Egyptian Journal of Animal Health

P-ISSN: 2735-4938 On Line-ISSN: 2735-4946
Journal homepage: https://ejah.journals.ekb.eg/

Impact assessment of some mineral feed additives on *O.niloticus* with special reference to potential effect on the immunological status and fish tissue quality

Jehan, I. Abdellatief[*] and Asmaa, E. Hassan[**]

[*]Fish diseases department
[**]Reference Lab for Safety Analysis of Food of Animal Origin,
Animal Health Research Institute (AHRI) 12618, Agricultural Research Center (ARC), Giza, Egypt.

Received in 12/5/2025
Received in revised from 2/6/2025
Accepted in 17/6/2025

Keywords:
Nile tilapia
Mineral feed additive
Immunity
Meat quality

ABSTRACT:

This study evaluated the addition of functional minerals as feed additives for Nile tilapia (*Oreochromis niloticus*). These minerals include calcium, phosphorus, magnesium, sodium and potassium and certain trace elements (iodine, iron, manganese and zinc) to enhance fish immunity and fish quality. Three groups (triple /group) experimented for 30 days provided a balanced commercial diet amounting to 3% of their body weight daily. These groups control, G2: fed with 20g/kg of feed additive to regular ration while G3: fed with 40g/kg of feed additive to regular ration. The study indicated immunologically, lysozyme activity and a significant up regulation of IL-10 gene expression were noticed. Meat safety parameters showed lower bacterial loads, with no detection of *Salmonella*, *Listeria*, or *Staphylococcus aureus*. Meanwhile, chemical indicators for freshness, including histamine, total volatile basic nitrogen (TVB-N), and thiobarbituric acid (TBA), remained within acceptable levels. Furthermore, the meat quality of treated fish exhibited improved protein content and reduced moisture, thus enhancing both meat freshness and nutritional values which make it a preferred choice among consumers.

INTRODUCTION

Nutrition from fish fulfills the body's requirements for high-quality proteins, essential amino acids, minerals, and vitamins. **(Ackman, 2000)** and polyunsaturated fatty acids "PUFA" **(Kmínková et al. 2001)**. Omega-3 polyunsaturated fatty acids (n-3 PUFAs) are particularly significant because the human body cannot produce them, necessitating their intake through food **(Alasalvar et al. 2002)** . Fish serves as a vital component of the human diet, offering significant nutritional advantages. In aquaculture, achieving high meat quality depends on maintaining healthy animals, and quality assessments help enhance the market potential of fish products. Understanding the impact of muscle properties on

Corresponding author: Jehan, I. Abdellatief, Reference Lab for Safety Analysis of Food of Animal Origin, Animal Health Research Institute (AHRI) 12618, Agricultural Research Center (ARC), Giza, Egyp.
Email address:
DOI: 10.21608/ejah.2025.456919

fish meat quality is crucial for driving advancements in the aquaculture industry.

The rising global demand for fish, especially those cultivated in aquaculture, is largely attributed to the nutritional and health benefits of fish meat. Among aquaculture species **(Burger and Gochfeld, 2009)**. Nile tilapia *(Oreochromis niloticus)* has quickly gained prominence due to its adaptability and commercial value **(Kayan et al. 2015)**.

Additionally, the importance of macro minerals such as calcium, phosphorus, magnesium, sodium, potassium, and chloride, along with trace elements like cobalt, copper, iodine, iron, manganese, selenium, and zinc, has been well-established in sustaining fish health and growth (**NRC, 2011 & Lall and Kaushik, 2021).**

An essential element of effective aquaculture is the development of fish rations carefully balanced diets designed to ensure optimal growth, health, and productivity in farmed fish.

Mineral absorption can differ between species due to variations in gastric acid secretion, which is influenced by whether the fish possess a stomach or are stomach less **(Lall, 2002**). along with the absorption of minerals from water. Variations also exist in the techniques employed to evaluate mineral demands and response parameters, such as growth, feed efficiency, mineral concentrations in the whole body, vertebrae, plasma/serum, or tissues, hematological markers, and alterations in specific enzyme activities. In teleost fish, the anterior kidney and spleen play a crucial role as primary immune organs **(Press & Evensen, 1999 and Grove et al. 2006)**

As international fish trade continues to expand alongside globalization, the risk of cross-border transmission of infectious agents becomes more pronounced. This poses heightened threats to human health and can have profound effects on global trade dynamics. To address these challenges, it is essential to adopt a harmonized global strategy that ensures fish safety and quality while preventing unfair trade practices and hidden technical barriers **(Ababouch, 2006).**

When addressing food quality, laws generally cover the following aspects:
- **Safety concerns**: Ensuring hygiene standards are upheld, while regulating and controlling the presence of undesirable substances, additives, and contaminants.
- **Composition matters**: Requiring producers to disclose the nutritional and energy content of food, alongside identifying allergenic components.
- **Fair trade and consumer protection**: Safeguarding consumers from fraud by prohibiting the sale of altered, impure, or substandard food products.

Food quality is fundamentally tied to meeting specific requirements that ensure products comply with established specifications, standards, and satisfy consumer expectations **(Nicolae, et al. 2016).**

This study explores the impact of feed additives on fish immunity, safety, and quality, using various microbiological, chemical, and biochemical tests to ensure the fish meet consumer expectations for quality.

2– MATERIALS AND METHODS: -

2-1- collection of samples: -

One hundred apparent healthy of *O. niloticus* (average weight, 60 ± 20 g), were transported from a private fish farm (history indicate free from diseases). They were placed in aerated containers and brought to the wet-lab of the Fish Diseases Department at AHRI in Dokki. They reared in glass aquaria provided with a balanced commercial diet at a daily rate of 3% of their body weight. The aquarium water was partially replaced each day, with the temperature consistently maintained at 25°C. Fish were kept under observation for two weeks; fifteen fish were randomly selected to be examined for bacterial and parasitic infection **(Austin and Austin, 2012)** and also for meat quality determination.

Fish were divided into 3 groups (triple /group) for experimental for 30 days

G1: control fish fed in basal diet

G2: fed with 20g/kg of feed additives to basal diet

G3: fed with 40g/kg of feed additives to basal diet

The Summary of Information on Mineral Requirements of Fish according to NRC (1977):

Mineral element	Principal metabolic activities	Requirement / kg dry diet	Feed additives added / kg dry diet
Calcium	Bone and cartilage development, blood coagulation, and muscle contraction	5g	4g
Phosphorus	Bone formation; high energy phosphate esters; other organo-phosphorus compounds	7g	5g
Magnesium	Enzyme co-factor that plays a crucial role in the metabolism of fats, carbohydrates, and proteins is essential for facilitating biochemical reactions within the body.	500 mg	300 mg
Sodium	The primary monovalent cation found in intracellular fluid plays a crucial role in maintaining acid-base balance and regulating osmoregulation.	1-3g	2g
Potassium	The primary monovalent cation found in intracellular fluid plays a crucial role in nerve signal transmission and maintaining osmotic balance within cells.	1-3g	2g
Iron	Essential constituent of haeme in haemoglobin, cytochromes, peroxidases, etc.	50-100 mg	50 mg
Manganese	Co-factor for arginase and certain other metabolic enzymes; involved in bone formation and erythrocyte regeneration	20-50 mg	30 mg
Zinc	Essential for insulin structure and function; co-factor of carbonic anhydrase	30-100 mg	50 mg
Iodine	Constituent of thyroxine; regulates oxygen use	100-300 mg	200 mg

2-2- Microbiological analysis:

Microbiological testing was conducted on fish samples to determine the average values for Total Aerobic Mesophilic counts and *Staphylococcus aureus* counts, as well as to identify the presence of *Salmonella spp.*, *Listeria monocytogenes*, and *Shigella spp*. The preparation of test samples, initial suspensions, and serial decimal dilutions was performed in accordance with established protocols. **ISO (6887-1:2017)**.

Subsequent enumeration and isolation were conducted with the following methods:

Total Aerobic Mesophilic count (TAMC): ISO (4833-1:2013 Amd. 1:2022).

Standard Plate Count Agar (PCA, Oxoid) was maintained at a temperature of 30°C for an incubation period of 72 hours. The percentage of microbial reduction was determined using the formula: Microbial reduction percentage (%) = [(control CFU - treated CFU) / control CFU] × 100. Furthermore, the logarithmic scale reduction factor (Log10) was computed using the equation RF = Log10(A) - Log10(B), where A represents the colony count from the control sample and B denotes the colony count from the treated sample.

Coagulase positive Staphyaureus and other species: (ISO 6888-1: 2021 Amd. 1:2023).

Baird Parker agar (Oxoid) should be incubated at a temperature of 34–38°C for a period

of 24 to 48 hours.

Isolation of *Shigella spp.* (ISO 21567:2004)
Using Hektoen enteric agar. (HEA, Oxoid) incubated at 37 ± 1°C for between 20 h and 24 h.

Isolation of *Salmonella spp.* (ISO 6579-1:2017(E)) Amd. 1:2020
Xylose Lysine Deoxycholate agar (XLD agar, Oxoid) was incubated at 37 °C and observed after 24 hours.

Enumeration of *E. coli* and the coliform bacteria: (FDA:2020)
Using violet red bile agar (VRBA) and incubating it at 35°C for a period of 18 to 24 hours.

Isolation of *Listeria monocytogen*(FDA:2022)
Using oxford agar and incubated at 35ºC and reviewed after 24 hours.

2-3- Chemical analysis:
Measurement of Protein Content (ISO 1871: 2009):
Evaluation of protein content using Kjeldahl digestion followed by distillation, carried out with VELP Scientifica equipment, model DK.

Determination of fat % (ISO 1443:1973).
The process involves determining the fat content by first digesting the samples with concentrated hydrochloric acid through acid hydrolysis. This is then followed by extracting the fats using petroleum ether in a Soxhlet apparatus for 4 hours.

Total volatile basic nitrogen (TVB-N): (ES 63-9: 2006)
The TVB-N measurement was conducted in accordance with the procedure specified in the Egyptian standard. The TVB-N value was expressed as milligrams per 100 grams of sample.
Thiobarbituric acid values: (ES 63-10:2006).
Measurement of TBA was conducted in accordance with the procedure specified in the Egyptian standard. The TBA value was expressed in milligrams of malonaldehyde (Mal) per kilogram of sample.
Determination of Ash Content (AOAC, 2016):
Approximately 3 to 5 grams of the prepared sample were placed in a dry, clean, and preweighed crucible. The sample was then ignited in a muffle furnace (manufactured by DAIHAN Scientific, model FH-14, serial number 10002491300002) at a temperature of 550°C until it transformed into a light gray ash or stabilized at a constant weight. Once the process was complete, the crucible was cooled in a desiccator and immediately weighed upon reaching room temperature. The ash content was determined using the following calculation:

$$\text{Ash \%} = \frac{\text{Weight of Ash (g)}}{\text{Weight of the sample (g)}} \times 100$$

Determination of Carbohydrate Content (FAO, 2003):
Carbohydrate % = 100 - (moisture % + ash % + protein % + fat %)

Determination of Histamine Content using ELISA technology:
Histamine levels in various groups were assessed through ELISA screening. **Sadeghi et al. (2019)** according to the procedure applied in ELISA Kits RIDASCREEN (R-Biopharm, Darmstadt, Germany). Samples were read at 450 nm on a microplate reader (BioTeK®, PMT 49984, U.S.A.) expressed as mg/100g and Log was 0.25mg/ 100g.

Determination of Trimethylamine (ES 2760 -1:2006):
The determination of TMA was carried out following the Egyptian standard method. The TMA value was expressed in milligrams per 100 grams of the sample.

Determination of Moisture Content (AOAC, 2016):
Approximately 2.7 to 3 grams of the prepared sample were placed in a covered dish with dimensions of 50 mm in diameter and 40 mm in depth. The sample was then dried in a hot air oven (manufactured by MMM Group, model Venticell, Serial No. 007151) at a temperature of 125°C for a duration of 2 to 4 hours, or until a constant weight was achieved. The moisture percentage was determined using the following formula:

$$\text{Moisture\%} = \frac{\text{Weight loss (g)}}{\text{Weight of sample (g)}} \times 100$$

2.4. Immunological examination:

Lysozyme activity:

Fish were humanely euthanized following established protocols. (**Fernandes et al. 2017**) Blood samples were obtained from the caudal vein of the fish and centrifuged at 3000 rpm to separate the serum from the coagulated blood. A 100 µl volume of serum was placed in a flat-bottomed microplate and combined with Micrococcus lysodeikticus. The microplate was examined using an ELISA reader set to a wavelength of 450 nm. Lysozyme activity was assessed by measuring a decrease of 0.001/ minute in the optical density of the cell suspension (**Abu-Elala et al. 2015**).

Serum analysis: Serum total protein and albumin levels were measured using test kits provided by Spectrum Diagnostics, Germany, at wavelengths of 546 nm and 578 nm, respectively. Serum globulin levels and the A/G ratio were determined through mathematical calculations.

Gene expression: IL10

RNA extraction

RNA extraction from the anterior kidney of tilapia from each group was carried out using the QIAamp RNeasy Mini Kit (Qiagen, Germany, GmbH). A tissue sample weighing 30 mg was homogenized in 600 µl of RLT buffer, to which 10 µl of β-mercaptoethanol per milliliter had been added. During the homogenization process, the tubes were placed in adapter sets securely fastened within the clamps of the Qiagen Tissue Lyser. Disruption was achieved through a high-speed shaking step at 30 Hz for 2 minutes. An equal volume of 70% ethanol was then added to the clarified lysate, and the subsequent steps were carried out according to the instructions detailed in the protocol for Purification of Total RNA from Animal Tissues included with the QIAamp RNeasy Mini Kit.
Note: On-column D Nase digestion was performed to remove any remaining traces of DNA.

Oligonucleotide Primers. Primers used were supplied from **Metabion (Germany)** are listed in table (1).

Table 1. Primers sequences, target genes, amplicon sizes and cycling conditions for SYBR green -PCR

Target gene	Primers sequences	Reverse transcription	Primary denaturation	Amplification (40 cycles)			Dissociation curve (1 cycle)		
				Secondary denaturation	Annealing (Optics on)	Extension	Secondary denaturation	Annealing	Final denaturation
*EF-1α	CCTTCAACGCTCAGGTCATC	50˚C 30 min.	94˚C 15 min.	94˚C 15 sec.	62˚C 30 sec.	72˚C 30 sec.	94˚C 1 min.	62˚C 1 min.	94˚C 1 min.
	TGTGGGCAGTGTGGCAATC								
**IL10	CTGCTAGATCAGTCCGTCGAA				60˚C 30 sec.			60˚C 1 min.	
	GCAGAACCGTGTCCAGGTAA								

*Gröner et al. 2015----**Standen et al. 2016

C. SYBR green rt-PCR. The reaction setup utilized primers in a 25-µl mixture, which included 12.5 µl of 2x QuantiTect SYBR Green PCR Master Mix (Qiagen, Germany, GmbH), 0.25 µl of RevertAid Reverse Transcriptase (200 U/µL) from Thermo Fisher, 0.5 µl of each primer at a concentration of 20 pmol, 8.25 µl of water, and 3 µl of RNA template. The real-time PCR analysis was performed using a Stratagene MX3005P machine.

D. Analysis of theSYBR green rt-PCR results. Amplification curves and Ct values were analyzed using Stratagene MX3005P software. To assess variations in gene expression among RNA samples, the Ct values from each sample were analyzed relative to those of the positive control group. This comparison followed the ΔΔCt method described by **Yuan et al. 2006**, utilizing the formula: 2^(-ΔΔCt).
Whereas ΔΔCt = ΔCt reference – ΔCt target
$\Delta Ct\ target = Ct\ control - Ct\ treatment$ and $\Delta Ct\ reference = Ct\ control - Ct\ treatment$

2.5. Statistical analysis:
The statistical analysis of the collected data was performed in triplicate using the Statistical Package for the Social Sciences (SPSS) version 20. The results were presented as mean values along with their standard errors (Mean ± SE). Data analysis was conducted through one-way analysis of variance (ANOVA), and a p-value of 0.05 or less ($p < 0.05$) was regarded as statistically significant.

3- **Results**:

Table 2. Statistical analysis of Microbiological screening of Nile Tilapia fish (control and treated) samples:

fish samples		Positive fish samples		Fish groups			Maximum Permissible Limit
Microbiological parameters	No. of samples	No.	%	Control	2% treated group	4% treated group	Acc. to. ES: 3494/2005
APC	A total of			3.82 [a]	3.6[a]	2.3[a]	No more than
G1	45 samples (15 samples of each group)	13	86.6	log CFU.g-1	log CFU.g-1	log CFU.g-1	10^6 colonies/g fish muscles.
G2		11	73.3				
G3		14	93.3				
E. coli		<1 log	0.0	<1 LogCFU.g-1	<1 LogCFU.g-1	<1 LogCFU.g-1	2 log CFU.g-1 ČSN 56 9609 (2008)
S. aureus		<1 log	0.0	<1 Log CFU.g-1	<1 Log CFU.g-1	<1 LogCFU.g-1	not exceed 3 log 10 CFU.g-1
Shigella		ND	0.0	ND	ND	ND	Free of 25g sample
salmonella		ND	0.0	ND	ND	ND	Free of 25g sample
L. monocytogenes		ND	0.0	ND	ND	ND	Free of 25g sample

There were significances differences ($P < 0.05$) between means having the same letters in the same row.
NB. <1 log calculated zero counting statistical analysis
ND = Not detected in any sample
All groups were free from *S. aureus, E. coli, Salmonella, Shigella & L. monocytogenes*

Table 3. Chemical parameters that indicate the quality of Nile Tilapia fish (control and treated) samples:

fish groups	control			2% treated group			4% treated group		
test (mg/100g)	Min.	Max.	Mean ±SE	Min.	Max.	Mean ±SE	Min	Max	Mean ±SE
Histamine	0.051	0.057	0.054±0.001[a]	0.042	0.051	0.047±0.001[a]	0.032	0.045	0.038±0.001[a]
TVBN	12	12.5	12.22±0.049[b]	10.6	11.2	10.87±0.074[b]	8.2	8.6	8.31±0.046[b]
TBA	0.28	0.29	0.285±0.0006[ab]	0.197	0.21	0.21±0.001[a]	0.195	0.24	0.22±0.003[b]
TMA	4.99	5.04	5.02±0.005[a]	5.39	5.544	5.46±0.02[ab]	4.97	5.04	5.01±0.007[b]

There were significances differences (P < 0.05) between means having the same letters in the same row.

Table 4. Acceptability of the examined Nile tilapia fish samples of according to their TVBN, TBA and TMA values (n=15)

Chemical Test parameters	Maximum Permissible Limit (mg %)	Accepted samples of treated groups		Unaccepted samples of treated groups	
		NO.	%	NO.	%
Histamine	5 Acc. to FDA(2011)	30	100%	0	0 %
TVBN	30*	30	100 %	0	0 %
TBA	1*	30	100 %	0	0 %
TMA	10*	30	100 %	0	0 %

* Egyptian Organization for Standardization "EOS" (2005).

Table 5. chemical comparison between the control and the fish treated groups

Fish groups	Nile Tilapia Fish groups								
Chemical test	control			2% treated group			4% treated group		
	Min.	Max.	Mean ±SE	Min.	Max.	Mean ±SE	Min	Max	Mean ±SE
Fat %	3.89	3.95	3.92 ±0.006[a]	3.75	3.81	3.78 ±0.005[ab]	3.75	3.85	3.78 ±0.004[b]
Carbohy--drates %	0.59	0.61	0.60 ±0.0006[a]	0.51	0.52	0.516±0.0005[a]	2.59	2.66	2.63 ±0.006[a]
Proteins %	18.19	18.28	18.23±0.009[a]	18.65	18.73	18.69±0.008[a]	19.89	19.97	19.94±0.008[a]
Moisture%	76.01	76.04	76.03±0.006[a]	74.39	74.49	74.44±0.01[a]	73.28	73.39	73.34±0.01[a]
Ash%	1.39	1.44	1.41±0.004[a]	1.49	1.56	1.52 ±0.007[a]	1.73	1.78	1.76±0.005[a]

There were significances differences (P < 0.05) between means having the same letters in the same row

Nile Tilapia groups were monitored daily, showing no clinical signs or instances of mortality.

Serum analysis

The serum analysis results indicated that lysozyme activity was significantly higher ($P<0.05$) in the control group compared to the 2% treated Nile tilapia samples (G2). However, in the 4% treated samples (G3), the lysozyme activity showed a slight increase compared to the control group, as illustrated in Fig. (1).

Total protein decrease in the control group fish samples than fish samples of G2 and G3, while A/G increase in G2 than G3 and control group fish samples fig (1)

IL10 cytokine

The mRNA expression levels of the immune-related cytokine IL-10 showed values of 2 in the control group, 5.4 in G2, and 7.5 in G3. After 30 days of administration, inflammation in *O.niloticus* subsided rapidly as the expression of the anti-inflammatory cytokine IL-10 showed a notable increase in fish consuming diets enriched with feed additives.

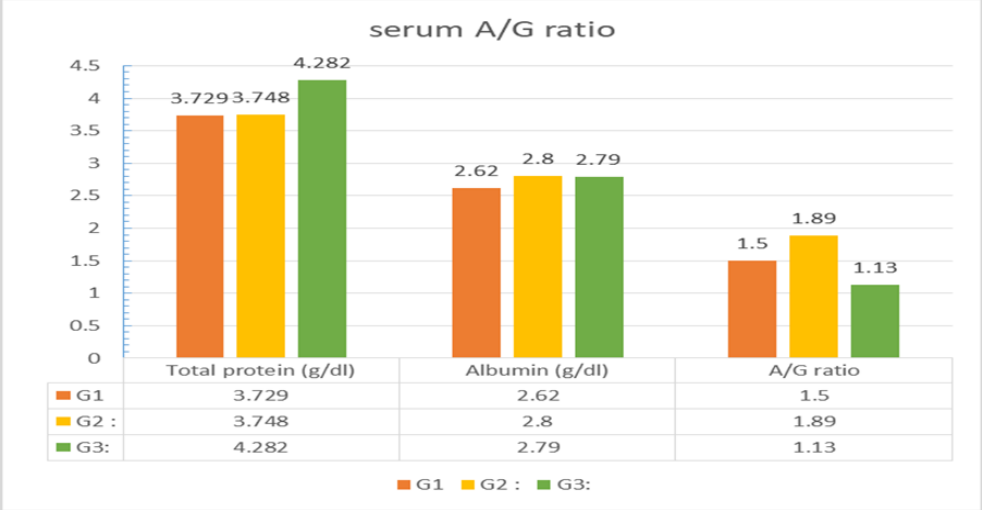

Fig. (1) Serum analysis of *O. niloticus* groups

DISCUSSION:

The safety and quality assurance of fish products represent a major challenge for the food industry today. The occurrence of food borne pathogens in fish products depends largely on factors such as the conditions of the harvest environment, the hygiene standards maintained, and the practices related to equipment and personnel within the processing facilities **(Grigoryan, et al. 2010)**.

Fish spoilage begins with autolytic degradation, followed by microbial activity that intensifies the breakdown of fish tissues, giving rise to unpleasant odors and flavors. While various microorganisms are commonly present in seafood after harvest, specific spoilage organisms (SSOs), such as psychrotrophic bacteria and coliforms, are identified as the primary culprits **responsible** for fish degradation and the reduced shelf life. To preserve the quality and freshness of fish products and extend their shelf life, it is essential to effectively control the growth of these SSOs. (**Ray and Bhunia, 2008**).

The total aerobic count.

The total aerobic count (table 2) observed in the samples from both the control and treated groups was comparable ($p < 0.05$). In the control, the total aerobic count was 3.82 logs CFU.g-1. In the treated group of 2% the total aerobic count was 3.6 logs CFU.g-1 while in the treated group by 4% the count was 2.3 logs CFU.g-1. The analysis revealed highly significant differences among the samples studied

(P< 0.05). All samples were approved in accordance with **ES. No. (3494: 2005)** which stated that, the permissible limit for the total bacterial count for fish not exceeding 10^6 colonies per gram of fish muscle.

The total aerobic count (TAC) observed in the samples was lower across all treated fish samples as well as the control group. When comparing the obtained TAC values with the results of ***Cwiková* , O (2016)**.

Nespolo et al. (2012) results for TAC are closely aligned with those observed in our study, ranging from 1.1 x 10^3 to 3.9 x 10^6 $CFU.g^{-1}$.

Miks-Krajnik et al. (2016) and **Parlapani and Boziaris (2016)** revealing that the total aerobic count at the start of storage was 3.5 log CFU.g-1. After storage at 5 °C, this increased to 8.1 logs CFU.g-1, a value higher than what was observed in our analysis.

E. coli and the coliform bacteria count:

Coliform bacteria, part of the Enterobacteriaceae family, are significant contributors to food spoilage through their capacity to break down amino acids into foul-smelling volatile compounds **(Remenanta et al. 2015)**.

Table (2) found in the samples of control and the treated groups of fish there were less than 10 CFU.g-1 and thus acceptable completely with ČSN **569609 (2008)** which set the maximum allowable quantity of E. coli in fresh fish and their parts was 2 log CFU per gram.

Isolation of Salmonella spp.:

Salmonella can be found in seafood due to contamination that originates from the natural aquatic environment, aquaculture practices, or cross-contamination during storage, transportation, and processing stages. **(Amagliani et al. 2012)**. According these study salmonella-negative results is in agreement with **ES. No. (3494: 2005)** The law demands absolute absence of salmonella in 25g food samples—a non-negotiable safety standard designed to protect public health. The samples under review clearly violate this essential regulatory requirement, necessitating immediate action. Conversely, **Patil et al. (2013)** found no salmonella in fish samples stored at 4°C during their microbiological quality assessment.

Enumeration of Staphylococcus Aureus:

Table (2) revealed that the control group and the two treated groups less than 10 CFU.g-1., so the results of fish examined samples accepted with the level that mentioned in **ES. No. (3494/2005)** *(Staph. aureus*count should not exceed 3 log 10 cfu/g of fish).

Isolation of Listeria Monocytogens :

ES. No. (3494: 2005) Microbiologic food criteria require zero *Listeria monocytogenes* in 25 g samples. That confirmed with the result in our study which revealed no *listeria monocytogens* were detected.

Isolation of Shigella spp.:

Moreover in table (2) there weren't *shigella* spp. detected in both control and the treated groups. Looking at European food safety regulations, there's a clear standard for fishery and aquaculture products: absolutely no *Shigella* bacteria can be present in 25g of sample. This zero-tolerance policy applies across all product types—whether they're fresh, salty, refrigerated, frozen, dry, salty-dry, semi-pickled, or smoked. **ES. No. (3494: 2005)**.

Fish decomposition is very complex (**Hungerford, 2010**) therefore; Sensory evaluation alone is often insufficient to accurately determine the quality of most fish. As a result, chemical testing becomes essential, focusing on parameters such as volatile amines and lipid oxidation, which are widely recognized as key indicators for assessing fish freshness and quality (**Gulsun et al. 2009 and Prester, 2011).**

Histamine in fish is produced by specific bacteria that generate the enzyme histidine decarboxylase. This enzyme transforms the naturally occurring histidine, found in the muscle tissue of certain fish, into histamine **(FAO/ WHO, 2013) .**

Table (3) revealed that the amount of histamine with mean values 0.054 ±0.001, 0.047±0.001 and 0.038±0.001 mg/100g with minimum values of 0.051, 0.042 and 0.032 mg/100g and of maximum values of 0.057, 0.051 and 0.045 mg/100g in the control fish , treated fish 2% group and treated fish 4% group, respectively. There were high significant differences among examined samples (P< 0.05).These results were lower than the detection level 5 mg/100 g given by **FDA (2011)** in table (4), that's mean, the samples of high quality and freshness.

Higher results obtained by **Refai et al. (2020)** who found histamine amount 8.97-9.76 mg/100mg in tilapia.

FAO/WHO (2013) Stated that when fish that form scombrotoxin are just pulled from the water, their histamine levels are remarkably low—typically less than 2 mg/kg, fresh as can be.

The FDA regulation dictates that the maximum allowable limit for histamine in seafood is 100 mg/kg, equivalent to 100 ppm. Low concentrations of biogenic amines are generally not a significant health concern, as enzymes such as monoamine oxidase and diamine oxidase in the human intestine are capable of detoxifying these compounds **(Biji et al. 2016).**

Once the fish dies, various deterioration processes begin to take effect in all types of meat. These include the breakdown of proteins and ATP, a drop in pH levels, lipid oxidation, and the formation of unwanted compounds like trimethylamine (TMA-N) and low molecular weight volatile bases (TVB-N), typically generated through bacterial activity. At the same time, noticeable changes occur in the texture and color of the muscle **(Li et al. 2011) .** These changes can be categorized into biochemical, physical, and microbiological factors, all of which influence consumer acceptance. When combined with a nutritional assessment, these factors help determine the shelf life of the meat **(McMillin, 2008)**.

Moreover table (3) revealed that the mean values of TVB-N mg/100g of fresh fish were 12.22±0.049, 10.87±0.074 and 8.31±0.046 mg/ 100 gm with minimum values were 12, 10.6 and 8.2 mg/100 gm and also the maximum values were 12.5, 11.2 and 8.2 mg/100 gm in the control group, treated fish group 2% feed additives and treated fish group 4% feed additives, respectively. Examined samples showed highly significant differences (P<0.05). In table (3) all fish samples were acceptable with values below maximum permissible limits per **EOS (2005).**

Omayma et al. (2013) measured TVB-N values in fresh fish which started at 5.03±1.12, 7.71±0.58, 7.14±0.47, and 6.42±0.43 mg/100g on day 1, rising to 66.5±0.39, 73±0.1, 70.67±1.48, and 70.2±1.04 by day 11 when spoilage occurred. This gradual increase with storage time indicates good initial freshness and quality.

Trimethylamine (TMA) values in table (3) ranged from 4.99 to 5.04mg /100g with an average of 5.02±0.005 mg /100g in the control group, while from 5.39 to 5.54 mg /100g with an average of 5.46±0.02 mg /100g in the treated fish group 2% with feed additives, moreover TMA in the treated fish group 4% with feed additives ranged from 4.97 to5.04 with an average of 5.01±0.007 mg /100g. There were significant differences among examined samples (P<0.05) of the treated fish group 2% with feed additives with the control group and with the treated fish group 4% with feed additives.

The comprehensive study by **EOS (2005)** confirms the safety of all examined fish samples, with every sample meeting regulatory standards by falling below maximum permissible limits. This finding is especially significant because TMA—recognized by **Baixas-Nogueras et al. (2002)** as a critical quality indicator across numerous fish species—validates the reliability of these results for consumer confidence.

Lower results obtained by **Hassan et al. (2019)** who revealed that TMA value in O. niloticus varied from 1.92 to 4.67 mg /100g with an average of 3.54 ± 0.26 mg /100g.

Table (3) revealed also the mean values of

thiobarbituric acid (TBA) 0.285±0.0006, 0.21±0.001 and 0.22±0.003 mg /100g with values ranged from 0.28 to 0.29, from 0.197 to 0.21 and from 0.195 to 0.24 mg /100g in the control, 2% treated fish group and 4% treated fish group, respectively. There were significant differences among examined samples (P<0.05) of the control group with 2% treated fish group and with 4% treated fish group.

Moreover table (4) mentioned that by **EOS (2005)** every fish sample examined passed safety standards, with all values comfortably below the maximum permissible limits.

Hassan et al. (2019) obtained TBA values lower than our study as they were from 0.087 to 0.151 mg /100g with an average of 0.117 ± 0.08 mg /100 g in O. niloticus. TBA effectively demonstrates fat rancidity.

Fish meat primarily consists of water (66-81%), protein (16-21%), carbohydrates (less than 0.5%), lipids (0.2-25%) and ash (1.2 to 1.5%) (**FAO, 1999)**; and is regarded as having significant biological value **(Molina et al. 2001 and Santaella et al. 2007)**, due to the contribution of essential amino acids **(Hatae et al. 1990).** Additionally, it contains high levels of omega-3 and omega-6 fatty acids, surpassing the concentrations found in most meats consumed by humans **(Gjedrem et al. 2012)**.

Table (5) illustrated the chemical comparison between the control group and the treated groups where fat % were the mean values 3.92 ±0.006, 3.78 ±0.005 and 3.78 ±0.004 % within range from 3.89 to 3.95 , from 3.75 to 3.81 and from 3.75 to 3.85 % in control , 2% treated group and 4% treated group, respectively. There were significant differences among examined samples (P<0.05) of 2% treated fish group with the control group and with4% treated fish group.

That's mean the amount of fat identical in all samples according to (**FAO, 1999).**

Moreover table (5) revealed that the carbohydrates % ranged from 0.59 to 0.61 with average 0.60 ±0.0006 in the control fish and ranged from 0.51 to 0.52 in 2% treated group but the percentage increase in 4% treated group to became from 2.59 to 2.66 with an average 2.63±0.006 %, so, 4% treated fish group were exceed the permissible limit according to (**FAO, 1999)**.

Protein % in table (5) ranged from 18.19 to 18.28% in the control group with similar results in 2% treated group which ranged from 18.65 to 18.73 % while protein % slightly increased in 4% treated group to became 19.89 to 19.97% but overall the all samples were have adequate amount of protein that indicate high quality fish meat (**FAO, 1999)**. There were high significant differences among examined samples (P<0.05).

The moisture content in fish as mentioned in table (5) with average 76.03±0.006, 74.44±0.01 and 73.34±0.01% in the control, 2% treated group, 4% treated group, respectively. Where the results indicate the amount of moisture decreased in the treated groups than the control one that's revealed enhancing to the quality of fish meat as mentioned in **FAO, (1999)**. There were high significant differences among examined samples (P<0.05)

The average value of Ash content as table (5) discussed were 1.41±0.004, 1.52 ±0.007 and 1.76±0.005 % in the control, 2% treated group, 4% treated group, respectively. There were high significant differences among examined samples (P<0.05). These results were acceptable limits by **FAO, (1999)**.

The anterior kidney plays a crucial role in supporting systemic immunity and is essential for removing foreign or antigenic substances from the bloodstream of teleost fish **(Grove et al. 2006).** When activated, these innate immune cells perform various functions to eliminate an antigen, including degranulation, phagocytosis, and the release of cytokines and chemokines. These substances help activate or recruit other leukocytes to the site of action **(Pandey et al. 2021)**.

Lysozyme measured was reduced in the control group similarly (**Möck and Peters (1990**). Strong stressors, such as transport or acute water pollution, significantly reduced

rainbow trout's lysozyme level. But increase in G2 than control group

One of the key biochemical indicators used to assess the health of fish is protein., Increase in total protein concentration in G2 and G3 aligned with **Abd Elmonem et al. (2004)**, though interestingly, **El-Houssiny et al. (1999**) observed no meaningful variations in either non-specific immunity factors like serum proteins or humoral immune responses when studying Nile tilapia.

Our findings reveal how dietary minerals stimulated the gene expression of IL-10 in *O. niloticus* that agreed to **Sherif et al. (2022)** Cytokine il-10 was significantly and rapidly increased in *O. niloticus* fed on diets containing 100-fold FFC plus *S. platens is* compared to the other treatments.

CONCLUSION:

It is the fundamental duty of all producers to ensure the health and safety of food. It is crucial to control bacterial contamination at every stage, from capture and handling to processing and final consumer consumption.
Microbial criteria and monitoring levels are essential to food inspection, determining whether fish and seafood are acceptable for consumers.
In conclusion, the evidence clearly demonstrates that the quality of fish that treated with feed additives with higher quality than the control one.

REFERENCES

Ababouch L. 2006. Assuring fish safety and quality in international fish trade. Marine Pollution Bulletin, (53):561–568.

Abd Elmonem AI, Shalaby SMM, El-Dakar AY, Sadrak OW. 2004. Nutritional evaluation of sesame hulls by-product as a non-conventional feedstuff in diets of red tilapia, *Oreochromis niloticus*× *Oreochromis mosambicus*. Alex. J. Agric. Res., 49 (2): 1 – 13.

Abu-Elala NM, Mohamed SH. Zaki MM, Eissa AE. 2015. Assessment of the immune-modulatory and antimicrobial effects of dietary chitosan on Nile tilapia (*Oreochrmis niloticus)* with special emphasis to its bio-remediating impacts, Fish Shellfish Immunol., 46 (2): 678–685.

Ackman RG. 2000. Nutritional composition of fats in seafood. Progress in Food and Nutrition Science, (13): 161-241.

Alasalvar C, Taylor KDA, Zubcov E, Shahidi F, Alexis M. 2002. Differentiation of cultured and wild sea bass (Dicentrarchuslabrax): total lipid content, fatty acid and trace mineral composition. Food Chemistry, (79): 145-150.

Amagliani G, Brandi G, Schiavano GF. 2012. Incidence and role of *Salmonella* in seafood safety (Review). *Food Research International*,45(2):780-788.

Association of Official Analytical Chemists "AOAC" 2016. Official methods of analysis, 20th Ed., (On-line). AOAC International, Rockville, MD. This reference contains the official methods for many specific food ingredients.

Austin B, Austin AD. 2012. Bacterial fish pathogens: diseases offarmed and wild fish. 5th ed. Chichester, UK: Springer/ Prazis Publishing ; 2012

Baixas-Nogueras S, Bover-Cid S, Veciana – Nogue T, Vidal-Carou MC. 2002. Chemical and sensory changes in Mediterranean hake (Merlucciusmerluccius) under refrigeration (6-8 oC) and stored in ice. Journal of Agriculture and Food Chemistry (50): 6504-6510.

Biji KB, Ravishankar CN, Venkateswarlu R, Mohan CO, SrinivasaGopal TK. 2016. Biogenic amines in seafood: a review. J Food Sci Technol., 53(5): 2210 – 2218.

Burger J, Gochfeld M. 2009. Perceptions of the risks and benefits of fish consumption: Individual choices to reduce risk and increase health benefits. Environmental Research, (109): 343-349.

Commission Regulation (EC) No 2073/2005 of 15 November (2005): on Microbiological Criteria for Foodstuffs. OJ L 338, 22/12/2005, p. 1-6.

ČSN (Czech technical standard) 569609 2008. Guides to good hygiene and manufacturing

practice-Microbiological criteria for foods. Principles for the establishment and application.

Cwiková O. 2016. Microbiological Evaluation of Fish. Potravinarstvo® Scientific Journal for Food Industry, 10(1): 407-412. doi:10.5219/617.

El-Houssiny OM, Shehata TM, Arafa SA, Goda AMA. 1999. Effect of dietary protein level and feeding regime on the humoral immune response of Nile tilapia (*Oreochromis niloticus*). Egypt. J. Aquat. Biol. & Fish., 3 (1): 25-38.

EOS. 2005. Chilled fish, ES, 3494:2005. ICS: 67.120.30. Egyptian Organization for Standardization and Quality Control, Arab Republic of Egypt.

ES 2760-1: 2006. Egyptian Standard, method of analysis and testing for fish and fish products, part1; frozen fish.

ES 63-10: 2006. Egyptian Standard, method of analysis and testing for meat and meat products, part10; determination of thiobarbituric acid, no 63-10/2006.

ES 63-9: 2006. Egyptian Standard, method of analysis and testing for meat and meat products, part9; determination of total volatile nitrogen, no 63-9/2006.

FAO. 1999. El pescado fresco: Su calidad y cambios de sucalidad. Documentotécnico de pesca 348. H.H. Huss (ed.). FAO, Roma. 220 p.

FDA (Food and Drug Administration) 2011. Fish and Fishery Products Hazards and Controls Guidance, 4th edn. Department of Health and Human Services, Food and Drug Administration, Center for Food Safety and Applied Nutrition, Washington DC.

FDA (Food and Drug Administration) 2020. Bacteriological Analytical Manual Chapter 4: Enumeration of Escherichia coli and the Coliform Bacteria, October 2020 Edition.

FDA (Food and Drug Administration) 2022. Bacteriological Analytical Manual. Chapter 10: Detection of *Listeria monocytogen* in foods and environmental samples, and Enumeration of *Listeria monocytogen* in foods, April 2022.

Fernandes IM, Bastos YF, Barreto DS, Lourenço LS, Penha JM. 2017. The efficacy of clove oil as an anaesthetic and in euthanasia procedure for small-sized tropical fishes, Braz. J. Biol. 77 (3): 444 –450

Food and Agriculture Organization of the United Nations "FAO" 2003. Food energy – methods of analysis and conversion factors. Food and Nutrition Paper, Rome, (77): ISSN 0254-4725R.

Food and Agriculture Organization of the United Nations/World Health Organization (FAO/WHO). 2013. Public Health Risks of Histamine and other Biogenic Amines from Fish and Fishery Products. Meeting report.

Gjedrem T, Robinson N Rye M. 2012. The importance of selective breeding in aquaculture to meet future demands for animal protein: A review. Aquaculture 350 (35):117-129.

Grigoryan K, Badalyan G, Andriasyan D. 2010. Prevalence of *Staphylococcus aureus*in Fish Processing Factory. *Potravinarstvo*, 4, (2):25-28. http:// dx.doi.org/10.5219/47

Gröner F, Ziková A, Kloas W. 2015. Effects of the pharmaceuticals diclofenac and metoprolol on gene expression levels of enzymes of biotransformation, excretion pathways and estrogenicity in primary hepatocytes of Nile tilapia (*Oreochromis niloticus*). Comparative Biochemistry and Physiology, Part C 167 (2015) :51–57.

Grove S, Johansen R, Reitan JL, press C. 2006. Immune and enzyme histochemical characterization of leukocyte populations within lymphoid and mucosal tissues of Atlantic halibut (Hippoglossus hippoglossus). Fish & Shellfish Immunology, vol. (20):693-708.

Gulsun O, Esmeray K, Serhat O, Fatih O. 2009. Sensory, microbiological and chemical assessment of the freshness of red mullet (Mullusbarbatus) and goldband goatfish (Upeneusmoluccensis) during storage in ice. Food Chem, (114): 505-10.

Hassan MA, Engy FAM, Elbahy Mai, F, Elsherief. 2019. Evaluation of some quality indices in farmed fish from KafrElshiekh

governorate. Benha veterinary medical journal,37, (2): 210-218.

Hatae K, Yoshimatsu F. Matsumoto JJ. 1990. Role of muscle fibers in contributing firmness of cooked fish. Journal Food Science 55(3): 693-696.

Hungerford JH. 2010. Scombroid poisoning: a review. Toxicon.J, (56): 231-43.

ISO (1443:1973): Meat and meat products — Determination of total fat content.

ISO (1871:2009): Food and feed products, general guidelines for the determination of nitrogen by the kjeldahl method.

ISO (4833-2:2013 Amd 1:2022): Microbiology of the food chain- Horizontal method for the enumeration of microorganisms-Part 2: Colony count at 30° C by the surface plating technique. Amendment 1: clarification of scope.

ISO (6579-1:2017, Amd 1:2020): Microbiology of the food chain — Horizontal method for the detection, enumeration and serotyping of Salmonella — Part 1: Detection of Salmonella spp. — AMENDMENT 1: Broader range of incubation temperatures, AMENDMENT to the status of Annex D, and correction of the composition of MSRV and SC.

ISO (6887-1:2017): preparation of test samples, initial suspension and decimal dilution for microbiological examination, part 1; general rules for the preparation of the initial suspension and decimal dilution.

ISO (6888-1: 2021, Amd 1: 2023): Microbiology of food and animal feeding stuffs -- Horizontal method for the enumeration of coagulase positive staphylococcus aureus and other species, part 1: Method using Baird- Parker agar medium.

ISO (21567:2004): Microbiology of food and animal feeding stuffs — Horizontal method for the detection of shigella spp.

Kayan A, Boontan I, Jaturssitha S, Wicke M, Kreuzer M. 2015. Effect of slaughter weight on meat quality of Nile tilapia (*oreochromis niloticus*). Agriculture and Agriculture science procedia (5):159-163.

Kmínková M, Winterová R, Kučera J. 2001. Fatty acids in lipids of carp (Cyprinuscarpio) tissues. Czech Journal of Food Sciences, (19): 177-181.

Lall SP. 2002. The minerals. In Fish Nutrition, 3rd ed.; Halver, J.E., Hardy, R.W., Eds.; Elsevier/Academic Press: San Diego, CA, USA, pp. 259–308.

Lall SP, Kaushik SJ. 2021. Nutrition and Metabolism of Minerals in Fish. Animals, (11):2711.

Li, X, Li, J, Zhu, J, Wang Y, Fu, L, Xuan W. 2011. Postmortem changes in yellow grouper (*Epinephelusawoara*) fillets stored under vacuum packaging at 0 °C. Food Chemistry 126(3): 896-901.

McMillin KW. 2008. Where is MAP going? A review and future potential of modified atmosphere packaging for meat. Meat Science 80(1): 43-63.

Miks-Krajnik M, Yoonc YJ, Ukukud DO, Yuka HG. 2016. Volatile chemical spoilage indexes of raw Atlantic salmon (*Salmosalar*) stored under aerobic condition in relation to microbiological and sensory shelf lives. *Food Microbiology*, vol. 53, Part B, p. 182-191. http://dx.doi.org/10.1016/j.fm.2015.10.001 PMid: 26678146.

Möck A, Peters G. 1990. Lysozyme activity in rainbow trout, *Oncorhynchusmykiss* (Walbaum), stressed by handling, transport and water pollution Volume Journalof fish Biology 37, Issue 6 December : 873–885.

Molina MR, Garroy OA, Judis MA. 2001. Calidad oxidativay estabilidad oxidativa de *Pseudoplatystoma curruscans*. Ciencia y Tecnología de los Alimentos 3(2): 89-95.

National Research Council (NRC) 1977. Subcommittee on Warm water Fishes, Nutrient requirements of warm water fishes. Washington, D.C., National Academy of Sciences, (Nutrient requirements of domestic animals) 78 p.

National Research Council (NRC) 2011. Nutrient Requirements of Fish and Shrimp; The National Academies Press: Washington, DC, USA, 2011.

Nespolo NM, Martineli TM, Rossi OD. 2012. Microbiological Quality of Salmon (*Salmosalar*) Sold in Cities of the State of

Sao Paulo, Brazil. *Brazilian Journal of Microbiology*, 43(4):1393-1400.

Nicolae GC, Bahaciu VG, ELIA E, Dumitrache F, Marin PM, Pogurschi E, Bădulescu L. 2016. A Review Of The Quality Standards For Frozen Beef Meat And Fish .Scientific Papers. Series D. Animal Science.Vol. LIX, 2016ISSN 2285-5750; ISSN CD-ROM 2285-5769; ISSN Online 2393-2260; ISSN-L 2285-5750.

Omayma MT, LabibMaghraby Hassouba MM, El-Mossalami EE. 2013. Effect of Methodology on the Determination of Total Volatile Basic Nitrogen as an Index of Quality of Meat and Fish Egyptian Journal of Food Safety, vol. (1): 23-34. ISSN: 2314-5676 © 2012 issued by Egyptian Veterinary Association for Food Control and Consumer Protection.

Pandey S, Stockwell CA, Snider MR, Wisenden BD. 2021. Epidermal club cells in fishes: A Case of Ecoimmunological Analysis. International Journal of Molecular sciences, 22(3): 1440.

Parlapani FF, Boziaris IS. 2016. Monitoring of spoilage and determination of microbial communities based on 16S r RNA gene sequence analysis of whole sea bream stored at various temperatures. LWT-Food Science and Technology, (66):553-559.

Patil SS, Koli JM, Sharangdher ST, Sharangdher MT. 2013. Storagecharakteristice of fish pakoda“ from pink perch (Nemipterusjaponicus) meat at 4 °C. Ecology, Environment and Conservation, 19, (3): 877-880.

Press CM, Evensen Ø. 1999. The morphology of the immune system in teleost fishes. Fish and Shellfish Immunology, (9):309–318.

Prester L. 2011. Biogenic amines in fish, fish products and shellfish: a review. *Food Add Contam*, (28): 1547-60.

Ray B, Bhunia A. 2008. Fundamental food microbiology; 4th ed. CRC Press, New York.

Refai EAM, El-Hariri M, Zakia AM, Ahmed and Jakeen El Jakee1. 2020. Histamine Producing Bacteria in Fish. Egyptian Journal of Aquatic Biology & Fisheries Zoology Department, Faculty of Science, Ain Shams University, Cairo, Egypt, 24(7): 1 – 11.

Remenanta B, Jaffrèsa E, Dousseta X, Pileta MF, Zagoreca M. 2015. Spoilers, wonder spores and diehard microorganisms: New insights to integrate these super foes in food spoilage risk management. *Food Microbiology*, vol. 45, Part A, 45-53.

Sadeghi N, Behzad M, Jannat B, Reza- Oveisi M, Hajimahmoodi M, Mozafazri M. 2019. Determination of histamine in canned tuna fish available in Tehran market by ELISA method. J Food Safe &Hyg., 5 (1): 46-50.

Santaella M, Martínezy G, Periago MJ, 2007. Comparación entre Ludubina (*Dicentrarchuslabrax*) salvaje y cultivada: composiciónquímica y variacióndelcontenido en ácidosgrasostras el cocinado. Anales de Veterinaria de Murcia (23): 105-119.

Sherif AH, Abdellatif JI, Elsiefy MM, Gouda MY, Mahmoud AE. 2022. Occurrence of infectious *Streptococcus agalactiae*in the farmed Nile tilapia, Egyptian Journal of Aquatic Biology & Fisheries, 26(3): 403 – 432

Standen BT, Peggs DL, Rawling MD, Foey A, Davies SJ, Santos GA, Merrifield DL. 2016. Dietary administration of a commercial mixed-species probiotic improves growth performance and modulates the intestinal immunity of tilapia, *Oreochromis niloticus*. Fish & Shellfish Immunology (49): 427-435

Yuan JS, Reed A, Chen F, Stewart CN. 2006. Statistical analysis of real-time PCR data. *BMC Bioinformatics*, (7):85.