Effects of supplementation of algae (*Sargassum ilicifolium*) on growth, survival and body composition of rainbow trout *Oncorhynchus mykiss*

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Abstract

The effects of substituting dietary protein sources with different levels of *Sargassum ilicifolium* on growth, survival and body composition of rainbow trout *Oncorhynchus mykiss* were investigated over the course of a 60 day experiment. A total of 360 juveniles (75±2.8 g) were randomly allotted to four treatment groups including (control: with 100% basal diet (BD); T_1: 5% sargassum meal (SM)+95% BD; T_2: 7.5% (SM)+92.5% BD and T_3: 10% (SM)+90% BD. Each treatment group was divided into three replicates of 30 fish per replicate. At the end of the experiment, our results showed that replacement of 5% and 7.5 % of basal diet with sargassum meal showed significant differences in average weight and total length, feed conversion ratio (FCR), specific growth rate (SGR), weight gain percent (WG), condition factor (CF) and survival rate (SR). There were significant differences between calorie content of carcass in T_2 and control with T_1 and T_3 (p<0.05). The highest values of carcass protein content were observed in T_1 (p<0.05). There were significant differences between lipid content of fish in T_2 and control with that of fish in T_1 and T_3 (p<0.05). The highest values of carcass ash content were observed in T_1, (p<0.05) which were not significantly different from that in other treatments (p>0.05). Levels all of amino acids were higher in fish in T_2.

Keywords: Protein sources, *Sargassum ilicifolium*, Growth, Survival, Body composition, Rainbow trout

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Introduction

Rainbow trout is one of the most widely cultured species in the world, including Iran. Due to the rapid expansion of rainbow trout culture in several parts of Iran, the demands for commercial diets have increased as well. At present, fish meal is used widely as an integral part of commercial diets to provide the protein requirements of cultured rainbow trout. Nevertheless, the use of fish meal is very expensive and increases the costs of rainbow trout aquaculture. During the past decades, numerous attempts have been carried out to substitute fish meal with other inexpensive complementary resources including protein resources such as soybean (Hardy, 1982; Webster *et al*., 1992; El-Dahhar and El-Shazly, 1993; Pongmaneerat and Watanabe, 1993; Oliva-Teles *et al*., 1994; Kaushik *et al*., 1995), maize gluten meal (Wu *et al*., 1995), lupins (Fontainhas-Fernandes *et al*., 1999), rapeseed (Davies *et al*., 1990), cottonseed meal (Rinchard *et al*., 2002), corn gluten (Moyano *et al*., 1992; Robaina *et al*., 1995; Jahanbakhshi *et al*., 2012), canola meal (Yurkowski *et al*., 1978; Hardy and Sullivan 1983; Lim and Klesius 1998; Thiessen *et al*., 2004; Abbas *et al*., 2008). In addition to these sources, *Dunaliella salina* meal has been found to be one of the best substitutes for fish meal in the diets of some cultured fish species especially rainbow trout (Bagheri, 2009). These studies have reported that the dietary fish meal could be replaced with various levels of Sargassum meal with significant differences in growth and survival parameters with that in the control group. To our knowledge, there was no information on the possible replacement of sargassum meal as a complement in rainbow trout diet. To this respect, in this study, we investigated the possibility of using different levels of sargassum meal as a constituent complement source in rainbow trout diets. Such studies could be useful for the recognition of cost-effective amino acid complementary sources for rainbow trout.

Materials and methods

The experiment was carried out for a period of 60 days at the Fisheries Research Center, Khojir, Iran. A total of 360 rainbow trout juveniles (75±2.8 g) were randomly allotted to four treatment groups with three replicates of 30 fish per replicate including (control: basal diet (BD); T_1: 5% sargassum meal (SM)+95% BD; T_2: 7.5 % (SM)+92.5 % BD and T_3: 10 % (SM)+90% BD. The juveniles were distributed into 2400 liter concrete tanks at a stocking rate of 30 fish per tank. During the experiment, the water temperature was 17±1 °C, dissolved oxygen was 8.3±0.81 mg/L and pH 6.3±0.15. The experimental diet was prepared according to National Research Council recommendations for rainbow trout (Table1). To prepare the experimental diet, at first, all ingredients were pulverized and then mixed to homogenize. After that, the homogenized ingredients were mixed

again with some 60 °C water for 30 min in order to shape them. At the end, dry pellets of 6 mm in diameter were made by the pellet-making machine. A feeding rate of 2.4 %/kg body weight was considered for daily feeding on the basis of a standard feeding schedule. Also, the daily feeding frequency during the experiment period was calculated according to Takeuchi *et al*. (1978) as follows:

Table 1: The composition of dietary ingredients in the experimental diets.

Ingredients (%)	Control	T1	T2	T3
Wheat flour	7	5	2.5	0
Soybean floure	28	27.5	27.5	27.5
Fish meal	35	35	35	35
Sargassum meal	0	5	7.5	10
Canola oil	10	10	10	10
Mineral supplementary mixture	0.1	0.1	0.1	0.1
Vitamin supplementary mixture	0.4	0.4	0.4	0.4
Binder	2	1.755	1.755	1.755
Bud wheat flour	17.25	15	15	15
Vitamin C	0.075	0.075	0.075	0.075
Choline chloride	0.25	0.25	0.25	0.25
Total	100	100	100	100

Feeding frequency

$$= 40 \times \frac{\sqrt{W}}{T^{1/1}}$$

Where W refers to the total weight and T refers to water temperature (°C).

To investigate the growth and survival of fish and also determine feeding rate, 4 biometric measurements were carried out at 15 day intervals throughout the experiment. In each biometry, all of the fish were captured and some parameters including average weight and total length, specific growth rate (SGR), weight gain (WG), conditon factor (CF), body weight gain (BWG), daily growth rate (DGR), feed conversion ratio (FCR) and survival rate (SR) were calculated as follows:

SGR= ln BW_F- ln BW_I / t×100 (Helland *et al*., 1996)

Where BW_F refers to the final weight of rainbow trout, and BW_I refers to the initial weight of rainbow trout.

WG= BW_F- BW_I (Ghosh *et al*., 2003)

BWG= BW_F- BW_I / BW_I × 100 (Ghosh *et al*., 2003)

CF= BW/TL^3 × 100 (Lagler *et al*., 1962)

Where BW refers to the weight of rainbow trout, and TL refers to the total length of rainbow trout.

FCR= F/W_F-W_I (Helland *et al*., 1996)

Where F refers to the amount of consumed food.

SR= (total number of alive fish/ total number of fish)×100.

DGR= (BW_F- BW_1)/ T (Helland *et al*., 1996)

Where BW_F refers to the final weight of rainbow trout, and BW_I refers to the initial weight of rainbow trout.

Body composition assays

After the 60 day experiment, 3 fish from each treatment were selected randomly for analysis of body composition. Body composition analysis was carried out according to procedures described by AOAC (1995); total crude protein by Kjeldahls method with the multiplier 6.25; lipid content by Soxhlet method, with petroleum ether as solvent for 8h; total ash content was measured by the mineralization of sample at temperature of 550 °C for 8 hours (Linn Electo-therm Furnace).

Amino acid profile assay

At the end of the 60 day experiment 2 fish from the control group and treatment 2 (amino acid analyses are of high cost in Iran) were selected randomly for amino acid profile assay through HPLC device. Amino acid analysis was carried out by Waters 2487, dual absorbance detector, HPLC in the chemistry laboratory at Atomic Energy Organization.

All data were subjected to one way analysis of variance (ANOVA) and means were separated by Duncan's multiple range test using SPSS software.

Results

Throughout the 60 day experiment, there were significant differences between experimental treatments and control group in terms of weight (Fig. 1), length (Fig. 2), DGR (Fig. 9), SGR (Fig 5), WG (Fig. 6), CF (Fig. 3), SR (Fig. 4), FCR (Fig. 7), BWG (Fig. 8). Accordingly, average weight and total length during 2 months were statistically higher in T_2 and T_1 (p<0.05). In this regard, the vales of DGR, SGR, WG, CF, SR were statistically higher in fish in T_2 and T_1 as compared to that in other experimental groups (p<0.05). The FCR values were statistically different between T_1, T_2, T_3 and that in the control group (p<0.05) (Fig. 5). There were significant differences between carcass calorie (Fig. 10) in fish in T_2 and control and that in T_1 and T_3 (p<0.05). The highest values of carcass protein content were observed in fish in T_1 (p<0.05). There were significant differences between lipid content (Fig. 10) of fish in T_2 and control and that of fish inT_1 and T_3 (p<0.05). The highest values of carcass ash content belonged to fish in T_1 (Fig. 11, p<0.05) which were not significantly different with that in other treatments (p>0.05). Amino acids content in fish in T_2 were higher than that in the control group (Table 2).

Table 2: The Rainbow trout Amino acid profile of control group and T2 after 60 days of experiment (mg/g).

	Amino acid	Content		Amino acid	Content
Control	Asp	16.2	**Treatment 2**	Asp	18.3
	Glu	25.3		Glu	27.6
	Ser	6.9		Ser	7.4
	Gly	9.4		Gly	9.8
	His	6.0		His	6.8
	Arg	11.3		Arg	13.3
	Thr	7.6		Thr	9.8
	Ala	8.8		Ala	10.4
	Pro	5.3		Pro	7.5
	Tyr	5.5		Tyr	6.1
	Val	8.4		Val	9.3
	Met	3.0		Met	3.5
	Cys	0.4		Cys	0.6
	Ileu	6.9		Ileu	7.8
	Leu	13.3		Leu	14.6
	Phe	6.8		Phe	7.3
	Lys	13.5		Lys	16.8

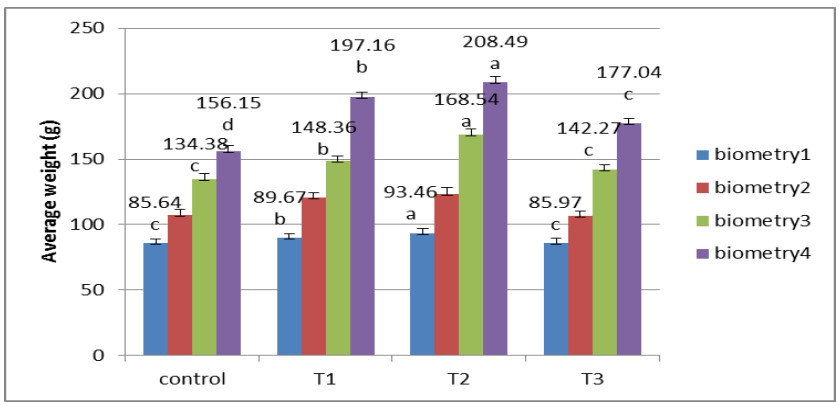

Figure 1: Comparison of weight values between experimental fish groups fed by various levels of sargassum meal in 4 biometry during 2 months (control: with 100%basal diet (BD); T_1: 5% sargassum meal (SM)+95% BD; T_2: 7.5 % SM+ 92.5 % BD and T_3: 10 % SM + 90% BD). Bars (Mean±SD) with different letters are significantly different (p<0.05).

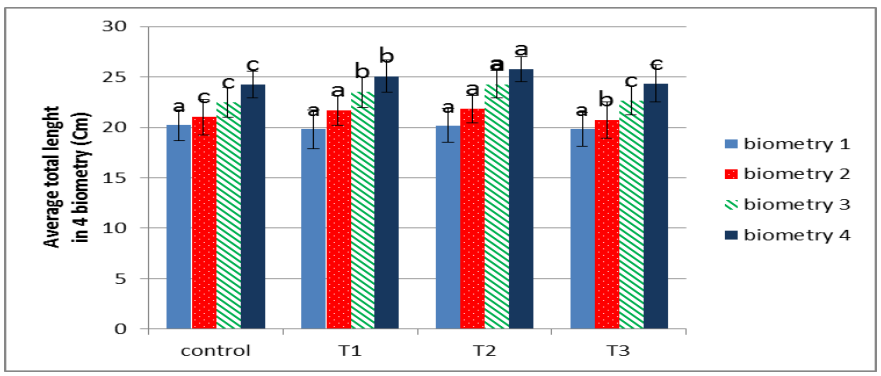

Figure 2: Comparison of total lenght values between experimental fish groups fed by various levels of sargassum meal in 4 biometry during 2 months (control: with 100% basal diet (BD); T_1: 5% sargassum meal (SM)+95% BD; T_2: 7.5 % SM+ 92.5 % BD and T_3: 10 % SM + 90% BD). Bars (Mean±SD) with different letters are significantly different (p<0.05).

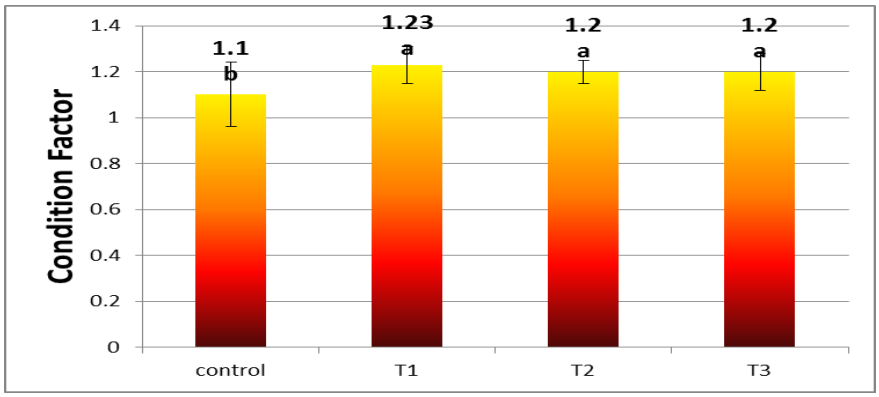

Figure 3: Comparison of CF values between experimental fish groups fed by various levels of sargassum meal (control: with 100% basal diet (BD); T_1: 5% sargassum meal (SM)+95% BD; T_2: 7.5 % SM+92.5 % BD and T_3: 10 % SM+90% BD. Bars (Mean±SD) with different letters are significantly different (p<0.05).

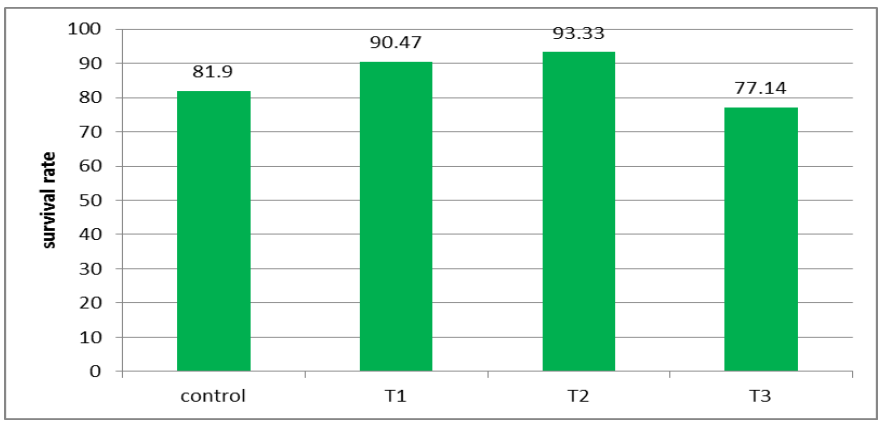

Figure 4: Comparison of SR values between experimental fish groups fed by various levels of sargassum meal (control: with 100% basal diet (BD); T_1: 5% (SM)+95% BD; T_2: 7.5 % SM+ 92.5 % BD T_3: 10 % SM+90% BD). Bars (Mean±SD) with different letters are significantly different (p<0.05).

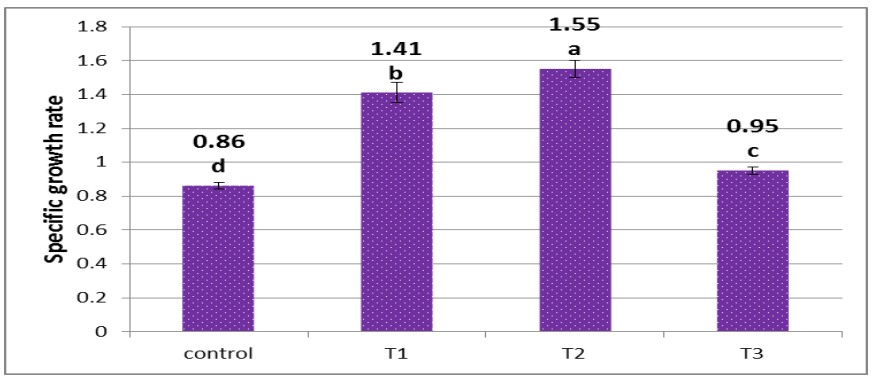

Figure 5: Comparison of SGR values between experimental fish groups fed by various levels of sargassum meal (control: with 100% basal diet (BD); T_1: 5% (SM)+95% BD; T_2: 7.5 % SM+92.5 % BD and T_3: 10 % SM+90% BD). Bars (Mean±SD) with different letters are sargassum meal significantly different (p<0.05).

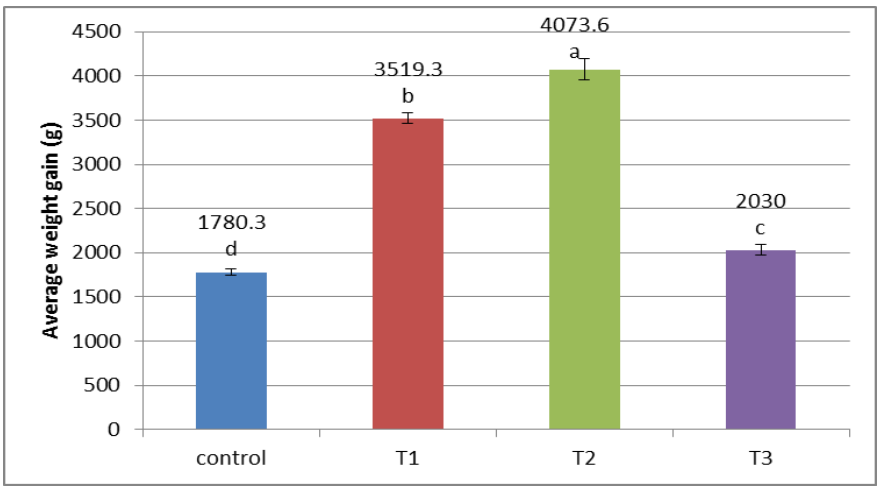

Figure 6: Comparison of WG values between experimental fish groups fed by various levels of sargassum meal (control: with 100 % basal diet (BD); T_1: 5% (SM) + 95% BD; T_2: 7.5 % SM+ 92.5 % BD and T_3: 10 % SM+90% BD). Bars (Mean±SD) with different letters are significantly different (p<0.05).

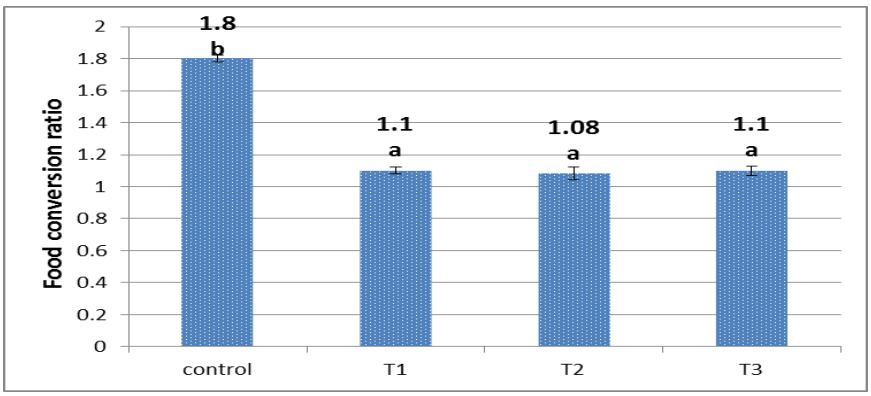

Figure 7: Comparison of FCR values between experimental fish groups fed by various levels of sargassum meal (control: with 100% basal diet (BD); T_1: 5% sargassum meal (SM) + 95% BD; T_2: 7.5 % SM+ 92.5 % BD and T_3: 10 % SM+90% BD). Bars (Mean±SD) with different letters are significantly different (p<0.05). (a) showed highest value.

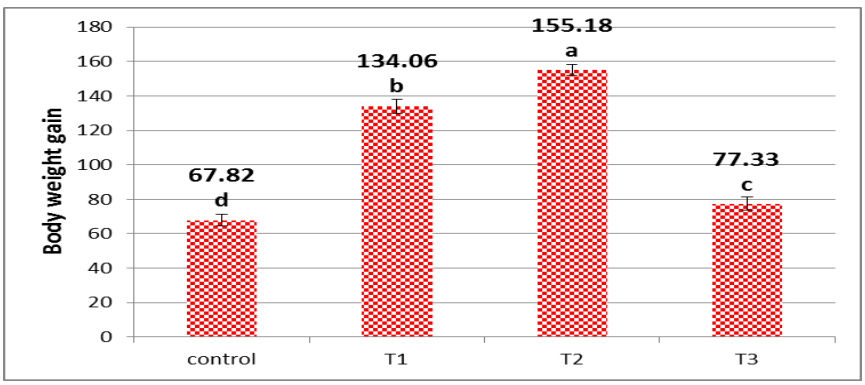

Figure 8: Comparison of BWG values between experimental fish groups fed by various levels of sargassum meal (control: with 100% basal diet (BD); T1: 5% sargassum meal (SM) + 95% BD; T2: 7.5 % SM+92.5 % BD and T3: 10 % SM+90%BD). Bars (Mean±SD) with different letters are significantly different (p<0.05).

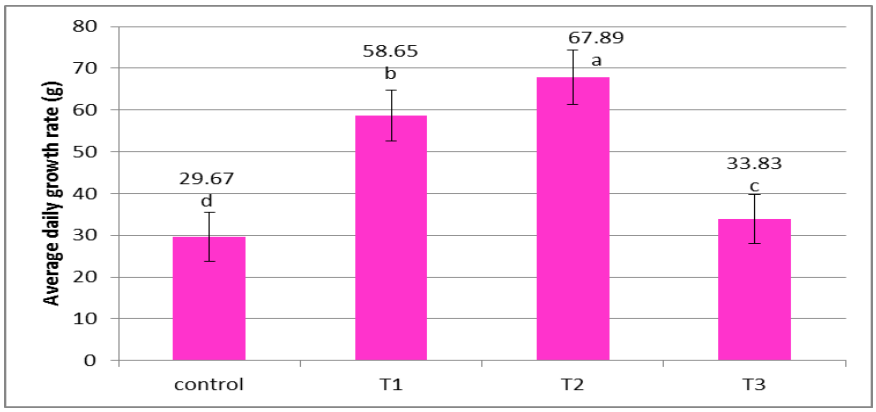

Figure 9: Comparison of DGR values between experimental fish groups fed by various levels of Sargassum meal (control: with 100% basal diet (BD); T1: 5% Sargassum meal (SM)+95% BD; T2: 7.5 % SM+ 92.5 % BD and T3: 10 % SM+90% BD). Bars (Mean±SD) with different letters are significantly different (p<0.05).

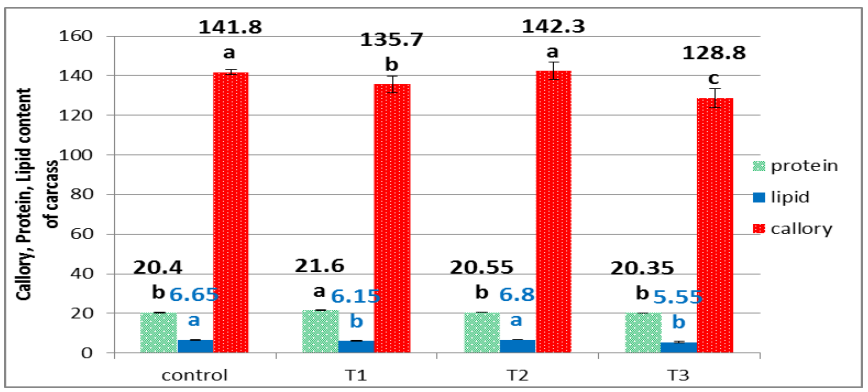

Figure 10: Comparison of Calorie percent values, protein percent values, lipid content values between experimental fish groups fed by various levels of sargassum meal(control: with 100% basal diet (BD); T_1: 5% sargassum meal (SM)+95% BD; T_2: 7.5 % SM+ 92.5 % BD and T_3: 10 % SM+90% BD). Bars (Mean±SD) with different letters are significantly different (p<0.05).

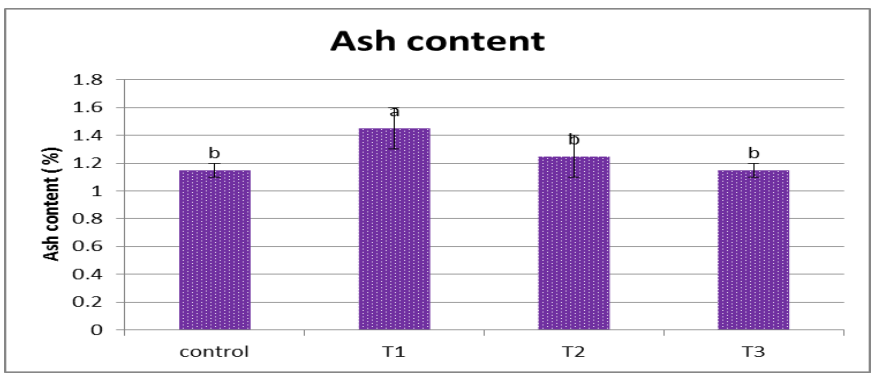

Figure 11: Comparison of Ash content values between experimental fish groups fed by various levels of sargassum meal(control: with 100% basal diet(BD); T1: 5% sargassum meal (SM)+95% BD; T2: 7.5 % SM+ 92.5 % BD and T3: 10 % SM+90% BD). Bars (Mean±SD) with different letters are significantly different (p<0.05).

Discussion

In the aquaculture industry, feed comprises the greatest costs because of the extended reliance on marine animal protein sources such as fish meal to meet the high dietary protein, and amino acids requirements of fish. On other hand, worldwide production of fish meal cannot meet the continuous demands for developing aquaculture. Thus, the use of complementary protein sources is required for fish meal. Several studies have reported useful effects of *Dunaliella salina* meal as feed for rainbow trout (Bagheri, 2009). To our knowledge, there is no information on the effects of sargassum meal on rainbow trout. In this study, we investigated the possible supplementation of different levels of sargassum meal as a protein source substitute for trout fish dietary protein sources. Our results showed that 5 and 7.5 % of BD can be replaced with sargassum meal with significant effects on FCR, SGR, DGR, WG, CF and SR values. In addition, the incorporation of more than 7.5% sargassum meal increased growth and FCR respectively but not as much as that reported in T_1 and T_2 treatments. This clearly indicates that dietary incorporation of sargassum meal up to 7.5 % of BD can adequately meet the essential requirements of rainbow trout for growth. It was found that sargassum meal has an appropriate ratio of essential amino acids, fatty acids and other required compounds for growth including: antioxidants, minerals and vitamins (Mendiola *et al*, 2008; Erulan *et al*., 2009; Abou-El-Wafaa *et al*., 2011; Peng *et al*., 2013). The analyses of the carcass reveal that the dietary sargassum meal protein has been utilized by the fish since the values of protein content were similar to fish fed only BD and those fed a diet containing sargassum meal up to 10 % of BD except T_1. Amino acid profile assay (Table 2) through HPLC device showed higher values in fish in T_2 as compared to the control. It is reported that the body lipid concentration of fish is positively related to the level of dietary lipid and energy (Takeuchi *et al*., 1978). In our study, different lipid levels were observed between experimental treatments especially in T_2, indicating this fact that the sargassum fat probably has important energetic roles for rainbow trout and it was accumulated mostly in the meat. Also, in rainbow trout, it was found that the short chain unsaturated fatty acids of plant fats were utilized for energy demands. The body ash content of rainbow trout increased in T_2 and T_1 treatments. It is reported that the body ash increased in *Cirrhinus mrigala* fed soybean meal, indicating a positive effect on meat quality (Jose *et al*., 2006). In starved fish, the lipid content of body tissues is used for providing energy and fish in T_2 showed significant differences in energy content as compared to that in other groups.

In conclusion, our results indicate that sargassum meal can be incorporated even up to 7.5% of rainbow trout feed formulation with different significant effects on meat quality, growth, survival and FCR of

rainbow trout. Therefore, use of this meal in rainbow trout diets can save some costs of feed preparation and be a good complementary source of dietary protein.

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