BOLETIM DO INSTITUTO DE PESCA
Scientific Article

Effects of fermented grape pomace (*Vitis* sp.) inclusion on growth performance, lipid peroxidation, and hematology of juvenile *Mugil liza*

Cybele Guimarães Pinheiro[1], Luis Alberto Romano[1], José María Monserrat[2], Juan Rafael Buitrago Ramírez[2], Marcelo Borges Tesser[1]

[1]Universidade Federal do Rio Grande – Instituto de Oceanografia – Programa de Pós Graduação em Aquacultura – Rio Grande (RS), Brazil.

[2]Universidade Federal do Rio Grande – Instituto de Ciência Biológicas – Programa de Pós-Graduação em Aquacultura – Rio Grande (RS), Brazil.

*Corresponding author: marcelotesser@furg.br

ABSTRACT

This study investigated the potential use of fermented grape pomace (FerGP) as a dietary ingredient for juvenile mullet (*Mugil liza*). For this purpose, growth perfomance, hepatic lipid peroxidation, and hematology of juvenile mullet fed various levels of FerGP were evaluated. A total of 225 fish (0.57 ± 0.01 g) were randomly distributed into 15 tanks and fed *ad libitum* three times a day for 65 days with different levels of FerGP (2.5, 5, 10, and 15%). There were no significant differences ($p > 0.05$) in hematological parameters. Regarding the zootechnical parameters, FerGP 15% showed higher specific growth rate compared to the other treatments. Dietary inclusion of FerGP significantly reduced hepatic lipid peroxidation, as indicated by a decrease in thiobarbituric acid reactive substances (TBARS) values. The results demonstrated that the inclusion of FerGP in the diet is feasible and safe, promoting benefits to the productive performance of juvenile mullet.

Keywords: Vegetable co-products; Nutrition; Marine fish.

Efeitos da inclusão de bagaço de uva fermentado (*Vitis* sp.) no desempenho de crescimento, peroxidação lipídica e hematologia de juvenis de *Mugil liza*

RESUMO

Este estudo investigou o uso potencial de bagaço de uva fermentado (FerGP) como ingrediente dietético para tainhas juvenis (*Mugil liza*). O desempenho de crescimento, a peroxidação lipídica hepática e a hematologia de tainhas juvenis alimentadas com vários níveis de FerGP foram avaliados. O total de 225 peixes (0,57 ± 0,01 g) foi distribuído aleatoriamente em 15 tanques e alimentado *ad libitum* três vezes ao dia por 65 dias com diferentes níveis de FerGP (2,5, 5, 10 e 15%). Não houve diferenças significativas ($p > 0{,}05$) nos parâmetros hematológicos. Em relação aos parâmetros zootécnicos, FerGP 15% apresentou diferença significativa em comparação com os outros. A inclusão dietética de FerGP reduziu significativamente a peroxidação lipídica hepática, conforme indicado pela diminuição dos valores de substância reativa ao ácido tiobarbitúrico (TBARS), e não foram observadas diferenças significativas nos parâmetros hematológicos. Este estudo demonstrou que 15% de FerGP pode ser usado para aumentar o crescimento e reduzir a peroxidação lipídica no fígado.

Palavras-chave: Subprodutos vegetais; Nutrição; Peixes marinhos.

Received: May 16, 2025 | **Approved:** March 24, 2026
Section editor: Leonardo Tachibana

INTRODUCTION

Viticulture is an economic activity specialized in the production of grapes, which are destined to produce juice, wine and derivatives, in addition to consumption *in natura*. According to data from the Food and Agriculture Organization of the United Nations (FAO), provided by FAOSTAT (2025), world production of *Vitis* sp. is led by China, with approximately 29 million tons in the period 2022–2023, followed by Italy with approximately 15 million tons and Spain with 10 million tons.

The grape production chain generates a significant amount of waste or co-products (Santos & Leite, 2020), which can reach 20–30% of the total processed volume (Bordiga et al., 2019; Bustamante et al., 2009). This biomass contains significant amounts of ethanol and organic acids, including tartaric acid and malic acid, as well as volatile organic compounds (VOCs) such as propionic acid, which can cause soil acidification, pollute water bodies, and consequently degrade the environment (Niculescu & Ionete, 2023; Zhang et al., 2017). Another destination for grape waste is fertilizer; however, if this waste is not previously treated, there is a possibility of environmental impact (Lopes et al., 2020). Therefore, it is important to mitigate the disposal of these materials by improving their functionalities. Given this context, the reuse of the co-products generated by winemaking makes it possible to obtain inputs with economic and sustainable potential for other sectors of the agroindustry.

The fermented grape pomace (FerGP), the main residue from winemaking, contains bioactive compounds, such as polyphenols, flavonoids, and tannins (Albuquerque et al., 2019; Beres et al., 2019; Bordiga et al., 2019; Chen et al., 2022), which have antioxidant properties and can be reused for applications in several areas, including aquaculture (Beres et al., 2019; Chen et al., 2022).

Research has highlighted the importance of including phenolic compounds and their benefits in the diet of aquatic organisms, such as improving the immune response (Morante et al., 2021; Peng et al., 2022a), growth (Arslan et al., 2018; Rosas et al., 2022), digestibility (Peña et al., 2020), antioxidant capacity (Arslan et al., 2018; Dawood et al., 2020; Mohammadi et al., 2021; Peng et al., 2022b), and antimicrobial activity (Baldissera et al., 2019). In this context, the inclusion of FerGP in the diet may represent a sustainable strategy to improve the zootechnical performance and health of farmed species.

However, it is crucial to recognize that these co-products may also contain antinutrients (Kumanda et al., 2019) that can negatively affect animal performance. Antinutrients such as tannins can interfere with the absorption of essential nutrients in the diet of animals, thereby harming their health and productivity (Vastolo et al., 2022). Therefore, understanding the balance between biocompounds and antinutrients is essential to optimize animal nutrition and ensure sustainable agricultural practices.

Among the species of interest for marine fish farming, mullet (*Mugil liza*) stands out for its cultivation potential, since it accepts artificial feed and occupies a lower trophic level (Cardona, 2016). This characteristic allows the use of diets with lower protein content, contributing to the reduction of feed costs (Ramos et al., 2015). In this context, the present study aimed to reuse FerGP, a co-product of the wine industry, and evaluate the effects of its inclusion in the diet on the growth performance and nutritional parameters of mullet.

MATERIAL AND METHODS

Fermented grape pomace

FerGP was donated by the Enology course of the Universidade Federal do Pampa, Dom Pedrito (RS), Brazil. The fermentation process began with grape must, defined as the juice extracted after crushing or pressing the grapes, before alcoholic fermentation, in which reducing sugars were converted into ethanol and carbon dioxide. Fermentation was carried out at 20 °C, using active dry yeast *Saccharomyces cerevisiae* (MAURIVIN AWRI 796), at the concentration of 20 $g \cdot L^{-1}$. After the fermentation was complete, the material was filtered to obtain the final co-product (FerGP) and stored at -18 °C.

Local and juvenile *Mugil liza*

This study was conducted at the Laboratório de Nutrição de Organismos Aquaticos, which is linked to the Estação de Aquicultura Marinha of the Instituto de Oceanografia of the Universidade Federal do Rio Grande. To carry out the experiment, 225 juvenile mullets (*M. liza*) were collected from Cassino Beach and kept for acclimation under controlled laboratory conditions: dissolved oxygen (6.52 $mg \cdot L^{-1}$ ± 0.62), alkalinity (146.90 $mg \cdot L^{-1}$ of $CaCO_3$ ± 5.07), pH (7.84 ± 0.27), temperature (27.42 °C ± 0.54), salinity (30 ppt ± 0.26), ammonia (0.08 $mg \cdot L^{-1}$ ± 0.04), nitrite (0.04 $mg \cdot L^{-1}$ ± 0.02), and photoperiod (12 h light: 12 h dark), for 30 days, in a recirculation system. Juveniles were fed *ad libitum* (8, 12, and 16 h) with commercial NUTRIPISCIS feed (36% crude protein) according to the recommendations of Calixto da Silva et al. (2020). This study was approved by the Animal Use Ethics Committee of the Universidade Federal do Rio Grande under protocol number 23116.005442.

Experimental design

After 30 days of acclimation, the juvenile mullets (n = 225; mean weight = 0.57 ± 0.01 g) were randomly distributed into

15 tanks (effective volume of 65 L), to start the feeding trial with five levels of FerGP inclusion, in triplicate. For 65 days, the animals were kept in a recirculating aquaculture system (RAS) with physical and biological filters and a constant water flow (1 L every 27 s). The water quality parameters were monitored daily. Temperature and dissolved oxygen were measured using a multiparameter monitor (YSI®-550 A), salinity was assessed with a refractometer (Atago®), and pH was measured with a digital pH meter (YSI®-pH100). The photoperiod was maintained at 12 hours of light and 12 hours of darkness. Ammonia (UNESCO, 1983), nitrite (Strickland & Parsons, 1972), and alkalinity (APHA, 2005) levels were measured every three days. System maintenance was also performed every three days by siphoning to remove feces and feed residues.

Preparation of diets

The experimental diets consisted of a control formulation, without FerGP (0%), and formulations containing 2.5, 5, 10, and 15% of FerGP. All diets were isonitrogenic, as described by Carvalho et al. (2010). The composition of all ingredients in the feed formulation (Tables 1 and 2) was checked in the laboratory according to AOAC International methodology (2006). The diets were pelletized using a meat grinder (Metalúrgica 9000, PC-22) and dried at 60°C for 8 h in an oven (Marconi, MA035). The particle size of the pellets used was 700–850 µm, and the diets were stored in hermetically sealed plastic bags at -18°C until use. The experimental diets were tested in triplicate, and the juveniles were fed *ad libitum* three times a day (8, 12, and 16 h) (Calixto da Silva et al., 2020).

Table 1. Proximal composition of fermented grape pomace (*Vitis* sp.).

Composition	%
Crude protein	17.55
Moisture	3.46
Fiber	25.78
Lipid	8.57
Mineral matter	5.96
Non-fibre carbohydrates	38.69

Zootechnical performance variables

Weight was measured at the beginning and end of the experiment. Animals were anesthetized with 50 ppm benzocaine (da Silva Braz et al., 2017) in an aquarium (3 L). Subsequently, the weight was measured using a digital scale (BIOPRECISA, model JH2102, precision 0.1 g). Food supplied and mortality were also registered. Data were used to calculate the weight gain (WG), survival, specific growth rate (SGR), and feed conversion (FCR). Equations 1, 2, 3, and 4 was used:

Table 2. Composition of the experimental diets and proximate analysis of diets containing different levels of fermented grape pomace meal (*Vitis* sp.) fed to mullet juveniles.

Ingredients	Fermented grape pomace (%)				
	0	2.5	5	10	15
Fishmeal[a]	41.0	41.0	41.0	41.0	41.0
Soybean meal[b]	15.0	14.5	14.0	13.0	12.0
Wheat bran[c]	15.0	14.0	13.0	11.0	9.0
FerGP meal	0.0	2.5	5.0	10.0	15.0
Fish oil[d]	4.0	3.8	3.6	3.2	2.8
Gelatin[e]	2.0	2.0	2.0	2.0	2.0
Starch[f]	20.0	19.2	18.4	16.8	15.2
Premix (mineral + vitamin)[g]	1.0	1.0	1.0	1.0	1.0
Cellulose[h]	2.0	2.0	2.0	2.0	2.0
Total	100.0	100.0	100.0	100.0	100.0
Proximate analysis (%)					
Crude protein	35.27	35.24	35.21	35.15	35.1
Moisture	4.68	6.77	5.59	7.01	6.11
Fiber	3.65	4.17	4.7	5.75	6.79
Lipid	7.87	7.87	7.87	7.88	7.89
Mineral matter	15.29	16.23	15.42	15.54	15.67
Non-fibre carbohydrates[i]	33.24	29.72	31.21	28.66	28.44

[a]Leal Santos, Rio Grande, RS, Brazil. FM composition: crude protein 55.60, lipids 8.80, ash 33, moisture 5.57, fiber 7.46; [b]Sulino, RS, Brazil; [c]Sulino, RS, Brazil; [d]Campestre, São Paulo, SP, Brazil; [e]gelatin powder, Exodus scientific; [f]Maizena, Brazil; [g]Premix M, Cassab, São Paulo, SP, Brazil [Vitamin A (500,000 U·kg^{-1}), vitamin D3 (250,000 U·kg^{-1}), vitamin E (5,000 mg·kg^{-1}), vitamin K3 (500 mg·kg^{-1}), vitamin B1 (1,000 mg·kg^{-1}), vitamin B2 (1,000 mg·kg^{-1}), vitamin B6 (1,000 mg·kg^{-1}), vitamin B12 (2,000 mg·kg^{-1}), niacin (2,500 mg·kg^{-1}), calcium pantothenate (4,000 mg·kg^{-1}), folic acid (500 mg·kg^{-1}), biotin (10 mg·kg^{-1}), vitamin C (10,000 mg·kg^{-1}), choline (100,000 mg·kg^{-1}), inositol (1,000 mg·kg^{-1}). Trace elements: selenium (30 mg·kg^{-1}), iron (5,000 mg·kg^{-1}), copper (1,000 mg·kg^{-1}), manganese (5,000 mg·kg^{-1}), zinc (9,000 mg·kg^{-1}), cobalt (50 mg·kg^{-1}), iodine (200 mg·kg^{-1})]; [h]microcrystalline cellulose PA, Synth; [i]determined by difference.

$$\text{WG (g)} = \text{Final weight (g)} - \text{Initial weight (g)} \quad (1)$$

$$\text{Survival (\%)} = (\text{Final number of fish/Initial number of fish}) \times 100 \quad (2)$$

$$\text{SGR (\% day -1)} = [(\ln \text{final weight (g)} - \ln \text{initial weight (g)}) / \text{experimental period (days)}] \times 100 \quad (3)$$

$$\text{FCR} = \text{feed provided (g)} / \text{weight gain (g)} \quad (4)$$

Carcass proximal composition

The proximal composition of the fish was determined in triplicate at Laboratório de Nutrição de Organismos Aquáticos following the methods described by AOAC (2006). The fish carcasses were dried in an oven at 105 °C to obtain dry matter. Crude protein was determined by the Kjeldahl method using a nitrogen distiller, and crude fat content was evaluated using a Soxhlet extractor and a solvent (petroleum ether). The mineral matter content was determined by gravimetry after the calcification of the samples at 600 °C in a muffle furnace, and the crude fiber content was determined using the method described by Silva and Queiroz (2009).

Hematological analysis

For blood collection, at the end of experimental period three fish from each experimental unit were randomly selected and anesthetized (Silva & Queiroz, 2009). Collection was performed by puncturing the caudal vein using heparinized syringes. After collection, the blood extensions were prepared on glass slides, dried, and stained (eosin, methylene blue and azure B) using the Romanowsky principle (Laborclin, Pinhais, PR, Brazil). Blood extension was evaluated using an optical microscope (Olympus, model CX21FS1; Tokyo, Japan) with an immersion objective (1,000 × magnification). Counts were performed to establish the differential profiles of blood leukocytes (monocytes, lymphocytes, erythrocytes, and total granulocytes).

Biochemical analysis of the liver

To analyze lipid peroxidation levels, the thiobarbituric acid reactive substances (TBARS) method was used as described by Oakes and Van Der Kraak (2003). Three fish per tank were euthanized with a megadose of benzocaine (500 ppm), and liver samples (50 µL) were combined with 20 µL of BHT solution (67 µM), 150 µL of 20% acetic acid, 150 µL of 0.8% thiobarbituric acid (TBA), 50 µL of Milli-Q water, and 20 µL of 8.1% sodium dodecyl sulfate (SDS). After heating at 95°C for 30 min, the samples were centrifuged (3,000 × g for 10 min at 15°C), and 100 µL of Milli-Q H_2O and 500 µL of n-butanol were added to the final solution. The resulting supernatant was used to measure the fluorescence (excitation at 520 nm and emission at 580 nm) using a BioTek microplate reader (Synergy HT). The results were expressed in nanomoles of tetramethoxypropane (TMP, supplied by ACROS Organics) per gram of wet tissue, using TMP (1,1,3,3-tetramethoxypropane) as the standard for calibration.

Statistical analysis

Data are expressed as the mean and standard deviation. Data normality and homogeneity were assessed using the Shapiro-Wilk's and Levene's tests, respectively. After checking the assumptions, the respective data were subjected to analysis of variance (one-way ANOVA), and when necessary, Tukey's post-hoc test was performed to compare the means. Linear and quadratic regressions were performed for all the variables. All analyses were performed with a significance level of 5%.

RESULTS

Water quality parameters remained stable throughout the 65-day experimental period, presenting average values of dissolved oxygen (DO) of 6.36 ± 0.46 mg·L^{-1}; temperature of 27.46 ± 0.77 °C; total ammonia (N–NAT) of 0.08 ± 0.05 mg·L^{-1}; nitrite (N–NO_2) of 0.06 ± 0.07 mg·L^{-1}; alkalinity of 144.84 ± 9.69 mg·L^{-1} $CaCO_3$; salinity of 30.48 ± 1.10 ppt; and pH of 7.73 ± 0.27.

The zootechnical parameters of juvenile mullet are presented in Table 3, with no significant effect observed from

Table 3. Zootechnical parameters of mullet (*Mugil liza*) fed diets containing different levels of fermented grape pomace*.

Parameters	Treatments (%)					*p*-value	Regression	
	0.0	2.5	5.0	10.0	15.0		linear	quadratic
Initial weight (g)	0.57 ± 0.13^a	0.56 ± 0.15^a	0.57 ± 0.12^a	0.57 ± 0.12^a	0.56 ± 0.14^a	> 0.05	$p > 0.71$ $r^2 = 0.003$	$p > 0.51$ $r^2 = 0.107$
Final weight (g)	9.55 ± 0.24^a	8.90 ± 0.15^a	8.97 ± 0.86^a	8.75 ± 0.66^a	9.57 ± 1.62^a	> 0.05	$p > 0.81$ $r^2 = 0.001$	$p > 0.34$ $r^2 = 0.166$
Weight gain (g)	8.99 ± 0.24^a	8.34 ± 0.14^a	8.40 ± 0.86^a	8.18 ± 0.64^a	9.00 ± 1.63^a	> 0.05	$p > 0.90$ $r^2 = 0.001$	$p > 0.33$ $r^2 = 0.168$
Survival (%)	100.00 ± 0.00^a	95.67 ± 7.50^a	88.67 ± 14.01^a	100.00 ± 0.00^a	86.67 ± 23.09^a	> 0.05	$p > 0.10$ $r^2 = 0.063$	$p > 0.67$ $r^2 = 0.063$
Food conversion	2.47 ± 0.11^a	2.69 ± 0.31^a	2.79 ± 0.29^a	2.70 ± 0.25^a	2.97 ± 0.35^a	> 0.05	$p > 0.06$ $r^2 = 0.246$	$p > 0.18$ $r^2 = 0.248$
Specific growth rate (% day^{-1})	4.35 ± 0.04^a	4.24 ± 0.01^a	4.24 ± 0.17^a	4.20 ± 0.07^a	4.84 ± 0.27^b	< 0.05	$p < 0.02$ $r^2 = 0.349$	$p < 0.0003$ $r^2 = 0.744$

*Values expressed as mean ± standard deviation, n = 3, $p > 0.05$ (no significant difference).

the different dietary levels of FerGP ($p > 0.05$), except for the specific growth rate. As illustrated in Fig. 1, the specific growth rate showed a quadratic response as a function of FerGP inclusion levels, with the best performance observed at the 15% inclusion level. Statistically significant differences ($p < 0.05$) were observed among treatments for fish body content of ether extract (EE%) and mineral matter (MM%). EE% levels were significantly higher in treatments with higher FerGP inclusion compared to the control ($p < 0.05$). For mineral matter, FerGP 0% and FerGP 2.5% were different than the other treatment ($p < 0.05$) (Table 4).

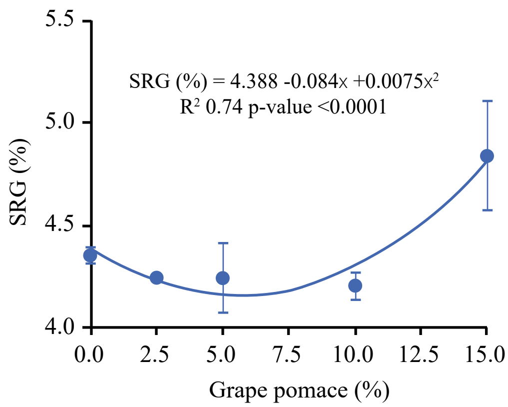

Figure 1. Relationship between the inclusion of grape pomace in the diet and the specific growth rate (SGR, % of live weight per day).

Table 5 shows the values for the erythrocytes, lymphocytes, granulocytes, and monocytes. There were no significant hematological variations ($p > 0.05$) with the inclusion of the FerGP.

Regarding the TBARS values in the liver, ANOVA showed no statistically significant differences ($p > 0.05$) among treatments. However, a statistically significant, albeit weak, linear relationship was observed between increased dietary FerGP levels and reduced hepatic lipid peroxidation ($R^2 = 0.15$; Fig. 2).

Table 4. Analysis of the proximal composition of the mullet carcass (*Mugil liza*) fed with diets containing different levels of fermented grape pomace*.

Proximal composition (%)	Treatments (%)					*p*-value	Regression	
	0.0	2.5	5.0	10.0	15.0		linear	quadratic
Moisture	65.60 ± 0.50[a]	65.55 ± 0.19[a]	66.23 ± 0.36[bc]	65.83 ± 0.76[ab]	66.65 ± 0.32[c]	< 0.05	$p < 0.05$ $r^2 = 0.305$	$p < 0.05$ $r^2 = 0.31$
Crude protein	17.10 ± 0.41[a]	17.07 ± 0.58[a]	16.76 ± 0.91[a]	17.39 ± 0.56[a]	16.78 ± 0.83[a]	> 0.05	$p > 0.70$ $r^2 = 0.003$	$p > 0.79$ $r^2 = 0.011$
Ether extract	6.20 ± 0.45[a]	11.41 ± 0.81[c]	10.40 ± 0.64[c]	8.15 ± 1.12[b]	11.19 ± 1.86[c]	< 0.05	$p < 0.01$ $r^2 = 0.138$	$p < 0.02$ $r^2 = 0.164$
Mineral matter	1.89 ± 0.17[b]	1.69 ± 0.11[b]	1.37 ± 0.12[a]	1.34 ± 0.23[a]	1.26 ± 0.13[a]	< 0.05	$p < 0.05$ $r^2 = 0.561$	$p < 0.05$ $r^2 = 0.678$

*Values expressed as mean ± standard deviation, n = 9, $p < 0.05$ (significant difference). Different letters indicate differences between treatments ($p < 0.05$).

Table 5. Hematological analysis of mullet (*Mugil liza*) fed with diets containing different levels of fermented grape pomace*.

Cell type	Treatments (%)					*p*-value	Regression	
	0.0	2.5	5.0	10.0	15.0		linear	quadratic
Erythrocytes	1,480.6 ± 738.6[a]	1,131.20 ± 263.0[a]	1,066.4 ± 347.9[a]	1,192.3 ± 354.1[a]	1,130.4 ± 388.6[a]	> 0.05	$p > 0.30$ $r^2 = 0.025$	$p > 0.06$ $r^2 = 0.059$
Monocytes	35.2 ± 23.3[a]	40.8 ± 53.3[a]	18.2 ± 6.8[a]	49.4 ± 49.9[a]	25.7 ± 16.2[a]	> 0.05	$p > 0.82$ $r^2 = 0.001$	$p > 0.90$ $r^2 = 0.005$
Lymphocytes	45.3 ± 35.8[a]	24.2 ± 24.2[a]	21.3 ± 12.4[a]	32.40± 26.5[a]	34.9 ± 23.9[a]	> 0.05	$p > 0.90$ $r^2 = 0.0004$	$p > 0.26$ $r^2 = 0.061$
Granulocytes	36.0 ± 19.7[a]	19.6 ± 11.8[a]	17.4 ± 6.9[a]	32.13 ± 14.9[a]	20.2 ± 13.7[a]	> 0.05	$p > 0.95$ $r^2 = 0.00007$	$p > 0.38$ $r^2 = 0.046$

*Values expressed as mean ± standard deviation, n = 9, $p > 0.05$ (no significant difference).

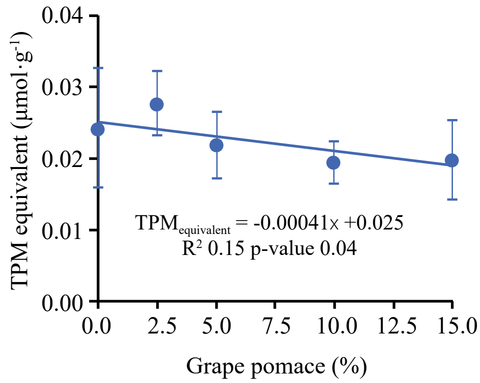

Figure 2. Content of thiobarbiturate-reactive substances in µmol TMP/g per tissue in the liver of *Mugil liza* fed with differences in fermented grape pomace inclusion. Values are expressed as mean and standard deviation (n = 9).

DISCUSSION

In the present study, the average final weight, feed conversion, and survival did not show significant differences, except for SGR, which showed improvement with an increase in the inclusion of FerGP at the level of 15%, indicating a promising response in terms of its inclusion in diets for juvenile mullet. The same was observed for juveniles of the common carp (*Cyprinus carpio*), which showed better zootechnical performance with the highest level of grape pomace inclusion evaluated (15%) (Mahmoodi et al., 2023).

Furthermore, studies have reported that the inclusion of grape pomace in fish diets influences the fatty acid composition (Mahmoodi et al., 2023) and reduces lipid oxidation in fish muscle (Barbacariu et al., 2024). Research on rainbow trout fry has indicated that diets containing grape pomace led to an increase in several polyunsaturated fatty acids, which are beneficial to human health (Peña et al., 2020). In our study, there was greater lipid deposition in mullet carcasses of the FerGP-treated groups. In contrast, Mahmoodi et al. (2023) found that the inclusion of grape pomace at high concentrations reduced lipid levels in common carp muscle with increasing feeding levels. These differences can be explained not only by the bioavailability of the bioactive compounds present in the ingredients used, but also by species-specific metabolic responses, considering that the present study evaluated a marine species (*M. liza*), while Mahmoodi et al. (2023) investigated a freshwater species (*C. carpio*). Understanding the interactions that regulate feeding behavior in fish still represents a significant challenge, especially in aquaculture species, in which physiological and metabolic responses to food intake can exhibit high variability (Plagnes-Juan et al., 2025). Marine and freshwater fish exhibit differences in lipid metabolism, osmotic physiology, and micro and macronutrient requirements, especially regarding the synthesis and utilization of long-chain polyunsaturated fatty acids (LC-PUFAs) such as arachidonic acid (ARA, 20:4n-6), eicosapentaenoic acid (EPA, 20:5n-3), and docosahexaenoic acid (DHA, 22:6n-3) (Baldisserotto, 2025), which may contribute to the divergent effects observed.

Furthermore, the inclusion of grape pomace can significantly alter the carbohydrate, fiber, lipid, and protein content in the diet of organisms (Taladrid et al., 2023). The fibers present in grape pomace can improve the overall digestibility and promote intestinal health, whereas the composition of lipids and proteins can directly influence the growth and nutritional quality of fish (Peña et al., 2020; Silva et al., 2022). However, an excessive amount of carbohydrates can reduce the digestibility of other nutrients owing to their lower absorption efficiency (Costa et al., 2019). This highlights the importance of considering the specificities of cultured organisms and the feeding context when evaluating the nutritional effects of grape pomace, as well as understanding how different levels of inclusion and processing can affect the efficiency of nutrient absorption and overall fish health, contributing to the development of more balanced and beneficial diets (Antonić et al., 2020; Peña et al., 2020).

It is interesting to note that despite presenting greater carcass lipid deposition in mullets fed with FerGP, a negative linear relationship was observed between the concentration of TMP equivalents and the increase in FerGP inclusion in liver tissue, suggesting that the greater the inclusion, the lower the lipid peroxidation. This finding indicates that the antioxidant compounds present in FerGP, especially the phenolic compounds, can act as free radical scavengers, including the hydroxyl radical—one of the main initiators of lipid peroxidation due to its high reactivity and ability to attack membrane lipids, forming lipid hydroperoxides. Neutralizing these radicals can interrupt oxidative chain reactions, contributing to the reduction of lipid peroxidation and oxidative stress (Aziz et al., 2019; Peixoto et al., 2018; Shahab et al., 2023; Vuolo et al., 2019). Chien et al. (2023) investigated the impact of dry grape extract on the diet of white shrimp *Litopenaeus vannamei*, focusing on establishing the ideal dietary level. The results indicated that increasing the dietary level of dry grape extract to 500 ppm resulted in an increase in superoxide dismutase (SOD) activity and a reduction in the levels of reactive substances such as TBARS in the shrimp hepatopancreas. In a study conducted by Rosas et al. (2022), lower lipid peroxidation and greater antioxidant capacity in the hepatopancreas of juvenile white shrimp (*L. vannamei*) was recorded when FerGP was included in the diet.

Studies on the effects of grape pomace in fish diets on hematological parameters are scarce, although there are studies on vegetable co-products. For example, a significant improvement in hematological parameters was observed in carp fed a pomegranate peel (*Punica granatum*) supplement (Sayed-Lafi et al., 2023). In the present study, the increasing inclusion of FerGP in the diet did not promote significant changes in the hematological parameters of juvenile mullet. Considering that hematological parameters are widely used as sensitive indicators of health, stress, and metabolic response in fish (Ahmed et al., 2020; Witeska et al., 2022), the observed balance suggests that the inclusion of FerGP, at the levels evaluated, is safe from a hematological point of view. However, given the relevance of these variables for the assessment of health in aquaculture species, further studies are recommended to deepen the understanding of the hematological responses of *M. liza* to the prolonged inclusion of agro-industrial by-products in the diet.

Barbacariu et al. (2024) observed changes in carp fed diets containing grape pomace, highlighting an increase in the number of monocytes, in addition to lymphopenia at moderate inclusion doses. These changes were attributed to the modulation of the intestinal microbiota and the activation of innate immune mechanisms, especially associated with the presence of phenolic compounds. In another study, dietary supplementation with a plant-derived prebiotic in juvenile pacu (*Piaractus mesopotamicus*) resulted in an increase in the number of leukocytes and thrombocytes, indicating an improvement in the overall health of the fish (Deon et al., 2021). These findings highlight the importance of expanding studies on the different levels and forms of processing of plant co-products, especially considering the relevance of hematological parameters as indicators of health and immune response, in addition to their application in functional diets for marine species.

This study demonstrated that the inclusion of grape pomace, a byproduct of the wine industry, did not compromise the growth performance of juvenile mullet (*M. liza*), indicating its viability as an ingredient in diets for this species. The absence of significant differences between dietary treatments for most of the parameters evaluated reinforces the potential of grape pomace as an alternative and environmentally sustainable ingredient in aquaculture feed formulations. In this context, the use of this byproduct represents an opportunity for the valorization of agro-industrial waste, contributing to more sustainable practices in aquaculture. Future studies are needed to evaluate its long-term effects, as well as different levels and forms of winemaking byproducts.

CONCLUSION

This study demonstrated that FerGP can be included in the diet of mullet at levels up to 15 g per 100 g of feed, without negative effects on growth, thus establishing itself as an alternative and environmentally sustainable ingredient for aquaculture.

CONFLICT OF INTEREST

Nothing to declare.

DATA AVAILABILITY STATEMENT

The data will be available upon request.

AUTHORS'CONTRIBUTIONS

Writing — original draft: Pinheiro, C.G.; **Data curation:** Pinheiro, C.G.; **Writing — review & edition:** Pinheiro, C.G., Romano, L.A., Monserrat, J.M., Ramírez, J.R.B., Tesser, M.B.; **Formal analysis:** Romano, L.A., Monserrat, J.M., Ramírez, J.R.B.; **Resources:** Tesser, M.B.; **Investigation:** Tesser, M.B.; **Supervision:** Tesser, M.B.; **Final approval:** Tesser.M.B.

FUNDING

Coordenação de Aperfeiçoamento de Pessoal de Nível Superior

Financial code 001

Conselho Nacional de Desenvolvimento Científico e Tecnológico

Grant No.: 305112/2023-6

Fundação de Amparo à Pesquisa do Estado do Rio Grande do Sul

Grant No.: 19/2551-0001874-6

DECLARATION OF USE OF ARTIFICIAL INTELLIGENCE TOOLS

Artificial intelligence was used for language correction in the manuscript translation. All scientific content remains the responsibility of the authors.

ACKNOWLEDGMENTS

Not applicable.

REFERENCES

Ahmed, I., Reshi, Q., & Fazio, F. (2020). The influence of the endogenous and exogenous factors on hematological parameters in different fish species: a review. *Aquaculture International*, 28, 869-899. https://doi.org/10.1007/s10499-019-00501-3

Albuquerque, M. A. C., Levit, R., Beres, C., Bedani, R., Moreno de LeBlanc, A., Saad, S. M. I., & LeBlanc, J. G. (2019). Tropical fruit by-products water extracts of tropical fruit by- products as sources of soluble fibers and phenolic compounds with potential antioxidant, anti- inflammatory, and functional properties. *Journal of Functional Foods*, 52, 724-733. https://doi.org/10.1016/j.jff.2018.12.002

Antonić, B., Jančíková, S., Dordević, D., & Tremlová, B. (2020). Grape pomace valorization: A systematic review and meta-analysis. *Foods*, 9(11). 1627. https://doi.org/10.3390/foods9111627

AOAC (2006). *Official methods of analysis of AOAC International*. AOAC International.

APHA (2005). *Standard Methods for the Examination of Water and Wastewater 1496*. APHA.

Arslan, G., Sönmez, A. Y., & Yank, T. (2018). Effects of grape *Vitis vinifera* seed oil supplementation on growth, survival, fatty acid profiles, antioxidant contents and blood parameters in rainbow trout *Oncorhynchus mykiss*. *Aquaculture Research*, 49, 2256-2266. https://doi.org/10.1111/are.13686

Aziz, M. A., Diab, A. S., & Mohammed, A. A. (2019). Antioxidants: antioxidant categories and mode of action. https://doi.org/10.5772/intechopen.77838

Baldissera, M. D., Souza, C. F., Descovi, S. N., Verdi, C. M., Zeppenfeld, C. C., Silva, A. S., Santos, R. C. V., & Baldisserotto, B. (2019). Grape pomace flour ameliorates *Pseudomonas aeruginosa* -induced bioenergetic dysfunction in gills of grass carp. *Aquaculture*, 506, 359-366. https://doi.org/10.1016/j.aquaculture.2019.03.065

Baldisserotto, B. (2025). *Fisiologia de peixes aplicada à piscicultura* (4ª ed.). Editora UFSM.

Barbacariu, C.-A., Dîrvariu, L., Șerban, D. A., Rîmbu, C. M., Horhogea, C. E., Dumitru, G., Todirașcu-Ciornea, E., Lungoci, C., & Burducea, M. (2024). Evaluating the use of grape pomace in *Cyprinus carpio* nutrition: effects on growth, biochemistry, meat quality, microbiota, and oxidative status. *Fishes*, 9(6), 219. https://doi.org/10.3390/fishes9060219

Beres, C., Freitas, S. P., Godoy, R. L. O., de Oliveira, D. C. R., Deliza, R., Iacomini, M., Mellinger-Silva, C., & Cabral, L. M. C. (2019). Antioxidant dietary fiber from grape pomace flour or extract: Does it make any difference on the nutritional and functional value. *Journal of Functional Foods*, 56, 276-285. https://doi.org/10.1016/j.jff.2019.03.014

Bordiga, M., Travaglia, F., & Locatelli, M. (2019). Valorization of grape pomace: an approach that is increasingly reaching its maturity – a review. *International Journal of Food Science and Technology*, 54(4), 933-942. https://doi.org/10.1111/ijfs.14118

Bustamante, M. A., Paredes, C., Morales, J., Mayoral, A. M., & Moral, R. (2009). Study of the composting process of winery and distillery wastes using multivariate techniques. *Bioresources Technology*, 100(20), 4766-4772. https://doi.org/10.1016/j.biortech.2009.04.033

Calixto da Silva, E., Sterzelecki, F. C., Alves Musialak, L., Sugai, J. K., Castro, J. J. P., Pedrotti, F. S., Magnotti, C., Cipriano, F. S., & Cerqueira, V. R. (2020). Effect of feeding frequency on growth performance, blood metabolites, proximal composition and digestive enzymes of Lebranche mullet (*Mugil liza*) Juveniles. *Aquaculture Research*, 51, 1162-1169. https://doi.org/10.1111/are.14466

Cardona, L. (2016). Biology, ecology and culture of gray mullets (Mugilidae). In D. Crosetti & S. Blaber (Ed.), *Biology, ecology and culture of gray mullets (Mugilidae)* (pp. 165-195). Taylor & Francis Group.

Carvalho, C. V. A., Bianchini, A., Tesser, M. B., & Sampaio, L. A. (2010). The effect of protein levels on growth, postprandial excretion and tryptic activity of juvenile mullet *Mugil platanus* (Günther). *Aquaculture Research*, 41, 511-518. https://doi.org/10.1111/j.1365-2109.2009.02340.x

Chen, B., Qiu, J., Wang, Y., Huang, W., Zhao, H., Zhu, X., & Peng, K. (2022). Condensed tannins increased intestinal permeability of Chinese seabass (*Lateolabrax maculatus*) based on microbiome-metabolomics analysis. *Aquaculture*, 560, 738615. https://doi.org/10.1016/j.aquaculture.2022.738615

Chien, A., Chou, C. Y., Cheng, Y. C., Sheen, S. S., & Kirby, R. (2023). The optimal dietary level of dry grape extract and its effect on the growth performance and antioxidant activity of the white shrimp *Litopenaeus vannamei*. *Aquaculture Reports*, 29, 101527. https://doi.org/10.1016/j.aqrep.2023.101527

Costa, J. R., Amorim, M., Vilas-Boas, A., Tonon, R. V., Cabral, L. M. C., Pastrana, L., & Pintado, M. (2019). Impact of: *In vitro* gastrointestinal digestion on the chemical composition, bioactive properties, and cytotoxicity of *Vitis vinifera* L. cv. Syrah grape pomace extract. *Food and Function*, 10(4), 1856-1869. https://doi.org/10.1039/c8fo02534g

Da Silva Braz, R., Oliveira Silva, I., Borges Tesser, M., André Sampaio, L., Vieira Rodrigues, R. (2017). Benzocaine, MS-222, eugenol and menthol as anesthetics for juvenile *Mugil lisa*. *Boletim do Instituto de Pesca*, 43(4), 605-613. https://doi.org/10.20950/1678-2305.2017v43n4p605

Dawood, M. A. O., Eweedah, N. M., Khalafalla, M. M., Khalid, A., El, A., Fadl, S. E., Amin, A. A., Ahamad, B., & Ahmed, H. A. (2020). *Saccharomyces cerevisiae* increases the acceptability of Nile tilapia (*Oreochromis niloticus*) to date palm seed meal. *Aquaculture Reports*, 17, 100314. https://doi.org/10.1016/j.aqrep.2020.100314

Deon, M. P. P., Bicudo, Á. J. A., & Sado, R. Y. (2021). Performance, hematology, and immunology of pacu in response to dietary supplementation with fructooligosaccharides. *Pesquisa Agropecuária Brasileira*, 56, e02460. https://doi.org/10.1590/S1678-3921.pab2021.v56.02460

FAOSTAT (2025). *Crops and livestock products*. FAOSTAT. Retrieved from: https://www.fao.org/faostat/en/#data/QCL

Kumanda, C., Mlambo, V., & Mnisi, C. M. (2019). Valorization of red grape pomace waste using polyethylene glycol and fibrolytic enzymes: Physiological and meat quality responses in broilers. *Animals*, 9(10), 779. https://doi.org/10.3390/ani9100779

Lopes, A. M., Filippi, A. C. G., & Streit, J. A. C. (2020). Solid waste management from wine culture: a case study in the Federal District and surroundings. *Revista Razão Contábil & Finanças*, 13(2), 1-19. https://doi.org/10.71136/rrcef.v13i2.327

Mahmoodi, B., Aberoumand, A., Ziaei-nejad, S., & Seyyedi, S. (2023). Effects of diets containing grape pomace on the growth, nutrition indices, and the quality traits of common carp (*Cyprinus carpio*). *Food Science & Nutrition*, 11(10), 6660-6669. https://doi.org/10.1002/fsn3.3614

Mohammadi, Y., Kamangar, B. B., & Zarei, M. A. (2021). Effects of diets containing grape seed proanthocyanidin extract on the growth and oxidative capacity of common carp (*Cyprinus carpio*). *Aquaculture*, 540, 763389. https://doi.org/10.1016/j.aquaculture.2021.736689

Morante, V. H. P., Copatti, C. E., Souza, A. R. L., Costa, M. M., Braga, L. G. T., Souza, A. M., Melo, F. V. S. T., Camargo, A. C. S., & Melo, J. F. B. (2021). Assessment the crude grape extract as feed additive for tambaqui (*Colossoma macropomum*), an omnivorous fish. *Aquaculture*, 544, 737068. https://doi.org/10.1016/j.aquaculture.2021.737068

Niculescu, V. C., & Ionete, R. E. (2023). An overview on management and valorization of winery wastes. *Applied Sciences*, 13(8), 5063. https://doi.org/10.3390/app13085063

Oakes, K. D., & Van Der Kraak, G. J. (2003). Utility of the TBARS assay in detecting oxidative stress in white sucker (*Catostomus commersoni*) populations exposed to pulp mill effluent. *Aquatic Toxicology*, 63(4), 447-463. https://doi.org/10.1016/S0166-445X(02)00204-7

Peixoto, C. M., Dias, M. I., Alves, M. J., Calhelha, R. C., Barros, L., Pinho, S. P., & Ferreira, I. C. F. R. (2018). Grape pomace as a source of phenolic compounds and diverse bioactive properties. *Food Chemistry*, 253, 132-138. https://doi.org/10.1016/j.foodchem.2018.01.163

Peña, E., Badillo-Zapata, D., Viana, M. T., & Correa-Reyes, G. (2020). Use of grape pomace in formulated feed for the rainbow trout fry, *Oncorhynchus mykiss* (Walbaum, 1792). *Journal of World Aquaculture Society*, 51, 542-550. https://doi.org/10.1111/jwas.12669

Peng, K., Chen, B., Wang, Y., Zhao, H., Zheng, C., & Chen, X. (2022a). Condensed tannins protect against aflatoxin B1-induced toxicity in *Lateolabrax maculatus*, restoring intestinal integrity and regulating the bacterial 555 microbiota. *Aquaculture*, 555, 738255. https://doi.org/10.1016/j.aquaculture.2022.738255

Peng, K., Chen, B., Zhao, H., Wang, Y., Zheng, C., Lu, H., Huang, M., Zhao, J., & Huang, W. (2022b). Reevaluation of dietary condensed tannins on growth, antioxidant and immune response and muscle quality of Chinese seabass (*Lateolabrax maculatus*). *Aquaculture*, 558, 738413. https://doi.org/10.1016/j.aquaculture.2022.738413

Plagnes-Juan, E., Martinat, M., Baranek, E., Surget, A., Heraud, C., & Roy, J. (2025). Temporal hedonic regulation of feeding behavior in rainbow trout: a role for the opioid system under plant-based diets from first feeding onward. *Water Biology and Security*, 100529. https://doi.org/10.1016/j.watbs.2025.100529

Ramos, L. R. V., Romano, L. A., Monserrat, J. M., Abreu, P. C., Verde, P. E., & Tesser, M. B. (2015). Biological responses in mullet *Mugil liza* juveniles fed with guar gum supplemented diets. *Animal Feed Science and Technology*, 205, 98-106. https://doi.org/10.1016/j.anifeedsci.2015.04.004

Rosas, V. T., Mureb, R. A., Monserrat, J. M., Wasielesky, W. J., & Tesser, M. B. (2022). Inclusion of grape bagasse (*Vitis* sp.) in the diet of white shrimp (*Litopenaeus vannamei*) and its effects on growth and antioxidant system. *Aquaculture Research*, 1-9. https://doi.org/10.1111/are.15972

Santos, P. V. S., & Leite, Â. A. M. (2020). Identification of secondary winemaking products: a case study. *Environmental Management & Sustainability Magazine*, 9(1), 650-666. https://doi.org/10.19177/rgsa.v9e12020650-666

Sayed-Lafi, R. M., Sultan, F. A. M., & Al-Tameemi, R. A. (2023). Changes in hematological parameters of common carp (*Cyprinus carpio*) fingerlings fed on pomegranate (*Punica granatum*) peel supplement. *Al-Mukhtar Journal of Sciences*, 38(1), 69-77. https://doi.org/10.54172/mjsc.v38i1.1120

Shahab, M., Roberto, S. R., Adnan, M., Fahad, S., Koyama, R., Saleem, M. H., Nasar, J., Saud, S., Hassan, S., & Nawaz, T. (2023). Phenolic compounds as a quality determinant of grapes: a critical review. *Journal of Plant Growth Regulation*, 42, 5325-5331. https://doi.org/10.1007/s00344-023-10953-w

Silva, M. E. S., Grisi, C. V. B., Silva, S. P., Madruga, M. S., & Silva, F. A. P. (2022). The technological potential of agro-industrial residue from grape pulping (*Vitis* spp.) for application in meat products: A review. *Food Bioscience*, 49, 101877. https://doi.org/10.1016/j.fbio.2022.101877

Silva, D. J., & Queiroz, A. C. (2009). *Análise de alimentos: métodos químicos e biológicos* (3rd ed.). Universidade Federal de Viçosa.

Strickland, J. D. H., & Parsons, T. R. (1972). *A practical handbook of seawater analysis* (2nd ed.). Fisheries Research Board of Canada. https://doi.org/10.25607/OBP-1791

Taladrid, D., Rebollo-Hernanz, M., Martin-Cabrejas, M. A., Moreno-Arribas, M. V., & Bartolomé, B. (2023). Grape pomace as a cardiometabolic health-promoting ingredient: activity in the intestinal environment. *Antioxidants*, 12(4), 979. https://doi.org/10.3390/antiox12040979

United Nations Educational, Scientific and Cultural Organization (UNESCO) (1983). *Chemical Methods for use in marine environmental monitoring*. Intergovernmental Oceanographic Commissionn.

Vastolo, A., Calabrò, S., & Cutrignelli, M. I. (2022). A review on the use of agro-industrial CO-products in animals' diets. *Italian Journal of Animal Science*, 21(1), 577-594. https://doi.org/10.1080/1828051X.2022.2039562

Vuolo, M. M., Lima, V. S., & Maróstica Junior, M. R. (2019). Phenolic compounds. *Bioactive Compounds*, 33-50. https://doi.org/10.1016/b978-0-12-814774-0.00002-5

Witeska, M., Kondera, E., Ługowska, K., & Bojarski, B. (2022). Hematological methods in fish – Not only for beginners. *Aquaculture*, 547, 737498. https://doi.org/10.1016/j.aquaculture.2021.737498

Zhang, N., Hoadley, A., Patel, J., Lim, S., & Li, C. (2017). Sustainable options for the utilization of solid residues from wine production. *Waste Management*, 60, 173-183. https://doi.org/10.1016/j.wasman.2017.01.006