Interactive effects to previous feed, low water temperature and nutrient restriction on metabolic response of male Nile tilapia (*Oreochromis niloticus*)

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Abstract: A growth trail and metabolic response were conducted to interactive effects for previous feed, low water temperature and nutrient restriction on male Nile tilapia. After the end experimental diets, male Nile tilapia returned to their original nine experimental diets fish oil replacement and alternative flaxseed oil and beef tallow in two replicates in to outdoor cements ponds (30 fish/pond) periodical sampling was conducted after 30, 60 and 90 days under lower temperature(.from 16.5 ± 1.8 ° C (November to December ; 12.4 ± 0.7°C (December to January) to 13.6 ± 1.1 °C (January to February respectively . After 90 days with nutrient restriction, a significant (P<0.05) reduction was observed in weight gain, thermal growth coefficient protein deposition, lipid deposition, energy deposition, total lipid in liver and hepatosomatic index between treatment, while between periodical sampling, no change we seen after 30 days in weight reduction %, protein, lipid and energy deposition. However, with total lipid in liver a significant reduction (P<0.05) was seen after 30 days. No significant reductions (P>0.05) in hematological parameters between periodical sampling. Fish treatment inter periodical sampling, the significant reduction (P<0.05) was observed in weight reduction, total lipid in liver and energy deposition. Fish previous fed 25 % fish oil and 75% beef tallow or fish previous fed 50% fish oil + 50% beef tallow its indicating that mixed fish oil with beef tallow in dietary male Nile tilapia, may was the cause in improve survival rate during lower water temperature with nutrient restriction appeared little reduction in weight gain (%) and higher reduction in total lipid in liver.
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1. Introduction:

As a tropical fish species, they are well adapted to worm waters, but mass mortalities of cultured tilapia have often been reported due to sever cold current in winter (Hsiew *et al.* , 2007).

Changes in environmental conditions challenge an organism to maintain homeostasis (Fiess*et al.* ,2007).

Winter temperature in the main culture areas are usually favorable yield cold spells do occur and cause considerable mortalities (Viola *et al.* ,1988) and the lethal temperature for tilapia hybrid (*Oreochromis aureus*×*O. niloticus*) is about 9°C for spells of 6-8 hours . In several studies it has been demonstrated that an increase in the content of poly unsaturated fatty acids (PUFA) in membrane phospholipids raised the membrane fluidity and lowered the phase transition temperature (Bell *et al.* ,1986) .

It was postulated that these changes may increase viability of fish at temperatures near zera (Farkas, 1979; Brenner, 1984). Hsieh *et al.* (2007) with grass carb, and with common carb (Truema*et al.* ,2000) .

Evidented when fish adapting to cold stress is the increased level of unsaturated fatty acids to maintain homeoviscousfluidity . Though, Rios *et al.* (2002) reported that the adaptive reductions in metabolic rate that are induced by starvation during overwintering may involve changes in respiratory frequency, which is closely related to the maintenance metabolism of an fish. Can *et al.*(1996) showed that the gastrointestinal tract and associated organs can account for approximately 40% of the resting metabolic rate of an animal.

Though, exposure to low temperatures usually leads to a reduction in the proportion of saturated fatty acids and a corresponding increase in unsaturated fatty acids (Hsieh *et al.*, 2007). Martins *et al.*(2009) showed that vegetable oils are characterized by a high C18 – fatty acid content , where as terrestrial animals fats are particularly rich in saturates fatty acids. Tocher (2003) found that fatty acids play vital roles in many physiological functions, from energy supply, to membrane integrity to eicosanoid production.

Qiang*et al.*(2013) reported that the healthy cultivation of tilapia depends on nutritional status and environmental conditions, and they suggested that temperature is a well – known principal environmental cue. Sun *et al.*(1995) found that tilapias stop feeding when temperature goes below 15° C and are unable to reproduce below 20° C.

The aim of the present study was to investigate the effect of feeding and subsequent under low water temperature nutrient restriction exposure of male Nile tilapia in an attempt for increase survival rate under cold shock. Three different periods were conducted to

assess; effect of previous feed, low water temperature and fasting on growth performance, chemical body composition , Hepatosomatic index, total lipid in liver and some hematological parameters (hematocrit value , hemoglobin concentration and red blood cells count.)

2. Materials and methods

Experimental conditions

This experiment was conducted at El-kanater El-Khayria .Research station of the National Institute of Oceanography and Fisheries, Egypt.

Experimental design :

The remaining male Nile tilapia (*Oreochromis niloticus*) from previous experiment diet by El-Hammady *et al.* (in press) , Fish oil replacement and alternative flaxseed oil and beef tallow in male Nile tilapia (*Oreochromis niloticus*)diets.

After the end previous experiment , fish were returned to their original experimental ponds into a cement ponds consisting of 18 (10m3 /unit) Fish with an initial weight ranged from 180.25 to 201.03g according to previous studs by El- Hammady *et et al.*(in press) with treatments which were: Fish oil (Fo); Flaxseed oil (Fxo) ; beef tallow (BT) ; blends of 50% fish oil + 50% flaxseed oil (Mix.1); 50% fish oil + 50% beef tallow (Mix.2); 50% flaxseed oil + 50% beef tallow (Mix.3),25%Fish oil+37.5 % flaxseed oil + 37.5% beef tallow (Mix.4) ; 25 % fish oil + 75% flaxseed oil (Mix.5) ; 25% fish oil + 75% beef tallow(Mix.6) . All fish groups were subjected to fasting forgo days from November 2012to February 2013(90days)

Periodical sampling was conducted after 30, 60 and 90 days to follow the growth performance; chemical body composition ,hepatosomatic index ; some hematological Parameters , and survival fish were recorded.

Analytical methods:

The test diets and fish fillets were analyzed for chemical composition following AOAC (1996) methods : dry matter by weight loss after 24 h in an oven at 105 ◦C for 16 h; crude protein (% × 6.25) determined using the Kjeldahl method , dietary lipids extracted was measured by ether extraction using Soxhlet method using Petroleum ether (40-60 ◦C boiling point) , however total lipid in fish fillets and livers sample were measured according to Folch *et al.* (1957) ,gross energy MJ kg-1 was calculated 1g crude protein = 23.6 K.J 1g crude lipid = 39.5 kj. ,1g nitrogen free extract = 17.2 kj according to Thanuthong *et al.* (2011).

Blood sampling:

At the end of the feeding trial , and blood was collected from the caudal vein used as an anticoagulant for hematology (hemoglobin, haematocrit and redblood cells counting). Haematocrit values (Ht) were immediately determined after sampling by placing fresh blood in glass capillary tubes and centrifuging for 15 min in a microhematocrit centrifuge Hemoglobin (Hb) level was determined colorimetrically by measuring the formation of cyanomethaemoglobin according to van Kampen and Zilstra (1961).

Calculations:

Specific growth rate of weight (SGR , % day^{-1})

$= (L_n W_f - L_n W_i) \times 100 / t$

Where ,W_f and W_i are final and initial mean weight g.

Hepatosomatic index (HSI) = 100 X (liver weight) / (body weight)

Survival = final fish number – initial fish number .

Statistical analysis:

Data are presented as the mean ±sd two- way analysis of variance (ANOVA) and Duncan's multiple testes were also conducted to determine the significance of differences among the time-course measurement for fish previous fed the same diet, and differences among the fish previous fed diets. Differences were regarded as significant when $P<0.05$. SPSS(spss 11.0) statistical software was used.

3.Results

In this study , fasting with lower water temperature in significant reductions in body weight(Fig .1) , specific growth rate and hepatosomatic index(Fig.3a) in the tilapia. 90 days of fasting also caused significant reductions in total lipid in liver (Fig.3b)and haematology parameters (Hct, Hb and RBC) (Fig4.a.b.c). After 30 days of fasting caused significant reductions ($p<0.05$) hepato-somatic index and total lipid in liver and also, body deposition (Fig.2 a,b,c) while hematology parameters were unchanged significant ($P> 0.05$). However, after 60 days noted an decrease significantly ($P<0.05$) in HSL , total lipid in liver , lipid body in fillet and hematological parameters (Hct, Hb, RBC). HSl , and total lipid in the difference in growth reduction liver became little apparent during period 60 to 90 days fast .Survival number was ranged between 26 (Mix. 6 and Mix.2) to 22 (Mix. 2 and BT.) fish from 30 fish / treat.

At the end of the 90 days fasting, there were significant differences ($P<0.05$) observed for thermal growth coefficient (Table .1). Fish pervious fed Mix 1 and Mix 4 recorded negative increased in TGC (-0.266; - 0.256) , while fish in treat BT and Mix 6 recorded negative decreased in TGC (-0.181; -0.197) .

After 90 days fasting with lowest in water temperature may be decrease growth (Table . 2)from – 16.51 (Mix. 1) to – 12.73% (Mix. 6); protein deposition – 63.01% (Fxo.) to – 50.09% (Mix 1), lipid deposition from -44.89% (Fo) to – 32.70% (BT), energy deposition from – 81.55% (Fxo) to- 69.60% (Mix. 5) ,

heptosomatic index – 51.02% (Mix. 1) to – 39.08% (BT); total lipid in liver ranged from -58.06% (BT) to – 48.06% (Mix. 5) ; haematocrit value from – 7.91% (BT) to – 4.21% (Mix. 4) hemoglobin concentrate from -8.96% (BT) to – 6.30% (Mix. 5) ; and red blood cells count ranged from – 5.18% (Mix. 5) to – 4.26% (Mix. 4) respectively.

Table 1:Growth performance of male Nile tilapia (*Oreochromis niloticus*)after being fed diets during fluctuation water temperature

	FO	FXO	BT	Mix.1	Mix.2	Mix.3	Mix.4	Mix.5	Mix.6
Initial body weight(g)	192.76 ± 3.34	189.41± 3.78	171.24 ± 3.66	197.22 ± 3.49	186.04± 3.57	190.96 ± 2.47	201.03± 4.11	190.74± 2.84	180.25 ± 3.56
Final body (weight(g)	164.14± 2.70	161.50± 3.05	150.72 ± 3.12	164.66± 2.77	161.93 ± 2.99	164.06 2.02	169.30± 3.24	162.97± 2.31	157.30 3.00±
Weight gain(g)	28.62± 1.17 a	27.91± 1.25 ab	22.52 ± 1.14 c	32.56± a -1.41	24.11 ± 1.15 bc	26.90 ± 1.23b	31.73± 1.12 a	27.77 ± 1.05b	22.95 ± 1.13 c
Weight reduction (%)	14.85± 0.61 a	14.74 ± 0.82 ±	12.98± 0.53 b	16.51 ± 0.95 a	12.96 + -0.64 b	14.09± 0.76 b	15.78 ± 0.88 a	14.056 ± -0.79 a	12.73 ± 0.56 b
Specific growth rate (%/day)	-0.179 ±0.021 a	-0.177 ±0.019 c	-0.142 ±0.017 bc	-0.025 a ±0.200	-0.154 b ± 0.015	-0.169 ab ±0.024	-0.191 a ± 0.027	-0.175 a ± 0.019	-0.151 b ± 0.013
Survival number / 30Fish	24	24	22	24	26	22	24	25	26
Thermal growth coefficient	-0.236 ± 0.03	-0.233 ± 0.04	-0.181 ± 0.02	-0.266 ± 0.03	-0.202 ± 0.04	-0.222 ± 0.03	-0.256 10.04	-0.231 ± 0.03	-0.197 ± 0.04

Values with different superscripts in each row significantly differ (P< 0.05)different letters (a,b, and C) indicates that differences between the diets were significantly (P < 0.05) for tilapia .

Table.2. Percentage change in some metabolic response of male Nile tilapia during nutrient restriction and lower water temperature (mean ± Sd.)

	Weight gain	Protein deposition	Lipid deposition	Energy deposition	Heoatosomatic index	Total lipid in liver	Hematocrit value	Hemoglobin concentration	Red blood cells
FO	14.85±1.22a	55.18±2.51b	44.89±2.06a	73.60±2.69b	44.57±1.85b	55.40±2.47ab	5.34±0.96b	8.63±1.41a	4.57±0.89a
FXO	14.74±1.16a	63.01±2.36a	42.06±1.75a	81.55±2.83a	47.69±2.16a	48.39±2.19c	6.65±1.14a	7.00±1.25ab	4.86±0.74a
BT	12.98±1.09b	51.63±1.82c	32.70±2.12c	73.83±3.05b	39.08±2.55b	58.06±2.85a	7.91±1.24a	8.96±1.68a	4.62±0.92a
mix. 1	16.51±1.25a	50.09±2.05c	39.62±1.67b	71.30±2.74bc	51.02±1.87a	48.97±2.91c	5.48±0.78b	7.58±1.49a	4.77±1.04a
mix. 2	12.96±1.17b	56.96±1.76b	40.23±2.15ab	73.07±2.65b	42.62±2.63b	57.48±2.75a	6.13±0.88ab	8.39±2.05a	4.81±1.13a
mix. 3	14.09±1.28ab	53.32±1.53bc	39.29±1.83b	71.50±2.84b	47.56±2.19a	51.29±2.81bc	5.73±1.04b	7.34±1.65a	4.38±0.92a
mix. 4	15.78±1.11a	52.45±2.08c	43.93±2.05a	72.92±3.14b	45.42±2.07ab	54.68±2.61b	4.21±0.75b	8.42±1.97a	4.26±0.75a
mix. 5	14.56±1.17a	55.83±1.76b	37.38±1.65b	69.60±2.56c	47.51±2.19a	48.06±2.46c	6.44±0.89a	6.30±1.65b	5.18±0.89a
mix. 6	12.73±1.08b	56.33±2.14b	39.56±1.84b	71.41±2.82b	43.00±2.85b	57.16±2.17a	6.78±0.9a	6.47±1.83b	4.64±0.88a

Value with different superscripts in each column significantly differ (*P*<0.05) different letters (a,b and c) indicate that different between the diets were significant (*P*<0.05)

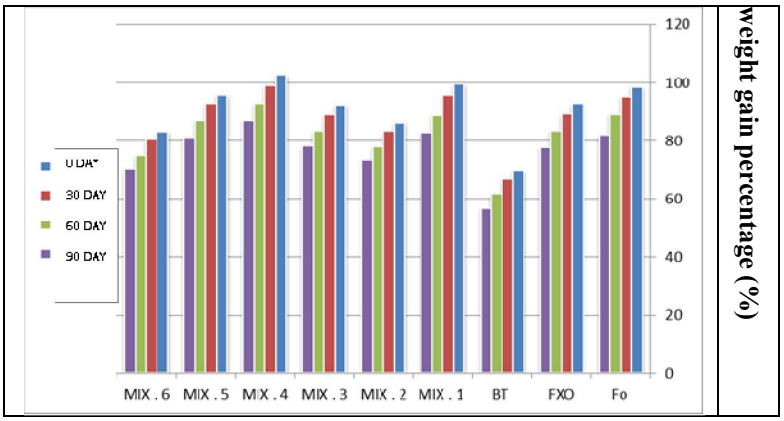

Fig.1Changes in weight gain percentage (%) of Male Nile tilapia previous fed diets containing different sources of lipids at various times under lower temperature and nutrient restriction .

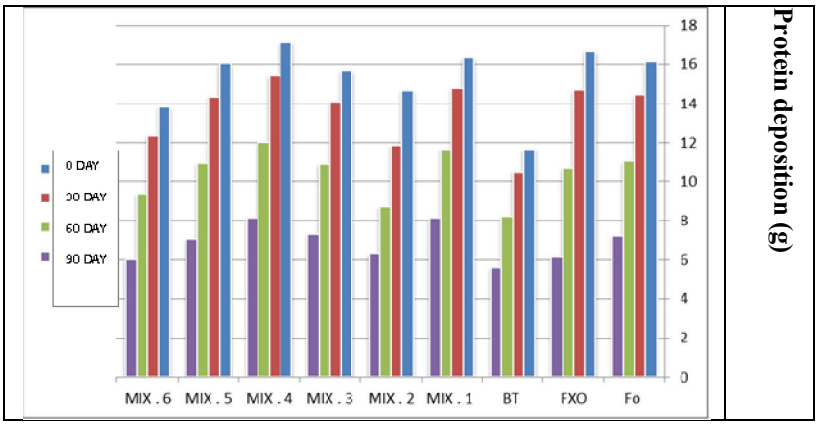

Fig.2 (a) Changes in protein deposition (g) of Male Nile tilapia previous fed diets containing different sources of lipids at various times under lower temperature and nutrient restriction .

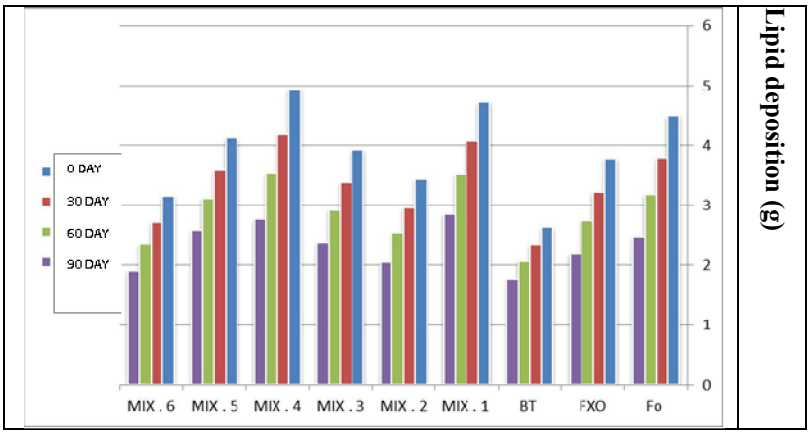

Fig.2 (b) Changes in Lipid deposition (g) of Male Nile tilapia previous fed diets containing different sources of lipids at various times under lower temperature and nutrient restriction .

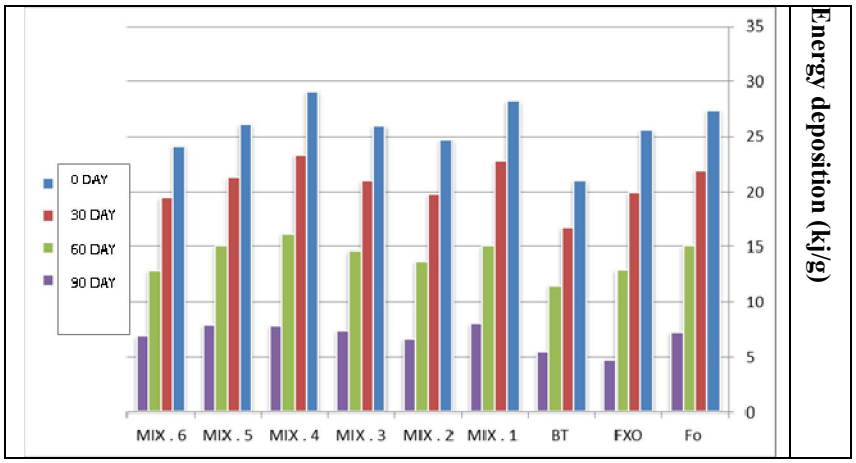

Fig.2 (c) Changes in energy deposition (kj/g) of Male Nile tilapia previous fed diets containing different sources of lipidsat various times under lower temperature and nutrient restriction .

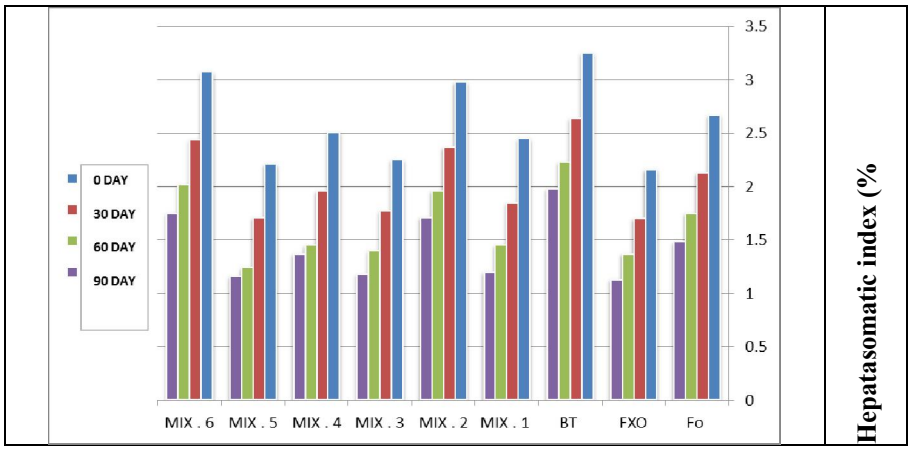

Fig.3 (a) Changes in hepatasomatic index (%) of Male Nile tilapia previous fed diets containing different sources of lipids at various times under lower temperature and nutrient restriction .

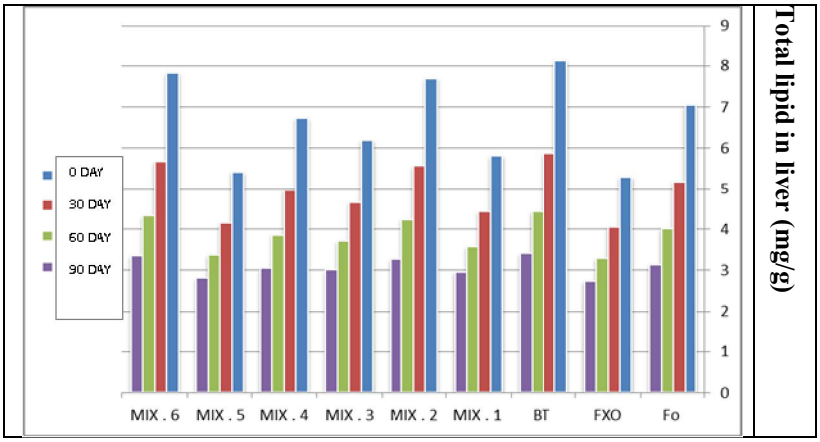

Fig.3 (b) Changes in total lipid in liver (mg/g) of Male Nile tilapia previous fed diets containing different sources of lipids at various times under lower temperature and nutrient restriction .

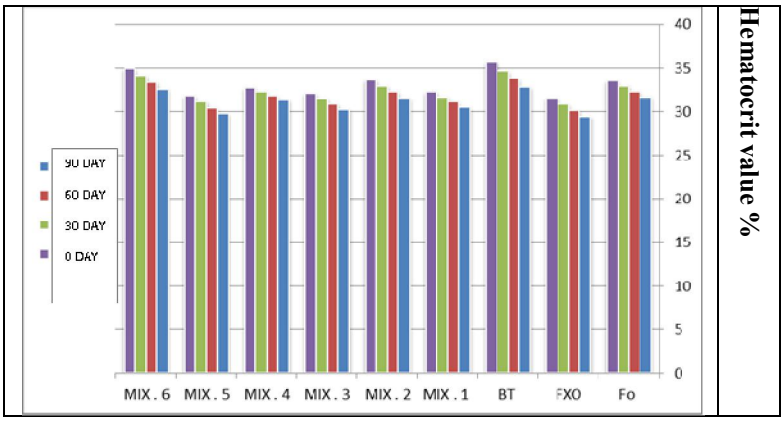

Fig.4 (a) Changes in Hematocrit value % of Male Nile tilapia previous fed diets containing different sources of lipids at various times under lower temperature and nutrient restriction .

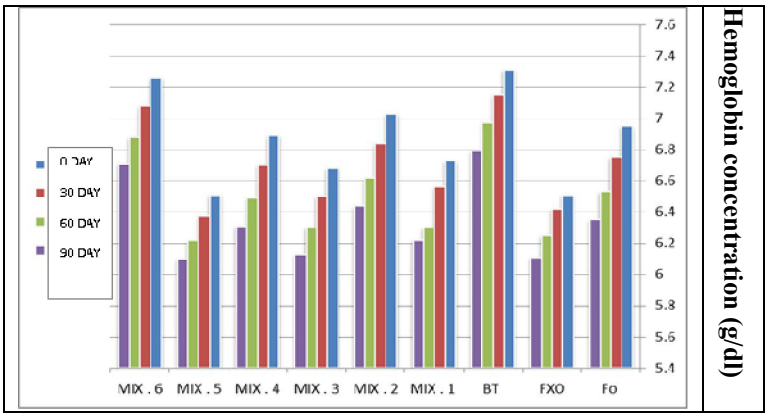

Fig.4 (b) Changes in Hemoglobin concentration (g/dl)of Male Nile tilapia previous fed diets containing different sources of lipids at various times under lower temperature and nutrient restriction

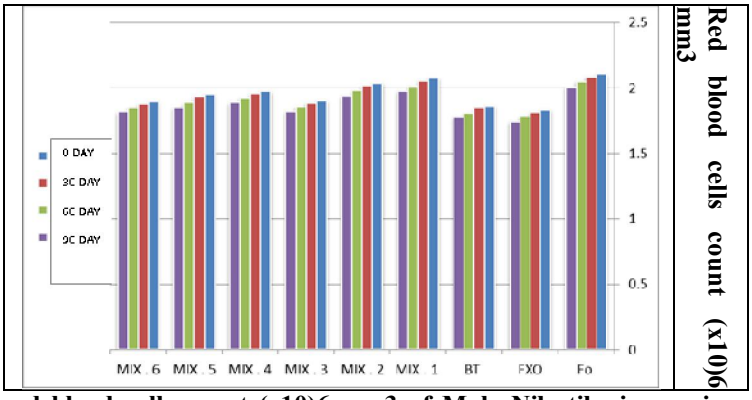

Fig.4 (c) Changes in red blood cells count (x10)6 mm3 of Male Nile tilapia previous fed diets containing different sources of lipids at various times under lower temperature and nutrient restriction.

4.Discusition

Fish, like poikilotherms, are capable of withstanding relatively long periods of food deprivation, during which body reserves are utilized for maintenance of routing body functions (De Silva *et al.,* 1997).

The ability of fish to adapt to environmental stress may depend on fish nutritional status (Salte*et al.,*1988).

When subjected to starvation conditions, animals employ various behavioral , physiological and biochemical responses to reduce maintenance metabolism, which can prolong the period during which the energy reserves can cover metabolic needs (Wang *et al.,*2006). Some poikilothermic animals such as snakes and fish can endure long periods of extreme

starvation during hibernation or overwintering (Hur *et al.,*2006, McCue,2010).

The same authors showed that starvation in known to effect fatty acid composition of body tissues in particular in the liver, muscle and viscera fat deposited.

Though, in consideration that the experimental diets were isonitrogenous and iso- lipidic in the experiment one (El- Hammady *et al.,*in press) the initial weight in the experimental two (Table. 2) were differences attributable to differences source lipid in diets.In fish, reduced or negative growth is commonly observed under conditions of stress and cortisol is known to inhibit somatic growth by stimulating energy consumption gluconeogenesis and lipogenesis in several aspects of teleost's (Schrech, 1981; Wendelaar – Bonga, 1997). During the period of experiment, the water temperature ranged from 16.5 ± 1.8 ° C (November to December ; 12.4 ± 0.7°C (December to January) to 13.6 ± 1.1 °C (January to February) respectively .The effect of fasting during decrease water temperature on growth are shown in table (1). These results revealed that decrease temperature may have been cause to restricted feeding or starvation for fish in all treatments

The growth ratio relationship and energy allocation are affected by many factors , such as feed type or composition (Yang *et al.,*2003), and water temperature (Sogard and Spencer, 2004) . As with starvation, central

component of stress adaptation seems to be the reallocation of metabolic energy from growth and reproduction toward activates that restore homeostasis , such as respiration , locomotion and hydromineral balance (Uchida *et al.,*2003).

Though , Zhang *et al* ., (2008)and Liu *et al.*(2011) showed that fasting leads to a loss of body weight , and accelerated oxidation to produce energy.

Results presented in table (1) are in agreement with obtained with Uchida *et al.*(2003) who found that fasting for 2 weeks resulted in significant reductions in body weight , specific growth rate in both male and female tilapia (*Oreochromis mossambicus).* Zeng *et al.* (2012) reported when facing the food deprivation, exhibited a great reduction in body weight for southern catfish.

Though, Tian *et al* . (2013) examined changed in the growth of Nile tilapia which were starved for 28 days in a controlled indoor environment and supplied with water temperature range 28± 2° C . They observed during the fasting phase, body weight decrease from 62.59 to 49.42 g (20.93% from initial respectively). However, in the present study the percentage decrease was between 12.73 % (Fish previous fed) (Mix.6, 25%fishoil +75% beef tallow) .

(Mix.1, (50% fish oil +50% flaxseedoil).respectively (Table.1) these variance in the % decrease between present study and results by Tian *et al.* (2013) may be attribute to different feed diets previous fasting phase , water temperature during starved , and long period of fasting phase (90 days) in the present study.

Satoh *et al.* (1984) showed that in a 30- day starvation study with tilapia *(Oreochromis niloticus*) of 40 g at a constant temperature of 15°C , found a reduction of 7% weight . Though, viola *et al.* (1988) found during the cold season, growth dropped to very low rates for tilapia (*Oreochromis niloticus*).

Percentage hepatosomatic index (HSl) showed significant differences increased initially and then decreased over the 90 day experimental period (table. 2) decreasing (%) ranged between 51.02 % , with fish previous fed Mix .1 to 39- 08% with fish previous fed BT. In the end period (90 days) under lowest water temperature with starvation whiled the total lipid in liver (as percentage decreasing / initial total lipid in liver) ranged 58.06 % with fish previous fed BT (100% beef tallow) , to 48.06% with fish previous fed Mix. 5 (25% fish oil + 75% flaxseed oil), Tabl.2.

In this connection , results obtained by Tian *et al.* (2013) suggested that HSI decreased from 2.02 to 0.88 (60%) after fasting Nile tilapia for 28 days, and fat content in liver significantly decreased ($P<0.05$) from 7 days of fasting onwards. The same authors, indicated that the liver is the critical organ under fasting . In this connection .Furne *et al.* (2008) showed that protein is used as the energy source in the initial stages of fasting , but for Atlantic cod (*Gadus morhua*), lipids are the major energy source. In this trends, Zeng *et al.* (2012) reported that the liver was a site containing energy substance (e.g. , lipid droplets and glycogen) and HSI exhibited reduction when facing the food deprivation.

These reduction in total lipid of liver may be to due low temperature according to by Qiang *et al.* (2014) found that hepatic gluconeogenesis and lipogenesis in *Oreochromis niloticus* were increased by low temperature (22◦ C). while 28◦ C is close to optimum temperature for growth of G1Fl (strain) tilapia juveniles (Qiang *et al.,*2012) , 22 ◦ C and 34◦ C were clearly below and above optimum range respectively (Qiang *et al.,*201 .Though, Liu *et al.* (2011) showed lipids are the predominant source of energy for fish and fatty acids stored in the form triacylglycerol are the main source of energy with absence of a dietary substrate . Teoh *et al.* (2011) reported that most of dietary LNA was oxidized to produce energy and its B-oxidation was greater than that of LA. However, Jezierska *et al.* (1982) found that preferential utilization of monoenes during food deprivation may be energetically advantageous because long chain PUFA are produced at considerable cost to

the fish and that it may be to the organism's advantage to delay the utilization of these substrates.

Starvation or restricted feeding generally leads to a reduction of the percentage lipid content and an increase in the water content of fish tissues (Weatherley and Gill. , 1987)..

Though, Tian *et al* . (2013) found that the percentage of crude fat decreased significantly ($P<$ 0.05) after 14 days fast of Nile tilapia.

Percentage body lipid of fillet decrease in fish previous fed Mix.6 (14.18%) were significantly lower ($P<$ 0.05) than those fish previous fed Mix .5(18.34%) (25% fish oil + 75% flaxseed oil).

Stickney and Andrew (1971) showed feeding saturated and monounsaturated lipids (beef tallow) produced high levels of oleic acid and low levels of carcass PUFA and increased liver levels of the palmitoleic and oleic series w7 and w9 and decreased levels of w3 and w6 series of fatty acids . Though, the same authors found that fish feeding highly unsaturated w3 fatty acids (Menhaden oil) resulted in high carcass levels of these acids and high liver levels of 20: 5 w3 , 22: 5 w3 and 22: 6 w3 were obtained . Similarly , Viola *et al.*(1988) found that body fat for tilapia fed fish oil were 10.7% , then decreased to 9.5% towards the end of October and dropped to 4.9 in mid-February .

Lall (2000) suggested that manipulation has been used as a tool to alleviate the damage of ammonia on fish because environmental stress may increase the requirement of essential fatty acids in fish such as rainbow trout (*Oncorhynchus mykiss*).

(Calabretti *et al.,*2003) and European seabass (*Dicentrarchus labrax)* (Person – le Ruyet *et al.,*2004).

Hsieh *et al.*(2007) found that dietary lipids a strong effect on the hepatic fatty acid composition of fish (hybrid tilapia) under cold shock. Though, the same authors, show that exposure to low temperatures leads to a reduction in the proportion of SFAs and a corresponding increase in UFAs (unsaturated fatty acids).

Murata and Wada (1995) suggested that fatty acid composition in membrane lipids is mainly regulated by the actions of SCD (Stearoyl - CoA) desaturase, EC 1.14 - 99.5, one of the acyl CoA desaturases representing the first regulatory step in the formation of long – chain unsaturated fatty acids (Enoch *et al.,*1976). However, Polley *et al.*(2003) found that diets with elevated proportions of SFAs was increased SCD activity .

Though , Hsieh *et al.*(2007) observed that the tilapia fed coconut oil (higher proportions of saturated fatty acids) as source lipid in diets , caused higher SCD gene expression.

The changes in haematological parameters (Hct, Hb, RBC) from the beginning until the end of the fasting period are shown in Fig.(4 a,b,c).

The haematocrit value (Hct), hemoglobin concentration (Hb) and red blood cells count (RBC) were further reduced until the end of the fasting period , while a percentage decreasing 7.91 % (BT) 4.21% (Mix. 4) for Hct, (PL 0.05) ; 8.96% (BT) until 6.30% (Mix. 5) for Hb (PL 0.05) 5.18% (Mix. 5) until 4.26 % (Mix. 4) for RBC ($P>$ 0.05), respectively (Table.2).

The reduction was more pronounced for haematocrit value than red blood cells (Table.2) might be attributed to decrease in water temperature. In this connection ,Bowden *et al.*(2007) showed that low temperature may reduce RBC products . Though ,Guijarra *et al.*(2003) found that RBC count and Hb concentration of were mainly affected by water temperature and increased as water temperature increases , and hence showed that RBC count and Hb concentration in summer were higher than in winter. Meanwhile, dissolved oxygen would be more sufficient in low temperature water; the blood may become more viscous , and hence require less RBC to transport oxygen (Morgan *et al.,*2008). In this connection, Qiang *et al.*(2013) found that RBC count and Hb concentration in blood were decreased significantly when water temperature was above 30◦ C , they also reported that high water temperature may pose physiological stress in tilapia, thereby causing cellular injury in kidney and liver as well as reducing the lever of erythropoietin, thus leading to a decrease in RBC count (Abdel – Tawwab *et al.,*2010). Slater and Schreck (1998) suggested that , acute temperature differences such as winter – summer temperature dynamics may inhibit immunological indices.

However, Lermen *et al.* (2004) showed that when water temperature ranged over 15 – 31 ◦ C, temperature had no influence on RBC count and haematocrit in silver catfish (*Rhamdia quelen*).

The results from the present study (table.2) showed reduced percentage change in haematocrit value and RBC count (4.21%)in fish fed Mix.4 (25% fish oil + 37.5% flaxseed oil +37.5% beef tallow) to 90 days last fasting , than other treatments.

These results may be related to increased lipid deposition and energy deposition during feeding period. Satoh *et al.* (1989) reported that 2% linseed oil is recommended for nutritional requirement in channel catfish, while 4% linseed oil is necessary when fish are under environmental stress (Calabretti *et al.,*2003).

Kelley and Daudu (1993) showed that polyunsaturated fatty acids (PUFAs) are important in cell membrane function and eicosanoids synthesis, regulate prostaglandin and keep more adhesive sites on cell membranes for antibodies to modulate immunological recognition and response (Obach *et al.,*1993). Though, stress response (decrease water temperature or high concentration of ammonia) in aquatic organisms can lead to increase energy demand

(Iwama, 1998) and fish may need to supplement more energy substrates from feed (Paust *et al.,*2011). Similar, Fish exposed to stressors tend to show high glucose mobilization in the glycolysis pathways, and then lipid and proteins can be utilized as an energy source (Mommsen *et al.,*1999)

Present study has suggested that RBC count (Table no) in the end fasting phase were lower percentage change than other haematocrit value and haemoglobin concentration. The hematology reaction to stress has been considered a strategy to enhance oxygen carrying capacity of blood cells for body energy consumption Barcellos *et al*.,2004 and Trenzado *et al.,*2006;). Calder (2001) reported that linolenic acid is an important constituent of blood energy consumption (Barcellos *et al*., 2004 and Trenzado *et al.,*2006;). Calder (2001) reported that linolenic acid is an important constituent of blood cell membrane, which is capable of maintaining the integrity of structure and functions of cell membrane, accelerating proliferation of lymphocyte. Though, Li *et al.*(2013) found that fish exposed to high stress (ammonia raise) requirement of linseed oil (rich of PUFA) for maintaining the structure and function of blood cells (Foss *et al.,*2009.)

References :

1. Abdel –tawwab , M., Ahmed , M.H. Khattab, Shalaby, A.M.E. (2010). Effect of dietary protein level, initial body weights and their interaction on the growth , feed utilization ,and physiological alterations of nile tilapia, *Oreocrhomis niloticus* (L.) .Aquaculture , 298 : 267-274.
2. AOAC , (1996) Official methods of analysis of the association of official analytical chemists, association of official analytical chemists, Arlington , VA, USA. Bell, M.V. Henderson , R.J., Sargent , J.R. 1986 , the role of polyunsaturated fatty acids in fish . comp. Biochem. Physio, 83B, 711-719.
3. Barcellos, L.J.G. kreuz , L.C. souza , C . rodifgues, LB.. Fioreze, I, Quevedo, R.M..Cericato, L, Soso, AB, Fagundes, M.. Conrad, J., lacerda. LA. Terra, S., 2004. Hematological changes in jundi3 (Rliamdiaquelen Quay and Caimard Pimelodidae) after acute and chronic stress caused by usual aquacultural management, with emphasis on immunosuppressive effects. Aquaculture 237, 229-236.
4. Bowden TJ, Thompson KD, Morgan AL. Gratacap RML. Nikoskelanien S. Seasonal variation and the immune response: i fish perspective. Fish Shellfish Immunol 2007:22:695-706.
5. Brenner, R.R. (1984) Effect of unsaturated acids on membrane structure and enzyme kinetics.Prog. Lipid ,Res., 23:69-96.
6. Calabretti , A.; Careni , F., procida , G., Favretto, L.G.(2003) influence of environment temperature on composition of lipids in edible flesh of rainbow tout (*Oncorhynchs mykiss*) , J.SC, Food Agric , 83 : 1493-8.
7. Cant , J.P. Mcbride , B.W. , and Croom, W.J. (1996) . The regulation of intestinal metabolism and its impact on whole animal energetics . J. anim. Sci, 74 : 2541-2553.
8. De silva, S., Gunasekera, R.M. and Austin , C.M. (1997) . Changes in the fatty acid profiles of hybrid red tilapia , *Oreochromis mossambicus X O , niloticus,* subjected to short –term starvation and a comparison with changes in seawater raised fish. Aquaculture , 153: 273-290.
9. Enoch, H.G., Catala, A. and Strittmatter, P. (1976) Studies of the substrate specificity, enzyme- substrate interacfions, and the function of the lipid. J. Biol. Chem. 251: 5095-5130 .
10. Farkas, T., (1979) Adaptation of fatty acid composition to temperature – a study of planktonic crustaceans. comp. Biochem.,Physiol, 64B: 71-76.
11. Fiess, J.C., Patterson , A.K, Mathias, L.Riely , L.G. , Yancey, P.H., Hirano – T. and Grau , E.G., (2007) . Effects of environmental salinity and temperature osmoregulatory ability , organic osmolytes, and plasma hormone profiles in the Mozambique tilapia (*Oreochromis mossambicus*). Comparative Biochemistry , and Physiology , part A., 146: 252-264.
12. Folch , J. Less , M. Sloanne –Stanely , G.H. (1957) A simple method for the isolation and purification of total lipids from animal tissues, J. Biol, Chem. 226, 497-509.
13. Foss , A., Insland , A.K. Vikingstad, Stefansson, S.O. Norberg, G.; Pedersen , S. Sandvik, T. and Roth . B. (2009). Compensatory growth in Atlantic halibut : effect of starvation and subsequent feeding on growth , maturation, feed utilization and flesh quality , Aquaculture , 290: 304-310.
14. Fotch , J. less , M., and Sloane – Stanely , G.H.(1957). a simple method for the isolation and purification of total lipid from animal tissues. J. Boil .Chem. , 226 : 497-509.
15. Guijarro Al. Lopez – Patino MA, Pinillos ML, Isorna E, De Pedra N, Alonso-Gomez AL. (2003).Seasonal changes in hematology and metabolic resources in the tench. J Fish Biol.,:22:S03-15.
16. Hsieh , S.L, Hu, C.Y, Hsu, Y.T., and Hsieh , T.J(2007). Influence of dietary lipids on the fatty acid composition and stearoyl – COA desaturase

expression in hybrid tilapia (*Oreochromi sniloticus* X *O. aureus*) under cold shock. Comparative Biochemistry and Physiology , part, B. 147: 438-444.
17. Hur, J.W, Jo. J.H. and Park, l. s (2006) Effects of long- term starvation on hepatocyte ultrastructure of olive flounder (*Paralichthys olivaceus*. 1 ch th. Gol Res 53:306-310 .
18. Iwanu G. Nakanishi T. The fish immune system. California: Academic Press;1996.
19. Jezierka, B. Hazel ,J.R., Gerking , S.D. 1982, lipid mobilization during starvation in the rainbow trout, *Salmo gairdneri* , Richardson , with attention to fatty acids, J. Fish Biol., 21.681-692.
20. Kelly , D.S. and Daudu, P.A. (1993) Fat intake and lmmune response, progress in food and Nutrition science 17,41-63 .
21. Lall , S.P.(2000) . nutrition and health of fish . in: cruz – suarez , L. ; ricque– marie , D. tapia – salazar, M. editore , avancesen nutricionacuicola , V.. merida ; memorias del. V. symposium international de nutricionacuicol ; 13- p.23.
22. Lermen CL, lappe R, Crestani M, Vieira VI', Cioda CR, Schetinger MRC(2004*)*.Effect of different temperature regimes on metabolic and blood parameters of silver catfish *Rhamdia quelen*. Aquaculture:239:497-507.
23. Liu, W., wei, Q.W. , Wen , H. Jiang , M., Wii, F. and Shi, Y.(2011). compensatory growth in juvenile Chinese sturgeon (*Acipensers ineusis*) effects of starvation and subsequent feeding on growth and body composition . journal of applied ichthyology , 27: 749-754.
24. McCue, M.D. , (2010) Starvation physiology , reviewing the different strategies animals use to survive a common challenge comp. Biochem. Physiol, A. 156.1-18.
25. Mommsen ,T.P., Vijayan ,M.M, Moon T.W. (1999) Cortisol in teleosts: dynamics , mechanisms of action , and metabolic regulation , Reviews in Fish Biology and Fisheries 9, 211-268.
26. Morgan AL, Thompson KD. Auchinachie MA. Migaud H. 2005 ,The effect of seasonality on normal hematological and innate immune parameters for rainbow trout *Oticoihynchus mykiss* L. Fish Shellfish Immunol;25:791-9.
27. Ng. ,W.K, Wang , Y. ketchimenin , P. and yuen , K. H.(2004). replacement of dietary fish oil with palm fatty acid distillate elevates tocopherol and toctrienol concentrations and increases oxidative stability in the muscle of African catfish , *Clarias gariepinus*. Aquaculture , 233: 423-437.
28. Obach, A.: quintel . C. and Laurencin , F.B.(1993). Effects of alpha-tocopherol and dietary oxidized fish oil on the immune response of sea bass ; *Dicentrarchus labrax*. Dis. Aquat. Org. 15: 175-85.
29. Paust, LO., Foss, A, Imsland, A.K.(, 2011). Effects of chronic and periodic exposure to amrfionia on growth, food conversion efficiency and blood physiology in juvenile Atlantic halibut [*Hippogfosm shippoglossut* L).Aquacuiture 315.'100 406.
30. Person – le ruyet .A. skallib , B., Dulaua N. le Bayona , H. and le Dellioua , JHK , (2004) . Does dietary n-3 highly unsaturated fatty acids level influence the European sea bass (*Dicentrarchus labrax*) capacity to adapt to a high temperature? . aquaculture , 242 : 571-88.
31. Polley, S.D., Tiku, P.E., Trueman, R.T., Caddick, M.X., Morozov , l.y. and Cossins A.R. (2003) Differential expression of cold and diet- specific genes encoding two carp liver Delta a- acyl. Co A. desaturase iso forms Am. J. Physil . 284, R 41-R50
32. Qiang , J. He, J., yang , H., Wang , H., Kpundeh, M.D., Xu, p. and Zhu Z.K.(2014) Temperature modulates hepatic carbohydrate metabolic enzyme activity and gene expression in juvenile GIFT tilapia (*Oreochromis niloticus*) fed a carbohydrate – enriched diet. Journal of Thermal Biology , 40: 25-31.
33. Qiang , J. Yang , Hwang , H, Kpundehs M. D. and Xu.(2013) Interacting effects of water temperature and dietary protein level on hematological parameters in Nile tilapia juveniles , *Oreochromis niloticus* (L.) and mortality under streptococcus iniae infection .Fish and Shellfish Immunology, 34-:8-16.
34. QiangJ, Yang , H. Wang , H. kpundeh , M D , X u , P.(2012b) growth and IGF-I response of juvenile nile tilapia (*Oreochromis niloticus*) to changes in water temperature and dietary protein level, J., Therm. Biol. 37, 686-695.
35. Rios, F.S, Kalinin, A.l., Fernandes, M.N and Rantin, F.T. (2002). The effects of long- term food deprivation on respivation and haematology of the neotropical fish (*Hoplias malabaricus*, J.Fish. Biol. 61;81-91.
36. Salte ,R. Thomassen , M.S and Wold , D. (1988). Do high levels of dietary polyunsaturated fatty acids (EPA / DHA) prevented diseases associated with member degeneration in farmed *Atlantic salmon* at a low water temperature 2bull . EurAssoc Fish Pathol , 8 : 63-6
37. Salter, C.H., Schreck,.B(1998) Season and physiological parameter modulate Salmonid leucocyte androgen receptor affinity and abundance. Fish Shellfish Immunol; 8:379-91.
38. Satoh , S., Takeuci , T , and Watanabe, T.(1984) Effects of starvation and environmental

temperature on psoximate and fatty acid composition of *Tilapia nilotica*, Bull , Jpn. , Soc., Sci. Fish , 30: 79-84.
39. Satoh, S., Poe, W.E., Wilson.R.P., 1989. Studies on the essential fatty acid requirement of channel catfish, *Ictalurus punctans* , Aquaculture, 79, 121-128.
40. Sheldon , W.H. and blazer . V.S. (1991) .influence of dietary lipid and temperature on bactericidal activity of channel catfish macrophages J. Aqua, Anim, Health , 3: 87-93.
41. Sogard, S.M and Spencer, M.J. (2004) . Energy allocation in juvenile, sable fish : effects of temperature , ration and body size. J. Fish Biol. 64 (3) : 726-738.
42. Stickney , R.R and Andrews , J. W (1971). Combined effect of dietary lipids and environment temperature on growth. Metabolism and body composition of chemical catfish (*Ictalurus puctatus*) . J. Nutr.,101 : 1703 -1710.
43. SUN IT, Clieng GR, Clung CF.(1995) Acute responses of blood parameters and comatose effects in salt-acclimated tilapias exposed to low temperatures. J TherBiol.,: 20 :299-306.
44. Teoh, C.Y. Turchini , G.M. and Ng. W.K (2011).Genetically improved farmed nile tilapia and red hybrid tilapia showed differences in fatty acid metabolism when fed diets with added fish oil or a vegetable oil blend. Aquaculture , 312 : 126-136.
45. Thanuthong , T., Francis , D.S. , Senadheera , S.D. Jones , P.L. and Turchini, G.M. (2011) .Fish oil replacement in rainbow trout diets and total PUFA content : 1) effects on feed efficiency , fat deposition and the efficiency of finishing strategy, Aquaculture , 320: 82-90.
46. Tian, J. Wen, H. Zeng , L.B , Jiang , M. Wu, F. Liu, W , and Yang , C.G. .(2013) . Changes in the activities and m RNA expression levels of lipoprotein lipase(LPL) , hormone sensitive lipase(HSL) and fatty acid synthetase (FAS) of Nile tilapia *(Oreochromis nilolticus*) during fasting and re-feeding .Aquaculture,400-401:29-35.
47. Toucher , D.R. bell , J.G ; Mcghee , F. ; Dick , J.R. and Fonseca – Madrigal ,J. (2003) . Effects of dietary lipid level and vegetable oil on fatty acid metabolism in atlantic salmon (*Slom solar* L.) over the whole the whole production cycle .Fish Physiology and Biochemistry, 29, 193-209.
48. Trenzado , C.E., Morales, A.E., HUGUERA, M.(2006) Physiological effects of crowding inrainbow trout, *Oncorliyitchus mykiss,* selected for low and high stress responsiveness, aquaculture , 258. 533-593.
49. Twibell , R.G. Gannam, A.L., Hyde, N. M., Holmes, S.A. and Poole, J.B (2012) Effects of fish meal and fish oil- free diets on growth responses and fatty acid composition of juvenile coho salman (*Oncorhynchus kisutch*) . Agriculture , 360-361 : 69-77
50. Van kampen , E. J and Zijlstra , N.C (1961) Determination of hemoglobin, Clin, Chemacta 5 , 719 -720.
51. Viola , S., Mokady , S., Behar, D., and Cogan, u.(1988). Effects of polyunsaturated fatty acids in feed of tilapia and carp body composition and fatty acid profiles at different environmental temperatures . Aquaculture, 75: 127-137.
52. Wang T., Hung , C. Randall, D.J.(2006) The comparative physology of food deprivation from feast to famine , Annu, Rev. Physiol, 68, 223-251.
53. Weatherley , A.H. and Gill, H.S.(1987) . The biology of fish growth. academic press. London , 443 P.P.
54. XU, J. nakamura ,M.T. , Cho, H.P. and Clarke , S.D. (1999). Sterol regulatory element binding protein-1 expression is suppressed by dietary polyunsaturated fatty acids. A mechanisms for the coordinate suppression of lipogenic genes by polyunsaturated fats. J. Biol .Chem., 214: 23577-23588.
55. Yang, YG.Cuiy.B., Xiong , B.X , Yang , Y.X. and Yie , S.Q. (2003) Comparative studies on nitrogen budget and energy budged of jian carp (Cyprinus carpio var. jian) and gibel carp (*Carassiusa uratus gibelia*) fed diets with different qualities. Acta Hydrobiolsin . 26(6) 572-579.
56. Zeng, .L.Q. , Li , F.J., Li, X.M., Cao , Z.D., Fu, S.J.,and Zhang , Y.G.(2012). The effect of starvation on digestive tract function and structure in juvenile southern catfish (*Silurusmeridion alis*chen) . Comparative Biochemistry and Physiology , part A., 162: 200-211.
57. Zhang , X.D. Zhu, Y.F, Cai, L.S , and Wu, T.X.(2008). Effects of fasting on the meat quality and antioxidant defenses of market size farmed large yellow croaker. (*Pseudosciaen acracea*) .Aquaculture , 280: 136-139.