



## Effect of slaughter weight on the quality of Nile tilapia fillets

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### ABSTRACT

The objective of this work was to evaluate the influence of slaughter weight on the expression of calpastatin, m-calpain and the RyR3 gene, and on the chemical composition, morphometric measurements, fillet yield and sensorial characteristics of Nile tilapia fillets. In the experiment, 90 Nile tilapia were divided into three experimental treatments regarding slaughter weight ( $n = 30$ ): in treatment 1, tilapia aged 140 days were slaughtered with an average body weight of  $665 \pm 85$  g; in treatment 2, the animals were slaughtered at 182 days and weighed  $1000 \pm 177$  g; and in treatment 3, they were slaughtered at 238 days and weighed  $1325 \pm 167$  g. There was no significant difference ( $P > .05$ ) between the treatments for the chemical composition and fillet yield. Fillets of tilapia slaughtered with a weight of 665 g presented higher expression of m-calpain and lower expression of calpastatin gene, lower pH values of the thawed fillet, lower drip and thawing loss, and lower shear force than animals slaughtered with the highest evaluated weight. Tilapias slaughtered at 665 g also presented higher flavor and general acceptance. These results show that slaughter weight may influence important aspects of the quality of Nile tilapia fillets and that the slaughter of Nile tilapia with a body weight of 665 g allows fillets that serve the consumer market to be obtained.

### 1. Introduction

The world fish production in 2016 was about 171 million tonnes, equivalent to 362 billion dollars. Nile tilapia represented 8% of total of finfish produced in world aquaculture in 2016, and together with freshwater species, tilapias are expected to represent about 62% of total world aquaculture production in 2030 (FAO, 2018). Tilapia presents several advantageous characteristics that allow success in the production process, such as tolerance to high density rates, rusticity, success in polycultures, and ability to accept low-cost diets (FAO, 2011; Fitzsimmons et al., 2011). Besides that, the characteristics of the animal such as white meat, firm texture, delicate flavor and the absence of intramuscular spines attract the consumer preference (Souza and Maranhão, 2001).

Fish meat is rich in high quality protein, essential amino acids, essential fats (omega-3), vitamins (D, A and B) and minerals such as calcium, iodine, zinc, and selenium. However, despite its high

nutritional value, because it has fragile and easily degraded muscle tissue, fish meat is one of the most perishable and vulnerable to handling and processing (Cheng et al., 2015). The quality of fish meat may be influenced by intrinsic factors such as race, age and gender (Huff-Lonergan et al., 1995; Horcada et al., 1998), and by a number of environmental factors, such as slaughter conditions for example (Kristoffersen et al., 2006). Thus, changes in the appearance, texture, and chemical composition of meat can be observed under unfavorable environmental conditions (Castro et al., 2017).

There have been few studies dealing with the quality of tilapia meat (Kayan et al., 2015; Mohamed et al., 2016). From the consumer perspective, the quality of the meat can be subjective, varying between people, societies and cultures. In this way, a major challenge faced by the meat industry is obtaining information about the production process that guarantees the supply of qualified product (Damez and Clerjon, 2008).

Around the world, tilapia fillets represent the preference in the form

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of meat consumption for this species (Boscolo and Feiden, 2007). The producers of Nile tilapia in the European market are paid according to the fillet weight and their characteristics are truly important for the commercialization process (Rutten et al., 2005). The requirement for the weight of fillets sold worldwide is directly linked to the consumer's eating habits: in the United States of America and Brazil, European Union, and China, tilapias are slaughtered with average weights of 600, 800 and 1200 g, respectively (Department of Fisheries, 2009).

As the characteristics related to fish meat quality are complex and influenced by several factors that can determine the degree of freshness and acceptance of the product in the market, and considering the different demands of the consumer market, this work aims to evaluate the effect of slaughter weight of Nile tilapia on physical-chemical and sensory characteristics that are directly related to the quality of their meat.

## 2. Material and methods

### 2.1. Animals and experimental design

In this experiment, 90 Nile tilapia (*Oreochromis niloticus*) were evaluated with three different slaughter weights. All of the fishes were acquired from Sobradinho Fish Breeders Association, Sobradinho, Bahia, Brazil at the same time and with the same physiological condition with exception of age. Until the moment of acquisition, all the animals were raised under the same conditions in a net tanks system, with a volume of 2m<sup>3</sup> and 450 fish per tank. The water-quality parameters remained within the optimal comfort range of the species according to Kubitzka (2000): dissolved oxygen > 5 g/l, pH = 7; temperature of 29 °C, and ammonia NH<sub>3</sub> < 1 mg/l. All the tilapias were fed with commercial feed that was adequate to meet the nutritional requirements for each phase of the farming and had 55, 32 and 28% of crude protein at fingerling, juvenile and termination phase, respectively (TOTAL AQUOS, Três Corações, MG, Brazil).

Each of the three experimental groups related to slaughter weight was composed of 30 specimens. In treatment 1, tilapia aged 140 days presented with a body weight of 665 ± 85 g at slaughter; in treatment 2, the animals were slaughtered at 182 days weighing 1000 ± 177 g, and in treatment 3, animals were slaughtered at 238 days with a weight of 1325 ± 167 g.

Tilapias from all the three experimental groups were transported in boxes for live fish (Marine Equipment, SC, Brazil) from the tank to the laboratory. Before slaughter, the animals were divided into nine subgroups of 10 individuals per box, respecting the treatments defined above. These remained for 24 h in a closed recirculation system, composed of nine 1000 l polyethylene boxes with a biofilter and artificial oxygenation until the moment of slaughter. This time was used to reduce the effect of stress caused by transportation. All of the animals were slaughtered by concussion. The same procedures were applied for all three groups during the entire experiment.

### 2.2. Morphometric measurements and fillet yield

The following morphometric measures were evaluated according to Pires et al. (2011): weight in grams, total length (TL), standard length (SL), head length (HL), body height (BH) and body width (BW) in centimeters.

To obtain the fillet, the skin of the animals was removed manually with a knife and pliers, and filleting was carried out in sequence. After filleting, the height (FH) and length (FL) of fillets were measured in centimeters, and weight was measured in grams. The fillet yield was calculated as a percentage according to the formula described by Costa et al. (2014): Yield of fillet (%) = (fillet weight) / (total weight) × 100.

The loin eye area (LEA) was measured using an A4 transparency slide and black permanent marker pen according to the pig carcass evaluation methodology standardized by the Brazilian Association of

Pig Breeders (ABCS, 1973). From the transparencies, the ImageJ® IJ1.46r software was used to obtain the dimensions of the loin, length and height of the eye area as described by Teixeira et al. (2006).

### 2.3. Physical characteristics

Immediately after slaughter, the pH of the fresh fillet (pHFF) was measured using a Hanna HI99163 meat pH meter and piston with a penetration spit.

After that, the fillets were washed with running drinking water, packed in vacuum sealer bags (30 × 25 cm, 100 μm, Selovac 200S, São Paulo, Brazil) and frozen in a freezer at -18 °C. For the measurement of pH of the thawed fillet and the color parameters, the fillets were thawed in refrigerator for 24 h at 4 °C, separated and identified according to slaughter weight.

The pH evaluation was performed as described above, and color analyses involved two distinct points of the fillet (1; 2) being measured for each color muscle fiber, white (B) and red (V). The evaluations were carried out using color read equipment (CR-10, Japan), and the parameters evaluated were L\*: luminosity; a\*: red-green component; and b\*: yellow-blue component (Minolta, 1998).

In order to obtain the values of water loss by thawing, the frozen samples were weighed in a digital analytical balance (initial weight) and then samples were weighed again after thawing (final weight). Thawing loss results were obtained by calculating the difference between the initial and final weights. Thawing occurred in a refrigerator at 4 °C for 24 h. During this time, the samples were kept in a clean and sterile tray.

For drip loss (DL) analyses, raw fillet samples were weighed *in natura* (initial weight), after which the samples were placed in net-type bags and sealed in permanent plastic bags for 48 h in a refrigerator at 4 °C. Following that time, they were again weighed (final weight). Drip loss was calculated using the formula described by Ramos and Gomide (2007): DL = [(Initial weight- final weight) / Initial weight] × 100.

The evaluation of shear force was performed using the Brookfield texturometer CT3 (Texture Technologies 15 Corp., UK) fitted with a Warner-Bratzler blade. The speed used was 2 mm/s, the target distance was 30 mm and the force used was 10 g. For evaluation, samples of 3.0 cm in height × 2.5 cm in width and 2.5 cm in length were taken from the dorsal area of the fillet. The results were expressed in Kg.

### 2.4. Chemical analysis

Analyses of the chemical composition of fillets were carried out on samples of ten fillets from each slaughter weight (*n* = 10). For these analyses, half of each fillet was used. After thawing, the samples were ground individually in an electric mixer respecting the defined treatments. The samples were used to determine moisture (%), dry matter (%) and ash (%), and crude protein (%) according to AOAC (1998). Total lipid (%) was determined according to Bligh and Dyer (1959).

### 2.5. Sensory analysis

For sensory analysis, 10 fillets of each treatment were thawed at 4 °C for 24 h, separated, immersed in 1% saline solution and roasted with constant temperature monitoring through a spit-type thermometer until reaching the internal temperature of 70 °C. The samples were divided into 2 × 2 cm sizes, packed in aluminum foil identified with random numbers, and kept in an oven at 60 °C.

The samples were given to 80 untrained tasters who then assessed their color, odor, texture, flavor, juiciness, and general appearance. During the test, the tasters received all samples simultaneously (three treatments), and were instructed to assess each sample individually. The tasters were instructed to use water and water-and-salt wafers to remove any residual flavor from the mouth between the samples.

A 10 cm scale was used, with unnumbered intervals, representing

extremes: minimum (absence of sensation) and maximum (extremely intense sensation). The results were measured using a millimeter ruler to indicate the point corresponding to the intensity of their different sensations according to Rodrigues and Teixeira (2009). Each attribute ranged from zero (minimum intensity) to 10 (maximum intensity).

## 2.6. Gene expression

For gene expression analyses, white muscle samples were collected from five fish per treatment. The samples were stored in RNAlater® solution (Invitrogen, Carlsbad, CA, USA) at  $-20^{\circ}\text{C}$  until total RNA was extracted. Total RNA was extracted using Trizol® (Invitrogen) according to the manufacturer's instructions (1 ml/100 mg tissue). The total RNA concentration was measured using a spectrophotometer at a wavelength of 260 nm. RNA integrity was analyzed by 1% agarose gel electrophoresis followed by staining with ethidium bromide (10 mg/ml), and visualization under UV light. The RNA samples were treated with DNase I (Invitrogen) according to the manufacturer's instructions to remove any potential genomic DNA contamination.

The SuperScript™ III First-Strand Synthesis Super Mix kit (Invitrogen Corporation, Brazil) was used to synthesize cDNA from 1  $\mu\text{g}$  DNase-treated total RNA, according to the manufacturer's instructions. The cDNA concentration was measured using a spectrophotometer at a wavelength of 260 nm. The cDNA samples were diluted to 40 ng/ $\mu\text{l}$  and stored at  $-20^{\circ}\text{C}$  until further use as the template in the amplification reaction. Real-time PCR reactions were performed using the fluorescent SYBR Green dye (SYBR® Green PCR Master Mix, Applied Biosystems, USA). The amplification reaction consisted of 5  $\mu\text{l}$  diluted cDNA, 0.5  $\mu\text{l}$  each primer (forward and reverse) at a concentration of 10  $\mu\text{M}$  (final concentration: 200 nM), 12.5  $\mu\text{l}$  SYBR® Green PCR Master Mix, and water to make up a total volume of 25  $\mu\text{l}$ . To measure the efficiency of each primer/gene set, a series of 25  $\mu\text{l}$  reactions was performed as described above, using 5  $\mu\text{l}$  of a serial dilution of pooled cDNA as the template. The thermal cycling parameters for all genes were as follows: hot-start at  $95^{\circ}\text{C}$  for 10 min, followed by 40 cycles of denaturation at  $95^{\circ}\text{C}$  for 15 s and annealing/extension at  $60^{\circ}\text{C}$  for 1 min, and ending with a melt curve from 65 to  $95^{\circ}\text{C}$ .

The primers utilized for the amplification of gene expression levels of calpastatin, m-calpain, and ryanodine receptor 3 (RyR3) were designed based on gene sequences deposited at [www.ncbi.nlm.nih.gov](http://www.ncbi.nlm.nih.gov) (accession numbers XM\_005450336.3, XM\_019359456.1, and XM\_019349095.1, respectively) using the site [www.idtdna.com](http://www.idtdna.com).  $\beta$ -Actin was employed as the housekeeping gene. The amplification efficiency of  $\beta$ -actin was similar to that of the target genes (Table 1). All of the analyses were performed in duplicate.

Amplification efficiencies (90–110%) were similar for the genes of interest. Analysis of dissociation curves did not reveal any non-specific PCR products, such as the formation of primer dimers,

**Table 1**  
Primer sequences used for quantitative real-time polymerase chain reaction.

Gene	Amplicon (bp) <sup>a</sup>	Primer sequence (5'-3')
<i>CAST</i> <sup>b</sup>	133	AAGGTCAGAAGTGTGGAGAAAG TTTAGGGACATCCACAGGTTTAG
<i>m-CALP</i>	123	GACGCAGGTTTCACTCTCAATA GAACATCATCTCCAGCCTCATC
<i>RyR3</i>	140	TGTTTCATCTGTGGGATCGG GTGTGCTCTCTCTCCTTG
<i><math>\beta</math>-actin</i>	217	TGGTGGGTATGGGTCAGAAAG CTGTGGCTTTGGGGTTCA

<sup>a</sup> Amplicon size in base pairs.

<sup>b</sup> Calpastatin (*CAST*), m-calpain catalytic subunit (*m-CALP*), ryanodine receptor 3 (*RyR3*).

demonstrating the reliability of the data for estimating mRNA expression of the examined genes. The endogenous control,  $\beta$ -actin, did not show any significant differences between treatments, confirming its suitability as a control.

## 2.7. Statistical analyses

The  $2^{-\Delta\text{CT}}$  method (Livak and Schmittgen, 2001) was utilized to analyze the relative change in expression of the examined genes. The Shapiro-Wilk test was applied to assess the normality of the data. The data were analyzed by one-way ANOVA. Where statistical significance was obtained, means were compared using Tukey's test ( $P < .05$ ) (SAS Version 9.0, SAS Inst. Inc., Cary, NC, USA). The results were presented as the mean and standard error.

## 3. Results

### 3.1. Morphometric measures

As expected, fish that were slaughtered with higher weights presented higher total length, standard length, head length, body width, body height, fillet weight, fillet height, and fillet length. We also observed treatment effects on loin eye area height (LEAH), loin eye area length (LEAL) and loin eye area (LEA); animals slaughtered weighing 1325 g presented higher LEAH, LEAL, and LEA than animals slaughtered weighing 625 g. There was no difference between the other treatments. Also, there was no difference between treatments for fillet yield (Table 2).

### 3.2. Physical-chemical characteristics and sensory analysis

The results of the physical characteristics are shown in Table 3. We observed treatments effect on the pH of thawed fillets: there was a significant difference between the highest and the lowest slaughter weight, with pH values of 6.53 and 6.30 g, respectively.

There was a significant treatment effect on shear force, drip loss and thawing loss. The lowest shear force was observed in fillets of fish slaughtered weighing 665 g. Regarding drip loss and thawing loss, fish slaughtered at 1325 g presented the highest values. There was no difference for drip and thawing loss between the other treatments.

There was no treatment effect on color parameters (Table 3) and on the chemical composition of Nile tilapia fillets (Table 4).

**Table 2**  
Morphometric measurements and fillet yield of Nile tilapia slaughtered with different weights.

	Slaughter weight			P-value
	665 g	1000 g	1325 g	
TL <sup>†</sup>	32.6 $\pm$ 0.2 <sup>c</sup>	37.3 $\pm$ 0.36 <sup>b</sup>	41.4 $\pm$ 0.82 <sup>a</sup>	0.0001
SL	27.6 $\pm$ 0.2 <sup>c</sup>	31.5 $\pm$ 0.36 <sup>b</sup>	34.1 $\pm$ 0.45 <sup>a</sup>	0.0001
HL	7.9 $\pm$ 0.05 <sup>c</sup>	8.8 $\pm$ 0.09 <sup>b</sup>	9.7 $\pm$ 0.10 <sup>a</sup>	0.0001
BW	4.5 $\pm$ 0.05 <sup>c</sup>	5.1 $\pm$ 0.05 <sup>b</sup>	5.3 $\pm$ 0.06 <sup>a</sup>	0.0001
BH	9.8 $\pm$ 0.09 <sup>c</sup>	11.2 $\pm$ 0.13 <sup>b</sup>	12.6 $\pm$ 0.14 <sup>a</sup>	0.0001
LEAH	1.8 $\pm$ 0.06 <sup>b</sup>	2.07 $\pm$ 0.061 <sup>ab</sup>	2.1 $\pm$ 0.084 <sup>a</sup>	0.0090
LEAL	7.4 $\pm$ 0.15 <sup>b</sup>	7.9 $\pm$ 0.23 <sup>ab</sup>	8.4 $\pm$ 0.27 <sup>a</sup>	0.0190
LEA	8.5 $\pm$ 0.4 <sup>b</sup>	11.3 $\pm$ 0.63 <sup>a</sup>	11.7 $\pm$ 0.62 <sup>a</sup>	0.0001
FW	192.8 $\pm$ 5.5 <sup>c</sup>	290.3 $\pm$ 10.51 <sup>b</sup>	377.06 $\pm$ 14.4 <sup>a</sup>	0.0001
FH	8.2 $\pm$ 0.16 <sup>c</sup>	9.4 $\pm$ 0.21 <sup>b</sup>	10.3 $\pm$ 0.21 <sup>a</sup>	0.0001
FL	19.4 $\pm$ 0.27 <sup>c</sup>	22.1 $\pm$ 0.35 <sup>b</sup>	23.7 $\pm$ 0.27 <sup>a</sup>	0.0001
FY	28.9 $\pm$ 0.36	28.7 $\pm$ 0.43	28.2 $\pm$ 0.64	0.6469

<sup>a,b,c</sup> Different letters in the same row represent significant difference by the Tukey test ( $P < .05$ ).

<sup>†</sup> TL- Total Length (cm); SL- Standard Length (cm); HL- Head Length (cm); BW- Body Width (cm); BH- Body Height (cm); LEAH- Loin Eye Area Height (cm); LEAL- Loin Eye Area Length (cm); LEA- Loin Eye Area (cm<sup>2</sup>); FW- Fillet Weight (g); FH- Fillet Height (cm); FL- Fillet Length (cm); FY- Fillet Yield (%).

**Table 3**  
Physical characteristics of fillets of Nile tilapia slaughtered with different weights.

Slaughter weight				
	665 g	1000 g	1325 g	P-value
pHFF <sup>†</sup>	6.75 ± 0.04	6.78 ± 0.03	6.71 ± 0.04	0.5854
pHTF	6.30 ± 0.07 <sup>b</sup>	6.43 ± 0.03 <sup>ab</sup>	6.53 ± 0.03 <sup>a</sup>	0.0142
White fibers				
L*	41.55 ± 0.81	41.61 ± 0.98	41.42 ± 1.11	0.8467
a*	2.38 ± 0.33	1.68 ± 0.23	2.77 ± 0.42	0.09935
b*	17.79 ± 0.45	17.1 ± 0.44	18.40 ± 0.61	0.35325
Red fibers				
L*	35.95 ± 0.9	38.06 ± 1.22	36.95 ± 1.25	0.45385
a*	11.03 ± 0.78	9.71 ± 1	10.19 ± 1.13	0.6477
b*	21.11 ± 0.45	20.34 ± 0.58	20.31 ± 0.72	0.4483
Shear force (Kgf)	1.85 ± 0.23 <sup>c</sup>	3.92 ± 0.23 <sup>a</sup>	3.08 ± 0.23 <sup>b</sup>	< 0.0001
Drip loss (%)	3.86 ± 0.27 <sup>b</sup>	6.43 ± 0.43 <sup>a</sup>	6.73 ± 0.30 <sup>a</sup>	< 0.0001
Thawing loss (%)	3.7 ± 0.32 <sup>b</sup>	4.5 ± 0.45 <sup>ab</sup>	5.5 ± 0.41 <sup>a</sup>	0.0132

<sup>a,b,c</sup> Different letters in the same row represent significant difference by the Tukey test ( $P < .05$ ).

<sup>†</sup> pHFF –pH Fresh Fillet; pHTF– pH Thawing Fillet; L\*– luminosity, a\* – red-green component, and b\* –yellow-blue component.

**Table 4**  
Chemical composition of fillets of Nile tilapia slaughtered with different weights.

Slaughter weight				
	665 g	1000 g	1325 g	P
Protein (%)	20.2 ± 0.36	20.3 ± 0.218	19.9 ± 0.27	0.6607
Moisture (%)	75.5 ± 0.49	74.1 ± 0.68	74.3 ± 0.63	0.2005
Dry matter (%)	24.4 ± 0.49	25.8 ± 0.68	25.6 ± 0.63	0.2876
Ash (%)	2.4 ± 0.07	2.2 ± 0.04	2.2 ± 0.07	0.0808
Total lipid (%)	2.38 ± 0.76	2.19 ± 0.82	2.71 ± 0.45	0.4678

**Table 5**  
Sensory analysis of fillets of Nile tilapia slaughtered with different weights.

Slaughter weight				
	665 g	1000 g	1325 g	P-value
Juiciness	6.97 ± 0.23	7.17 ± 0.23	7.42 ± 0.24	0.4094
Texture	7.47 ± 0.21	7.54 ± 0.21	7.80 ± 0.22	0.5390
Odor	6.85 ± 0.28	6.94 ± 0.28	7.35 ± 0.29	0.4254
Flavor	7.59 ± 0.26 <sup>a</sup>	7.23 ± 0.26 <sup>ab</sup>	6.56 ± 0.26 <sup>b</sup>	0.0215
General Appearance	7.93 ± 0.24 <sup>a</sup>	7.58 ± 0.23 <sup>ab</sup>	7.11 ± 0.23 <sup>b</sup>	0.0532

<sup>a,b</sup> Different letters in the same row represent significant difference by the Tukey test ( $P < .05$ ).

Fillets from tilapia slaughtered weighing 665 g presented the highest flavor and general appearance values, of 7.59 and 7.93, respectively (Table 5).

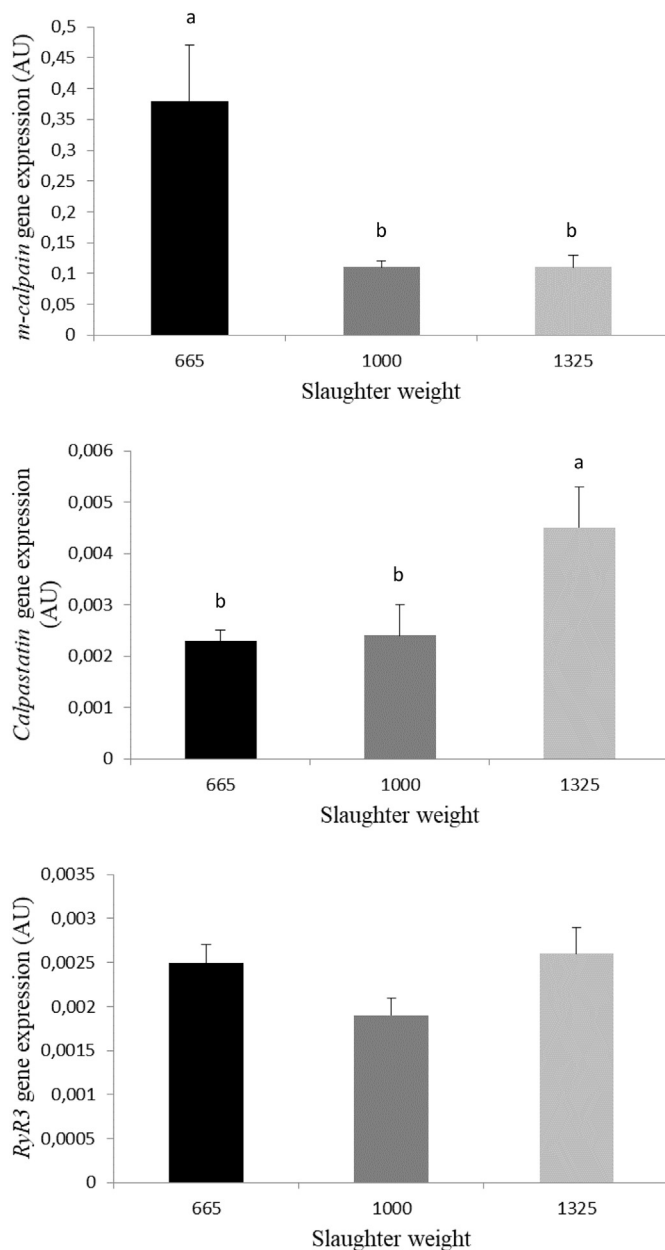
### 3.3. Gene expression

We observed the effect of slaughter weight on calpain and calpastatin gene expression (Fig. 1). Fishes slaughtered weighing 665 g presented higher calpain and lower calpastatin gene expression.

There was no treatment effect on RyR3 gene expression.

## 4. Discussion

Among the main parameters related to the consumer's acceptance of



**Fig. 1.** Effect of slaughter weight on m-calpain (A), calpastatin (B) and ryanodine receptor 3 (RyR3) (C) gene expression on muscle of Nile tilapia. Different letters above the bars indicate significant differences ( $P < .05$ ) by the Tukey's test; data are expressed as mean ± SEM.

the product are the color, general appearance, water retention capacity, texture and juiciness (Fonseca et al., 2013; Listrat et al., 2016), which can generally be influenced by several factors such as blood residue (Roth et al., 2007), fat content and lipid oxidation (Ruff et al., 2002), storage conditions (Guillerm-Regost et al., 2006), and even slaughter conditions (Kristoffersen et al., 2006). Kayan et al. (2015) observed that the slaughter weight of Nile tilapia can influence the characteristics of the meat. Thus, the authors suggest that animals should be slaughtered at different weights depending on the consumer's preference.

In our work, evaluating fillets weighing 665, 1000 or 1325 g, we did not observe a significant effect of slaughter weight on the parameters L\*, a\*, and b\* measured in the two regions evaluated in the Nile tilapia fillets, or on the pH value of the fresh fillets; however, we did observe that the fillets of fish slaughtered with the lowest weight had a lower pH value after thawing. The pH of the fish fillet is related to the rigor

mortis process characterized by the decrease in postmortem pH associated with the rapid transformation of glycogen into lactic acid (Nakayama et al., 1992). One of the factors that can influence the duration and development of the rigor mortis process is the stress caused at the pre-slaughter moment. The stress is related to the rapid drop in pH and low ultimate pH value, which can result in greater water loss, and poorer meat texture (Thiansilakul et al., 2011).

In our study, all animals were kept under the same conditions prior to slaughter, and it was still observed that animals with lower slaughter weight had lower pH values after thawing, even though there was no difference between the pH values of the fresh fillets. It is important to note that animals with lower slaughter weight also presented lower water loss due to thawing and dripping, and lower shear force. The texture and water retention capacity are pH-related characteristics (Kristoffersen et al., 2007). It is known that the pH value after rigor mortis implantation and the rate of drop are fundamental for the processes that transform the muscle into meat. As observed in our work, shortly after slaughter, the pH value is around 7. When the pH value is reduced rapidly, lower water retention capacity associated with increased protein denaturation is observed (Huff-Lonergan and Lonergan, 2005). On the other hand, when there is no reduction in pH value due to a lack of energy reserves, the meat may have darker coloration accompanied by less tenderness. It is also important to note that the pH value and the speed taken to reach the ultimate pH are species-specific.

In addition to pH, many other characteristics may influence water retention capacity, such as the structure of muscle cells and their components as well as genetic factors. Studies have shown that mutations in the ryanodine receptor genes (RyR) and alterations in the activity of these receptors are related to lower capacity in the control of calcium release into the sarcoplasm. This accelerated release of calcium into the cytoplasm allows increased muscle metabolism, which results in lower pH values in a shorter time and thus lower water retention capacity (reviewed by Huff-Lonergan and Lonergan, 2005). Goes et al. (2015) showed the relationship between the expression of the *RyR1* and *RyR3* genes and the ability of water retention in animals submitted to different stress conditions prior to slaughter.

In our study, the observed changes in drip and thawing loss were not accompanied by alterations in *RyR3* gene expression since there was no effect of slaughter weight on the expression of this gene. Thus, the reduced loss and the lower shear force observed in fillets of fish slaughtered with 665 g may be related to the higher expression of calpain and reduced expression of calpastatin also observed in this group. The postmortem degradation of intermediate filament proteins such as desmin and talin by the action of calpain has been associated with higher water retention capacity (Zhang et al., 2006). Activation of calpains can also result in softening of fish muscle (Salem et al., 2004). Calpastatin acts as a regulator of calpain activity, and similar to the observed in mammals (Casas et al., 2006; Bagatoli et al., 2013; Ropka-Molik et al., 2014), calpastatin has been negatively associated with tenderness traits in fish, as lower calpastatin gene expression is associated with strains that have the slowest growth rate and yielded the softest fillet (Salem et al., 2005). Despite calpain/calpastatin system is the most extensively studied enzyme system involved in meat tenderization (Bhat et al., 2018), the role of these enzymes in fish flesh tenderization remains not fully understood.

The water retention capacity may be related to the chemical characteristics of the meat produced since the loss of water is also related to the loss of muscle proteins (Savage et al., 1990). In our work, despite the results observed for water loss by thawing and dripping, we did not observe any effect of treatments on the chemical composition of fillets. The average protein and moisture content of the treatments was 20% and 74.6%, respectively. These values are within the range reported by Listrat et al. (2016), who cited values of 20% and 75% for protein and moisture, respectively.

The water retention capacity also influences the sensory characteristics of the meat, such as texture and taste (Pelicano et al., 2003).

In the sensory analysis, the significant effect of slaughter weight on juiciness, tenderness and odor was not verified. However, in relation to taste and general acceptance, we observed that higher values were significantly attributed to fillets of fish with lower slaughter weight. This phenomenon was also cited by Johansson et al. (2000) in the sensory analysis of rainbow trout fillets of different aged fish, and may be related to the results of pH, shear force and water loss discussed in our work.

Our results show that slaughter weight may influence important physical and sensory aspects of the quality of Nile tilapia fillets. In general, the results suggest that the slaughter of Nile tilapia with an average weight of 665 g makes it possible to obtain fillets that serve the consumer market.

## Declaration of Competing Interest

The authors declare no competing financial interests.

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