J.Animal and Poultry Prod., Mansoura Univ., Vol. 7 (11): 433 - 438, 2016

Effect of Different Sources of Carotenoids on Growth Performance, Stress Response, and Flesh Quality in Fingerlings Grey Mullet, *Liza ramada* in Cultured Floating Cages

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ABSTRACT

The present study was conducted to evaluate the sutability of carotenoid sources (tomato powder, 20, 40 and 60 g / kg; pepper powder, 30, 60 and 120 g / kg and Synthetic Astaxanthin (vit A) at 70, 75 and 80 mg / kg, respectively on growth performance, stress response, feed utilization, body composition, and final flesh quality of *Liza ramada*. A total number of 20 *Liza ramada* (4 g) were randomly distributed into 20 hapas, and were fed daily at a rate of 5 % of fish live body weight through 90 days, each treatment group was applied at two replicates. The results showed that the effect of Synthetic Astaxanthin (vit A) (80 mg) was highly significant among all the other treatment in FBW, ADG, BWG, SGR, and PER. Pepper powder at 30g showed a high significant crude protein % (58.15±0.15) among all the other treatments. The TBARS values in *Liza ramada* flesh showed a significant decrease among all carotenoid sources as the time of frozen storage elapsed as compared to control. The effect of Paper powder at 120 g was highly significant from the other treatment in carotenoid flesh content while the control didn't record any deposit of carotenoids. The study demonstrated that the carotenoids sources showed significant effect on survival, growth performance, feed utilization and TBARS values.

Keywords: *Liza ramada*, Tomato powder, Pepper powder, Astaxanthin, growth performance, Survival, Feed utilization, carotenoids, and TBARS.

INTRODUCTION

Liza ramada is a catadromous fish with a wide distribution (Mediterranean, Black Sea, Azov Sea and Eastern Atlantic from Cape Verde and Senegal to southern Baltic and British Isles), frequently prevailing in polluted waters (Freyhof and Kottelat, 2008). Carotenoids constitute one of the most widely distributed groups of naturally occurring pigments. More than 700 different carotenoid molecules have been described and they can be found in all photosynthetic organisms, in addition to several non-photosynthetic organisms (Britton *et al*., 2004). The colors of these pigments range from yellow to red, and some well-known examples are lycopene (in tomato fruit), zeaxanthin (in maize corn) and β-carotene. In photosynthetic organisms, carotenoids function as accessory light-harvesting pigments, structural components for photosystem assembly, moderators of non-photochemical quenching and as scavengers of reactive oxygen species reviewed in (Britton *et al*., 1999). These antioxidant properties, in addition to their color, have resulted in important industrial applications of carotenoids. In the food, feed and cosmetic industries, carotenoids such as B-carotene are widely used as dyes. In addition, studies claiming that carotenoids can prevent several human diseases (Giovannucci, 1999; Ribaya- Mercado and Blumberg, 2004 and Elliott, 2005) and are used in various nutraceutical products containing carotenoids. However, contradictory studies demonstrate no positive effect on health or even have a negative effect of carotene supplementations (Bjelakovic *et al*., 2007 and Kavanaugh *et al*., 2007). Kotkov *et al*. (2011) reported possible efforts to minimize negative effect of fish oil supplementation by adding antioxidant. Tomato is one of the potential natural antioxidant sources. Tomato contains some phytochemicals including lycopene, folic acid, vitamin C, vitamin A, vitamin E, and phenolics which possess antioxidant activity. Hancz *et al*. (2003) cited that feeding diets containing paprika was reported to improve the skin pigmentation of goldfish and koi carp. Rodrigues *et al*. (2012) reported that astaxanthin protects membrane phospholipids and other lipids against peroxidation and was more effectively than β-carotene and lutein and shows higher scavenging capacity against peroxyl and hydroxyl radicals than that of α-tocopherol, lutein, lycopene and β-carotene. This study aimed to investigate the effect of using carotenoid sources tomato powder, pepper powder and Astaxanthin on growth performance, chemical composition, feed utilization, and final flesh quality of *Liza ramada*.

MATERIALS AND METHODS

Apparently healthy one thousand fingerlings of grey mullet (*Liza ramada*) were obtained from a commercial fish farm in EL-Behera Governorate, Egypt. Fish were acclimated before starting the feeding experimental, and transferred to an earthen pond and reared in hapa prior to the experiment.

Fish and culture conditions:

After two-week acclimation period, fish were selected and randomly stocked into 20 hapa (2*1*1 m), the stocking density was 20 fish per hapa and each treatment was applied in 2 hapas. Mean initial body weight was 4 ± 0.30 g for all treatments. Fish from each replicate were weighted every 2 weeks. All hapas were cleaned weekly. The pond was supplied with fresh water from Edku area. The water exchange rate was 15% of total ponds water volume/day.

Basal diet and experimental design:

Four hundred fingerlings grey mullet were randomly allocated in 20 hapas (2 hapa/ treatment) fish were subjected to the same environmental condition, and the daily amounts of food were introduced as percentage of live body weight at feeding rate of 5 % for 90 days at 2 meals/day.

Preparation of the experimental diets:

The diets were prepared by mixing thoroughly the dry ingredients (Table 1) at first and with oil thereafter. The basal diet was used as a control diet without addition of carotenoids sources. Other tested

diets were prepared by adding different levels of different sours of carotenoid, mainly tomato powder (20 ,40 and 60 g / kg), pepper powder (30, 60 and 120 g / kg), and Synthetic Astaxanthin (70, 75 and 80 mg / kg), respectively. A basal diet was formulated from the commercial ingredients (fish meal, shrimp meal, soybean meal, flour, starch, vit & min , oil and tomato , pepper powder, and Astaxanthin). The dry ingredients were grounded through a feed grinder to very small particle size (0.5 mm). The ingredients were weighed and mixed by a dough mixer for 20 minutes to homogeneity of the ingredients. The estimated amount of oil was gradually added (few drops) and the mixing operation was continued for minutes. After homogenous mixture was obtained , forty ml water per hundred g diet were slowly added to the mixture according to Shimeino *et al*. (1993). The diets were cooked on water evaporator for minutes. The diets were pelleted through fodder machine and the pellets were dried.

Biological parameters:

Mean final body weight (FBW) of each experimental treatment was determined by dividing total fish weight in each hapa by number of fish. Weight gain (WG), specific growth rate (SGR), feed conversion ratio (FCR), protein efficiency ratio (PER), protein productive value (PPV %), energy retention (ER %) and survival (SR %) were calculated using the following equations according to Castell and Tiews (1980):

SGR (%/day) = 100× (ln W1-ln W0)/days.

SR= (No. of fish at end / No. of fish at the start) ×100.

FCR= dry matter feed intake (g)/body weight gain (g)

PER (%) = weigh gain (g) / protein intake (g)

PPV (%) = Retained protein (g) /protein intake (g) ×100.

Stress tolerance:

At the termination of the feeding experiment (90days), stress test has been conducted on fish to evaluate salinity (saline water ca. 35 ‰) effect on treated fish.

Chemical analysis:

Analysis of the experimental formulated diets and whole fish body were carried out for moisture, crude protein, ether extract, crude fiber, ash, and nitrogen free extract (NFE) according to the procedures of association of official analytical chemists (A.O.A.C, 2000) using duplicate samples.

TBARS determination:

At the end of the feeding experiment, samples of *Liza ramada* were put into sacks and stored frozen at -18°C for four months. Samples of different treatments were periodically tested on monthly bases for TBARS starting from October.

Thiobarbituric acid reactive substances (TBARS):

The Thiobarbituric acid value was determined calorimetrically by the method described by kirk and sawyer (1991) using two grey mullet flesh replicates. A portion (200mg) of sample was mixed with a constant volume of 1-butanol. A portion (5.0ml) of the mixture was pipetted into a dry stoppered test tube and 5 ml of TBA reagent (prepared by dissolving 200 mg of 2-TBA in100 ml 1-butanol, filtered, stored at 4°C for not more than7 days) were added. The test tubes were stoppered, vortexed and placed in a water bath at 95°C for 120 min, then cooled. Absorbance (As) was measured at 530 nm against water blank. A reagent blank was run and absorbance (Ab) recorded. Using spectrophotometer (T80 UV/VIS Spectrometer PG instruments Ltd) for estimating absorbance of grey mullet flesh samples. TBA value (mg of malonaldehyde) equivalents/ kg of tissue) was obtained by the formula: TBA = 50 x (As-Ab) / 200

Statistical analysis:

The data collected were statistically analyzed using General Linear Models Procedure (GLM) adapted by SPSS (1997) for users guide. Means were statistically compared for the significance ($P<0.05$) using Duncan'smultiple range test (1955).

Table 1. Shows the feed ingredients and chemical composition of the basal diet:

Ingredients composition	T1	T2	T3	T4	T5	T6	T7	T8	T9	T10
Fish meal	25	25	25	25	25	25	25	25	25	25
Shrimp meal	18	18	18	18	18	18	18	18	18	18
Soybean	27	27	27	27	27	27	27	27	27	27
Corn flour	23	21	19	17	20	17	11	23	23	23
Starch	6	6	6	6	6	6	6	6	6	6
Vitamin and minerals mixture*	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
Corn oil	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.7
Tomato	-	2	4	6	-	-	-	-	-	-
Pepper	-	-	-	-	3	6	12	-	-	-
Astaxanthin	-	-	-	-	-	-	-	70	75	80
Chemical composition										
Dry matter (DM)	92.43	91.83	92.58	90.18	92.8	92.88	95.22	89.73	95.74	89.61
Crude protein(CP)	39.45	39.10	39.15	39.40	39.15	39.60	38.80	39.30	39,30	39.20
Ether extract(EE)	5.37	5.42	4.33	4.33	4.33	5.22	5.67	5.42	4.78	5.31
Ash	10.31	10.85	9.69	11.19	11.19	10.83	9.20	9.72	11.54	8.93
Crude fiber (CF)	2.78	3.05	2.70	2.76	2.89	2.73	2.95	2.75	2.83	2.88
Nitrogen free extract (NFE)	42.09	44.85	44.13	42.32	42.44	41.62	43.38	42.81	41.55	43.68
Gross energy** (Kcal/100gm)	446.22	442.62	443.06	437.04	436.15	443.67	450.62	448.81	437.57	450.73
N:C ratio mg CP: Kcal GE	88.41	88.34	88.36	90.19	89.78	89.26	86.11	87.57	89.81	86.97

T1 : control; T2,T3,T4: Tomato 20-40-60 g/Kg; T5,T6,T7 : Pepper 30-60-120g/Kg),T8,T9,T10 Astaxanthin 70-75-80mg/Kg) add by mg.

***Premix Composition:- Each 3 kg contains Vit A 1200000 i.u., Vit D 300000 i.u., Vit E 700 mg, Vit K3 500 mg, Vit B1 500 mg, Vit B2 200mg, Vit B6 600mg, Vit B12 3mg, Vit C 450mg, Niacin 3000mg, Methionine3000mg, Cholin chloride 10000mg, Folic acid 300mg, Biotin 6mg, Panthonic acid 670mg, Magnesiam salphate 3000mg, Copper sulphate 3000mg, Iron sulphate 10000mg, Zinc sulphate , 1800mg, Cobalt sulphate 300mg, Carrier upto 3000mg.**

****Gross energy, calculated on the basis of 5.64, 4, 11 and 9, 44 kcal GE / g protein, NFE and Ether Extract respectively (NRC, 1993).**

RESULTS AND DISCUSSION

Growth performance and feed utilization:

Growth performance and feed utilization of *Liza ramada* fingerlings has been studied for 90 days and presented in Tables 2 and 3. There were no significant differences in initial body weight among all tested treatment. Which mean that a random distribution of fish was performed in the different treatments at the beginning of the experiment. During the period of the study data indicated that among all the tested treatments Astaxanthin 80mg/Kg diet has been improved FBW, ADG, BWG, and SGR significantly (25.30±0.30, 0.24±0.00, 21.14±0.31, 2.01±0.02, respectively).

Table 2. Effect of different dietary carotenoids sources on final body weight (FBW), body weight gain (BWG), specific growth rate (SGR) and survival rate (%) for *Liza ramada*s fingerlings (mean ± S.E.).

Items	Initial W	Final W	Weight Gain	ADG	SGR	Survival
T1	4.19±0.02	13.19±0.04^{g}	9.01±0.03^{g}	0.10±0.00f^{g}	1.28±0.00^{gh}	92.50±2.50
T2	4.16±0.00	13.63±0.09^{fg}	9.48±0.08^{fg}	0.11±0.01^{efg}	1.33±0.01f^{g}	92.50±7.50
T3	4.21±0.01	15.15±0.14^{d}	10.95±0.12^{d}	0.12±0.00^{d}	1.43±0.01^{d}	85.00±5.00
T4	4.21±0.00	14.53±0.33^{def}	10.33±0.32^{def}	0.12±0.01^{de}	1.38±0.02^{def}	100.00±0.00
T5	4.17±0.01	14.85±0.21^{de}	10.68±0.22^{de}	0.12±0.00^{d}	1.41±0.02^{de}	90.00±0.00
T6	4.19±0.02	13.96±0.21^{efg}	9.77±0.22^{efg}	0.11±0.00^{def}	1.34±0.02^{efg}	90.00±0.00
T7	4.21±0.01	12.97±0.45^{g}	8.76±0.46^{g}	0.10±0.01^{g}	1.25±0.04^{h}	100.00±0.00
T8	4.23±0.00	16.67±0.53^{c}	12.44±0.53^{c}	0.14±0.01^{c}	1.53±0.04^{c}	92.50±7.50
T9	4.18±0.00	22.93±0.32^{b}	18.75±0.32^{b}	0.21±0.00^{b}	1.90±0.02^{b}	95.00±5.00
T10	4.17±0.01	25.30±0.30^{a}	21.14±0.31^{a}	0.24±0.00^{a}	2.01±0.02^{a}	92.50±7.50

a,b,c,d,e,f, and g means in the same column with different letters differ significantly at 0.05 level .
T1 : control (T2,T3,T4: Tomato 20-40-60 g/Kg), (T5,T6,T7 : Pepper 30-60-120g/Kg),T8,T9,T10 Astaxanthin 70-75-80mg/Kg).

Data of growth parameters and feed utilization of *Liza ramada* summarized in Tables 2 and 3 showed that the response of fish to different levels of tomato, pepper, and astaxanthin in diet is different. Data show that all fish fed different carotenoid sources diet resulted in higher growth performance than the control diet, suggesting that the addition of carotenoid (Astaxanthin 80mg/Kg) to the diets enhanced the growth performance and survival (%) of *Liza ramada* which gave the highest values of final body weight (FBW), weight gain (WG), specific growth rate (SGR) and survival (%) while the lowest values was observed for fish fed the control diet. The same trend was observed for PER, PPV (%), ER (%) and the best FCR vales. These results are in partial agreement with the results of Maeda *et al*. (2013) and Sajjad *et al.* (2013), who reported the effect of placement carrot and red pepper on growth, pigmentation and blood factors of rainbow trout and grey mullet. The results showed a significant difference between treatments in terms of weight and length ($P<0.05$) and no significant differences ($P>0.05$) in food intake and growth performance parameters. The successful use of *Liza ramada* (Strati and Oreopoulou, 2014) of carotenoids from tomato processing by-products indicated that tomato waste should be regarded as potential nutraceutic resource and may be used as a functional food ingredient. Plant carotenoids can be stated with greater accumulation in the tissues, due to its vitamins, carotene, and immersed color (Amaninejad, 2009 and Faghani *et al.,* 2009) has been reported for *Liza ramada* feeding. Carotenoids in dry tomato by-product and pepper have positive effect on growth rate and survival rate may be due to well credited with important health promoting functions, such as provitamin A and antioxidant activity enhancement of the immune system and reduction of the degenerative diseases such as cancer (Wang *et al*., 2006 and Hernandez *et al*., 2007). Kalinowsky *et al*. (2005) found that carotenoids have appositive effect on growth performance of pagers. A similar study by Hu *et al.* (2006) on young hybrid tilapia, was indicative of the fact that dietary β- carotene resulted in an increase in growth performance of the fish. These results are in partial agreement with the findings of Yew-hu and Wen (2005) and Strati and Oreopoulou (2014) who reported that the control diet and diet containing dried tomato, pepper (60 Mg/Kg) and astaxanthin, all being a rich source of natural carotenoids and astxanthin levels (50.and 100Mg/Kg) fed to *Liza ramada* produced significantly higher PER, FCR and PPV values compared to diets (control) without tomato, pepper and Astaxathin.

Table 3. Effect of dietary carotenoids on feed intake, feed conversion ratio (FCR), protein utilization (PER-PPV), energy gain and utilization for *Liza ramada* fingerlings.

Items	Feed intake	FCR	PER	PPV	Energy Gain	Energy U
T1	36.31±0.16^{d}	3.84±0.02^{b}	0.66±0.01f^{g}	12.98±0.16d^{e}	16.33± 0.26^{d}	10.52± 0.25^{c}
T2	35.31±0.06^{c}	3.23±0.02^{bc}	0.67±0.01^{fg}	12.05±0.11^{e}	16.56± 0.22^{d}	10.31± 0.03^{c}
T3	36.65±0.07^{cd}	3.56±0.11^{ef}	0.79±0.00^{cd}	15.01±0.07^{cd}	19.53± 0.41^{cd}	12.49± 0.23^{bc}
T4	36.58±0.16^{bc}	3.53±0.05^{cd}	0.72±0.02^{ef}	12.90±1.05^{de}	18.39± 1.27^{cd}	11.50± 0.99^{c}
T5	34.87±1.04^{bc}	3.57±0.03^{de}	0.75±0.02^{de}	14.29±0.26^{ced}	18.10± 0.49^{cd}	11.35± 0.53^{c}
T6	36.78±0.35^{d}	4.21±0.18^{cd}	0.71±0.00^{ef}	14.11±0.37^{ced}	17.52± 1.12c^{d}	11.32± 0.49^{c}
T7	38.01±0.34^{bc}	3.06±0.16^{a}	0.62±0.03^{g}	13.41±2.12^{ced}	16.87± 2.57^{d}	10.18± 1.55^{c}
T8	51.37±0.07^{b}	2.74±0.04^{f}	0.83±0.04^{c}	16.16±0.94^{bc}	21.41± 0.89^{c}	12.56± 0.72^{bc}
T9	50.54±0.58^{a}	2.39±0.01^{g}	0.93±0.03^{b}	18.01±0.01^{ab}	33.21± 1.43^{b}	14.77± 0.64^{ab}
T10	34.82 ±0.58^{a}	3.39±0.03^{h}	1.07±0.01^{a}	19.83±0.25^{a}	38.28± 0.74^{a}	16.80± 0.13^{a}

a,b,c,d,e,f, and g means in the same column with different letters differ significantly at 0.05 level .
T1 : control (T2,T3,T4: Tomato 20-40-60 g/Kg), (T5,T6,T7 : Paper 30-60-120g/Kg),T8,T9,T10 Astaxanthin 70-75-80mg/Kg)

Results of this experiment conducted with studies on trout fingerling with plant carotenoids (Mehrobi *et al.,* 2008). Carotenoids in salmon fishes (Bjerkeng *et al.,* 2000) were similar, but studies on shrimp and trout fed with red pepper (Harpaz *et al.,* 2000 and Yanar *et al.,* 2007) this positive effects on feed efficiency may be due to tomato, pepper waste extracts contained considerable amounts of carotenoids (lycopene and β-carotene), and exhibit good antioxidant and anti – proliferation activities. Therefore, because of their low costs and bio- renewable nature, tomato waste could be alternative source of valuable bioactive compounds. Maede *et al.* (2013) showed that the use of vegetable ingredients in diets for rainbow trout is an effective and affordable and the diet of 55 mg/Kg red bell pepper is available diet for rearing rainbow trout.

The data illustrated in Table 4 showed the effects of deferent (carotenoids) on carcass composition and gross energy of *Liza ramada* fingerlings. The results indicated significant (P<0.05) differences among the body percentage of crude protein (CP), ether extract (EE), ash and carcass energy. Meanwhile, the treatment fed with diet pepper (6%) increased CP% and decreased the value of EE and carcass energy. As for dry matter, there were no significant differences among the treatments. As the concentration of (crude protein, CP) increased in the diet, the carcass water content was reduced. The lowest fat and highest ash contents were recorded in the fish fed the 20% CP diet. Furthermore, Richter *et al*. (2003) and Abdelhamid *et al.* (2004) studied the effect of dried moringa leaf meal and water hyacinth inclusion level (20 and 30%) as alternative protein source for Nile tilapia on body chemical composition. They reported that increasing the level of moringa leaves in the diet increased moisture body, while lipid and gross energy values decreased significantly (p<0.05). Crude protein and crude ash contents remained constant in all experimental groups.

Table 4. Effect of different carotenoids sources on carcass composition for *Liza ramada* fingerlings.

Items	Dry matter	% on dry matter basis			Carcass energy
		Protein	Ether extract	Ash	
T1	29.81±0.27	56.02±0.38^{bc}	25.10±0.08^{a}	18.89±0.47	552.85±2.95^{a}
T2	30.73±0.23	56.85±0.15^{ab}	24.07±0.81^{ab}	19.09±0.96	547.81±8.44^{ab}
T3	30.70±1.15	54.55±0.35^{c}	25.24±0.64^{a}	20.21±0.29	545.93±4.07^{ab}
T4	30.41±0.80	57.90±0.10^{a}	21.77±0.57^{c}	20.33±0.47	532.07±4.82^{b}
T5	31.03±0.76	58.15±0.15^{a}	22.62±0.52^{bc}	19.23±0.37	541.50±4.06^{ab}
T6	33.05±2.70	57.80±0.60^{a}	21.80±0.60^{c}	20.40±0.01	531.78±2.28^{b}
T7	30.90±0.25	58.00±0.10^{a}	21.74±0.46^{c}	20.26±0.36	532.35±3.77^{b}
T8	31.61±1.23	58.06±0.64^{a}	22.55±1.05^{bc}	19.39±0.41	540.33±6.30^{ab}
T9	31.73±0.08	56.00±0.10^{bc}	24.87±0.47^{a}	19.14±0.37	550.57±3.82^{a}
T10	31.12±0.56	57.31±1.11^{ab}	23.25±0.45^{abc}	19.44±0.66	542.71±2.01^{ab}

a,b,c,d,e,f, and g means in the same column different letters differ significantly at 0.05 level .

T1 : control (T2,T3,T4: Tomato 20-40-60 g/Kg), (T5,T6,T7 : Paper 30-60-120g/Kg),T8,T9,T10 Astaxanthin 70-75-80mg/Kg.

The effect of salinity of *Liza ramada*:

During stress period, survival of control group was very high under salinity stress condition compared with all diets containing β-carotene supplemented diets. Pepper supplemented diet (120 g/kg) at time 34.15 hours followed by that containing tomato and astaxanthin compared with the control. The effects of dietary supplementation of carotenoids (tomato, and pepper) and astaxanthin on low and high salinity stress resistance (Table 5) when subjected to change salinity stress *Liza ramada* fed dietary supplementation of carotenoids. These results agree with those of Yen-hu and Wen (2005) and similar results were reported by Chien *et al.* (1999) on juvenile fed with diet without astaxanthin supplement compared with those fed with 360 mg/kg astaxanthin for one week.

Table 5. The effect of dietary supplementation of tomato, pepper, and astaxanthin on salinity (35 mg /l) stress resistance of *Liza ramada*.

No of dead fish	T1	T2	T3	T4	T5	T6	T7	T8	T9	T10
1	3.13	2.08	2.70	2.40	7.15	6.15	29.20	4.20	3.13	5.13
2	3.10	2.18	3.80	3.22	9.28	7.25	32.55	4.40	3.15	5.15
3	3.28	2.55	4.05	4.15	12.35	8.20	35.15	5.15	3.38	5.43
4	3.45	3.13	6.05	4.35	13.18	9.15	35.48	5.43	4.38	6.23
5	3.75	3.18	6.25	5.05	13.48	9.15	36.28	6.20	5.15	6.35
MEAN*	3.26	2.46	4.53	3.77	11.25	8.18	34.15	5.42	4.16	5.58

***Average number of hours incurred by fish of high salinity .**

T1 : control (T2,T3,T4: Tomato 20-40-60 g/Kg), (T5,T6,T7 : Paper 30-60-120g/Kg),T8,T9,T10 Astaxanthin 70-75-80mg/Kg)

The effect of storage on the quality of fish flesh:

The TBA reactive substance is a test which indicates the oxidative changes in muscle foods during storage (Egan *et al*., 1981). Thiobarbituric acid reactive substance (TBARS) is widely used as indicator for the assessment of the degree of lipid oxidation (Jeon *et al*., 2002). In the present study (Table 6) the TBA values of the different treatment recorded a steady rise throughout the storage period (3 months at -18°C). As a matter of fact, the feed containing tomato powder treatment at 20 and 40% (T1 and T2) exhibited a significant decrease in TBA value of flesh being 5.41 and 5.09, mg melanodialdehyde / kg tissue as compared to the T3 treatment and control which reached 7.63 and 6.33 melanodialdehyde / kg tissue at the end of the storage period, respectively. On the other hand, the feed containing 30 g/kg (T4) paprika recorded the best TBA value at the end of the frozen storage period being 5.16 mg malondialdehyde /kg tissue, whereas the TBA value of T5 and T6 reached 7.82 and 10.00 mg malondialdehyde /kg tissue at the end of the storage period even though higher than the control samples. It is well documented that the high concentration of natural antioxidants may exhibit an attenuating effect or a pro – oxidant effect (Aruoma *et al*., 1992). On contrary to the aforementioned results, the synthetic vitamin (A) recorded a significant decrease (p< 0.05) in TBA value of the fish tissue which was more pronounced at the higher levels used (T8 and T9) being 4.38 and 4.61 mg melanodialdehyde/kg tissue as compared to the control (6.33) and all the other treatments at the end of the storage period. It may be concluded that tomato powder at T1 and T2 addition and paprika at T4 addition achieved significant potential in lowering the onset of lipid oxidation as compared to synthetic vitamin A and control samples. Notwithstanding, it was reported that the maximum level of TBARS value indicating good

quality of fish tissue frozen, chilled or stored in ice is 3-5 mg melanodialdehyde/ kg Tissue (Egan *et al*., 1981).

Table 6. the Effect of different concentrations of plant ingredients and vitamin(A)on thiobarbituric acid reactive substances (TBARS) of grey mullet flash during frozen storage at -18°C.

Treatment	Storage time (months)			
	Zero	1 month	2 month	3 month
Tomato powder				
T2	4.50 ±0.05 ^{bc}	4.67±0.05^{bc}	5.11±0.33^{bcd}	5.41±0.39^{cd}
T4	4.22±0.33 ^{bc}	4.46±0.36 ^{bc}	4.82± 0.27^{cd}	5.09± 0.28^{cd}
T6	6.11±0.63^{ab}	6.28±0.66 ^{ab}	7.16±0.35^{ab}	7.63±0.31^{b}
Paprika				
P3	4.13 ±0.51^{bc}	4.27±0.55 ^{bc}	4.89±0.98^{cd}	5.16 ±1.08^{cd}
P6	5.97±0.28^{ab}	6.21 ±0.33 ^{ab}	6.83±0.49 ^{abc}	7.82±0.00^{b}
P12	6.94±1.07^{a}	7.34±1.34 ^{a}	8.40±1.39^{a}	10.00± 0.72^{a}
Vitamin A pure (ml)				
A70	5.07±1.19^{abc}	5.22±1.14 ^{abc}	6.74±0.16 ^{abc}	6.06±0.58^{bcd}
A75	3.53± 0.24^{c}	3.77±0.14^{c}	4.06±0.26^{d}	4.38±0.15^{d}
A80	4.09± 0.51b^{c}	4.25±0.44 ^{bc}	4.43±0.51^{d}	4.61± 0.60^{cd}
Control	5.38±0.43ab^{c}	5.70±0.25 ^{abc}	6.01 ±0.22^{bcd}	6.33 ±0.16^{bc}

a,b,c,d,e,f, and g means in the same column different letters differ significantly at 0.05 level .

(T2,T3,T4: Tomato 20-40-60 g/Kg), (P4,P5,P6 : Paper 30-60-120g/Kg),(A70,A75,A80Astaxanthin 70-75-80mg/Kg).

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تأثير مصادر مختلفه من الكاروتينات على النمو والإستجابه للإجهاد فى سمك الطوباره فى الاقفاص العائمة

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اجريت هذه الدراسة لتقييم تأثير التغذية بمصادر كاروتين مختلفة وهى مسحوق الطماطم (٢٠،٤٠،٦٠ جم/كجم)، مسحوق الفلفل (٣٠،٦٠،١٢٠ جم/كجم) والاستاكزانسين الصناعى (٧٠،٧٥،٨٠ مللى/كجم) على التوالى على معدلات النمو والإجهاد وجودة اللحم النهائية، حيث استخدم ٢٠ اصبعيه بمتوسط وزن ٤ جم وضعت فى هابات (٢×١×١م) لمدة ٩٠ يوما وكانت عدد المعاملات ١٠ معاملات و لكل معاملة مكررتين، وقد أظهرت النتائج أن أفضل معاملة هى استخدام الاستاكزانسين الصناعى (فيتامين أ) ٨٠ مللى/ كجم بالمقارنه بالمعاملات الاخرى والكنترول فى الوزن النهائى ، معدل الزيادة اليومية ، الزيادة فى وزن الجسم، معدل النمو النوعى وكفاءة الإستفادة من البروتين (٢٥,٣٠، ٠,٢٤ ، ٢١,١٤ ، ١,٠٧) بالترتيب، وفى معاملة مسحوق الفلفل ٢٠ جم/كجم وجد ارتفاع معنوى فى البروتين عن المعاملات الأخرى (٥٨,١٥). وانخفضت قيم TBARS إنخفاضا معنويا فى المعاملات التى تغذت على مصادر الكاروتينات وكانت أقل معاملة فى مسحوق الفلفل ١٢٠ جم/ كجم.