

Short Communication

Effect of mint powder, *Mentha longifolia*, and vitamin D administration on growth indices of juvenile rainbow trout, *Oncorhynchus mykiss*

Albab Fawwaz Alfarras^{1*}, Noora M. Hameed², Huda Sabah Jabr³, Salam Ahjel⁴, Mohamed Kazem Alaraji⁵, Ahmed S. Abed⁶

¹Medical Lab. Techniques Department/ College of Medical Technology, Al-Farahidi University, Iraq.

²Anesthesia techniques, Al-Nisour University College, Iraq.

³Department of Anesthesia Techniques, Al-Mustaqbal University College, Babylon.

⁴Department of Pharmacy, Al-Zahrawi University College, Karbala, Iraq.

⁵Department of Pharmacy, Ashur University College/Baghdad, Iraq.

⁶Department of Prosthetic Dental Technology, Hilla University college, Babylon, Iraq.

Abstract: Numerous studies have shown that herbs can be used as growth stimulants in aquaculture. This study aimed to investigate the effect of combined consumption of mint powder and vitamin D on the growth and health of juvenile rainbow trout, *Oncorhynchus mykiss*. For this purpose, a total of 200 juvenile rainbow trout were divided into four groups and treated for 60 days as follows: The 1st group (control) used a standard diet; the 2nd group had 1.5% mint powder in their diet; The 3rd group having an additional amount of 120 mg/kg of vitamin D in their diet; The 4th group fed a diet having mint and vitamin D supplements as 2^{ed} and 3rd groups. All groups' growth and health indices viz. condition factor, hepatic steatosis index, visceral sensitivity index, spleen somatic index, weight gain percentage, specific growth rate, feed conversion ratio, satiety index, food conversion efficiency, and protein efficiency ratio were measured and compared on days 20, 40, and 60. The results revealed that the use of mint powder in the juvenile rainbow trout diet not only had no remarkable effect on the growth and health of fish but also improved their growth. In addition, all the analyzed indications of the mint powder group outperformed than the vitamin D group.

Article history:

Received 17 March 2022

Accepted 20 June 2022

Available online 25 August 2022

Keywords:

Trout

Mint

Vitamin D

Fish Diet

Antioxidants

Introduction

The application of the plant essential oils and herbs in aquatic and livestock diets has attracted much attention in recent years (Soñta et al., 2019; Pouladi et al., 2020; Molajou et al., 2021; Paray et al., 2021; Mucha and Witkowska, 2021; Nehme et al., 2021; Angane et al., 2022). Antioxidant properties are found in almost all essential oils and herbs, helping prevent microbial growth and toxin production in feed (Ceylan et al., 2019; Dorra et al., 2019). Plant compounds also improve the performance of livestock and aquatic products, strengthen their immune system, and have antimicrobial properties (Ahmadifar et al., 2021). By effectively releasing digestive enzymes, they increase nutrient digestion and absorption, and by enhancing nitrogen uptake, they help protein absorption into the cell (Wainstein et al., 2012).

Several studies have shown adding herbs to food improves aquatic growth performance (Manhas and Gill, 2010; Promya and Chitmanat, 2011; Hai, 2015; Prabu et al., 2017). Medicinal plants have emerged as an alternative and promising agent for controlling disease in fish (Zam et al., 2019). Medicinal herbs are used in aquaculture not only as chemotherapeutics but also as feed supplements. This is because they include a diverse range of nutrients in addition to chemical compounds (Kalita and Borthakur, 2010; Dutta et al., 2020). Therefore, they have been shown to promote growth, hunger stimulation, immunological stimulation, antibacterial activity, and anti-stress activity in aquaculture (Shakya, 2017; Caipang, 2020). The ease of access to many plants and their low cost encourages their application in aquaculture on a large scale (Khosravi et

*Correspondence: Albab Fawwaz Alfarras
E-mail: a.ibrahim@uofarahidi.edu.iq

Table 1. Contents of the fish feed ingredients.

Content	Amount (%)
Protein	39
Fiber	3.7
Raw fat	12
Ash	11
Moisture	10
Phosphorus	1.3

al., 2018; Bharathi et al., 2019; F Ayoub et al., 2019).

Spices and condiments such as mint are used in foods and are natural sources of antioxidants and antibacterial chemicals in aquaculture (Azizkhani and Tooryan, 2015; Abdul Qadir et al., 2017; Khan et al., 2018). *Mentha longifolia* (mint), a member of the Lamiaceae family, is found in the Mediterranean region and North Africa, Australia, and Europe (Mahmoud et al., 2022). It has wide application in the food processing and pharmaceutical industries. Mint's seeds, bark, flowers, stems, and leaves have all been used in traditional folk medicine as carminatives, stimulants, antispasmodics, antimicrobials, and antioxidants, and for the treatment of various ailments like digestive problems and headaches (Asghari et al., 2018). The addition of mint in the diet of poultry improves growth performance and physiological conditions and raises antioxidant activity and feed conversion ratio (Kumar and Patra, 2017; Vargas-Sánchez et al., 2019). Based on the above-mentioned background, this study aimed to investigate the effects of mint powder and dietary vitamin D on rainbow trout's (*Oncorhynchus mykiss*) health and growth performance.

Methods and Material

In March 2021, 200 rainbow trout juveniles with an average weight of 36 ± 2 g and a total length of 15.2 ± 0.7 cm were purchased and transferred to a wet Lab, at Al-Farahidi University. The fish were maintained in a 1500-liter tank for two weeks to acclimatization. During the adaption period, they were fed a conventional diet of trout twice a day at a rate of 3% body weight, and 20% of the tank water was replaced daily. The dietary composition of the ratio used is presented in Table 1.

The fish were divided into four groups each with three replications and introduced randomly into twelve 300L tanks, each with a density of twelve fish. The 1st group (control) was fed a standard diet, the 2nd group fed 1.5% mint powder in their diet, the 3rd group fed a diet having 120 mg/kg of vitamin D and the 4th group fed a combination of mint and vitamin D supplements as

mentioned in 2^{ed} and 3rd treatments. The desired amount of mint powder (1.5%) and vitamin D (120 mg/kg dry weight) were mixed and converted as pellets with a diameter of approximately 3.5 mm by a meat grinder. The pellets were frozen at -18°C after being dry at 45°C for 30 hours (with 10% humidity). On days 20, 40, and 60, two fish from each tank (6 fish per treatment) were randomly sampled.

After anesthesia using MS222, the fish were killed and following growth parameters viz. Condition Factor (CF) (Osho and Usman, 2019), the Hepatic Steatosis Index (HSI) (Ribeiro et al., 2013), Visceral Sensitivity Index (VSI) (Torstensen et al., 2011), Spleen Somatic Index (SSI) (Abdel-Tawwab et al., 2021), Weight Gain Percentage (WG%) (Besson et al., 2016), Specific Growth Rate (SGR) (Korzen et al., 2016), Feed Conversion Ratio (FCR), Satiety Index, Food Conversion Efficiency (FCE) (Zhang et al., 2010), and Protein Efficiency Ratio (PER) (Moogouei, 2014; Tae et al., 2017; Adeshina et al., 2018) were measured.

After verifying the data for normality with the Kolmogorov-Smirnov test, a one-way analysis of variance (ANOVA) was used to analyze the data. Using Duncan's multiple range test (MRT), mean differences between the groups were obtained. Data analysis was performed using SPSS software version 25. Data analysis was performed using SPSS software version 25.

Results and Discussion

No signs of disease or mortality were observed in the treatments during the experimental period. The findings revealed no differences between the experimental groups except for visceral and satiety indices on the 20th day. On this day, when comparing vitamin D treatment to others, VSI and satiety had increased significantly ($P < 0.05$) (Fig. 1). VSI shows gastrointestinal-specific anxiety, affective, and cognitive response to the fear of gastrointestinal symptoms, sensations, and the circumstances in which these visceral symptoms and sensations manifest themselves (Torstensen et al., 2011). In addition, the satiety Index describes the sensation of being full and losing one's appetite after eating (Zhang et al., 2010)

Significant increases in the VSI, satiety coefficient, WG%, PER, SGR, and FCE were observed in the treatment of mint powder on the 40th day. On this day, other treatments revealed no significant differences from the control one ($P < 0.05$) (Fig. 2). There are reports on the positive growth effects of mint on the broiler and aquatic

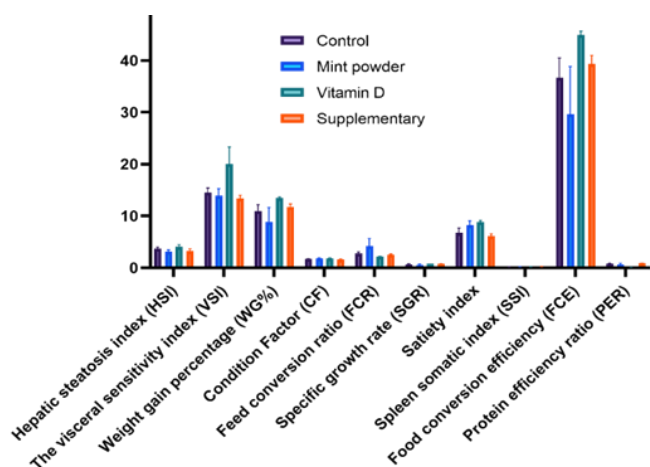


Figure 1. A comparison of growth indicators in juvenile trout fed with a variety of diets on day 20 of the experiment.

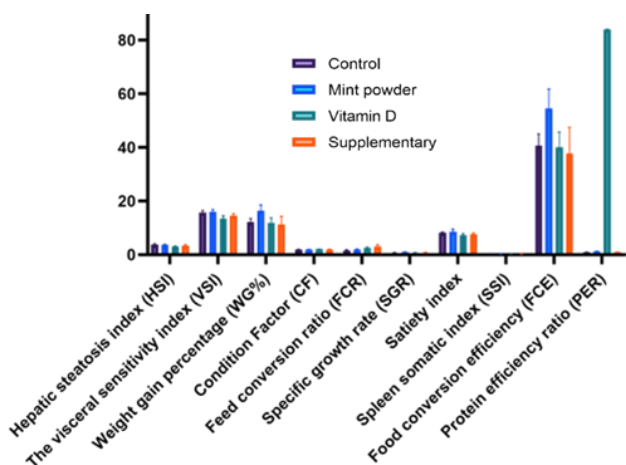


Figure 2. A comparison of growth indicators in juvenile trout fed with a variety of diets on day 40 of the experiment.

animals (Hong et al., 2012). In line with our findings, Emami et al. (2012) reported similar results. Also, SSI in mint and vitamin D supplemented treatments was significantly lower ($P < 0.05$). Spleen Somatic Index is a critical metric for determining a fish's relative vulnerability to various stressors (Serrat et al., 2019; Abdel-Tawwab et al., 2022).

The VSI increased in the mint powder supplemented treatments on day 60 ($P < 0.05$) (Fig. 3). The satiety index for mint powder treatment was higher than others. Compared to the control group, mint powder treatments significantly improved SGR, PER, WG%, and FCE ($P < 0.05$). In these indices, there was no difference between the vitamin D group and the control one. Regarding HSI and VSI, the supplement of the mint powder showed a nearly uniform trend over time. While little changes were found in the control and vitamin D

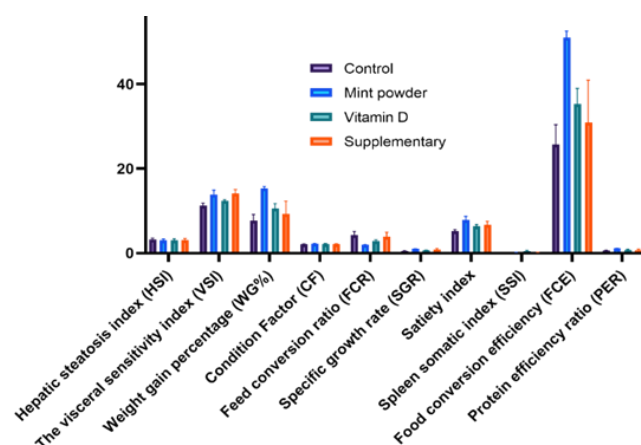


Figure 3. A comparison of growth indicators in juvenile trout fed with a variety of diets on day 60 of the experiment.

groups regarding these indices, i.e. a significant decline was found in HSI and VSI in the vitamin D group on days 40 and 60. HSI is diagnosed when the amount of fat within the liver is greater than 5% of the total weight (Eguchi et al., 2012; Machado and Cortez-Pinto, 2013; Abd El-Kader and El-Den Ashmawy, 2015; Liu et al., 2017), on day 60, VSI in the control group decreased significantly. On this day, there was a substantial decline in the satiety index in the control and vitamin D groups. In previous works, vitamin D has caused an increase in the immune system and other hematological factors (Dehghanizadeh et al., 2014), despite a decrease in the studied growth parameters.

No differences were found in the supplemented mint powder groups. Except in the control group on day 40, no other changes in the SSI were seen. Although there is no remarkable change in the weight gained in all study groups, the trajectory of change in this index in the mint powder group had a distinct tendency in the 40th and 60th days compared to the 20th day. Until the end of the experiment, the condition factor increased significantly in all of the groups, including the control group. The condition factor of a fish reflects the fish's biological and physical circumstances, as well as oscillations in those circumstances caused by the interaction between feeding environments, parasite diseases, and physiological factors (Osho and Usman, 2019; Jafari-Patcan et al., 2018; Mouludi-Saleh and Eagderi 2019; Eagderi et al., 2020). Although there were no significant differences in any of the studied groups regarding the feed conversion ratio, the reduction in this index was noticeable on days 40 and 60. But in the mint treated groups, the indices of specific growth rate, feed conversion efficiency, and protein

efficiency ratio exhibit showed increasing trends, with a significant difference on the 40th day compared to the 20th day.

Conclusion

According to the findings of this study, mint powder-receiving juveniles exhibit a considerable improvement in growth and health indices at the end of the trial period, i.e., day 60, compared to other groups. Furthermore, the trend of changes in the group receiving mint powder during the experiment revealed that mint powder has a proper effect on the growth of juvenile rainbow trout.

References

- Abd El-Kader S.M., El-Den Ashmawy E.M.S. (2015). Non-alcoholic fatty liver disease: The diagnosis and management. *World Journal of Hepatology*, 7(6): 846.
- Abdel-Tawwab M., Abdulrahman N.M., Ahmad V.M., Ramzi D.O., Hassan B.R. (2021). Effects of dietary oak (*Quercus aegilops* L.) acorn on growth performance, somatic indices, and hemato-biochemical responses of common carp, *Cyprinus carpio* L., at different stocking densities. *Journal of Applied Aquaculture*, 1-17.
- Abdel-Tawwab M., Abdulrahman N.M., Baiz A.I., Nader P.J., Al-Refaiiee I.H. (2022). The using of *Chlorella pyrenoidosa* and *Daphnia magna* as feed supplements for common carp, *Cyprinus carpio*: growth performance, somatic indices, and hemato-biochemical biomarkers. *Journal of Applied Aquaculture*, 34(1): 64-78.
- Abdul Qadir M., Shahzadi S.K., Bashir A., Munir A., Shahzad S. (2017). Evaluation of phenolic compounds and antioxidant and antimicrobial activities of some common herbs. *International Journal of Analytical Chemistry*, 2017(2): 28316626.
- Adeshina I., Sani R.A., Adewale Y.A., Tihamiyu L.O., Umma S.B. (2018). Effects of dietary *Moringa oleifera* leaf meal as a replacement for soybean meal on growth, body composition and health status in *Cyprinus carpio* juveniles. *Croatian Journal of Fisheries*, 76(4): 174-182.
- Ahmadifar E., Yousefi M., Karimi M., Fadaei Raieni R., Dadar M., Yilmaz S., Dawood M.A., Abdel-Latif H.M. (2021). Benefits of dietary polyphenols and polyphenol-rich additives to aquatic animal health: an overview. *Reviews in Fisheries Science and Aquaculture*, 29(4): 478-511.
- Angane M., Swift S., Huang K., Butts C.A., Quek S.Y. (2022). Essential oils and their major components: An updated review on antimicrobial activities, mechanism of action and their potential application in the food industry. *Foods*, 11(3): 464.
- Asghari B., Zengin G., Bahadori M.B., Abbas-Mohammadi M., Dinparast L. (2018). Amylase, glucosidase, tyrosinase, and cholinesterases inhibitory, antioxidant effects, and GC-MS analysis of wild mint (*Mentha longifolia* var. *calliantha*) essential oil: A natural remedy. *European Journal of Integrative Medicine*, 22: 44-49.
- Ayoub H., Abdelghany M., El-Sayed A.E.-K. (2019). Effects of Diatoms *Amphora coffeaeformis* on growth parameters, non specific immunity and protection of the Nile tilapia (*Oreochromis niloticus*) to *Aeromonas hydrophila* infection. *Egyptian Journal of Aquatic Biology and Fisheries*, 23(1): 413-426.
- Azizkhani M., Tooryan F. (2015). Antioxidant and antimicrobial activities of rosemary extract, mint extract and a mixture of tocopherols in beef sausage during storage at 4°C. *Journal of Food Safety*, 35(1): 128-136.
- Besson M., Aubin J., Komen H., Poelman M., Quillet E., Vandeputte M., Van Arendonk J.A.M., De Boer I.J.M. (2016). Environmental impacts of genetic improvement of growth rate and feed conversion ratio in fish farming under rearing density and nitrogen output limitations. *Journal of Cleaner Production*, 116: 100-109.
- Bharathi S., Antony C., Cbt R., Arumugam U., Ahilan B., Aanand S. (2019). Functional feed additives used in fish feeds. *International Journal of Fisheries and Aquatic Studies*, 7(3): 44-52.
- Caipang C.M.A. (2020). Phyto-genics in aquaculture: a short review of their effects on gut health and microflora in fish. *The Philippine Journal of Fisheries*, 27(2): 11-22.
- Ceylan S., Cetin S., Camadan Y., Saral O., Ozsen O., Tutus A. (2019). Antibacterial and antioxidant activities of traditional medicinal plants from the Erzurum region of Turkey. *Irish Journal of Medical Science*, 188(4): 1303-1309.
- Dehghanizadeh S., Zamini A.A., Khara H. (2014). Effects of vitamin D diets in the fingerling rainbow trout on blood parameters and immune system. *Journal of Aquatic Animals and Fisheries*, 4(16): 33-44.

- Dorra N., El-Berrawy M., Sallam S., Mahmoud R. (2019). Evaluation of antiviral and antioxidant activity of selected herbal extracts. *Journal of High Institute of Public Health*, 49(1): 36-40.
- Dutta M.P., Singh M.K., Borah D. (2020). Piscicidal plants of northeast India and its future prospect in aquaculture - a comprehensive review. *Indian Journal of Natural Products and Resources (IJNPR) [Formerly Natural Product Radiance (NPR)]*, 10(3): 165-174.
- Eagderi S., Mouludi-Saleh A., Cicek E. (2020). Length-weight relationship of ten species of Leuciscinae sub-family (Cyprinidae) from Iranian inland waters. *International Aquatic Research*, 12(2): 133-136.
- Eguchi Y., Hyogo H., Ono M., Mizuta T., Ono N., Fujimoto K., Chayama K., Saibara T. (2012). Prevalence and associated metabolic factors of nonalcoholic fatty liver disease in the general population from 2009 to 2010 in Japan: a multicenter large retrospective study. *Journal of Gastroenterology*, 47(5): 586-595.
- Emami N.K., Samie A., Rahmani H.R., Ruiz-Feria C.A. (2012). The effect of peppermint essential oil and fructooligosaccharides, as alternatives to virginiamycin, on growth performance, digestibility, gut morphology and immune response of male broilers. *Animal Feed Science and Technology*, 175(1-2): 57-64.
- Hai N.V. (2015). The use of probiotics in aquaculture. *Journal of Applied Microbiology*, 119(4): 917-935.
- Hong J., Steiner T., Aufy A., Lien T. (2012). Effects of supplemental essential oil on growth performance, lipid metabolites and immunity, intestinal characteristics, microbiota and carcass traits in broilers. *Livestock Science*, 144(3): 253-262.
- Jafari-Patcan A., Eagderi S., Mouludi-Saleh A. (2018). Length-weight relationship for four fish species from the Oman Sea, Iran. *International Journal of Aquatic Biology*, 6(5): 294-295.
- Kalita P.C., Borthakur S.K. (2010). Studies on some highly utilized important medicinal plants among the rural people of Madankamdev hill region, Assam. *Journal of Economic and Taxonomic Botany*, 34(2): 257-261.
- Khan A., Jan G., Khan A., Jan F.G., Danish M. (2018). Evaluation of antioxidant and antimicrobial activities of *Bergenia ciliata Sternb* (Rhizome) crude extract and fractions. *Pakistan Journal of Pharmaceutical Sciences*, 31(1): 31-36.
- Khosravi S., Bui H.T.D., Herault M., Fournier V., Kim K.-D., Lee B.-J., Kim K.-W., Lee K.-J. (2018). Supplementation of protein hydrolysates to a low-fishmeal diet improves growth and health status of juvenile olive flounder, *Paralichthys olivaceus*. *Journal of the World Aquaculture Society*, 49(5): 897-911.
- Korzen L., Abelson A., Israel A. (2016). Growth, protein and carbohydrate contents in *Ulva rigida* and *Gracilaria bursa-pastoris* integrated with an offshore fish farm. *Journal of Applied Phycology*, 28(3): 1835-1845.
- Kumar P., Patra A.K. (2017). Beneficial uses of black cumin (*Nigella sativa* L.) seeds as a feed additive in poultry nutrition. *World's Poultry Science Journal*, 73(4): 872-885.
- Liu J., Ghaziani T.T., Wolf J.L. (2017). Acute fatty liver disease of pregnancy: updates in pathogenesis, diagnosis, and management. *Official Journal of the American College of Gastroenterology| ACG*, 112(6): 838-846.
- Machado M.V., Cortez-Pinto H. (2013). Non-invasive diagnosis of non-alcoholic fatty liver disease. A critical appraisal. *Journal of Hepatology*, 58(5): 1007-1019.
- Mahmoud E.A.-M., Al-Askalany S.A., Hanafy E.A. (2022). Antioxidant, antibacterial and cytotoxic effect of *Cymbopogon citratus*, *Mentha longifolia*, and *Artemisia absinthium* essential oils. *Egyptian Journal of Chemistry*, 65(2): 287-296.
- Manhas S.S., Gill B.S. (2010). Effect of planting materials, mulch levels and farmyard manure on growth, yield and quality of turmeric (*Curcuma longa*). *Indian Journal of Agricultural Sciences*, 80(6): 501-506.
- Molajou A., Afshar A., Khosravi M., Soleimani E., Vahabzadeh M., Variani H.A. (2021). A new paradigm of water, food, and energy nexus. *Environmental Science and Pollution Research*. pp: 1-11.
- Moogouei R. (2014). A SWOT analysis of aquaculture development in rural areas of Iran, an application to Rainbow trout (*Oncorhynchus mykiss*). *International Journal of Aquatic Biology*, 2(1): 36-42.
- Mouludi-Saleh A., Eagderi S. (2019). Length-weight relationship and condition factor of ten fish species (Cyprinidae, Sisoridae, Mugilidae, Cichlidae, Gobiidae and Channidae) from Iranian inland waters.

- Journal of Wildlife and Biodiversity, 3(4): 12-15.
- Mucha W., Witkowska D. (2021). The applicability of essential oils in different stages of production of animal-based foods. *Molecules*, 26(13): 3798.
- Nehme R., Andrés S., Pereira R.B., Ben Jemaa M., Bouhallab S., Cecilian F., López S., Rahali F.Z., Ksouri R., Pereira D.M. (2021). Essential oils in livestock: From health to food quality. *Antioxidants*, 10(2): 330.
- Osho F.E., Usman R.A. (2019). Length-weight relationship, condition factor and fecundity of African snakehead *Parachanna obscura* from the Anambra River, South East Nigeria. *Croatian Journal of Fisheries*, 77(2): 99-105.
- Paray B.A., El-Basuini M.F., Alagawany M., Albeshr M.F., Farah M.A., Dawood M.A. (2021). *Yucca schidigera* usage for healthy aquatic animals: Potential roles for sustainability. *Animals*, 11(1): 93.
- Pouladi P., Afshar A., Molajou A., Afshar M.H. (2020). Socio-hydrological framework for investigating farmers' activities affecting the shrinkage of Urmia Lake; hybrid data mining and agent-based modelling. *Hydrological Sciences Journal*, 65(8): 1249-1261.
- Prabu E., Felix S., Felix N., Ahilan B., Ruby P. (2017). An overview on significance of fish nutrition in aquaculture industry. *International Journal of Fisheries and Aquatic Studies*, 5(6): 349-355.
- Promya J., Chitmanat C. (2011). The effects of *Spirulina platensis* and *Cladophora* algae on the growth performance, meat quality and immunity stimulating capacity of the African Sharptooth Catfish (*Clarias gariepinus*). *International Journal of Agriculture and Biology*, 13(1): 77-82.
- Ribeiro C.A. de O., Katsumiti A., França P., Maschio J., Zandoná E., Cestari M.M., Vicari T., Roche H., Assis H.C.S. de, Filipak Neto F. (2013). Biomarkers responses in fish (*Atherinella brasiliensis*) of paranaguá bay, southern Brazil, for assessment of pollutant effects. *Brazilian Journal of Oceanography*, 61: 1-11.
- Serrat A., Lloret J., Frigola-Tepe X., Muñoz M. (2019). Trade-offs between life-history traits in a coldwater fish in the Mediterranean Sea: the case of blue whiting *Micromesistius poutassou*. *Journal of Fish Biology*, 95(2): 428-443.
- Shakya S.R. (2017). Effect of herbs and herbal products feed supplements on growth in fishes: A review. *Nepal Journal of Biotechnology*, 5(1): 58-63.
- Soñta M., Rekiel A., Batorska M. (2019). Use of duckweed (*Lemna* L.) in sustainable livestock production and aquaculture—a review. *Annals of Animal Science*, 19(2): 257-271.
- Tae H.M., Hajimoradloo A., Hoseinifar S.H., Ahmadvand H. (2017). The effects of dietary Myrtle (*Myrtus communis* L.) supplementations on growth performance and some innate immune responses in rainbow trout (*Oncorhynchus mykiss*). *International Journal of Aquatic Biology*, 5(4): 252-259.
- Torstensen B.E., Espe M., Stubhaug I., Lie Ø. (2011). Dietary plant proteins and vegetable oil blends increase adiposity and plasma lipids in Atlantic salmon (*Salmo salar* L.). *British Journal of Nutrition*, 106(5): 633-647.
- Vargas-Sánchez R.D., Ibarra-Arias F.J., del Mar Torres-Martínez B., Sánchez-Escalante A., Torrescano-Urrutia G.R. (2019). Use of natural ingredients in Japanese quail diet and their effect on carcass and meat quality-A review. *Asian-Australasian Journal of Animal Sciences*, 32(11): 1641.
- Wainstein J., Ganz T., Boaz M., Bar Dayan Y., Dolev E., Kerem Z., Madar Z. (2012). Olive leaf extract as a hypoglycemic agent in both human diabetic subjects and in rats. *Journal of Medicinal Food*, 15(7): 605-610.
- Zam S.I., Agustien A., Djamaan A., Mustafa I. (2019). The diversity of endophytic bacteria from the traditional medicinal plants leaves that have anti-phytopathogens activity. *Journal of Tropical Life Science*, 9(1): 53-63.
- Zhang L., Zhang X., Cao D., Yang J., Chu Z., Sun X. (2010). QTL analysis related to feed conversion efficiency in common carp (*Cyprinus carpio*) using SSR and EST markers. *Journal of Agricultural Biotechnology*, 18(5): 963-967.