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EFFECTS OF GINGER (*Zingiber officinale*) AND GARLIC (*Allium sativum*) SUPPLEMENTED DIETS ON GROWTH AND FEED UTILIZATION OF RAINBOW TROUT (*Oncorhynchus mykiss*) JUVENILE

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ABSTRACT
Incorporation of herbal additives into fish diets has emerged as a promising strategy to boost growth and bolster immunity in aquaculture. This study aimed to evaluate the impact of two such additives, ginger (*Zingiber officinale*) and garlic (*Allium sativum*), on the growth and feed efficiency of rainbow trout (*Oncorhynchus mykiss*). For this, 3600 trout averaging 3.52 ± 0.15 g, allocated to 15 raceway tanks, housing 240 fish per tank were fed diets containing varying levels of ginger (1 and 2%) and garlic (1 and 2%), alongside a control group with no additives over a 60-days period at 5% of their body weight. Results revealed that the final weight (22.0±0.8g), weight gain (18.3±0.8g), specific growth rate (SGR) (2.97±0.04%/day) and condition factor (2.39±0.36) were significantly higher ($p< 0.05$) in 1% ginger-supplemented groups compared to the control and garlic-supplemented groups. Similarly, fish fed with 2% ginger exhibited significantly higher SGR than control and 1% garlic fed groups. However, there was no significant difference between 2% ginger, 1 and 2% garlic, and control group in terms of weight gain. The survival rates remained consistent across the treatments. Consequently, this study advocates for the inclusion of 1% ginger in rainbow trout diets as a viable, cost-efficient, and eco-friendly means of enhancing growth.

Keywords: Herbal additives, medicinal plants, immunostimulant

INTRODUCTION
Rainbow trout (*Oncorhynchus mykiss*) is a popular species to grow in hilly and mountainous regions of Nepal due to the availability of cold-water resources, which is considered as one of the most important aspects for the successful cultivation of this species (Nepal et al. 2021). At higher altitudes, warm water species have not yet flourished as biologically and economically sustainable ventures in Nepal (Gurung et al. 2017). After comprehensive research and development of rainbow trout farming technological packages suitable for mountainous region, rainbow trout farming offers substantial opportunities for commercial culture in hilly areas of Nepal. In addition, several job and income opportunities have been generated by the promotion of trout farming in the highlands of Nepal. It has been made possible because farmers can now prepare cost-effective feed using locally available ingredients (Voorhees 2011; Mahato et al. 2023). With the advancement in the technology of breeding and fry production coupled with the popularity of rainbow trout among consumers and motivated farmers, rainbow trout farming in the private sector is increasing trend. Moreover, interest in growing rainbow trout is increasing in the hilly parts of the country resulting in a total of 120 farms throughout the country, producing more than 550 metric tons of rainbow trout (NFRC 2021).

The success of rainbow trout farming is related to the high-quality protein feed required by carnivorous fish while lowering the FCR (Barimani et al. 2013) and promoting the immunity of the fish, which otherwise can be severely suppressed at higher stocking density in raceway tanks, a commonly practiced production method for rainbow trout (Nepal et al. 2021). In this context, especially when public perception is changing and attention has been increased towards the farming of the trout, the welfare of the farmed trout is a critical aspect to be considered. A study has shown that fish can have impaired immunity, making them susceptible to bacterial infections even at lower stocking densities when they are fed inadequately (Winfree et al. 1998). Therefore, researchers have constantly focused on the development of a feed strategy that can promote the immune response and growth of rainbow trout, regardless of the stocking density. At higher stocking densities, bacterial infection causing tail and fin rot is one of the major causes of production halts, making this condition an operational and welfare indicator for trout farms (Ellis et al. 2009). Due to the intensive farming of rainbow trout and the common occurrence of bacterial infections in such farms, farmers often resort to indiscriminate antibiotic use without fully understanding the potential consequences such as antibiotic resistance in bacteria, as highlighted by Timalsina et al. (2022). Therefore, to address this issue, the development of functional feed containing plant-based ingredients that serve as growth promoters, immunostimulants, antioxidants, and anti-pathogenic agents should be explored to support the intensive culture of rainbow trout while minimizing antibiotic usage. Plant extract-based functional feed has ameliorated the immune response in Atlantic salmon (*Salmo salar*), thereby improving the health status of the fish (Reyes-Cerpa et al. 2018). Moreover, studies have proved that natural plant-derived products can be used in aquaculture as substitutes for vaccines and synthetic chemicals in order to prevent disease and enhance growth and immune responses due to their richness in bioactive compounds (Citarasu 2010; Reverter et al. 2017). Garlic (*Allium sativum*) and ginger (*Zingiber officinale*) are the most commonly used spices and additives in aquaculture for their nutritional, physiological, and pharmacological properties. Hence, the use of these compounds as growth promoters and immunostimulants in the feed for rainbow trout might be a promising way to improve the performance of rainbow trout.

Garlic (*Allium satium*), a plant that grows as a bulb, is a popular member of Liliace family and has been used for decades as a flavoring agent and traditional medicine to improve physical and mental being of humans (Paulin et al. 2021). Earlier studies have shown that garlic, as a functional feed additive in fish feed, promoted growth and improved antioxidant, immunological, and hematological parameters (Diab et al. 2008; Yılmaz and Ergün 2012). The garlic-supplemented feed, either in aqueous or dried powder form contains several organic sulfur compounds like ajoene, allicin, diallyl disulfide, and S-allylcysteine (Chi et al. 1982). It also contains minerals like calcium and phosphorous, vitamin A, C, and B complexes, linolenic acid, and other important compounds like iodine and silicate, which makes it a suitable candidate for incorporating as a functional additive in fish feed (Chi et al. 1982; Corzomartinez et al. 2007). Similarly, ginger (*Zingiber officinale*), a plant that grows as an underground stem or rhizome, is a widely used food as a spice around the world. It is one of the most studied and common members of the Zingiberaceae family. The biologically active compounds like alkaloids, polyphenols, flavonoids, saponin, carotenoids, and nutrients such as carbohydrates, vitamins, and minerals, along with the potent antioxidant component Zingerone, are highly abundant in this plant (Paulin et al. 2021). Therefore, several earlier studies have successfully incorporated ginger in the diet of rearing fish to promote growth, immune response, digestion, metabolism, and antimicrobial and antiparasitic properties (Mohammadi et al. 2020; Shakya 2015). Although these studies have shown that garlic and ginger can be a potential functional additive for promoting fish growth and overall health, such information on the effect of ginger and garlic in the context of rainbow trout farming in flow-through systems in Nepal is limited. Hence, the aim of the present study was to evaluate the effect of garlic and ginger powders as feed additive on growth performance and feed efficiency of juvenile rainbow trout. Ultimately, the findings of this study can be applicable in intensive rainbow trout culture to ensure food and nutritional security while enhancing the livelihood of the farmers.

MATERIALS AND METHODS

Study location and experimental conditions

The study was conducted at the Fishery Research Station (FRS), Trishuli from August to October 2022. A total of 4000 healthy rainbow trout juveniles were purchased from the private hatchery and were acclimatized in two raceway tanks at FRS for two weeks. Fish were fed a farm-made basal diet with 34.9% crude protein (CP) at a rate of 5% of body weight during acclimation. After the acclimation, 3600 rainbow trout juveniles (3.52±0.15 g) were randomly distributed into 15 nursery raceway tanks (3m × 0.5m × 0.2m) at a stocking density of 240 fish per tank. Fish were randomly divided into 5 experimental groups as follows: fish fed with basal diet without ginger and garlic (control) (T1), fish fed with 1% ginger in the basal diet (T2), fish fed with 1% garlic in the basal diet (T3), fish fed with 2% ginger in the basal diet (T4), and fish fed with 2% garlic in the basal diet (T5). The experiment was performed in triplicate. Fish were fed at 5% body weight twice a day for 60 days. The water source was the Trishuli River, from which the water was first collected in a siltation tank then drawn to the experimental tanks. The water quality parameters, including temperature, pH, and dissolved oxygen, were monitored daily using multimeter probes.

Acquisition of feed ingredients and preparation of experimental diets

All feed ingredients used to prepare the experimental diets were obtained from the local market, including raw ginger and garlic. Raw ginger and garlic were first dried and crushed into powdered form mechanically, followed by sieving with fine mesh to obtain powdered ingredients, which were then stored in an airtight container until the formulation and preparation of the experimental diets. Five isonitrogenous (45% CP) diets were formulated using the Pearson's square method. Feed ingredients used for the preparation of experimental diets were ginger powder, garlic powder, soya full, small shrimp meal (jwala), wheat flour, rice bran, vitamin and mineral premix, and additional materials. The formulation was based on the percentage composition of the ingredients, as shown in Table 1. To prepare the formulated experimental diets, all the dry ingredients in their powder form were weighed and mixed thoroughly, put into the electric feed pelleting machine to make pellets. The pellets were dried for 24 hours and stored in an airtight container at room temperature until used. The proximate composition of experimental diets was determined following the standard method of AOAC (1990). Dry matter was analyzed by drying the sample feed in a hot-air oven at 105 °C overnight. Crude protein was analyzed by following the Kjeldahl method after acid digestion (% crude protein = % nitrogen × 6.25). Crude lipid was determined by the Soxhlet extraction method using petroleum ether. The total ash content in the experimental diet was determined by the combustion of samples in a muffle furnace at 600 °C for 6 hours (Table 1).

Table 1: Formulation and proximate composition (%) of experimental diets.

Ingredient (%)	T1 (Control)	T2 (1% Ginger)	T3 (1% Garlic)	T4 (2% Ginger)	T5 (2% Garlic)
Soya full	30	30	30	30	30
Jwala (small shrimp)	44	44	44	44	44
Wheat flour	10	9	9	9	9
Rice bran	7	7	7	6	6
Vitamin premix[a]	1	1	1	1	1
Mineral mix[b]	1.45	1.45	1.45	1.45	1.45
Yeast	3	3	3	3	3
Additional					
Liver tonic	2.5	2.5	2.5	2.5	2.5

Phytase	0.03	0.03	0.03	0.03	0.03
Butylated hydroxyl toluene	0.02	0.02	0.02	0.02	0.02
Salt	1	1	1	1	1
L-Lysine	0.2	0.2	0.2	0.2	0.2
Ginger (%)	0	1	0	2	0
Garlic (%)	0	0	1	0	2
Proximate composition (%)					
Dry matter	94.69±0.31	96.48±0.01	96.21±0.12	96.27±0.22	95.55±0.33
Crude protein	45.8±0.34	45.07±0.01	45.38±0.14	45.75±0.52	45.19±0.31
Crude lipid	6.67±0.4	6.31±0.11	6.45±0.21	6.45±0.41	6.66±0.29
Total Ash	10.6±0.28	10.8±0.04	10.05±0.11	10.46±0.17	10.19±0.04
Energy (MJ/Kg)	19.88±0.13	19.71±0.21	19.89±0.01	19.85±0.18	19.90±0.64

[a] Vitamin mixture/kg premix containing the following: 33000IU vitamin A, 3300IU, vitamin D3, 410IU vitamin E, 2660mg Vitamin BI, 133mg vitamin B2, 580mg vitamin B6, 41mg vitamin B12, 50mg biotin, 9330mg choline chloride, 4000mg vitamin C, 2660mg Inositol, 330mg para-amino benzoic acid, 9330mg niacin, 26.60mg pantothenic acid. [b] Mineral mixture/kg premix containing the following: 325mg Manganese, 200mg Iron, 25mg Copper, 5mg Iodine, 5mg Cobalt.

Growth, survival rate and feed utilization parameters

On every 15-day interval from stocking, fish were starved for 24 hours prior to the sampling. Sampling was done by netting 20% of the fish from each tank to determine their growth and daily ration needs. Fish were anesthetized with clove powder (200 mg/L) before each sampling (Naderi et al. 2017). At the end of the experiment, on the 60th day, all fish were sampled, anesthetized, and measured for their individual weight and length to calculate the weight gain (WG), specific growth rate (SGR), condition factor (K), feed conversion ratio (FCR), and survival rate (SR) according to Aqmasjed et al. (2023):

WG (g) = Final weight (g) - Initial weight (g)
SGR (%/day) = 100 × [ln (final weight) – ln (initial weight)]/days
K = 100 × [final weight/ (final length)^3]
FCR = Dry feed intake (g)/ weight gain (g)
SR (%) = 100 × (final number of fish/initial number of fish)

Statistical analysis

All the results (mean ± SE) were analyzed by using SPSS software (Version 25, IMB, Armonk, NY, USA). Data were checked for normality and homogeneity of variance with the Shapiro-Wilk and Levene tests, respectively before analysis. Data collected throughout the experiment were subjected to one-way analysis of variance (ANOVA), followed by Tukey's multiple comparison test to compare the means among treatments. Means were regarded as significantly different when $p < 0.05$.

RESULTS

Water quality parameters

Throughout the experiment, the natural light-dark cycle was maintained, and daily monitoring of water quality parameters such as temperature, pH, and dissolved oxygen was conducted. These parameters were recorded as 18.2±1.4 °C for temperature, 7.4±0.1 for pH, and 7.7±0.8 mg/L for dissolved oxygen, respectively.

Fish growth, feed utilization and survival

As shown in table 2, inclusion of ginger and garlic induced a significant change in both weight body and length in the juvenile rainbow trout. Final weight was significantly higher in 1% ginger group (T2) than in Control (T1), 1% garlic (T3), and 2% garlic (T5) fed group (p = 0.003). Weight gain was significantly higher in the 1% ginger group (T2) group than control (T1), 1% garlic (T3), and 2% garlic (T5) (p = 0.004) group. Final length was significantly higher in the 2% garlic group (T3) than 1% and 2% ginger (T2 and T4) (p = 0.01) fed group. Specific growth rate (SGR) was significantly higher in 1% ginger group (T2) than in control (T1), 1% garlic (T3), and 2% garlic (T5) (p = 0.001) group. Average daily weight gain (ADWG) was significantly higher in 1% ginger group (T2) than in control (T1), 1% garlic (T3), and 2% garlic (T5) (p =0.004) (Figure 1). The condition factor in 1% ginger group was significantly higher than control (T1), 1% garlic (T3), and 2% garlic (T5) (p = 0.003). Similarly, feed conversion ratio was significantly lower in 1% ginger group than control (T1), 1% garlic (T3) (p=0.002). In this study, the survival rate was not affected by the inclusion of dietary garlic or ginger at any level (p > 0.05).

Table 2: Growth performance, feed utilization, and survival of *O. mykiss* in different treatment at the end of the feeding trial. T1 (Control), T2 (1 % Ginger), T3 (1% Garlic), T4 (2% Ginger), and T5 (2% Garlic)

Treatment	T1	T2	T3	T4	T5	
Parameters	Control	1% Ginger	1% Garlic	2% Ginger	2% Garlic	*p*
Initial weight (g)	3.34±0.11	3.69±0.09	3.65±0.05	3.50±0.14	3.41±0.11	0.14
Final weight (g)	15.13±0.84^{b}	22.00±0.84^{a}	17.17±0.58^{b}	19.80±0.80^{ab}	17.00±0.95^{b}	0.003
Final length (cm)	11.56±0.30^{ab}	9.83±0.56^{b}	12.90±0.58^{a}	10.20±0.71^{b}	10.97±0.04^{ab}	0.01
Weight gain (g)	12.80±1.09^{b}	18.31±0.76^{a}	13.52±0.60^{b}	16.31±0.82^{ab}	13.59±0.87^{b}	0.004
Specific growth rate (%/day)	2.41±0.05^{c}	2.97±0.04^{a}	2.58±0.05^{c}	2.89±0.10^{ab}	2.67±0.07^{bc}	0.001
Condition factor	1.05±0.11^{bc}	2.39±0.36^{a}	0.82±0.12^{c}	1.94±0.31^{ab}	1.29±0.07^{bc}	0.003
Feed conversion ratio	2.04±0.25^{a}	1.07±0.11^{c}	1.67±0.12^{ab}	1.29±0.14^{bc}	1.49±0.16^{bc}	0.002
Survival rate (%)	79.33±5.20	84.33±7.37	79.32±7.94	79.00±4.19	83.83±4.76	0.94

Values in each row with different superscripts letters are significantly different at p < 0.05 (Mean ± SE).

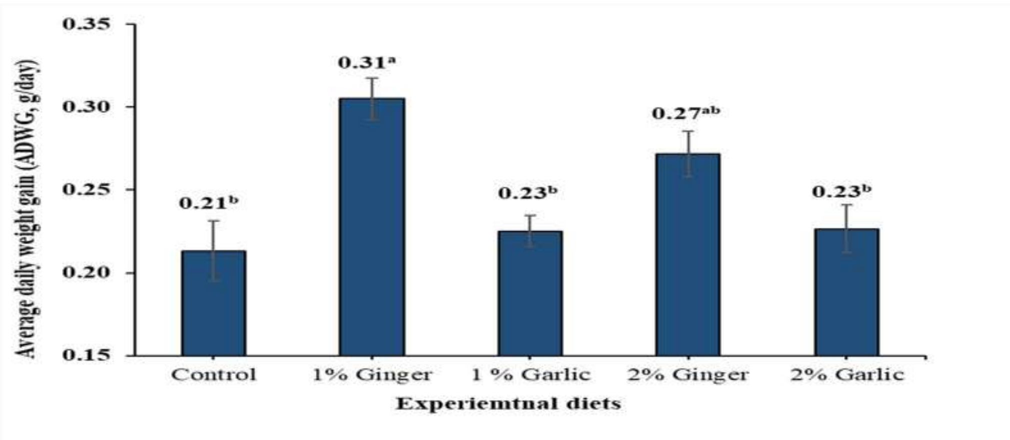

Figure 1: Average daily weight gain (ADWG, g/day) of *O. mykiss* fed diet supplemented with different levels of ginger and garlic. Values with different superscripts letters are significantly different at p < 0.05. Values are mean ± SE of three replications.

DISCUSSION

In the present study, it was found that the growth performance of rainbow trout was significantly higher in 1% ginger-supplemented groups compared to the garlic-supplemented groups and control group. These results were consistent with findings from previous studies conducted in other fish species, which also demonstrated that body weight gain, SGR, and condition factors were significantly increased in the fish fed with 1% ginger supplemented feed compared to the 1% garlic supplemented feed and control groups in juvenile *Huso huso* fish after 60 days of experiment (Gholipour et al. 2014). Sukumaran et al. (2016) also reported the positive effect of 0.6-1% inclusion of ginger on the growth performance of Rohu (*Labeo rohita*) when fed for 60 days. However, the present results were in contradiction with those obtained by Aqmasjed et al. (2023), who observed no improvement in the growth rate, condition factor, or feed utilization in rainbow trout fed with a diet containing 0.5% ginger after 60 days of culture in a fiberglass tank as compared to the control. The conflicting results of the present study with those of Aqmasjed et al. (2023) could therefore be attributed to the difference in the inclusion level of ginger in the diet or the culture condition. Studies had reported the beneficial effects of ginger on the growth performance, immune system, blood parameters, and antioxidant status of several other fish species (Ahmadifar et al. 2019; Nya and Austin 2009; Sukumaran et al. 2016; Talpur et al. 2013) as it is a medicinal herb and an excellent source of nutrients (Thanikachalam et al. 2010). The ginger in a dry powder form generally contains around 60-70% carbohydrates, 9% protein, 3-6% lipid, 3-8% fibers, protease about 2-6%, 1-3% volatile compounds such as zingiberol, gingerol, zingiberene, shogaol, and vitamin A, C and B3 (Mohammadi et al. 2020). Therefore, in the present study, the growth enhancement and lower fed conversion ration after the supplementation of ginger, especially at 1%, have been attributed to the nutrient richness of ginger rhizomes, which may have enhanced digestion and nutrient absorption in the gastrointestinal tract. Ginger can also positively affect the intestinal morphology and bacterial micro flora, which further enhances nutrient availability, thereby promoting the overall performance of the fish (Ali et al. 2008).

On the other hand, in this study, garlic fed trout performed apparently better yet statistically similar to the control group in terms of growth performance, feed utilization, and survival. However, in contrast to these results, Nya and Austin (2009) indicated that rainbow trout fingerlings (15g average body weight) when fed with dietary garlic at 0.1-1% significantly improved the growth and feed conversion rate. Similarly, at the end of 90-day feeding trail, the specific growth rate and feed conversion efficiency were significantly higher than the control group when rainbow trout (64.1±0.3g) were fed with garlic at a rate of 1-2% for 120 days (Öz and Dikel 2022). They found these results when the rainbow trout used in their experiment was either bigger in average size or the number of culture days was longer than the present study. However, in slight agreement with the results of present study, garlic has been linked previously with growth suppression in fish as shown in juvenile tilapia at 1% level with no significant improvement in growth performance (Ndong and Fall 2007). Similarly, Nwabueze (2012) and Thanikachalam et al. (2010) found no significant effect on the weight gain, SGR, and FCR of African catfish (*Clarias gariepinus*) when fed garlic at the rate of 0.5-15%. Likewise, when garlic powder was fed at 2-4% for 60 days, blood parameters such as red blood cell count (RBC), hemoglobin (Hb) concentration, hematocrit (%), and mean corpuscular hemoglobin were significantly lowered in European Sea Bass (*Dicentrarchus labrax*) as reported by Irkin et al. (2014). Based on the findings in different fish species, it may be concluded that the dose of garlic, size of fish, and duration of exposure to garlic may alter the effects of garlic on the growth performance, feed utilization, and physiology of fish. Moreover, the differences in the results may also be due to the differences in diet formulation, purity of the plant, form of the garlic (powders, oils, or extracts) used in the diet, physiological characteristics, and culture condition of the fish (Dadgar et al. 2017). Despite the constant controversy about the effects of garlic as growth enhancer for fish, few studies have shown that garlic is an important medicinal herb with organosulfur compounds, such as allicin, widely known to improve digestion and promote the growth of fish (Büyükdeveci et al. 2018; Lee et al. 2012). Therefore, the present study suggests that dietary garlic does not decrease the growth of rainbow trout and may positively affect the growth of trout, taking into consideration the size and culture duration.

This study clearly indicated that 1% ginger supplement in the feed for juvenile rainbow trout elicited weight gain, specific growth rate, and condition factor. Ginger inclusion in rainbow trout feed at the 1% inclusion level is therefore suggested for juvenile rainbow trout to promote growth performance as a cost-effective and environmentally friendly feed additive. On the other hand, it is also concluded that garlic does not affect the growth of rainbow trout when included at 1-2% in feed. However, it remains for further research to consider the appropriate size of fish and dose for the application of garlic and the duration of culture for favorable garlic performance and to assess its impacts on growth performance. With the established history of garlic and ginger in dietary and medicinal applications as anti-infective agents, their potential to protect the rainbow trout against a specific pathogen is also another aspect that can be considered in future research to enhance the production of rainbow trout in the Nepalese context.

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REFERENCES

Ahmadifar, E., Sheikhzadeh, N., Roshanaei, K., Dargahi, N., & Faggio, C. (2019). Can dietary ginger (*Zingiber officinale*) alter biochemical and immunological parameters and gene expression related to growth, immunity and antioxidant system in zebrafish (*Danio rerio*)? Aquaculture, 507, 341–348. doi: https://doi.org/10.1016/ j. aquaculture.2019.04.049

Ali, B.H., Blunden, G., Tanira, M.O., & Nemmar, A. (2008). Some phytochemical, pharmacological and toxicological properties of ginger (*Zingiber officinale Roscoe*): A review of recent research. Food and Chemical Toxicology, 46(2), 409–420. doi: https://doi.org/10.1016/j.fct.2007.09.085

AOAC. (1990). Official Methods of Analysis (15th Ed.). Association of Official Analytical Chemist.

Aqmasjed, B.S., Sajjadi, M.M., Falahatkar, B., & Safari, R. (2023). Effects of dietary ginger (*Zingiber officinale*) extract and curcumin on growth, hematology, immunity, and antioxidant status in rainbow trout (*Oncorhynchus mykiss*). Aquaculture Reports, 32, 101714. doi: https://doi.org/10.1016/j.aqrep.2023.101714

Barimani, S., Kazemi, M.B., & Hazaei, K. (2013). Effects of Different Photoperiod Regimes on Growth and Feed Conversion Rate of Young Iranian and French Rainbow Trout (*Oncorhynchus mykiss*). World Applied Sciences Journal, 21(10), 1440–1444.

Büyükdeveci, M.E., Balcázar, J.L., Demirkale, İ., & Dikel, S. (2018). Effects of garlic-supplemented diet on growth performance and intestinal microbiota of rainbow trout (*Oncorhynchus mykiss*). Aquaculture, 486, 170–174. doi: https://doi.org/10.1016/ j. aquaculture.2017.12.022

Chi, M.S., Koh, E.T., & Stewart, T.J. (1982). Effects of Garlic on Lipid Metabolism in Rats Fed Cholesterol or Lard. The Journal of Nutrition, 112(2), 241–248. doi: https://doi.org/10.1093/jn/112.2.241

Citarasu, T. (2010). Herbal biomedicines: a new opportunity for aquaculture industry. Aquaculture International, 18(3), 403–414. doi: https://doi.org/10.1007/s10499-009-9253-7

Corzomartinez, M., Corzo, N., & Villamiel, M. (2007). Biological properties of onions and garlic. Trends in Food Science & Technology, 18(12), 609–625. doi: https://doi.org/10.1016/j.tifs.2007.07.011

Dadgar, S., Seidgar, M., Neukuiefard, A., Valipour, A.R., Sharifian, M., & Hafezieh, M. (2017). Oral administration of garlic powder (*Allium sativum*) on growth performance and survival rate of *Carassius auratus* fingerlings. Iranian Journal of Fisheries Sciences, 18(1), 71–82.

Diab, A.S., Aly, S.M., John, G., Abde-Hadi, Y., & Mohammed, M.F. (2008). Effect of garlic, black seed and Biogen as immunostimulants on the growth and survival of Nile tilapia, *Oreochromis niloticus* (Teleostei: Cichlidae), and their response to artificial infection with *Pseudomonas fluorescens*. African Journal of Aquatic Science, 33(1), 63–68. doi: https://doi.org/10.2989/AJAS.2007.33.1.7.391

Ellis, T., Hoyle, I., Oidtmann, B., Turnbull, J.F., Jacklin, T.E., & Knowles, T.G. (2009). Further development of the "Fin Index" method for quantifying fin erosion in rainbow trout. Aquaculture, 289(3–4), 283–288. doi: https://doi.org/10.1016/ j. aquaculture.2009.01.022

Gholipour Kanani, H., Nobahar, Z., Kakoolaki, S., & Jafarian, H. (2014). Effect of ginger- and garlic-supplemented diet on growth performance, some hematological parameters and immune responses in juvenile *Huso huso*. Fish Physiology and Biochemistry, 40(2), 481–490. doi: https://doi.org/10.1007/s10695-013-9859-6

Gurung, T.B., Wagle, S.K., Nepal, A.P., & Lamsal, G.P. (2017). Rainbow trout (*Oncorhynchus mykiss*) based mountain aquaculture in Nepal. Nepalese Journal of Aquaculture and Fisheries, 3(4), 12–20.

Irkin, L.C., Yigit, M., Yilmaz, S., & Maita, M. (2014). Toxicological Evaluation of Dietary Garlic *(Allium sativum*) Powder in European Sea Bass (*Dicentrarchus labrax*) Juveniles. Food and Nutrition Sciences, 05(11), 989–996. doi: https://doi.org/10.4236/fns.2014.511109

Lee, D.-H., Ra, C.-S., Song, Y.-H., Sung, K.-I., & Kim, J.-D. (2012). Effects of Dietary Garlic Extract on Growth, Feed Utilization and Whole Body Composition of Juvenile Sterlet Sturgeon (*Acipenser ruthenus*). Asian-Australasian Journal of Animal Sciences, 25(4), 577–583. doi: https://doi.org/10.5713/ajas.2012.12012

Mohammadi, G., Rashidian, G., Hoseinifar, S.H., Naserabad, S.S., & Doan, H. Van. (2020). Ginger (*Zingiber officinale*) extract affects growth performance, body composition, haematology, serum and mucosal immune parameters in common carp (*Cyprinus carpio*). Fish & Shellfish Immunology, 99, 267–273. doi: https://doi.org/10.1016/j.fsi.2020.01.032

Naderi, M., Keyvanshokooh, S., Salati, A.P., & Ghaedi, A. (2017). Effects of chronic high stocking density on liver proteome of rainbow trout (*Oncorhynchus mykiss*). Fish Physiology and Biochemistry, 43(5), 1373–1385. doi: https://doi.org/10.1007/ s10695-017-0378-8

Ndong, D., & Fall, J. (2007). The effect of garlic (*Allium sativum*) on growth and immune responses of hybrid tilapia (*Oreochromis niloticus* x *Oreochromis aureus*). Aqua Docs, *5*(3), 1–23.

Nepal, A.P., Sharma, S., Bhujel, R.C., & Gurung, T.B. (2021). Condition factor, growth performance, and production of Rainbow trout (*Oncorhynchus mykiss*) in floating cages in a shallow reservoir in Panauti, Nepal: A preliminary study. International Journal of Fisheries and Aquatic Studies, 9(3), 12–21. doi: https://doi.org/10.22271/ fish.2021.v9.i3a.2471

NFRC. (2021). Annual Report 2077/78 (2020/21). National Fishery Research Centre, NARC, Godawari, Lalitpur, Nepal. 118.

Nwabueze, A.A. (2012). The Effect of Garlic (*Allium sativum*) on Growth and Haematological Parameters of *Clarias gariepinus* (Burchell, 1822). Sustainable Agriculture Research, 1(2), 222. doi: https://doi.org/10.5539/sar.v1n2p222

Nya, E.J., & Austin, B. (2009). Use of dietary ginger, *Zingiber officinale Roscoe*, as an immunostimulant to control *Aeromonas hydrophila* infections in rainbow trout, *Oncorhynchus mykiss* (Walbaum). Journal of Fish Diseases, 32(11), 971–977. doi: https://doi.org/10.1111/j.1365-2761.2009.01101.x

Öz, M., & Dikel, S. (2022). Effect of garlic (*Allium sativum*) - supplemented diet on growth performance, body composition and fatty acid profile of rainbow trout (*Oncorhynchus mykiss*). Cellular and Molecular Biology, 68(1), 217–225. doi: https://doi.org/10.14715/cmb/2022.68.1.26

Paulin, N., Noel Arlette, T.N., Doriane Divine, M.Y., Nesrine Yolande, C.D., Georges, F., Ghislain Nguimdop, N., & Minette Eyango, T.-T. (2021). Dietary inclusion effect of ginger (*Zingiber officinale*) and garlic (*Allium sativum*) blend on growth, feed nutrients utilization and retention in African Catfish (*Clarias gariepinus*) fry in intensive system. International Journal of Aquaculture and Fishery Sciences, 14–23. doi: https://doi.org/10.17352/2455-8400.000068

Reverter, M., Tapissier-Bontemps, N., Sasal, P., & Saulnier, D. (2017). Use of Medicinal Plants in Aquaculture. Diagnosis and Control of Diseases of Fish and Shellfish, 223–261. Wiley. doi: https://doi.org/10.1002/9781119152125.ch9

Reyes-Cerpa, S., Vallejos-Vidal, E., Gonzalez-Bown, M.J., Morales-Reyes, J., Pérez-Stuardo, D., Vargas, D., Imarai, M., Cifuentes, V., Spencer, E., Sandino, A.M., & Reyes-López, F.E. (2018). Effect of yeast (*Xanthophyllomyces dendrorhous*) and plant (Saint John's wort, lemon balm, and rosemary) extract based functional diets on antioxidant and immune status of Atlantic salmon (*Salmo salar*) subjected to crowding stress. Fish & Shellfish Immunology, 74, 250–259. doi: https://doi.org/10.1016/j.fsi.2017.12.061

Shakya, S.R. (2015). Medicinal uses of ginger (*Zingiber officinale Roscoe*) improves growth and enhances immunity in aquaculture. International Journal of Chemical Studies, 3(2), 83-87.

Sukumaran, V., Park, S.C., & Giri, S.S. (2016). Role of dietary ginger *Zingiber officinale* in improving growth performances and immune functions of *Labeo rohita* fingerlings. Fish & Shellfish Immunology, 57, 362–370. doi: https://doi.org/10.1016/j.fsi.2016.08.056

Talpur, A.D., Ikhwanuddin, M., & Ambok Bolong, A.-M. (2013). Nutritional effects of ginger (*Zingiber officinale Roscoe*) on immune response of Asian sea bass, *Lates calcarifer* (Bloch) and disease resistance against *Vibrio harveyi*. Aquaculture, 400-401, 46–52. doi: https://doi.org/10.1016/j.aquaculture.2013.02.043

Thanikachalam, K., Kasi, M., & Rathinam, X. (2010). Effect of garlic peel on growth, hematological parameters and disease resistance against *Aeromonas hydrophila* in African catfish *Clarias gariepinus* (Bloch) fingerlings. Asian Pacific Journal of Tropical Medicine, 3(8), 614–618. doi: https://doi.org/10.1016/S1995-7645(10)60149-6

Timalsina, P., Gurung, B., Thapa, R., Kunwor, P., Rayamajhi, A., Pradhan, N., Bhandari, M., Singh, S., & Sharma, A. (2022). Tail and fin rot disease and antibiotics resistance pattern in major rainbow trout farms of Nepal. International Journal of Fisheries and Aquatic Studies, 10(1), 33–37. doi: https://doi.org/10.22271/fish.2022.v10.i1a.2628

Voorhees, T. M. (2011). The potential to expand the rainbow trout (*Oncorhynchus mykiss*) commercial aquaculture industry in the mid-and high-hills of Nepal. Ecological Aquaculture (Ed. Barry Costa-Pierce), *27*.

Winfree, R.A., Kindschi, G.A., & Shaw, H.T. (1998). Elevated Water Temperature, Crowding, and Food Deprivation Accelerate Fin Erosion in Juvenile Steelhead. The Progressive Fish-Culturist, 60(3), 192–199.

Yılmaz, S., & Ergün, S. (2012). Effects of Garlic and Ginger Oils on Hematological and Biochemical Variables of Sea Bass *Dicentrarchus labrax*. Journal of Aquatic Animal Health, 24(4), 219–224. doi: https://doi.org/10.1080/08997659.2012.711266