 | Experimental conditions

The trial was performed at the Batam Dae Hae Seng Indonesia (Batam, Riau Island province, Indonesia) and lasted for 90 days. Post-larvae of Pacific white shrimp (PL8, weighing 0.03–0.05 g) were obtained from PT Prima

Akuakultur Lestari (PAL, Rajabasa, Lampung, Indonesia) and tested negative for WSSV, TSV, AHPND, YHD, and bacterial infection. The shrimp were stocked in 12 semi-indoor concrete tanks (8 × 8 × 1.38 m), with a density of 500 post larvae (PL) m^{-2} arranged in a completely randomized design. The four treatments consisted of the addition of 2 Kg ha^{-1} (OY-1), 2.5 Kg ha^{-1} (OY-2), 3 Kg ha^{-1} (OY-3) of the commercial product (OY) and a control treatment with no addition of the commercial product (OY-0) in the shrimp rearing media. Each treatment was applied in triplicate tanks. OY was applied by dispersing it on the water surface of the corresponding tanks. Daily water replenishment at a 2–3% rate was applied to all the tanks to compensate for water loss via evaporation and daily siphoning activities to collect feces, uneaten feed, and dead shrimp. The addition of OY was done every 7 days during the culture period. Weekly water exchange of 30–40% was applied in the control group tanks parallel to the addition of OY in the other treatment groups. The measurement of Ammonia (NH_3-N), Nitrite (NO_2-N), and Nitrate (NO_3-N) was performed on the second and sixth days after each addition of OY. The primary source of mechanical aeration was an air disc fine bubble diffuser working in tandem with a 0.5 HP paddlewheel (Minipadd™) per tank as an additional aeration system. Water exchange was set 5–10% throughout the 90 days trial.

2.3 | Feed management

Shrimp in all the tanks were fed with the same commercial diet (33–35% crude protein and 5% crude lipids - Evergreen Feed, Indonesia Evergreen Agriculture, Lampung Selatan, Indonesia) for 90 days. The amount of feed used in this experiment was calculated based on the expected weight gain of 1 g/week, a feed conversion ratio (FCR) of 1.4, and a weekly mortality of 3% during the grow-out period. During the trial, shrimp were fed six times per day, and the daily ration was adjusted based on the percentage of body weight after the weekly sampling of the shrimp.

2.4 | Growth sampling, water quality, and total bacteria analysis

Shrimp were sampled weekly throughout the production cycle using a hand net (0.5 m in diameter and 1 cm mesh size) to collect approximately 20–30 individuals per tank. Water quality (DO, pH, temperature, salinity, total dissolved solids, conductivity, and oxidative redox potential) was monitored four times/day (06.00–07.00 a.m.; 2.00–3.00 p.m.; 5.00–6.00 p.m., and 11.00–12.00 p.m.) using real-time water quality sensors (Aqua Troll 500, In-Situ Inc., Fort Collins, CO, USA). The water quality data were stored, processed, displayed in real-time using AquaEasy Smart Aquaculture apps (BOSCH, Singapore) available both in Android and iOS operating systems. Secchi disk readings were recorded once a week. Ammonia nitrogen (NH_3-N) was analyzed with an ultraviolet/visible spectrophotometer (PerkinElmer, Lambda XLS, USA) once a week (Table 1). Nitrite nitrogen (NO_2-N) and nitrate-nitrogen (NO_3-N) were analyzed using a HACH DR890 colorimeter (Hach Company, Love- land, CO, USA) twice a week (Table 2). At the end of the growth trial, shrimp were entirely harvested, counted, and batch-weighed to calculate the final biomass, final body weight (FBW), percentage weight gain (PWG), FCR, survival, and Protein Retention Ratio (PRR), shown in Table 2.

$$\text{PWG}\,(\%) = [\text{average individual final weight}\,(\text{g}) - \text{average individual initial weight}\,(\text{g})] \div [\text{average individual initial weight}\,(\text{g})] \times 100$$

$$\text{FCR} = \text{feed given}\,(\text{g}) \div \text{alive weight gain}\,(\text{g})$$

$$\text{Survival rate}\,(\%) = (\text{final number of shrimp} \div \text{initial number of shrimp}) \times 100$$

$$\text{PRR}\,(\%) = [(\text{final weight of shrimp} \times \text{percent final protein} - \text{initial weight} \times \text{percent initial protein}) \div \text{total protein intake}\,(\text{dry matter})] \times 100$$

TABLE 1 Water quality of Pacific white shrimp (*Litopenaeus vannamei*) cultured intensively using concrete tanks treated with three different doses of the commercial product (OY, Tanin Sevnica, Slovenia)

Treatment[a]					
Parameter	**OY-0**	**OY-1**	**OY-2**	**OY-3**	***p*-value**
pH	7.82 ± 0.31	7.85 ± 0.28	7.79 ± 0.38	7.81 ± 0.34	
Salinity (‰)	32.3 ± 1.08	32.1 ± 0.94	32.2 ± 1.04	32.2 ± 0.98	
Temperature (°C)	28.9 ± 2.04	29.1 ± 1.88	28.9 ± 1.95	29.0 ± 1.93	
Dissolved oxygen (mg L^{-1})	5.67 ± 0.77	5.72 ± 0.82	5.69 ± 0.76	5.75 ± 0.92	
Ammonia - NH_3-N (mg L^{-1})	0.0392 ± 0.0115[a]	0.0223 ± 0.0129[b]	0.0190 ± 0.0049[b]	0.0165 ± 0.0058[b]	<0.0001
Nitrate – NO_3-N (mg L^{-1})	5.4978 ± 0.1506[a]	3.4377 ± 0.1550[b]	3.3148 ± 0.1041[b]	3.1153 ± 0.11115[b]	<0.0001
Nitrite – NO_2-N (mg L^{-1})	0.4863 ± 0.0406[a]	0.3097 ± 0.0114[b]	0.2878 ± 0.0249[b]	0.2656 ± 0.0486[b]	<0.0001

Note: Values are the means ± sd of three replicates per dose of OY. Results in a row with different superscript letter are significantly different ($p < 0.05$) based on analysis of variance followed by the Tukey's multiple comparison test.
[a]OY-0 = no treatment applied. Weekly water exchange at a rate of 30–40%; OY-1 = treated with the commercial product OY (2 kg Ha^{-1}); OY-2 = treated with the commercial product OY (2,5 kg Ha^{-1}); OY-3 = treated with the commercial product, OY (3 kg Ha^{-1}).

TABLE 2 Growth performance of Pacific white shrimp (*Litopenaeus vannamei*) (PL8, initial mean weight of 0.03–0.05 g) treated with three different doses of the commercial product (OY, Tanin Sevnica, Slovenia) for 90 days

	Treatment[a]					
Items	**OY-1**	**OY-2**	**OY-3**	**OY-0**	***p*-value**	**PSE[b]**
FBW[c] (g)	10.27[b]	10.57[a]	10.13[b]	9.63[c]	<0.0001	0.0333
PWG[d] (%)	34122[b]	35122[a]	33677[b]	32011[c]	<0.0001	111.0001
FCR[e]	1.50[b]	1.41[c]	1.55[b]	1.71[a]	<0.0001	0.0140
Survival (%)	75.76[ab]	78.12[a]	74.01[bc]	70.94[c]	0.0017	0.7993
Biomass (kg)	233.33[b]	247.67[a]	225.00[b]	205.00[c]	<0.0001	2.1344
PRR[f]	568.395	653.802	553.057	573.298	0.1176	27.6394

Note: Values are the means ± sd of three replicates per dose of OY. Results in a row with different superscript letters are significantly different ($p < 0.05$) based on analysis of variance followed by Tukey's multiple comparison test.
[a]The nomenclature of the treatments is as described in Table 1.
[b]PSE, pooled standard error.
[c]FBW, final body weight.
[d]PWG, percentage weight gain.
[e]FCR, feed conversion ratio.
[f]PRR, protein retention rate.

2.5 | Whole body composition analysis

Upon termination of the trial, four shrimp from each tank, or 12 shrimp per dose of the product, were randomly sampled and stored at −60°C proximate body compositions analysis. Prior to the analysis, dried whole shrimp were chopped and rigorously blended in a mixer according to the standard methods established by the Association of

Official Analytical Chemists (AOAC, 1990). Proximate composition and mineral contents of the whole shrimp body were analyzed at the Fish Nutrition Laboratory, Faculty of Fisheries, and Marine Sciences, Bogor Agricultural University (Bogor, West Java, Indonesia).

2.6 | Statistical analysis

All growth parameters were analyzed using a one-way analysis of variance (ANOVA) to determine the significant differences among the treatments. Data were subjected to a post hoc test using Tukey's multiple comparison tests to determine the difference between the treatment means in each trial. All statistical analyses were conducted using the SAS system (V9.4. SAS Institute, Cary, NC, USA).

3 | RESULTS

3.1 | Water quality of the culture environment

The addition of the commercial product, OY, at three different doses into the culture tanks had a significant effect on the levels of ammonia-nitrogen (NH_3-N), nitrite-nitrogen (NO_2-N), and nitrate-nitrogen (NO_3-N) in the water. As the culture time increased, the levels of ammonia-nitrogen (NH_3-N), nitrite-nitrogen (NO_2-N), and nitrate-nitrogen (NO_3-N) in the tanks treated with 2, 2.5, and 3 Kg Ha^{-1} of OY were significantly lower than those in the control group ($p < 0.0001$). The lowest NH_3-N, NO_2-N and NO_3-N values were found in the tanks treated with OY-3 (3 Kg Ha^{-1} of OY). From the ammonia-nitrogen (NH_3-N) measurement displayed in Figure 1, it is demonstrated that the NH_3-N concentrations in water treated with OY decreased significantly ($p < 0.0001$) compared to the control, during and after the treatment. Levels of NO_2-N, NO_3-N, and NH_3-N in the treatment tanks tended to decrease significantly ($p < 0.0001$) after each cycle addition of OY (every seventh day). This trend was shown during the analysis the second day after the addition of OY extracts. The sixth day, or 1 day before the addition of the product, all nitrogen substances had risen despite being within the acceptable range and lower than that of in the control group. The latter showed a similar trend after the weekly water exchange at a rate of 30–40% the seventh day. The levels of nitrogen decreased the second day and then rose again on the sixth day. However, the levels of all the nitrogen substances in the control tanks were significantly higher ($p < 0.0001$) compared to that of the tanks treated with OY. Other parameters, such as pH, salinity, and dissolved oxygen, were within the acceptable range in all the tanks. In addition, the temperature was relatively stable (± 29°C) in all the tanks throughout the 90 days.

3.2 | Growth performance of shrimp

At the end of the growth trial, the average FBW, PWG, and biomass (kg) were significantly higher in shrimp from OY-treated groups compared to those from the control group ($p < 0.0001$). The FCR of shrimp from groups OY-1, OY-2 and OY-3 were significantly lower ($p < 0.0001$) (1.50, 1.41, and 1.55, respectively) than that of shrimp from the control group (OY-0:1.71), with that of group OY-2 being the lowest ($p < 0.0001$). Based on the quantitative value of the growth parameters, the optimum results were obtained by shrimp reared in the tanks treated with OY-2 (2.5 Kg Ha^{-1} of the commercial product), and slightly decreased in shrimp treated with 3 Kg Ha^{-1} of OY (OY-3). Shrimp reared in OY-treated tanks exhibited a significant increase ($p < 0.005$) in the survival rate (%) (74.01–78.12%), compared to the control group (70.94%).

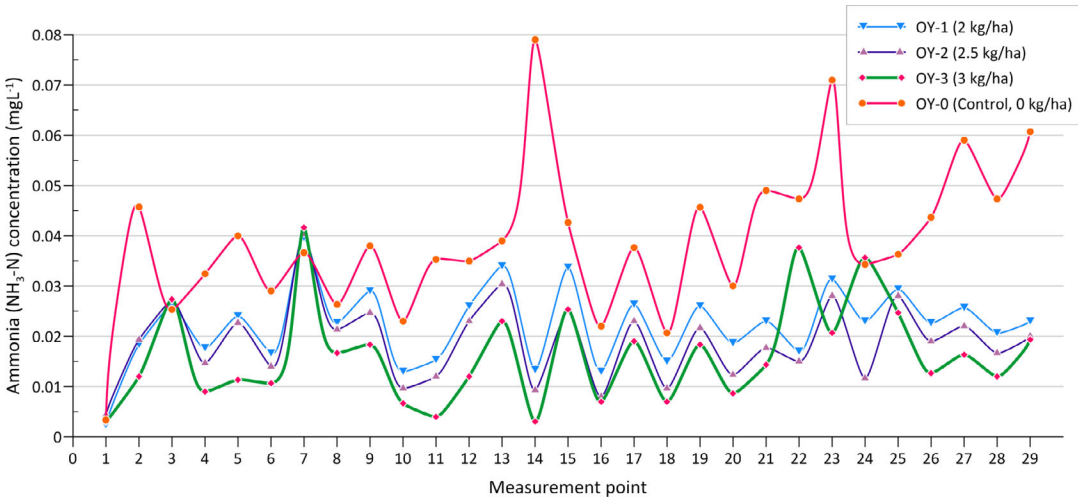

FIGURE 1 Trend of the ammonia (NH_3-N) level in the intensive culture environment of Pacific white shrimp (*Litopenaeus vannamei*) treated with three different doses of the commercial product, OY (Tanin Sevnica, Slovenia). The nomenclature of the treatments is as described in Table 1

3.3 | Whole body composition analysis

The different doses of the commercial product had no significant effect on the water, dry fiber, protein, fat, and ash contents of the whole body of shrimp.

4 | DISCUSSION

Recently, saponins extracted from plants such as yucca and quillaja have been gaining attention due to their benefits as antiprotozoal, growth promoter, and ammonia binding agents in aquaculture and livestock production systems (Adegbeye et al., 2019; Castillo-Vargasmachuca et al., 2015; Chepete, Xin, Mendes, Li, & Bailey, 2012; Hassan, Yusuf, Badran, Griesh, & Zidan, 2017; Matusiak et al., 2016). In the case of Pacific white shrimp, the biological function of yucca extracts was to enhance the rearing water quality, the shrimp growth performance, and the nonspecific immunity, with promising results (Hernández-Acosta et al., 2016; Santacruz-Reyes & Chien, 2012; Yang et al., 2015). Santacruz-Reyes and Chien (2012) demonstrated that the addition of commercial product containing only 30% yucca extract at ratios of 18, 36, and 72 mg L^{-1} in the effluent from a shrimp culture system containing total ammonia nitrogen (TAN) of 0.592, 0.672, and 0.718 mg L^{-1}, was able to reduce TAN by 78.7–99.7% after 12 hr and 88.1–99.7% after 24 hr. In this research, the addition of Sapotan Powder™ containing oak-yucca combination (OY) at 3 Kg Ha^{-1} or 19.2 mg L^{-1} resulted in the lowest concentrations of NH_3-N compared to other treatments. In comparison to the control group, addition of 2 and 2.5 Kg Ha^{-1} of OY or 12.8 and 16 mg L^{-1}, respectively were also able to reduce the NH_3-N concentration level in the culture environment. The underlying mechanisms, whether of yucca alone or in combination with other plant substances, which play a role in reducing the ammonia level in shrimp production systems, have not been fully described. However, several researchers suggest that some components of yucca directly bind ammonia or mediate the conversion of ammonia to nitrite and nitrate and eventually protect the aquatic organisms from the toxicity of waterborne ammonia (Dawood et al., 2021; Kelly & Kohler, 2003; Yang et al., 2015). These processes might be responsible for reducing the ammonia concentration levels within the shrimp culture environment.

Interestingly, our research showed that higher levels of NO_3-N corresponded to higher administration amounts of OY and lower levels of NH_3-N in the culture environment. These patterns indicate that the nitrification process that converts ammonia to nitrite (NO_2-N) and nitrite (NO_2-N) to nitrate (NO_3-N) is likely facilitated by the presence of oak and yucca, resulting in an increase in the level of NO_3-N as the final product. According to Alves Neto, Brandão, Furtado, and Wasielesky Jr (2019), NO_3-N is a relatively less toxic form among the nitrogen compounds for shrimp. It does not cause any health hazard if maintained at a level between 60.05 and 127.61 mg L^{-1} for Pacific white shrimp cultured in 5 and 10 g L^{-1} salinity levels, respectively. Currently, there is no published study evaluating the chronic level of NO_3-N in shrimp intensive culture. However, a study by Furtado et al. (2015) demonstrated that a level of up to 177 mg L^{-1} of NO_3-N is acceptable for rearing Pacific white shrimp with a stocking density of 333 shrimps m^{-3}, at a salinity of 23 g L^{-1}. The reduction of NH_3-N and other nitrogen metabolites in our study indicates that the addition of OY could increase the productivity and carrying capacity of the shrimp culture environment, as well as reduce the amount of pollution released from the culture system.

In our study, the performance parameters (FBW, PWG, FCR, and biomass) of shrimp cultured intensively with a density of 500 PL m^{-2} were better in the groups of shrimp treated with OY than those of the control group. This result is in line with the results from Yang et al. (2015), who reported an increase in FBW and PWG of Pacific white shrimp when using powdered yucca supplemented as part of the diet at a 0.2% per unit feed. Similarly, the dietary administration of yucca in combination with *Quillaja saponaria* enhanced the FBW, PWG. and FCR of shrimp cultured in 140 L fiberglass tanks with a density of 10 shrimp L^{-1} (Hernández-Acosta et al., 2016). In addition, these authors reported that the inclusion of yucca and quillaja in shrimp diet at the level of 1–2 g Kg^{-1} significantly increased the feed utilization efficiency, as shown by the lower FCRs. The ability of triterpenoid and steroidal saponins in dietary quillaja, oak, and yucca to enhance shrimp growth remains unclear. Goetsch and Owens (1985) suggested that steroidal saponins and other bioactive natural substances can change the cell membrane structure of an animal digestive tract epithelial cell. Such change will lead to the reduction of the surface tension and promote the absorption of nutrients. Several authors argue that yucca-supplemented feed can stimulate the active digestive enzymes for a better digestion process of protein and carbohydrates, enhance aerobic metabolism, promote nutrient absorption, and increase protein synthesis. These benefits of yucca compounds are suggested to be responsible for the animals' improved growth (Francis et al., 2002; Serrano et al., 2000). In the present study, the addition of OY up to 3 Kg Ha^{-1} in the rearing media did not cause any adverse effect on the diet palatability and shrimp survival. Based on the quantitative values displayed in Table 2, the optimum results for FCR were obtained by shrimp in the tanks treated with 2.5 Kg Ha^{-1} of the commercial product, being lower in shrimp from the tanks treated with the highest dose of OY (OY-3 - 3 Kg Ha^{-1}). Concerning the survival rate, shrimp exhibited higher survivals with the addition of OY compared to the control group. Conversely, survival rates were lower in the tanks treated with 3 Kg H^{-1} of the commercial product than in those

TABLE 3 Proximate composition of the whole body of Pacific white shrimp (*Litopenaeus vannamei*) treated with three different doses of the commercial product (OY, Tanin Sevnica, Slovenia) for 90 days

	Treatment[a]					
Compositions	**OY-1**	**OY-2**	**OY-3**	**OY-0**	***p*-value**	**PSE[b]**
Proximate composition (g kg^{-1})						
Water	72.670	73.117	72.890	73.393	0.3483	0.2749
Dry fiber	1.670	1.870	1.917	1.880	0.6054	0.1381
Protein	20.120	20.347	20.013	19.887	0.6151	0.2453
Fat	2.020	1.643	2.040	2.060	0.3758	0.1830
Ash	3.013	3.143	3.277	3.140	0.6811	0.1493

Note: Values are the means ± std of three replicates per dose of OY.
[a]The nomenclature of the treatments is as described in Table 1.
[b]PSE, pooled standard error.

treated with 2.5 Kg Ha^{-1}. This indicates that shrimp might be sensitive to the excessive amounts of OY applied in the culture environment. A different situation was observed in the water quality, as a higher administration of OY significantly reduced the NH_3-N level and other nitrogen metabolites within the culture environment. Future research in this area should concentrate on understanding the physiological mechanisms by which the triterpenoid and steroidal saponins contained in oak and yucca, along with the polyphenols, help improve shrimp growth.

The amount of nutrient deposition per unit of live weight gain in aquatic organisms is not constant but instead changes with size and type of feed used during the production system (Bureau, Azevedo, Tapia-Salazar, & Cuzon, 2000). In this study, OY applied in the water had no significant effect on the dry fiber, protein, fat, ash, and water content of shrimp. However, all values of protein content in the whole body of shrimp in OY treatments were higher than that of the control. Similarly, Yang et al. (2015) reported that the addition of 0.2% dietary *Yucca schidigera* significantly increased the shrimp serum protein content in shrimp (*L. vannamei)* cultured for 100 days. It is argued here that the shorter period of growth trial used in the present study might be responsible for the insignificant effect of OY treatments on the nutrient deposition in shrimp (Table 3).

5 | CONCLUSION

The present study successfully reported significant reductions in ammonia and other nitrogen metabolites within the culture environment, coupled with a substantial increase in the growth parameters of shrimp when OY was administered. Our study showed that the use of 2.5 Kg Ha^{-1} of OY was optimal for an intensive shrimp culture system with a density of 500 PL m^{-2}. Therefore, the addition of a mixture of oak and yucca (OY) to the rearing water of Pacific white shrimp has beneficial effects on shrimp productivity. Further research in understanding the underlying mechanism of OY in reducing ammonia in the rearing media and its commercial-scale application in shrimp production systems are recommended to determine its viability.

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CONFLICTS OF INTEREST

Clara Trullàs is used by Tanin Sevnica, Slovenia. The rest of the authors state no conflict of interest.

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