Journal of Science, Technology, and Humanities : 2013, Vol. 11, No. 2 pp. 95-103

EFFECTS OF DIETARY PROTEIN, LIPID AND ASTAXANTHIN LEVELS ON GROWTH AND CAROTENOID ACCUMULATION IN ANEMONE FISH, *Amphiprion ocellaris*.

Amonrat Kanokrung[*1,3], Rawiwan Watanadilok[1], Vorathep Muthuwan[1] and Smartachai Santawanpas[2]

[1]*Institute of Marine Science, Burapha University, Chon Buri 20131, Thailand.*
[2]*Department of Biochemistry, Burapha University, Chon Buri 20131, Thailand.*
[3]*Biological Science Program, Faculty of Science, Burapha University, Chon Buri 20131, Thailand.*

ABSTRACT

Fish fed diets containing astaxanthin have been shown to accumulate carotenoid while those receiving a diet containing optimum protein level display increased growth. The purpose of the present study was to formulate a diet that ensured good growth and the accumulation of carotenoid in cultured stocks of the anemone fish, *Amphiprion ocellaris*. To test this, two trials were conducted. The first explored different inclusion rates of protein (40, 50 and 60%) on growth, whereas the second 3-month feed trial assessed different levels of lipid, i.e.,13, 16, and 20%, and astaxanthin, i.e., 25, 30, and 35 mg/kg and the subsequent deposition of carotenoid in the fish. The first trial indicated that a diet containing 50% protein gave better specific growth rates than the other diets ($P \leq 0.05$) and higher standard length increments ($P \leq 0.05$). From the second trial, diets containing 16% lipid and 30 mg/kg astaxanthin produced fish with the best standard length ($P \leq 0.05$). The accumulation of beta-carotene was highest in those fed 16% lipid and 35 mg/kg astaxanthin ($P \leq 0.05$); the cantaxanthin was highest in those fed 13% lipid and 35 mg/kg astaxanthin ($P \leq 0.05$). Lutein and zeaxanthin, however, were highest in those fed 20% lipid and 35 mg/kg astaxanthin ($P \leq 0.05$). The trial demonstrated that the diets with the higher protein content were better for growth while those containing either 16% or 20% lipid with 35 mg/kg astaxanthin were better able to accumulate carotenoid, beta-carotene, lutein and zeaxanthin.

Keywords: Dietary, astaxanthin, carotenoid, anemonefish, and *Amphiprion ocellaris*.

*Corresponding author. E-mail address: a_chomrung@yahoo.com

INTRODUCTION

Anemone fish are popular ornamental species with marine aquarium enthusiasts and are now being commercially bred in Thailand on a farm scale. As household aquaria have become popular globally, the trade in ornamental fish species, which includes the anemone fish market, has increased. Anemone fish are now reared under intensive aquaculture conditions to meet this rise in popularity and so their demand. This popularity is demonstrated through the figures provided by the Food and Agriculture Organization (FAO) which suggest that the annual international trade in ornamental fish is worth in excess of US$ 3 billion per annum (Lem, 2001).

The quality and value of anemone fish, however, is dependent upon their coloration. Fish are usually graded according to the vibrancy of their color and patternation, which are the most important criteria linked to their market value (Wang et al., 2006). The inclusion of synthetic astaxanthin within the fish's diet is the main factor contributing to its vibrant colors. Studies with Atlantic salmon, *Salmo salar*, fed a diet containing astaxanthin, demonstrated that the fish used the astaxanthin and accumulated it within their flesh (Quinton et al., 2005). Likewise, carotenoids are an important factor in fish coloration (Choubert, 2006), as are other dietary factors such as lipid and protein which are essential for good growth. From a study conducted by Boonyaratpalin (1997) on seabass, *Lates calcarifer*, optimal growth was achieved when fish were fed an artificial diet containing between 40 – 60% proteins. *Amphiprion ocellaris* is noted for its dominant orange coloration and the purpose of the current study was to investigate the dietary constituents contributing to optimal growth and a vibrant coloration in larval anemone fish.

MATERIALS AND METHODS

A total of 1,440 three-week old *A. ocellaris*, supplied from the aquaculture research unit of the Institute of Marine Science, Burapha University, were randomly assigned to one of thirty-six 80 L circular glass flow-through tanks supplied with 30-32 ppt seawater at a rate of 1.2 L/min. The fish were acclimated for a period of seven days under control experimental conditions before the first feed trial was started. At the start of the experiment, the fish had a standard length of 1.14 ± 0.01 cm, a total length of 1.40 ± 0.01 cm, and an average weight of 0.0503 ± 0.001 g. Of the 36 tanks, nine tanks were used to dietary protein (Trial 1), and 27 tanks were used to investigate the effect of different inclusion rates of lipid and synthetic astaxanthin on growth and coloration (Trial 2).

The three experimental diets used to assess growth in the first trial included different inclusion rates of protein, i.e., 40%, 50%, and 60% (see Table 1) and were tested in triplicate. Diets were pelleted by machine and extruded at 2 mm. The fish were fed at 2% body weight/day given in three rations at 0800, 1100, and 1400 h and fed to satiation at each feed; the trial duration was up to three months. Any uneaten proportion of the diet was recorded and used for subsequent calculations of feed and protein intake. At the end of the first month of feeding, the fish in the first tank were euthanized with an overdose of MS-222 (ethyl 3-aminobenzoate methanesulfonate salt, 60 mg/L in saltwater; Ytrestøyl, et al, 2004). Thereafter, individual fish were measured (standard length and total length) and weighed before they were pooled together, freeze dried and reweighed before storing for future evaluation.

For the second trial using 27 tanks (40 fish per tank), nine diets were prepared which included three levels of dietary lipid, i.e., 13%, 16%, and 20%, and three levels of astaxanthin, i.e., 25, 30, and 35 mg/kg. The diets were prepared and administered as detailed above, and the fish processed in the same manner.

The study followed and adhered to the guidelines detailing the ethical use of animals in scientific experiments as laid out by Burapha University, Thailand. The moisture, protein, and fat content of each sample were determined following the standard methods of the Association of Official Analytical Chemists (AOAC, 1990). The moisture content was estimated by heating samples in an oven at 110°C until a constant weight was achieved and the weight loss from original sample weight

calculated. The nitrogen content of the samples was determined by the Kjeldahl method. The total lipid content was determined by using an ether extraction for 36 h.

A one-way analysis of variance (ANOVA) using the statistics package SPSS version 17.0 was used to analyze the data. The probability level for rejecting hypotheses was 0.05. Significant differences between means were tested for using Duncan's new multiple range tests.

The carotenoid content of the freeze-dried samples were determined following the method detailed by Britton (1995) with slight modification. Each sample was first ground with acetone and then filtered. The residue was recovered and subjected to a further extraction with acetone. This was repeated four to five times until no color in the filtrate was seen. The combined extracts were then treated with a 1:1 of hexane: distilled water (v/v) mix to partition the carotenoids. The upper phase was collected and placed in a rotary evaporator until dry. The dried extract was then re-dissolved in hexane and separated into two aliquots. The first aliquot was dried under nitrogen gas and then stored at -80 ˚C in the dark until a time when the astaxanthin, cantaxanthin, zeaxanthin, and lutein concentration of each could be determined. The second aliquot was saponified with a 10% KOH solution in water for 10-12 h at room temperature in the dark. Thereafter, the beta-carotene content of the sample was determined.

The beta-carotene content was determined by HPLC on a 5μm *ACE*-AR column (4.6x250 mm; Fortune Scientific Co., Ltd.) by using an isocratic mobile phase of acetonitrile:methanol :dichloromethane (70:20:10, v/v) with 1 ml/min flow rate. Astaxanthin, canthaxanthin, zeaxanthin, and lutein were analyzed on normal phase 5 μm silica (L) column (4.6x150 mm; Venusil XBP, Columnex, Bonna-Agela Technologies Inc.) by using an isocratic mobile phase of hexane: acetone (82:18, v/v) at a flow rate of 1.2 ml/min. The concentrations of a series of standard carotenoid solutions (i.e., lutein, zeaxanthin, beta-carotene, canthaxanthin, and astaxanthin) against the concentration of the test samples were determined spectrophotometrically on a UV- visible Spectrometer; model UV 300, Unicam. The extinction coefficient ($E^{1\%}_{1cm}$) of all carotenoids followed the values reported by Britton (1995). The detector was set at a wavelength of 470 nm for both systems.

The peaks corresponding to each pigment were identified based on retention times and by comparison with the standards. Co-chromatography was also conducted with the standard curve of external standard concentration and peak height.

RESULTS

The first trial evaluated the inclusion of three different levels of dietary protein (i.e. 40-60%) on the growth of *A. ocellaris* over a period of three months. The initial standard length, total length, and weight of the fish were 1.14 ± 0.009 cm, 1.40 ± 0.013 cm, and 0.0503 ± 0.001 g, respectively. The trial showed that diets containing 50+% protein resulted in greater standard and total lengths ($P \leq 0.05$) than those fish receiving a 40% protein diet. The weights of the fish receiving the higher protein diet were also greater but were not significantly different. There was no loss of stock in the first two months of the trial but there were some mortality in the final month, i.e., 85% in the 40% protein group, 78% in the 50% protein group, 80% in the 60% protein group. These losses were not significantly different between the test groups.

The specific growth rate of the fish in the 50% and 60% protein groups were similar, i.e., 2.90% and 2.89% per day, respectively, and significantly higher ($P \leq 0.05$) than the fish in the 40% protein group, i.e., 2.71% per day.

In the feed trial, fish were fed 2% body weight/day split into three rations given to satiation. Any uneaten proportion of the diet was used to calculate protein intake. There was a decreasing trend in food intake with the fish receiving a low protein diet consuming less feed than those receiving higher protein diets. The feed intakes for the three groups were 0.428 mg/day (40% protein group), 0.385 mg/day (50% protein group), and 0.338 mg/day (60% protein group). The amount of protein intake followed a similar trend with each group

consuming 0.168 mg/day (40% protein group), 0.192 mg/day (50% protein group), and 0.200 mg/day (60% protein group). The feed conversion was inversely proportional to this with values determined as 0.342, 0.456, and 0.516 for the 40%, 50%, and 60% protein groups, respectively (Table 3).

Table 1. The composition of the three experimental protein diets modified formulation from Hansen et al. (2007).

Ingredient	g / 100g		
	40% protein	50% protein	60% protein
Tuna fish meat[1]	22	30	48
Krill	12	19	18
Rice flour	26	12	6
Seaweed[2]	19	18	10
Yeast[3]	7	8	7
Fish oil[4]	8	7	5
Vitamin C[5]	0.005	0.005	0.005
Mineral premix[6]	1	1	1
Vitamin premix[7]	1	1	1
Wheat gluten[8]	4	4	4

[1]Tuna semi-refined oil (T.C. Union Agrotech Co., LtD) Values are approximate and were adjusted to maintain constant protein levels.
[2]Seaweed (purchased from the local grocery store)
[3]Yeast (Saf-Instant, S.I. Lesaffre, France)
[4] Semi-refined tuna fish oil (T.C. Union Agrotech Co., Ltd.)
[5]Vitamin C (Pharmatech F.C. Co., Ltd.)
[6]Mineral premix (as g / kg): 54.50 g sodium; 2.00 g magnesium; 25.50 g potassium; 5.25 g zinc; 10.00 g pantothenic acid; 12.5 g nicotinic acid; 45.00 g antioxidants; 5.00 g additives in feeding materials (Octamix mineral compound, Octa Memorial Co.,Ltd.)
[7]Vitamin premix: vitamin A, 5,000,000 units; vitamin D_3 24 g; vitamin B_6 49 g; vitamin B_{12} 0.01g; 19.90 g niacin; 2.27 g pantotinic acid; 0.95 g folic acid; 28.27 g vitamin C; (Zebra-0.02vit®, Union Shrimp Trading, Co, Ltd.)
[8]Wheat gluten (Tariko Co., Ltd., China)

For the second trial investigating different levels of lipid and astaxanthin in the diet, the diets containing the higher lipid fractions produced fish which had longer standard and total lengths than those fed the 13% lipid diet. Only the total length of the fish fed the 16% and 20% lipid diets in the first two months of the trial were significantly different ($P \leq 0.05$). The weight of both these higher lipid groups were also higher in the third month of the trail but were not significantly different. Fish survival during this second trial was high, but those receiving the 16% diet were significantly higher ($P \leq 0.05$) (i.e. 98% survival) than the 13% and 20% lipid groups (both had 94% survival).

Table 2. The standard length of *A. ocellaris* fed an experimental diet containing either 40%, 50% or 60% protein for a period of three months.

	40% protein	50% protein	60% protein
Standard length (cm±SE)			
0 month	1.14 ± 0.01	1.14 ± 0.01	1.14 ± 0.01
1 month	2.15 ± 0.05^{a}	2.28 ± 0.04^{ab}	2.36 ± 0.04^{b}
2 month	2.34 ± 0.04^{a}	2.57 ± 0.05^{b}	2.62 ± 0.05^{b}
3 month	2.43 ± 0.04^{a}	2.78 ± 0.04^{b}	2.82 ± 0.07^{b}
Total length (cm±SE)			
0 month	1.40 ± 0.01	1.40 ± 0.01	1.40 ± 0.01
1 month	2.52 ± 0.06^{a}	2.75 ± 0.04^{b}	2.82 ± 0.05^{b}
2 month	2.92 ± 0.06^{a}	3.11 ± 0.07^{b}	3.23 ± 0.06^{b}
3 month	2.98 ± 0.06^{a}	3.31 ± 0.06^{b}	3.34 ± 0.07^{b}
Weight (gram+SE)			
0 month	0.050 ± 0.001	0.050 ± 0.001	0.050 ± 0.001
1 month	0.479 ± 0.028^{a}	0.502 ± 0.023^{a}	0.511 ± 0.022^{a}
2 month	0.555 ± 0.031^{a}	0.624 ± 0.036^{a}	0.625 ± 0.029^{a}
3 month	0.578 ± 0.037^{a}	0.682 ± 0.051^{a}	0.679 ± 0.031^{a}

abc; values in the same row with different superscript letter are significantly different $P \leq 0.05$). Data are mean ± SE, diet 40,50,60 protein, n= 34,37,36, respectively, at the end of experiments.

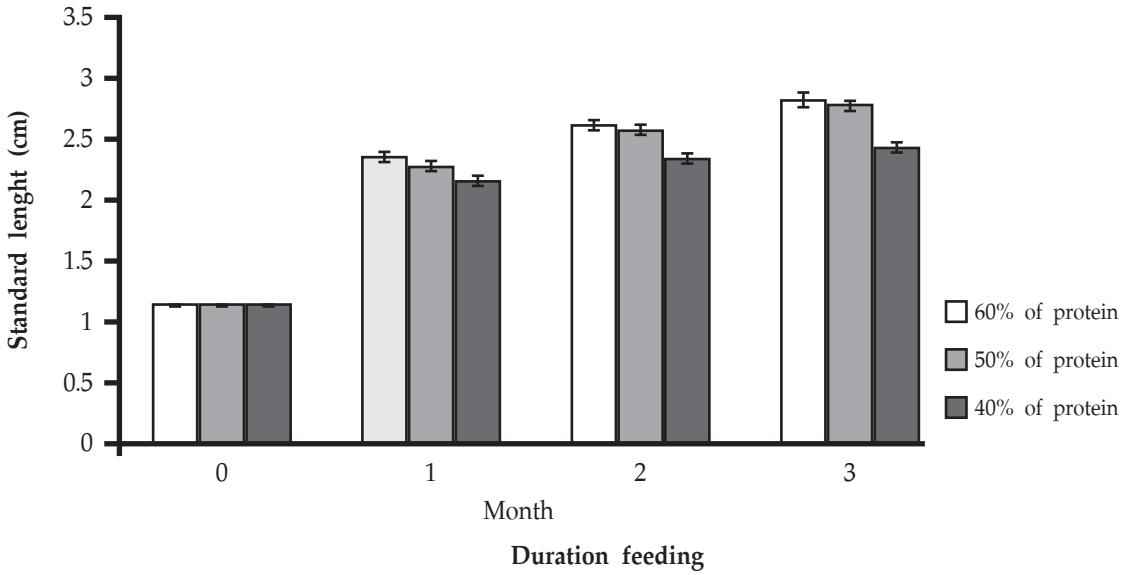

Figure 1. Standard length of *A. ocellaris* fed a diet containing either 40%, 50% or 60% protein.

Table 3. Diet and protein intake, feed conversion efficiency and specific growth rate of *A. ocellaris* by the end of the three-month experiment.

Treatment	Amount of food intake (mg/day)	Amount of protein intake (mg/day)	Feed conversion efficiency	Specific growth rate per day
40% protein	0.428	0.168	0.342	2.71%
50% protein	0.385	0.192	0.456	2.90%
60% protein	0.338	0.200	0.516	2.89%

*Specific growth (SGR%) = $\frac{(\ln Wt) - (\ln Wi)}{Wi}$

Where: Wt = mean final weight and Wi = mean initial weight

*Feed conversion efficiency (FCE) = (Wt) (Wi)/ total feed fed (g)

Where: Wt = mean weight of fish at T2 time (day) and Wi - mean weight of fish at T1 time (day)

*Amount of protein intake = $\frac{\text{amount of food intake x \% protein}}{100}$

Table 4. The survival rate (%) of *A. ocellaris* fed an experimental diet containing either 40%, 50% or 60% protein.

Treatment	1 month	2 months	3 months
40% protein	100[a]	100[a]	85[a]
50% protein	100[a]	100[a]	78[a]
60% protein	100[a]	100[a]	80[a]

a: values in the same column with same superscript letters are not significantly different ($P > 0.05$)

The analysis of the fish samples for pigments revealed that the fish fed the 13% and 16% lipid diets for just one month had accumulated greater amounts of astaxanthin and beta-carotene than those fed the 20% lipid diet. At the end of the experiment, the fish fed with diets containing 30 and 35 mg/kg synthetic astaxanthin had a higher ($P \leq 0.05$) concentration of cantaxanthin than the fish fed the 25 mg/kg diet due to higher concentration of synthetic astaxanthin in the experimental diets. Similarly, the accumulation of zeaxanthin and lutein was found to increase with increasing dietary lipid and synthetic astaxanthin levels.

The results for the fish that were sampled after two months after being on the experimental diets were not dissimilar in their levels of astaxanthin, beta-carotene, zeaxanthin, and lutein which increased with increasing levels of synthetic astaxanthin in the diet. The concentration of cantaxanthin, however, was higher in the 25 and 30 mg/kg astaxanthin diets than in the fish fed the 35 mg/kg astaxanthin diet. The concentration of astaxanthin in the fish samples fed the highest lipid and astaxanthin diet for three months, however, was found to decrease significantly ($P \leq 0.05$).The level of beta-carotene was higher ($P \leq 0.05$) in the fish fed the 35 mg/kg astaxanthin diet than in the fish receiving the other diets. Likewise the measured levels of cantaxanthin, lutein, and zeaxanthin were found to increase ($P \leq 0.05$) with increasing levels of synthetic astaxanthin in the diet.

Table 5. The accumulation of carotenoids in *A. ocellaris* after the first month of having been fed with nine diets (n=20).

Treatment	1 month (mg/Kg)				2 month (mg/Kg)				3 months (mg/ Kg)			
	Astaxanthin	Beta-carotene	Cantaxanthin	Lutein and zeaxanthin	Astaxanthin	Beta-carotene	Cantaxanthin	Lutein and zeaxanthin	Astaxanthin	Beta-carotene	Cantaxanthin	Lutein and zeaxanthin
13% lipid, staxanthin												
25 ppm	0.265	1.463	0.069	0.285	0.089	2.435	0.023	0.620	0.065	13.538	0.014	0.859
30 ppm	0.285	2.928	0.076	0.406	0.176	4.118	0.071	0.799	0.115	28.198	0.042	1.290
35 ppm	0.286	3.864	0.082	1.053	0.107	4.623	0.084	0.916	0.092	33.370	0.062	1.349
16% lipid, astaxanthin												
25 ppm	0.235	2.490	0.100	0.812	0.091	2.943	0.062	0.786	0.071	20.506	0.035	1.115
30 ppm	0.222	3.642	0.123	0.911	0.123	5.026	0.085	0.937	0.102	40.461	0.051	2.401
35 ppm	0.237	4.756	0.097	1.466	0.098	6.874	0.052	1.387	0.079	44.614	0.040	3.001
20% lipid, astaxanthin												
25 ppm	0.252	3.190	0.096	0.424	0.071	4.436	0.079	0.722	0.000	18.244	0.034	2.126
30 ppm	0.178	4.503	0.118	1.317	0.085	6.627	0.054	1.854	0.000	41.050	0.046	3.226
35 ppm	0.079	5.613	0.145	2.253	0.000	7.123	0.038	2.352	0.000	42.964	0.037	3.345

DISCUSSION

This study explored different levels of protein inclusion in the diet and their effect on the growth of juvenile *A. ocellaris*. From the first trial, the best specific growth rates, feed conversion efficiencies and longest standard length increments were seen in those fish fed the diets containing 50% and 60% protein. Although there was better weight gain in the fish groups receiving the high protein content diets, these differences were not significantly different.

In the current study, diets containing higher protein content contribute to optimal growth. These findings agree with the studies conducted by others, for example, Chong et al. (2004), working with swordtails, *Xiphophorus melleri,* found that increasing dietary protein from 20% to 60% had a beneficial effect which produced optimum growth. Although the current trial found no additional benefits in increasing the protein content from 50% to 60%, these rates have been shown elsewhere to produce significant differences, e.g., in European seabass, *Dicentrarchus labrax* (Peres and Oliva-Teles, 1999). From the second trial, the diet containing 16% lipid produced the best growth increments in the juvenile *A. ocellaris.* According to Chatzifatis et al. (2010), increases in the lipid content of the diet beyond 16% given to juvenile seabass and meager, *Argyrosomus regius*, did not result in any additional growth beyond those receiving a 16% lipid diet. The findings of this study are that a 16% lipid content results in optimal growth but beyond this there appears to be no beneficial effects on the growth of juvenile anemone fish.

Likewise, the accumulation of astaxanthin in *A. ocellaris* after the first month was higher in those receiving the 13% and 16% lipid diets. These levels subsequently decreased throughout the duration of the trial. According to Torrissen (1985), the increased level of lipid in the diet dilutes astaxanthin in fish. A later study conducted by Torrissen and Naevdal (1988) suggested that the accumulation of astaxanthin is influenced by the genetic background of the fish and their life stage. These findings would suggest that the accumulation of astaxanthin is determined by lipid level, and that high levels of lipid in the diet have no benefit in the levels that are accumulated.

Zeaxanthin and lutein were found to accumulate with increasing concentration of synthetic astaxanthin. Both pigments were observed to rise throughout the culture period and these may be influenced by lipoproteins in the skin that have an affinity for lutein and zeaxanthin, more so than other carotenoids (Gomes et al. 2002). Additionally, dietary astaxanthin may have been into lutein and zeaxanthin, as demonstrated by Schiedt et al. (1988) who found that in Atlantic salmon, zeaxanthin is one of the main metabolites of astaxanthin. This study found that the levels of zeaxanthin and lutein in the skin of anemone fish was different in the different test groups of fish fed diets containing different levels of synthetic astaxanthin. Zeaxanthin and lutein are important carotenoids in the characteristic orange-red skin coloration of *A. ocellaris*. Other red colored species of fish, however, have not been found to contain the same high level of zeaxanthin as found in anemone fish (Tanaka et al., 1992). The findings of the current study agree with those of Adeljean et al. (2013), confirming that the concentration of astaxanthin in the diet resulted in an accumulation of carotenoid which enhanced the bright coloration of the experimental anemone fish.

ACKNOWLEDGEMENT

This project was supported by the Institute of Marine Science, Burapha University and the Office of the Higher Education Commission, Thailand for Partial Research funding.

REFERENCES

Adeljean, L.F.C., Stephen, K.O., and Harold, F.P. 2013. Dietary esterified astaxanthin effect on color, carotenold concentration, and composition of clown aremonefish, *Amphiprion ocellaris*, skins. *Aquaculture* 21:361-374.

AOAC (Association of Official Analytical Chemists). 1990. *Official methods of analysis of association of official analysis chemists*. Association of Analytical Chemist, Arlington, VA, USA, 648.

Boonyaratpalin, M. 1997. Nutrient requirements of marine food fish cultured in Southeast Asia. *Aquaculture* 151: 283-313.

Britton, G., Liaaen-Jensen, S., and Pfander, H. 1995. *Carotenoids volume 1A: Isolation and analysis*. ISBN 3-7643-2908-4, Basel, Switzerland. 328.

Chong, A.S.C., Ishak, S.D., Osman, Z., and Hashim, R. 2004. Effect of dietary protein level on the reproductive performance of female swordtails *Xiphophorus melleri*_(Poeciliidae). *Aquaculture* 234: 381-392.

Chatzifotis, S., Panagiotidou, M., Papaioannou, N., Pavlidis, M., Nengas, L., and Mylonas, C.C. 2010. Effect of dietary lipid levels on growth, feed utilization, body composition and serum metabolites of meagre (*Argyrosomus regius*) juveniles. *Aquaculture* 307: 65-70.

Choubert, G., Mendes-Pindo, M.M., and Morais, R. 2006. Pigmenting efficacy of astaxanthin fed to rainbow trout *Oncorhynchus mykiss*: effect of dietary astaxanthin and lipid sources. *Aquaculture* 257: 429-436.

Gomes, E., Dias, J., Silva, P., Valente, L., Empis, J., Gouveia, L., Bowen, J., and Young, A. 2002. Utilization of natural and synthetic sources of carotenoids in the skin pigmentation of gilthead seabream (*Sparus aurata*). *European Food Research and Technology* 214: 287-293.

Hansen, A., Rosenlund, G., Karlsen, ø., Koppe, W., and Hemre, G. 2007. Total replacement of fishmeal with plant protein in diets for Atlantic cod (*Gadus morhua* L.) I-Effects on growth and protein retention. *Aquaculture* 272:599-611.

Lem, A. 2001. International trade in ornamental fish. In: *Second International Conference on Marine Ornamental: Collection, Culture and Conservation*. Programs and Abstracts. Florida, United States of America, 26 pp.

Quinton, C. D., McMillan, I., and Glebe, B. D. 2005. Development of an Atlantic salmon (*Salmo salar*) genetic improvement program: Genetic parameters of harvest body weight and carcass quality traits estimated with animal models. *Aquaculture* 247: 211-217.

Peres, H., and Oliva-Teles, A. 1999. Effect of dietary lipid level on growth performance and feed utilization by European sea bass juveniles (*Dicentrarchus labrax*). Aquaculture 197: 325-334.

Schiedt, K., Vecchi, M., Glinz, E., and Storebakken, T. (1988). Metabolism of Carotenoids in Salmons Part3 Metabolites of Astaxanthin and Canthaxanthin in the Skin of Atlantic Salmon (*Salmo sala*, L.). *Helvetica Chimica Acta* 71(4): 887-896.

Tanaka, Y., Yamamoto, A., Kamata, T., and Simpson, K.L. 1992. Biochemical study on the carotenoid in the anemonefish, *Amphiprion spp. Memoirs of Faculty of Fisheries* 41: 1-8.

Torrissen, O., 1985. Pigmentation of salmonids: Factors affecting carotenoid deposition in rainbow trout (*Salmo gairdneri*). *Aquaculture* 46(2):133-142.

Torrissen, O.J., and Naevdal, G. 1988. Pigmentation of salmonids-variation in flesh carotinoids of Atlantic salmon. *Aquaculture* 68:305-310.

Wang, Y., Chien, Y., and Pan, C. 2006. Effects of dietary supplementation of carotenoids on survival, growth, pigmentation, and antioxidant capacity of Characins, *Hyphessobrycon callistus. Aquaculture* 261: 641-648.

Ytrestøyl, T., Coral-Hinostroza, G., Hatlen, B., Robb, D.H.F., and Bjerkeng, B. 2004. Carotenoid and lipid content in muscle of Atlantic salmon, Salmo salar, transferred to seawater as 0+ or 1+ smolts. *Comparative Biochemistry and Physiology Part B* 138(1): 29-40.