International Journal of Fisheries and Aquatic Studies 2015; 2(6): 170-174

ISSN: 2347-5129
IJFAS 2015; 2(6): 170-174

www.fisheriesjournal.com
Received: 18-05-2015
Accepted: 20-06-2015

Jayarama Naik N
Department of Food Technology,
Davangere University,
Davangere, Karnataka, India,
577002.

Vanita S. Bhat
Department of Food Technology,
Davangere University,
Davangere, Karnataka, India,
577002.

Abhilash V
Department of Food Technology,
Davangere University,
Davangere, Karnataka, India,
577002.

Basavaraj Madhusudhan
Department of Food Technology,
Davangere University,
Davangere, Karnataka, India,
577002.

Correspondence
Basavaraj Madhusudhan
Department of Food Technology,
Davangere University,
Davangere, Karnataka, India,
577002.

Influence of flaxseed on the body weight, biochemical constituents and histology of muscle and liver tissues of Common Carp, *Cyprinus carpio*: A comparative study

Jayarama Naik N, Vanita S. Bhat, Abhilash V, Basavaraj Madhusudhan

Abstract
Of 16 fish, 4 different groups were made as rice bran (RB), flaxseed (FL), garden cress seed (GC) and flaxseed: Garden cress seed (FL: GC) fed groups. The FL supplemented group had the weight reduction the most and RB exhibited the least, whereas two other groups GC and FL: GC showed the marginal change in the weight parameter. Significant changes in lipid, protein and glycogen contents were seen in muscle and liver tissues. Histology of the experimental groups exhibited supporting relevance to the weight reduction (FL and FL: GC) and so lipid accumulation in tissues (RB). We hypothesized that the weight-loss appeared in FL was may be due to the suppression of hunger inducing satiety centers through signaling molecules by leading to weight loss as evidenced by the biochemical constituents and histology scores. This data would provide additional information to consider flaxseed as another potential ingredient while preparing dietary regimen.

Keywords: Feed, common carp, satiety, biochemical constituents, body-weight, histology.

1. Introduction

Oil seeds and their polyunsaturated fatty acids play a significant role in contributing for weight reduction. Oil seeds are not only affecting on the heath and disease, but also on the quality of cultured fish by improving their fatty acid composition to benefit the consumers [26]. The protein sparing action of lipids and fatty acids are well documented apart from their satiety inducing properties. Existing research studies related to pulse consumption, satiety and body weight have been summarized in a review paper [16]. For the past 30 years, research studies have examined the potential associations between pulse consumption and weight status [21]. The data from National Health and Nutrition Examination Survey (NHANES) 1999-2002, has provided a new insight that individuals who regularly consumed beans weighed less, had a 23 % reduced risk of increased waist size and a 22 % reduced risk of being obese [20]. Particularly, there is a great interest in the health benefits of both garden cress seed and flaxseed for their medicinal properties and nutraceuticals. Nutritionally, garden cress seed and flaxseed offer excellent sources of nutrients and energy [19]. Garden cress seeds are rich source of proteins, dietary fiber, omega-3 fatty acids, iron, other essential nutrients and phytochemicals and more so flaxseed. Garden cress is widely used in folk medicine for the treatment of hyperactive airways disorders, such as asthma, bronchitis and cough. Seeds are considered to be galactogogue, emmenagogue and therefore recommended in inflammation, bronchitis, muscular pain and rheumatism [22]. Economically, garden cress seed and flaxseed offer consistence, dependable and affordable supply of ingredients from year to year. Recent investigation has revealed that flaxseed soluble fibers appear to induce satiety [27].They can affect multiple aspects of the gastrointestinal function such as gastric emptying rate and nutrient absorption rate in the small intestine, which offers numerous opportunities to influence satiation and satiety [13]. In concordance with this, researchers have observed that doses of 5 and 10 g of flaxseed fibers increased satiety and gave a prolonged decrease in ghrelin a hunger-signaling gut peptide [12]. This investigation has evoked research interest among us to gather more supporting information to strengthen the already existing data on pulses and more precisely to know whether flaxseed especially has any independent effect(s) on body weight. Due to nutritional and therapeutic credentials aforementioned reasons and anticipating a future consumption of this ingredient, it becomes necessary to consider and evaluate whether it is feasible in terms of antiobesity diet, to incorporate the flaxseed as well as garden cress seed either individually or in combination as additional ingredient(s) of food. Rice bran is a good choice to play role in therapeutic intervention studies due to its potential efficacy in disease prevention, especially in reducing cardiovascular disease risk [3]. Despite the use of flaxseed, scanty information is available on the effect of flaxseed or its fractions on fish model. This comparative study reports the flaxseed's influence on the body-weight, biochemical and histological parameters, which may promote the flaxseed consumption and to consider its incorporation while preparing dietary formulations.

2. Materials and Methods

Two potential seeds of nutritional importance include flaxseed and garden cress seed were selected to design feed study on body-weight, biochemical and histological parameters of common carp fishes. The study was planned for twenty five days of feeding trials with rice bran as control diet. The NL-115 variety of FL was purchased from University of Agricultural Sciences, Raichur, India. The GC and RB were collected from the local market. Upon arrival, the samples were cleaned and stored at 4 °C until further use. All chemicals for biochemical analyses were of analytical grade purchased from authorized dealers (India).

2.1 Methodology

Fish and Experimental Conditions

16 freshwater common carp fish between 118 ± 2g were procured from the State Fisheries Department, Bhadra Reservoir Project, Shimoga, Karnataka, India, use for this study. Upon arrival, fish were washed with 0.1 % $KMnO_4$ solution to avoid dermal infection. All the precautionary measures were followed for maintaining the fish [2]. Prior to the start of the experiment, fish were individually weighed and allowed to acclimatize for 2 weeks at 24 ± 1 °C in the experimental semi-intensive aquaria (3 sq.mtr) containing dechlorinated tap water of the quality used in the test. The aquarium was fitted with waste filtration facility. Compressed air was used to maintain oxygen supply. The fish were randomly assigned to 4 different groups based on 3 experimental diets and 1 control diet. The fish were fed regularly with commercial fish food pellets (kind gift by State Fisheries Department) during acclimatization. Fish were fed twice daily at approximately 07.00 and 17.00 h to apparent satiation for a period of 25 days. Regular monitor of water quality parameters was carried out. Water supplied continuously via semi re-circulating system and a 12-h and 12-h of night and day photoperiod was maintained during acclimatization and test periods. Feeding was stopped just two days prior to exposure to the test feed samples only.

2.2 Experimental Feeds

The samples were formulated from 100 % FL, 100 % GC and 100 % RB and 1:1 blends of the two seeds (FL: GC). The RB was used as control. Besides this, The seeds were moistened, crushed into a fine paste, added with binding agent (agar-agar, 1 %) and extruded through an empty syringe (10 ml) and converted into pellets ~ 2 mm diameter by rolling between the fingers. The pelleted feeds were dried in a hot air driven oven at 60 °C and stored at -20 °C throughout the experimental period. The feeds were offered (4 % of body wt.) twice daily for 25 days. In order to quantify the exact amount of feed intake or any refusal was monitored by siphoning out immediately after 2-h, dried to 60 °C and weighed. The fish were randomly assigned to 4 different groups based on 3 experimental feeds and 1 control feed. Feeding was stopped just two days prior to exposure to the test feed samples only.

2.3 Sampling and Analytical Methods

Sampling was carried out at the end of the experiment. The time elapsed between the last meal and sampling was within 2-h. A set of 16 fish (four fish per group) was taken and anesthetized in excess using anesthetic ether for biochemical parameters and another set of four fish (one fish from each group) used for histological study. Euthanization was carried out according to regulations and their mean body weight was recorded to assess the significant difference between dietary groups. Three fish from each group were dissected to collect their muscle and livers. The weights of the specimen were recorded immediately. The specimen were cut into fine pieces and minced through a meat mincer. The homogenized samples were immediately frozen at -30 °C until further analysis. The proximate compositions of diets and fish fillets were analyzed. Total lipid estimation was carried out using methanol-chloroform method [10] total protein by the Biuret method [1] and total glycogen by modified phenol-H_2SO_4 method [25]. Analyses were carried out in triplicate, unless otherwise stated and the general precautions recommended for isolation and handling of specimen [5] was followed.

2.4 Statistical Analysis

For determining the significant inter group difference, data from each parameter was analyzed with one-way ANOVA. A significance level of $P < 0.05$ and $P > 0.01$ was used to determine statistically significant differences between means.

3. Results

Table 1: Shows types of feed used, change in body-weight, liver weight and feed consumptions of common carp after 25 days feed trial.

Types of feed	Fish	Initial weight (g)	Final weight (g)	Weight loss (g)	Liver weight (g)	Percent average weight	Feed refused (%)
RB	F1	118.50	104.40	14.10	1.63		
	F2	120.00	103.50	16.50	1.88	11.27	10
	F3	119.00	106.30	12.70	1.54		
	F4	119.50	109.00	10.50	1.79		
Total		477.00	423.20	53.80			
FL	F1	118.70	98.70	20.00	1.66		
	F2	119.30	95.40	23.90	1.89	17.67	30
	F3	119.50	98.50	21.00	1.69		
	F4	120.00	100.50	19.50	1.80		
Total		477.50	393.10	84.40			
GC	F1	118.90	104.95	13.95	1.78		
	F2	119.10	104.45	14.65	1.79	13.51	15

	F3	119.90	101.95	17.95	1.70		
	F4	119.70	101.70	18.00	1.87		
Total		477.60	413.05	64.55			
FL:GC	F1	119.60	101.60	18.00	1.79		
	F2	119.60	102.10	17.50	1.76	14.52	25
	F3	118.50	102.50	16.00	1.69		
	F4	119.40	102.70	16.70	1.80		
Total		597.10	510.40	86.70			

The common carp fish fed for 25 days on control (RB) and experimental (FL, GC and FL: GC) diets exhibited interesting results. Differences in initial weight before the experiment and final weight after 25 days were recorded and shown in Table1. From the muscle and liver tissues the concentrations of total lipid, total protein and total glycogen were measured are presented in Figure 1.

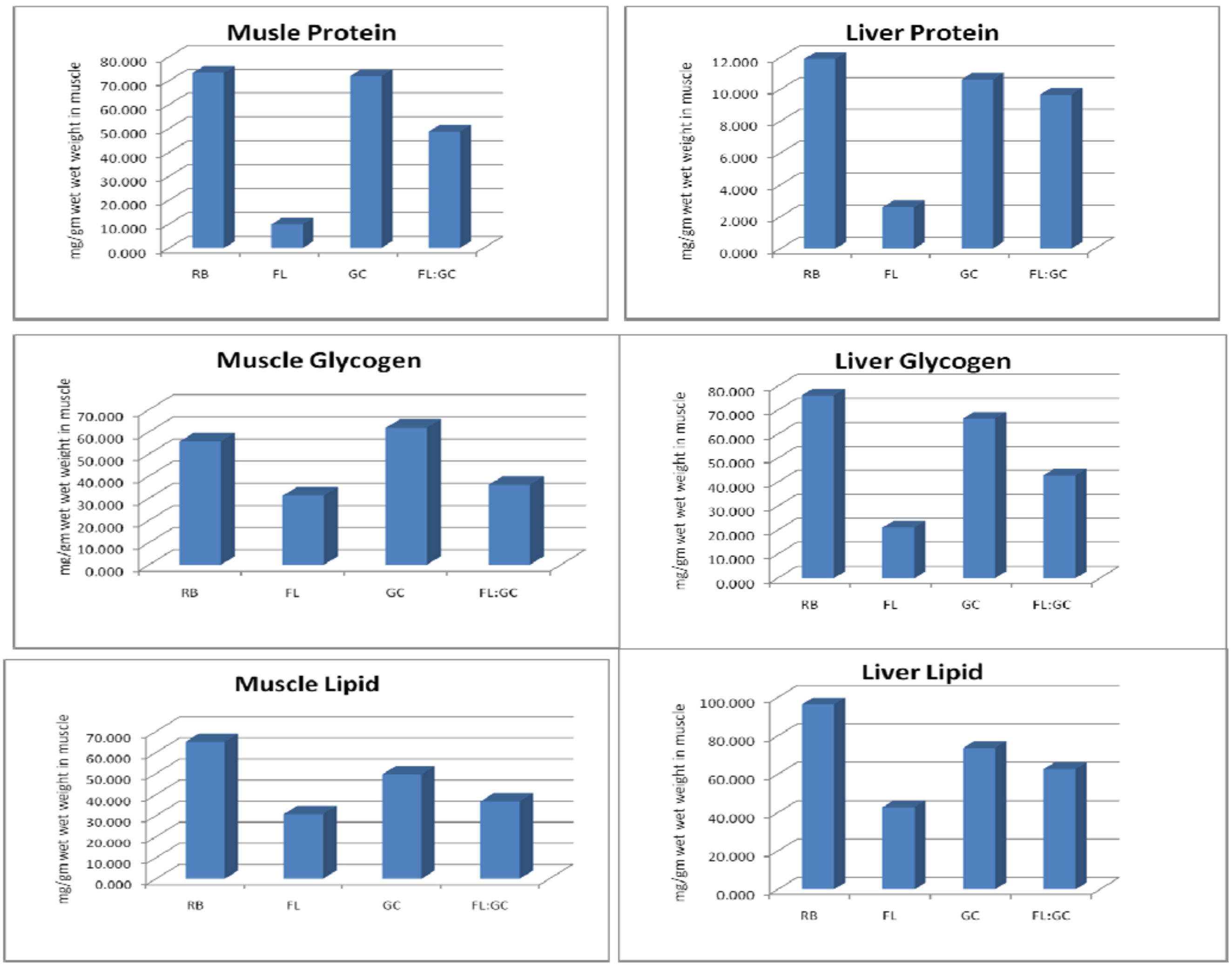

Fig 1: Changes in the levels of total protein (mg/g), total glycogen (mg/g) and total lipid (mg/g) are shown in muscle and liver tissues. Feeds used were RB, FL, GC and FL: GC.

Surprisingly, there was a significant decrease ($P < 0.05$) in the total lipid content of muscle in FL fed fish group in comparison to fish fed with other 3 feeds, where they fell in the order of FL:GC < GC < RB. We noticed that the total lipid content of muscle of FL showed 52.69 % reduction over RB, whereas FL: GC exhibited 43.45 % reduction over RB. While comparing the total protein contentin the muscle, we observed the similar reducing trend in FL (86.33 %) over RB, and the order appeared to be remained same FL: GC < GC < RB as in the case of lipid content. The FL: GC feed fish exhibited 33.59 % difference over RB (Figure 1). The total glycogen content of muscle exhibited a different trend than that of total lipids and total proteins contents from each dietary regimen used. This was exceptional to GC which in comparison with RB did follow the similar trend as in total lipid and total protein contents. Apparently FL and FL: GC feeds showed significant reduction in total glycogen content of the muscle by 44.09% and 35.25 % over RB, respectively (Figure 1). To our surprise, we noticed a similar trend and no significant difference between the muscle constituents and liver constituents. Overall, the FL feed fed fish constantly maintained the reduced trend of total lipid, total protein and total glycogen constituents than the RB feed fed fish (Figure 1).

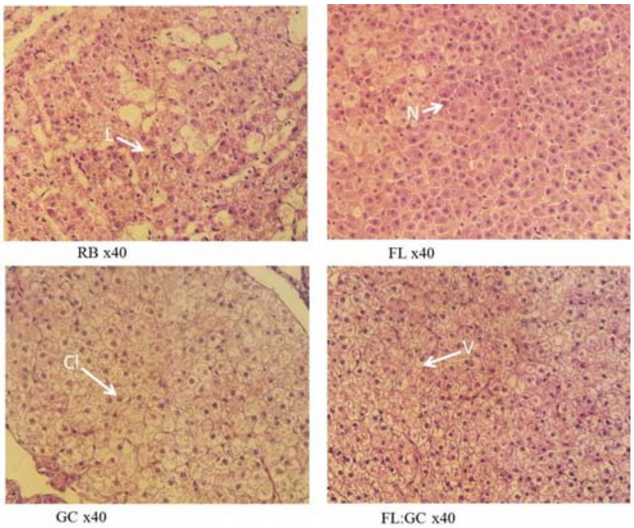

Fig 2: Liver lipidosis (steatosis) was found in 4 different feeds.

RB (control): Lipid accumulation with swollen cells; FL: No lipid accumulation; GC: Lipid accumulation with swollen cells; and FL: GC. Extensive vacuolar degeneration with no fatty change. Additionally, N: Hepatocytes nucleus, L: Large lipid droplet, Cl: Cytoplasmic clarification V: Microvesicle, were seen.

From the histological point of view, the deciding feeds revealed the supporting evidences of lipid accumulations in the liver of RB feed fed fish and tissues were overloaded with fat globules (steatosis). The RB fed fish liver tissues also indicated swollen morphological appearance. Whereas, the fish fed GC showed vacuolization in liver tissues and exhibited swellings due to accumulation of water (edematous). However, the FL: GC fed fish showed extensive vacuolar degeneration, where the nucleus in their cells was found to be slightly pushed towards the periphery (Figure 2). Similarly, the photomicrograph of the muscle tissues of fish fed with FL depicted the presence of normal myotomes associated with equidistance muscle bundles. In comparison, the muscle tissues of the RB, GC and FL: GC fed fish showed distinctly marked and thickened linings. In which, the muscle tissues exhibited gaps in between the bundles and intracellular edema with minor dystrophic changes were observed (Figure 3).

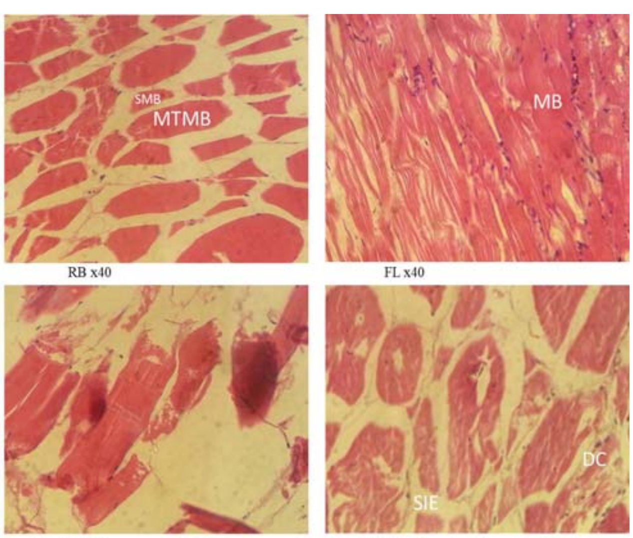

Fig 3: Photomicrograph of muscle tissues.

RB (control): Muscle tissues exhibiting MTMB and SMB; FL: Muscle tissues exhibiting MB; GC: Muscle tissues exhibiting MTMB and SMB; and FL: GC: Muscle tissues exhibiting SIE, SMB and DC. MB: Muscle bundles, MTMB: Marked thickening of muscle bundles, SMB: Shortening of muscle bundles, SIE: Severe intramuscular oedema, DC: Dystrophic changes.

4. Discussion

Earlier experiments in fish have demonstrated beneficial effects of dietary ingredients. Generally, the major constituents found in dietary sources are rich in fibre, fat, protein and other nutraceutical agents. Some of these nutraceuticals are considered to be the protective molecules that play an important role in health and diseases [24]. It is evident from the investigation by [5] that fish fed with elevated levels of YG (yellow grease) attained higher body weight than that of fish fed SAO (soy-acid oil). The results indicated the under utilization of dietary SAO compared to YG in common carp fingerlings may be due to the certain signaling molecules that come in the way of nutrients utilization. Similar experiments carried out by other researchers on catfish (*Ictalurus punctatus)* and tilapia (*Tilapia zillii)* have also exhibited the identical results demonstrating lower utilization of lipids on par with carbohydrates as source of energy [6,4]. In addition, African catfish (*Clarias gariepinus)* and sunshine bass (*Morone chrysops)* have been found to utilize dietary lipid in higher level as reported by [15, 11] respectively. The results reported by Lim *et al.* 2001 [15], are agreeable with our present investigation, where the level of dietary lipid may influence body weight and protein utilization. Protein retention is influenced by a variety of factors including the digestibility of feed ingredients, and anti-nutritional factors [24]. Presence of monounsaturated and n-6 fatty acids has their specific influence on protein sparing action. Investigation on common carp by revealed that the fish require both n-6 and n-3 fatty acids and supplementation of these fatty acids (1 % each) would have positive bearing on growth and utilization of feed [23]. Utilization of FL and GC in our experiments have demonstrated the under utilization of feed which in turn allowed the fish utilize bodily fat and protein for energy needs and to cope up with the metabolism [8]. Daily feed consumption of fish slightly decreased with respect to FL and followed by GC. Data from earlier research on most cultured fish species have supported the lower feed or energy intake even in the presence of high lipid diets [9, 14, 17] have reported that FL had suppressed hunger by inducing satiety *in vivo*. This could be resulting from lipid accumulation in control feed [18] and might be attributed to poor utilization of the FL and GC compared to the fish fed RB. No lipoid accumulations in fish fed with FL feed and GC feed and negligible amount of lipid was observed in fish fed with FL: GC feed, in comparison to that of control fish fed with RB feed. The results published by Genc *et al*., (2005) [07] are encouraging in line with our investigations inviting the additional insight on feed induced hepatic lipidosis.

5. Conclusion

The present study involving experimental fish (common carp) fed with FL alone and FL: GC feed in combination exhibited weight loss. Histological findings on muscle and liver tissues have supported the present investigation. From these preliminary results, FL alone and FL: GC in combination may be considered while recommending feed supplementation in weight reduction intervention studies as an alternative.

6. Acknowledgements

The authors wish to thank Mr. Prasanna for timely supplying fish (common carp) and his invaluable suggestions in aquaculture (staff of State Fisheries Department, Bhadra Reservoir Project, India) and Dr. E. Keshamma for suggesting the garden cress seed elaborating with nutraceutical properties and therapeutic role in weight loss. We also thank Dr. Shashikala P Krishnamurthy (Head, Pathology Dept. SS Medical College and Research Centre, Davangere, India) for helping in histology work and Mr. Y.H. Kundargi for providing fish aquarium to establish the experimentation.

7. References

1. Allan GG, Charles J, Maxima MD. Determination of serum proteins by means of the biuret reaction. J Biol Chem 1949; 177:751-766.
2. APHA, Standard Methods for the Examination of Water and Waste Water, 20th Edition, American Public Health Association, Washington DC, 1998.
3. Cicero AFG, Derosa G. Rice bran and its main components: Potential role in the management of coronary risk factors. Curr Top Nutraceutical Res 2005; 3:29-46.
4. El-Sayed AM, Jr Garling DL. Carbohydrate-torpid ratio in diets for *Tilapia zittii* fingerlings, Aquacult. 1988; 73:157-163.
5. Erdal Yılmaz, Ercument Genc. Effects of Alternative Dietary Lipid Sources (Soy-acid oil and Yellow grease) on Growth and Hepatic Lipidosis of Common Carp (*Cyprinus carpio*) Fingerling: A Preliminary Study. Turk J Fish Aquat Sci 2006; 6:37-42.
6. Garling DL, Wilson RP. Effects of dietary carbohydrate to lipid ratios on growth and body composition of fingerling channel catfish. Prog Fish Cult 1977; 39:43-47.
7. Genc E, Yilmaz E, Akyurt I. Effects of dietary fish oil, soy-acid oil, and yellow grease on growth and hepatic lipidosis of hybrid tilapia fry, Isr J Aquacult 2005; 57:90-96.
8. Henderson RJ. Fatty acid metabolism in freshwater fish with particular reference to polyunsaturated fatty acids. Arch Anim Nutr. 1996; 49:5-22.
9. Hernandez MD, Egea MA, Rueda FM, Aguado F, Martinez FJ, Garcia B. Effects of commercial diets with different P/E ratios on sharpsnout seabream (*Diplodus puntazzo*) growth and nutrient utilization. Aquacult 2001; 195:321-329.
10. Jordi Folch, Lees M, Sloane Stanley GH. A simple method for the isolation and purification of total lipides from animal tissues. J Biol Chem 1957; 226:497-509.
11. Keembiyehetty CN, Wilson RP. Effect of water temperature on growth and nutrient utilization of sunshine bass (*Morone chrysops* x *M. saxatilis*) fed diets containing different energy/protein ratios. Aquacult. 1998; 166:151-162.
12. Kristensen M, Savorani F, Christensen S, Engelsen SB, Bugel S, Toubro S *et al.* Flaxseed dietary fibers suppress postprandial lipemia and appetit sensation in young men. Nutr Metab Cardiovasc Dis 2013; 23:136-43.
13. Lairon D, Play B, Jourdheuil-Rahmani D. Digestible and indigestible carbohydrates: interactions with postprandial lipid metabolism. J Nutr Biochem. 2007; 18:217-227.
14. Lee ML, Jeon IG, Lee JY. Effects of digestible protein and lipid levels in practical diets on growth, protein utilisation and body composition of juvenile rockfish (Sebastes schlegeli). Aquacult 2002; 211:227-239.
15. Lim PK, Boey PL, Ng WK. Dietary palm oil level affects growth performance, protein retention and tissue vitamin E concentration of African catfish, *Clariasgari epinus"*, Aquacult 2001; 202:101-112.
16. McCrory MA, Hamaker BR, Lovejoy JC, Eichelsdoerfer PE. Pulse consumption, satiety, and weight management. Adv Nutr 2010; 1:17-30.
17. Mette K, Morten GJ, Julie A, Kristina P, Lise S, Mette SM, *et al.* Flaxseed dietary fibers lower cholesterol and increase fecal fat excretion, but magnitude of effect depends on food type. Nutr & Metab 2012; 9:8.
18. Murai T, Akiyama T, Takeuchi T, Watanabe T, Nose T. Effects of dietary protein and lipid levels on performance and carcass composition of fingerling carp. B Jpn Soc Fish 1985; 51:605-608.
19. Nidhi A, Sheel S. Garden cress (*Lepidium sativum* L.)-A non-conventional traditional plant item for food product. Indian J Traditional knowledge 2013; 12:699-706.
20. Papanikolaou Y, Fulgoni VL. Bean consumption is associated with greater nutrient intake, reduced systolic blood pressure, lower body weight, and a smaller waist circumference in adults: results from the National Health and Nutrition Examination Survey 1999-2002. J Am College Nutr 2008; 27:569-576.
21. Roberts SB. High-glycemic index foods, hunger, and obesity: Is there a connection? Nutr Rev 2000; 58:163-169.
22. Snehal D, Manisha G. Garden cress Seed - An Important Medicinal Source: A. J Nat Prod Plant Resour 2014; 4:69-80.
23. Takeuchi T, Watanabe T. Requirement of carp for essential fatty acids, B Jpn Soc Fish, 1977; 43:541-551.
24. Thompson LU. Experimental studies on lignan and cancer. braillierres clin endocrinology metab 1998; 12:691-705.
25. Vishu Kumar and Pattabiraman TN. Standardization of a colorimetric method for the determination of fructose using o-cresol: Sulphuric acid reagent, Indian J Clin Biochem 1997; 12:95-99.
26. Wagboo R, Sandnes K, Torrissen OJ, Sandvin A, Lie O. Chemical and sensory evaluation of fillets from Atlanatic salmon fed three levels of n-3 PUFAs at two levels of vitamin E. Food Chem. 1993; 46:361- 366.
27. Wanders AJ, van den Borne JJ, de GC, Hulshof T, Jonathan MC, Kristensen M *et al.* Effects of dietary fibre on subjective appetite, energy intake and body weight: a systematic review of randomized controlled trials. Obesity Rev 2011; 12:724-39.