



Effect of *Ulva lactuca* and *Sargassum cinereum* Supplemented Diets on Growth Performance of Koi Carp (*Cyprinus carpio* Linnaeus, 1758) Fry in Laboratory Conditions

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ABSTRACT

There is a potential to formulate cost effective aquafeeds using seaweeds in the littoral zone of the coastal belt of Sri Lanka. This study was designed to evaluate the effect of seaweed supplemented diets on the growth of Koi carp. The experimental set up consisted of a control (C) and two experimental treatments (T₁ and T₂). The control diet (without seaweed) and the two test diets, of which 30% of the weight of control diet was replaced by *Ulva lactuca* and *Sargassum cinereum* were fed to Koi carps in C, T₁ and T₂ respectively. Proximate analyses were carried out for both ingredients, and the diets were prepared following standard methods. Koi carp in fry stage belonging to the same cohort (weight 2.20±0.08 g, length 3.89±0.04 cm) were assigned to C, T₁ and T₂ in fiber tanks filled with 150L of water (n=20 per tank) with four replicates each. The fish were fed 5% of their body weight twice a day (09.00h and 15.00h) for 14 weeks. The length and weight measurements were recorded once in two weeks. Fish performance was compared using Body Weight Gain (BWG), Daily Growth Rate (DGR), Relative Growth Rate (RGR), Feed Conversion Ratio (FCR), Feed Efficiency (FE), Fulton's Condition Factor and survival rates. At the end 14th week of the experiment, mean values of the weight, length, BWG, DGR showed a significant difference ($p<0.05$) among treatments. Tukey test revealed that FCR of the T₂ and T₁ were significantly different ($p<0.05$) from the Control and FE of T₂ was significantly different ($p<0.05$) from the C. Condition Factor and water quality parameters did not show a significant difference ($p>0.05$) among treatments. There was a 100% survival rate in all groups. Aquafeeds supplemented with seaweeds showed promising results in terms of increasing growth rates of young Koi carps.

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INTRODUCTION

The ornamental carp *Cyprinus carpio* Linnaeus, 1758 commonly known as Koi, is very popular in the freshwater ornamental fish industry. The marketable size of the Koi carps is considered as over 4 g which can be obtained during 3-5 months of growing period (Jha and Barat, 2005; Yeşilayer *et al.*, 2011). The type of diets that used for feeding the fish plays an important role in the aquaculture industry (FAO, 2017). Nutritional requirements of ornamental fish are important in maintaining colouration, breeding, health, and growth (Sales and Janssens, 2003).

Different food types including herbs have been tested for better growth and immunity of ornamental fish industry (Madhusudan *et al.*, 2011; Radhika and Mohaideen, 2017). Production of cost-effective nutritionally balanced diets of fish is the main factor affecting intensive aquaculture due to its influence on growth, health and production cost (Yeşilayer *et al.*, 2011). It has been reported that different seaweed types have been utilized in feed preparation in the ornamental fish industry in other countries due to their growth promoting and antimicrobial activity (Vallinayagam *et al.*, 2009; Raghunathan *et al.*, 2014; Abdel-Warith *et al.*, 2016; Rizzo *et al.*, 2017). Seaweeds or marine algae are one of the abundant natural resources in marine ecosystems classified as red algae (Rhodophyta), brown algae (Phaeophyta) and green algae (Chlorophyta), depending on their nutrient composition and chemical composition. They are rich in proteins, vitamins, minerals, iodine, bromine and bioactive substances (Christobel, 2008; Domettila *et al.*, 2013; Parmar *et al.*, 2016). Utilization of seaweed as supplementary material for preparation of diets in ornamental fish industry is not popular in Sri Lanka (Coppejans *et al.*, 2009; Tacon and Metian, 2013). Therefore, the objective of the current study was to determine the effect of two types of seaweeds; *Ulva lactuca* and *Sargassum cinereum* supplemented diets on growth performance of fry stage Koi carp (*Cyprinus carpio* Linnaeus, 1758).

METHODOLOGY

Ethical Approval

Ethical clearance for the study was obtained from the Institute of Biology (IOB), Sri Lanka (Permit No: ERC IOBSL 181 11 18).

Formulation of Test Diets

Seaweeds were collected from the Kamburugamuwa beach (5° 56' 22.64996" N, 80° 29' 51.19994" E) located on the southern province, Matara, Sri Lanka. The collection was thoroughly washed with tap water to remove extraneous materials. Then the samples were sun-dried, fine-milled and sieved (mesh size = 500 µm) in order to remove the fibrous portion. Control diet was prepared using the ingredients shown in Table 1.

Table 1: Ingredients used in the formulation of Control diet

Ingredients	Amount (g kg ⁻¹ of diet)
Fish meal	415.72
Soybean	415.72
Coconut meal	77.78
Wheat flour	77.78
Vitamin and Mineral mixture	13.00

Ingredients were weighed using electronic balance (Mettler PE 3600) and mixed using Kenwood mixer. Gelatin was used as a binding agent in different formulations. Based on the previous findings (Zhou *et al.*, 2011; Anh *et al.*, 2018; Perez-Velazquez *et al.*, 2019), 30% of the weight of the control diet was replaced by the *Ulva lactuca* and *Sargassum cinereum* powder to prepare test diets T₁ and T₂ (Table 2). The protein level of 40% was maintained for formulation both the test diets and the control diet using the Pearson square method (Wagner and Stanton, 2012). The pellets (2 mm diameter) were prepared by pressing the mixture and dried in a drying cabinet (40°C) until moisture dropped around 10%. The dried pellets were manually broken into small pieces to facilitate to consumption and were packed in sealed polythene bags before storing in a refrigerator at 4 °C.

Table 2: Types of diets and ingredients used in the preparation of each diet

Types of diets	Ingredients
Control diet (C)	Fish meal, Wheat flour, Coconut meal (Coconut poonac), Soybean, Vitamin & mineral mixture and gelatin
<i>Ulva</i> diet (T ₁)	70% Control diet + 30% <i>Ulva</i> sp. Powder
<i>Sargassum</i> diet (T ₂)	70% Control diet + 30% <i>Sargassum</i> sp. Powder

Proximate Analysis of Dietary Ingredients and Diets

Proximate analyses of experimental diets and ingredients were performed for dry matter, ash, crude protein, lipid using standard methods described by AOAC (2000) and Ergün *et al.* (2010).

Experimental Design and Setup

The research was carried out at the animal house of the Department of Zoology, University of Ruhuna under the controlled laboratory conditions. The experimental setup consisted of 12 circular fiber tanks of 216 L capacity (79.5 cm in depth, 43.5 cm in width) with a centrally located outflow. Each fiber tank was filled with previously aerated water up to 30 cm level to get 150 L capacity of water. The experimental set up consisted of two treatments (T₁ and T₂) and a control group (C) in four replicates arranged in Completely Randomized Design. A random number table was used for random assigning of tanks.

Fish Tanks and Sampling

Koi carp fry belonging to the same cohort were purchased from Ornamental Fish Breeding and Training Center (NAQDA), Rambadagalla. Fish were acclimatized while feeding a commercial diet (Prima aquafeed - Crude Protein 36%) for 2 weeks. After the acclimatization period, each tank was stocked with 20 fry. Before assigning fry into tanks initial length and weight of each fish were taken. The average weight and length of the fry were 2.20±0.08 g and 3.89±0.04 cm, respectively. Then fish in untreated control tanks (C), *Ulva* treatment (T₁) and *Sargassum* treatment (T₂) were fed with prepared diets approximately 5% of their body weight daily twice a day (two rations at 09.00h and 15.00h) for 14 weeks. Their weight and length measurements were recorded once in two weeks using electronic balance (Mettler PE 3600) and measuring board. Continuous aeration was

maintained throughout the experimental period. Tanks were siphoned to remove fecal matter of fish at the end of each day during the experimental period.

Evaluation of Fish Performance

Body Weight Gain (BWG) (Yigit *et al.*, 2002), Specific Growth Rate (SGR) (Yesilayer *et al.*, 2011), Daily Growth Rate (DGR) (Nekoubin and Sudagar, 2012), Relative Growth Rate (RGR) (Yesilayer *et al.*, 2011), Feed Conversion Ratio (FCR) (Yılmaz *et al.*, 2013), Feed Efficiency (FE) (Yigit *et al.*, 2002) and Fulton's Condition Factor (Froese *et al.*, 2006), Survival Rate (Goure-bi *et al.*, 2018) were calculated to evaluate the fish performance.

Physicochemical Parameters of Water

Temperature, pH, conductivity, dissolved oxygen (Montgomery *et al.*, 1964), NO₃⁻ (Monteiro *et al.*, 2003) and PO₄³⁻ (Murphy and Riley, 1962) were measured in treatment tanks twice a week during the course of the experiment.

Statistical Analysis

The data were analyzed using Minitab 17.0 statistical package and Microsoft Excel 2016 for Windows 8.1. The One-way ANOVA and Tukey multiple range test were used to compare the differences between treatments and control at the significance level of $p=0.05$.

RESULTS AND DISCUSSION

The average weight and length of the Koi carp fry were 2.20±0.08 g and 3.89±0.04 cm, respectively. The results of the proximate analyses of dietary ingredients and experimental diets are presented in the Table 3 and Table 4, respectively.

Table 3: Summary of proximate analysis of dietary ingredients

Proximate Analysis	Ingredient					
	Fish meal	Soybean	Coconut meal	Wheat flour	<i>Ulva</i> powder	<i>Sargassum</i> powder
DM (%)	78.0±0.2 ^d	88.0±0.0 ^a	88.1±0.0 ^a	87.5±0.5 ^a	83.5±0.0 ^c	86.2±0.1 ^b
Ash (%)	16.5±0.1 ^c	4.2±0.0 ^e	7.8±0.1 ^d	1.6±0.0 ^f	43.8±0.3 ^a	35.7±0.2 ^b
Crude Protein (%)	57.6±0.1 ^a	30.6±0.1 ^b	20.7±0.1 ^c	15.4±0.0 ^d	20.0±0.3 ^c	12.8±0.3 ^e
Lipid (%)	13.5±0.0 ^a	2.7±0.1 ^e	6.8±0.1 ^c	3.7±0.0 ^d	8.6±0.1 ^b	3.6±0.0 ^d

Values are means ± SEM (n=3). Different letters in same row indicate significant differences within groups ($p<0.05$).

Table 4: Types of diets and proximate compositions of experimental diets

Proximate Analysis	Diet		
	C	T ₁	T ₂
Dry Matter (%)	90.0±0.1 ^b	92.7±0.2 ^a	92.1±0.4 ^a
Ash (%)	23.1±0.1 ^c	38.2±0.3 ^a	26.7±0.2 ^b
Crude Protein (%)	36.3±0.6 ^a	24.1±0.3 ^b	21.3±0.0 ^c
Lipid (%)	7.0±0.0 ^a	4.6±0.1 ^c	5.6±0.2 ^b

Values are means ± SEM (n=3). Different letters in same row indicate significant differences within groups ($p < 0.05$).

At the end of the 14 weeks of experimental period, mean weight, mean length, mean weight gain and

daily growth rate showed a significant difference ($p < 0.05$) among treatments (Figure 1).

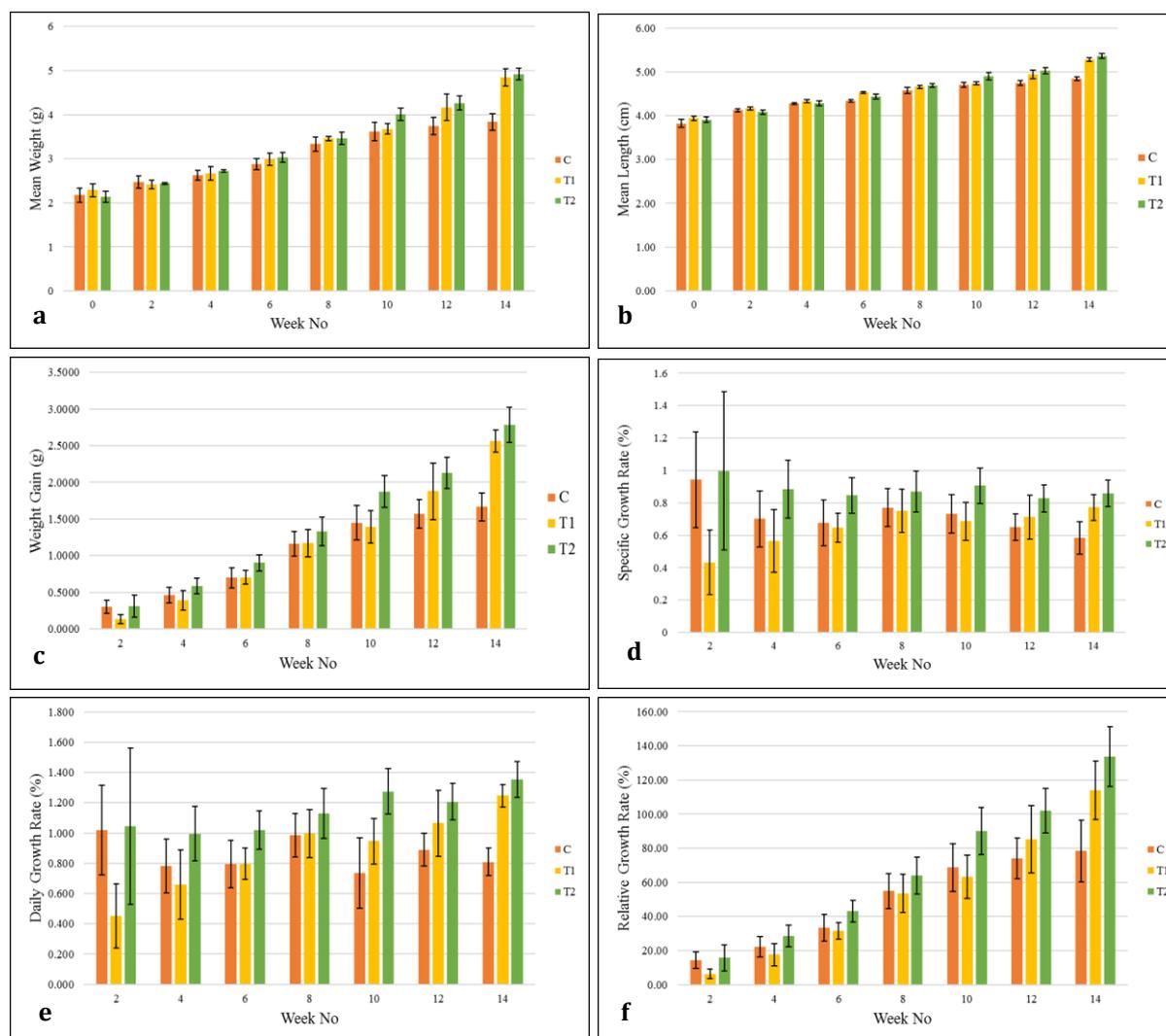


Figure 1: Fluctuation of mean values of different growth parameters (a-f) of Koi carps fed with control and two experimental diets during 14 weeks. Note: a - Body weight, b - Body length, c - Body weight gain, d - Specific growth rate, e - Daily growth rate, f - Relative growth rate values of Koi carps fed with control and two experimental diets during 14 weeks

Estimation of Growth Performance of Koi Carps in Three Treatment Groups

Tukey test revealed that FCR of T₂ and T₁ were significantly different ($p < 0.05$) from C (Figure 2a) and FE% of T₂ was significantly different ($p < 0.05$) from C (Figure 2b). At the beginning of the experiment, the mean Fulton's condition factor in the Control group was slightly higher than that in

Ulva and *Sargassum* treatments. During the experimental period of 14 weeks, mean values in the control and two treatments have shown random fluctuations. At 2nd and 3rd weeks, *Sargassum* treatment has obtained the highest mean Fulton's Condition Factor values. A similar pattern of fluctuations can be observed in the 8th, 10th, 12th, 14th weeks reflecting random variations in survival, reproduction, maturity and health of fish (Figure 3).

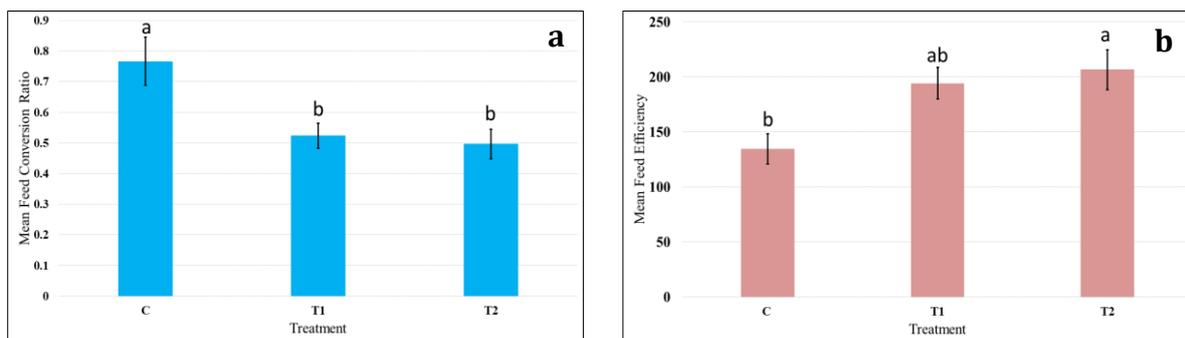


Figure 2: (a) Mean Feed Conversion Ratio; (b) Mean Feed Efficiency values of Koi carps fed with control and two experimental diets during 14 weeks

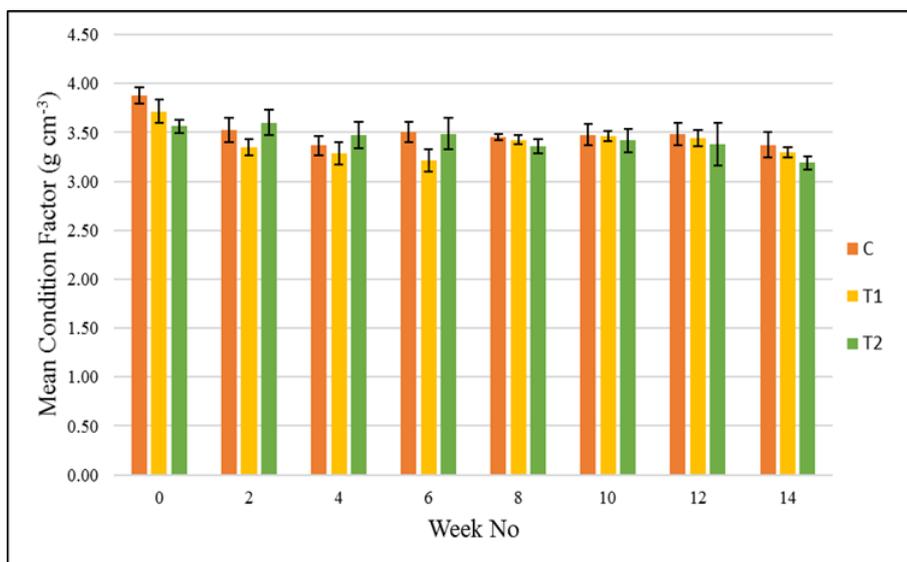


Figure 3: Fluctuation of mean Fulton's condition factor values of control and two treatment tanks during the 14 weeks of experimental period

The summary of the results of some selected growth parameters and survival rate of Koi carp in control and two treatment groups for the 14 weeks period are given in Table 5. The mean values of Temperature, pH, Conductivity, Dissolved Oxygen (DO), Nitrate and Phosphate were normally distributed. Therefore, the one-way ANOVA revealed that there was no significant difference

($p > 0.05$) among C, T₁, and T₂ treatments during the 14 weeks period (Table 6).

In the proximate analysis of dietary ingredients, dry matter content ranged from 78% - 88%. When the experimental diets were considered, the dry matter content varied from 90% - 92.72%. Samuelson and Oterhals (2016) suggested that the moisture content is important to pellet hardness,

Table 5: Growth parameters and survival rate (Mean ± SEM) of juvenile Koi carp (*Cyprinus carpio* Linnaeus, 1758) fed with control and two experimental diets

Growth indices	Treatments		
	C	T ₁	T ₂
Initial length (cm)	3.82±0.09 ^a	3.94±0.05 ^a	3.91±0.06 ^a
Final length (cm)	4.84±0.04 ^b	5.28±0.04 ^a	5.36±0.06 ^a
Initial weight (g)	2.17±0.16 ^a	2.29±0.15 ^a	2.13±0.13 ^a
Final body weight (g)	3.84±0.38 ^b	4.85±0.39 ^a	4.92±0.27 ^a
Feed Conversion Ratio (FCR)	0.77±0.08 ^a	0.52±0.04 ^b	0.50±0.05 ^b
Feed Efficiency (FE)	134.63±13.76 ^b	194.20±14.32 ^{ab}	206.54±17.95 ^a
Fulton's Condition Factor	3.52±0.06 ^a	3.41±0.06 ^a	3.43±0.05 ^a
Survival Rate (SR%)	100±0.00 ^a	100±0.00 ^a	100±0.00 ^a

Means followed by same letters in a row are not significantly different at $p=0.05$ level.

Table 6: Water quality parameters (Mean ± SEM) of control and two treatment tanks

Parameter	Treatment		
	C	T ₁	T ₂
Temperature (°C)	27.80±0.10 ^a	27.90±0.10 ^a	27.90±0.10 ^a
pH	8.09±0.02 ^a	8.05±0.06 ^a	7.96±0.03 ^a
Conductivity (µS)	126.43±1.68 ^a	129.07±1.04 ^a	131.65±1.57 ^a
DO (mg/L)	7.47±0.14 ^a	7.90±0.47 ^a	7.63±0.26 ^a
Nitrate (mg/L)	0.15±0.01 ^a	0.16±0.01 ^a	0.16±0.02 ^a
Phosphate (µg/L)	6.85±0.21 ^a	6.70±0.21 ^a	7.25±0.31 ^a

Means followed by same letters in a row are not significantly different at $p=0.05$ level.

physical pellet quality, specific mechanical energy, bulk density and oil adsorption capacity of fish feed. However, Halver and Hardy (2002), pointed out that the high moisture contents in fish diets lead to microbial growth on feed and tanks. Bhuiyan *et al.* (2018) showed that moisture content of fish feed ingredients varied from 5% - 13.28%. Therefore, ingredient moisture should be lowered in future studies in order to achieve optimal moisture contents in fish feed formulations.

According to the experimental results, it was revealed that at the end of the experiment at 14th week, the Koi carps in the seaweed supplemented treatments (T₁ and T₂) have achieved a significantly high ($p<0.05$) mean weight, mean length compared to the Control group (Table 5). Between two experimental diets, *Sargassum* treatment has achieved higher growth parameters during the experimental period and performed significantly better at the 14th week of the experiment in terms of mean weight, mean length. It was also found that at the 14th week the DGR and BWG of fish fed with *Sargassum* diet was significantly different ($p<0.05$) from the Control

group. Throughout the experimental period of 14 weeks *Sargassum* treatment (T₂) has maintained the highest DGR and BWG (Figure 1). Koi carps in the *Sargassum* treatment also have shown a higher SGR and RGR than the Control group during the period. This could be due to the high palatability of *Sargassum* supplemented diet.

Ulva treatment has also shown the second highest overall growth performance, but *Sargassum* treatment has indicated the highest overall growth performance during the experimental period (14 weeks). Thirty percent of *Ulva* powder was incorporated with the standard diet in diet formulation. It seems that high levels of *Ulva* incorporation in the diet leads to reduce the growth performance of Koi carps as they have shown retardation of growth performance compared to *Sargassum* treatment.

A similar study was carried out by Abdel-Warith *et al.* (2016) by incorporating green macroalgae *Ulva lactuca* as a feed supplement in diets on growth performance, feed utilization and body composition of the African catfish (*Clarias gariepinus*). They suggested that fish fed with a diet

containing 20% and 30% *Ulva lactuca* meal had poorer growth performance and feed utilization than those fed with diet containing 10% *Ulva lactuca* meal. It may be due to the decrease in palatability of the diets with a high amount of *Ulva lactuca*. Presence of food particles in the *Ulva* treatment tanks observed in the present study also supports the fact that fish preferred *Sargassum* diet over *Ulva* diet. According to these observations, it could be suggested that, if a lower percentage of *Ulva lactuca* had been incorporated into the basal diet, it would have performed even better than the *Sargassum* treatment. According to the suggestions of Azaza et al. (2008), there is a possibility for diminished growth at higher levels of *Ulva* meal (30%) due to the high fiber content which could affect on digestibility of protein and dry matter. It is possible that fiber structures reduce the accessibility of intestinal enzymes to food nutrients such as starch, protein, and lipids and thus act as physical barriers between nutrients and digestive enzymes in the intestine (Potty, 1996), thereby making the enzymes less active.

Zamannejad et al. (2016) carried out a study on the effect of supplementation of *Sargassum ilicifolium* on growth, survival and body composition of rainbow trout (*Oncorhynchus mykiss*) and revealed that 5% and 7% replacement of *Sargassum ilicifolium* in the basal diet made significant effects on feed conversion ratio, specific growth rate, daily growth rate, weight gain, condition factor and survival rate values. Zamannejad et al. (2016) clearly showed that dietary incorporation of *Sargassum ilicifolium* meal up to 7.5 % of basal diet was sufficient to meet the essential requirements for the growth of Rainbow trout. Makkar et al. (2016) suggested that *Sargassum* spp. could be introduced at up to 30% in the farm diets without depressing intake, growth performance and diet digestibility. Based on the results obtained from the present study it can be suggested that for Koi carp juveniles, 30% replacement of *Sargassum cinereum* of the basal diet was successful.

Koi carps in the *Sargassum* treatment have recorded the highest feed efficiency and the lowest feed conversion ratio (Table 5 and Figure 2). Given the high cost involved in feeding, the feed

conversion ratio which is the amount of feed it takes to grow a kilogram of fish, is an important factor for the producer (Craig et al., 2017). Therefore, if a feed has a low feed conversion ratio, it takes less feed to produce one kilogram of fish than a feed with a higher feed conversion ratio. Thus, low feed conversion ratio, indicates that the quality of feed is high (Tuominen, 2003). The acceptability, palatability, and digestibility of feeds vary depending on the ingredients and the quality of the feed. Fish farmers pay special attention to feeding activities to determine feed acceptance, calculate feed conversion and efficiency rates, monitor feed costs, and track feed demand throughout the year (Craig et al., 2017). Therefore, *Sargassum* supplementation in fish feed could be an effective option in formulating nutritionally balanced diets for Koi carps.

CONCLUSION

Supplementation of diets of Koi carps with *Sargassum* and *Ulva* showed beneficial effects on their growth indices where *Sargassum* supplemented diet performed better than that of *Ulva* supplemented diet. Considering the high palatability and low feed conversion ratio indicating the high feed quality shown it can be concluded that the *Sargassum* supplemented diet is more beneficial than the *Ulva* supplemented diet for juvenile Koi carp. The results showed the potential use of seaweeds as a dietary supplement or in developing low-cost diets in aquaculture programmes. Further studies are suggested to incorporate other substances (such as digestive enzymes) when formulating seaweed supplemented diets to increase the effectiveness of the diets.

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