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INSTITUTO DE INVESTIGACIONES OCEANOLÓGICAS

MAESTRÍA EN CIENCIAS EN OCEANOGRAFÍA COSTERA

Efecto de la suplementación dietaría con L-arginina sobre el desempeño, salud, y expresión de genes del sistema antioxidante e inmune en tilapia del Nilo (*Oreochromis niloticus, Linnaeus, 1758*) expuesto a estrés crónico por salinidad

T E S I S

QUE PARA CUBRIR PARCIALMENTE LOS REQUISITOS NECESARIOS PARA OBTENER EL GRADO DE MAESTRO

PRESENTA

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FACULTAD DE CIENCIAS MARINAS
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Presenta

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Resumen

A causa del estrés hídrico, y la escasez de alimento, se ha considerado como una posible solución el cultivo de organismos dulceacuícolas en ambientes salinos. Sin embargo, esto puede representar un estrés crónico para algunas especies, lo que puede comprometer la calidad e integridad del cultivo. Es por ello que se ha indagado más sobre suministrar suplementos dietarios que mitiguen los efectos derivados del estrés. En el presente estudio se evaluaron los efectos de cuatro dietas con distintas inclusiones de arginina en el crecimiento, salud y expresión de marcadores moleculares relacionados con el sistema antioxidante e inmune, en juveniles de tilapia del Nilo expuestos a estrés crónico por salinidad. Los organismos fueron alimentados con cuatro dietas experimentales suplementadas con 0, 1, 2 o 3% de arginina. Las tilapias fueron cultivadas durante 57 días a una salinidad de 20 UPS. Se observó un incremento en el crecimiento con respecto al incremento de arginina. Sin embargo, las concentraciones medias de arginina tuvieron un efecto positivo en hemoglobina, recuento leucocitario, diferenciación de leucocitos y presencia de aberraciones nucleares. Así mismo, los parámetros de química sanguínea, como proteína total, albumina, globulina y triglicéridos también tuvieron un efecto positivo ante concentraciones medias de arginina. Los marcadores moleculares del sistema inmune (Il-10) y estrés (SOD y HSP70) mostraron alta correlación a tendencia cuadrática. En conclusión, la suplementación dietaría con L-arginina puede ayudar a la tilapia del Nilo a disminuir los efectos del estrés crónico por salinidad.

Palabras clave: Suplementos, estrés, *Oreochromis niloticus*, L-arginina, salud, inmunidad, estrés oxidativo.

Abstract

Due to water stress and food scarcity, growing freshwater organisms in saline environments has been considered a possible solution. However, this is a chronic stress that can affect the quality and integrity of the organisms. For this reason, the use of dietary supplements to mitigate the effects of stress has been further investigated. In the present study, we evaluated the effects of four diets with different levels of arginine inclusion on growth, health, and expression of molecular markers related to the antioxidant and immune systems in juvenile Nile tilapia exposed to chronic salinity stress. The organisms were fed four experimental diets supplemented with 0, 1, 2, or 3% arginine. The tilapia were grown for 57 days at 20 UPS. An increase in growth was observed with the rise in arginine. However, the mean arginine concentrations positively affected hemoglobin, leukocyte count, leukocyte differentiation, and the presence of nuclear aberrations. Blood chemistry parameters such as total protein, albumin, globulin, and triglycerides also had a positive effect on mean arginine concentrations. Molecular markers of the immune system (IL-10) and stress-related (SOD and HSP70) showed a high quadratic trend. In conclusion, arginine supplementation can alleviate the chronic stress caused by salinity.

Keyword: Supplements, stress, *Oreochromis niloticus*, L-arginine, health, immunity, oxidative stress.

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CAPÍTULO I: Introducción

1.1 La acuicultura frente al cambio climático

El aumento poblacional y la seguridad alimentaria son unos de los mayores retos en la actualidad (FAO, 2018; 2020; 2022; 2024a). En la actualidad se estima que más del 40% de la población mundial carece de una dieta adecuada (FAO, 2024a). Este problema desencadenó la búsqueda inmensurable por una manera sostenible de producir alimento (FAO, 2020). Dado que es complicado encontrar un balance entre abastecimiento de alimento y bajo impacto al ecosistema. La mayoría de las industrias alimentarias generan gran producción de CO_2, tala de flora, acidificación de suelo, alteración de integridad edafológica, entre otros (FAO, 2022). En años recientes, la FAO (2020) cataloga la industria acuícola como "la opción más prometedora". Esta actividad proporciona proteína de alta calidad (al considerar los criterios de textura y palatabilidad), tiene un menor impacto al ecosistema, puede ser realizada en cuerpos de agua naturales y artificiales (FAO, 2020; Li *et al.*, 2023). Pese a que esta actividad pudiese ser una solución para el abastecimiento de alimento, existe otro problema, la disponibilidad de agua dulce.

El estrés hídrico cada vez es un problema más palpable, con el aumento poblacional, el cambio climático y la intrusión salina, este recurso cada vez es más limitado (Kummu *et al.*, 2016; Pueppke, 2025). Es por esto que se busca aprovechar en mayor medida los cultivos en cuerpos de agua marinos, y con ello disminuir costos totales de producción, así como llevar a cabo esta actividad en zonas con estrés hídrico (SADER, 2018; Norambuena Cleveland *et al.*, 2024, FAO, 2024a).

Las actividades de cultivo realizadas en el mar pueden ser nombradas como maricultura (uso de jaulas flotantes o líneas en caso de almejas) o acuicultura costera (estanques o jaulas en la zona costera) (FAO, 2020; FAO, 2024a). En el año 2022, estas actividades estimaron una producción de 35.3 millones de toneladas de animales acuáticos a nivel mundial (FAO, 2024a). Pese a ser considerada esta práctica como la posible solución ante la escasez de agua dulce, no todas las especies de importancia comercial son aptas para ser cultivadas en ambientes salinos. El cultivo de organismos en salinidades que no son óptimas representa un estrés crónico difícil de mitigar (Sun *et al.*, 2023; Tang *et al.*, 2025). A causa de ello se puede ver disminuida las capacidades reproductivas, la calidad del cultivo e inmunocomprometer a los organismos (Butler, 2018; Gattuso *et al*., 2018; Sun *et al.*, 2023; Norambuena Cleveland *et al.*, 2024; Tang *et al.*, 2025).

La dieta juega un papel muy importante en el manejo y mitigación del estrés en la acuacultura, esto mediante la incorporación de suplementos dietéticos (Ciji & Akhtar, 2021; Sanchez-Velázquez *et al.*, 2024). Es por esto que se busca ampliar la investigación de suplementos/aditivos dietéticos que mitiguen el estrés, y con ello asegurar la integridad de los organismos (Ciji & Akhtar, 2021; Bartlett *et al*., 2022; Norambuena Cleveland *et al.*, 2024; Sánchez-Velázquez *et al.*, 2024).

1.2 *Oreochromis niloticus*

El organismo *Oreochromis niloticus (Linnaeus, 1758),* conocido como tilapia del Nilo, es un teleósteo perteneciente a la familia Cichlidae (NCBI taxonomy, 2020; Froese & Pauly, 2024; ITIS, 2003). Esta especie uno de los cíclidos más cultivados a nivel mundial y nacional (Vajargah, 2021; Munguti *et al.*, 2022).

La presencia de la tilapia del Nilo data desde hace 3,000 años, sin embargo, sus primeros registros de cultivo a nivel productivo data del año 1924 en Kenia, comenzando su distribución y extensión en 1930. En la actualidad, esta especie se distribuye en aproximadamente 140 países, con la finalidad de cultivarlas, comercializarlas y consumirlas (Fitzsimmons, 2019; Vajargah, 2021; Munguti *et al.*, 2022; Shuai & Li, 2022).

Una de las razones por las que la tilapia del Nilo es un organismo altamente utilizado en los cultivos es su amplio régimen nutricional. Su dieta se compone de plancton, larvas de peces, micrófitos, insectos, nematodos y detritos, sin embargo, recibe alimento comercial sin ningún problema (Popma & Masser, 1999; Beardmore *et al*., 2001; FAO. 2009; Vajargah, 2021; Munguti *et al.*, 2022; Tesfahun & Alebachew, 2023).

Los requerimientos nutricionales de la tilapia dependen de la etapa de desarrollo y los parámetros de cultivo al que se expongan, por ejemplo la salinidad y temperatura (FAO, 2024 b; Larumbe-Morán *et al.*, 2010; Wu *et al.*, 2021). El requerimiento proteico en larvas de tilapia (<0.02g) es de 45-50%, tilapias juveniles de 0.02-10 g, requieren de 35- 40%, en juveniles de más de 25 g no reproductores se requiere de 28-30% y en

organismos reproductores de 40-45% (FAO, 2024b.; Kpundeh *et al.,* 2015; Magbanua & Ragaza, 2023).

La popularidad e importancia de cultivar tilapia del Nilo (*Oreochromis niloticus*) se debe a su bajo costo, rápido crecimiento, gran adaptabilidad a condiciones adversas, resistencia a enfermedades, euritermo, rápida reproducción y capacidad de cultivarse en agua salobre (Fitzsimmons, 2019; Munguti *et al.,* 2022; Rairat *et al.,* 2022). Si bien, este organismo es dulceacuícola, y su crecimiento óptimo es en agua con salinidad de 0 a 8 unidades prácticas de salinidad (UPS), tiene una tasa de supervivencia aproximada de 66% a 20 UPS, y puede tolerar salinidades de 30 UPS (Foroutan *et al*., 2022; Rairat *et al*; Metwaly *et al,* 2025). Sin embargo, el poder tolerar altas salinidades no lo exime de estrés (Martins *et al*., 2022). Withyachumnarnkul y colaboradores (2017), reportaron un incremento en mortalidad con respecto a la salinidad. Un incremento en salinidad por arriba de 15 UPS incrementa la mortalidad en esta especie (Withyachumnarnkul et al., 2017; Martins *et al.,* 2022; Metwaly *et al.,* 2025). Sin embargo, la tasa de mortalidad puede reducirse a 78% si el incremento de salinidad se hace de manera gradual (5 UPS/9 días) (Mirera & Okemwa, 2023).

Así mismo, tiene la capacidad de desarrollarse de manera óptima en temperaturas de 20.2 a 31.7 °C, y resistir temperaturas de 13 y 39°C (Leonard & Skov, 2022; Rairat *et al.*, 2022). Sin embargo, se estima que su temperatura letal es de 11-12 °C como mínimo y máximas de 42°C (Leonard & Skov, 2022; Rairat *et al.*, 2022).

De acuerdo con la FAO, la tilapia del Nilo es de los organismos de mayor importancia acuícola, posicionándose en cuarto lugar a nivel mundial (por debajo del camarón

patiblanco, ostiones y la carpa china), con una producción acuícola anual de 5 millones de toneladas a nivel mundial (SADER, 2022; FAO, 2024a).

1.3 Estrés

El término estrés ha evolucionado a lo largo de la historia, teniendo como primera definición la brindada por Hans Selye, a raíz de su experimento en 1936, como "la respuesta no específica del cuerpo a cualquier demanda presente" (Selye, 1973, Pág. 692). Para otros científicos, el concepto podría definirse como una respuesta fisiológica compensatoria, donde un organismo intenta restablecer su homeostasis a consecuencia a alguna alteración mental, emocional o física (De Ocampo & Cambreros, 1999; Arturo-Rodriguez, 2012; Munoz *et al*., 2015; Schreck & Tort, 2016; Ramanathan & Desrouleaux, 2022). En la actualidad, algunos autores complementan el concepto de estrés con el de "alostasis" y "carga alostática" (Balasch, J. & Tort, 2019; Virtanen *et al.,* 2023). La alostasis se podría definir como los procesos o cambios de un organismo para adaptarse o recuperarse a los retos percibidos, mientras que la carga alostática se podría definir como el gasto requerido para llevar a cabo estas acciones (Balasch, J. & Tort, 2019; Khansari *et al.,* 2019; Valencia-Florez *et al.,* 2023; Virtanen *et al.,* 2023). Para este trabajo definiremos estrés como las respuestas o mecanismos fisiológicos activados a nivel sistémico ante un peligro o daño a la integridad del organismo (Schreck & Tort, 2016; Balasch & Tort, 2019; Petitjean *et al.,* 2019; Khansari *et al.,* 2019; Valencia-Florez *et al.,* 2023; Virtanen *et al.,* 2023).

Al hablar de estrés debemos tomar en cuenta la intensidad del evento, ya que esto va a determinar el nivel de daño que tiene el organismo, por lo que debemos introducir otros

dos conceptos, eustrés y distrés (Schreck & Torl, 2016; Balasch & Tort, 2019). El eustrés se podría definir como un estrés de bajo nivel al que, el organismo expuesto se puede adaptar y no altera su homeostasis de manera significativa (Schreck & Torl, 2016; Balasch & Tort, 2019; Burren & Pietsch, 2021). Por otra parte, el distrés es aquel estrés de mayor nivel que interfiere con el buen funcionamiento de un organismo, dado que no se logra adaptar ni restablecer la homeostasis, por lo que se generan alteraciones o inhibiciones de mecanismos reguladores (Schreck & Torl, 2016; Balasch & Tort, 2019; Burren & Pietsch, 2021; Demin *et al.*, 2021).

1.3.1 Tipos de estresores

El estrés, como se mencionó anteriormente, es un mecanismo derivado de alguna amenaza o peligro a la integridad del organismo en cuestión, a lo que esta amenaza o peligro se le denomina estresor (Schreck & Tort, 2016; Balasch & Tort, 2019; Petitjean *et al.*, 2019; Khansari *et al.*, 2019; Valencia-Florez *et al.*, 2023; Virtanen *et al.*, 2023). Los estresores se dividen en abióticos (pH, temperatura, salinidad, oxígeno disuelto, etc.) y bióticos (patógenos, alta densidad de siembra, depredación, etc.) (Gutha *et al.*, 2018; Canosa & Bertucci, 2023). Existen diversos estresores, entre los principales se encuentran las variables biogeoquímicas del agua, cambios en temperatura y agentes patógenos (Schreck & Torl, 2016; Balasch & Tort, 2019; Martos-Sitcha *et al.*, 2020; Menon *et al.*, 2023; Sures & Nachev, 2022; Kate *et al.*, 2023). En los cultivos acuicolas, los estresores son más controlados, sin embargo, el hacinamiento, altas densidades de siembra, mala calidad de agua o salinidades inadecuadas son los estresores más comunes (Martos-Sitcha *et al.*, 2020; Sures & Nachev, 2022; Kate *et al.*, 2023).

Los estresores, dependiendo la presencia o prevalencia, pueden clasificarse en dos tipos: agudo y crónico (Munoz *et al*., 2015; Schreck & Torl, 2016; Arteaga-Vasquez, F. G., 2023). El estrés agudo se caracteriza por estresores muy breves, lo que incrementa la actividad neuronal de manera rápida para la liberación de hormonas y neurotransmisores, y con ello restablecer la homeostasis del organismo (Munoz *et al*., 2015; Schreck & Torl, 2016; Demin *et al.*, 2021). El estrés crónico es definido por algunos autores como un estrés constante y prolongado, lo que desencadena una alteración neuroendocrina y génica, por lo que se considera que representa mayor carga alostática (Munoz *et al*., 2015; Schreck & Torl, 2016; Burren & Pietsch, 2021; Demin *et al.*, 2021).

Si bien, los estresores de manera individual son los más estudiados (por ejemplo, únicamente un incremento en la salinidad), en la actualidad el ecosistema acuático natural presenta varios estresores de manera simultánea (por ejemplo, incremento de temperatura derivado del cambio climático, y la presencia de contaminantes químicos) (Schreck & Torl, 2016; Gandar *et al.*, 2017; Petitjean *et al.*, 2019). La respuesta del organismo ante estos múltiples estresores puede ser variable, depende principalmente de la ruta reguladora implementada (estrategia de conservación o de compensación), por lo que puede alterar la tolerancia al estresor (Schreck & Torl, 2016; Petitjean *et al.*, 2019).

La presencia de estresores desencadena el mecanismo regulador de la homeostasis, los cuales se dividen en tres niveles (Barton, 2002; Evans & Claiborne, 2005; Gorissen & Flik, 2016; Virtanen *et al.*, 2023). En la respuesta primaria, la regulación es a nivel neuroendocrino (Barton, 2002; Evans & Claiborne, 2005; Gorissen & Flik, 2016). La

respuesta secundaria, lleva a cabo la regulación a nivel bioquímico, metabólico y fisiológico (Barton, 2002; Evans & Claiborne, 2005; Gorissen & Flik, 2016; Virtanen *et al.,* 2023). La respuesta terciaria involucra al organismo completo, en la que se realizan modificaciones en su comportamiento, y con ello lograr sobrevivir (Barton, 2002; Evans & Claiborne, 2005).

1.3.2 Respuesta primaria

La ruta del estrés inicia con la recepción del estímulo mediante órganos o células sensoriales (el sistema nervioso central recibe y manda la primera señal mediante ramificaciones en células cromafines) (Barton, 2002; Balasch & Tort, 2019). Posterior a eso, la señal estimula el eje simpático cromafin, en las que las células cromafines tienen mayor almacenamiento de catecolaminas, esta estimulación provoca la liberación rápida de catecolaminas (epinefrina y norepinefrina), esto provoca un aumento de frecuencia cardiaca y ventilatoria, mayor suministro de glucosa y movilidad de ácidos grasos libres (Parte de la respuesta secundaria) (Wendelaar Bonga, 1997; Barton, 2002; Kalamarz-Kubiak, 2018; Balasch & Tort, 2019). A la par de esto, en el eje hipotálamo hipofisario intrarrenal se realizará la activación de hormonas liberadoras de corticotropina en el hipotálamo, lo cual enviará estímulos a células corticotrópicas en la glándula pituitaria, lo que provoca la liberación adrenocorticotropina (Barton, 2002; Kalamarz-Kubiak, 2018; Virtanen *et al.,* 2023).

La circulación de adrenocorticotropina estimulará células intrarrenales en cascada, como mediadores algunas hormonas (hormona liberadora de tirotropina, hormona estimulante de melanóforos) para la liberación y síntesis de corticoesteroides (principalmente

cortisol) (Wendelaar Bonga, 1997; Barton, 2002; Kalamarz-Kubiak, 2018; Balasch & Tort, 2019; Virtanen *et al.,* 2023). Esta activación puede provocar un aumento de catabolismo, suministro de glúcidos, uso de ácidos grasos, disminuir costos energéticos e incluso inmunosupresión (respuestas secundarias y terciarias) (Wendelaar Bonga, 1997; Barton, 2002; Kalamarz-Kubiak, 2018; Balasch & Tort, 2019).

1.3.3 Respuesta secundaria

Posterior a la liberación de adrenalina, se hace activación de la respuesta secundaria al estrés, en la que se busca satisfacer la demanda energética que requiere el organismo para huir o pelear, por lo que se fomenta la glicogenolisis y gluconeogenesis (Evans & Claiborne, 2005; Gorissen & Flik, 2016). La adrenalina glucogenolítica es liberada y fomenta la hiperglucemia, lo que desencadena la liberación de adrenocorticotropina (Gorissen & Flik, 2016). Sin embargo, al sintetizarse los corticoesteroides, se mantiene la hiperglucemia para redistribuir la energía durante un periodo más largo (Evans & Claiborne, 2005; Gorissen & Flik, 2016). Si bien la glucosa es el indicador de estrés más implementado, las respuestas secundarias también se expresan en un aumento de frecuencia respiratoria y cardiaca, liberación de otros sustratos energéticos en el sistema circulatorio, alteraciones de otros elementos suspendidos en el plasma, cambios hematológicos e iónicos, y alteración en proteínas de choque térmico (Barton, 2002; Evans & Claiborne, 2005; Gorissen & Flik, 2016).

1.3.4 Respuesta terciaria

Dado que el organismo en cuestión está destinando mucha energía para restablecer homeostasis, se hacen cambios en el crecimiento, capacidad reproductiva, sistema inmune, capacidad natatoria, comportamiento, condición y supervivencia (De Ocampo & Cambreros, 1999; Barton, 2002; Evans & Claiborne, 2005; Gorissen & Flik, 2016; Mohamed *et al.,* 2021).

1.3.5 Estrés oxidativo

Las respuestas primarias al estrés, como se mencionó anteriormente, hacen liberaciones hormonales, las cuales serán captadas por receptores celulares para poder continuar con los mecanismos de respuesta al estrés (Schreck & Torl, 2016; Özbey *et al.*, 2021). Algunos elementos como el tipo, duración, y cantidad de estresores pueden influir en la ruta que la célula lleva a cabo, como podria ser un aumento en las proteínas de shock térmico y la disminución del sistema antioxidante, lo que desencadena en estrés oxidativo (Evans & Claiborne, 2005; Schreck & Torl, 2016; Petitjean *et al.*, 2019; Özbey *et al.*, 2021; Bortoletti *et al.*, 2023).

El estrés oxidativo es un desequilibrio entre el sistema de defensa antioxidante y las especies reactivas de oxígeno (ROS) y/o especies reactivas de nitrógeno (RNS), esto por una sobreproducción de ROS/RNS o por un "apagado metabólico" del sistema antioxidante como mecanismo conservativo (Vinagre *et al*. 2012; Petitjean *et al.*, 2019; Özbey *et al.*, 2021; Bortoletti *et al.*, 2023; Song *et al*. 2023). Las principales ROS involucradas son el superóxido ($O_2^{\cdot -}$), el peróxido de hidrógeno (H_2O_2) y los radicales

hidroxilos (HO), siendo este último el más reactivo (Mugoni *et al.,* 2014; Lushchak, 2016; Di Meo *et al.,* 2016; Shi *et al.,* 2022; Liu *et al.,* 2023; Da Silva et al., 2024; Melo *et al.,* 2024). Los principales RNS son el óxido nítrico ($NO^{\cdot}$), dióxido de nitrógeno ($^{\cdot}NO$), trióxido de dinitrógeno (N_2O_3) y peroxinitrito ($ONOO^-$), este último el más peligroso de este grupo (Mugoni *et al.,* 2014; Lushchak, 2016; Di Meo *et al.,* 2016; Shi *et al.,* 2022; Liu *et al.,* 2023).

Pese a que los ROS y los RNS son agentes involucrados en el estrés oxidativo, los ROS son el foco principal ante el estrés oxidativo, esto por ser más perjudícales y el papel como precursores de otros oxidantes secundarios, por ejemplo, el O_2^- que interactúa con el $NO^{\cdot}$ para formar el $ONOO^-$ (Lushchak, 2011; Mugoni *et al.,* 2014; Lushchak, 2016; Di Meo *et al.,* 2016; Shi *et al.,* 2022; Liu *et al.,* 2023). La formación de los ROS y RNS tiene origen en distintas zonas celulares, como las mitocondrias (por el 2-10 % de oxigeno no usado en la cadena de respiración), en los lisosomas (por el sistema de trasporte de electrones), en peroxisomas y el retículo endoplasmático (Di Meo *et al.,* 2016). Pese a que cada uno da origen a distintas especies reactivas, las rutas de los ROS y RNS son muy similares (Lushchak, 2011; Mugoni *et al.,* 2014; Lushchak, 2016; Di Meo *et al.,* 2016; Biller & Takahashi, 2018; Liu *et al.,* 2023). La ROS inicial, que es precursora para las otras especies (ROS/RNS), es el O_2^- la cual pasa por un proceso de dismutación mediante la enzima superóxido dismutasa (SOD) y da origen a dos moléculas, O y H_2O_2, sin embargo, puede reducirse mediante el mecanismo de sucesión de electrones, dando origen al H_2O_2 (Lushchak, 2016; Di Meo *et al.,* 2016; Biller & Takahashi, 2018; Liu *et al.,* 2023). Posterior a esto, se realiza la reducción de H_2O_2 en

agua y oxígeno molecular, mediante la catalasa, aunque también puede ser realizado por la peroxidasa dependiente de glutatión (GPx) (Lushchak, 2011; Mugoni *et al.*, 2014; Lushchak, 2016; Di Meo *et al.*, 2016; Biller & Takahashi, 2018; Jena *et al.*, 2023; Liu *et al.*, 2023). Por último tenemos la formación de OH^-. Este se puede originar por dos vías, la primera es por reacción de fenton, en la que al interactuar el H_2O_2 con iones metálicos resulta en la formación del OH^-, mientras que la segunda es por respuesta de Haber- Weis, en la que la formación de este radical se da por la interacción de H_2O_2 con O_2 (Mugoni *et al.*, 2014; Di Meo *et al.*, 2016; Biller & Takahashi, 2018; Jena *et al.*, 2023).

Este desequilibrio puede inducir a daños tisulares, daños en el ADN e incluso muerte celular (Di Meo *et al.*, 2016; Petitjean *et al.*, 2019; Özbey *et al.*, 2021; Bortoletti *et al.*, 2023). En organismos acuáticos, uno de los órganos más susceptible al estrés oxidativo es el hígado, dado que es en donde se llevan a cabo la mayoría de procesos metabólicos, transforma, almacena y excreta sustancias derivadas del proceso alostático (Da Silva *et al*., 2024; Melo *et al.*, 2024).

1.3.6 Métodos diagnósticos de salud

Debido a las repercusiones en la salud que tienen los distintos niveles de respuesta al estrés, se tienen diversos métodos de evaluación, desde el estudio fisiológico del organismo, hasta análisis moleculares (Petitjean *et al.*, 2019). Los indicadores de salud van a depender de qué tipo de respuesta queremos captar, si es de respuesta primaria, secundaria o terciaria (Mallett *et al.*, 2024).

El estudio de parámetros sanguíneos, en la actualidad, tienen mayor relevancia para determinar el estado de salud de peces en el sector acuícola (De Ocampo & Cambreros, 1999; Sáez *et al*., 2018; Del Rio-Zaragoza *et al.,* 2021). Los parámetros sanguíneos son contemplados como biomarcadores óptimos para la determinación de respuestas al estrés (Del Rio-Zaragoza *et al*., 2008; Al-Asgah *et al.,* 2015; Witeska *et al.,* 2022; Witeska *et al.*, 2023; Mallett *et al.,* 2024). Los parámetros sanguíneos se pueden dividir en dos tipos de estudios, hematología (hematocrito, hemoglobina, recuento celular, recuento diferencial leucocitario, etc.) y química sanguínea (metabolitos como glucosa, lactato, proteína, colesterol, etc.) (Al-Asgah *et al.,* 2015; Witeska *et al.,* 2022; Witeska *et al.,* 2023; Mallett *et al.,* 2024). Estos estudios son implementados para la detección temprana de la salud de organismos (Mallett *et al.,* 2024).

Entre los análisis hematológicos se encuentran el recuento celular de leucocitos y diferenciación leucocitaria, los cuales permiten corroborar el impacto de estresores sobre el sistema inmune (Witeska *et al.*, 2022; Witeska *et al.*, 2023; Mallett *et al.*, 2024). Sin embargo, existen otros estudios para determinar la actividad del sistema inmune, como lo sería la detección de citocinas (Zhu *et al.*, 2023), una amplia familia de proteínas que fungen como señalizadoras para la distribución de células inmunes (Zou & Secombes, 2016; Huo *et al.,* 2019; Kirsten *et al.,* 2021; Zhu *et al.,* 2023). Entre las citocinas más relevantes se encuentran las interleucinas, las cuales se dividen en proinflamatorias (IL-6, IL-1β, IL-8) y antiinflamatorias (IL-10) (Huo *et al.,* 2019; Vallejos-Vidal *et al.,* 2016; Kirsten *et al.,* 2021; Zhu *et al.,* 2023). La interleucina 10 (IL-10) ha sido una de las citocinas antiinflamatorias más estudiadas en los últimos años (Karan *et al.,* 2016). Esta

interleucina es expresada principalmente en monocitos, macrófagos, neutrófilos y células T CD4, a su vez que suprime elementos ROS y regula el óxido nítrico, los cuales cumplen un papel antimicrobiano (Karan *et al.,* 2016; Zou & Secombes, 2016; Huo *et al.,* 2019; Vallejos-Vidal *et al.,* 2016; Kirsten *et al.,* 2021; Zhu *et al.,* 2023).

Otro marcador altamente utilizado para determinar el estado de salud de organismos son las proteínas del shock térmico (Eissa *et al*., 2017; Ikwegbue *et al*., 2017; Jeyachandran *et al*., 2023). Son proteínas auxiliares de actividades metabólicas proteicas y lipídicas, fundamentales en el plegamiento de proteínas, y gran relación con el sistema inmunológico, así como en la apoptosis y en reestablecer la homeostasis del organismo (Eissa *et al*., 2017; Ikwegbue *et al*., 2017; Szyller & Bil-Lula, 2021; Jeyachandran *et al*., 2023). La variedades más estudiadas son la HSP70 y HSP90, las cuales son altamente conservadas, por lo que se aprecia un aumento en su expresión ante la presencia de distintos estresores (Schreck & Torl, 2016; Ikwegbue *et al*., 2017; Szyller & Bil-Lula, 2021; Jeyachandran *et al*., 2023).

Las respuestas terciarias al estrés pueden ser evaluadas mediante los índices de condición, viscerales, cambios conductuales, las respuestas del sistema inmune y antioxidante, así como las modificaciones estructurales de ADN, proteínas y lípidos (Ighadaro *et al*., 2018; Santana *et al.*, 2022; Bizoń *et al.,* 2023; Menon *et al.*, 2023; Da Silva *et al*., 2024; Mallett *et al.,* 2024; Melo *et al.,* 2024).

En años recientes, la presencia de alteraciones nucleares (micronúcleos y aberraciones nucleares) se ha implementado como un método de detección de alteraciones celulares, esto como respuesta terciaria al estrés (Melo et al., 2014; Xu *et al*., 2014; Braham *et al*.,

2017; Hussain *et al.,* 2018; Anifowoshe *et al.*, 2022). Las aberraciones nucleares y los micronúcleos son alteraciones nucleares formadas durante el proceso de división celular, sin embargo, la presencia de agentes estresores o elementos tóxicos puede fomentar una mayor presencia de estos (Bolognesi & Hayashi, 2010; Melo et al., 2014; Xu *et al*., 2014; Braham *et al*., 2017; Hussain et al., 2018; Anifowoshe *et al.,* 2022).

1.4 Suplementación dietaría

En la actualidad, derivado del incremento en la demanda animales para consumo, así como la búsqueda de elementos que mejoren la calidad de los cultivos, se ha implementado la adición de suplementos alimenticios (Vallejos-Vidal *et al.,* 2016; Herrera *et al.,* 2019; Ciji & Akhtar, 2021; Mahdavi-Roshan *et al.,* 2021; Vijayaram *et al.,* 2023; Sanchez-Velázquez et al., 2024). Un suplemento alimenticio es un elemento complementario a la alimentación, el cual aumenta la concentración de dicho componente nutricional (vitaminas, minerales, aminoácidos, etc.) con el fin de conferir algún efecto benéfico al organismo que lo consume (Vallejos-Vidal *et al.,* 2016; Herrera *et al.,* 2019; Mahdavi-Roshan *et al.,* 2021; Chakravarty *et al.,* 2023; Vijayaram *et al.,* 2023; Shastak, & Pelletier, 2024).

1.4.1 Inmunoestimulantes

Una función de los suplementos alimenticios es su uso como estimulantes del sistema inmune, mejor conocidos como inmunoestimulantes (Vallejos-Vidal *et al.,* 2016; Kord *et al.,* 2021; Vijayaram *et al.,* 2023; Shastak, & Pelletier, 2024). Los inmunoestimulantes son elementos (bióticos, químicos o sintéticos) que permiten resistir y hacer frente a

patógenos, mediante la activación del sistema inmune innato y adaptativo (Vijayaram *et al.*, 2023; Dev *et al.*, 2024). En la actualidad, el implementar inmunoestimulantes como las vitaminas, minerales y aminoácidos resulta ser una mejor solución contra los brotes infecciosos, en el que se priorizan los suplementos que actúan como cofactores, que intervienen en la síntesis de elementos del sistema y actúan como reguladores de mecanismos inmunitarios (Vallejos-Vidal *et al.*, 2016; Dawood *et al.*, 2017; Farooqi & Qureshi, 2018; Vijayaram *et al.*, 2023; Bahi *et al.*, 2024).

1.4.2 Antioxidantes

Otro tipo de suplementación dietaría que ha tomado mayor fuerza son los antioxidantes, que son elementos que neutraliza las especies reactivas (ROS/RNS) y contrarresta los radicales libres (He *et al.*, 2017; Abdel-Rahman Mohamed *et al.*, 2021; Habotta *et al.*, 2022). Los antioxidantes como suplemento alimenticio pueden encontrarse como enzimas antioxidantes o como elementos no enzimáticos, los cuales actúan a manera de cofactor o donantes de electrones (Habotta *et al.*, 2022; Kotha *et al.*, 2022; Zhang *et al.*, 2022; Aramli *et al.*, 2023). Otra manera de clasificar los antioxidantes es por la actividad que realiza, ya sea la prevención de formación de estas especies (primera línea, enzimáticos), interrupción de las reacciones de oxidación (segunda línea, no enzimáticos) o la inactivación de productos derivados de la oxidación de radicales libres (tercera línea, enzimáticos) (He *et al.*, 2017; Hoseini et al., 2020; Kotha *et al.*, 2022; Pruteanu *et al.*, 2023). En la suplementación dietaría con antioxidantes, se hace mayor énfasis en los antioxidantes no enzimáticos, provenientes de fuentes naturales y/o sintéticas para contrarrestar el efecto del estrés oxidativo (He *et al.*, 2017; Hoseini et al.,

2020; Abdel-Rahman Mohamed *et al.,* 2021; Habotta *et al.,* 2022; Kotha *et al.,* 2022; Pruteanu *et al.,* 2023).

1.5 La arginina

La arginina es un aminoácido esencial y versátil, altamente estudiado como suplemento dietario (Hoseini *et al.,* 2020; Pervin *et al*. 2021; Ahmed *et al.,* 2022). La arginina es un aminoácido de cadena lateral alifática, de tres carbonos, en terminación de un grupo guanidino (Oliveira *et al.,* 20217; Wang *et al.,* 2021). Los requerimientos dietarios de arginina varía entre especies de peces, sin embargo, el requerimiento oscila entre 2 y 8.1 % de proteína cruda, o 1 hasta 6% de la dieta seca (Hoseini et al, 2020; Cheng *et al.,* 2021; Wang *et al.,* 2021). El uso de arginina para mitigar estresores y los requerimientos dietarios ya han sido reportados en *Lateolabrax maculatus* (Cheng et al., 2021), *Caenorhabditis elegans* (Furuhashi *et al*., 2016), *Oreochromis niloticus* (Fujimoto *et al.,* 2019; Li et al., 2020; Li et al., 2022; Vianna et al., 2020; Kamal et al., 2024). Entre los efectos reportados de la arginina como suplemento dietario se ha observado la resistencia al estrés, un aumento en el crecimiento, la inmunoestimulación y la oxidación de ácidos grasos intestinales (Yue et al., 2015, Neu et al., 2016; Li et al., 2020; Vianna et al., 2020; Li et al., 2022; Furuya et al., 2023; Kamal et al., 2024).

1.5.1 Importancia de la arginina

En peces, la arginina es considerado como aminoácido esencial, lo que significa que debe ser administrado mediante la dieta, dado que estos organismos tienen limitación o

baja actividad en la biosíntesis endógena en ciertos estadios, la cual es llevada a cabo por enzimas como la ornitina trascarboxilasa, carbamoil fostato sintasa III y la pirrolina- 5-carboxilato (Oliveira *et al.,* 20217; Wang *et al.,* 2021; Ding *et al.,* 2024). La arginina influye en distintos aspectos metabólicos, inmunitarios, antioxidantes y de crecimiento en los peces (Wu *et al.,* 2018; Wang *et al.,* 2021).

El principal aspecto estudiado de la arginina, es su efecto en el crecimiento de los peces, dado que este aminoácido es estimulador de la vía de señalización TOR a manera de promotor para síntesis proteica y miogénesis. Así mismo, genera la activación de la proteína quinasa activada por adenosina 5´ monofosfato, e incrementa la expresión del factor de crecimiento insulinoide (IGF-1) y la hormona del crecimiento (GH), los cuales regulan el crecimiento del organismo (Hoseini *et al*, 2020; Cheng *et al.,* 2021; Wang *et al.,* 2021; Ahmed *et al.,* 2022; Ding *et al.,* 2024).

Otros procesos en los que interviene la arginina a nivel metabólico son la síntesis de creatina, que al otorgar su grupo amino participa en la recuperación de ATP a nivel muscular (Ahmed *et al.,* 2022). La arginina interviene en la síntesis de ornitina, ácidos grasos, glucosa, y poliaminas (Wu *et al.,* 2018; Hoseini *et al*, 2020; Ding *et al.,* 2024). Es por ello que la arginina tiene un papel importante en el sistema inmune, la proliferación celular, crecimiento celular y su diferenciación (Hoseini *et al*, 2020; Ding *et al.,* 2024). Así mismo, tiene papel en el sistema digestivo, al mejorar el proceso digestivo de proteínas, estimular la migración de celular y síntesis de proteínas intestinales, al igual que regula molecularmente las citocinas pro y antiinflamatorias (Wu *et al.,* 2018; Hoseini *et al*, 2020; Wang *et al.,* 2021).

1.5.2 Arginina como inmunoestimulante y antioxidante

Como se mencionó en el apartado anterior, la arginina promueve la síntesis de poliaminas, las cuales participan en la diferenciación celular, promueven la secreción de citocinas y la respuesta leucocitaria, y hematopoyesis (Hoseini et al, 2020). La arginina se conoce como un modulador importante del sistema inmune, regula los genes de citocinas pro inflamatoria (disminuye la expresión de mRNA) y antiinflamatorias, promueve el número de leucocitos, los lisosomas, el sistema de complemento, la producción de inmunoglobulina y la producción de óxido nítrico (He *et al.,* 2017; Hoseini *et al*, 2020; Cheng *et al.,* 2021; Ding *et al.,* 2024)

El papel de la arginina en la sintesis de óxido nítrico es fundamental, ya que esta va a ser catalizada por las distintas isoformas del óxido nítrico sintetasa (NOS), en el que se obtiene l-citrulina y NO (óxido nítrico) (He *et al.,* 2017; Herrera *et al.,* 2019; Hoseini *et al*, 2020; Cheng *et al.,* 2021; Wang *et al.,* 2021; Vijayaram *et al.,* 2023; Ding *et al.,* 2024). Se sabe que en mamíferos existen tres NOS, la neuronal (nNOS), la endotelial (eNOS) y la inducible (iNOS), sin embargo, en la mayoría de peces se desconoce la existencia de eNOS por el momento (He *et al.,* 2017; Hoseini *et al*, 2020; Wang *et al.,* 2021). Cuando hablamos del sistema inmune, el iNOS es el más relevante, ya que este es sintetizado por diversas formas celulares del sistema inmune (Hoseini *et al*, 2020; Giordano *et al*., 2022; Peter *et al.,* 2022; Locascio *et al.,* 2023). La iNOS es calcio independiente, aumenta la citotoxicidad de los leucocitos, la expresión de CD3 en

linfocitos T (Hoseini et al, 2020; Wang *et al.*, 2021; Giordano *et al*., 2022; Vijayaram *et al.*, 2023).

La capacidad antioxidante reportada por arginina se teoriza que sea por la estimulación a la síntesis de glutatión (GSH) y la modulación de la vía Nrf2-Keap1 (regulada por NO), al igual que un aumento en la actividad enzimática de SOD, GPx y CAT (Wu *et al.*, 2018; Hoseini et al, 2020; Cheng et al, 2021; Ahmed *et al.*, 2022; Peter *et al.*, 2022; Ding et al, 2024)

La ruta de la arginina en su totalidad sigue siendo desconocida, sin embargo, se ha visto que interviene en distintos mecanismos, como lo sería su papel ante el estrés (Wu *et al.*, 2018; Hoseini et al, 2020; Peter *et al.*, 2022; Locascio *et al.*, 2023; Ding *et al.*, 2024).

La presencia de un estresor desencadena una serie de respuestas que pueden ser monitoreadas, y con ello evaluar la salud de los organismos. Es por ello que en este estudio se busca analizar los efectos de suplementar con L-arginina juveniles de tilapia del Nilo expuestos a estrés crónico salino durante 57 días. Con ello se busca analizar si la suplementación dietaría puede mitigar los efectos perjudiciales del estrés. Para ello, se considerarán variables como crecimiento, índices de condición y viscerales, parámetros hematológicos, química sanguínea, recuento de anormalidades nucleares, así como expresión de genes asociados al sistema antioxidante e inmune. Los métodos y resultados están estructurados en forma de manuscrito (manuscrito sometido y en revisión, con número de seguimiento ID: fishes-3676546) y se presentan en el capítulo II.

CAPÍTULO II: **Efectos de la suplementación dietaría con L-arginina en el desempeño, salud, y expresión de marcadores moleculares de estrés en tilapia del Nilo bajo estrés salino**

L-arginine effect as an additive on growth performance, health status, and expression of stress molecular markers in Nile tilapia (*Oreochromis niloticus*) under chronic salinity exposure

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Article

L-arginine effect as an additive on growth performance, health status, and expression of stress molecular markers in Nile tilapia (*Oreochromis niloticus*) under chronic salinity exposure

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Abstract: Growing freshwater fish in saline environments is being considered as a solution to the freshwater shortage. To achieve this goal, it is crucial to enhance the fish's immune and antioxidant system through dietary supplements. In the present study, it was evaluated the effects of four dietary levels of arginine supplementation on the growth, health status, and expression of stress-related molecular markers in juveniles of Nile tilapia exposed to chronic salinity stress. The tilapia were fed four experimental diets supplemented with 0, 1, 2, and 3% of L-arginine. The tilapias were exposed to a salinity level of 20 ‰ for 57 days after an acclimatization period. Our findings showed that growth performance parameters were significantly influenced by L-arginine supplementation, with the exception of the condition factor, viscerosomatic index, and hepatosomatic index. Additionally, the mid-levels of L-arginine supplementation positively affected some blood parameters, including, hematology profiles (hemoglobin and leukocytes), blood chemistry (protein total, albumin, globulin, and triglycerides), and the frequency of certain nuclear abnormalities. Moreover, L-arginine supplementation regulated the expression of molecular markers related to stress and the immune system. In conclusion, this study indicates that L-arginine supplementation can help alleviate the chronic stress caused by salinity in juvenile Nile tilapia.

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Key Contribution: This study suggests that supplementation with 3% arginine increases weight gain and specific growth rate in Nile tilapia. Also, arginine supplementation increases the immune system in Nile tilapia under chronic salinity stress and showed a quadratic trend in the expression of stress and immune system molecular markers. Consequently, a diet containing 7-9% arginine in crude protein could reduce the effects of chronic salinity stress in Nile tilapia.

39 **1. Introduction**

Climate change and population growth have recently been identified as two of the world's most pressing challenges [1-2]. These issues contribute to a range of related problems that impact human well-being, including water shortage, ocean acidification,

and food insecurity [1-3]. To address these challenges, international organizations such as the United Nations (UN) and the Food and Agriculture Organization (FAO) have proposed specific solutions. These include the implementation of aquaculture practices and the pursuit of blue transformation, which refers to the adoption of environmentally sustainable practices in the maritime sector [1,4]. The implementation of aquaculture has the potential to address food security issues by providing high-quality meat at a low cost.

Aquaculture can be developed in various regions, including natural and artificial bodies of water [1,5,6]. However, this practice can be counterproductive in areas experiencing water shortages. Therefore, the goal is to expand mariculture [1-7]. Globally, the four most produced and consumed species are the white-legged shrimp (*Penaeus vannamei*), oysters (*Crassostrea* spp.), Chinese carp (*Ctenopharyngodon idella*), and Nile tilapia (*Oreochromis niloticus*). The latter two are freshwater organisms [1].

Among the organisms important for aquaculture, the Nile tilapia stands out due to its versatility in feeding, low maintenance cost, rapid growth, wide adaptability to adverse conditions, wide range of temperature tolerance, and easy reproduction [8-10]. Although the Nile tilapia is primarily a freshwater fish, with an optimum salinity of 0-8 parts per thousand (‰), it also has a remarkable tolerance at higher salinity levels. However, to survive in elevated salinities, the Nile tilapia requires a higher protein intake [10-15], besides it can be stressful [15-17]. It has been documented that prolonged exposure to suboptimal or elevated salinity levels leads to increased cortisol and glucose levels, along with reduced growth and survival rates [16-19]. Furthermore, freshwater organisms experience chronic stress when kept in salinity that exceeds their optimal range [17–18, 20–21].

Chronic stress, in aquaculture, is considered particularly harmful because it demands a significant amount of energy from the organisms [20-23], which can compromise their overall performance. The consequences of this energy demand may include tissue damage, alteration in metabolic processes, reduced growth, changes in the antioxidant system, weakened immune response, and death in severe cases [19-23]. To mitigate these negative effects, researchers have sought to implement dietary additives that help organisms cope with adverse conditions [23-25].

Dietary additives are additional components added into the diet not considered nutrients but are added in lower concentrations to confer some beneficial effect on the organism [23, 25, 26]. Among the most studied dietary additives are vitamins, minerals, certain amino acids, and fatty acids [23-27]. Amino acids play a crucial role not only in constructing tissues but also in various metabolism processes. As a result, there is a growing interest in using amino acids as dietary additives to enhance resistance to stress. This interest stems from their involvement in neuroendocrine responses and their indirect function in energy storage to produce ATP production and control blood circulation [25, 28, 29].

Specific amino acids like tryptophan, phenylalanine, methionine, taurine, glutamine, glycine, and arginine have been the focus of interest since they mitigate the detrimental effects of different stressors [25, 27-29]. In particular, arginine is valuable as a dietary additive in aquatic organisms due to its role in stimulating the production of the growth hormone (GH) insulin-like growth factor 1 (IGF-1), a precursor of nitric oxide (NO), and polyamine synthesis [29-37]. Furthermore, arginine helps modulate immune responses, supports the antioxidant system, and reduces serum lipid levels [29-30, 25, 35-39].

The use of arginine as a dietary additive has been shown to enhance resistance to stressors in several species, including *Eriocheir sinensis* [33] (stressed with high pH), *Cirrhinus mrigala* [32] (stressed with hypoxia), S*cophthalmus maximus* [38] (stressed with hypoxia and handled), *Solea senegalensis* [34], *Oreochromis niloticus* [40] (stressed with

Streptococcus agalactiae), and *Cyprinus carpio* [41] (stressed with ammonia toxicity). Additionally, the effects of arginine supplementation on fatty acid oxidation have been investigated [38-39], along with its impact on hematological parameters, antioxidant levels, and immune system function [40, 42-43]. Nevertheless, the degree to which dietary supplementation with L-arginine may provide similar benefits to Nile tilapia cultured in brackish water is still unclear.

Therefore, this study aims to assess the effect of varying levels of L-arginine supplementation on growth, health status, and the expression of stress and immune system molecular markers in Nile tilapia (*Oreochromis niloticus*) exposed to chronic saline stress.

2. Materials and Methods

2.1 Experimental design

The Nile tilapia (*Oreochromis niloticus*) juveniles were donated by an Aquamol aquaculture producer in Jamay, Jalisco. México. The experiment was conducted at the Instituto de Investigaciones Oceanológicas at the Universidad Autónoma de Baja California (IIO-UABC, Ensenada, B.C., México). A total of 25 juveniles per tank were randomly distributed. The juveniles (4.04 ± 0.72 g) were acclimatized to 20 ‰ over 4 days, with an increase of 5 ‰ per day. The experimental system consisted of 12 tanks, 500-L each, connected to a recirculation system (RAS) under a constant aeration system. The RAS system was integrated by a biofilter (PolyGeyser®; Pneumatic Drop Bead Filter model PG7 International Filter Solutions, TX, USA), a protein skimmer, a UV lamp, settlers in each tank, and a 1000-L reservoir with a heater at constant temperature (27.41±0.9 °C). Dissolved oxygen was monitored daily with a multi-parameter (YSI-55, YSI Inc., Yellow Springs, OH, USA), registering 8.0 ± 0.2 mg/L. The salinity was monitored with a portable refractometer (Portable refractometer WL0020-ATC, Agriculture Solutions LLC, Kingfield, ME, USA) and registered 20.5±2.5 ‰. Ammonium (0.05 ± 0.1 mg/L) and nitrite (0 ± 0 mg/L) levels were monitored once a week (API test kits, Mars Fishcare Inc., Chalfont, PA, USA). The tanks were siphoned daily, whereas the RAS system was backwashing once a week. The experimental diets were fed four times a day (8:00, 11:00, 14:00, and 17:00 hrs) seven days a week, at apparent satiety.

2.2 Experimental diet design

Four isoproteic diets, each containing 45% crude protein and 10% crude lipid, were formulated as shown in Table 1. The diets varied based on the levels of arginine (Arg) included, with glycine (Gly) as a replacement. The diets were assigned as follows: 0% (control diet), 1% Arg, 2% Arg, and 3% Arg.

Table 1. Ingredient composition and proximate analysis of experimental diets with different arginine levels to feed Nile tilapia (*Oreochromis niloticus*). The arginine was calculated from the ingredient content.

	Treatments			
Ingredients	**0% Arg**	**1% Arg**	**2% Arg**	**3% Arg**
Poultry byproducts meal (68% CP) [a]	10	10	10	10

Bovine byproducts meal (50% CP) [a]	10	10	10	10
Fish meal (70% CP) [b]	24	24	24	24
Corn gluten meal (65% CP) [c]	10	10	10	10
Corn starch [d]	28.3	28.3	28.3	28.3
Glycine[e]	3	2	1	0
Arginine[e]	0	1	2	3
Beef tallow [f]	2.5	2.5	2.5	2.5
DHA Nature ™ (24% DHA)[g]	5	5	5	5
Gelatin [h]	2.5	2.5	2.5	2.5
Methionine[e]	1	1	1	1
Rovimix [i]	3	3	3	3
Taurine [j]	0.1	0.1	0.1	0.1
Stay c[k]	0.5	0.5	0.5	0.5
TOTAL	100	100	100	100
Arginine on diet (%)	2.6	3.6	4.6	5.6
Arginine on crude protein (%)	4.3	5.91	7.54	9.16
Proximal composition (%)				
Crude protein	45.72±1.89	45.52±0.16	44.92±0.47	45.83±1.61
Crude fat	10.40±0.42	10.57±0.21	10.35±0.14	10.46±0.28
Ash	10.58±0.01	10.06±0.01	10.06±0.01	10.23±0.01
NFE	34.95	33.85	35.16	33.46

NFE(%)= 100-(% crude protein + % crude fat + % ash). [a] Pet food grade (65% CP) Scoular de México S. de R.L. de C.V. Originally from the USA (National Renderers Association); [b] Baja Marine Foods S.A.P.I. de C.V, El Sauzal de Rodríguez, Ensenada, Baja California, México; [c] INGREDION S.A. de C.V., México, 65% CP; [d] Maicena™, Unilever Food Solutions, México; [e] Future Foods, México; [f] Kindly donated by Grasas y Derivados de Tijuana; [g] kindly donated by ADM; [h] Progel Mexicana SA de CV, León, Guanajuato, México (commercial grade, 85% CP); [i] la vitamin and mineral mixture from DSM; [j] NUBIOT S.A. de C.V., Mexico; [k] Stay C from DSM.

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Diets were prepared at the LINDEAACUA plant at the II0-UABC facilities (Ensenada, Mexico), according to their internal protocols. Macronutrients were ground to 0.5 mm (Inmimex M-300, Mexico) and sieved (Kemutek-Gardner K300, USA). Mixing was then performed in a vertical cutter/mixer (Robot Coupe R-60, USA). Incorporation was performed in four steps. The first consisted of the incorporation of the macronutrients, the second of the incorporation of the micronutrients and arginine/glycine; the third consisted of the gelatin and cooked starch, and the last consisted of the incorporation of the fat source (beef tallow). The dough was mixed until a homogenous mixture was achieved. It was then pelletized to 5 mm in a commercial feed mill (Tor-Rey, model M32-5, Mexico). They were dried in a forced air oven at 60°C for 24 hours and stored at 4°C in plastic bags.

2.3 Proximal composition

Experimental diets were analyzed in triplicate according to AOAC [44]. The dry weight of the diet was determined by the gravimetric method, drying the samples at 60°C for 24 hours. After that, the samples were ashed in a muffle furnace (Thermolyne 62700 Muffle furnace) at 550°C for 6 hours and then weighed. Crude protein was determined by the micro-Kjeldahl method (rapid distillation unit, Labconco), and a nitrogen conversion (N*6.25) was made to calculate the protein content. Crude lipid was extracted and quantified by the Soxhlet method (Labconco, Kansas City, MO, USA), using petroleum ether as the solvent carrier. Finally, the nitrogen-free extract (NFE) was determined by the difference.

2.4 Sampling

Following a 57-day feeding trial, the organisms were quantitatively assessed and weighed. These data were used for the quantification of the following metrics.

Specific growth rate (SGR, %/d) = 100 * ((ln final weight–ln initial weight) * days).

Feed Conversion Ratio (FCR) = total feed consumed/wet weight gained.

Condition Factor (CF) = (final body weight/body length^3) * 100.

Survival rate (SR, %) = Final number of fish*100/initial number of fish

At the end of the biometry, three organisms per tank were euthanized according to UABC protocols for the utilization of animals for experimental purposes. The process to anesthetize fish was carried out with 2-phenoxyl-ethanol (100 ppm) at a dilution of 0.5 mL/L. A blood sample was collected from the caudal vein using tuberculin syringes (1 mL). The blood sample was subsequently divided into three parts: a tube with EDTA-K2 (Becton Dickinson®) for hematological analysis, a 1.5 mL microcentrifuge tube (Eppendorf®) for blood chemistry, and a drop in an object holder for blood smears (hematology). The sample without anticoagulant was centrifuged at 7000 rpm for 10 minutes. Subsequently, the serum was stored at -20°C. Following the collection of blood samples, the organisms were dissected to quantify morphometric parameters.

Hepatosomatic index (HSI, %) = (liver weight/body weight) × 100.

Viscerosomatic index (VSI, %) = (visceral weight/body weight) × 100.

A tissue sample of approximately 1 cm^3 was extracted from the liver and transferred into a centrifuge tube free of RNAases containing RNA later (Ambion). The tubes were left at room temperature for 24 hours and then stored at - 80 °C.

2.5 Hematology assay

The blood from the EDTA tube was used to calculate the hemoglobin (Hb), hematocrit (Hct), and blood cell count. The Hct was estimated using a 2/3 filled capillary tube (Leex Equipment, Mexico). The tubes were sealed and placed in a micro-hematocrit centrifuge at 7000 rpm (Premiere® XC-3012, Mexico) for 10 min. The packed cells were measured using the hematocrit reader and reported as percentages [45]. The Hb was measured using the HemoCue ® Hb 201 (HemoCue AB; Angelholm, Sweden). Red blood cell (RBC) and white blood cell (WBC) counts were made with the Natt-Herrick method [46]. The blood sample was then placed in a Neubauer hemocytometer (Marienfeld-Superior, Lauda-Königshofen, Germany) using 20 µl of blood mixed with the Natt-Herrick solution in a 1:200 ratio, and the cells were counted using an optical microscope (Karl Zeiss, Primo Star, México) at 40X. The mean corpuscular volume, mean corpuscular hemoglobin and mean corpuscular hemoglobin concentration were calculated using standard formulas with data of Hct, RBC, and Hb.

$$\text{Mean Corpuscular Volume (MCV, fL)} = (\text{Hct} / \text{RBC}) \times 10$$

$$\text{Mean Corpuscular Hemoglobin (MCH, pg)} = (\text{Hb} / \text{RBC}) \times 10$$

$$\text{Mean Corpuscular Hemoglobin Concentration (MCHC, g/dL)} = (\text{Hb} / \text{Hct}) \times 100$$

For leukocyte cellular differentiation and micronucleus assay, we use the samples of the blood smears and implement the method of [45-48]. The staining was made with the hemocrom-fix kit ®, following the manufacturer´s instructions. Blood smears were mounted with cytoseal™ 60 resin and observed in an optical microscope. From each blood smear, the percentage of each of the leukocyte cellular types was calculated.

Nuclear morphological alterations in erythrocytes were classified as follows: blebbed, notched, and binucleate. Only erythrocytes with intact nuclear and cytoplasmic membranes were taken into account, discarding those superimposed or damaged.

2.6 Serum biochemical parameters

The serum biochemical parameters assay was calculated using colorimetric kits (MexLab Group, Jalisco, Mexico) following the manufacturer's instructions. Total protein was determined with the Biuret method at 540 nm. Albumin, using the bromocresol green method (BCG) at 620 nm. The glucose oxidase method (God-Pap) was measured at 505 nm for glucose concentration. While the Triglycerides assay was performed with the glycerol-3-phosphate-oxidase method (GPO-PAP) at 505 nm, and cholesterol was analyzed with cholesterol esterase (CHOD-PAP) at 505 nm. The globulin was calculated by contrast of total protein and albumin. All blood parameters analyzed in each sample were performed in triplicate, and the reader with a microplate reader (Multiskan GO, Thermo Scientific).

2.7 RNA extraction and quantitative PCR

The protocols followed for the RNA extraction and qPCR are described in detail in [49]. The liver tissue preserved in RNAlater was analyzed individually to extract the RNA with the PureLink RNAlink Minikit (Ambion). To homogenize the tissue, a micropistill was used. The quantity and quality of RNA were measured by

spectrophotometry (Nanodrop® LITE, Thermo Fisher Scientific INC., Wilmington, USA). Only the samples with an optical density ratio of 260 at 280 nm within the range of 1.90 to 2.10 were used.

The RNA extracted (500 ng) was reverse-transcribed with the High-Capacity cDNA reverse transcription kit in a 20 μL reaction, according to the manual (Applied Biosystems; Carlsbad, CA, USA). The qPCR reaction was performed using the SYBR® Select Master Mix (Applied Biosystems). The specific primers used in this study are shown in Table 2.

Table 2: Primer sequences used for the real-time PCR.

Gene	Forward sequence (5′-3′)	Reverse sequence (5′-3′)	NCBI reference
HSP 70	CCGGTTTGATGACACAGTTG	CGAGGTAGGCTTCAGCAATC	XM_023404852
SOD	GACGTGACAACACAGGTTGC	TACAGCCACCGTAACAGCAG	XM_003449940.5
IL-10	CTGCTAGATCAGTCCGTCGAA	GCAGAACCGTGTCCAGGTAA	XM_013269189.3
B-Actin	TGGTGGGTATGGGTCAGAAAG	CTGTTGGCTTTGGGGTTCA	ENSONIG00000008505

2.8 Statistical Analysis

The values are expressed as the mean ± standard deviation. Normality and homogeneity of variance were assessed using the Shapiro-Wilk and Levene tests, respectively. If the statistical assumptions were met, a one-way analysis of variance (ANOVA) was applied, with a post hoc Tukey test. Otherwise, a Kruskal-Wallis test was used. All tests were with the significance level set at 0.05. The relationship between dietary treatment, weight gain, specific growth rate (SGR), Feed conversion rate (FCR), Survival rate, and gene expression was evaluated by polynomial regression analysis that was selected for best fit based on R^2 values. All statistical analyses were performed in the SPSS STATICS® V26.0.0® IBM Corporation 1989, 2011, USA program.

3. Results

3.1 Performance and biological index

As a result of a 57-day nutritional trial, organisms fed the 3% additional arginine diet had higher weight gain and SGR in contrast to the control treatment ($p < 0.05$; Table 3). However, the survival rate was higher in the 2% treatment ($p < 0.05$). The FCR was found to be significantly lower in the 3% treatment, in contrast to the 1% and 2% treatments. The observed trend of weight gain was linear, and SGR quadratic, with an increase in a higher percentage of arginine in both. On the other hand, the trend of FCR and survival rate was quadratic, with the highest peaks observed in treatments 1 and 2 (Fig. 1). A lack of statistical significance was observed in the total length and final weight of the organisms ($p > 0.05$).

Table 3. Productive performance of Nile tilapia (*Oreochromis niloticus*) fed diets with different levels of L-arginine and cultured at 20 ‰. Data are mean ± SD.

Treatments	Initial Weight (g)	Final weight (g)	Final length (cm)	Weight gain (g)	Specific growth rate (%/d)	Feed conversion ratio	Survival rate
0% Arg	4.06±0.76	48.03±19.17	13.43±1.82	42.53±4.38 A	4.27±0.15^{A}	23.15±1.65 AB	76.0±4.0 ^{A}
1% Arg	4.05±0.76	56.14±23.09	13.96±1.95	44.4±3.23^{A} B	4.35±0.13^{AB}	24.75±0.38^{A}	83.7±0.38^{AB}
2% Arg	4.05±0.73	45.87±9.25	13.43±1.11	48.34±0.43 AB	4.49±0.02^{AB}	24.25±1.44^{A}	94.0±2.82^{B}
3% Arg	4.01±0.66	68.16±18.98	14.91±1.39	52.44±3.84 B	4.63± 0.11^{B}	19.42±2.56^{B}	76.0±8.0^{A}
p-value	0.437^{A}	0.098^{A}	0.247^{K}	0.046^{A}	0.042^{A}	0.014^{A}	0.007^{A}

Superscript letters show differences between the same column ($p < 0.05$). Superscript A or K letters in *p* values represent ANOVA or Kruskal-Wallis analysis, respectively.

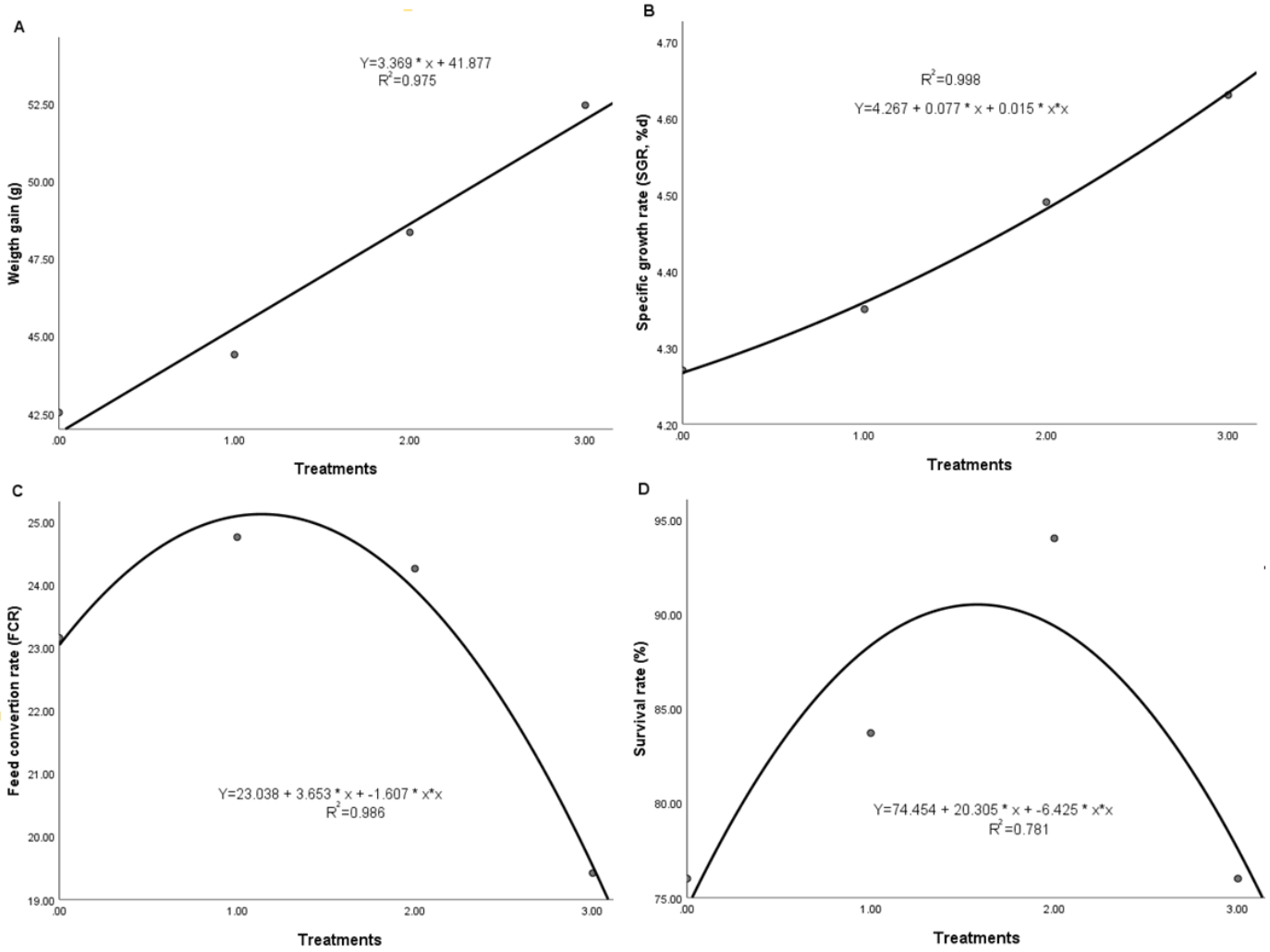

Figure 1: Polynomial regression analysis of the effects of different arginine inclusions on weight gain (A), specific growth rate (B), feed conversion rate (C), and survival rate (D).

The organisms of the different experimental diets did not show significant differences in condition index, hepatosomatic index, and visceral index ($p > 0.05$; Table 4).

Table 4. Biological indices of Nile tilapia (*Oreochromis niloticus*) fed diets with different levels of L-arginine and cultured at 20 ‰. Data are mean ± SD.

Treatments	Hepatosomatic index	Visceral index	Condition index
0% Arg	2.16±0.47	12.65±1.60	1.86±0.15
1% Arg	2.18±0.87	13.55±1.05	1.99±0.39
2% Arg	1.8±0.43	13.72±1.92	1.88±0.15
3% Arg	2.29±0.34	13.30±2.36	2.01±0.22
p-value	0.246^{K}	0.628^{K}	0.542^{K}

3.2 hematology

The hematological results are summarized in Table 5. Hematocrit, RBC, and MCH do not show differences among treatments. The study found that the hemoglobin levels in the control group were significantly lower compared to the other treatment groups. The white blood cell count was found to be significantly higher in the 3% group when compared to the control group. The VMC had a considerable decrease in the 2% and 3% treatments in contrast to the control and 1% groups. On the other hand, MCHC showed a significant increase in the 2% treatment in contrast to the control group and 1%.

Table 5. Blood hematological values of Nile tilapia (*Oreochromis niloticus*) fed diets with different levels of L-arginine and cultured at 20 ‰. Data are mean ± SD.

Treatments	Hb (g/ dL)	Hct (%)	RBC (x 10^6 /mm^3)	WBC (x10^3 /mm^3)	MCV (fL)	MCH (pg)	MCHC (g/dL)
0% Arg	6.76±1.37^{A}	23.88±5.79	1.39±0.55	11.20±2.11^{A}	183.21±37.82^{A}	53.41±17.11	28.88±4.8^{A}
1% Arg	8.15±0.92^{B}	28.1±4.13	1.59±0.24	12.40±1.13^{AB}	179.91±39.41^{A}	52.22±10.62	29.30±3.1^{A}
2% Arg	8.16±0.53^{B}	23.5±2.66	1.77±0.22	13.00±0.58^{AB}	134.18±20.05 ^{B}	46.69±6.18	34.90±1.6^{B}
3% Arg	8.25±0.56^{B}	25±4.20	1.80±0.27	13.60±0.99^{B}	140.87±32.25^{B}	46.55±7.82	34.20±8.8^{AB}
p-value	0.006^{A}	0.142^{K}	0.162^{K}	0.021^{K}	0.02^{K}	0.669^{K}	0.017^{K}

Superscript letters show differences between the same column ($p < 0.05$). Superscript A or K letters in *p* values represent ANOVA or Kruskal-Wallis analysis, respectively.

3.3 Blood cell differentiation

The percentage of neutrophils, basophils, and eosinophils did not demonstrate significant differences (see Table 6). The 2% arginine treatment resulted in a significantly higher lymphocyte count compared to the other treatments. A significant decrease in monocyte number was observed in the 2% arginine treatment group. A statistically significant increase in thrombocytes was observed in the control treatment group.

Table 6: Blood cell types (leukocytes and thrombocytes) of Nile tilapia (*Oreochromis niloticus*) fed diets with different levels of L-arginine and cultured at 20 ‰. Data are mean ± SD.

Treatments	Lymphocytes (%)	Monocytes (%)	Neutrophils (%)	Basophil (%)	Eosinophils (%)	Thrombocytes (%)
0% Arg	64.95±13.73^{A}	27.25±9.19^{A}	7.42±5.91	0±0	1.16±0.55	75.78 ± 6.14^{A}
1% Arg	57.54±14.18^{A}	29.23±11.62^{A}	11.43±5.18	0.19±0.40	2.55±1.05	66.25±6.14 ^{B}
2% Arg	75.50±10.69^{B}	16.79±4.51^{B}	7.70±7.002	0±0	0.000±0.00	66.36±5.77^{B}
3% Arg	61.57±19.49^{A}	27.77±12.29^{A}	8.92±7.13	0.29±0.47	2.69±1.62	59.71±5.86^{B}
p-value	0.037^{K}	0.015^{K}	0.166^{K}	0.069^{K}	1^{K}	0^{A}

Superscript letters show differences between the same column ($p < 0.05$). Superscript A or K letters in *p* values represent ANOVA or Kruskal-Wallis analysis, respectively. The lymphocytes, monocytes, neutrophils, basophils, and eosinophils expression is in percent of each of the leukocyte cellular types, without thrombocytes. Thrombocyte expression is in the percent of total leukocytes and thrombocyte count.

3.4 Micronucleus and nuclear aberrations assay

The different diets did not affect the presence of micronuclei, blebbel, and notched nuclear aberrations of erythrocytes (see Table 7). A reduced incidence of binucleated nuclear aberrations has been observed in the 1% arginine diet, in contrast to the other treatments.

Table 7: Micronucleus and nuclear aberrations of erythrocytes of Nile tilapia (*Oreochromis niloticus*) fed diets with different levels of arginine and cultured at 20 ‰. Data are mean ± SD.

Treatments	Micronucleus (%)	Binucleated (%)	Blebbel (%)	Notched (%)
0% Arg	0.488±0.25	0.333±0.14 ^{A}	0.600±0.47	2.950±2.01
1% Arg	0.190±0.01	0.200±0.00 ^{B}	0.311±0.14	2.238±2.19
2% Arg	0.197±0.01	0.500±0.50 ^{AB}	0.289±0.22	2.717±1.32
3% Arg	0.177±0.04	0.418±0.26 ^{A}	0.357±0.27	2.479±1.76
p-value	0.134^{K}	0.034^{K}	0.089^{K}	0.223^{A}

Superscript letters show differences between the same column ($p < 0.05$). Superscript A or K letters in *p* values represent ANOVA or Kruskal-Wallis analysis, respectively.

3.5 Serum biochemistry parameters

The investigation revealed that cholesterol and glucose levels remained unchanged across all experimental groups (see Table 8). Triglyceride levels were statistically similar for the control and 2% Arg treatments. However, a significant increase was observed in the 3% Arg treatment, in contrast to the 1% Arg group, which exhibited a decrease. The total protein content was found to be considerably higher in the 1% Arg group compared to the 2% Arg group. In contrast, the control and 3% Arg treatments failed to

show a significant difference. Albumin and globulin levels exhibited a marked increase in the 1% Arg treatment group, in contrast to the 2% Arg treatment group.

Table 8: Serum biochemistry of Nile tilapia (*Oreochromis niloticus*) fed diets with different levels of L-arginine and cultured at 20 ‰. Data are mean ± SD.

Treatments Su	Cholesterol (mg/dL)	Triglycerides (mg/dL)	Glucose (mg/dL)	Total protein (g/dL)	Globulin (g/dL)	albumin (g/dL)
0% Arg	106.9±12.72	138.26±55.86 AB	105.85±24.65	2.68±0.37 AC	1.39±0.39 AB	1.29±0.26 AB
1% Arg	100.83±14.31	115.93±39.98 A	92.17±20.78	3.17±0.59 B	1.78±0.48 B	1.38±0.17 A
2% Arg	94.89±11.61	120.87±45.25 AB	93.59±18.63	2.22±0.49 A	1.13±0.54 A	1.08±0.13 B
3% Arg	97.63±17.61	182.75±99.97 B	93.72±27.76	2.74±0.56 BC	1.41±0.36 AB	1.32±0.24 A
p-value	0.103[K]	0.039[K]	0.482[K]	0.001[K]	0.002[A]	0.004[K]

letters show differences between the same column ($p < 0.05$). Superscript A or K letters in *p* values represent ANOVA or Kruskal-Wallis analysis, respectively.

3.6 Gene relative expression

The polynomial regression of the relative expression of genes related to the immune system (IL-10), stress response (HSP70), and antioxidant defense (SOD) in the liver of *O. niloticus* are presented in Figure 2.

No significant differences were observed between arginine inclusion levels and the relative expression of IL-10, HSP70, and SOD. However, a quadratic trend was observed: expression levels of HSP70 and SOD increased at intermediate arginine levels, while IL-10 expression decreased at those same levels.

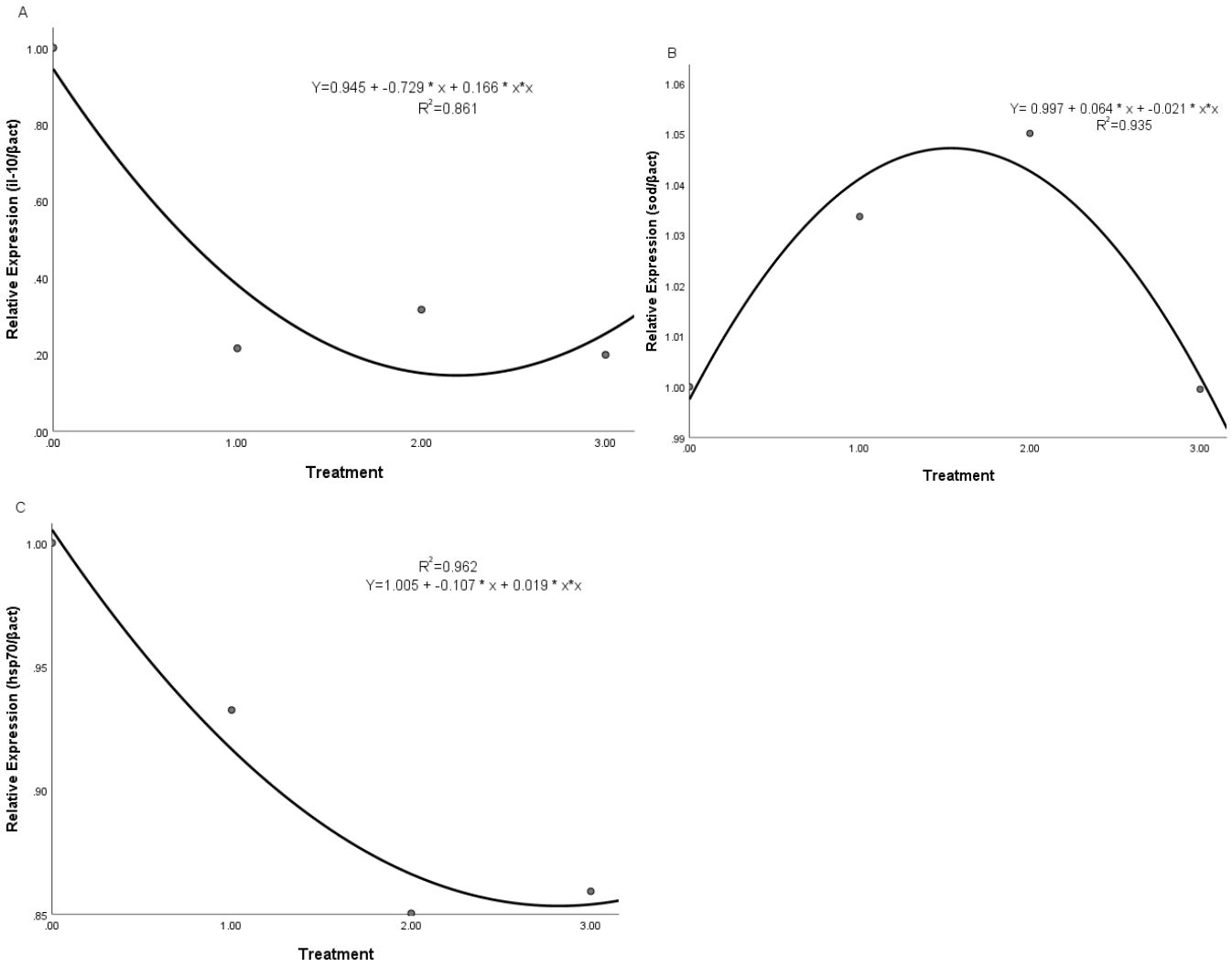

Figure 2: Polynomial regression analysis of the effects of different L-arginine inclusions on relative gene expression of a) *il-10*, b) *sod,* and c) *hsp70* in the liver of Nile tilapia (*O. niloticus*), cultured at 20 ‰.

4. Discussion

The results of this study demonstrate an increase in Specific Growth Rate (SGR) and greater weight gain with higher levels of arginine. The highest SGR was observed in the 3%Arg treatment. A trend that aligns with that observed in *Cirrhinus mrigala* [32], *Siniperca chuatsi* [30], *Eriocheir sinensis* [33], and Nile tilapia [43]. However, in the present work, maximum growth was observed at concentrations of total 9.16% Arg of crude protein, with differs from previous reports suggesting that in Nile tilapia grown in freshwater [43] and *Siniperca chuatsi* [30], achieved maximum growth at arginine concentrations of 6.24% crude protein and 2.61% dry diet respectively. A difference that may explain arginine´s role counteracting saline stress as an insulinotropic agent [36].

In Nile tilapia culture, salinity stress greater than 15 ‰ can increase the mortality rate to 16.67%, which can reach 33.33% at salinity levels of 20 ‰ [50]. In the present study, it was observed that organisms supplemented with 1% and 2% Arg had an increased survival rate, with mortality rates of 15% and 8%, respectively. The improved survival at these arginine concentrations (3.6% and 4.6% of the dry diet) is similar to those reported in *Siniperca chuatsi* [30] and *Eriocheir sinensis* [33], where the highest

survival rate was observed at concentrations of 3.37% [30] and 4.01% [33] of dry diet, despite not having significant differences along treatments.

The hematological parameters are important indicators of the health status of fish, helping to identify the impact of stressors on an organism's health [17, 45]. In this study, hemoglobin concentrations were significantly lower in the control group, consistent with observations by Mohamed *et al.* [51], who reported decreased hemoglobin levels at high salinity. The mean corpuscular volume (MCV) along the arginine concentration increased, while the Mean Corpuscular Hemoglobin Concentration (MCHC) increased with rising arginine concentration. This pattern aligns with previous findings for juveniles of the same species [42].

One of the most widely studied functions of dietary arginine supplementation is its role as an immunostimulant [25, 29, 36, 52]. Arginine serves as a precursor for the synthesis of nitric oxide (NO) and polyamines that promote cell proliferation. It also plays a role in modulating the inflammatory response, influencing the TOR signaling pathways, and supporting lymphocyte proliferation [25, 29, 35-36, 52]. Supplementing arginine promotes the biosynthesis of polyamines, resulting in an increased number of leukocytes [35, 36]. Here the white blood cells (WBC) significantly increased along with the concentration of arginine. This effect has been previously observed in Jian carp [53], *Scophthalmus maximus* [37], and Nile tilapia [54]. Notably, the diet containing 2%Arg led to an increase in leukocytes and a decrease in monocytes, contrasting with the other dietary treatments. This result is consistent with that reported in *Scophthalmus maximus* [37], where organisms supplemented with 2.3% Arg, exhibited a similar response, closely to the 2% Arg supplementation in the present study.

A study on *Ictalurus punctatus* [55] evaluated the components of the innate immune system and found an increase in lysozyme levels when a 4% arginine in dry diet was supplemented. An increase that could be associated with a potential rise in monocytes and granulocytes [55]. However, no significant differences were observed in the presence of micronuclei, blebbels, and notched cells. The number of binucleated erythrocytes was higher in the control and 3%Arg dietary treatments. Responses that are similar to those reported in *Barbonymus gonionotus* exposed to sublethal concentrations of profenofos for one day [56]. This resemblance may indicate that the control group experienced a molecular level due to the saline stress, while the result in the 3%Arg group could be attributed to excessive arginine intake. Fujimoto *et al.* [54] reported that arginine concentrations greater than 4.1% in dry matter can cause liver necrosis in Nile tilapia. However, insufficient information exists to verify the effects of excessive arginine intake.

No significant differences were found in glucose levels along the dietary treatments. The glucose response at different arginine concentrations resembled findings reported in Nile tilapia [42] and Nile tilapia GIFT [57], where increased arginine resulted in decreased serum glucose. Glucose concentrations in the present study were slightly higher than those reported in freshwater mossambica and Nile tilapias [45, 50]. Nevertheless, the glucose levels were comparable to those reported in Nile tilapia cultured at 20 ‰ by Metwaly *et al.* [50]. The increase in glucose at salinities over 20 ‰ likely reflects a stress response in Nile tilapia. When the organism faces stress, it releases catecholamines and cortisol as a primary response, stimulating the release of energy substrates through glycolysis [25, 36]. Consequently, elevated glucose levels and insulin are required to promote the absorption of glucose by tissues [58]. An increase in insulin in response to higher arginine availability has been reported in *Largemouth bass* [59], *Salmo salar* [60], and other salmonids [36]. Likewise, the role of arginine in glucose uptake has been linked to the activation of glucose transporter-4 translocation [35] and its subsequent oxidation [36, 60]. However, further studies are required to clarify this interaction.

No significant differences were found in cholesterol levels among the dietary treatments. However, triglyceride levels were significantly lower in the 1% Arg diet and higher in the 3% Arg diet. The decrease in triglyceride concentrations observed in the 1% and 2% Arg treatments aligns with that reported by Li *et al*. for the same species [39]. Conversely, organisms fed arginine containing 3.1% and 4.5% of dry matter exhibited reduced triglycerides [39] as well. Nonetheless, information regarding triglyceride responses at higher arginine levels is lacking. The diet with 1% Arg supplementation resulted in the highest concentrations of total protein, albumin, and globulin. These results are comparable to those observed in *Cirrhinus mrigala* [32] and GIFT [61], where higher concentrations of these metabolites were noted in diets with 2.78-3.4% Arg for carp and 1.75-2% of dry diet for Nile tilapia GIFT.

No statistical differences were observed in molecular markers of the immune system (IL-10), stress response (HSP70), and antioxidant defense (SOD). However, the cytokine IL-10 exhibits a quadratic trend. The control group had higher IL-10 expression than those found in the dietary treatments supplemented with Arg, where the lowest level corresponded to 1% Arg and a tendency to increase afterwards. This result is similar to that reported in Jian carp [53], in which the highest IL-10 expression was observed in the basal diet, increasing after reaching an arginine level of 1.61% of the dry diet. The decrease in the expression of this gene could be because it is expressed after the expression of pro-inflammatory cytokines [62]. This response is consistent with the idea that an excess of arginine can lead to tissue necrosis, although more research is needed to confirm this. The highest levels of SOD expression were noted in the 1%Arg and 2%Arg dietary treatments, while the control and 3%Arg groups had very similar concentrations. An increase in expression of this antioxidant was observed with rising dietary arginine has been reported in species such as the juvenile *Eriocheir sinensis* [32], *Scophthalmus maximus* [37], *Sciaenops ocellatu*s [35], and *Cyprinus carpio* [41]. HSP70 expression showed a trend similar to that of IL-10, with the control having the highest expression, followed by decreases in the 1% Arg and 2% Arg groups, and a slight increase in the 3% Arg group. In Cyprinus carpio [41], a diet containing 4–4.63% arginine of dry matter underexpressed the HSP70 gene after exposure to ammonia stress.

Overall, dietary supplementation with L-arginine at moderate concentrations may offer beneficial effects on Nile tilapia cultured in brackish water. However, the molecular-level effects of the control and 3% Arg dietary treatments raise questions regarding the impact of high concentrations. The presence of binuclear aberrations, along with the expression levels of IL-10 and HSP70 at elevated concentrations, suggests a potential stress response in the organism, warranting further investigations to confirm these findings.

5. Conclusions

Supplementing the diet with L-arginine may help alleviate the negative effects of saline stress on Nile tilapia. A diet with a 7-9% arginine concentration in the total crude protein content could improve the growth of Nile tilapia while lowering the feed conversion ratio (FCR). Additionally, it may stimulate their immune system, resulting in better health under chronic salinity stress. However, further studies are recommended to investigate the effects of excessive dietary arginine, and whether arginine supplementation has the same effect on other types of abiotic stressors or higher salinities. Although this study was conducted on Nile tilapia, further investigations into dietary L-arginine supplementation to alleviate stress in native species and promote sustainable aquaculture practices that preserve ecosystem integrity are advisable.

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Abbreviations

The following abbreviations are used in this manuscript:

‰	Parts per thousand
RAS	Recirculation system
GLY	glycine
Arg	Arginine
CP	Crude protein
NFE	Nitrogen-free extract
SGR	Specific growth rate
FCR	Feed Conversion Ratio
CF	Condition Factor
SR	Survival rate
HSI	Hepatosomatic index
VSI	Viscerosomatic index

Hb	Hemoglobin
Hct	Hematocrit
RBC	Red blood cell
WBC	White blood cell
MVC	Mean Corpuscular Volume
MCH	Mean Corpuscular Hemoglobin
MCHC	Mean Corpuscular Hemoglobin Concentration
HSP 70	Heat shock protein 70
SOD	Superoxide dismutase
IL-10	Interleukin-10

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CAPÍTULO III: Conclusiones

El estrés crónico por salinidad en tilapia tiene fuertes repercusiones a nivel fisiológico. La suplementación con arginina puede mitigar este efecto, sin embargo, no lo erradica en su totalidad. El suplementar a los organismos con arginina en concentraciones aproximadas de 7-9% de proteína cruda coadyuva a mitigar el estrés. Se observó una tendencia lineal con respecto al crecimiento e inclusión de arginina, con el mayor crecimiento en los organismos alimentados con el tratamiento de 3%, además de presentar la menor tasa de conversión alimenticia. Así mismo, la suplementación con arginina influyó en el recuento celular leucocitario y la hemoglobina, con un incremento gradual conforme incrementa la inclusión de arginina. Las concentraciones de glucosa y colesterol, pese a no ser estadísticamente distintas, sí se aprecia un decremento gradual conforme incrementa la concentración de arginina. La regulación de estos parámetros ante la adición de arginina puede significar que este aminoácido funge como mitigador del estrés.

Las dietas con inclusión de arginina al 1 y 2%, pese a no tener la mayor tasa de conversión alimenticia o el mayor crecimiento, sí mostraron mejores respuestas compensatorias, lo cual se vio reflejado en la concentración de proteínas totales, globulina, albumina, triglicéridos y expresión relativa de SOD. Estos parámetros demuestran el papel de la arginina como antioxidante y mitigador de estresores.

Se observó mejoría en los organismos suplementados con arginina, sin embargo, la inclusión de 3% de arginina en la expresión relativa de SOD, expresión relativa de IL- 10, el recuento de linfocitos y monocitos tuvo un resultado similar a los expuestos en la

dieta control y 1%. Aunque esté resultado no afectó la integridad del organismo, sí es un resultado a considerar en la búsqueda de una concentración intermedia que proporcione los beneficios del crecimiento, inmunoestimulación y función antioxidante.

Este estudio abre la puerta a futuros trabajos con tilapia del Nilo, en los que se evalúe los efectos de una dieta con concentraciones entre 7-9% de arginina en proteína cruda. Esta investigación se realizó en una especie de importancia acuícola a nivel mundial. Así mismo se recomienda implementar este suplemento en especies nativas, así como implementarlo ante otras situaciones de estrés, y con ello promover la acuacultura sustentable en la zona costera.

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