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MORPHOMETRIC, BROMATOLOGICAL, AND SENSORY ANALYSIS OF NILE TILAPIA VACCINATED AGAINST *Streptococcus agalactiae*

Rovena Lebarch ROCHA[1]
Julia Merlo FERREIRA[1]
Taciana Onesorge MIRANDA LOPES[1]
Levy Carvalho GOMES[1]

[1]Universidade Vila Velha, Laboratório de Ictiologia Aplicada, Rua Comissário José Dantas de Melo, 21, CEP 29102-770, Vila Velha, ES, Brazil. E-mail: taci_miranda@yahoo.com.br (corresponding author)

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ABSTRACT

The objective of this study was to evaluate the effect of vaccination against *Streptococcus agalactiae* on the morphometry, bromatology, and sensory traits of tilapia bred in net-tanks. Tilapia juveniles were bred in net-tanks separated into two groups: vaccinated and unvaccinated fish. Ten fish from each group were collected from different weight classes: 400-500, 501-600, 601-700, and 701-800 g. Measurements and weighing of whole fish and fillets did not show significant differences between the two groups. Fillet thickness was significantly greater in unvaccinated fish in the weight range of 601-700 g. Significant differences were not found in protein, lipid, ash, or moisture content between the two groups in any of the weight classes studied. Significant preferences between unvaccinated and vaccinated fish were not observed in the paired preference test, regardless of weight class. The hedonic scale analysis showed that tasters moderately liked the tilapia fillets regardless of weight class or whether the fish had been vaccinated. In net-tank breeding experimental conditions, vaccinated and unvaccinated Nile tilapia weighing between 400 and 800 g showed similar morphometric, bromatological, and sensory characteristics.

Key words: *Oreochromis niloticus*; nutritional quality; protein; net-tank; bacteria.

ANÁLISE MORFOMÉTRICA, BROMATOLÓGICA E SENSORIAL DE TILÁPIA DO NILO VACINADA CONTRA *Streptococcus agalactiae*

RESUMO

O objetivo desse trabalho foi avaliar o efeito da vacinação contra *Streptococcus agalactiae* na morfometria, bromatologia e análise sensorial de tilápia criada em tanque-rede. Juvenis de tilápia foram criadas em tanque-rede separados em dois grupos: peixes vacinados e não vacinados. Foram coletados 10 peixes de cada grupo em diferentes classes de peso: 400-500, 501-600, 601-700 e 701-800 g. As medidas e pesagens do peixe inteiro e do filé não apresentaram diferença significativa entre os dois grupos. A espessura do filé foi significativamente maior nos peixes não vacinados na faixa de 601-700 g. Não teve diferença significativa nos valores de proteína, lipídio, cinza e umidade entre os peixes dos dois grupos em nenhuma das classes de peso estudada. Para o teste pareado preferência, não houve uma preferência significativa entre peixes não vacinados e vacinados independente da classe de peso analisada. A análise de escala hedônica apontou que os provadores gostaram moderadamente do filé de tilápia independente da classe de peso e de ter sido vacinado ou não. Nas condições experimentais de criação em tanques-rede, tilápias do Nilo vacinados e não vacinados, com peso entre 400 e 800 g, apresentaram características morfométricas, bromatológicas e sensoriais semelhantes.

Palavras-chave: *Oreochromis niloticus*; qualidade nutricional; proteína; tanque-rede; bactéria.

INTRODUCTION

The Nile tilapia is an attractive species for aquaculture due to its rapid growth, high reproductive efficiency, feeding at low trophic levels, and low production costs (GARCIA *et al*., 2016; BOSISIO *et al*., 2017; COA *et al*., 2017). The fish has characteristics desirable to the consumer market, such as firm white flesh, delicate flavor, absence of Y-shaped bones and unpleasant odor, low fat and calorie content (SILVA *et al*., 2016), and disease resistance (HAI, 2015). However, the high fish density in net-tank systems causes stress in the cultured animals, resulting in a higher occurrence of infectious and parasitic diseases and, thus, higher fish mortality (RORIZ *et al*., 2017).

Bacterial diseases, especially from *Streptococcus*, most affect the cultivation of tilapia (SILVA *et al*., 2009a; SALVADOR *et al*., 2013). *Streptococcus agalactiae* is the most common species in hot climates and is associated with freshwater fish (PRETTO-GIORDANO *et al*., 2010). According to SILVA *et al*. (2009a), the use of vaccines to avoid tilapia mortality from bacterial diseases has increased among breeders. Tilapia vaccinated against *S. agalactiae* show significant increase in specific antibody levels (PASNIK *et al*., 2006) and reduce economic losses caused by mortality, with a primary focus on prevention (ZHANG *et al*., 2017).

The increased occurrence of bacterial diseases in net-tank breeding has led producers to vaccinate tilapia in the pre-fattening phase. However, producers observed that vaccinated tilapia appear to have more fat and less utilization of the fillet at the time of processing. This worry is well founded, given that AFONSO *et al*. (2005) observed an increase in the amount of adipose tissue in the abdominal cavity of vaccinated sea bass (*Dicentrarchus labrax*) due to the appearance of granulomas. Therefore, this study aimed to evaluate the effect of vaccination against *S. agalactiae* on the morphometry, bromatology, and sensory traits of tilapia bred in net-tanks.

METHODS

Tilapia juveniles of the Genetically Improved Farmed Tilapia (GIFT) strain (n = 7000; ± 5 g) were purchased from a private nursery and transported to an 18 m^3 rearing net-tank installed in a fish farm located in Aguiar Lake, municipality of Linhares, state of Espírito Santo, Brazil. After the rearing period, a 50 g (n = 3500) lot of fish was anesthetized with eugenol (50 mg L^{-1}) and vaccinated against *Streptococcus agalactiae* with an intraperitoneal injection of 0.05 mL AQUAVAC® STREP per fish. A second group of fish from the same lot was not vaccinated (n = 3500). After the vaccination, vaccinated and unvaccinated fish were placed separately in two 18 m^3 pre-fattening net-tanks until they reached 200 g. Then, fish from each pre-fattening tank were distributed into two fattening tanks (18 m^3) at a density of 100 fish m^{-3}. Thus, there were two fattening net-tanks for vaccinated fish and two for unvaccinated fish. During fattening, fish were fed three times each day with extruded commercial feed containing 32% crude protein at a rate of 2-3% of live weight/day. Fish were raised under this scheme until they reached an average weight of approximately 800 g (*ca.* five months of fattening).

Fish were captured for commercialization and slaughtered by hypothermia in isothermal boxes containing ice and water when they reached the following average weight classes: 400-500 g (3 months of fattening), 501-600 g (3 months and 15 days of fattening), 601-700 g (4 months and 10 days of fattening), and 701-800 g (5 months of fattening). Once slaughtered, ten vaccinated and ten unvaccinated fish (five fish from each net-tank) per weight class were collected for a total of 80 fish analyzed (40 vaccinated and 40 unvaccinated). The fish were weighed then filleted manually, the carcass (representing the remainder of the fish after filleting) was reweighed, and the fillets were packed separately and frozen at -20°C. Total length (from the tip of the head to the end of the caudal fin) and height (body height, as measured from the lower part of the first ray of the dorsal fin) measurements of whole fish were taken with calipers as described by SANTOS *et al*. (2007) and CREPALDI *et al*. (2008). Filleting was done manually by a single, well-trained individual. Fish were filleted, skinned with a knife (SOUZA, 2002), and weighed. Fillet length, width, and thickness were measured. Width was measured at 6 cm after the start of the fillet. Once measured and weighed, the fillets were washed, packed individually in plastic bags, and frozen at -20°C. Samples were thawed at the time of bromatological and sensory analyses.

Bromatological analyses were performed according to the methods described by the Association of Official Analytical Chemists (AOAC, 1995). Moisture was determined using a digital moisture analyzer (MOC63u; Shimadzu Corp; Japan). Total protein was measured by the Kjeldahl method. The Soxhlet extraction method was employed to determine total lipid content. Sample ash percentage was determined in a muffle furnace at 550°C.

The hedonic scale acceptance method and paired preference test were employed in the sensory analysis, both as described by ZENEBON *et al*. (2008). A total of 80 samples were prepared (10 for each weight class and vaccination status) on four different days, with one weight class for each day. Samples were cooked in an oven at 180°C for 10 minutes and offered to trained tasters. Each judge had to taste the samples while avoiding sensory fatigue, so judges wash their mouths with filtered water or ate apple slices to neutralize their sense of taste. A total of 20 tasters were used for the acceptance test, with each expressing their degree of aversion. This test used a hedonic scale of 5 points, with 5 representing the highest score and 1 the lowest. For the paired preference test, the fillets were placed in two different trays (one with vaccinated fish and one with unvaccinated fish), and each judge had to test two pieces from each tray and indicate the tray with the preferred sample.

Morphometric and bromatological results are expressed as the mean ± standard deviation and were compared between vaccinated and unvaccinated fish using Student's t-test. Hedonic scale test results are expressed as median and were compared using the Mann-Whitney test, while the paired preference test results were assessed with the chi-squared test. All tests were performed with Sigmaplot 12.5 software.

RESULTS

Significant differences were not found in total weight, carcass weight, total length, or height between vaccinated and unvaccinated fish from any of the weight classes analyzed (Table 1).

Fillet thickness was significantly greater in unvaccinated fish in the weight range of 601–700 g. However, significant variations were not found for fillet yield, weight of fillet without skin, or height in any of the weight classes analyzed (Table 2).

Significant differences were not found in protein, moisture, ash, or lipid content between vaccinated and unvaccinated fish, regardless of weight class. Even when results from different weight

Table 1. Body morphometric variables of Nile tilapia of different weight classes from fish vaccinated and unvaccinated against *Streptococcus agalactiae*.

Variable	Unvaccinated	Vaccinated
400-500 g		
Total weight (g)	436.6±26.21	426.19±19.75
Carcass weight (g)	258.7±16.6	263.1±12.81
Total length (cm)	26.75±0.97	26.59±0.59
Height (cm)	8.66±0.28	8.77±0.23
501-600 g		
Total weight (g)	541.23±22.53	543.44±23.48
Carcass weight (g)	323.59±18.7	332.95±15.92
Total length (cm)	28.69±1.18	28.78±0.56
Height (cm)	9.31±0.2	9.36±0.28
601-700 g		
Total weight (g)	667.01±31	652.59±37.22
Carcass weight (g)	397.84±18.91	390.25±31
Total length (cm)	29.81±0.73	29.91±0.86
Height (cm)	10.21±0.35	10.11±0.41
701-800 g		
Total weight (g)	748.9±28.89	754±35.71
Carcass weight (g)	451.4±27.5	459.8±19.83
Total length (cm)	32.39±0.93	32.71±0.92
Height (cm)	10.17±0.49	10.24±0.50

Indicates significant difference between vaccinated and unvaccinated fish by Student's t-test (P<0.05).

classes were grouped, significant differences were not observed in the bromatological analysis between the two groups (Table 3).

In the paired preference test in the range of 400-500 g, 50% of tasters preferred unvaccinated fish, which was a trend maintained with slight variations in the other weight classes. Therefore, significant preferences between unvaccinated and vaccinated fish were not observed, regardless of the weight class analyzed. Of the eighty tested samples of each treatment (grouping all weight classes), tasters preferred unvaccinated fish in 42 samples (52.5%) and vaccinated fish in 38 samples (47.5%), which were similar according to the chi-squared test (Table 4).

Hedonic test results showed a median of 4-4.5 in all weight classes, without significant differences between vaccinated and unvaccinated fish for any weight class, showing that tasters moderately liked the tilapia fillets. In all weight classes and regardless of vaccination, over 50% of tasters liked or moderately liked the tilapia fillets. The lowest score was 1 for vaccinated fish, in 5% of the 400-500 g samples and 10% of 501-600 g samples, and 2 for unvaccinated fish, in 15% of the 400-500 g samples and in 10% of samples of 501-600 g. This scenario was reversed for weight classes 601-700 g and 701-800 g, with the lowest score being 1 for unvaccinated fish (10% of the samples in these two weight classes) and 3 (10% of the samples) and 2 (15% of the samples) for vaccinated fish of 601-700 g and 701-800 g, respectively (Figure 1).

Table 2. Morphometric variables of Nile tilapia fillets of different weight classes from fish vaccinated and unvaccinated against *Streptococcus agalactiae*.

Variable	Unvaccinated	Vaccinated
400-500 g		
Fillet yield (%)	31.80±0.66	30.82±1.61
Weight of fillet without skin (g)	138.87±10.05	131.33±8.92
Length of fillet (cm)	12.89±0.49	13.48±0.82
Thickness (cm)	1.05±0.09	1.13±0.11
Width (cm)	6.7±0.34	6.72±0.47
501-600 g		
Fillet yield (%)	30.52±0.77	30.21±1.20
Weight of fillet without skin (g)	165.15±7.33	164.27±11.10
Length of fillet (cm)	15.4±0.12	14.93±0.87
Thickness (cm)	1.04±0.09	0.98±0.09
Width (cm)	6.69±0.40	6.93±0.54
601-700 g		
Fillet yield (%)	32.18±1.64	31.03±1.06
Weight of fillet without skin (g)	214.8±16.87	202.61±14.78
Length of fillet (cm)	17.79±1.06	17.9±2.92
Thickness (cm)	1.58±0.08	1.34±0.11*
Width (cm)	8.57±0.96	8.5±0.54
701-800 g		
Fillet yield (%)	32.76±1.42	32.35±1.55
Weight of fillet without skin (g)	245.2±10.71	244.2±20.34
Length of fillet (cm)	18.22±0.77	18.11±0.72
Thickness (cm)	1.25±0.15	1.31±0.22
Width (cm)	8.06±0.64	7.89±0.48

*Indicates significant difference between vaccinated and unvaccinated fish by Student's t-test (P<0.05).

Table 3. Bromatological analysis of Nile tilapia fillets of different weight classes from fish vaccinated and unvaccinated against *Streptococcus agalactiae*.

Variable	Unvaccinated	Vaccinated
Protein (%)	19.65±1.72	19.85±0.53
Moisture (%)	77.35±1.11	77.11±0.13
Ash (%)	1.07±0.19	1.09±0.22
Lipid (%)	4.91±2.42	4.19±1.96

Indicates significant difference between vaccinated and unvaccinated fish by Student's t-test (P<0.05).

Table 4. Paired preference test of Nile tilapia fillets of different weight classes from fish vaccinated or unvaccinated against *Streptococcus agalactiae.*

Weight class (g)	Preference (%)	
	Unvaccinated	Vaccinated
400-500	50	50
501-600	60	40
601-700	35	65
701-800	65	35
Total	52.5	47.5

Indicates significant difference between vaccinated and unvaccinated fish by the chi-squared test (P<0.05).

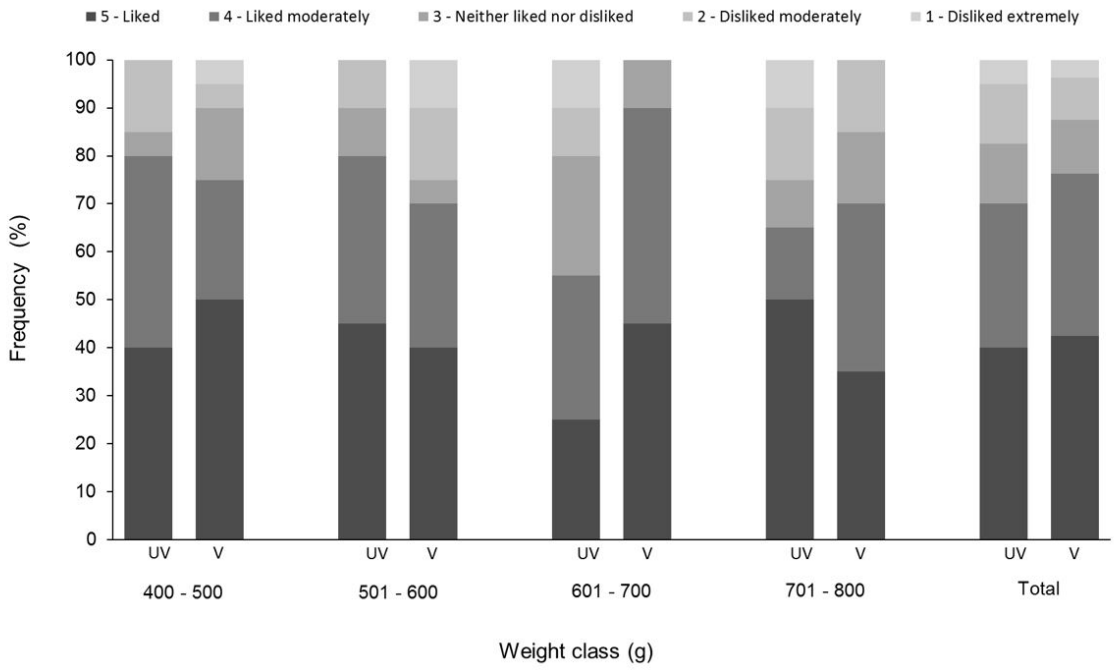

Figure 1. Frequency of occurrence of hedonic scale categories for Nile tilapia fillets of different weight classes from fish vaccinated or unvaccinated against *Streptococcus agalactiae*. UV = unvaccinated and V = vaccinated.

DISCUSSION

As shown by the above results, the vaccine does not affect the morphology of the fish, which is beneficial in the commercialization of the whole fish and for mechanical filleting. ROCHA *et al*. (2012) observed that breeding system (net-tank and pond) has a significant effect on the morphology of Nile tilapia. This morphological difference may impair the mechanical filleting process, given the fine adjustment of the machinery to obtain higher fillet yield. The only difference found between vaccinated and unvaccinated fish is an oily layer, barely perceptible to the consumer, on the peritoneum wall of vaccinated fish, which had been previously observed in sea bass by AFONSO *et al*. (2005). However, most of the tilapia produced in Brazil is destined for filleting (FURLANETO *et al*., 2010), which discards the peritoneum region.

Fillet thickness was significantly greater in unvaccinated fish in the weight range of 601-700 g. SILVA *et al*. (2009b) found a yield of 33.83% in tilapia weighing 450 and 500 g, slightly higher than that found by this study (31.80%). However, BOSCOLO *et al*. (2001), when evaluating the performance of common and Thai Nile tilapia strains, found that the yield of the common lineage was 33.37% in tilapia weighing 600–700 g, which are results closer to those of the same weight class observed in this study (32.18%).

Our results for protein, moisture, ash, and lipid content values were similar to those of BOTARO *et al*. (2007) in their evaluation of the chemical composition of Nile tilapia bred in net-tanks. LEONHARDT *et al*. (2006) analyzed Nile tilapia collected from a fishery and found similar results in their bromatological evaluation.

The idea that vaccinated animals have more fat is related to the application of the vaccine. All vaccinated fish have an oily layer on the peritoneum wall that lasts for life, which producers associate with more fat. However, the initial hypothesis that vaccinated fish would have more fat was not corroborated here, as significant differences were not seen in fillet lipid contents between the vaccinated and unvaccinated animals. Moreover, the visceral fat was weighed together with the fish carcass, which again did not show significant differences between vaccinated and unvaccinated fish, implying that vaccinated fish do not present greater fat deposition.

According to the paired preference test in fish of 400–500 g, 50% of tasters preferred unvaccinated fish, which was a trend maintained with slight variations in the other weight classes. Therefore, significant preferences between unvaccinated and vaccinated fish were not observed, regardless of the weight class analyzed. ROCHA *et al*. (2012), when testing tilapia fillets from ponds and net-tanks, found that tasters moderately liked the fillets and did not show preference related to breeding system.

It is widely known by the population that vaccination is common in the breeding of several animal species and groups, such as cows, buffalos, goats, swine, and birds, and this procedure is accepted by most consumers. However, the vaccination of fish is recent and still makes consumers, and even producers, suspicious. From the perspective of nutritional quality and utilization of the fillet, the initial hypothesis that vaccinated fish have more fat and a smaller fillet was not corroborated by this study.

CONCLUSION

In net-tank breeding experimental conditions, vaccinated and unvaccinated Nile tilapia weighing between 400 and 800 g showed similar morphometric, bromatological, and sensory characteristics.

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