AMERICAN UNIVERSITY OF BEIRUT

REPLACING FISHMEAL BY SAFFLOWER MEAL IN THE DIET OF THE MARINE ALGAEVOROUS TELEOST, *SIGANUS RIVULATUS*

by
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AN ABSTRACT OF THE THESIS OF

Nisrine Ali Assaf for Master of Science
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Fish meal has been used for a long time as the main protein source for animal feeds used in aquaculture. Unfortunately, this practice exerts pressure on wild fish resources. In addition, the price of fish meal is in continuous increase. Thus, it is imperative to replace fish meal by plant protein sources to ensure the sustainable success of commercial aquaculture operations. The aim of the present work was to assess the effect of replacing fish meal protein with safflower meal (SFM) on survival, growth, proximate composition, feed efficiency and protein efficiency of the marine herbivore *Siganus rivulatus*. For that purpose, five experimental diets were formulated with an increasing replacement of fishmeal by safflower meal. The percentages of fishmeal replacement were 0, 25, 50, 75 and 100 % for D1, D2, D3, D4 and D5 respectively. Groups of fifteen size sorted fish were stocked in each of fifteen aquaria. Fish were fed at 5% body weight three times daily. Weight gain, specific growth rate, feed efficiency ratio and protein efficiency ratio significantly decreased as the proportion of SFM increased with 100% SFM inclusion resulting in the least growth among treatments. The results showed no significant effect of SFM inclusion on viscerosomatic index, whole body protein, body ash and packed cell volume while Hepatosomatic index and lipid content were significantly different among the five treatments. In conclusion, the replacement of fish meal by safflower meal in the diets of *Siganus rivulatus* led to decreased growth performance and hence it is not suitable to replace fish meal by safflower meal in the diets of this marine herbivore.

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CHAPTER I

INTRODUCTION

A. Statement of the Problem

Aquaculture is the science, art and business of farming aquatic organisms including fish, crustaceans, molluscs and aquatic plants in controlled and selected environment. It is considered the fastest growing sector of global food production as it expanded with an average rate of 6.2% per annum in the period 2000-2012 (FAO 2014). Aquaculture production is steadily increasing, thus relieving stress off global capture fisheries production which has been static at 90 million metric tons per year for the last two decades (Pauly and Froese, 2012). By 2030, the human consumption of fisheries production is predicted to reach 186 million tons (Pauly and Froese 2012). Accordingly, aquaculture is called upon to fill this gap by providing high quality and affordable marine protein for the expanding human population.

Although aquaculture is considered the most promising solution for food security and safety issues, aquaculture practices are subjected to a variety of criticisms. In fact, the feed used in aquaculture has attracted much of the criticism because it depends on marine ingredients usually in the form of fish meal (FM) and fish oil. The usage of wild fish in the production of FM exerts pressure on fisheries resources. This pressure will definitely limit fish meal supply that is being exacerbated by increased global demand. Increased fishing pressures and rising demand for fish meal resulted in a dramatic increase in price (Tacon

and Metian, 2008), which was expected to range between US$ 1200 per ton and US$ 1600 per ton in 2017 (Jane Byrne 2016). If alternatives are not found, the cost of fishmeal will threaten the continued rapid growth of aquaculture production and hence weaken its role in global food safety and security.

Fish meal is an excellent nutritive feed ingredient for several reasons. It is rich in highly digestible proteins characterized by an excellent amino acid profile especially the ten essential amino acids (Watanabe 2002). In addition, it is rich in omega-3 poly unsaturated fatty acids, notably Docosahexaenoic acid (DHA) and Eicosapentaenoic acid (EPA). Moreover, FM is a rich source of essential vitamins like B1, B2, B6 and B12, and minerals such as calcium, phosphorous, magnesium, and potassium (Windsor, 2001). Moreover, fish meal is not known to contain any anti-nutritional factors and is low in carbohydrates (Zhou *et al*., 2004). It contains ingredients that make it palatable to fish and thus allows for high nutrient uptake. These properties have made fish meal the benchmark diet ingredient for the aquaculture industry. Nevertheless, as mentioned earlier, FM is environmentally threatening and economically very expensive.

Because feed is responsible for the majority of operating costs in aquaculture and fish meal is the most expensive feed ingredient, research on partial or total replacement of fish meal by more sustainable and less expensive plant-based diets is imperative. Aquaculture nutritionists are challenged to prepare diets that meet the nutritional and energy requirements of culture species, reduce feeding costs, and minimize environmental impacts with no detriment to fish health and flesh quality. The formulation of these diets requires not only knowledge of the nutritional requirement of the species but also

understanding the nutritional value and digestibility of the ingredients contained in the developed diet.

Diverse plant protein and energy sources such as soybean, cottonseed, sunflower, canola, lupin and linseed are used to prepare fish diets with partial or total replacement of fish meal. However, many of these diets have negative aspects on fish performance because of deficiencies in their amino acid profile and presence of high level of carbohydrates and anti-nutritional factors (Booth *et al*., 2001). Safflower meal (SFM) is a plant based protein source with good potential to replace fishmeal because it is a high protein supplement. It is economically competitive and available in large quantities. Additionally, safflower meal is already being used as a protein ingredient for terrestrial animal feeding.

B. Safflower meal as an alternative to fish meal.

Safflower, *Carthamustinctorius L.*, is a broadleaf annual herbaceous plant that belongs to the kingdom: Plantae, order: Asterales and family: Compositae or Asteracea. It is a draught-tolerant oilseed crop originating from the eastern Mediterranean (Farran *et al*. 2010) and cultivated mainly in tropical and dry regions as it requires a dry atmosphere during flowering and maturation (Knowles, 1975). Yau (2004) noted that safflower can be grown in the Bekaa Plain of Lebanon. Safflower is a diploid (2n=24) plant (Knowles, 1975) that can reach a height of 150 cm. It is a coarse, erect and highly branched herb with up to five yellow, red, orange or white flower heads carried by each branch. Safflower has a strong extensive taproot system that can reach deep-lying water (Aase and Pikul, 2000)

which enables it to be more tolerant to dry climates than small grain (Singh *et al*., 1996). The Safflower (*Carthamustinctorius*) was originally grown for its flowers which were used in making red and yellow dyes for clothing and food preparation. Presently, safflower is primarily cultivated for its oil which is used for food and industrial purposes. Safflower is a minor crop with a world production of about 591,997 tons of seeds in 2011(FAO, 2013). It is resistant to saline (Francois and Bernstein, 1964) and moisture stress (Bassiri *et al*., 1977) conditions. India, USA and Mexico are the major producers of safflower (FAOSTAT 2008). Today, safflower is used to provide four major products: oil, flowers, birdseed and meal (Galicia-González *et al*., 2010).Safflower seed contains 35–50% oil, 15–20% protein, and a 35–45% hull fraction (Betschart, 1975; Rahamatalla *et al* 1988, 2001). Bright white safflower seeds are used as birdseeds for domestic and wild birds as well as for some pets (Peterson, 1996).

The nutritional composition of safflower meal depends on the amount of hull removed and the extent of oil extraction. The oil can be extracted from the seeds by cold pressing, expeller pressing or solvent extraction (GRDC, 2010). The undecorticated (hulled) meal is referred to as whole seed meal whereas the decorticated (dehulled) meal is known as a safflower meal. However, Crude protein content in safflower meal varies from 20-25% in the hulled meal to up to 40% in dehulled meal (Dajue *et al*.,1996; Göhl,1982) whereas crude fiber content varies from 30-40% in undecorticated meal to as low as 10% in dehulled meal (GRDC 2010). Thus, meal sifting and hull removal result in a high protein less fibrous safflower meal (Alvarez-Gonzalez *et al*., 2007). Still, safflower meal has certain disadvantages. Safflower meal is a poor source of lysine and methionine, two essential amino acids (Betschart and Saunders, 1978). The low content of lysine is the main

reason for the overall low nutritive value of the protein (Darroch 1990). The most abundant amino acid in safflower meal is arginine (Galicia-González *et al*., 2010). Safflower meal is an excellent source of phosphorus and a good source of iron and zinc. It has a relatively poor vitamin profile, but when compared to soybean meal it is a good source of biotin, riboflavin and niacin (Darroch 1990). Safflower meal has a slightly bitter taste that makes it less palatable than other common plant meals (Smith, 1996). However, its taste was accepted by ruminants when mixed with other feeds (Göhl, 1982). Early trials showed that beef cattle did not like the taste of safflower meal, but dairy cows found it palatable (Smith, 1996).

C. Anti-Nutritional Properties of SFM

Most plant protein sources contain anti- nutritional factors (ANFs). Anti- nutrients are naturally occurring substances that interfere with feed utilization and affect the health of animals either directly or by their metabolic products (Francis *et al*. 2001). Their biological effects on animals vary from mild reduction in their growth performance to death (Nalle, 2009). Plant meal anti-nutritional factors include protease inhibitors, phytates, glucosinolates, saponins, tannins, lectins, oligosaccharides and non-starch polysaccharides, phytoestrogens, alkaloids, antigenic compounds, gossypols, cyanogens, mimosine, cyclopropenoid fatty acids, canavanine, antivitamins, and phorbol esters. Any of these compounds could have negative effects on fish growth, health and physiology (Ketola, 1982; Sandholm *et al*., 1976; Francis *et al*., 2001; Rumsey and Ketola, 1975). Amino acid digestibility decreases in diets containing tannins due to the binding of dietary tannins with

feed proteins and with digestive enzymes (Bressani *et al*., 1988 and Nalle, 2009). Protease inhibitors (trypsin and chymotrypsin inhibitors) are plant proteins that inhibit proteolytic enzymes of the gastrointestinal tract by forming complexes with the digestive enzymes (Cabrera-Orozco *et al*. 2013). When trypsin is inhibited by active trypsin inhibitors, proteins are poorly digested, and the availability of amino acids is reduced. Lectins (or haemaglutinnins) are proteins that have one or more binding sites for carbohydrates containing glycoprotein found in the plasma membrane of cells (Nalle, 2009). The binding of lectins to cell surface glycoprotein may cause agglutination, mitosis, or other biochemical changes in the cell (Nalle, 2009). When experimental animals were offered diets containing plant lectins, they showed loss of appetite and decreased body weight (Liener, 1989; Duranti & Gius 1997). Oxalate is an anti-nutrient salt formed when oxalic acid binds with different minerals such as calcium, magnesium, sodium and potassium rendering them inaccessible to the body. Calcium oxalate is insoluble and has tendency to precipitate in the kidney forming crystals that play a role in the formation of stones in the kidney (Gemede and Ratta, 2014). The cyanogenic glycosides are derived from the five protein amino acids: Valine, Isoleucine, Leucine, Phenylalanine and Tyrosine and from the non-protein amino acid cyclopentenyl glycine. B-glycosidase activates the function of cyanogenic glycosides to release toxic volatile HCN as well asketones or aldehydes when the plants are attacked by herbivore or pathogen (Gemede and Ratta, 2014). Processing methods such as dehulling, aqueous extraction, extrusion (De Silva and Anderson, 1995), dietary incorporation of enzymes to break down phytate, and the use of genetically modified grains and oilseeds can reduce the negative effects of these anti-nutritional factors.

Ingale and Shrivastava (2011) compared the content of anti-nutritional factors like cyanogenicglucoside, tannin, and oxalate and the hemagglutinin activity in new oilseeds varieties: Sunflower (*Helianthus annuus L.)* variety LSF-11 & LSF-8, Safflower (*Carthamustinctorius L.)* variety PBNS-12 & PBNS-40, and the Groundnut (*Arachishypogaea L.)* variety JL-24. They found that safflower seeds had the least Cyanide content (3.458%) and the smallest oxalate content (0.079%). They also found that the tannin content of safflower seeds ranged from 0.51 to 0.53 %. Furthermore, no trypsin inhibitor activity or hemagglutinin activity was reported in safflower seeds. The protein fraction of the safflower meal contains two phenolic glucosides: the matairesinol-β-glycoside owing to the bitter flavor and the 2-hydroxyarctiin-β-glucoside owing to the cathartic activity. The two glycosides can be removed by extraction with water or methanol, by the addition of β-glycosidase (Darroch, 1990), or by a combination of physical and enzymatic treatments. Additional research is needed to determine anti-nutritional compounds in safflower meal and study their effect on the nutritional value of safflower meals, especially if used as a fishmeal alternative in fish diets.

Although several studies have evaluated the effects of safflower meal in animal diets, few (Nagaraj *et al*., 1990; Alvarez -Gonzalez *et al*., 2007; Ustaoglu *et al*., 2015) tested safflower meal in fish diets. Ustaoglu*et al*. (2015) studied the effect of three inclusion levels (10%, 15%and 20%) of safflower meal in the diet of rainbow trout. The results showed no negative aspects of the three diets on growth performance, nutrient digestibility, feed efficiency and body composition of the rainbow trout. To our knowledge,

there are no published studies that elucidate the effect of inclusion of safflower meal in diets of rabbitfish, *Siganus rivulatus*.

D. Marbled Spinefoot, *Siganus rivulatus*

1. Species' Biology

Siganidae is a relatively small family of marine algaevorous (Suyehiro 1942) fish. This family is composed of one genus *Siganus* and two subgenera: *Siganus* (22 species) and *Lo* (5 species). Siganids are widely distributed in the tropical Indo-West Pacific Ocean (Woodland, 1983). The opening of the Suez Canal in 1869 allowed the migration, Lessepsian migration (Por, 1978), of many marine species from the Red Sea to the Eastern Mediterranean where few native herbivorous fish species existed (Verlaque, 1990).

Siganids are potential candidates for marine aquaculture because of their herbivorous feeding habit (Tacon *et al*. 1990). They feed on low energy and low protein algae in their natural environment (El-Dakar, 2011). Their mouth shape helps them remove algae from in between rock crevices, or coral branches (El-Drawany, 2015) by a nibbling action in which the upper jaw is fixed and only the tip of the mouth can move up or down (El-Drawany 2015). Several rabbitfish species such as *Siganus argenteus*, *Siganus canaliculatus*, *Siganus guttatus*, *Siganus javus* and *Siganus rivulatus* are considered good candidates for mariculture (El-Dakar, 2011).

Two rabbitfishes, the marbled spinefoot, *Siganus rivulatus* and the dusky spinefoot, *Siganus luridus*, out of the five Siganidae species that live in the northwestern

area of Red Sea (Hashem, 1983) invaded the Mediterranean and established themselves successfully in their new environment (Ben-Tuvia 1964). *Siganus rivulatus* is one of the most successful Lessepsian fish (George 1972, Ben-Tuvia 1985, Papaconstantinou 1990a, Bariche *et al*. 2004) having been first recorded in the Levant Basin in 1927 (Tortonese, 1970). The lack of genetic differentiation among Red Sea and Mediterranean *Siganus rivulatus* populations implies that the Lessepsian migration of these species is a continuous process (Bonhomme *et al*.2003). Marbled spinefoot is a warm-water herbivorous (Tacon *et al*., 1990; Bariche 2005) fish that usually browses in schools of dozens or hundreds of individuals (George 1972, Popper and Gunderman 1975) in different overgrown habitats (rocks with algae, sand with algae and grass with algae). It mainly inhabits shallow coastal water (Bariche, 2005) at depths of 1-50 meters which is expected as algae are widespread at these depths. *Siganus rivulatus* changed their diet in their new habitat to adapt to the new algal resources that differ from those found in the Red Sea (Lundberg 1989). It is a selective species when macrophytes are diverse and abundant and non- selective in the cold season (October and November) as they feed on what are available (Lundberg and Lipkin 1993, Lundberg et al. 1999a). Histochemical studies on the intestinal bulb of *Siganus rivulatus* show weak lipid, moderate protein enzyme activities and strong carbohydrate β glucorinidase activity (Rizkalla *et al*., 1988). *Siganus rivulatus* can reach a maximum length and body weight of 32 cm and 318 g respectively (Bariche 2005; Anastasiades 2011) and their sexual maturity is reached at about 107 g (14-16cm) (Hashem 1983). (Bariche *et al*. 2003) noted that the spawning of *S. rivulatus* in Lebanese waters occurred within a temperature range of 24-29˚C but was not observed during the warmest period (30-31˚C).

George (1972) reported that *S. rivulatus* spawned when water temperature reaches 27˚C in the Lebanese coast.

2. Culture Potential

Farmers need to shift their emphasis from fresh water to saltwater culture and from carnivores that feed high on the food web to herbivorous marine species that feed low on the aquatic food chain (El-Dakar *et al*., 2010; Stephanou, 2007). *Siganus rivulatus* is an important marine primary consumer that attracted the attention of aquaculturists since the early 1970s. Herbivorous marine species exhibit better digestibility of plant based diets (Bowyer 2013b) and better energy utilization (Anastasiades 2011) than carnivorous fish which potentially helps in the replacement of fish meal by alternative ingredients from plants. Rabbitfishes hold particular promise for marine aquaculture development (Stephanou, 2007) as they are relatively easy to rear by virtue of their biological potentials and favorable aquaculture characteristics. The herbivorous feeding habit of marbled spinefoot can be altered to accept artificial feed in captivity (Parazo, 1990) and hence they can be omnivorous in culture system (El-Dakar *et al*., 2010). They are euryhaline and eurythermal species that can tolerate wide range of salinity (10 – 50ppt) (Saoud *et al*. 2007) and temperature (17 – 32°C) (Saoud *et al*. 2008b) with an optimal salinity (35ppt) and an optimal temperature (27°C) for growth and survival. Rabbitfish can handle stress and high stocking density (Carumbana & Luchavez, 1979).Saoud *et al*. (2008b) reported that *S. rivulatus* can grow well at high stocking densities (up to 770 fish/m^3). This is could be due to their gregarious nature that suggests little competition among fish reared at high densities. Furthermore, preliminary studies showed that marbled spinefoot is highly tolerant

to nitrite (Saoud *et al*. 2014) and ammonia (Roumieh *et al*. 2013). The marbled spinefoot is an appreciated food item in markets along the Levantine coast (Lebanon, Cyprus, Saudi Arabia) (Anastasiades 2011) because it is tasty and meaty with an average fillet yield of 37 % of body weight (Saoud *et al*. 2008a). In Lebanon, the fish is considered a healthy and affordable food item when comparing the edible yield to its price in market (U.S. $6-7 per kg) (Saoud *et al*. 2008a).

3. Nutrient Requirements for Siganus rivulatus

a. Protein

Protein is the most expensive and significant macronutrient in manufactured fish diets. Protein quality and quantity affect fish growth, health and physiology (Kaushik and Seiliez 2010, Lazo *et al*. 1998). In general, the protein requirement for fish is higher than in terrestrial livestock (National Research Council, 1993) because the fish energy requirements from carbohydrates are lower (Smith, 1989) as fish derive more energy from catabolism of proteins than do terrestrial animals (Brett and Groves 1979). The optimal dietary protein level in a fish feed is influenced by the amino acid composition, the digestibility of the protein and the amount of non-protein energy source in the feed (Halver and Hardy 2002). If the diet does not contain enough non-protein energy, protein is utilized for maintenance rather than growth; however, excess energy limits amino acid intake needed for growth (NRC 1993). Cowey 1979 noted that the optimal protein requirement of the fish can be modified by modifying the energy content of the developed diet. Dietary protein requirement varies depending on the fish species, age, size and water temperature (Halver and Hardy 2002). Although carnivorous fish are assumed to have greater protein

requirement as a proportion of the diet than herbivorous fish (Cowey 1979; Wee and Tacon 1982), all studied siganids seem to have a protein requirement close to that of carnivorous fish (Abou-Daoud *et al*. 2104). Parazo (1990) reported that 35g CP/100g diet was optimal for the growth of *S. guttatus* when dietary energy density was 16 MJ/kg. Tacon*et al*. (1990), Bwathondi (1982) and Ismail *et al*. (1986) found that 31g CP/100g was optimal for *S. canaliculatus* growth. El- Dakar *et al*. (2011) reported that the minimal protein requirement for optimal growth of juvenile *S. rivulatus* is 40 g CP/100g of a diet containing gross energy level 17 MJ/kg. The protein requirement of marbled spinefoot was also studied by Abou-Daoud *et al*. (2014) who found that 43g CP/100g diet was optimal for good growth of juvenile *S. rivulatus* when digestible energy level is 14.2 MJ/kg.

b. Lipid

Proteins and lipids are well utilized sources of energy for fish (Cruz, 1975; Smith, 1976). In formula fish diets, lipid is considered a concentrated, cost-effective energy source. However, dietary lipids have other important nutritional functions as they provide essential fatty acids, phospholipids, sterols and fat-soluble vitamins that play a role in various physiological processes and are used for biological structure and functions of cell membrane (Watanabe 1982; Sargent *et al*. 1989). The addition of lipids to a diet contributes to effective dietary protein utilization by sparing protein and thus improves feed efficiency and growth (Watanabe 1982, Skalli *et al*. 2004). However, growth can also be retarded by excess dietary lipid due to reduced feed consumption (Page and Andrews, 1973; Watanabe, 1982; Ellis and Reigh, 1991). Growth can also be slowed by insufficient dietary lipid because of insufficient digestible energy or deficiencies in essential fatty acids (Ghanawi *et*

al. 2011). Furthermore, excessive lipid intake can result in fat deposition in visceral cavity, liver, and muscle tissues of fish (Wang *et al*., 2005; Martins *et al*., 2007; Song *et al*., 2009).Ghanawi *et al*. (2011) studied the effect of dietary lipid levels on growth performance and body composition of juvenile marbled spinefoot. The authors reported that marbled spinefoot rabbitfish have less dietary lipid requirements than typically cultured carnivorous fish. They noted that in the marbled spinefoot 52.2–68.9% of total body lipids are stored in the viscera with little lipid stored in the muscles (6.4–8.9%) and in the liver (2.1–3.9%). Accordingly, the authors suggested diet formulations that contain a greater protein to energy (lipid) ratio to accelerate growth rates of the marbled spinefoot without excessive fat deposition.

c. Carbohydrate

Carbohydrate utilization among fish species is extremely variable. The potential for incorporation of carbohydrates into the diets of fish depends on the species of fish, source and complexity of carbohydrate and the environmental conditions available (Wilson, 1994; Hemre and Hansen, 1998; Rawles and Gatlin, 1998). For example, water temperature affects carbohydrate digestibility as warm-water fish can utilize higher levels of carbohydrates than cold-water and marine species (National Research Council, 1993). Furthermore, herbivorous and omnivorous fish can utilize carbohydrates better than carnivorous fish (El-Sayed and Garling, 1988). For example, Sabapathy and Teo (1993) reported greater abundance and activity of carbohydrases (amylase, laminarinase, maltase, sucrase and trehalase) in the herbivorous rabbitfish *Siganus canaliculatus* than in the

carnivorous sea bass *Lates calcarifer* and suggested that the higher amylase activity in rabbitfish indicates an important role for carbohydrate in energy metabolism.

E. Aim of present study

The field of finfish nutrition plays an essential and central role in the sustained development of aquaculture. However, the main challenge in this field is to formulate diets that are nutritive, palatable, not expensive and showing good quality of the end product. For this purpose, various studies are performed to replace expensive fish meal used in aquafeeds by plant protein sources. The present study was performed to assess the dietary suitability of safflower meal as partial and total replacement of fish meal in diets of the marine algaevore, *Siganus rivulatus*.

CHAPTER II

MATERIALS AND METHODS

A. Feed Formulation Strategy:

Five experimental diets were formulated based on current data and information about juvenile rabbitfish, nutritional requirements developed at the fish physiology lab at AUB. The diets were designed to contain increasing amounts of SFM and concomitant decreasing amounts of FM. Replacement levels for FM with SFM were formulated at 0,25,50,75, and 100%. All five diets were formulated to be isonitrogenous and isoenergetic such that gross energy level of the each of the diets is 17 MJ/kg. The diets contained 40% crude protein and 4.5% crude lipid content on a dry-matter basis. The diets were supplemented with crystalline amino acid (methionine and lysine) as per typical fish requirement. The prepared diets were kept isoenergetic by adjusting the starch and cellulose contents in the diets. The diets water stability was improved by adding α-cellulose. All ingredients were thoroughly mixed using a dough mixer before the gradual addition of gelatin dissolved in boiling water. The mixture was then cold pelleted through a meat mincer fitted with a 3.5mm die. After pelleting, diets were dried in a forced-air oven at 40°C for approximately 24 hours until all diets had moisture content of circa 8%. The pellets were then ground to a size suitable for consumption by the juvenile rabbitfish and stored in airtight zip-lock bags at -20°C until used. Protein, lipid, and ash proportions (% dry matter) in the five diets were determined to be approximately 42%, 5%, and 11%

respectively. **Table 2.1** below depicts the actual composition of diets with an increasing inclusion of SFM.

Table 2.1. Composition of isonitrogenous and isoenergetic diets with safflower meal protein replacing fishmeal protein at 0, 25, 50, 75 and 100%. Diets were offered to rabbitfish juveniles for eight weeks.

Diet	D1	D2	D3	D4	D5
Fish Meal (g/Kg diet)	802.5	599.3	408.0	198.0	0.0
Dehulled press extruded SFM (g/Kg diet)	0.0	255.0	495.0	757.5	1005.0
Menhaden Fish Oil (g/Kg diet)	40.4	32.0	24.2	15.6	10.5
Wheat Flour (g/Kg diet)	405.0	375.0	345.0	315.0	277.5
Vit+ Mineral premix (g/Kg diet)	45.0	45.0	45.0	45.0	45.0
Choline Chloride (g/Kg diet)	7.5	7.5	7.5	7.5	7.5
Vitamin C (g/Kg diet)	1.5	1.5	1.5	1.5	1.5
Cap- Dibasic (g/Kg diet)	3.0	24.0	46.5	67.5	90.0
Lysine (g/Kg diet)	0.0	6.3	12.8	19.5	26.0
α - Cellulose (g/Kg diet)	171.5	130.7	91.4	49.7	13.1
Methionine (g/Kg diet)	8.7	8.9	8.3	8.3	9.0
Gelatin (g/Kg diet)	15.0	15.0	15.0	15.0	15.0
	1500.0	1500.0	1500.0	1500.0	1500.0

[1] FF Skagen Denmark. Havnevagtvej 12.9990 Skagen.
[2] De-hulled solvent extracted safflowermeal, Southern Sates Cooperative Inc., Richmond VA, USA.
[3] Seven Seas LTD., Great Britain. Mar Fleet, Hull, England HU9 5NJ.
[4] The vitamin and mineral premix provided the following per kg of experimental diet: vitamin A retinyl acetate 1 million IU, vitamin D3 cholecalciferol 0.1 million IU, vitamin E alpha-tocoph acet 7 g, vitamin K 0.5 g, folic acid niacin 0. 1 g, niacin 4 g, calcium pantothenate 2.5 g, riboflavin (B2) 0.6 g, vitamin B12 0.001 g, thiamine (B1 nitrate) 0.5 g, pyridoxine (B6 HCl) 0.5 g, biotin 0.0125 g, vitamin C (ascorbic acid) 0.25 g, inositol 5 g, selenium (as sodium selenite) 0.0045 g, iodine (as calcium iodate) 0.25 g, iron (as sulphate monohydrate) 2 g, zinc (as oxide) 5 g, copper (as sulphate pentahydrate) 0.25 g, manganese (as sulphate monohydrate) 3.5 g, chlorine chloride 75, phosphorus (as monodicalcium phosphate) 2.5, sodium chloride (salt) 225 g, and cellulose 75 g. Calcium carbonate carrier to balance.
[5] 250 mg kg − 1 active vit C supplied by Stay C®, (L-ascorbyl-2-polyphosphate 25% Active C), Roche Vitamins Inc., Parsippany, New Jersey, USA.
[6] Alpha-cellulose, Unites States Biochemical Corporation, Cleveland, Ohio, USA.
[7] Himedia laboratories Pvt. Ltd., 23, Vadhani Ind. Est., LBS Marg, Mumbai, India.
[ŧ] Based on a calculated value.

B. Experiment 1: Growth

1. Fish Acquisition and Experimental Design

Juvenile rabbitfish, *Siganus rivulatus*, were caught in traps off the Beirut beach and transported to the aquaculture research laboratory at the American University of Beirut (AUB) where they were quarantined in an outdoors recirculating system composed of 6 circular 1000-L tanks filled with filtered seawater. The fish were acclimated to culture conditions and trained to accept artificial diets (36% Crude Protein, 6% Crude Fat; Rangen EXTR 350, Rangen Inc., Buhl, Idaho) for at least two weeks. After the fish were fasted for a day, they were transferred to an indoor flow-through research system composed of 15 rectangular fiberglass tanks (65×64×55cm; L×W×H) connected to a biological filter and settling tank. Experimental fish were size sorted by hand to a uniform size and 15 groups of fifteen fish within same range of weight were randomly stocked into 15 tanks. The flow rate of filtered seawater into each tank was 55 L/h. Aeration was supplied by a regenerative blower and submerged diffusers. Photoperiod was fixed at 14:10 h (light: dark) with the light phase starting at 6:00 and ending at 20:00. An additional thirty-five fish randomly selected from the hand-sorted fish were euthanized using Tricaine-S(Tricaine Methane sulfonate, MS 222, Western Chemical Inc., Ferndale, WA, USA). These fish were individually assessed for weight and length to calculate initial Fulton's condition indices ($K= 10^5 \times W/L^3$ where W is weight (g) and L is length (mm)). Fifteen fish were then stored at -20 C^o for later proximate analysis.

2. Environmental Conditions.

Throughout the 9-week experimental period, water temperature, salinity, and dissolved oxygen were measured using a YSI 85 oxygen meter (Yellow Springs Inc., Ohio, and USA). Dissolved oxygen was maintained at (6.1 ± 0.04 mg/L). Salinity was maintained at 35.7 ± 0.05 ppt (mean ± SE) by adding freshwater when salinity increased. Water temperature was maintained at 26.4 ± 0.4 °C (mean ± SE) using a submersible heating element. pH, Total Ammonia Nitrogen (TAN) and Nitrite nitrogen (NO_2-N)) were measured using a HACH Saltwater Aquaculture Test Kit, Model FF-3 and recorded weekly. They averaged 7.8 ± 0.05 (mean ± SE) pH, 0.06 ± 0.00 mg/L TAN and 0.06 ± 0.02 mg/L NO_2-N.

3. Feeding

The experiment consisted of three replicates for each of the five diets. Treatments were randomly allotted to triplicate tanks. Diets were offered by hand three times daily at 7.00, 13.00 and 19.00 for 63 days. The amount of feed offered daily was calculated to be 5% of the average weight of the group weights of fish in each tank then divided into three equal feeding events. Feed consumption by the fish was checked within thirty minutes after offering the feed. Following one day of starvation, fish in each tank were harvested and group weighed biweekly and a new amount of feed to be offered was calculated according to the new average group weight. One week after group weighing, the amount of feed offered was increased by 10%. The daily ration supplied to the mentioned tanks was

calculated based on 15 fish in tank. The feeding part of the experiment was terminated after 63 days.

4. Sampling and chemical analysis

At the end of grow out period, all fish were harvested, placed in labeled buckets corresponding to each tank and anaesthetized with a solution of Tricaine-S. The fish in each bucket were group weighed and the individual weight and length of each fish was measured. The final condition index of every fish in each tank was calculated. Three fish were taken at random from each tank. The weight of each fish was measured before the fish was dissected to measure separately the weight of its liver and viscera. Hepatosomatic (HSI) and viscerosomatic (VSI) indices of each fish were calculated. The three fish that belong to each tank were pooled together along with their dissected organs and minced into a homogenous sample. The samples were dried in an oven at 95°C until constant weight and moisture content of each sample calculated. The dried samples were then ground using a mortar and pestle into fine granules and stored at -20°C for further analysis.

Proximate analysis of the stored samples was performed to estimate protein, lipid and ash content of the fish offered the various treatments. Nitrogen content in the samples was determined by a modified Kjeldahl method using Digesdahl Digestion Apparatus and was multiplied by 6.25 (Albanese and Orto 1963) to estimate protein content in the sample. Crude lipid content was estimated using a Reflux extractor (ANKOM XT20 Fat Analyzer and ANKOMXT Recovery System, ANKOM Technology Corporation, Macedon, NY, USA) and a mixture of petroleum ether/diethyl ether (9:1) as solvent.Ash content was

measured by burning 0.5g samples in crucible in a furnace at 500°C. All results of proximate analysis were reported on a wet weight basis.

C. Experiment 2: Hematology

At the experiment termination, three or four (as needed) anaesthetized fish were randomly selected from each tank for blood sampling. Blood was drawn from the fish to determine the packed cell volume, total plasma protein, and glucose content. Blood samples (0.5 – 1 ml) were collected from each fish by cardiac puncture using a 1 ml syringe with 27 gauge and 2.5 cm heparinized needles. The blood was then placed into heparinized 3ml Eppendorf tubes. Since drawing blood by cardiac puncture stressed the fish and potentially affected glucose level, we decided to perform glucose test on other selected fish two days later. Each Eppendorf tube was used to fill two heparinized capillary tubes that were centrifuged at 10,000 g for 5 minutes in a microhematocrit centrifuge at room temperature (Morris & Davey 2001) to determine PCV. The plasma obtained in capillary tubes used for hematocrit measurement was used to measure the total plasma protein (TPP). A drop of the plasma was placed on the glass plate of a veterinary refractometer (RHC-200ATC, Westover Scientific, Inc., Mill Creek, WA, USA) that was used to assess total plasma protein in (g/dl).

D. Data Analysis and Statistics.

Survival was calculated as S (%) = 100* (number of fish at the end of the experiment/number of fish at the start of the experiment). Feed Efficiency was calculated as FE = weight gain of fish in g / weight of feed offered in g. Protein efficiency was calculated as PE= weight gain in g/ protein offered in g. Viscerosomatic index (VSI) and hepatosomatic index (HSI) were calculated as VSI= 100* (Viscera weight in g/ body weight in g) and HSI= 100*(liver weight in g / body weight in g).

The mean ±SD values of the three replicates of a treatment were compared using one-way ANOVA. Results were considered statistically significant at $P<0.05$. Significant differences among means were further analyzed using the Student Newman-Keuls multiple-range mean separation test. Analyses of the data were performed using the SAS 9.1 statistics software (North Carolina 27513, USA), and General Linear Model procedure of SPSS (v.23.0, SPSS Inc., Chicago IL, USA).

CHAPTER III

RESULTS

A. Experiment 1: Growth

Fish in all treatments grew but fish offered treatment D1 showed significantly the best growth performance while treatment D5 showed the worst growth performance. Growth curves of fish in the five treatments as average group and individual body weight are presented in **Fig 3.2a** and **Fig 3.2b** respectively. At the end of the feeding trial, results showed that PE and FE tended to decrease as the inclusion of SFM in the diet increased. Values for final body weights, final body length, condition index, feed efficiency, protein efficiency, and survival are shown in **Table 3.1.** During the experiment, water quality, chemical parameters, and temperature were maintained within suitable ranges for survival of *Siganus rivulatus.* Results showed no significant differences in survival among treatments.

At termination significant differences were observed in mean fish weight among the experimental groups. The average weight in treatment D1 (19.74 ± 4.021 g) was significantly greater than weight of fish in all other treatments. Mean weight of fish fed D2 was not significantly different from mean weight of fish fed D3 and D4. Fish offered D5 (13.82 ± 4.135g) were significantly smaller than fish in all other treatments. Best fit regressions were performed for all data. Results fit a straight-line model with an equation of

$y = -0.0063x + 19.484$ and $R^2 = 0.8703$ **(Fig 1).** In general, these results showed that fish gained less weight as SFM inclusion in experimental diets increased.

The final average length of fish in treatment D1 (11.63±0.269) was significantly greater than fish in all other treatments **(Table 3.1).** However, fish offered D5 (10.33±0.269) yielded the lowest average length among treatments. Differences in average length of fish offered D2 (11.13±0.269), D3 (11.24±0.269), and D4 (11.04±0.269) treatment were not significant.

Fulton's condition index (K) did not vary significantly among the various treatments. However,K in treatment D1 (1.25±0.0292) was greater than in all other treatments. K was least in fish offered D4 and D5 (1.21±0.0292).

Feed efficiency (FE) decreased as SFM inclusion in diets increased. Feed efficiency in treatment D1 (0.64±0.047) was significantly greater than that of all other treatments and FE in treatment D5 (0.37±0.047) was less than in all other treatments. There were no significant differences in FE among treatments D2, D3, and D4 **(Table 3.1).** Protein efficiency (PE) followed a trend similar to FE. PE of treatment D1 (1.58±0.119) was significantly greater than in other treatments whereas PE of treatment D5 (0.92±0.119) was significantly less than all other treatments. PE in treatments D2, D3, and D4 were not significantly different from each other.

Values of hepatosomatic and visceromatic indices are shown in **(Table 3.2)**. HSI of fish offered treatment D5 (0.027 ±0.0121) was not significantly different from HSI value in treatments D2 and D3 but was significantly different from HSI of treatments D1 (0.022±0.0121) and D4 (0.021±0.0121). HSI value was least in fish offered treatment D4.

Viscerosomatic index (VSI) did not show any significant differences among the five treatment diets.

Whole body proximate compositions as a proportion of wet weight of fish are shown in **Table 3.3**. Moisture proportion in fish (70.47±0.82) at stocking was significantly greater than moisture proportion in all experimental fish at termination. Fish offered D4 had a moisture proportion similar to that of fish offered D1 and D3 but significantly different from moisture proportions of fish in D2 and D5. Lipid content of fish at stocking (9.987±0.846) was not significantly different from body lipid of fish in D1, D2, D3, and D5. Fish offered D4 had significantly less body lipid content than fish in D1, D3, and D5. Protein proportions in wild fish and experimental fish were similar to each other. No significant difference was observed in body ash among wild fish and fish offered the various diets.

B. Experiment 2: Hematology

Values for packed cell volume and total plasma protein are shown in **Table 3.4.** PCV (hematocrit) did not vary significantly among treatments. Hematocrit exhibited a decreasing trend in fish offered D1, D2 and D3. However, fish offered D4 showed an unexpected increase in PCV which decreased again in fish offered D5. Similarly, TPP results showed no differences among fish in all treatments.

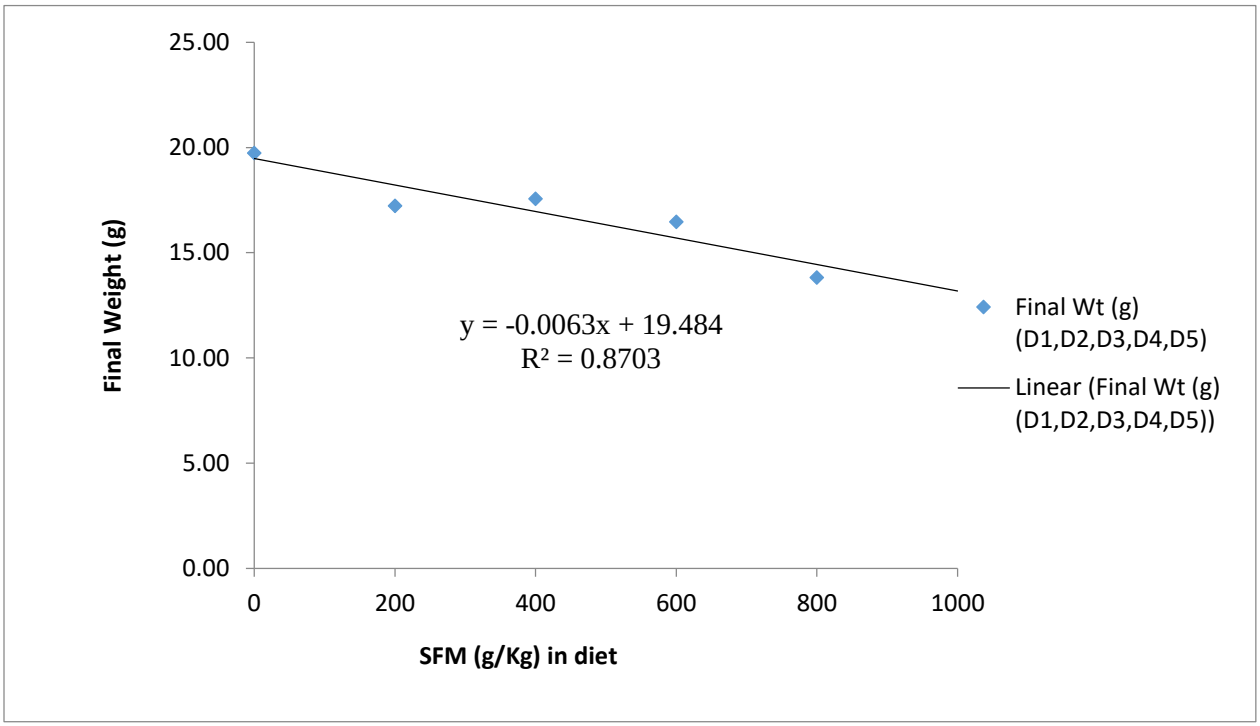

Fig. 3.1: Linear regression analysis of final body weight expressed in (g) as a function of increasing SFM inclusion in the diets (g/kg).

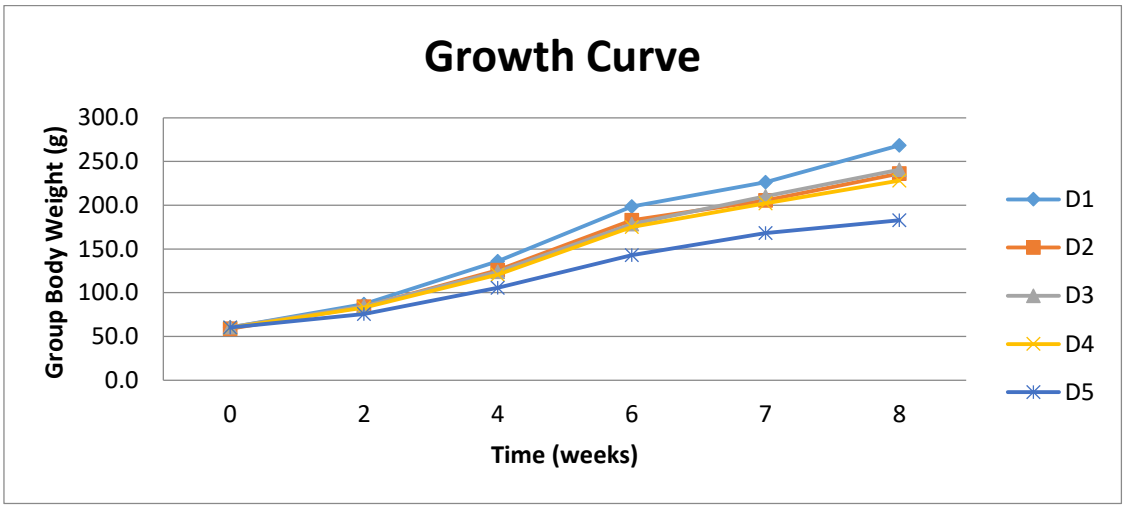

Figure 3.2a: Body weight (g) over experiment duration (8 weeks) of juvenile *S. rivulatus*, offered diets with increasing SFM inclusions. D1 (0%SFM), D2 (25%SFM), D3 (50%SFM), D4 (75%SFM) and D5 (100%SFM).

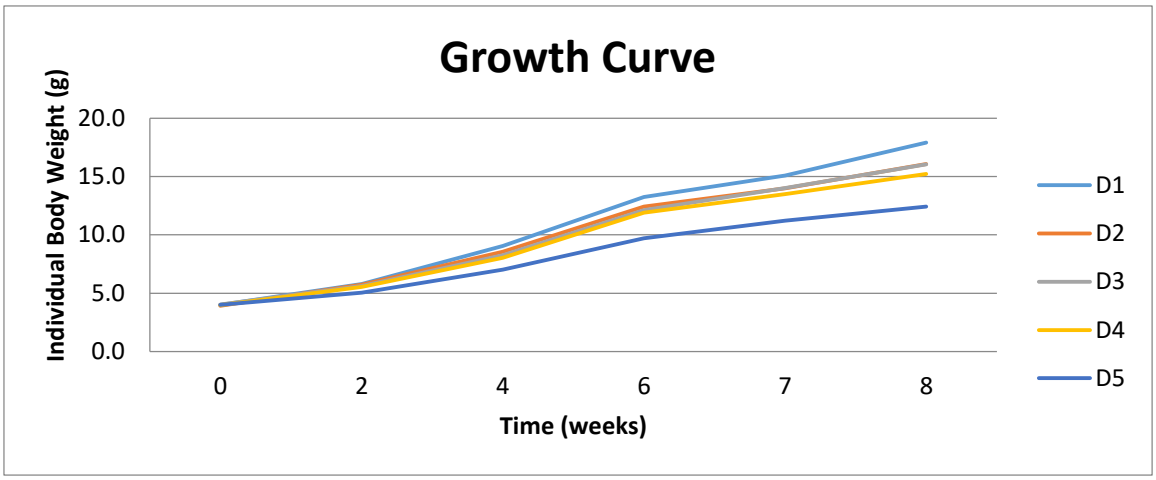

Figure 3.2b: Growth (g) over experiment duration (8 weeks) of juvenile *S. rivulatus*, offered diets with increasing SFM inclusions. D1 (0%SFM), D2 (25%SFM), D3 (50%SFM), D4 (75%SFM) and D5 (100%SFM).

Table 3.1 Values of juvenile *S. rivulatus* treated with increasing SFM inclusions as percentage of dietary protein. FBW (Final body weight) (g), FBL (Final body length) (cm), Fulton's condition index (K) ((g/mm3) ×105, FER (feed efficiency ratio), PER (protein efficiency ratio), and S (survival) (%). Values with different superscripts within the same column are considered significantly based on SNK test (P<0.05).

Treatment	FBW	FBL	K	FER	PER	S
D1	19.7^{a}	11.6^{a}	1.3	0.64^{a}	1.58^{a}	100
D2	17.2^{b}	11.1^{b}	1.2	0.55^{b}	1.38^{b}	97.77
D3	17.6^{b}	11.2^{b}	1.2	0.54^{b}	1.35^{b}	100
D4	16.5^{b}	11.0^{b}	1.2	0.50^{b}	1.23^{b}	100
D5	13.8^{c}	10.3^{c}	1.2	0.37^{c}	0.92^{c}	95.55
PSE*	1.32	0.27	0.03	0.05	0.12	3.14

[1]PSE: Pooled Standard Error

Table 3.2 Hepatosomatic and viscerosomatic indices (expressed in %) of *S. rivulatus* treated with diets of increasing inclusion of SFM as percentage of dietary protein. Values with different superscripts within the same column are considered significantly based on SNK test (P<0.05).

Treatment	HSI	VSI
D1	2.2^{b}	9.8
D2	$2.4^{a, b}$	9.4
D3	$2.3^{a, b}$	8.9
D4	2.1^{b}	9.9
D5	2.7^{a}	9.7
PSE[1]	0.012	0.003

[1]PSE: Pooled Standard Error

Table 3.3 Moisture, protein, lipid and ash content as percent of wet matter of juvenile *S. rivulatus* treated with increasing SFM inclusions as percentage of dietary protein. Values of different superscripts within the same column are considered significantly different based on SNK test (P<0.05).

Treatment	Moisture	Lipid	Protein	ASH
D1	68.3[a, b]	9.4[a, b]	17.9	3.9
D2	67.3[b]	9.8[a]	16.9	4.3
D3	68.1[a, b]	8.8[a, b]	16.7	4.0
D4	69.1[a]	8.4[b]	17.1	4.0
D5	67.5[b]	10.0[a]	17.8	4.0
PSE[1]	0.82	0.85	0.90	0.42

[1]PSE: Pooled Standard Error

Table 3.4 PCV (packed cell volume PCV) (expressed in %) and TTP (total plasma protein) (expressed in g/dl, mean ± SD) of juvenile *S. rivulatus* treated with increasing SFM inclusions diets as percentage of dietary protein. Values of different superscripts within the same column are considered significantly different based on SNK test (P<0.05).

Treatment	PCV	TPP
D1	32.0	4.1
D2	29.6	4.0
D3	28.9	4.0
D4	31.8	3.9
D5	30.4	4.4
PSE[1]	6.49	0.53

[1]PSE: Pooled Standard Error

CHAPTER IV

DISCUSSION

A. Growth

Although assessments of SFM in animal diets have been well studied, evaluations of SFM inclusion in fish feeds are relatively scant. The present study was performed to assess the efficiency of replacing FM with SFM in diets of juvenile rabbitfish, *Siganus rivulatus*, at inclusion levels of 25, 50, 75, and 100 (%). Growth performance of juvenile marbled spinefoot showed a decreasing trend with increasing SFM inclusion. Similarly, Alvarez Gonzalez *et al*. (2007) indicated that the final weight of tilapia (*Oreochromis niloticus*) offered diets containing SFM was less than the final weight of fish offered control diets. However, other researchers reported different results when SFM was used as an alternative feed ingredient. For example, Nagaraj *et al*. (1990) reported that carp (*Cyprinus carpio*) treated with SFM diets exhibited better growth than carp fed conventional fish feed. Also, Ustaoglu *et al*. (2015) reported that SFM can be supplanted to up to 20 % in rainbow trout diets with no adverse effect on growth. These studies contradict our results since juvenile rabbitfish showed growth reduction when SFM partially or totally replaced fish meal in the diets.

Final body weights of our experimental fish **(Table 3.1)** were significantly greater in D1 than in D2, D3 and D4, which were not statistically different from each other. Fish in D5 had significantly less growth than fish in all other treatments.

Thus, even partial replacement of FM by SFM in diets of *Siganus rivulatus* had adverse effects on growth performance. The strong correlation between SFM inclusion and reduction in final body weight shows no breakpoint beyond which SFM inclusion can significantly affect fish growth performance and indicates a continuous decrease of final body weight as the concentration of SFM in the diets of *Siganus rivulatus* increases.

Visual observations of unconsumed feed in tanks suggest reduction in feed intake in fish offered diets with increased SFM inclusion. Growth retardation might thus have been a result of low protein intake directly related to the low feed intake. SFM is known to have a bitter taste as it contains phenolic glucoside (Darroch, 1990), and the reduced feed intake could be attributed to low diet palatability. In fact, as mentioned earlier, marbled spinefoot grazes on different taxa of macrophytes in a selective manner. The selective behavior of the species while feeding in nature might suggest an influence of diet palatability on the selection of their preferred macrophytes species. Similarly, other researchers, working with juvenile red drum had suggested that poor fish performance and growth could be attributed to shifts in palatability when fish meal was replaced by other protein sources (Reigh and Ellis 1992; Davis *et al.* 1995; Meilahn *et al.* 1996). Fowler (1980) hypothesized that poor growth of juvenile Chinook salmon fed diets containing 80% full fat SBM was because of the presence of unpalatable compounds in SBM. However, Ustaoglu *et al.* (2015) reported that rainbow trout accepted diets containing up to 20 % SFM inclusion without palatability problems. I

thus postulate that unpalatability could be the reason for the reduction in intake but not necessary.

There were no differences in condition index (k) among the various treatments. However, K decreased as the amount of SFM in the diet increased. Furthermore, FE of marbled spinefoot decreased as the inclusion of SFM in the diets increased. Fish offered D5 had significantly lower values of FE than all other treatments. Similar results were obtained by a previous study performed at the aquaculture lab at AUB in which FM was replaced by SBM (Monzer *et al.* 2017). However, Ustaoglu *et al.* (2015) reported that up to 20% safflower meal can be used in rainbow trout diet with no negative effects on feed efficiency.The decreasing FE values along with the increased SFM inclusion might be attributed to the low digestibility of the diets because of presence of antinutrients that inhibit protein digestion or hamper the bioavailability of some minerals in SFM. Previous researchers working with Tilapia had reported that replacing fish meal with a single plant source containing 25-35% dry matter resulted in retarded growth. They mainly attributed poor performance and slow growth to the effects of anti-nutritional factors (Davies *et al*., 1990; Olvera-Novoa *et al*., 1988; 1990; Jackson *et al*., 1982).

Precise information regarding essential amino acid requirements of most finfish species is yet unknown (NRC 2011). Also, some studies showed that even when amino acid profile is balanced by adding deficient amino acids, growth performance declines as the inclusion of plant ingredients increases (Cai and Burtle 1996, Yamamoto *et al*. 2002). In fact, the nutritive value of proteins is not identical in various ingredients as proteins differ in their amino acid profile and digestibility.

According to Blair (2011), safflower meal is a poor source of lysine and methionine. Studies suggest that free amino acids can be as efficient as protein bound amino acids in meeting EAA requirements in fish (Espe*et al*. 2006). Accordingly, although methionine was added to the five experimental diets and lysine was added to the four diets including SFM, the limited information about finfish specific amino acid digestibility of safflower meal prevents us from making conclusions. Alternatively, retarded growth performance might be because of low digestibility of safflower proteins rather than lack of availability.

Protein efficiency in *S. rivulatus* decreased as the inclusion of SFM in the diets increased. Fish offered D5 had significantly the least PE value among treatments. As protein offered by fishmeal was replaced by SFM protein, fish had less protein body content. This result along with the decrease in FE and body weight suggests that a decline in the apparent protein digestibility (APD) with increasing SFM inclusion. Hernàndez *et al*.(2007) reported a significant difference in APD between diets containing 100% FM or 100% SBM. Forde- Skiaervik *et al*. (2006) stated that the decline in APD might be because of difference in structure between FM and SBM proteins.

In the present study, there were no significant differences of VSI values of fish in all five treatments. This observation is consistent with results obtained by the previous study at our lab when marbled spinefoot were offered diets containing various inclusion levels of SBM. Conversely, the HSI values of our experimental fish did not follow a specific trend. Fish offered D5 had significantly the highest HSI value among treatments. In fact, results showed that liver weight of each of

three randomly selected fish from each of the three replicates of the five treatments were relatively close to each other. However, as mentioned earlier, fish in D5 had significantly the least growth**.** Thus, the high value of HSI of fish in D5 is not because they have liver weight greater than other fish but because they have body weight significantly less than fish in all other treatments.

B. Proximate composition

No significant differences were observed in protein and ash composition among the various treatments whereas lipid and moisture content varied significantly among treatments. The results showed that body lipid content decreased with increasing inclusion of SFM, reaching a minimum lipid content in fish offered D4. Similar results were observed by Monzer *et al.* (2017) who reported that stored lipid in fish decreased upon the increase of SBM inclusion. The author suggested that marbled spinefoot could not utilize carbohydrates of SBM origin and thus relied on body lipid for part of energy requirement. However, the results showed that fish in D5 had the greatest lipid content among all treatments. Similarly, Kaushik *et al*. 2004 and Koumi *et al*. 2009 reported similar result of increase in lipid content when FM was replaced by plant protein source suggesting an increase in hepatic lipogenesis probably because of a paucity in essential amino acids for protein buildup. In general, results observed in the present study suggest that it is not possible to replace fish meal by safflower meal in diets for *Siganus rivulatus* without having negative effects on growth performance.

C. Hematology

Total plasma protein levels showed no significant differences among treatments and did not follow any specific trend. Variations of total plasma protein might indicate a relationship between offered dietary protein and its metabolism in fish body (Monzer *et al*. 2017).Ye *et al.* (2011) suggested that high plasma protein concentration could mean poor protein utilization and an increase in protein turnover and degradation.(Coz-Rakovac *et al*. 2005) stated that increased plasma protein could also indicate structural liver alterations and impairment of protein liver metabolism. Although TPP levels observed in the present study are greater than those reported earlier by Nasser (2012) for cultured *S. rivulatus*, the possibility of liver damage is improbable because no significant differences were observed among TPP results of fish in all treatments. Likewise, no significant differences in hematocrit (PCV) were observed among treatments. This contradicts with the results observed by Monzer *et al*. (2017) who reported a significant decrease of hematocrit (PCV) level with increase in SBM inclusion in rabbifish diets. Brown *et al*., (2008) working with SBM as replacement for FM, had reported that the anti-nutritional factors found in SBM could harm the liver, and thus biological factors such as PCV, TPP, and HB are negatively affected. Thus, this result suggested that all levels of SFM inclusion in experimental diets did not affect hematopoiesis in rabbitfish and hence did not alter liver morphology and metabolism.

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