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Effect of *Ulva lactuca* sap on the growth performance and mucosal immunity in Koi Carp (*Cyprinus carpio koi*)

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ABSTRACT

Cyprinus carpio koi is a beautiful ornamental fish known for its attractive patterns and colourations. Since it has a good demand for aesthetic purposes, its growth and the other factors are taken into consideration. In this study, algal sap of *U. lactuca* was used as a feed additive and coated along with commercial pellet diet of ornamental fish at various concentrations, i.e. 0.5%, 1.0% & 2.0% respectively. In parallel, a control diet lacking *U. lactuca* sap was maintained for analysis. Koi carp fed on U_1, U_2, & U_3 diets showed a decline in weight gain, i.e. 0.86 ± 0.02g, 0.64 ± 0.03g & 0.57 ± 0.01g, whereas control diet fed fish showed an elevated weight gain of 1.02 ± 0.04g. Alternatively, the mucosal Immune parameters showed an enhanced trend in U_1, U_2, & U_3 treated groups compared to the control diet-fed groups.

INTRODUCTION

Ornamental fish culture, also known as aquariculture, involves breeding and rearing attractive, colourful, and peaceful fish species in confined aquatic systems such as aquariums and garden ponds. Koi carp is one of the most preferred ornamental fish known for its beautiful colourations and patterns. Due to its friendly nature towards humans, it has a high demand in the ornamental fish market. Originating from Asia, these carp varieties have gained global prominence and are now recognised as one of the most significant ornamental species cultivated worldwide (Andriani *et al*., 2019; Horváth *et al*., 2023). However, the increasing demand for these ornamental fish, particularly koi carp, necessitates advanced aquaculture practices to ensure sustainable production (Legendre *et al*., 2012). In this view, the growth and mucosal immunity of the fish are taken into consideration.

U. lactuca, or sea lettuce, is a macroalgae found commonly on the rocky seashores, particularly in tidal regions, adhering to stones and rocks. It is recognisable by its thin, wavy leaf-like structure. It generally grows to 20-30 cm in length and is easily identified by its vibrant green colour (Korkmaz, 2025). It is essential in maintaining ecological balance, and it is found

in both tropical and temperate regions. Due to the broad spectrum of biological activities, this photosynthetic alga draws attention and plays a significant role in the food chain of the aquatic environment (Kaur *et al.* 2024; Hossain *et al.* 2021). Diets used to feed fish are very important in the field of aquaculture (FAO, 2017). *U. lactuca* is a marine resource valued for its extensive nutritional value, that includes essential nutrients, as well as bioactive components like carotenoids and polyphenols, that have antioxidant properties (Pappou *et al*., 2022; Rasyid, 2017). Therefore, this study focuses on evaluating the effect of *U. lactuca* sap on the growth performance and mucosal immunity in Koi carp reared in an indoor culture system by coating it with a commercial pellet diet at various concentrations.

MATERIALS AND METHODS

Koi Carp

Koi carp was procured from Jay Jay Aquarium, Nagercoil. The fish were brought to the culture site in oxygenated polytene bags with the least disturbance to minimise the stress of the fish. The fish were acclimatized in 100-litre capacity plastic troughs containing well-aerated fresh water for a six days time duration to monitor any signs of abnormality prior to the commencement of experimentation.

U. lactuca sap

U. lactuca was obtained from the seashore of Manavalakurichi, Tamil Nadu, India. The seaweed was washed thoroughly in freshwater to remove the unwanted debris and finally shade-dried for a duration of 2 weeks. The seaweed was further subjected to drying in a hot air oven at 50 °C in order to facilitate the grinding process. The ground *U. lactuca* powder was mixed with distilled water in a 1:2 ratio and agitated for 24 to 48 hours. The contents were centrifuged at 10000 rpm for 10 minutes, and finally, the supernatant was obtained. The supernatant was stored in plastic vials that served as the *U. lactuca* sap. The sap was coated along with a commercial pellet diet at various concentrations to test its efficiency in koi carp in terms of growth performance and modulation in mucosal immunity.

Diet preparation

The commercial pellet diet for experimentation consisted of 35% crude protein. The pellet diet was coated with *U. lactuca* sap at 0.5%, 1.0% and 2.0% and dried in a hot air oven at 45 $^{\circ}$C ie U_1, U_2, & U_3, respectively. In parallel, a control diet lacking *U. lactuca* sap was also maintained for analysis. The diet was stored in airtight plastic containers to be offered to the candidate fish.

Experimentation

Koi carp (20 nos) were segregated into four groups in triplicate, i.e. C, U_1, U_2, & U_3 tanks, respectively. The fish were fed on the respective experimental diets to satiation for a duration of 40 days. During the experimentation, unfed remains were siphoned regularly, and 50% water exchange was given to maintain optimum water quality parameters.

Water Quality Parameters

The temperature in the culture tanks was determined using a digital thermometer. The pH of the water samples was determined using pH meter, calibrated using standard buffers. The dissolved oxygen content in the culture tanks was determined using standard protocols of (APHA 1995). The ammonia content in the culture system was determined using the Biosol kit.

Growth Parameters

Growth parameters were calculated in fish according to the formula given below

Weight gain (WG)

$$Weight\ gain\ (g) = Final\ weight - Initial\ weight$$

Feed consumption (FC)

$$Food\ consumption\ (g) = Food\ provided - Unfed\ remains$$

Feed conversion ratio (FCR)

$$FCR = \frac{Total\ amount\ of\ feed\ given\ (g)}{Total\ production\ of\ fish\ (g)}$$

Feed conversion efficiency (FCE)

$$FCE\ (\%) = \frac{Wet\ weight\ of\ the\ fish\ produced\ (g)}{Dry\ weight\ of\ the\ feed\ given\ (g)} \times 100$$

Specific growth rate (SGR)

$$SGR\ (\%) = \frac{In\ final\ weight\ (g) - In\ initial\ weight(g)}{Experimental\ period} \times 100$$

Growth Percentage (G%)

$$G\ (\%) = \frac{Growth\ (g)}{Experimental\ duration} \times 100$$

Evaluation of Mucosal Immune Parameters

Mucus Collection

Koi carp were held carefully, and the mucus samples were obtained gently by scraping the skin of the Koi Carp using a cell scraper. Further, the mucus were pooled together for the analysis of mucosal immune parameters.

Alkaline Phosphatase Activity (ALP)

Alkaline Phosphatase activity was performed in the pooled skin mucus samples using the ERBA ALP kit. The ALP activity was read at the Systronics spectrophotometer (Model 104). The values were expressed as an increase in optical density (OD) read at 405 nm.

Lysozyme activity

Lysozyme activity was determined in the skin mucus of Koi carp, according to the method of Parry *et al.* (1965) with slight modifications. Briefly, about 100µl of the pooled skin mucus sample was added to 1.8 ml of *Micrococcus lysodeikticus* (sigma) dissolved in the required amount of sodium phosphate buffer, taken in a cuvette. The reduction in OD was read at 450 nm after regular time intervals. Lysozyme activity was expressed in U/ml.

Bactericidal assay

The bactericidal assay was performed using the MTT reduction protocol, according to the method of Welker *et al* (2007), with slight modifications. The bactericidal activity of the skin mucus was determined according to the formation of formazan, that was read in the spectrophotometer at 560nm.

RESULTS

Water Quality Parameters

The water quality parameters recorded in the culture system is represented in Table 1. The temperature in the culture tanks ranged from 29.00 ± 0.12°C to 29.00 ± 0.16 °C, whereas the pH and dissolved oxygen in the culture tanks ranged from 7.62 ± 0.12 to 7.66 ± 0.12 and 5.42 ± 0.10 mg/l to 5.68 ± 0.24 mg/l, respectively. The ammonia content in the culture tanks was negligible, i.e. < 0.1 mg/l.

Table 1. Water Quality Parameters in the culture system

Experimental tanks	Temperature (˚C)	pH	DO (mg/l)	Ammonia (mg/l)
Control	29.00 ± 0.14	7.62 ± 0.12	5.42 ± 0.10	< 0.1
U_1	29.00 ± 0.12	7.65 ± 0.14	5.59 ± 0.10	< 0.1

U_2	29.00 ± 0.16	7.64 ± 0.16	5.60 ± 0.19	< 0.1
U_3	29.00 ± 0.13	7.66 ± 0.12	5.68 ± 0.24	< 0.1

Growth Responses

Growth responses of Koi carp fed on control and treated diets (U_1 – U_3) are illustrated in Table 2. The production showed significant variation ($P < 0.05$) between the control and treated groups. For instance, the production peaked high 1.02 ± 0.03 g in the control group compared to a low production of 0.86 ± 0.02 g, 0.64 ± 0.03 g, and 0.57 ± 0.01 g, noticed in U_1, U_2, & U_3 treated fish. The food conversion efficiency (FCE) showed significant variations ($P < 0.05$) among the control & treated groups, i.e. it was high 36.17 ± 1.40% in the control group, whereas it declined to 30.49 ± 1.34 %, 22.69 ± 1.36 %, and 20. 21 ± 1.42 % in U_1, U_2, & U_3 treated groups. The SGR and growth percentage also displayed a similar trend, and it was notably high in the control group and showed significant variations ($P < 0.05$) in comparison with the treated groups.

Table 2. Growth performance of koi carp in response to *U. lactuca* sap

Parameters	Control	U_1	U_2	U_3
Initial weight (g)	5.71 ± 0.01	4.89 ± 0.02	4.78 ± 0.01	6.45 ± 0.02
Final weight (g)	6.73 ± 0.05	5.75 ± 0.04	5.42 ± 0.02	7.02 ± 0.04
Production (g)	1.02 ± 0.03	0.86 ± 0.02	0.64 ± 0.03	0.57 ± 0.01
Food Consumed (g)	2.82 ± 0.04	2.82 ± 0.02	2.82 ± 0.02	2.82 ± 0.02
FCR	2.76 ± 0.02	3.25 ± 0.04	4.40 ± 0.03	4.94 ± 0.02
FCE (%)	36.17 ± 1.40	30.49 ± 1.34	22.69 ± 1.36	20.21 ± 1.42
SGR (%)	4.01 ± 0.04	3.56 ± 0.02	3.37 ± 0.03	4.10 ± 0.02
(G) %	2.55 ± 0.02	2.15 ± 0.03	1.60 ± 0.01	1.42 ± 0.02

Alkaline Phosphatase Activity

ALP activity in the skin mucus of Koi carp is depicted in Fig. 3. The ALP activity showed significant variations ($P < 0.05$) among the control & treated groups. For instance, ALP activity peaked high 1.814 ± 0.04 OD in the U_3 diet-fed fish compared to a low activity of

1.075 ± 0.01 OD noticed in the control diet-fed group. In U_1 & U_2 treated groups, however, the ALP activity was high compared to control fish, i.e., 1.246 ± 0.02 OD and 1.414 ± 0.02 OD, respectively. The ALP activity corresponds to the increase in optical density.

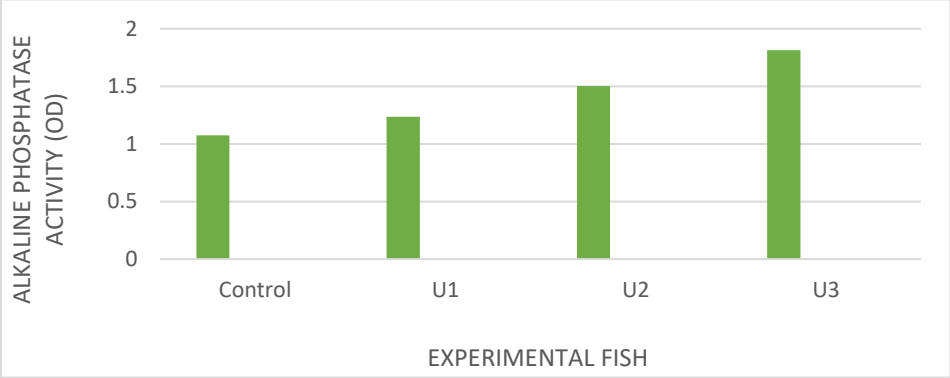

Fig. 3. Alkaline phosphatase activity in the skin mucus of control and experimental fish.

Lysozyme activity

Lysozyme activity in the skin mucus of Koi carp is depicted in Fig. 4. The lysozyme activity showed remarkable variations ($P < 0.05$) among the control & treated groups. Accordingly, the Lysozyme activity ranged high, 4.20 ± 0.04 U/ml in the mucus of U3-treated fish, whereas, in the control diet-fed fish, a least activity of 1.30 ± 0. 02 U/ml was noticed. In the U_1 & U_2 treated groups, the activity was 2.32 ± 0.03 U/ml and 3.64 ± 0.01 U/ml, better than the control groups.

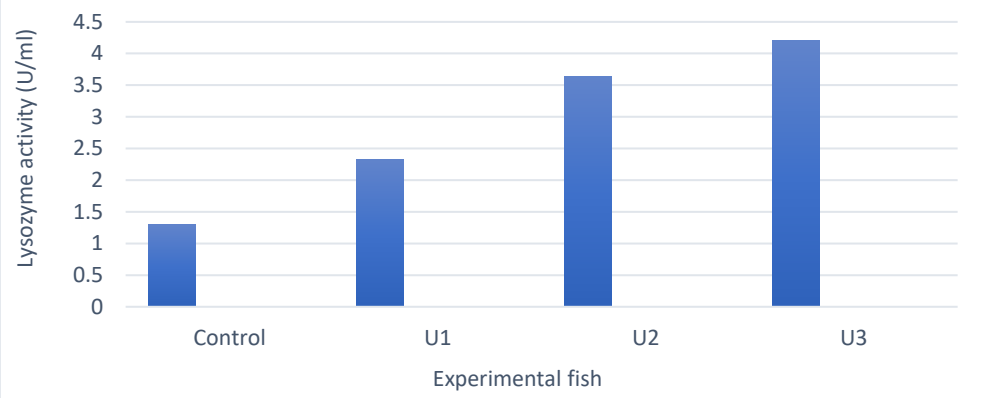

Fig. 4. Lysosome activity in the mucus of control and experimental fish.

Bactericidal activity

Bactericidal activity in the skin mucus of Koi carp is depicted in Fig. 5. The bactericidal activity displayed significant ($P < 0.05$) among the control & treated groups. Notably, the activity ranged high, i.e. 58.64 ± 1.82 % in the skin mucus of U_3 treated groups, whereas in the control, U_1 & U_2 treated groups, the activity declined, i.e., 32.46 ± 1 .04%, 40.04 ± 1.26 % and 52.63 ± 1.14 %, respectively.

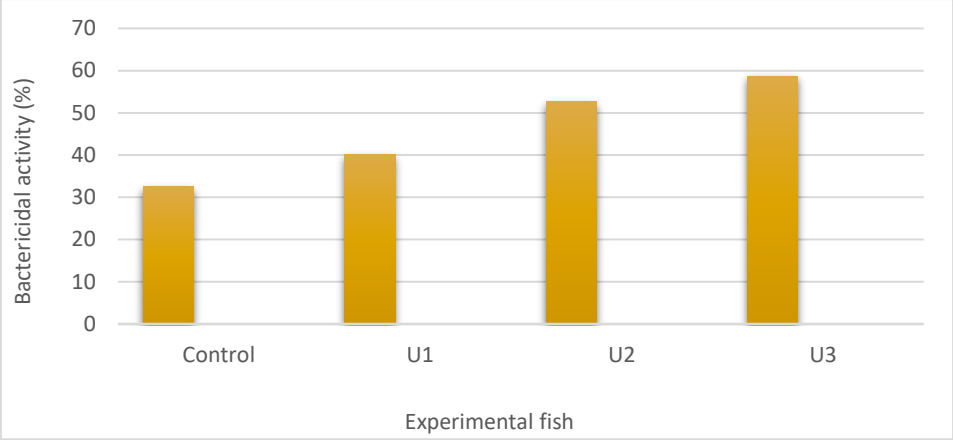

Fig. 5. Bactericidal activity in the mucus of control and experimental fish.

STATISTICAL ANALYSIS

The data obtained in the present investigation were expressed as mean ± SD and were analysed using one-way ANOVA test followed by Tukey's test at 5 % significant level.

DISCUSSION

Seaweeds contain significant amount of Vitamins, minerals, omega-3 fatty acids, and several other micronutrients & macronutrients (Penalver *et al*., 2020). Due to these multiple effects, they are much favoured as a feed additive in the aquaculture sector. *Ulva* species have an appreciable amount of protein, pigments, minerals, and vitamin C (Ortiz *et al*., 2006). It has attracted much in recent years as a feed additive for fish. Seaweed extracts, in general, when added as a feed additive in fish feed, render a positive effect, but in some cases there are reports indicating an improper growth rate compared to the control (Felix and Alan Brindo, 2014). Similarly, in the present investigation, *U. lactuca* sap coated as a feed additive resulted in a decline in weight gain in (U1, U2 & U3) treated groups, i.e., 0.86 ± 0.02 g, 0.64 ± 0.03 g, and 0.57 ± 0.01 g, compared to an elevated weight gain of 1.02 ± 0.03 g observed in control diet fed groups. *Ulva* species have a significant amount of vitamin C (Ortiz *et al*, 2006; Gracia Casal *et al*, 2007; Dermid & Stuercke, 2003) that plays a significant role in antioxidant activity. Accordingly, in the present investigation an enhanced mucosal immune parameters in *U. lactuca-treated* groups were noticed . For instance, an improved lysozyme activity of 0.82 ± 0.04 U/ml, 1.460 ± 0.07 U/ml and 3.425 ± 0.04 U/ml were noticed in U1, U2 & U3 treated groups compared to a low lysozyme activity of 0.450 ± 0.02 U/ml noticed in the skin mucus of control groups. Likewise, Alkaline phosphate (ALP) activity in the skin mucus was remarkably high in *U. lactuca-treated* groups, i.e., 1.814 ± 0.06 OD, 1.502 ± 0.01 OD and 1.235 ± 0.07 OD in U3, U2 & U1 treated groups compared to a low ALP activity of 1.075 ± 0.04 OD noticed in the control diet-fed group.

U. lactuca contains appreciable amounts of saponins, steroids, alkaloids and phenolic compounds that have wound healing and anti-inflammatory reactions (Ardita *et al,* 2021; Wang

et al, 2018; & Kim *et al,* 2011). Due to these compounds, they possess antioxidant properties (Guo *et al*, 2018; Aslan *et al*, 2019).Green macroalgae *Ulva rigida* holds a sulphated polysaccharide with an enormous amount of biological activities (Cunha and Grenha 2016). There are evidences suggesting the antibacterial and immune-enhancing properties of the hot water extract of *U. rigida* (Shannon and Abu-Ghannam 2016). Antibacterial activity of seaweed can be attributed to its phytochemical content (Anjali *et al*., 2019; Deepitra *et al*., 2021). In parallel with the above findings in the present study inclusion of *U. lactuca* sap in koi carp diet resulted in an enhanced bactericidal activity of skin mucus against *Aeromonas hydrophila* in U1, U2 and U3 treated groups i.e. a high bactericidal activity of 58.64 ± 1.82% was observed in skin mucus of U3 diet fed fish compared to a low bactericidal activity of 32.46 ± 1.04% noticed in control diet fed groups. Likewise, Karnjana *et al.* (2019) showed a decreased colonisation of *Vibrio* in the shrimp gut by supplementing the ethanolic extract of red algae *Gracilaria fisheri* in the shrimp diet.Due to the multiple benefits of seaweeds, they have been extensively used as a nutritional food supplement and as a traditional medicine (Lyu *et al*, 2017). In addition, they have some peculiar compounds that make them a good option for industrial applications. In addition, their cheap, non-toxic properties made them a good candidate alternative to synthetic compounds. In this view, there are several reports indicating that the inclusion of seaweeds in diets has rendered control against bacterial and viral infections (Arvinda Swamy *et al,* 2011; Gheda *et al,* 2016). Similarly, in the present scenario, the addition of *U. lactuca* sap in the Koi carp diet has shown a positive effect on fish skin mucus, rendering a better bactericidal activity compared to control treated groups.

CONCLUSION

The results inferred that *U. lactuca* sap supplemented as a feed additive in koi carp diet at different concentrations resulted in a decreased growth performance, but in parallel, increased the skin mucosal immune parameters. So, in future investigation, *U. lactuca* sap may be treated with growth promoters, expecting better growth and immunity in koi carp.

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