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ORIGINAL ARTICLE

Use of Pacific thread herring (*Opisthonema libertate*) protein hydrolysates in Nile tilapia (*Oreochromis niloticus*) feeds: Productive performance and antioxidant enzymes on organisms exposed to a heat-induced stress

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[Correction added on 7 September 2022, after first online publication: *Orechromis* has been corrected to *Oreochromis* in the article title.]

Abstract

The Nile tilapia (*Oreochromis niloticus*) industry represent the 71% of aquaculture total world production and demands for novel feeds and better proteins. In this sense, Pacific thread herring (*Ophistonema libertate*) contains high-quality proteins suitable to produce protein hydrolysates (PH) with antioxidant activity for tilapia feeds. In this study, two Alcalase herring muscle PH were prepared at 10% and 30% degree of hydrolysis (DH) and were supplemented on two formulated feeds, FH10 and FH30 respectively. One hundred eighty tilapia juveniles were fed with the experimental and a control feed (CF) for 6 weeks and then submitted to an acute stress by temperature increment for 24 hours. The results revealed that organisms fed with the FH10 shown the highest ($p < 0.05$) weight increment compared to the other feeds, showing a better protein yield. The enzymatic activity of superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) measured in Nile tilapia livers was influenced by the experimental feeds, the organisms fed with FH10 exhibited the highest activity in SOD and CAT and the best productive performance, while the tilapias fed with FH30 showed an elevated GPx activity, suggesting that herring muscle PH in feeds are a suitable option to bring a potential benefit for Nile tilapias.

KEYWORDS
antioxidant enzymes, feeds, heat stress, productive performance, protein hydrolysate

1 | INTRODUCTION

The Nile tilapia (*Oreochromis niloticus*) is a species of great commercial interest in aquaculture, during 2018 it was the most produced tilapia species, reaching 1.03 million tonnes and representing 71% of total world production (FAO, 2020). Mexico is the ninth largest producer of Tilapia worldwide, which represents 94.3% of the national fishery for the species for Mexico (CONAPESCA, 2018).

Currently, fishmeal has been the main source of protein used for feed formulation in aquaculture. More than 6 million tonnes of fish meal is produced globally each year from about 25–30 million tonnes of industrial fish, that is, 30% of the world's total catch is used for the production of fish meal (FAO, 2020). However, new alternatives are being sought for the formulation of diets for aquaculture organisms, one of them being the total or partial replacement, with protein sources of better quality and that provide greater benefits to the

organisms, being protein hydrolysates application one of the most promising protein source for aquafeeds (Ferraro et al., 2013).

Protein hydrolysates are a mix of peptides with different molecular weight; these compounds might have interesting functional and bioactive properties and can be used as ingredients in animal fed formulations (Hou et al., 2017; Soares et al., 2020). Furthermore, these peptides of short-chain and low-molecular-weight make them more digestible and attractive to animals, promoting growth performance and feed efficiency and also facilitate the absorption of the molecules by intestinal epithelial cells (Najafian & Babji, 2012). These benefits also influence the antioxidant defence of the enzymes superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx), preventing oxidative damage in cells (Yarnpakdee, Benjakul, Kristinsson, & Bakken, 2015; Yarnpakdee, Benjakul, Kristinsson, & Kishimura, 2015).

The Pacific thread herring (*Ophistonema libertate*), is one of the small pelagic species with the greatest commercial importance in terms of catch volume and represents one of the most important fisheries in north-western Mexico (Ruiz-Domínguez & Quiñonez-Velázquez, 2018). This resource is used mainly in the production of fishmeal and fish oil; however, the high-quality muscle protein of these pelagic fish in terms of content of essential amino acids and digestible compounds, is considered as a quality raw material, which can be used to produce high value commercial products, such as protein hydrolysates (PH; Sandoval-Gallardo et al., 2020).

Several studies have reported the use of fish protein hydrolysates in fish diets, in which an effect in several important features, such as productive performance, intestinal microbiology and antioxidant enzymes of organisms was observed. Regarding to antioxidant enzymes, the activity of superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) have been highlighted as important indicators of oxidative stress, since the catalytic performance of these enzymes aims to annul reactive oxygen species (ROS), maintaining their intracellular concentration at physiological levels (Joy et al., 2017).

For instance, Leal et al. (2010) applied protein hydrolysates from shrimp (*Litopenaeus vannamei*) in diets for Nile tilapia (*O. niloticus*), observing that the inclusion significantly affected ($p < 0.05$) the final fish body composition. Li et al. (2018), reported that shrimp survival significantly increased with the inclusion of low-molecular-weight sardine hydrolysate; also, phenoloxidase and superoxide dismutase activity enhanced when the organisms were feeding with the supplemented diet. In another study, Ha et al. (2019) observed that protein hydrolysates of sardine (*Sardinella* spp.) improved productive performance for South American catfish (*Rhamdia quelen*).

Nowadays, global change causes several environmental alterations that exerted negative effects on aquaculture, being water temperature one of the most important abiotic factors, which affect the metabolism, growth and even survival of fish. In fact, several studies have demonstrated that thermal stress leads oxidative damage of aquatic organisms (Cheng et al., 2018; Joy et al., 2017; Pinto et al., 2019; Yu et al., 2017).

Taking into account the relevance of the usage of protein hydrolysates in supplemented diets to improve productive performance and the antioxidant defence system of aquatic organisms, the aim of this study was to evaluate the effect of inclusion of protein hydrolysates produced from Pacific thread herring at different degree of hydrolysis on the productive performance and antioxidant enzymes of Nile tilapia, subjected to heat stress, which is also known to play a determining role in the metabolic processes of fish.

2 | MATERIALS AND METHODS

2.1 | Reagents

Alcalase 2.4 L, 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2′-azino-bis (3-ethylbenzothiazoline)-6-sulphonic acid (ABTS), 2,4,6-tris(2-pyridyl)-s-triazine (TPTZ), iron (III) chloride hexahydrated ($FeCl_3 \bullet 6H_2O$), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) and catalase assay kit were purchased from Sigma-Aldrich. Gel filtration standard was obtained from BIORAD (151-1901). Superoxide dismutase and Glutathione peroxidase assay kits were obtained from Cayman Chemicals.

2.2 | Production of Pacific thread herring muscle hydrolysates

The Pacific thread herring (*Ophistonema libertate*), (corporal weight of 102.1 ± 3.2 g; total longitude of 18.5 ± 1.4 cm) was captured in spring of 2016 and kindly donated from a fishmeal plant (Maz Industrial, S.A. de C.V.) located in Mazatlán, Sinaloa, Mexico. Two antioxidant herring protein hydrolysates were produced, with DH values of 10% and 30% (PH10 and PH30 respectively). Subsequently, they were included in tilapia feeds. Production of PH was following the process conditions described in a previous study by Sandoval-Gallardo et al. (2020) (pH = 8, temperature of 50°C and using the enzyme Alcalase at 3% w/v).

2.3 | Proximate chemical composition

The determinations of crude protein (Micro-Kjeldahl method), ash and moisture content of protein hydrolysates, ingredients and feeds were carried out according to methodologies of the Association of Official Analytical Chemists (AOAC, 2012). Lipid content was analysed using a micro–Foss Soxtec Avanti 2050 Automatic System (Foss Soxtec; Hoganas) using petroleum ether as the extractor solvent.

2.4 | Size-exclusion chromatography

The molecular weight distribution of peptides contained of protein hydrolysates was determined by size-exclusion chromatography (SEC). The samples were freeze-dried and further rehydrated (1 mg/ml) with 150 mM sodium phosphate buffer at pH 7.0. Then,

rehydrated samples were filtered through a 0.45 μm PTFE filter. The filtered samples were chromatographed in a Varian HPLC (Varian™ Pro Star) equipped with a Bio SEC-5™ size-exclusion column (4.6 × 300 mm; from Agilent). The samples were monitored with a diodes array detector (Varian™ Pro Star) using the software Galaxy™ (version 1.9.302.952). The samples were eluted using an isocratic flow of 0.4 ml/minute at room temperature and absorbance was monitored at 254 nm. A gel filtration marker kit (BIORAD, 151-1901) composed by thyroglobulin (M_W = 670 kDa), gamma-globulin (M_W = 158 kDa), ovalbumin (M_W = 44 kDa), myoglobulin (M_W = 17 kDa) and vitamin B-12 (M_W = 1.35 kDa), was used to determine peptide profiles (Zhou et al., 2012).

2.5 | Amino acid analysis

The amino acid (AA) profiles of defatted samples of protein hydrolysates (PH10 and PH30) and tilapia feeds (CF, FH10 and FH30) were determined. The samples were hydrolysed and derivatized for their chromatographic quantification. In summary, 3 ml of 6 N HCl was added to 3 mg of sample containing the same mass of solid sodium thioglycolate as antioxidant. The mixture was then digested for 6 hours at 150°C to form free amino acids in a closer vial under a nitrogen atmosphere. Then, hydrolysed samples were dried in a rotary evaporator and further rehydrated with 2 ml of citrate buffer at pH 2.2. The rehydrated samples were derivatized with *o*-phthalaldehyde (OPA; Fluoropa™, Pierce). Then, samples were chromatographed in an Agilent Technologies™ 1100 HPLC equipped with a reversed-phase column (4.6 × 150 mm; Zorbax 300 Extent-C18™, Agilent), using the 0.1 M acetate buffer (pH 7.2) with 1% tetrahydrofuran (solution A) and methanol (solution B) gradient at a flow rate of 1.2 ml/minute. A fluorescence detector was set up for excitation/emission wavelengths of 350 nm/450 nm. Amino acid concentration was calculated using α–aminobutyric acid as internal standard (Vázquez-Ortiz et al., 1995).

2.6 | Antioxidant activity of herring protein hydrolysates

2.6.1 | DPPH radical scavenging activity

The DPPH radical scavenging potential of the protein hydrolysates, was analysed according to Müller et al. (2011), with slight modifications, reported by Sandoval-Gallardo et al. (2020). The percentage of DPPH radical scavenging was calculated according to the following equation:

$$\text{DPPH scavenging}\,(\%) = \frac{1 - \text{ABS sample}}{\text{ABS control}} \times 100 \quad (1)$$

The trolox equivalent antioxidant activity was expressed in μmol of Trolox Equivalent (TE) per mg of dry sample.

2.6.2 | ABTS $^{\cdot+}$ free-radical scavenging activity

The 2,2′-azino-bis[3-ethylbenzothiazoline]-6-sulphonic acid (ABTS) radical scavenging activity was determined according to Przygodzka et al. (2014), with slight modification, reported by Sandoval-Gallardo et al. (2020). The percentage of ABTS$^{\cdot+}$ radical scavenging was calculated using the following equation:

$$\text{ABTS}^{\cdot+}\ \text{scavenging}\,(\%) = \frac{A - B}{A} \times 100 \quad (2)$$

Where *A* represents the absorbance value of the ABTS$^{\cdot+}$ control solution and *B* the absorbance value of the testing solution. Also, the mean effective concentration (EC_{50}) was calculated as the concentration of sample that reduced 50% of ABTS$^{\cdot+}$ radical under the assayed conditions. The trolox equivalent antioxidant activity was expressed in μmol of Trolox Equivalent (TE) per mg of dry sample.

2.6.3 | Ferric reducing power assay (FRAP)

The FRAP assay was determined according to modified method of Szôllôsi and Szôllôsi Varga (2002); with slight modification, reported by Sandoval-Gallardo et al. (2020). Trolox was used as a reference standard. The reducing power was expressed in in μmol of Trolox Equivalent (TE) per mg of dry sample.

2.7 | Experimental feeds

Three experimental feeds for Nile tilapia (*O. niloticus*; Table 1) were elaborated, following the reported by Hernández et al. (2011). The control feed (CF) did not contain herring protein hydrolysate. Protein hydrolysates were added with a concentration of 5% (w/w), substituting the total protein content of the fishmeal, based on the methodology described by Hernández et al. (2013). All the diets were isoproteic (35% protein) and isolipidic (8% lipids) and their formulation was made based on the bromatological analysis (protein, lipids, ash and moisture) performed.

2.8 | Nile tilapia (*O. niloticus*) organisms, facilities and experimental procedures

The Nile tilapia (*O. niloticus*) used during the experiment were randomly selected from a batch of 400 juveniles donated by the company Spring Genetics, located in Los Pozos, El Rosario, Sinaloa, Mexico. The feeding experiment was performed in an indoor, closed, freshwater system located at the Centro de Investigación en Alimentación y Desarrollo (CIAD Mazatlán).

The 6th week experiment was carried out with a batch of 180 juvenile Nile tilapia with an initial mean weight (±SD) of 0.5 g (±0.1 g). Fish were distributed in nine white-bottomed fibreglass tanks containing 150 L of freshwater (20 organisms were assigned per tank

TABLE 1 Ingredient and proximate composition of the experimental feeds for Nile tilapia (*Oreochromis niloticus*)

Ingredients (g/kg wet weight)	Feeds		
	CF	FH10	FH30
Fish flour[a]	43.00	40.86	40.86
Herring protein hydrolysate (PH)	0	1.73	1.59
Soybean paste flour[b]	11.56	11.56	11.56
Sardine Oil[a]	1.51	1.66	1.71
Wheat flour[c]	29.00	29.00	29.00
*Vitamins and minerals premix[d]	0.45	0.45	0.45
Carboxymethylcellulose[f]	14.48	14.74	14.83
Total	100.00	100.00	100.00
Proximal composition g/kg, Dry weight (%)			
Dry matter	94.18	93.84	93.62
Protein	35.22	35.69	35.88
Lipids	8.46	8.60	8.37
Ashes	9.43	9.41	9.48
NFE[e]	41.07	40.14	39.89

Note: FH10 and FH30 were supplemented with protein hydrolysate.

Abbreviations: CF, control feed; FH10, feed with a PH at 10% of DH; FH30, feed with a PH at 30% of DH.

[a]Maz Industrial S.A de C.V.

[b]La Casa del ganadero S.A de C.V.

[c]Harina de trigo Selecta.

[d]Donated by Trout Nutrition Mexico S.A. de C.V. *Vitamins and minerals premix composition: manganese, 100.00 g; magnesium, 45.00 g; zinc, 160.00 g; iron, 200.00 g; copper, 20.00 g; iodine, 5.00 g; selenium, 0.40 g; cobalt 0.60 g, vitamin A, 2400 IU/g; vitamin D3e, 2250 UI/g; vitamin E, 160.00 g; vitamin K3, 8.00 g; thiamine B1, 20.00 g; riboflavin B2, 40 g; pyridoxine B6, 16.00 g; vitamin B-12, 80.00 mg; pantothenic acid, 60.00 g; nicotinic acid, 160.00 g; folic acid, 4.00 g; biotin, 0.50 g; vitamin C, 100.00 g; choline 300.00 g and filler 1046.85 g.

[e]NFE (nitrogen-free extract) with fibre included = 100 − (% moisture + % crude protein + % crude lipids + % ash).

[f]Drogueria cosmopolita, S.A. de C.V.

and each treatment was evaluated in triplicate), with continuous aeration, under a natural photoperiod (12 hours light:12 hours dark) and maintaining a temperature of 28 ± 1°C using heaters. The dissolved oxygen was maintained at a range of 5.0 ± 0.3 mg/L. The levels of total ammonia (NH_3+ NH_4^+), NO_2 and NO_3 were daily evaluated using test strips Insta-Test® (LaMotte Company). The tilapias were fed with three rations per day (08:00, 13:00 and 18:00 hours) based on their biomass. The fish were weighed individually every 2 weeks to calculate their mean body weight and the biomass present in each tank. The variables determined were the following: Specific growth rate (SGE), survival, total feed intake (TFI), protein efficiency ratio (PER), viscerosomatic index (VI) and hepatosomatic index (HI); they were calculated as follow (Xia et al., 2018).

$$\text{SGE}(\%) = \frac{\ln \text{final weight (g)} - \ln \text{initial weight (g)}}{\text{time (days)}} \times 100 \quad (3)$$

$$\text{S}(\%) = \frac{\text{organisms final count}}{\text{organisms initial count}} \times 100 \quad (4)$$

$$\text{TFI}(\%) = \frac{\text{feed consumed (g)}}{\text{total weight gained (g)}} \times 100 \quad (5)$$

$$\text{PER} = \frac{\text{weight gain (g)}}{\text{protein consumed}} \quad (6)$$

$$\text{VI} = \frac{\text{viscera weight (g)}}{\text{total weight (g)}} \times 100 \quad (7)$$

$$\text{HI} = \frac{\text{liver weight (g)}}{\text{total weight (g)}} \times 100 \quad (8)$$

2.9 | Heat stress procedure

Once the 6-week feeding period was over, the heat stress challenge was immediately continued. For each treatment, 30 organisms were randomly selected and assigned to a white-bottom fibreglass tank with a capacity of 400 L, fish density was kept raising the volume of water to 225 L. Previously, the corresponding treatment was assigned (CF, FH10 or FH30) and 500-watt aquarium heaters were adapted. Once in the tanks, the organisms were acclimatized for 24 hours at an initial temperature of 28 ± 1°C and subsequently the temperature was increased by 2°C every 24 hours, until 36°C was reached.

The water temperature values used for the heat stress trial (28, 32 and 36°C) were selected taking into account that optimal temperatures reported for Nile tilapia culture ranging between 25 and 28°C (El-Sayed, 2006). Whereas it has been reported that temperatures higher than 36°C may be hazardous to tilapia affecting its metabolic and immune responses and reducing food appetite and growth performance (Islam et al., 2019; Mahmoud et al., 2020).

When the desired temperature was reached and after 24 hours of exposition, the organisms were sacrificed by introducing them into a clove extract solution (*Syzygiuma romaticum*) and then, six organisms per treatment, were measured in total length and weight. Finally, fish were carefully dissected at low temperature to obtain their livers, which were weighed and immediately stored and kept at −80°C until processing.

2.10 | In vivo determination of antioxidant enzymes activity, catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GPX) in Nile tilapia livers

2.10.1 | Obtention and preparation of liver tissue from Nile tilapia under heat stress conditions

The livers were cold washed (4°C) with a phosphate buffer saline (PBS) pH 7.4, then transferred to vials and homogenized with 500 µl of PBS buffer. The homogenized was centrifuged at 5000 *g* for 15 minutes at 4°C. As a result of centrifugation, three phases were obtained, an upper solid (lipid phase), liquid (cell concentrate) and a lower solid (cellular remains), only the liquid part was extracted and transferred to another vial, which was centrifuged again with the same conditions. The supernatant was used to perform subsequent enzymatic analyses.

2.10.2 | CAT activity determination

Catalase activity was determined using the Cayman kit (Cat. 707002). This method is based on the reaction of the enzyme catalase with methanol in the presence of H_2O_2; the formaldehyde produced is measured calorimetrically with the chromogen 4-amino-3-hydrazino-5-mercapto-1,2,4-triazole (purpald) at 540 nm.

2.10.3 | SOD activity determination

The SOD test was performed following the indications of a Sigma-Aldrich kit (19160), incubating the samples at 37°C for 20 minutes, and then the absorbance was recorded at 450 nm .

2.10.4 | GPX activity determination

The GPX test was carried out using a Cayman kit (Cat. 703102) following the supplier's instructions. The readings were made in a multi-modal microplate reader A unit is defined as the amount of enzyme needed for the oxidation of 1 nmol of NADPH to $NADP^+$ per minute at 25°C.

2.10.5 | Soluble protein quantification

The soluble protein content of tilapia liver extracts was determined by the Bradford method (1976) using bovine albumin serum (1 mg/ml) as protein standard.

2.11 | Statistical analysis

Data were reported as the mean ± standard deviation. All data were tested for normality and homogeneity of the variances using Kolmogorov–Smirnov and Bartlett's tests. Once the normality and homoscedasticity of the data (antioxidant activity and molecular weight distribution of PH, productive indexes and viscerosomatic and hepatosomatic index) were corroborated, they were processed by a one-way analysis of variance (ANOVA). The activity of antioxidant enzymes (SOD, CAT and GPx) were analysed by a two-way ANOVA, having as an independent variable: the feed supplied to the organisms (with three levels: FC, FH10 and FH30) and bioassay temperatures (with three levels: 28, 32 and 36°C). Thus, the interaction of independent variables was determined, Feed (A) and temperature (B) with the antioxidant enzymes activity as response variable. Statistical differences were considered significant at $p < 0.05$ values. The differences among the means were determined using Tukey's HSD tests (Zar, 2013). The statistical package SIGMAPLOT 12 was used for data analysis.

3 | RESULTS

3.1 | Biochemical and antioxidant characterization of herring protein hydrolysates

The antioxidant activity results of the PH analysed, showed that PH30 exhibited a higher antioxidant response ($p < 0.05$) compared to PH10 in all antioxidant tests performed, so it should be noted that a higher degree of hydrolysis (DH) had a positive effect in the increased antioxidant activity of the herring hydrolysates (Table 2). Regarding the distribution of molecular weight (Table 2), it was observed that PH10 did not contain peptide fractions at ranges of 158–44 and 44–17 kDa; also, the highest peptide content was shown at 17–1.35 kDa (73.3%) fraction, while the <1.35 kDa was in a smaller proportion. On the other hand, the PH30, contained a small proportion at ranges of 158–44 and 44–17 kDa, while the highest number of peptides were distributed between fractions 17–1.35 and <1.35 kDa, the latter being the fraction that contained the largest number of smaller peptides (56.2%), matching with the highest DH values in the PH30 sample. The amino acid (AA) composition of the

TABLE 2 Antioxidant activities assessed with DPPH, ABTS and reducing power (FRAP) and molecular weight distribution of peptide fractions present in herring hydrolysates

	Antioxidant activities			
Sample	DPPH		ABTS	FRAP
PH10	145.2 ± 3.4[b]		95.64 ± 1.5[b]	0.81 ± 0.01[a]
PH30	201.8 ± 2.2[a]		165.16 ± 1.1[a]	0.99 ± 0.02[b]
	Peptide distribution (%)[†]			
	158–44 kDa	44–17 kDa	17–1.35 kDa	<1.35 kDa
PH10	ND	ND	73.32 ± 0.9	26.67 ± 0.3
PH30	0.33 ± 0.011	1.46 ± 0.2	42 ± 0.3	56.22 ± 0.7

Note: Data are shown as means ± standard deviation of triplicate determinations. Values with different letter for each column are statistically different ($p < 0.05$). DPPH and FRAP were evaluated at concentration of 50 mg/ml while ABTS at 1 mg/ml. Antioxidant activity is expressed in μmol of Trolox equivalent (TE)/mg of dry sample.

Abbreviation: ND, Not detected.

[†]Data are expressed as percentages of the areas in the chromatograms.

protein hydrolysates (PH10 and PH30) is presented in Table 3. In both hydrolysates, Glu > Lys > Asp > Arg are the predominant AA in proportional quantities (Glu: 18.92/16.23; Lys: 14.71/13.20; Asp: 10.26/10.27 and Arg: 7.88/7.67 g/100 g protein [PH10 and PH30 respectively]).

3.2 | Productive performance of Nile tilapia (*O. niloticus*)

Table 4 shows the productive performance parameters obtained from organisms after 6 weeks maintained with the supplemented feeds containing herring protein hydrolysates. On all treatments a tilapia survival of 100% was achieved. The experimental feeds were widely taken by tilapias and all feed content offered was consumed. The weight of the organisms increased continuously throughout the experiment, reaching an increase in up to six times their initial weight. The organisms fed with FH10 shown a higher final weight ($p < 0.05$) respect to the fed with FH30 and the control feed. The same behaviour was observed in specific growth rate, protein efficiency ratio and the apparent nitrogen utilization (Table 4).

On the other hand, Table 5 showed the estimated values for the hepatosomatic (HI) and viscerosomatic (VI) indexes determined on tilapias fed with the different supplemented diets, where no significant differences ($p > 0.05$) were found in HI and VI values on organisms fed with CF, FH10 and FH30 respect to their initial condition.

TABLE 3 Total amino acid (AA) composition (g/100 g of protein) of protein herring hydrolysates and formulated feeds

AA	PH10	PH30	CF	FH10	FH30
EAA					
Arg	7.88	7.67	8.35	7.16	7.79
His	1.10	2.65	2.16	2.05	2.82
Ile	5.13	5.14	4.38	5.42	5.09
Leu	7.06	6.65	6.62	9.11	7.95
Lys	14.71	13.20	9.65	11.08	11.13
Met	2.65	2.90	1.52	0.89	1.72
Phe	4.31	4.65	3.20	2.30	3.71
Thr	2.65	3.84	3.49	2.72	3.59
Val	6.03	6.26	5.41	6.77	6.30
NEAA					
Ala	5.85	6.11	6.23	5.76	5.82
Asp	10.26	10.17	10.12	11.87	10.11
Glu	18.92	16.23	20.51	22.67	20.14
Gly	4.82	5.00	6.95	7.10	6.78
Ser	4.17	4.32	3.05	1.76	3.35
Tau	0.28	0.40	5.30	0.00	0.10
Tyr	4.19	4.80	3.06	3.34	3.58
Total EAA	51.51	52.97	44.78	47.49	50.12
Total NEAA	48.48	47.03	55.22	52.51	49.88

Abbreviations: CF, control feed; EAA, Essential amino acids; FH10, feed with a PH at 10% of DH; FH30, feed with a PH at 30% of DH; NEAA, non-essential amino acids.

3.3 | Effect of thermal stress on enzyme activity of catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GPX) in Nile tilapia livers

In Figure 1, CAT enzymatic activity present in liver tissues of tilapias under an acute heat stress is shown. The results show that the organisms fed with FH10 and FH30 exhibited an increase in CAT activity in the first rise of temperature (28–32°C); subsequently, in the second rise (from 32 to 36°C) at 48 hours, the activity decreased significantly ($p < 0.05$), returning to values like the initial ones. In the case of the organisms fed with the CF diet, they presented a

TABLE 4 Productive performance evaluated in Nile tilapia (*O. niloticus*) for 6 weeks ($n = 3$) and maintained with supplemented feeds containing herring hydrolysates with different degree of hydrolysis

	Feed		
Parameters	**CF**	**FH10**	**FH30**
Initial weight (g)	0.491 ± 0.001	0.491 ± 0.001	0.491 ± 0.001
Final weight (g)	3.53 ± 0.15^{b}	3.86 ± 0.06^{a}	3.63 ± 0.06^{b}
Weight increment (g)	3.04 ± 0.15^{a}	3.37 ± 0.06^{ab}	3.14 ± 0.09^{b}
TFI (mg/tilapia)	59.86 ± 1.92^{a}	60.94 ± 2.85^{a}	61.67 ± 1.48^{a}
PER	3.16 ± 0.1^{b}	3.28 ± 0.20^{a}	3.06 ± 0.15^{b}
FCR	1.22 ± 0.03^{a}	1.12 ± 0.07^{b}	1.22 ± 0.03^{a}
SGR (%/day)	3.18 ± 0.07^{b}	3.32 ± 0.03^{a}	3.23 ± 0.04^{b}
ANU (%)	64.21 ± 1.97^{b}	77.98 ± 4.63^{a}	62.12 ± 4.45^{b}
Survival (%)	100 ± 0	100 ± 0	100 ± 0

Note: Data are shown as means ± standard deviation of triplicate determinations. Values with different letter in the same line are statistically different ($p < 0.05$). Apparent nitrogen utilization (ANU %) = (carcass nitrogen deposition/nitrogen intake) × 100. Feed conversion ratio (FCR) = TFI/weight gain.

Abbreviations: CF, control feed; FCR, feed conversion ratio; FH10, feed with a PH at 10% of DH; FH30, Feed with a PH at 30% of DH; PER, protein efficiency ratio; SGR, specific growth rate; TFI, total feed intake.

TABLE 5 Viscerosomatic (VI) and hepatosomatic (HI) indexes evaluated in Nile tilapia (*O. niloticus*) for 6 weeks ($n = 3$) and maintained with supplemented feeds containing herring hydrolysates with different degree of hydrolysis

	Initial weight	CF	FH10	FH30
VI	8.35 ± 0.25^{a}	8.00 ± 0.29^{a}	8.23 ± 0.30^{a}	7.96 ± 0.15^{a}
HI	2.41 ± 0.13^{a}	3.03 ± 0.25^{a}	2.91 ± 0.36^{a}	2.94 ± 0.34^{a}

Note: Data are shown as means ± standard deviation of triplicate determinations. Values with different letter in the same line are statistically different ($p < 0.05$).

Abbreviations: CF, control feed; FH10, feed with a PH at 10% of DH; FH30, feed with a PH at 30% of DH.

significant increase in CAT activity ($p < 0.05$) only when the temperature of 36°C was reached.

The SOD activity is showed in Figure 2; the results indicated an activity increase with the rise in temperature. For the organisms fed with FH10, when kept at a temperature of 32°C, a greatest increase in SOD activity was shown; the same trend can be seen for the FH30 feed, showing a significant increase ($p < 0.05$) in the activity when temperature was kept at 32°C. In the CF treatment, a significantly lower activity was observed ($p < 0.05$) exhibiting the highest activity at 36°C.

Figure 3 shows the activity of GPx measured on tilapia livers samples. Unlike the activity of SOD and CAT, GPx exhibited enzyme activity values up to three times higher in the organisms that was feeding with supplemented diets respect to control feed. A significant increase ($p < 0.05$) in the response of enzymatic activity is shown with increases in temperature in all treatments. The greatest increases in activity occurred in the FH30 treatment at 32 and 36°C, showing no significant differences ($p < 0.05$) between these two temperatures.

4 | DISCUSSION

4.1 | Biochemical and antioxidant characterization of herring protein hydrolysates

Antioxidant activity has been reported to be dependent on several factors, mainly those related to chemical composition, such as amino acid (AA) content and molecular mass of peptides (Benjakul et al., 2014). In this study, the highest antioxidant activity exhibited by PH30, agrees with that reported by Sepúlveda and Zapata (2020) in red tilapia (*Oreochromis* spp.) protein hydrolysates; this effect would be related to the diversity of the smallest peptides produced at high DH values, where in this study the PH30 had a higher percentage of the smallest peptides (<1.35 kDa) with respect to PH10 (Table 2). Also, Farvin et al. (2016) observed for hydrolysates of cod (*Gadus morhua*) an elevated antioxidant activity on the peptide fractions of lowest molecular weights (<3 kDa), coinciding with the results obtained in this study. Regarding to amino acid (AA) composition, Morales-Medina et al. (2016), reported for sardine protein hydrolysates (*Sardine pilchardus*) an elevated content of Glu, Asp and Lys, affirming that those AA acts mainly as metal chelators and free-radical's neutralizers. Moreover, in this study was observed that PH30 contains more than twice as much (2.65%) of His as PH10 (1.1%), and its presence may be related to the elevated antioxidant activity of PH30. Regarding to essential amino acid (EAA) content more than a half of the total AA represents EAA (51.5% and 52.97% for PH10 and PH30 respectively). These results support the supplementation of diets with this kind of samples that are an excellent source of quality protein.

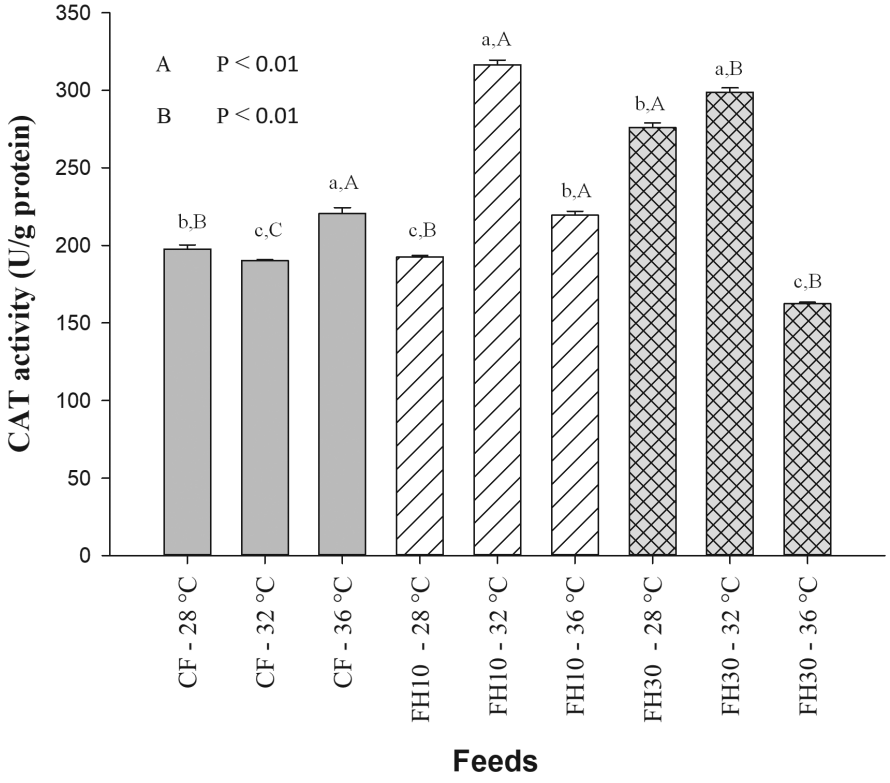

FIGURE 1 CAT activity in tilapia livers under heat stress. Different lowercase letters indicate the existence of significant differences ($p < 0.05$) between the same feed; capital letters indicate significant differences between the same temperature values ($p < 0.05$). The mean of the data is presented ($n = 3$). Vertical bars indicate standard error. CF, control feed; FH10, feed with a PH at 10% of DH; FH30, feed with a PH at 30% of DH. A = feed, B = temperature.

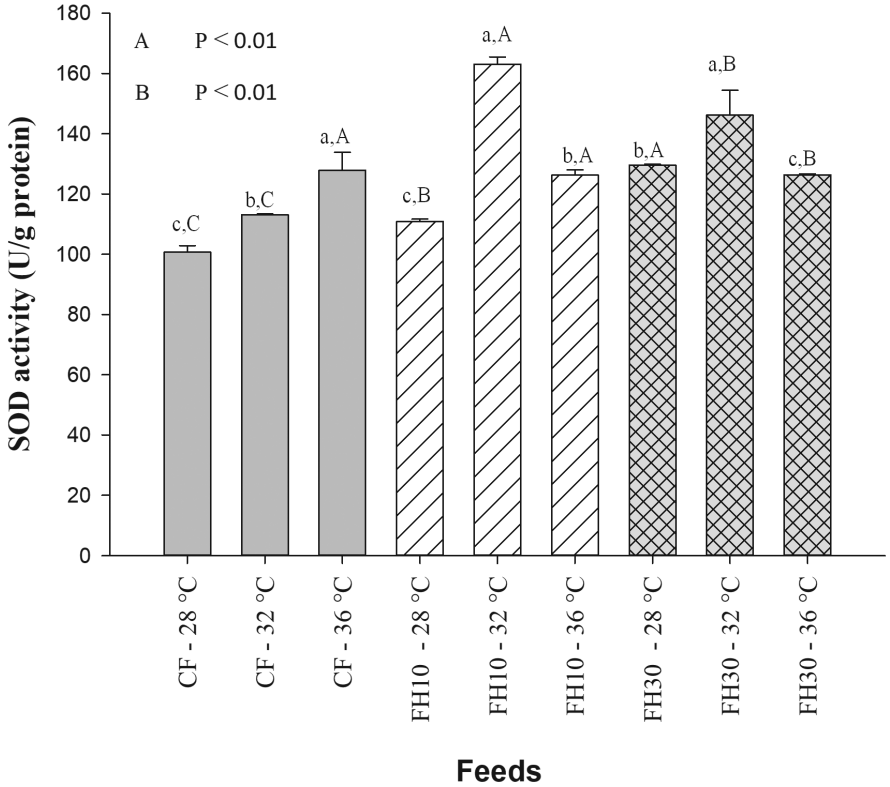

FIGURE 2 SOD activity in tilapia livers under heat stress. Different lowercase letters indicate the existence of significant differences ($p < 0.05$) between the same food; capital letters indicate significant differences between the same temperature values ($p < 0.05$). The mean of the data is presented ($n = 3$). Vertical bars indicate standard error. CF, control feed; FH10, feed with a PH at 10% of DH; FH30, feed with a PH at 30% of DH. A = feed, B = temperature.

4.2 | Productive performance of Nile tilapia (*O. niloticus*)

Interestingly, despite the similarity of productive performance values, it is observed that organisms fed with the supplemented diets registered the best responses compared to the control feed (without inclusion of protein hydrolysate); this positive effect could be related to the superior bioavailability of hydrolysates, enhancing their transport through the intestinal membrane of the evaluated organisms; Bui et al. (2014), stated this point observing an elevated final weight,

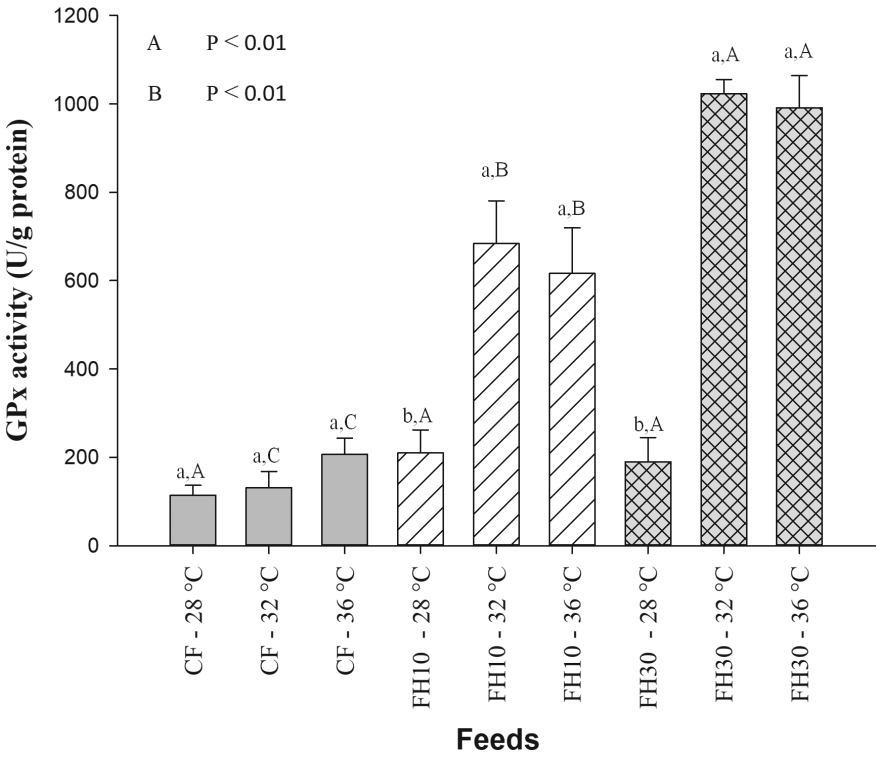

FIGURE 3 GPx activity in tilapia livers under heat stress. Different lowercase letters indicate the existence of significant differences ($p < 0.05$) between the same food; capital letters indicate significant differences between the same temperature values ($p < 0.05$). The mean of the data is presented ($n = 3$). Vertical bars indicate standard error. CF, control feed; FH10, feed with a PH at 10% of DH; FH30, feed with a PH at 30% of DH food with inclusion of sardine hydrolysate at 30% of DH. A = feed, B = temperature.

specific growth and protein efficiency rate on for Japanese snapper (*Pagrus major*) fed with tilapia hydrolysates.

Results like those obtained in this research were observed by Hernández et al. (2013), reporting better productive performance in Nile tilapia that was feeding with supplemented diets containing tuna protein hydrolysates, comparing to control diets (without tuna hydrolysate). In the same way, Sary et al. (2017), obtained a better performance in weight of Nile tilapia using diets supplemented with protein hydrolysates from tilapia viscera. These authors attribute this result to the fact that protein content in diets is more available for absorption and, in turn, stimulate the intracellular activity of peptidases, thus, allowing a better assimilation of amino acids.

The VI and HI indices allow us to identify certain conditions during the tilapia development due to the feeds supplied. These indices relate the weight gain of the viscera and liver by the fat accumulated around them, which must be kept as low as possible, because the weight of the organism must be increased mainly in muscle. Our results are consistent with the findings of Triana-García et al. (2013), reporting VI values near to 8.5 and 2.5 for HI, when fed hybrid tilapia (*Oreochromis* spp.) with different lipid sources. In the same way, Del Toro (2017), did not find significant differences between the VI and HI values when substituted fish meal with a mixture of animal and vegetable flours (Iberic pig meal, soybean meal, pea and sunflower meal) to fed gilthead sea bream (*Sparus aurata*).

Besides, according to Triana-García et al. (2013) HI value is related to the consumption of lipids; when aquatic organisms are fed with high concentrations of long-chain polyunsaturated fats an inhibition of enzymes responsible of lipid conversion (i.e. lipoprotein lipase and hepatic lipase) could occur, causing the utilization of proteins from muscle tissue to produce energy and consequently affect the final body's protein weight by the accumulation of fat in liver fish. Regarding to changes on VI values, Monge-Ortiz et al. (2016), observed an increment in VI on organisms fed with high proportion of vegetable protein, which is usually less digestible and can causes a lower availability of protein and a greater absorption of fat reserves. Considering that HI and VI values on tilapias fed with CF, FH10 and FH30 exhibited no differences ($p > 0.05$) respect to the initial weight, we can sight that herring protein hydrolysates had a positive effect on protein utilization of organisms and consequently in their weight (Tables 4 and 5).

Regarding the EAA content in formulated feeds utilized in this study (Table 3) meet the necessary requirements recommended by some authors (El-Sayed, 2006; Fitzsimmons, 2005; Lim & Webster, 2006; Shiau, 2002) for elaborations of Nile tilapia (*O. niloticus*) diets (AA% required in diet according to FAO: Arg: 1.18, His: 0.48, Ile: 0.87, Leu: 0.95, Lys: 1.43, Met: 0.75, Phe: 1.05, Thr: 1.05, Val: 0.78).

An unexpected result related to tilapia productive performance, was the slight highest productive performance (Table 4) exhibited by organisms fed with FH10, which contained 0.89 g/100 g of Met, whereas FH30 contained 1.72 g/100 g of this limiting AA. According to Nguyen and Allen Davis (2009), the sulphur amino acid Cys can replace Met in those diets where this is deficient; regrettably Cys content in diets of present study was not determined. Thus, we strongly suggest to determine essential AA as Cys and Trp to clarify anomalous results. Moreover, further study also is needed to establish Met absorption, utilization and its role as signalling metabolic pathways on fish fed with protein hydrolysates supplemented with Met at different inclusion levels. Some interesting researches related to this topic, are the conducted by Wu et al. (2017), Wang et al. (2021) and Teodósio et al. (2022).

Hernández et al. (2013), stated point that an aquafeed must contain an adequate concentration of EAA to promote the development and weight gain of organisms. In fact, the lack of adequate requirements of EAA in diets, could trigger important physiological problems, preventing growth and in many cases affecting survival of organisms. In this study, the content of EAA in FH10 was lower compared to NEAA; on FH30 the EAA content is slightly higher than NEAA. We can highlight those supplemented feeds exhibited a higher content of EAA than control feed (with no herring protein hydrolysate); therefore, the positive effect observed in productive performance of the evaluated organisms (Tables 3 and 4) could be related to the adequate content of EAA in FH10 and FH30. In fact, unlike of results obtained in this study, the lack of EAA in tilapia feeds has been reported. For instance, Met, Val and Thr deficiency cause a weight and feed intake loss in organisms (Diógenes et al., 2015); while the absence of Met and Thr can produce a reduction in growth and forfeit of nitrogen disposition (Marammazi et al., 2017). For the specific case of juvenile phase tilapias, Rodrigues et al. (2020), concluded that Met, Trp and Val are the most limiting EAA for nitrogen retention and consequently in the growth of organisms. Therefore, it is of vital importance to meet the adequate nutritional requirements for the development of tilapia.

Another interesting role of amino acids in fish formulated diets is their feeding stimulant effect, that strongly influence food consumption (Kolkovski et al., 2000). Leal et al. (2010) reported that Ala, Gly, Pro, Val, Thr, Tyr, Phe, Gly, Lys and His, act as a food stimulant for many fish species. In this regard, the amino acid composition of FH10 and FH30 presented an elevated concentration of some of these amino acids, such as Lys, Gly, Val and Ala (Table 3) and could influence the organisms behaviour observed in this study, consuming the supplemented feeds in an avid way.

4.3 | Effect of thermal stress on enzyme activity of catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GPX) in Nile tilapia livers

The SOD converts the anion superoxide to hydrogen peroxide, and CAT, in turn, displays its catalytic activity by neutralizing it to oxygen and water (Ahmed et al., 2017). In aquatic animals, SOD and CAT are widely distributed and exhibit a defensive mechanism against oxidative stress. Some authors (Abdel-Tawwab & Wafeek, 2017) have reported an increase in the activity of antioxidant enzymes (SOD and CAT) in tilapia livers, when the temperature in the organisms was increased, due to the formation of oxidizing species, attributing this effect to the raising in temperature, which provokes the production of oxidizing species and consequently the activation of the tilapia antioxidant system. In this sense, the same behaviour can be observed in this study, highlighting those organisms fed with FH10 and FH30 exhibited an increase ($p < 0.05$) on SOD and CAT values after thermal stress at 32°C (Figures 1 and 2).

Moreover, Xian-Quan et al. (2019) who determined the activity of SOD in tilapia livers fed with diets supplemented with yeast protein hydrolysates, showing a significant increase in the activity of SOD, concluding that protein hydrolysates improve the antioxidant enzyme system, because they exert a prebiotic effect on the blood and liver of aquatic organisms.

Since a biochemically point of view, there is a close interaction between SOD and CAT; in the case of CAT, the raise in temperature increases the enzymatic response of organisms due to a greater production of H_2O_2 by SOD, mainly in the mitochondria and peroxisomes (Ahmed et al., 2017). Interestingly, Hardy et al. (2020), reported that yeast protein hydrolysates could reduce the liver damage produced during the oxidative stress, by improving the activity of CAT and SOD in tilapia liver.

The increase in activity values of GPx, could be related to the scavenging of hydrogen peroxide (H_2O_2) mediated by GPx in the cytosol. This antioxidant defence system, transforms H_2O_2 to lipoperoxide (L-OOH), using reduced glutathione (GSH) as a reducing agent (Kurutas, 2015). In this sense, a positive effect of the antioxidant system of Nile tilapia that was fed with diets supplemented with herring protein hydrolysates is showed. An increase in GPx values in pearl spot (*Etroplus suratensis*) livers was observed by Joy et al. (2017), after an acute increment of temperature (38°C) at 24 and 48 hours of exposition.

Our results suggest that the elevated enzyme activity ($p < 0.05$) of CAT, SOD and GPx (Figures 1, 2 and 3) detected in tilapia livers that consumed supplemented feeds (FH10 and FH30), respect to control feed after thermal stress (mainly at 32°C), could be related to the interrelated catalytic performance of CAT, SOD and GPx to protect organisms against oxidative stress. According to Esfandi et al. (2019), since CAT, SOD and GPx are metalloenzymes, its activity could be strongly affected by peptides that are present in protein hydrolysates, which interact with metals (iron, selenium, zinc or manganese) protected them from oxidation, thus benefiting the activity of antioxidant enzymes.

In conclusion, the formulated feeds supplemented with herring protein hydrolysates contained acceptable requirements of essential amino acids and had a positive effect on the productive performance of Nile tilapia. The acute thermal stress applied to organisms (28, 32 and 36°C) triggered the catalytic activity of SOD, CAT and GPx measured in tilapia livers, exhibiting higher activity values at 32 and 36°C than 28°C. Fish that was feeding with FH10 presented a better productive performance and also a marked increase in SOD and CAT activity. These results support the fact, that herring protein hydrolysates can be considered as a functional ingredient that can provide nutritional and metabolic benefits (i.e. protection against oxidation induced by thermal stress) of aquatic organisms that are important commodities worldwide, such as Nile tilapia. Further research is recommended to study the mRNA expression of SOD, CAT and GPx in tilapia livers in order to elucidate the mechanisms of action of protein hydrolysates in the antioxidant defence system of tilapia.

AUTHOR CONTRIBUTIONS

Jorge Manuel Sandoval-Gallardo: Performed the experimental work and wrote the paper. Idalia Osuna-Ruiz, Emmanuel

Martínez-Montaño, Miguel Ángel Hurtado-Oliva, José Basilio Heredia, Crisantema Hernández, Gissel Daniela Rios-Herrera, Jorge Saúl Ramírez-Pérez and María Isaura Bañuelos-Vargas: Contributed data and analysis of samples. Jesús Aarón Salazar-Leyva: Conceived an designed the analysis. Drafted the manuscript and revised it critically for important intellectual content.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the results in this study are available from the corresponding author upon a reasonable request.

ETHICAL APPROVAL

Ethical approval was not required for this research.

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