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Research Article

Changes on the Development of *Rigor Mortis* in Cultured Tilapia (*Oreochromis niloticus*) Fed with a Mixture of Plant Proteins

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In recent years it has been pointed out that the feed of farmed fish has an effect on the quality of the final product. Therefore, this study evaluated the effect of fishmeal (FM) replacement by a mixture of plant protein (MPP) on the development of *rigor mortis* of tilapia (*Oreochromis niloticus*). One hundred and twenty fish at an initial average weight of 123 ± 6.3 g were fed with three extruded isonitrogenous and isolipidic 6.2% crude lipids experimental diets, in which FM were replaced by 0% (D0), 50% (D50), and 100% (D100) of MPP (soybean meal, corn meal, wheat meal, and sorghum meal). A reference diet (DC) containing FM as the main protein source was used as a control. The fish were divided into triplicate groups per dietary treatment. The experiment was conducted in a tank system at 26.8°C water temperature for 67 days. The chemical composition of experimental diets and muscle were determined. The glycogen, adenosine 5′-triphosphate (ATP) and related compounds, pH, shear force, and rigor index (RI%) were monitored during storage on ice for 48 h. The results indicated that FM replacement affected ($p \leq 0.05$) the muscle composition, where the fish fed with D100 presented the higher content of lipids and ash. Fish fed with D0 and DC presented a more pronounced onset of *rigor mortis* and also showed a higher IR%, a lower content of glycogen, ATP, adenosine 5′-diphosphate (ADP), adenosine 5′-monophosphate (AMP), pH, and shear force. The changes in chemical composition of muscle and other parameters evaluated indicated that FM replacement increases energy reserves (glycogen, ATP, ADP, and AMP) which delayed the onset of *rigor mortis*, as well as a lower pH and shear force in the muscle of tilapia. Therefore, the substitution of FM by MPP could contribute to delaying the onset of *rigor mortis* and with this, the quality and shelf life of tilapia could be increased.

1. Introduction

The Nile tilapia is a tropical fish with rapid growth rate, good quality flesh, high resistance to disease, adaptability to a wide range of environmental conditions, ability to reproduce and grow in captivity, and feeds efficiently on natural fauna and flora [1]. The world production of tilapia in 2015 was 5,576,800 mt and is expected to increase in the coming years [2]. Tilapia culture is considered as a dynamic activity and is increasing. Currently, consumption of tilapia fish has been

widely accepted by consumers. Besides being considered as an excellent food source, culture of tilapia represents a potential source of income [1, 3].

The development and profitability of fish cultivation such as tilapia depends inevitably on the availability of commercial foods that meet the essential nutrients requirements to ensure optimal growth and performance of the fish. However, a factor that represents the largest expenditure of operations in fish culture is the balanced feed [4]. The fish under culture require high levels of protein to meet their nutritional requirements. To accomplish this, fishmeal (FM) is used as the main ingredient because it has high palatability and high nutritional value; however, it is very expensive and is not always available [5]. For this reason the search for alternative sources that are suitable, inexpensive, and available to replace fishmeal with plant protein is under course.

At present, one plant protein source used for the partial replacement of fishmeal in feed is soybean meal, which has high protein content, it is abundant and at low cost [6]. Other alternative proteins used are wheat, sorghum, corn meal products, and byproducts of terrestrial animals such as blood meal, feather, and bone steak. These sources have been used because of their viability as a replacement and low cost [5]. Those alternative sources have been found to promote a performance similar or better than that obtained with formulations containing fishmeal [7].

Currently, research in fish cultivation has focused on improving production system, such as knowledge of reproductive physiology, genetic aspects, and nutritional requirements [5]. However, product quality aspects have been overlooked. It has been described that the muscle quality of any aquatic organism (fish, crustaceans, and molluscs) decreases immediately after the capture and death of the animal, and due to the fact that the blood circulation stops, the oxygen transport and the natural defenses against bacteria cease [8]. Consequently, an anaerobic condition is generated in the muscle and the tissue becomes more susceptible to deterioration. From there, a series of biochemical changes are developed, such as *rigor mortis*, energy production, autolysis by endogenous proteases, degradation of adenosine-5′-triphosphate (ATP), pH decrease, and protein denaturation. These changes cause an increase in the ammonia concentration, TMA, peptides, and other amines, as well as changes in color, texture, taste, and odor [9]. Of these changes, *rigor mortis* is one of the most important postmortem events in the muscle of different fish species; it starts immediately after death of fish, when the glycogen reserves and ATP are depleted, or if the fish is stressed, and is manifested by stiffness and muscle inextensibility [10, 11]. In fish muscle, ATP is metabolized according to the following sequence: ATP $\longrightarrow$ adenosine-5′-diphosphate (ADP) $\longrightarrow$ adenosine-5′-monophosphate (AMP $\longrightarrow$ inosine-5′-monophosphate (IMP $\longrightarrow$ inosine (HxR) $\longrightarrow$ hypoxanthine (Hx). ATP rapidly decreases within the first 24 h postmortem, and depending on the rate of degradation of the ATP, it will impact on the rate of onset of *rigor mortis*. As a consequence, the quality of the product is affected, modifying appearance, water holding capacity, color, and texture of the final product [12].

Different studies have evaluated factors related to the development of *rigor mortis* such as species, stress, fasting, acclimation temperature, and method of slaughter [13, 14]. However, to date there are no studies of how diet, composition, and origin of ingredients affect the *rigor mortis* of the organisms. This study was carried out to evaluate physics, chemistry, and biochemistry changes on the development of *rigor mortis* in tilapia (*Oreochromis niloticus*) fed with a mixture of plant proteins. Likewise, a growth trial was carried out to show that the fish were growing normally using the MPP (the results of this trial is not shown).

2. Materials and Methods

2.1. Experimental Diets. Three extruded isonitrogenous and isoenergetic experimental diets (37% crude protein) were formulated replacing 0% (D0), 50% (D50), and 100% (D100) of the protein from fishmeal (FM) by a mixture of plant proteins (soybean meal, corn, wheat, and sorghum) (Table 1). The D0 was formulated to simulate a commercial diet, while a commercial diet was used as a reference diet (DC) containing FM as the main protein source was used as control.

Prior to the preparation of the experimental diets, all ingredients were pulverized and sieved through a 500 μm mesh sieve. Dry ingredients of each diet were mixed thoroughly in a food mixer and oil (mixed fish oil and soybean lecithin) was added. Once the oil was dispersed in the dry ingredients, water was added to make a homogenous mixture. The resulting mixture was extruded using a simple Brabender laboratory screw extruder (Model E 19/25 D, Instruments Inc., South Hackensack, NJ, U.S.A) with the following characteristics: four heating zones, screw compression force 1 : 1, longitude/diameter relation (L/D) 20 : 1, and internal diameter of the exit die being 3 mm. The pellets were dried during the extrusion process, obtaining a moisture content between 8.8 and 8.9%, were manually reduced to approximately 0.35 mm, and stored in sealed polyethylene bags at 4°C until use.

2.2. Growth Trial. A 67-day growth trial was conducted in an outdoor tank water system with 90% daily water exchange, at the Wet Laboratory of Department of Scientific and Technological Research of the University of Sonora. The experimental system consisted of 16 tanks of 250 L capacity, which were filled with 230 L of water. Each dietary treatment was randomly assigned to four replicate tanks to evaluate the effects on biological performance of tilapia. *Oreochromis niloticus* adults were obtained from the fish farm Crilab at La Victoria, Sonora, Mexico. They were fed with 38% crude protein (CP) commercial diet three times daily for 10 days while acclimating to the laboratory conditions. Ten tilapia of similar size were randomly assigned in each tank at a density of 67 per m^3 with an average size and weight of 18.8 ± 0.3 cm and 123 ± 6.3 g. Tilapia were fed *ad libitum* three times daily (09 : 00, 13 : 00, and 17 : 00 h). The feeding rate was adjusted to 2.5% of the body weight, by weighing fish weekly. Uneaten feed and fecal wastes were removed daily before the next feeding.

Table 1: Formulation and chemical composition of experimental diets and muscle of cultured tilapia (*O. niloticus*).

	Diet			
	D0	D50	D100	Control
Ingredient ($g\ kg^{-1}$)				
Soy bean meal[1]	387.0	556.0	728.0	—
Fish meal (sardine)[2]	200.0	100.0	0.0	—
Corn meal[1]	178.0	74.9	42.9	—
Wheat flour[1]	134.4	140.0	112.0	—
Sorghum meal[1]	32.5	48.0	24.0	—
Fish oil[1]	37.0	50.0	62.0	—
Soy lecitin[3]	10.0	10.0	10.0	—
Vitamin premix[4]	8.0	8.0	8.0	—
Mineral premix[5]	5.0	5.0	5.0	—
Dibasic sodium phosphate[6]	5.0	5.0	5.0	—
Choline chloride[1]	2.0	2.0	2.0	—
Vitamin C[7]	1	1	1	—
Butylhydroxytoluene (BHT)[8]	0.1	0.1	0.1	—
Chemical composition of diet				
Moisture (%)	8.9 ± 0.2^a	8.8 ± 0.4^a	8.8 ± 0.6^a	8.8 ± 0.7^a
Protein (%)*	37.6 ± 0.2^a	37.6 ± 0.2^a	37.6 ± 0.2^a	38.0 ± 0.2^a
Lipids (%)*	6.2 ± 0.0^a	6.2 ± 0.0^a	6.2 ± 0.1^a	4.6 ± 0.1^b
Ash (%)*	5.6 ± 0.1^a	5.6 ± 0.0^a	5.6 ± 0.1^a	8.8 ± 0.0^b
Crude fiber (%)*	4.8 ± 0.1^a	4.8 ± 0.1^a	4.6 ± 0.1^b	4.5 ± 0.1^b
Chemical composition of muscle				
Moisture (%)	77.3 ± 0.7^a	77.3 ± 0.6^a	77.9 ± 0.6^a	77.0 ± 0.3^a
Protein (%)*	12.0 ± 0.8^a	12.7 ± 0.2^a	12.7 ± 0.0^a	13.3 ± 0.7^a
Lipids (%)*	4.3 ± 0.1^a	4.6 ± 0.3^a	5.3 ± 0.0^b	4.3 ± 0.4^a
Ash (%)*	2.7 ± 0.3^b	3.3 ± 0.0^a	3.3 ± 0.1^a	2.5 ± 0.2^b
Crude fiber (%)*	1.3 ± 0.2^a	1.3 ± 0.2^a	1.2 ± 0.1^a	1.3 ± 0.1^a

[1]Promotora Industrial Acuasistemas, S. A. de C. V., La Paz, BCS, México. [2]Pescaharina de Guaymas S. A de C. V. [3]ODONAJI. Distribuidora de Alimentos Naturales and Nutricionales S. A. de C. V., México, D. F. [4]Composition of the vitamin premix (g/kg premix): Vit. A_5, D_3 0.001, E_8, menadione 2, B_1 0.5, B_2 3, B_6 1, DL-Ca-pantothenate 5, nicotinic acid, H 0.05 inositol 5, B_{12} 0.002, folic acid 0.18, α-cellulose 865.266. [5]Composition of the mineral premix (g/100 g premix): $CoCl_2$ 0.004, $CuSO_4$. $5H_2O$ 0.25, $FeSO_4$. $7H_2O_4$, $MgSO_4$ $7H_2O$ 28.398, $MnSO_4$ H_2O 0.65, KI 0.067, Na_2SeO_3 0.01, $ZnSO_4$, $7H_2O$ 13.193, α-cellulose 53.428. [6]SIGMA Cat No. S-0876. SIGMA–ALDRICH Chemical Company, St. Louis, MO, USA. [7]Stay C (35% active agent). Roche, México, D. F. [8]butylated hydroxytoluene, ICN Cat. No.101162. Aurora, Ohio, USA. *% dry weight basis. Values are means of three replicates ± SD. Means within rows with different letter are significantly different ($p \leq 0.05$).

2.3. Sampling Procedures. Once the feeding trial was over, the water level in the tanks was reduced, and ice was added; therefore, the specimens were slaughtered by immersion in water/ice slurry. They had an average size and weight of 22.5 ± 0.1 cm and 223.1 ± 9.7 g, respectively. Immediately, ten slaughtered specimens per treatment were taken at random for the rigor index (RI%) evaluation. Slaughtered fish were placed on ice inside a hermetic cooler and transported to the Laboratory of Food Research at the University of Sonora. Fish samples were divided into two lots; one consisted of whole fish, which were stored for 48 h in ice and used to evaluate the effect of feed on rigor index in the whole fish. For the second lot, the fish was filleted, packed in polyethylene bags, and stored for 48 h in ice. At intervals of 6 h, samples of fillets were obtained, frozen, and stored at −80°C until analysis. ATP concentration, glycogen, pH, and shear force were determined for each sampling time. For each sampling time 6 fillets were analyzed.

2.4. Chemical Analysis. The chemical composition of the formulated diets and muscle of the tilapia after the bioassay were determined according to the standard methods reported in the AOAC [15]. Moisture, protein, lipid, ash, and crude fiber were determined. Samples were dried in a convection oven at 105°C for 5 h to determine moisture content. Crude protein (CP) was analyzed by Kjeldahl method and calculated from sample *N* content (total nitrogen × 6.25 = CP). Crude fat was analyzed using an FOSS semiautomatic extraction system (ST 243 Soxtec) with petroleum ether as the extracting solvent, and ash was determined by incineration at 550°C in a muffle furnace. Crude fiber was loss on ignition of dried residue remaining after digestion of sample with 1.25% H_2SO_4 (w/v) and 1.25% NaOH (w/v).

2.5. Glycogen. The glycogen content was determined in muscle samples by the anthrone method described by Racotta et al. [16]. One gram of muscle was homogenized with cold 10% trichloroacetic acid (TCA). The homogenate was centrifuged at 3000 × g at −5°C for 5 min. For glycogen, 0.1 mL of the supernatant was mixed with 1 mL of 95% ethanol and centrifuged under the same conditions. The precipitate (glycogen) obtained was resuspended in 0.1 mL distilled water. Then, 1 mL of anthrone reagent (0.1% dissolved in 76% sulphuric acid) was added to tubes that were

incubated at 90°C for 5 min. Absorbance was read at 620 nm in a Agilent Technologies spectrophotometer.

2.6. pH. The pH assays were carried out following the method described by Woyewoda et al. [17]. The measurement of pH was carried out using a Hanna HI 90140 penetration pH meter (Hanna Instruments, Inc.). Equipment was calibrated daily with commercial standard solutions.

2.7. ATP and Related Compounds. Quantification of ATP, ADP, AMP, IMP, HxR, and Hx was carried out by a reverse-phase high performance liquid chromatography procedure (HPLC) from a perchloric acid extract described by Ryder [18] The identification of nucleotides, nucleosides, and bases was made by comparing their retention times with those of commercially obtained standards and by adding or spiking of standards. Twenty μL of diluted extract was filtered through a 0.2 μm filter and then injected in a Agilent Technologies (Modelo 260 Infinity Series) chromatograph, using a C-18, 4.6 × 150 mm (Agilent Technologies) reverse-phase column. Mobile phase consisted of a phosphate buffer consisting of 0.04 M KH_2PO_4 and 0.06 M K_2HPO_4. A 1 mL/min flow was applied, carrying out the detection at 254 nm in a UV-Vis detector Varian Prostar 325 (Varian Inc., Lake Forest, CA). Results were expressed as μmol/g of sample.

2.8. Shear Force. Measurement of shear force was used to evaluate texture in tilapia muscle using a Warner–Bratzler blade in a testing machine (Model EZ TEST EZ-S, Shimadzu Corp.) equipped with a 50 kg cell. The crosshead speed was set at 20 cm/min and shearing force was transversally applied to the direction of the muscle fibers. Standardized cuts of 10 mm wide × 10 mm thick × 20 mm long of the upper back zone were used, and necessary force (N) to shear the muscle was recorded. Before the texture measurements, the cuts were acclimatized to room temperature and covered with cling wrap.

2.9. Rigor Index (RI%). Measurement of the *rigor mortis* was based on the tail curvature according to Bito et al. [19]. The fish was placed on a horizontal table with half the body (tail part) spread out from the edge of the table. At selected time intervals (2, 6, 12, 24, 36, 42, and 48 h), the rigor index was determined by the following equation: $\mathrm{IR} = [(L_o - L_t)/L_o] \times 100$, where L represents the vertical distance between the base of the caudal fin and the table surface measured immediately after death (L_o) and during storage (L_t).

2.10. Statistical Analysis. Analyses were performed with the NCSS 2000 statistics software (NCSS, Kaysville, UT). Descriptive statistics (mean and standard deviation), one-way ANOVA, and multiple comparison by Tukey's test were applied. A significance level of 5% was used. For the chemical composition three samples ($n = 3$) were analyzed, while for the growth trial and rigor index ten organisms ($n = 10$) were used, and for the rest of the determinations six samples ($n = 6$) were used.

3. Results and Discussion

3.1. Chemical Composition of Experimental Diets. The crude protein of experimental diets averaged was 37.6%, while the commercial diet had 38.0% (Table 1). Crude lipids averaged were 6.2% for D100, D50, and D0, compared to 4.6% in DC. Mean ash content of D0, 50, and 100 ranged from 5.6 to 5.7% compared to 8.8% in DC. Significant differences ($p \leq 0.05$) between the commercial (DC) and experimental diets were found with respect to lipid and ash content. Despite variations found in the different diets, all components are within the optimum range for this species [20]. It is important to mention that in the majority of the studies where FM replacement by mixtures of plant sources has been evaluated, supplementation of diets with essential amino acids has been used to achieve a good growth of organisms. However, in this study no supplementation was used and good growth of the organisms was obtained.

3.2. Chemical Composition of Tilapia Muscle. The proximate composition of tilapia muscle is shown in Table 1. No significant differences ($p > 0.05$) were found in moisture, protein, and fiber content, while muscle lipids content of fish fed with D100 (5.3 ± 0.1%) was significantly higher ($p \leq 0.05$) compared to the other treatments (4.3–4.6%). Ash showed a similar trend.

There are some discrepancies in studies where the effect of FM replacement by plant sources has been evaluated by changes in proximate composition. Some studies have not found any effects of MPP on the whole-body protein, lipid, and ash contents in turbot *Psetta maxima* [21], in rainbow trout *Oncorhynchus mykiss* [22], in carp [23], and in yellowtail [24]. Contrary to this, Wang et al. [25] described that muscle composition of grouper (*Epinephelus akaara*) was significantly affected by the dietary carbohydrate level and when the levels of carbohydrate were increased, this generated a linear increase in liver glycogen. In this regard, it has been described that Nile tilapia are capable of utilizing high levels of various carbohydrates in feed and are used efficiently as a source of energy, while excess is stored as body fat [26]. Moreover, it has been reported that muscle lipid composition of farmed fish is strongly influenced by the composition, digestibility, amount of food, and unbalance of nutrients [27], as well as feeding management strategy [26]. A possible explanation to the changes in composition of muscle of this study could be attributed to the variety, preprocessing, and good digestibility of plant protein sources used to replace FM. This reflected on the organisms fed with the highest percentages of replacement that presents a greater accumulation of lipids in the muscle compared with the control food; this is in accordance with the report by Kikuchi [27]. However, this study did not carry out the determination of the digestibility of the diets, so it would be necessary to carry out further research to be able to elucidate if the changes in composition were due to that factor or to other factors.

3.3. Glycogen. The main energy source to maintain the physiological level of ATP in the muscle tissue is the glycogen degradation. Figure 1 shows the results of the concentrations of glycogen in muscle of tilapia. Initial glycogen values between 5.2 ± 0.1 and $5.7 \pm 0.2\,\text{mg}\cdot\text{g}^{-1}$ muscle were found, with fish fed with the D50 and D100 diets where the highest glycogen concentrations were obtained. These results are superior to those reported by Cappeln and Jessen [28] who reported an initial value of $0.2\,\text{mg}\cdot\text{g}^{-1}$ muscle of cod (*Gadus morhua*). The variation of these results may be due to the species, as well as the location of the muscle area where the sample was taken [28].

In this study it was observed that after 48 h *postmortem*, glycogen concentration decreased significantly ($p \leq 0.05$), being fish fed with D0 and DC treatment who presented the lowest values, probably because there was fewer initial glycogen available. Montoya-Mejía et al. [29] described that even though carbohydrates of plant sources used in fish feed are not the main source of energy, they are vital for the organism since the quality and quantity of these could interfere with digestion of other nutrients and optimal development of fish. It has also been observed that omnivorous fish such as tilapia are highly tolerant of carbohydrates and are used as a source of energy and the excess can be stored as glycogen and body lipids [29]. According to this, the differences found in the glycogen content could be related to a greater digestibility and assimilation of the nutrients of D50 and D100 diets, and therefore a greater amount of energy was available for growth, while the excess was stored as glycogen and lipids [26].

3.4. ATP and Related Compounds. In this study, it was found that the initial concentration from ATP of muscle in all treatments was low ($0.12 \pm 0.0\,\mu\text{mol}\cdot\text{g}^{-1}$) (Figure 2(a)). These results are similar to those reported by Castillo-Yáñez et al. [9] and Tomé et al. [30] who found 0.08 and $0.2\,\mu\text{mol}\cdot\text{g}^{-1}$ of ATP in the muscle of tilapia (*O. niloticus*) and pacu (*Colossoma* sp.), respectively. However, it is less than $3.59\,\mu\text{mol}\cdot\text{g}^{-1}$ of ATP in the muscle of tilapia (*O. niloticus*) reported by Oliveira-Filho et al. [31]. The differences observed between studies may be due to the species and size of the fish, muscle type, season, or time of year and the site of capture or harvest, in addition to the degree of stress.

With respect to ADP and AMP concentration at the beginning of storage, an initial value of 0.19 ± 0.0 and $0.3 \pm 0.0\,\mu\text{mol}\cdot\text{g}^{-1}$ was found, respectively (Figures 2(b) and 2(c)). These results are similar to those reported by Castillo-Yáñez et al. [9] and Ocaño-Higuera et al. [32] who found $<0.5\,\mu\text{mol}\cdot\text{g}^{-1}$ of ADP and AMP in the muscle of tilapia (*O. niloticus*) and cazon fish, respectively. On the other hand, the predominant nucleotide in the tilapia fish muscle after the harvest was IMP with an initial value of $7.6 \pm 0.5\,\mu\text{mol}\,\text{g}^{-1}$ (Figure 2(d)). The high concentration of IMP in this study indicated a rapid degradation of ATP into IMP. The initial IMP value obtained is lower than that reported by Özogul et al. [33] for catfish ($12.6\,\mu\text{mol}\cdot\text{g}^{-1}$). With respect to HxR and Hx concentration, an initial value of 0.19 ± 0.0 and $0.3 \pm 0.0\,\mu\text{mol}\cdot\text{g}^{-1}$ was found, respectively (Figures 2(e) and 2(f)).

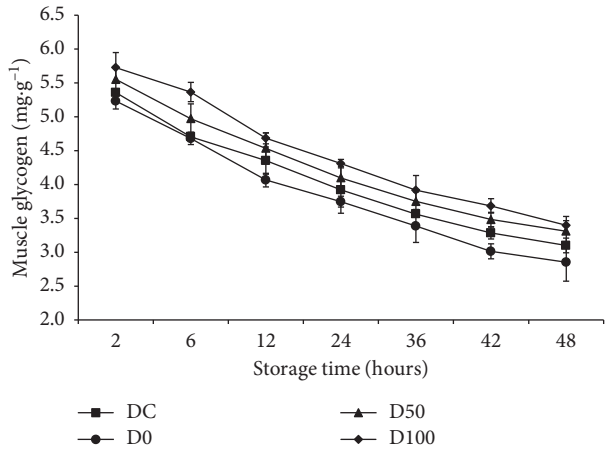

Figure 1: Glycogen in the muscle of tilapia (*Oreochromis niloticus*) stored on ice for 48 hours. Data points are the mean of $n = 6$ for each sampling hour. Bars represent the standard deviation of the mean. DC = control diet, D0 = diet with 0% substitution, D50 = diet with 50% substitution, and D100 = diet with 100% substitution.

After 48 h *postmortem*, ATP, ADP, AMP, and IMP levels decreased ($p \leq 0.05$) and the organisms fed with D0 and DC showed the lowest values, followed by D50 and D100. It has been reported that immediately after the fish dies, the muscles are relaxed, but as ATP content decreases, bond between actin and myosin is irreversible and the muscle goes into *rigor mortis*. In this study, this fact was reflected mainly in organisms of treatment D0, which showed a lower initial glycogen content, and subsequently a decrease of this component possibly due to stress generated before and during slaughter. An ATP-producing pathway in the initial *postmortem* stages is carried out by the enzyme adenylate kinase, which converts two molecules of ADP into one of ATP and another of AMP. This is related to the decrease in ADP concentration which was used as a supply of energy in the muscle during storage. On the other hand, the HxR and Hx concentration were significantly increased ($p \leq 0.05$).

The changes found in the ATP content and related compounds in the different treatments can be related to the content of energy reserves of the muscle, since the organisms fed D50 and D100 presented higher content of glycogen. This could probably be the result of higher digestibility and utilization of the nutrients of the diet and was reflected in the increase of the available energy reserves to the process of *rigor mortis*.

3.5. pH. Immediately after the death of the organism, the muscle has a near neutral pH [8], which can vary from 6.0 to 7.0 by different factors such as species, nutritional status of the fish, and stress suffered at the time of catching and the slaughter. Organisms initially presented a pH of 6.79 ± 0.02, as shown in Figure 3. This result is similar to that described by Tulli et al. [11] who found a pH of 6.74 in the muscle of European bass (*Dicentrarchus labrax*). Huss [8] described that usually *postmortem* initial pH varies by species and may be slight variations within the same species. In this study it

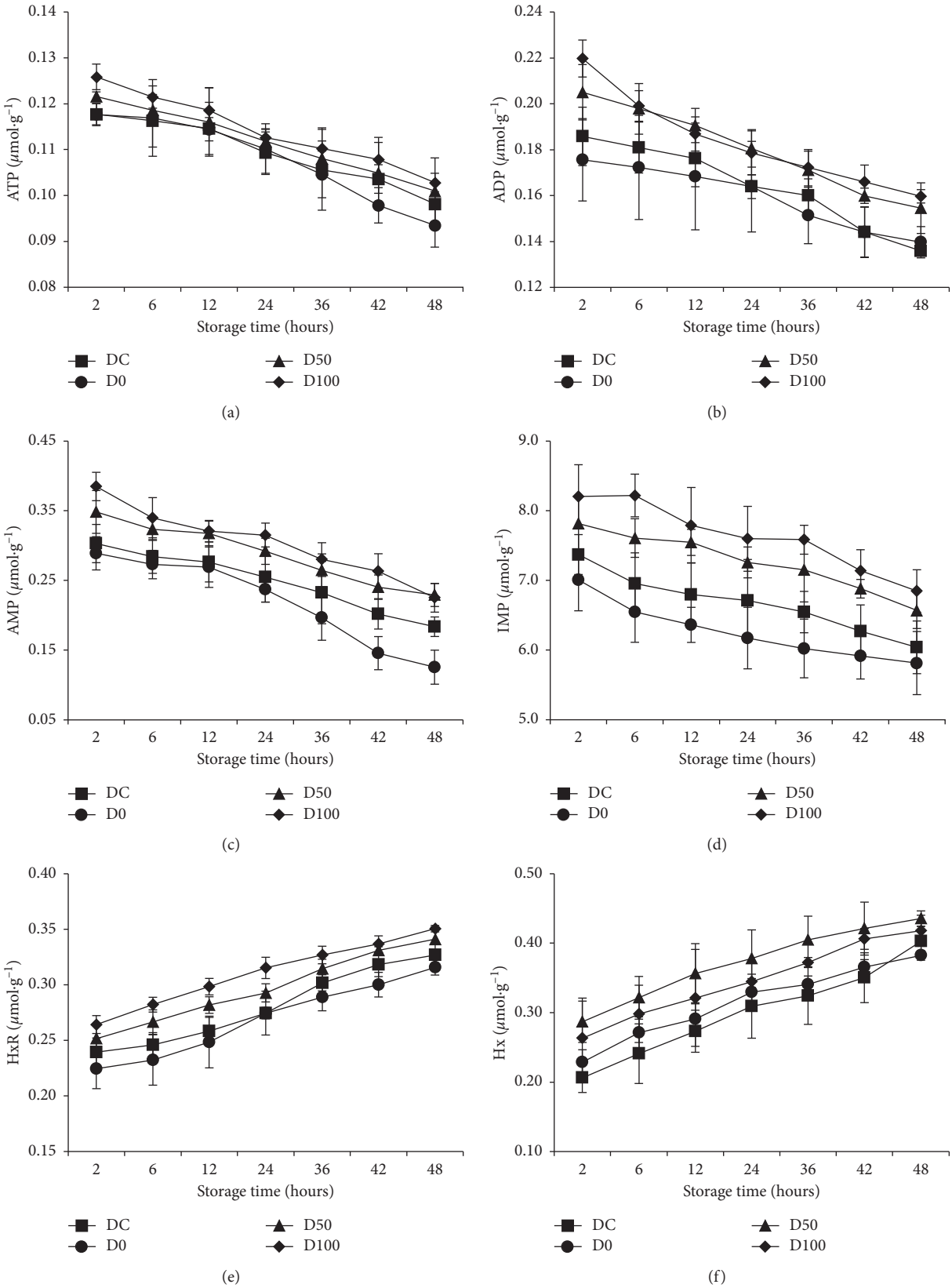

Figure 2: (a) ATP, (b) ADP, (c) AMP, (d) IMP, (e) HxR, and (f) Hx in muscle of tilapia (*Oreochromis niloticus*) stored on ice for 48 hours. Data points are the mean of $n = 6$ for each sampling hour. Bars represent the standard deviation of the mean. DC = control diet, D0 = 0% substitution, D50 = 50% substitution, and D100 = 100% substitution.

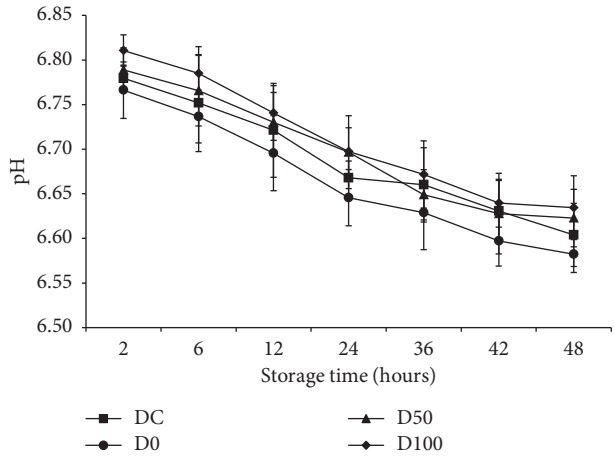

Figure 3: pH in the muscle of tilapia (*Oreochromis niloticus*) stored on ice for 48 hours. Data points are the mean of $n = 6$ for each sampling hour. Bas represent the standard deviation of the mean. DC = control diet, D0 = 0% substitution, D50 = 50% substitution, and D100 = 100% substitution.

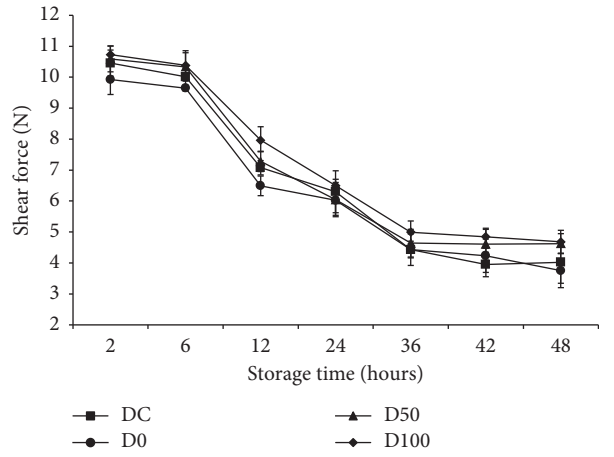

Figure 4: Shear force in the muscle of tilapia (*Oreochromis niloticus*) stored on ice for 48 hours. Data points are the mean of $n = 6$ for each sampling hour. Bars represent the standard deviation of the mean. DC = control diet, D0 = 0% substitution, D50 = 50% substitution, and D100 = 100% substitution.

was observed that after 48 h *postmortem*, muscle pH decreased significantly ($p \leq 0.05$), being the fish fed with D0 and DC who showed the lower pH values. This indicates an increase in the rate of *postmortem* events, where these include excessive muscle activity. A possible explanation for this is that having a lower final pH indicates a higher degree of stress, decreased energy reserves (glycogen and ATP), and thus a rapid acceleration of *rigor mortis* [34]. The *postmortem* pH decreases during *rigor mortis* due to the conversion of glycogen to lactic acid, which is the final product of anaerobic glycolysis in most fish products [31, 35]. Likewise, when the muscle pH decreases rapidly in the early hours *postmortem*, high intramuscular acidity reduces the net charge on the surface of muscle proteins causing them to partially denature and lose their ability to maintain a strong structure and water holding capacity [9]. The decrease rate of pH was very similar for all treatments and can be related to the content of energy reserves. However, the organisms fed D0 and DC were the ones that presented lower pH values, which correlates with the lower content of glycogen, ATP, ADP, and AMP. These reserves were exhausted faster and were reflected in a lower value of pH and a rapid onset of *rigor mortis*.

3.6. Shear Force. As shown in Figure 4, organisms initially presented a shear force mean of 10.43 ± 0.3 N. The shear force of organisms fed with D0 was significantly ($p \leq 0.05$) lower than the DC, D50, and D100 treatments. These differences may be due to the fact that fish fed with D0 had the lowest pH, which could affect the initial shear force. This result is similar to that reported by Suárez et al. [36] who found value of 11.82 N in the muscle of cultivated Denton (*Dentex dentex*). However, it is greater than 8.5 N reported by Duran et al. [12] in the muscle of rainbow trout (*Oncorhynchus mykiss*). The differences could be due to the species, fish size, and part of the muscle where the shear force was measured as well as the method of slaughter [12, 37].

In the same Figure 4, it can be observed that at 12 h *postmortem* all treatments showed a marked decrease ($p \leq 0.05$) of shear force; this behavior continued until 48 h, being fish fed with D0, the treatment that had the highest decrease in this parameter compared to the other treatments. This behavior was expected and was consistent with decreasing pH and energy reserves found in this treatment. Several studies have described that the changes in the texture of fish muscle are the result of the modification of the extracellular matrix and collagen degradation as a result of decreasing pH, as well as by the activity of endogenous enzymes (autolysis) on myofibrillar proteins [30].

3.7. Rigor Index (RI%). Figure 5 shows the results of rigor index behavior of all treatments stored during 48 h. The initial value of IR was $31 \pm 4.5\%$. Afterward, at 24 h this value increased in all treatments to 66–77% (minimum and maximum values), being the fish fed with D0 who showed the fastest rigor, and it was significantly different ($p \leq 0.05$) with respect to the other treatments. Subsequently, the RI increased significantly ($p \leq 0.05$) until 48 h and the maximum values achieved were 76–88% (minimum and maximum values). A possible explanation for the observed differences in *rigor mortis* could be related to the change in the chemical composition and energy reserves of the muscle. It was observed that the organisms fed with the highest proportions of plant sources (D100) had a higher content of muscle lipids, higher energy sources such as glycogen, ATP, ADP, and AMP, which could delay the *rigor mortis* process. This behavior is desired, since it has been observed that delaying the onset of *rigor mortis* generates products of better quality and longer shelf life. Contrary to this, it was observed that the fish fed with D0 and DC presented lower energy reserves, resulting in a lower pH value and a decrease

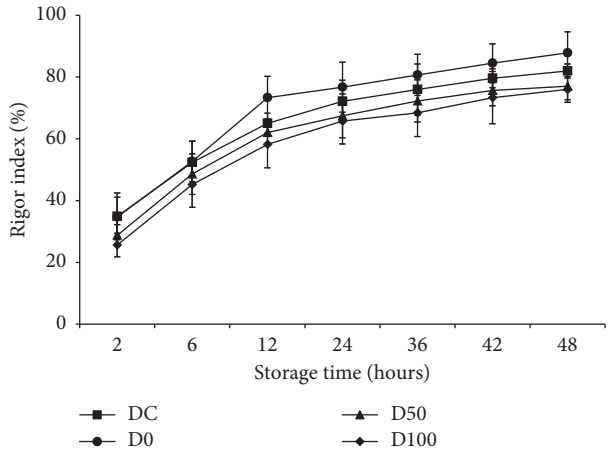

Figure 5: Rigor index in the muscle of tilapia (*Oreochromis niloticus*) stored on ice for 48 hours. Data points are the mean of $n = 10$ for each sampling hour. Bars represent the standard deviation of the mean. DC = control diet, D0 = 0% substitution, D50 = 50% substitution, and D100 = 100% substitution.

in shear force of the muscle and this contributed to a pronounced *rigor mortis*.

4. Conclusions

The results of the present study indicated that the substitution of FM by MPP modifies the chemical composition of the tilapia muscle as well as its energy reserves, which contributes to delaying the onset of *rigor mortis*, which could allow increasing the quality and shelf life of the tilapia muscle. In addition, these sources of vegetable protein (MPP) are available locally and at a lower cost than fishmeal (FM), so their use would reduce production costs and improve the sustainability of tilapia culture.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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