Veterinary and Animal Science 22 (2023) 100316

Contents lists available at ScienceDirect

Veterinary and Animal Science

journal homepage: www.elsevier.com/locate/vas

Microencapsulated essential oils influence the growth and foregut histomorphometry of Nile tilapia (*Oreochromis niloticus*) fingerlings

Jesús Roldan-Juarez [a,b], Rubén Pinares [c,*], Carlos E. Smith [a], Cielo A. Llerena [a], Virgilio Machaca [b], Dante M. Pizarro [a]

[a] *Universidad Peruana Cayetano Heredia, Facultad de Medicina Veterinaria y Zootecnia, Lima, Peru*
[b] *Universidad Nacional Micaela Bastidas de Apurímac, Facultad de Medicina Veterinaria y Zootecnia, Abancay, Peru*
[c] *Universidad Nacional de San Antonio Abad del Cusco, Escuela Profesional de Medicina Veterinaria, Sicuani, Peru*

ARTICLE INFO

Keywords:
Animal supplementation
Aquaculture
Histometry
Intestinal folds
Goblet cells
Fish size

ABSTRACT

Essential oils supplementation has potential growth-promoting, antibacterial, and immunostimulatory effects for various fish species. Dietary supplementation with essential oils improves Nile tilapia's growth and intestinal morphology. The aim of the study was to assess the effect of supplementation with microencapsulated essential oils (MEOs) containing cinnamaldehyde (53.9 %), thymol (24.2 %), and carvacrol (18.7 %) in Nile tilapia fingerlings on growth parameters and foregut histomorphometry. Six thousand fishes with initial body weights and lengths of 1.20 ± 0.32 g and 2.03 ± 0.40 cm, respectively, were reared in two separate 60 m^3 circular tanks (control and supplemented with 500 mg/kg of MEOs) at a 5 kg/m^3 density. Growth parameters included weight and length. Ten foregut samples per tank were collected at 0-, 15- and 30-days post-treatment. The histological analysis involved the size of intestinal folds and the number of goblet cells. Our results showed that fingerling growth parameters such as final body weight and length increased by 16.9 % and 10.43 %, respectively, with MEOs supplementation compared to the control group. Furthermore, histomorphometry results showed that the supplementation of MEOs led to a significant increase in the growth of both the width and length of intestinal folds and the number of goblet cells ($p < 0.05$). In conclusion, the early supplementation with MEOs improved the number, length, and width of intestinal folds and increased the number of goblet cells, positively influencing intestinal morphology and health. Additionally, MEOs improved growth parameters in Nile tilapia at 30 days of supplementation.

Introduction

The aquaculture sector is one of the main activities contributing to global food security (Dawood et al., 2022). Nile tilapia (*Oreochromis niloticus*), a freshwater fish greatly appreciated for its delectable taste and nutritional value, is cultivated worldwide due to its adaptability to various climatic conditions across extensive, semi-intensive, and intensive production systems (Mengistu et al., 2020). This species contributes 8 % of total aquaculture output, plays an important role in human nutrition due to its protein contribution, and holds economic significance for numerous families within local economies (FAO, 2020).

However, the rapid growth of aquaculture and intensified fish farming have made these populations more susceptible to diseases (Martos-Sitcha et al., 2020; Oddsson, 2020), leading to a pervasive use of antibiotics as a preventive or therapeutic strategy. Unfortunately, this indiscriminate antibiotic use has fostered bacterial resistance, environmental contamination, and residual presence in meat (Romero et al., 2012). Thus, a global push to reduce antibiotic dependence in aquaculture is imperative. One promising strategy involves adopting rearing practices that alleviate stress on fish, diminishing the likelihood of antibiotic-requiring infections (Ardó et al., 2008; Romero et al., 2012) and thus reducing antibacterial resistance and meat contamination (Brum et al., 2017). This approach advocates for the integration of novel nutritional additives-either natural (organic) or synthetic immunomodulators-to guarantee public health (Romero et al., 2012; Valladão et al., 2015; Elumalai et al., 2020; Abdel Rahman et al., 2022a, 2022b) and ensures sustainable fish farming.

Essential oils have potential growth-promoting, antibacterial, and immunostimulatory effects for various fish species. In addition, these oils enhance the antioxidant capacity and resistance against infectious

* Correspondence author at: Universidad Nacional de San Antonio Abad del Cusco, Escuela Profesional de Medicina Veterinaria, Sicuani, Peru.
E-mail address: ruben.pinares@unsaac.edu.pe (R. Pinares).

https://doi.org/10.1016/j.vas.2023.100316

Available online 28 September 2023

diseases (Abdollahzadeh et al., 2014; Brandão et al., 2021; Dawood et al., 2022), favoring better nutritional status and animal welfare (Dinardo et al., 2020). They also influence nutrient digestion and absorption (Mohamed et al., 2014), decreasing the pathogen adhesion to the intestinal mucosa (Jamroz et al., 2006).

The intestine is crucial for the immune system; its effectiveness depends on mucus production, epithelial cell integrity, and a balanced commensal bacterial population, which contributes to intestinal balance and fish health, curbing infectious diseases (Nicholson et al., 2012). Goblet cells produce mucopolysaccharides and synergize with immunoglobulins, and lysozymes act as a potent chemical defense against pathogens (Schroers et al., 2009). The density and size of intestinal folds are intrinsically tied to the number of cells that compose them, where more cells equate to larger folds and an increased nutrient absorption surface (Velasco et al., 2010).

Including essential oils in Nile tilapia diets could be an adequate strategy since they have natural products without residual effects. The objective of the study was to assess the foregut histomorphometry and growth parameters of Nile tilapia fingerlings supplemented with a microencapsulated mixture of clove, cinnamon, oregano, and palm essential oils.

Materials and methods

Fish rearing condition

The study was conducted at Hippocampus del Sur SAC, a company specialized in a semi-intensive tropical fish production system in the province of Barranca (Lima, Peru).

Six thousand sexually reverted Nile tilapia fingerlings from the Fish Research Center (CINPIS, Universidad Nacional Agraria La Molina, Lima, Peru) were distributed across two circular 6 m^3 geomembrane tanks at a stocking density of 5 kg/m^3 (3000 tilapia fingerlings in each tank, one tank per treatment). The selected fingerlings were healthy, free of lesions, and had an average initial weight and length of 1.20 $\pm$ 0.32 g and 2.03 $\pm$ 0.40 cm, respectively.

Nile tilapia fingerlings were provided a commercial extruded feed (1.5 $\times$ 2.0 mm) containing 45 % protein, 7 % fat, and 2.5 % fiber. The treatment group received a microencapsulated essential oils (MEOs) supplementation at 500 mg/kg (Valladão et al., 2019). The product used (EMERALD®, IGUSOL ADVANCE SA, La Rioja, Spain) contained a high concentration of the following compounds (Table 1): cinnamaldehyde (53.9 %), thymol (24.2 %), and carvacrol (18.7 %). The control group did not receive any supplementation. For the homogenization and mixing of the extruded feed with MEOs, a mechanical mixer was used at 540 RPM for 10 min; molasses was also added to facilitate the adherence of the micronutrients. To avoid MEOs oxidation, the mixture was prepared daily over 30 days. The final diet was weighed with a BL320S (Shimadzu Corporation, Kyoto, Japan) precision balance.

To prevent leaching, fingerlings were fed four times a day (8:00, 10:00, 14:00, and 16:00 h) at a rate of 5 % biomass. The feeding rate was adjusted according to the water temperature. This study evaluated three critical physical-chemical parameters of the water: Temperature, dissolved oxygen levels, and pH. Daily temperature readings were recorded using a mercury thermometer during the morning before feeding (7:30 h) and afternoon (13:30 h). The water's dissolved oxygen levels (YSI 550A YSI, Yellow Springs, OH, USA) and pH (TEC-5, TECNAL, Piracicaba, SP, Brazil) were measured at 7:30 and 13:30 h every two weeks. Water aeration was not performed as oxygen levels were within the normal range (6 to 7 mg/L). Water flow was conducted through an open system with constant exchange. The waste was cleaned automatically through a centrifugal force by a sump attached to the tank's base.

Table 1
Chemical composition of volatile compounds (essential oils) in EMERALD® microencapsulated by solid phase microextraction and gas chromatography coupled to mass spectrometry.

Volatile compounds (essential oils)	Gas retention time (minutes)	Sample content (%)
Styrene	14.35	0.29
Benzaldehyde	15.87	0.73
p-Cymene	17.01	0.81
Limonene	17.09	0.12
1-methyl-4-(1-methylethenyl)-benzene	18.17	0.09
3-phenyl-2-propenal	20.33	1.08
Cinnamaldehyde	21.17	53.93
Thymol	21.24	24.22
Carvacrol	21.40	18.73

Growth parameters and histomorphometry

To determine the fish count, the mean difference statistical test was used (Valladão et al., 2019), with a confidence of 95 % and a statistical power of 90 %. Ten individuals were obtained per treatment and control group. Ten random sampling was conducted on day 0 (control) and days 15 and 30 post-treatment. On each fish, body weight (g) and length (cm) were measured on days 0 (initial body weight and length), 15, and 30 (final body weight and length) as growth parameters. A benzocaine dose of 200 mg/L was used to euthanize fish. Followed by a necropsy, the foregut sections were collected and preserved in a 10 % formalin solution for 24 h. Subsequently, histological samples were obtained and fixed in paraffin. Staining processes with hematoxylin and eosin were performed for histomorphometry. Leica® Suite Version 3.4.0 software was used, with 10X and 40X objectives, to digitize the images and measure the length and width of the intestinal folds and the number of goblet cells, using periodic acid-schiff, alcian blue pH 2.5 and alcian blue pH 0.5 stains.

Statistical analyses

The *t*-test ($\alpha = 0.05$) was used to compare the effect of MEOs on the length and width of the intestinal folds, the number of goblet cells (histomorphometry), as well as on the length and weight of the fingerlings (growth parameters). The statistical analysis of both the growth parameters and histomorphometry was carried out separately on days 0, 15, and 30 using the Rcmdr package of R software version 4.1.1 (R Core Team, 2021).

Results

Water quality parameters

The average water quality parameters such as dissolved oxygen (mg/L), temperature (°C), and pH were 6.8 $\pm$ 0.72, 23.7 $\pm$ 0.20 and 7.6 $\pm$ 0.12, respectively. No differences were observed in any of these parameters ($p > 0.05$) between the control and MEOs groups. Additionally, Abdel-Tawwab et al. (2015) reported an adequate level of dissolved oxygen between 6.0 and 6.5 mg/L; in our study, this parameter, on average, has been maintained at a slightly higher level, which allowed an efficient metabolism under physiological conditions of the fish.

Nile tilapia growth parameters

Supplementation of fingerlings' diet with MEOs improved growth parameters (Table 2). Growth parameters were similar ($p > 0.05$) at 15 days, but significant differences ($p < 0.05$) were observed at 30 days. Among the fingerlings supplemented with MEOs, the final weight gain at 30 days was 16.9 % higher than the control group, and the final length was 10.4 % greater than the control group.

Table 2
Growth parameters (mean ± standard error) of Nile tilapia (*Oreochromis niloticus*) fingerlings supplemented with microencapsulated essential oils (MEOs).

Growth parameters	Days	Treatment Control	0.5 % MEOs (500 mg/kg)	Significance *p*-value
Weight (g)	0	1.20 ± 0.34	1.19 ± 0.35	0.93
	15	4.37 ± 0.94	4.57 ± 0.83	0.48
	30	5.70 ± 0.80	6.26 ± 0.74	0.02*
Length (cm)	0	2.03 ± 0.43	2.15 ± 0.51	0.97
	15	3.76 ± 0.65	4.05 ± 0.67	0.33
	30	4.87 ± 0.47	5.28 ± 0.61	0.02*

*: significant differences between the control and the MEOs group ($p < 0.05$).

Histomorphometry of Nile tilapia intestinal folds

Table 3 shows the changes in length and width of the intestinal folds among the Nile tilapia fingerlings. Differences ($p < 0.05$) were observed at 15 and 30 days of MEOs supplementation. However, the number of goblet cells did not show significant differences ($p > 0.05$) after 15 days of MEOs supplementation. In this study, MEOs supplementation only showed differences ($p < 0.05$) at 30 days.

Nile tilapia supplemented with MEOs showed evident improvement in the number, length, and width of the intestinal folds when compared to the histomorphometry of the intestinal folds in the fingerlings of the control group (Fig. 1(A) and (B)). Similarly, the number of goblet cells increased in fish supplemented with MEOs, as observed through different stains: periodic acid-Schiff, alcian blue pH 2.5 and alcian blue pH 0.5 (Fig. 2(A), (B) and (C), respectively).

Discussion

Essential oils are secondary plant metabolites containing various bioactive components such as polyphenols, organic alcohols, terpenes, ketones, esters, etc. (Pan et al., 2023). In this study, dietary supplementation of MEOs achieved higher growth parameters such as weight and length, similar to De Souza et al. (2020) findings, where *Aloysia triphylla* essential oils supplementation improved growth at 45 days. Our results align with previous studies involving different essential oils in Nile tilapia (Aanyu et al., 2018; El-Hawarry et al., 2018) and red tilapia (Verastegui & Fikoshima, 2009). Amer et al. (2018) indicated greater weight gain in Nile tilapia through supplementation with cinnamaldehyde and thymol at 1 and 2 ml/kg doses for 30 days. Additionally, a positive effect of basil and ginger essential oils on the growth of Nile tilapia after 55 days has been reported (Brum et al., 2017).

Table 3
Intestinal histomorphometry and goblet cell number (mean ± standard error) of Nile tilapia (*Oreochromis niloticus*) supplemented with microencapsulated essential oils (MEOs).

Histomorphometry	Days	Treatment Control	0.5 % MEOs (500 mg/kg)	Significance *p*-value
Intestinal fold length (μm)	0	127.78 ± 10.67	122.08 ± 11.66	0.40
	15	175.79 ± 19.01	201.67 ± 22.75	0.01*
	30	228.41 ± 38.09	269.39 ± 21.48	0.01*
Intestinal fold width (μm)	0	41.69 ± 5.66	42.14 ± 07.04	0.90
	15	64.38 ± 11.94	75.07 ± 06.34	0.02*
	30	76.09 ± 8.28	84.13 ± 08.06	0.04*
Goblet cell number (cells/area)	0	622 ± 116.95	628.2 ± 112.59	0.93
	15	604.3 ± 125.63	672.5 ± 167.03	0.32
	30	1065.3 ± 92.31	1159.5 ± 86.67	0.03*

Dietary supplementation of lemongrass (*Cymbopogon citratus*) and rose geranium (*Pelargonium graveolens*) essential oils showed a potential to improve fish growth performance, feed utilization, oxidative status, immune responses, and resistance to diseases (Al-Sagheer et al., 2018). Phytochemicals in essential oils have shown multiple biofunctionality and are less likely to induce bacterial resistance. Microencapsulation can be applied to solve the challenges of effectively utilizing essential oils (Pan et al., 2023). Improving the immune responses and intestinal morphology is crucial in increasing growth performance, nutrient absorption, and disease resistance in fish, important outcomes in such a competitive and developing aquaculture sector (Ramos et al., 2017).

The histomorphometry results agree with a prior study by Magouz et al. (2021), who used a mixture of essential oils to increase the number of intestinal folds, length, and goblet cells (Moreira et al., 2018). Moreover, lemon verbena essential oil in catfish at doses of 0.25, 1, and 2 ml/kg for 60 days showed significant changes in the length and number of intestinal folds (Zeppenfeld et al., 2016).

In Nile tilapia, Valladão et al. (2017) observed greater length of the intestinal folds when supplementing with tea essential oil for 60 days. However, when supplementing with thyme essential oil for 15 days, Valladão et al. (2019) only observed a 0.5 % increase in the width of the intestinal folds from 102.55 ± 6.42 (control) to 116.11 ± 16.00, with an increase in the number of goblet cells. Furthermore, the number of lymphocytes ($p < 0.05$) and leukocytes ($p < 0.05$) increased in the blood of fish fed with 1 % of thyme essential oil (Valladão et al., 2019). This supplementation stimulates the cellular components of the non-specific immune response of Nile tilapia without causing harmful effects or altering the population of important intestinal bacteria.

Essential oils act as growth promoters, improving the palatability of food and stimulating mucopolysaccharide secretion to facilitate the digestion and absorption of ingested nutrients (Hashemi & Davoodi, 2010). They also modulate the intestinal microbiota for greater activity of beneficial bacteria, which, in turn, secrete digestive enzymes promoting nutrient digestion and inhibiting pathogenic growth (Reverter et al., 2014; Zeng et al., 2015).

Cinnamaldehyde, a component of MEOs, acts on serotonin receptors in the fish gastrointestinal tract, increasing both the number and size of intestinal folds and thereby improving absorption (Mohamed et al., 2014; Lieder et al., 2020; Gupta et al., 2021). The greater intestinal fold surface area facilitates more nutrient digestion and absorption. Moreover, an increased number of goblet cells increases the secretion of mucins or glycoproteins, which allows modulation of the intestinal microbiota and inhibits pathogenic bacteria growth (Lazado & Caipang, 2014; Abdel Rahman et al., 2019).

The synergistic effect of essential oils improves immunity, histomorphology, bacterial digestion, weight yield, and growth (Roques et al., 2020; Magouz et al., 2021; Pan et al., 2023). By alternating the active principles of the essential oils, the rapid growth of the intestinal folds is promoted (Branco et al., 2011), therefore enhancing nutrient digestion and absorption (Mohamed et al., 2014), ultimately leading to higher yields in weight and size of fish.

The supplementation with MEOs improved Nile tilapia fingerlings' histomorphometry and growth parameters; however, further studies are required to evaluate the productive performance, such as feed conversion rate, daily weight gain, and mortality rates. In addition, it is recommended to perform assessments of animal welfare and economic profitability of families dedicated to aquaculture production.

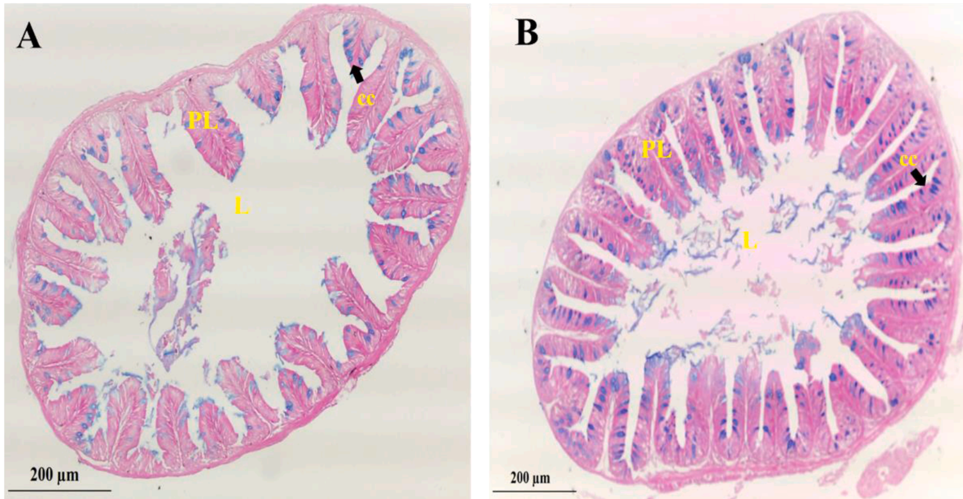

Fig. 1. Microscopic images at 10× of the foregut of Nile tilapia fingerlings at day 15, control (A) and treatment group (B). Alcian blue pH 2.5 stain showing changes in length, width and number of goblet cells with secretion of sulfated acid glycoproteins. PL = intestinal fold, L = lumen, CC = goblet cell producing sulfated acid glycoproteins (arrows).

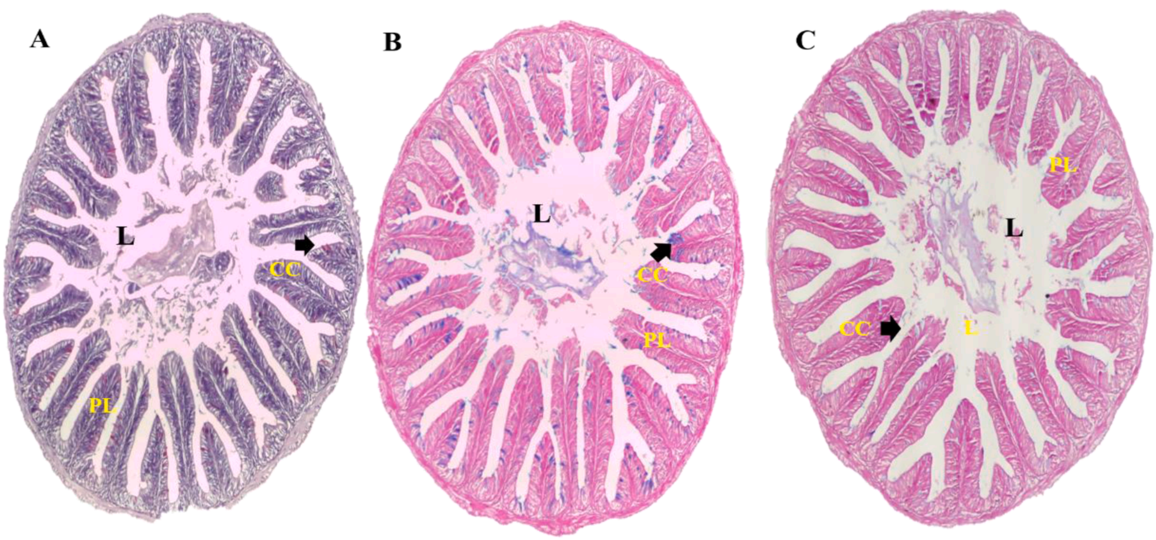

Fig. 2. Cross-sectional microscopic images at 10× of the foregut of Nile tilapia fingerlings on day 30 of the treatment group. Periodic acid-Schiff (A), alcian blue pH 2.5 (B) and alcian blue pH 0.5 (C) stains. PL = intestinal fold, L = lumen, CC = goblet cell (arrows).

Conclusions

The diet supplementation with MEOs enhanced the histomorphology of the intestines by improvements in width, length, and fold count. This supplementation also positively impacted the number of goblet cells and mucopolysaccharide production. These combined effects increase absorption area, enhancing growth parameters such as greater weight gain of about 16.9 % in Nile tilapia fingerlings after 30 days of supplementation.

Ethical statement

The research was conducted in compliance with the Institutional Ethics Committee for the Use of Animals (CIEA) regulations approved by the Universidad Peruana Cayetano Heredia Ethics Committee. The CIEA is registered in the Office of Laboratory Animal Welfare of the U.S. Department of Health and Human Services, National Institutes of Health, with Assurance Number F16-00076 (A5146-01).

Funding sources

This study was supported by the Fondo Nacional de Desarrollo Científico, Tecnológico y de Innovación Tecnológica (FONDECYT). Convenio no. 230-2015-FONDECYT-Esquema Financiero EF 230. Maestría en Sanidad Acuícola en la Universidad Peruana Cayetano Heredia.

CRediT authorship contribution statement

Jesús Roldan-Juarez: Conceptualization, Formal analysis, Investigation, Methodology, Supervision, Writing – original draft, Writing – review & editing. **Rubén Pinares:** Conceptualization, Formal analysis, Investigation, Methodology, Supervision, Writing – original draft, Writing – review & editing. **Carlos E. Smith:** Conceptualization, Formal analysis, Investigation, Methodology, Supervision, Writing – original draft, Writing – review & editing. **Cielo A. Llerena:** Conceptualization, Formal analysis, Investigation, Methodology, Supervision, Writing – original draft, Writing – review & editing. **Virgilio Machaca:** Writing –

review & editing. **Dante M. Pizarro:** Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

The authors are grateful to Programa Nacional de Innovación en Pesca y Acuicultura (PNIPA). Sub proyecto: "Capacitación en suplementación de alimento balanceado con aceites esenciales en la dieta, para aumentar los índices productivos en la crianza de tilapia en el distrito de Barranca PNIPA-ACU-SEREX-PP000115".

The authors thank IGUSOL ADVANCE SA for providing the micro-encapsulated essential oils (EMERALD®).

References

Abdel-Tawwab, M., Hagras, A. E., Elbaghdady, H. A. M., & Monier, M. M. (2015). Effects of dissolved oxygen and fish size on Nile tilapia, *Oreochromis niloticus* (L.): Growth performance, whole-body composition, and innate immunity. *Aquaculture International, 23*, 1261–1274. https://doi.org/10.1007/s10499-015-9882-y

Abdel Rahman, A., Hassanin, M., & ElHady, M (2019). Growth performance, haematology and intestinal histo-morphology of Nile tilapia fed on Indian Lotus (*Nelumbo nucifera Gaertn*.) leaf powder at different concentrations. *Aquaculture Research, 50*(11), 3211–3222. https://doi.org/10.1111/are.14276

Abdel Rahman, A. N., Elshopakey, G. E., Behairy, A., Altohamy, D. E., Ahmed, A. I., Farroh, K. Y., & Ibrahim, R. E (2022a). Chitosan-*Ocimum basilicum* nanocomposite as a dietary additive in *Oreochromis niloticus*: Effects on immune-antioxidant response, head kidney gene expression, intestinal architecture, and growth. *Fish and Shellfish Immunology, 128*, 425–435. https://doi.org/10.1016/j.fsi.2022.08.020

Abdel Rahman, A. N., Van Doan, H., Elsheshtawy, H. M., Dawood, A., Salem, S. M., Sheraiba, N. I., & Mahboub, H. H (2022b). Dietary Salvia officinalis leaves enhances antioxidant-immune-capacity, resistance to *Aeromonas sobria* challenge, and growth of Cyprinus carpio. *Fish and Shellfish Immunology, 127*, 340–348. https://doi.org/10.1016/j.fsi.2022.06.030

Abdollahzadeh, E., Rezaei, M., & Hosseini, H. (2014). Antibacterial activity of plant essential oils and extracts: The role of thyme essential oil, nisin, and their combination to control Listeria monocytogenes inoculated in minced fish meat. *Food Control, 35*, 177–183. https://doi.org/10.1016/j.foodcont.2013.07.004

Al-Sagheer, A. A., Mahmoud, H. K., Reda, F. M., Mahgoub, S. A., & Ayyat, M. S. (2018). Supplementation of diets for Oreochromis niloticus with essential oil extracts from lemongrass (*Cymbopogon citratus*) and geranium (*Pelargonium graveolens*) and effects on growth, intestinal microbiota, antioxidant, and immune activities. *Aquaculture Nutrition, 00*, 1–9. https://doi.org/10.1111/anu.12637

Aanyu, M., Betancor, M. B., & Monroig, O. (2018). Effects of dietary limonene and thymol on the growth and nutritional physiology of Nile tilapia (*Oreochromis niloticus*). *Aquaculture, 488*, 217–226. https://doi.org/10.1016/j.aquaculture.2018.01.036

Amer, S. A., Metwally, A. E., & Ahmed, S. A. (2018). The influence of dietary supplementation of cinnamaldehyde and thymol on the growth performance, immunity, and antioxidant status of monosex Nile tilapia fingerlings (*Oreochromis niloticus*). *The Egyptian Journal of Aquatic Research, 44*, 251–256. https://doi.org/10.1016/j.ejar.2018.07.004

Ardó, L., Yin, G., Xu, P., Váradi, L., Szigeti, G., Jeney, Z., & Jeney, G. (2008). Chinese herbs (*Astragalus membranaceus* and *Lonicera japonica*) and boron enhance the non-specific immune response of Nile tilapia (*Oreochromis niloticus*) and resistance against *Aeromonas hydrophila*. *Aquaculture, 275*, 26–33. https://doi.org/10.1016/j.aquaculture.2007.12.022

Brum, A., Pereira, S. A., Owatari, M. S., Chagas, E. C., Chaves, F. C. M., Mouriño, J. L. P., & Martins, M. L. (2017). Effect of dietary essential oils of clove basil and ginger on Nile tilapia (*Oreochromis niloticus*) following challenge with *Streptococcus agalactiae*. *Aquaculture, 468*, 235–243. https://doi.org/10.1016/j.aquaculture.2016.10.020

Branco, P. A. C., Soares, R., Vieites, F., Cabral, N., & Tavares, E. (2011). Efeito de óleos essenciais como promotores de crescimento em leitões recém-desmamados. *Archivos de Zootecnia, 60*(231), 699–706. https://doi.org/10.4321/S0004-05922011000300057

Brandão, F. R., Farias, C. F. S., de Melo Souza, D. C., de Oliveira, M. I. B., de Matos, L. V., Majolo, C., de Oliveira, M. R., Chaves, F. C. M., de Almeida O'Sullivan, F. L., & Chagas, E. C. (2021). Anesthetic potential of the essential oils of *Aloysia triphylla, Lippia sidoides* and *Mentha piperita* for *Colossoma macropomum*. *Aquaculture, 534*, Article 736275. https://doi.org/10.1016/j.aquaculture.2020.736275

Dawood, M. A., El Basuini, M. F., Yilmaz, S., Abdel-Latif, H. M., Alagawany, M., Kari, Z. A., & Van Doan, H. (2022). Exploring the roles of dietary herbal essential oils in aquaculture: A review. *Animals, 12*, 823. https://doi.org/10.3390/ani12070823

De Souza, R. C., Baldisserotto, B., Melo, J. F. B., da Costa, M. M., de Souza, E. M., & Copatti, C. E. (2020). Dietary *Aloysia triphylla* essential oil on growth performance and biochemical and haematological variables in Nile tilapia. *Aquaculture, 519*, Article 734913. https://doi.org/10.1016/j.aquaculture.2019.734913

Dinardo, F. R., Deflorio, M., Casalino, E., Crescenzo, G., & Centoducati, G. (2020). Effect of feed supplementation with *Origanum vulgare* L. essential oil on sea bass (*Dicentrarchus labrax*): A preliminary framework on metabolic status and growth performances. *Aquaculture Report, 18*, 100–511. https://doi.org/10.1016/j.aqrep.2020.100511

El-Hawarry, W. N., Mohamed, R. A., & Ibrahim, S. A. (2018). Collaborating effects of rearing density and oregano oil supplementation on growth, behavioral and stress response of Nile tilapia (*Oreochromis niloticus*). *The Egyptian Journal of Aquatic Research, 44*(2), 173–178. https://doi.org/10.1016/j.ejar.2018.06.008

Elumalai, P., Kurian, A., Lakshmi, S., Faggio, C., Esteban, M. A., & Ringø, E. (2020). Herbal immunomodulators in aquaculture. *Reviews in Fisheries Science and Aquaculture, 29*, 33–57. https://doi.org/10.1080/23308249.2020.1779651

[FAO]. (2020). *Organización de las naciones unidas para la alimentación y la agricultura. 2020. El estado mundial de la pesca y la acuicultura.* Roma, Italia: La Sostenibilidad en Acción.

Gupta, N., Rani Kar, S., & Chakraborty, A. (2021). A review on medicinal plants and immune status of fish. *Egyptian Journal of Aquatic Biology and Fisheries, 25*, 897–912. https://doi.org/10.21608/ejabf.2021.170062

Hashemi, S. R., & Davoodi, H. (2010). Phytogenics as new class of feed additive in poultry industry. *Journal of Animal and Veterinary Advances, 9*, 2295–2304. https://doi.org/10.3923/javaa.2010.2295.2304

Jamroz, D., Wertelecki, T., Houszka, M., & Kamel, C. (2006). Influence of diet type on the inclusion of plant origin active substances on morphological and histochemical characteristics of the stomach and jejunum walls in chicken. Journal of Animal *Physiology and Animal Nutrition,* 90(5–6), 255–268. 10.1111/j.1439-0396.2005.00603.x.

Lazado, C.C., & Caipang, C.M.A. (2014). Mucosal immunity and probiotics in fish. *Fish and Shellfish Immunology,* 39(1), 78–89. 10.1016/j.fsi.2014.04.015.

Lieder, B., Hoi, J., Burian, N., Hans, J., Holik, A. K., Beltran Marquez, L. R., & Somoza, V. (2020). Structure-dependent effects of cinnamaldehyde derivatives on TRPA1-induced serotonin release in human intestinal cell models. *Journal of Agricultural and Food Chemistry, 68*(13), 3924–3932. https://doi.org/10.1021/acs.jafc.9b08163

Magouz, F. I., Mahmoud, S. A., El-Morsy, R. A., Paray, B. A., Soliman, A. A., Zaineldin, A. I., & Dawood, M. A. (2021). Dietary menthol essential oil enhanced the growth performance, digestive enzyme activity, immune-related genes, and resistance against acute ammonia exposure in Nile tilapia (*Oreochromis niloticus*). *Aquaculture, 530*, Article 735944. https://doi.org/10.1016/j.aquaculture.2020.735944

Mengistu, S. B., Mulder, H. A., Benzie, J. A., & Komen, H. (2020). A systematic literature review of the major factors causing yield gap by affecting growth, feed conversion ratio and survival in Nile tilapia (*Oreochromis niloticus*). *Reviews in Aquaculture, 12* (2), 524–541. https://doi.org/10.1111/raq.12331

Martos-Sitcha, J. A., Mancera, J. M., Prunet, P., & Magnoni, L. J. (2020). Welfare and stressors in fish: Challenges facing aquaculture. *Frontiers in Physiology, 11*, 162. https://doi.org/10.3389/fphys.2020.0016

Mohamed, M. A., El-Daly, E. F., Abd El-Azeem, N. A., Youssef, A. W., & Hassan, H. M. A. (2014). Growth performance and histological changes in ileum and immune related organs of broilers fed organic acids or antibiotic growth promoter. *International Journal of Poultry Science, 13*, 602. https://doi.org/10.3923/ijps.2014.602.610

Moreira, A.P., Lima, J.J., Olivera, F.C., Chagas, E.C., & Campos, C.M. (2018). Histomorfometría intestinal de Pacus alimentados com dieta contendo óleo essencial de *Ocimum gratissimum*. 28 Congresso Brasileiro de Zootecnia. https://ainfo.cnptia.embrapa.br/digital/bitstream/item/188668/1/trab-1725.pdf.

Nicholson, J. K., Holmes, E., Kinross, J., Burcelin, R., Gibson, G., Jia, W., & Pettersson, S. (2012). Host-gut microbiota metabolic interactions. *Science, 336*, 1262–1267. https://doi.org/10.1126/science.1223

Oddsson, G. V. (2020). A definition of aquaculture intensity based on production functions: The aquaculture production intensity scale (APIS). *Water, 12*, 765. https://doi.org/10.3390/w12030765

Pan, J., Zhu, Y., Abdel-Samie, M. A., Li, C., Cui, H., & Lin, L. (2023). Biological properties of essential oil emphasized on the feasibility as antibiotic substitute in feedstuff. *Grain and Oil Science and Technology, 6*(1), 10–23. https://doi.org/10.1016/j.gaost.2022.11.001

R Core Team. (2021). *R: A language and environment for statistical computing.* Vienna, Austria: R Foundation for Statistical Computing. http://www.R-project.org/.

Ramos, M. A., Batista, S., Pires, M. A., Silva, A. P., Pereira, L. F., Saavedra, M. J., & Rema, P. (2017). Dietary probiotic supplementation improves growth and the intestinal morphology of Nile tilapia. *Animal, 11*(08), 1259–1269. https://doi.org/10.1017/s1751731116002792

Reverter, M., Bontemps, N., Lecchini, D., Banaigs, B., & Sasal, P. (2014). Use of plant extracts in fish aquaculture as an alternative to chemotherapy: Current status and future perspectives. *Aquaculture, 433*, 50–61. https://doi.org/10.1016/j.aquaculture.2014.05.048

Romero, J., Feijoó, C. G., & Navarrete, P (2012). Antibiotics in aquaculture – use, abuse and alternatives. In E. D. Carvalho, G. Silva, & R. J. Silva (Eds.), *Health and environment in aquaculture* (pp. 159–198). InTechOpen. https://www.intechopen.com/chapters/35141.

Roques, S., Deborde, C., Richard, N., Skiba-Cassy, S., Moing, A., & Fauconneau, B. (2020). Metabolomics and fish nutrition: A review in the context of sustainable feed development. *Reviews in Aquaculture, 12*, 261–282. https://doi.org/10.1111/raq.12316

Schroers, V., van der Marel, M., Neuhaus, H., & Steinhagen, D. (2009). Changes of intestinal mucus glycoproteins after peroral application of *Aeromonas hydrophila* to common carp (*Cyprinus carpio*). *Aquaculture, 288*, 184–189. https://doi.org/10.1016/j.aquaculture.2008.12.013

Valladão, G. M. R., Gallani, S. U., Kotzent, S., Assane, I. M., & Pilarski, F. (2019). Effects of dietary thyme essential oil on hemato-immunological indices, intestinal morphology, and microbiota of Nile tilapia. *Aquaculture International, 27*, 399–411. https://doi.org/10.1007/s10499-018-0332-5

Valladão, G. M., Gallani, S. U., Pala, G., Jesus, R. B., Kotzent, S., Costa, J. C., & Pilarski, F. (2017). Practical diets with essential oils of plants activate the complement system and alter the intestinal morphology of Nile tilapia. *Aquaculture Research, 48*, 5640–5649. https://doi.org/10.1111/are.13386

Valladão, G. M. R., Gallani, S. U., & Pilarski, F. (2015). Phytotherapy as an alternative for treating fish disease. *Journal of Veterinary Pharmacology and Therapeutics, 38*, 417–428. https://doi.org/10.1111/jvp.12202

Velasco, S., Alzueta, M. C., Rodríguez, M. L., Rebolé, A., & Ortiz, L. T (2010). Los prebióticos tipo inulina en alimentación aviar. I: Características y efectos a nivel intestinal. *Revista Complutense de Ciencias Veterinarias, 4*, 87–104.

Verastegui, A., & Fukushima, M. (2009). Observaciones preliminares del efecto de la canela (*Cinnamomum zeylanicum*) y clavo de olor (*Eugenia caryophyllata*) en el crecimiento de juveniles de tilapia roja (*Oreochromis spp*.), bajo condiciones de laboratorio. *Anales Científicos, 70*, 73. https://doi.org/10.21704/ac.v70i4.542

Zeng, Z., Zhang, S., Wang, H., & Piao, X. (2015). Essential oil and aromatic plants as feed additives in non-ruminant nutrition: A review. *Journal of Animal Science and Biotechnology, 6*, 1–10. https://doi.org/10.1186/s40104-015-0004-5

Zeppenfeld, C. C., Hernández, D. R., Santinón, J. J., Heinzmann, B. M., Da Cunha, M. A., Schmidt, D., & Baldisserotto, B. (2016). Essential oil of *Aloysia triphylla* as feed additive promotes growth of silver catfish (*Rhamdia quelen*). *Aquaculture Nutrition, 22*, 933–940. https://doi.org/10.1111/anu.12311