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Effect of ginger (*Zingiber officinale*) rhizome extract on growth performance, survival and gut flora of common carp (*Cyprinus carpio*)

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Abstract
Phytogenics, or plant-based additives, are pivotal for growth enhancement and disease resistance in cultured fish diets. A 60-days feeding trial was carried out in hapas (1m×1m×1.2m) within a grow tank (5 m×5 m×1.5 m) at the Department of Aquaculture, Agriculture hatchery complex. The study aimed to assess growth performance, survival, and gut flora of *Cyprinus carpio* fed with varying levels of *Zingiber officinale* rhizome extract. An initial cohort comprising 450 juveniles (fry) of *C. carpio*, averaging 2.55±0.03 g in weight and 4.9±0.68cm in length, were fed with diets containing 27.15% crude protein, included four concentrations of *Z. officinale* rhizome extract (1%, 2%, 3%, and 4%), along with a control group (0%); feeding three times a day for a total of 60 days, with each meal amounting to 5% of their body weight. Fish group receiving the diet containing 3% Ginger rhizome extract, showed the highest values for growth performance parameters ($p<0.05$), including average harvesting weight (10.33±1.32 g/fish), total harvesting weight (247.82±31.60 g/hapa), daily weight gain (0.13±0.01g/fish/day), specific growth rate (2.33±0.18%/ day) in comparison with control group. The mean initial total bacterial count of fries was observed (1.38×10^9 cfu/ml) and notable variances ($p<0.05$) were observed among groups fed with varying level of Ginger. The least total bacterial count was recorded in group fed 3% ginger rhizome extract on observation at 20 days ($1.75\times10^8\pm2.64\times10^6$ cfu/ml), 40 days ($1.9\times10^7\pm4.23\times10^6$ cfu/ml), and 60 days ($1.62\times10^6\pm3.60\times10^4$ cfu/ml). Similarly, the effects of varying level of ginger rhizome extract on the water quality parameters were not significantly different ($p>0.05$). The study concluded that supplementing the diet of cultured *C. carpio* with 3% *Z. officinale* rhizome extract effectively fosters growth and reduces the bacterial load in the gut of fish.

Keywords: Common carp, phytogenics, specific growth rate, total bacterial count

Introduction
The aquaculture and fisheries sectors in Nepal are pivotal contributors to national food security, economic output, and employment contributing 17% of global human consumption of animal protein.

Nepal's annual fish production amounts to 104,623 mt. of which 21,000 mt. are derived from capture fisheries, while aquaculture contributes 73,693 mt. accounting for 0.44% of the GDP and 1.44% of the Agricultural Gross Domestic Production (AGDP) (CFPCC, 2022) [1]. Intensified aquaculture can lead to water quality decline, stress, and disease (Hoseinifar, Soleimani, & Ringø, 2014) [10] while antibiotics and chemicals were traditionally used to mitigate these issues (Mohammadi *et al*., 2020) [13]. Concerns over resistance and environmental impact have shifted focus towards dietary supplements for improved fish health, particularly in larval and fry stages (Hoseinifar, Soleimani, & Ringø, 2014) [10]. Herbal plants, with medicinal properties, offer potential for enhancing fish health and growth in aquaculture (Gabriel, 2021). Research indicates various plant parts improve growth, antioxidant profiles, immunity, and survival in farmed fish (Jafarinejad *et al*., 2020) [11].

Common carp (*Cyprinus carpio*) are favored for their rapid growth and adaptability (Tokur *et al*., 2006) [12]. However, plant-based feeds, while desirable, contain anti-nutritional factors (ANFs) that can negatively impact gut microbes, digestibility, growth, immunity, and disease resistance in carp (Apines-Amar *et al*., 2016) [26].

Ginger (*Zingiber officinale*), used medicinally and as a supplement (Jafarinejad *et al*., 2020 [11], contains carbohydrates, protein, lipids, fiber, proteases, and volatile compounds like

gingerol, which enhances digestion (Ahmadifar *et al*., 2019) [3]. Ginger powder has shown positive effects on fish growth, hematology, immunity, and antioxidant profiles (Talpur *et al*., 2013; Sukumaran, Park & Giri, 2016; Ahmadifar *et al*., 2019) [14, 15, 3]. It also enhances digestion and regulates gut bacteria by stimulating pancreatic enzyme and bile secretion (Baliga *et al*., 2011) [16]. Gastro-intestinal tract (GIT) microorganisms improve digestion through enzyme provision and vitamin synthesis (Tovar-Ramírez *et al*., 2004) [25], with their composition heavily influenced by diet (Nayak, 2010) [24]. Adegbesan *et al*. (2019) [2] showed ginger reduces gut bacterial and fungal counts in *Clarias gariepinus* ($p<0.05$). A 2% ginger diet resulted in the lowest counts (7.4×10^5 bacteria, 2.0×10^5 fungi), followed by a 3% diet. Abbasi Ghadikolaei *et al*. (2017) [8] found that 2% dietary ginger powder significantly improved growth and biochemical composition in juvenile common carp. Adegbesan *et al*. (2019) [2] found *Streptococcus agalactiae*, *Bacillus subtilis*, and *Enterococcus faecalis* in *Clarias gariepinus* intestines fed ginger, suggesting increased gram-positive bacteria. This aligns with findings by Olojo *et al*. (2010) [17] and Nwabueze (2014) [19] regarding similar bacterial flora in *C. gariepinus*. Similarly, M Alsaiad, S., & M AL-Zayat, (2019) [18] found ginger increased feed costs, but improved weight gain. However, the impact of ginger on gut flora requires further study. This research aims to assess ginger's effects on common carp growth, survival, and gut flora.

Materials and Methods

Site Selection

The research was conducted at the Hatchery Complex within the Fisheries Program of Agriculture and Forestry University, spanning from June to August 2022. The site was located at 27°39'26.5"N longitude & 84°20'58.6"E latitude.

Experimental Setup

Experimental fish

Fries of Common Carp with average individual weight of 2.55±0.03 g and length 4.9±0.68 cm were procured from CAARP, Kathar, Chitwan (Private farm) which were kept in conditioning for 30 minutes prior to transferring them on holding tank.

Treatment design

A 60-day feeding trial, with three replicates per treatment, tested various ginger rhizome extract ratios across five treatment groups (Table 1).

Table 1: Treatment design with ratio of Ginger rhizome extract in experimental feed.

Treatment No.	Treatment	Ratio of Ginger rhizome extract (%)
T_1	Control	0
T_2	Pellet feed + Ginger rhizome extract	1
T_3	Pellet feed + Ginger rhizome extract	2
T_4	Pellet feed + Ginger rhizome extract	3
T_5	Pellet feed + Ginger rhizome extract	4

Tank preparation and hapas installation

Cement tanks were drained, fish and debris removed, and air-dried. Tanks were disinfected with 2 g/1000L $KMnO_4$, dried again, and refilled to 1.2 m. Nylon hapas, disinfected with 5% salt, were secured in tanks with bamboo and iron supports. Natural algal blooms eliminated the need for fertilization.

Experimental setup

A Complete Randomized Design (CRD) was used. 1 m x 1 m x 1.2 m nylon hapas were placed in 5 m x 5 m x 1.5 m cemented tanks. Treatments were randomly assigned to three tanks each via lottery. Each hapas contains 30 fries.

Experimental feed

Proximate composition

To prepare the experimental feed of CP (27.15%) the ingredients used are mentioned in Table 2.

Table 1: % CP and % DM of feed ingredients used in experiment.

SN	Feed ingredients	Proximate composition	
		CP %	DM %
1	Rice bran	13.7	90.8
2	Corn flour	12.4	89.9
3	Mustard oil cake	32.5	95.8
4	Soyabean meal	47.09	93

Preparation of ginger rhizome extract

Local ginger rhizomes were washed, peeled, and sliced before being dried at 40 °C for 72 hours. The dried slices were then ground, sieved, and 20 g of the resulting powder was macerated in 100ml of 35 °C distilled water for 48 hours. Following maceration, the extract was filtered, dried at 40 °C for another 72 hours, cooled to room temperature, and finally stored at 4 °C.

Experimental diet and feeding

A 27.15% CP pellet feed was formulated from crushed ingredients suitable for carp fry. Extracts, prepared according to treatment, were dissolved in 90% ethanol, sprayed onto the feed, and mixed thoroughly. The experimental diet was then air-dried for 3 hours and stored. Juvenile common carp were fed this diet three times daily at 5% of their body weight for 60 days.

Data collection

Fish growth was measured before and after treatment. Gut bacteria counts were taken from two fish per group every 20 days. Fish used for bacteria counts were excluded from growth performance measurements.

Growth parameters and Survival

$$\text{Daily weight gain (g/fish/day)} = \frac{\text{Mean final weight (g)} - \text{Mean initial weight(g)}}{\text{Culture period (days)}}$$

$$\text{Specific growth rate (\%/day)} = \frac{\ln W_1 - \ln W_0}{\text{Culture period}} \times 100$$

Where, W_0 and W_1 are the average initial and final body weights, respectively, and (t) time (days)

$$\text{Survival rate (\%)} = \frac{\text{Number of fish harvested}}{\text{Number of fish stocked}} \times 100$$

Where, Nf= final number of fish stock, Ni= Initial number of fish stock.

Examination of Gut flora

Collection of intestines

Fish were washed, disinfected with 90% ethanol, and anesthetized with 0.1 ml/L clove oil for 30 minutes. Under aseptic conditions, the gut was removed, homogenized, and 2 g of the homogenate was suspended in 25 ml normal saline (NS) to create a stock solution, stored at 4 °C.

Total aerobic heterotrophic plate count

A 1 ml gut suspension sample was serially diluted to 10^{-9}. 0.1 ml aliquots were spread onto nutrient agar plates in triplicate and incubated at 37 °C for 48 hours. Aerobic heterotrophic bacteria were counted, and results were expressed as cfu/ml.

Water quality analysis

Maintaining good water quality is of utmost importance for promoting growth and preventing diseases. The parameters of water quality, including Dissolved oxygen (DO), pH, and Temperature, were assessed on a daily basis

Proximate analysis

Random sampling was carried out for each batch of feed produced throughout the experiment. These samples, which represented the diets, were subjected to proximate analysis following the guidelines outlined in AOAC (2000) [4]. The analysis was conducted at the Animal Nutrition Laboratory of Agriculture and Forestry University in Rampur, Chitwan, Nepal.

Statistical analysis

Statistical analysis was conducted using R (version 3.6.1). ANOVA was used to assess treatment mean variations, with significance set at $p < 0.05$. Mean values are presented with their standard error of the mean (± SEM).

Result

Proximate composition of diet

Table 3: Proximate composition of experimental diet

SN	Proximate composition	Percentage (%)
1	Moisture	11.2
2	Crude protein	27.15
3	Crude fat	5.75
4	Crude fiber	4.00
5	Total ash	5.80
6	Metabolic energy	3239.63Kcal/kg

Growth Performance and survival

Table 2: Effect of varying levels of ginger rhizome extract on growth performance of Common carp fry (Mean±SEM)

Parameters	T_1	T_2	T_3	T_4	T_5
Average stocking weight (g/fish)	2.55±0.04^a	2.55±0.03^a	2.56±0.03^a	2.53±0.03^a	2.55±0.03^a
Average harvesting weight (g/fish)	5.76±0.13^c	5.83±0.09^c	7.39±0.90^b	10.33±1.32^a	9.61±1.16^a
Harvested number (Nos.)	22±0.57^b	23±0.00^{ab}	24±0.00^a	24±0.00^a	24±0.00^a
Total harvesting weight (g/hapa)	138.28±3.13^c	141.16±4.37^c	177.48±2.11^b	247.82±31.60^a	230.63±28.12^a
Daily weight gain (g/fish/day)	0.053±0.005^c	0.053±0.005^c	0.08±0.00^b	0.13±0.01^a	0.11±0.01^a
Specific growth rate (%/day)	1.35±0.06^c	1.38±0.04^c	1.76±0.03^b	2.33±0.18^a	2.20±0.22^a
Survival rate (%)	93.05±1.39^c	95.83±0.00^b	100±0.00^a	100±0.00^a	100±0.00^a

(Mean values in the same column sharing identical superscripts do not differ significantly at $p < 0.05$)

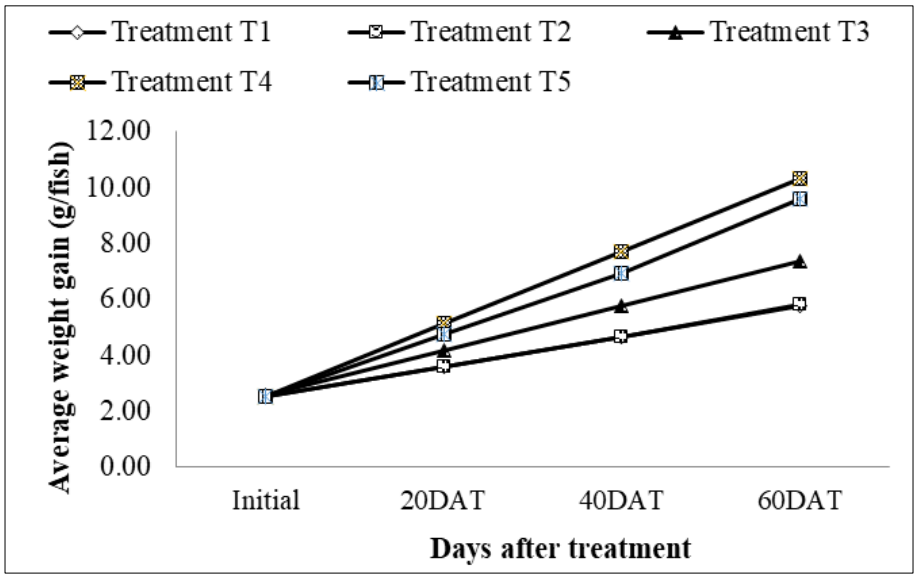

Fig 2: Graphical presentation of average weight gain (g/fish) of fish during treatment period

Microbial count

Table 5: Effect of varying levels of Ginger rhizome extract on gut flora (TBC) count of Common carp fry (Mean±SEM)

Treatment	Bacterial count (cfu/ml)			
	Initial	20DAT	40DAT	60DAT
T_1	1.38×10^{9}	$2.14\times10^{8}\pm6.55\times10^{6\ bc}$	$2.9\times10^{8}\pm2.78\times10^{7\ a}$	$2.71\times10^{9}\pm1.62\times10^{8\ a}$
T_2	1.38×10^{9}	$3.31\times10^{8}\pm3.91\times10^{7\ a}$	$3.1\times10^{8}\pm5.89\times10^{7\ a}$	$1.88\times10^{8}\pm9.50\times10^{7\ b}$
T_3	1.38×10^{9}	$2.24\times10^{8}\pm1.83\times10^{7\ b}$	$2.2\times10^{7}\pm4.36\times10^{6\ b}$	$6.30\times10^{6}\pm7.88\times10^{6\ c}$
T_4	1.38×10^{9}	$1.75\times10^{8}\pm2.64\times10^{6\ c}$	$1.9\times10^{7}\pm4.23\times10^{6\ b}$	$1.62\times10^{6}\pm3.60\times10^{4\ c}$
T_5	1.38×10^{9}	$2.06\times10^{8}\pm2.26\times10^{7\ bc}$	$2.2\times10^{7}\pm2.87\times10^{6\ b}$	$1.99\times10^{6}\pm1.68\times10^{5\ c}$

(Mean values in the same column sharing identical superscripts do not differ significantly at $p< 0.05$)

Water quality analysis

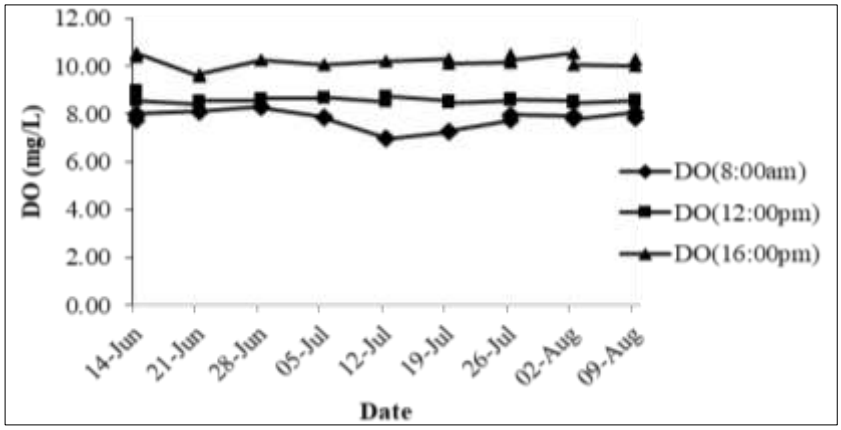

Fig 3: Weekly averages dissolve oxygen concentration (mg/L) during experimental period.

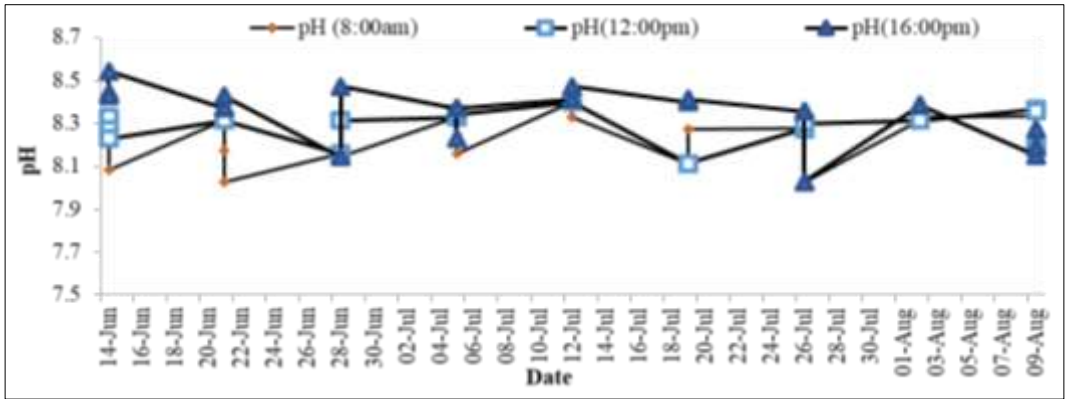

Fig 4: Weekly average pH of water during the experimental period.

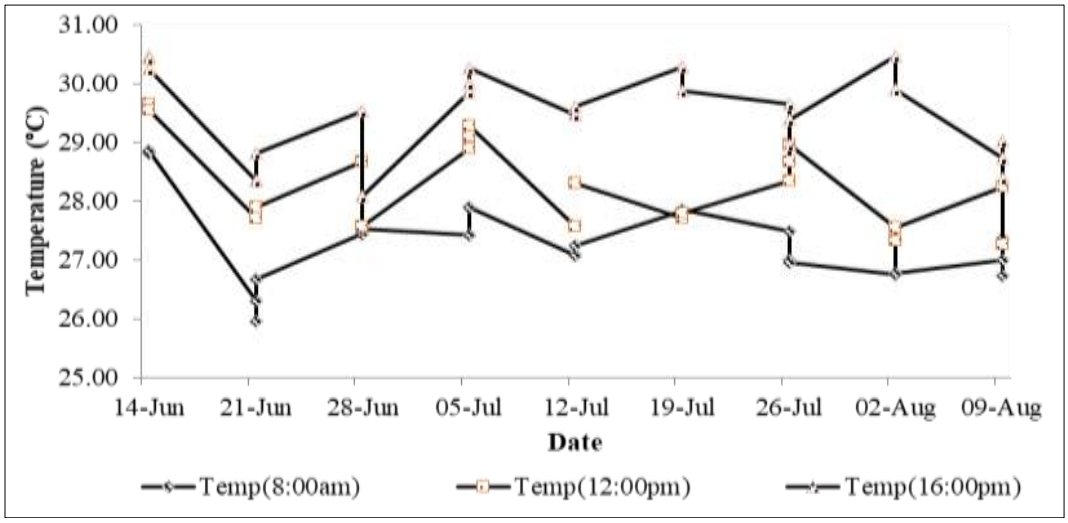

Fig 5: Weekly average temperature (°C) during experimental period.

Discussion

The water conditions, including temperature, dissolved oxygen levels, and pH, were maintained within the optimal range for Common carp culture, consistent with the research carried out by Nasir and Khalil in 2016 [20] and the guidelines from NSA (1999). Among the treatments, T_4 (3% Ginger rhizome extract) recorded the higher value of parameters followed by T_4 (4% Ginger rhizome extract) depicting the growth performance of Common carp fry. Adegbesan *et al*. (2019) [2] reported that fish (Clarias gariepinus) exhibited a consistent rise in weight gain, with the most substantial growth performance observed in those fed diets containing 1% and 3% Ginger rhizome powder. Likewise, in a research study conducted by Jafarinejad, Gharaei, and Mirdar (2020) [11], they investigated the impact of a ginger-infused diet on the growth characteristics of *Cyprinus carpio*. Their findings indicated that fish that were fed diets containing 2% to 5% ginger experienced a substantial rise in final weight, weight gain, specific growth rate, and improved feed conversion ratio (FCR) compared to the fish on the control diet. These findings align with the results of the present study, suggesting that the level of ginger inclusion in the diet plays a crucial role in promoting higher growth rates in *Cyprinus carpio*. The findings of present study on growth performance of *C. carpio* are congruent with Makode (2017) [22] who found similar report after incorporation of onion powder @ 3% had yield the higher growth performance. Similar results were also reported by Gabor *et al*. (2010) [6] and Gabor *et al*. (2012) [7]. Rawat *et al*. (2022) [23] reported the growth-promoting effects of ginger in the diet can be attributed to improved nutrient digestibility and enhanced digestion and absorption of protein components which is in line with study conducted by Talpur *et al*., (2013) [14] and Hassanin *et al*. (2014) [9]. Significant distinctions ($p< 0.05$) were noted in the reduction of the total bacterial count among fish fed different concentrations of *Zingiber officinale* extract, with the lowest bacterial count observed in fish that consumed a diet containing 3% Ginger rhizome extract, as compared to the control group. This result aligns with the research conducted by Adegbesan *et al*. (2019) [2], where the lowest bacterial count was found in the group fed with 2% ginger root powder ($7.4\pm5.31\times10^5$cfu/ml), followed by 3% ginger powder ($8.84\pm7.58 \times10^5$ cfu/ml). Similarly, Bello *et al*. (2012) [5] reported a decline in bacterial load in groups supplemented with onion bulb and walnut leaves, with increasing levels of inclusion and over time.

Conclusion

Aquaculture intensification necessitates sustainable strategies to combat disease and stress. This study demonstrated that a 3% ginger extract diet significantly improved common carp fry growth, survival, and reduced gut bacteria, offering a natural alternative to antibiotics.

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