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Original Article

Dietary conjugated linoleic acid (CLA) and lecithin affects levels of serum cholesterol, triglyceride, lipoprotein and hypoxic stress resistance in rainbow trout (*Oncorhynchus mykiss*)

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Abstract: Considering the role of conjugated linoleic acid (CLA) and soybean lecithin (SBL) in fat digestion, absorption and metabolism as well as stress resistance in aquatic organisms, the current study was a 74-day attempt to investigate the synergistic effects of these two compounds on stress resistance and blood lipoprotein levels of rainbow trout (*Oncorhynchus mykiss*) weighing 120±5 g. Nine isonitrogenous and isocaloric experimental diets containing 1, 2 and 3% CLA and 2, 3 and 4% soybean lecithin on the basis of the basal control diet (free from CLA and lecithin) were formulated. The fish were exposed to three ascending periods of hypoxic stress (7.5, 15 and 30 min) at the end of culture period. The dissolved oxygen was decreased to 1.96 ppm by injecting nitrogen gas. Significantly higher levels of cortisol and glucose were detected in fish fed on diets having higher levels of SBL (2%) and CLA (3%) at higher stress time improving the resistance to hypoxia stress ($P \leq 0.05$). Moreover, levels of triglyceride (TRG), cholesterol (COL), low density lipoprotein (LDL), very low-density lipoprotein (VLDL) and high density lipoprotein (HDL) also increased significantly compared to the control group ($P \leq 0.05$). This study reveals that using 2% SBL and 3% CLA in rainbow trout feed can promote resistance to hypoxic stress.

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Introduction

Conjugated linoleic acid (CLA) is a member of specific group of geometric isomers of linoleic acid that their double bonds are separated from each other by a single bond. CLA is produced as a result of isomerization and bio-hydrogenation of microbes in the rumen of ruminant (Wahle et al., 2008). In aquaculture nutrition, the two bioactive isomers of CLA viz. sis-9 trans-11 and sis-10 trans-12 are used (Zanqui et al., 2013). These two isomers affect lipid metabolism in aquatic's body affecting enzymes such as acyl-CoA oxidase, Carnitine acyl transferase I, malic enzyme, desaturases, elongase and lipoprotein lipase (Valente et al., 2005; Leaver et al., 2006). CLA can influence the ability of aquatic organisms in converting fatty acids to each other (bioconversion) and change the ratio of omega-3 to omega-6 fatty acids in muscle and liver (Leaver et al., 2006) and creates a tendency to increase the growth performance (Berge et al., 2004). The above-mentioned enzymatic effects can influence the level of blood lipoproteins of the fish fed diets containing CLA supplement. For example, it has been proven that CLA increases insulin resistance and decreased serum cholesterol and high-density lipoprotein (HDL) levels (Yang et al., 2014).

The positive effect of CLA on lipolysis in comparison to proteolysis and glycolysis can be attributed to the low amount of CO_2 produced in fish fed diets containing CLA supplement. Therefore, CLA is expected to affect RQ and consequently the amount of CO_2 produced in intensive fish culture systems and as a result increase fish resistance to

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hypoxia stress. CLA not only increased insulin resistance and decreased lipogenesis and lipoprotein lipase activity in mice, but also increased mice adaptation to hypoxic stress in vivo (Luo et al., 2011). The role of DHA when being exposed to stress as well as its role in cortisol secretion in fish has been proven by Kanazawa (1979) and Tago et al. (1999). On the other hand, CLA increases DHA level in fish (Berge et al., 2004; Makol et al., 2009), therefore, it indirectly influences the production of DHA affecting hypoxic stress resistance in fish. According to Rohmah et al. (2016), CLA can increase insulin resistance and affect blood glucose level of the fish under hypoxic stress.

To maximize intestinal absorption of dietary CLA, new methods should be taken into account when formulating diets. CLA has the capacity to bond with phospholipid compounds (Choi et al., 1999). Therefore, it seems that phospholipid compounds such as lecithin have specific emulsifier roles (Atar et al., 2009) and are capable of increasing the enterocyte absorption rate of CLA. Lecithin consists of phosphoric acid and choline, fatty acids, glycerol, glycolipid, triglycerides and phospholipids such as phosphatidylcholine, phosphatidylcholine ethanol amine, phosphatidyl serine and phosphatidylinositol. Lecithin has an interactive role in intestinal absorption of cholesterol and can improve the survival of aquatic species (ADM Specialty Ingredient, 2003). Since fish and crustaceans cannot produce enough phospholipid to maximize their growth, phospholipids should be added to their diets (Ketola, 1976; Hung and Lutes, 1988; Hung, 1989; Poston, 1991a, b). The main role of lecithin seems to be its synergic property in lipid metabolism.

The increased resistance to stress has been reported by supplementing of phospholipids to the diets (Salhi et al., 1999; Tocher et al., 2008). Under the hypoxic stress, i.e. salinity and temperature, phosphatidylcholine containing DHA has increased the resistance to the stress in Flounder (*paralichthys olivaceus*) more efficiently than triglycerides containing DHA (Tago et al., 1999). Therefore, since lecithin is a rich source of phosphatidylcholine, it can play a role in creating resistance to stress in fish. Moreover, Kanazawa (1979) stated that DHA and soy lecithin increases the stress tolerance of oxygen, temperature and salinity in Sea bream. Liu et al. (2002) also found lecithin and eicosapentaenoic acid (EPA) effective in producing cortisol or stress hormone in Gilthead Sea bream (*Sparus aurata*). Additionally, researchers pointed to the role of lecithin as an emulsifier in increasing the enterocyte absorption rate of lipids and increasing blood lipoproteins.

Several studies on marine fishes have shown the synergistic effects of phospholipids in fat metabolism and these studies attributed the increase in digestion and absorption efficiency of fat in intestinal cells (enterocytes) by phospholipids to the emulsification role of phospholipids in increasing the intestinal absorption of fat (Fontagne et al., 1998; Olsen et al., 1999; Salhi et al., 1999; Liu et al., 2002; Azarm et al., 2012). Hence considering the important role of CLA and lecithin in reducing environmental stress and digestive processes, absorption, transfer and metabolism of lipids, the present study aimed to investigate the synergistic effects of these two dietary supplements on coping with hypoxia stress and their effects on the concentration of lipoproteins in experimental rainbow trout, *Oncorhynchus mykiss*, under stress.

Materials and Methods

Experimental diets and fish: During a 74-day experiment, nine isonitrogenous (45% crude protein) and isocaloric (3.65 mega calories per kg) experimental diets with different levels of CLA and lecithin were formulated according to the requirements of cold-water fishes (Table 1). Experimental diets were proximately analyzed according to AOAC (1995). The control diet was free from CLA and lecithin. The required CLA with 58% purity was provided by Salamat Pakhsh-e-Yalda Company, Iran Pharmaceutical Products Technology Incubator. The used lecithin was soybean lecithin, the product of Vicentin Company, Argentina. The pellets were produced industrially and the amounts of CLA and lecithin were added to experimental diets (Table

Table 1. Formulation of experimental diets containing different levels of CLA & soy bean lecithin (%).

Ingredients	Control	D1	D2	D3	D4	D5	D6	D7	D8	D9
Fish Meal[1]	36	36	36	36	36	36	36	36	36	36
Fish Oil[2]	6	6	6	6	6	6	6	6	6	6
Safflower Oil	7	7	7	7	7	7	7	7	7	7
Soybean Meal	15	15	15	15	15	15	15	15	15	15
Wheat Gluten	15.5	15.5	15.5	15.5	15.5	15.5	15.5	15.5	15.5	15.5
Corn Gluten	7	7	7	7	7	7	7	7	7	7
Canola Meal	4	4	4	4	4	4	4	4	4	4
CLA[3]	0	1	2	3	1	2	3	1	2	3
Soybean Lecithin[4]	0	2	2	2	3	3	3	4	4	4
Starch	7	4	3	2	3	2	1	2	1	0
Vitamin Supplements[5]	1	1	1	1	1	1	1	1	1	1
Binder[6]	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Mineral Supplements[7]	1	1	1	1	1	1	1	1	1	1
Analyzed proximate composition (percent in dry matter)										
Crude protein (N*6.25)	40.42	40.31	40.23	40.15	40	40	39.97	40.12	40.12	40.1
Crude Lipid	15	15.01	15	15.25	15.12	15.18	15.26	15.08	15.1	15.36
Ash	3.13	13	13.10	12.13	12.28	12	12.3	12	13.13	12.03
Crude Fiber	3.40	3.36	3.40	3.44	3.40	3.42	3.36	3.42	3.40	3.37
Dry matter	94.2	94	94.21	94.1	94	93.98	94	93.97	94.15	94
Digestible Energy (Mcal kg^{-1} diet)	3.60	3.60	3.63	3.60	3.60	3.60	3.62	3.60	3.61	3.63

1, Kilka Meal. Iran; 2 Kilka Oil. Iran; 3, Conjugated linoleic acid. Salamat pakhsh yalda co, Iran purity 58%; 4 Soybean lecithin, Vicentin co, Argentina; 5, Vitamin mixture (Unit kg^{-1} of diet): Vitamin A, 1200 000 IU; D3, 400 000 IU; E, 3000 mg; K3, 1200 mg; C, 5400 mg; H2; 200 mg; B1, 200 mg; B2, 3600 mg; B3, 7200 mg; B5, 9000 mg; B6, 2400 mg; B9, 600 mg; B12, 4 mg; antioxidant 500 mg Career up to 1 kg; 6, Binder: Amet binder (Component: Crude protein: 71.98%, Crude fibre: 0.9%, Ash: 17.8%, Moisture: 9.55%) and 7, Minerals mixture (mg kg^{-1} of diet): Fe, 4500 mg; Cu, 500 mg; Co, 50 mg; Se, 50 mg; Zn, 6000 mg; Mn, 5000 mg; I, 150 mg; Choline chloride, 150 000 mg; Career up to 1 kg.

1).

Thirty 300-L tanks were used for this experiment, each containing 20 rainbow trout (with average weight of 120±5 gr). The fish were fed the control diet (2% of body weight) for two weeks (three times a day) during the adaptation period. Length and weight measurements were done every 14 days. To reduce the biometry stress, the experimental fish were not fed 24 hours before the biometry.

Hypoxic stress: All experimental fish were exposed to hypoxic stress for 7.5, 15 and 30 min by injecting nitrogen gas into the culture tanks (Bloom et al., 1993; Dabrowskia et al., 2004). After each stress period, the fish were recovered and feeding continued until second and third phases of stress. The concentration of oxygen gas during hypoxic stress fell to 1.96 ppm.

Sampling and measuring parameters: At the end of each hypoxic stress stage, four fish were caught from each tank and were anesthetized using clove powder (*Eugenia caryophyllata*) with a concentration of 200 ppm. Blood samples were taken from the caudal peduncle using 5 ml syringes without heparin. Blood samples were centrifuged at 3000 rpm for 10 min (Burel, 2001). Sera were separated by pipette and poured into 0.5 ml micro tubes and were frozen and kept at a temperature of -20°C till the determination of their biochemical parameters.

Cortisol was measured by ELISA method using Accubind kits made by Monobind Company. To measure cholesterol and glucose, Autoanalyzer (Alpha-6 Model) and the kits of the Man Company were used. Samples were read at a wavelength of 450 nm. Triglyceride was determined by enzymatically glycerophosphate dehydrogenase (GOD-PAP) method (Fukuda, 1958) and to measure the serum lipoproteins, the kits of Pishtazteb Company were utilized and all measurements were repeated three times.

Statistical analysis: The experiment was completely randomized in a factorial design of 10 treatments with 3 replications. At the end of the study, the data from the effect of experimental diets on biochemical parameters of hypoxic stress (cortisol levels, glucose) as well as cholesterol, triglyceride and blood

Table 2. Effect of different feeding treatments on the levels of glucose, cortisol, cholesterol, triglyceride, high-density, low-density and very low-density lipoproteins at the end of the experiment before stress (µg/dl).

Treatments	CORT	GLU	CHOL	TRY	HDL	LDL	VLDL
0	4.38±0.62^{a}	152.92±9.87^{a}	268.93±19.97^{d}	262.4±31.65^{d}	47.45±2.94^{c}	26.26±5.82^{d}	58.68±6.96^{d}
1	3.98±0.66^{ab}	150.1±9.23^{ab}	294.73±21.96^{dc}	317.19±36.2^{cd}	53.73±3.55^{bc}	29.41±6.33^{d}	63.24±7.02^{cd}
2	3.14±0.44^{abc}	142.48±5.98^{abc}	326.95±21.02^{bcd}	325.69±38.2^{bcd}	61.61±3.74^{ab}	37.32±6.42^{cd}	70.47±9.23^{abcd}
3	2.56±0.43^{abc}	118.12±10.98^{cd}	346.67±11.46^{abc}	423.87±52.3^{abc}	62.84±2.85^{ab}	50.61±6.66^{abc}	89.4±9.86^{a}
4	4.13±0.7^{ab}	140.58±11.04^{abc}	314.42±20.87^{cd}	323.7±35.76^{cd}	56.81±4.01^{bc}	44.34±8.5^{bcd}	62.95±6.29^{d}
5	2.78±0.56^{abc}	144.8±8.95^{ab}	346.08±17.24^{abc}	354.98±21.5^{bcd}	60±2.53^{ab}	43.9±6.16^{bcd}	74.6±5.83^{abcd}
6	2.2±0.37^{bc}	113.83±5.22^{d}	385.9±19.06^{ab}	518.1±84.25^{a}	69.59±3.9^{a}	64.62±7.21^{a}	82.2±7.01^{abc}
7	3.64±0.5^{ab}	145.53±9.8^{ab}	309.8±18.14^{cd}	338.6±26.6^{bcd}	60.93±2.57^{ab}	39.34±6.7^{cd}	67.38±5.27^{bcd}
8	2.53±0.65^{abc}	139.2±8.54^{abc}	336.57±17.28^{abc}	389.13±31.7^{bc}	63.59±2.94^{ab}	45.55±6.18^{abcd}	72.36±6.9^{abcd}
9	1.51±0.24^{c}	126.75±9.92^{bcd}	389.07±22.27^{a}	453.7±50.3^{ab}	69.96±4.25^{a}	60.9±8.08^{ab}	85.7±5.25^{ab}

Lack of common letters indicates significant differences between treatments (P<0.05).

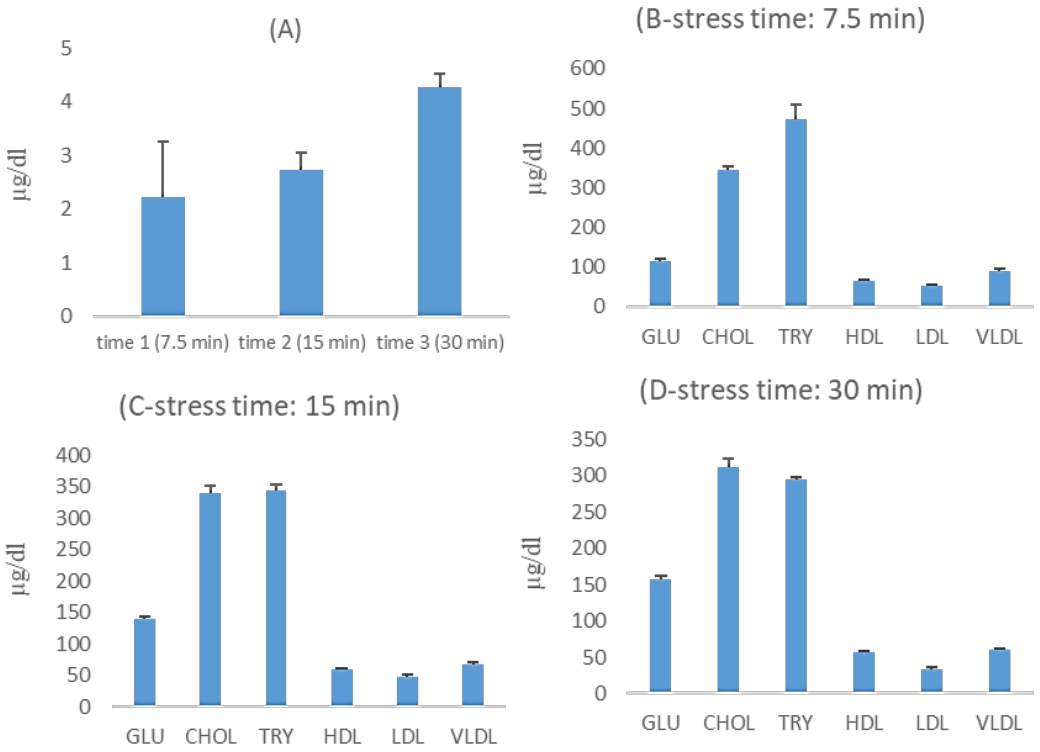

Figure 1. Effect of hypoxic stress on cortizol level (A); glucose; Cholesterol; triglycerides; high, low and very low density lipoprotein (B, C, D) at different stress timings.

lipoproteins were analyzed using SAS (9.1) Software, GLM procedure. Mean comparisons were performed by Duncan Test. Presence or absence of statistical differences between treatments was set at 0.05.

Results

The results of the blood analysis at the end of rearing period before application of stress showed significantly lower cortisol and glucose in treatments received 3% CLA and 2-4% SBL, but cholesterol, triglyceride, HDL, LDL and VLDL were significantly higher in most fish fed experimental diets compared to the control (Table 2). Cortisol level in fish exposed to hypoxic stress increased gradually with increasing the stress period but was significant only in the third phase (30 min stress) compared to others (Fig. 1, Table 3,

Table 3. The effect of duration of hypoxic stress (7.5, 15 and 30 min) on changes in levels of cortisol, glucose, cholesterol, triglyceride, high-density, low-density and very low-density lipoproteins (µg/dl).

	CORT	GLU	CHOL	TRY	HDL	LDL	VLDL
time1_T0	3.26±1.75^{abc}	123.75±9.6^{cdefgh}	255.25±44.1^{c}	323.8±84.4^{bcdef}	55.9±3.94^{bc}	34.78±8.9^{bcde}	83.37±12.1^{bcde}
time1_T1	2.66±1.5^{abc}	121.75±10.8^{cdefgh}	311±53.22^{abc}	401.7±88.7^{bcdef}	59.6±5.07^{bc}	33.18±5.7^{bcde}	81.5±18.4^{bcde}
time1_T2	2.57±1.3^{abc}	134±1.73^{abcdefgh}	358±14.98^{abc}	414±115.2^{bcdef}	66.33±8.04^{ab}	50.85±8.9^{abcde}	90.13±27.9^{bc}
time1_T3	2.13±1.08^{bc}	94±31.96^{h}	359.75±27.8^{abc}	549±143.1^{b}	66.53±7.4^{ab}	58.2±8.45^{abcd}	125.23±17.6^{a}
time1_T4	2.74±1.07^{abc}	122.5±30.7^{cdefgh}	352.75±43.2^{abc}	432.8±69.1^{bcde}	61.38±6.15^{ab}	45.8±15.9^{abcde}	82.2±12.5^{bcde}
time1_T5	2.06±1.03^{c}	116.8±17.03^{defgh}	355.75±19.9^{abc}	433.3±26.8^{bcde}	60.88±6.25^{ab}	56.25±8.66^{abcd}	89.45±8.22^{bc}
time1_T6	1.36±0.68^{c}	104.75±7.18^{gh}	374±29.17^{abc}	782±223.25 ^{a}	77.7±6.4^{a}	73.38±15.7^{a}	89.88±16.64^{bc}
time1_T7	2.92±1.2^{abc}	109.5±3.1^{efgh}	344.5±44.46^{abc}	428.3±9.7^{bcdef}	63.93±5.3^{ab}	49.5±8.25^{abcde}	84.6±3.36^{bcde}
time1_T8	1.48±1.03^{c}	117.5±19.1^{defgh}	361.75±31.4^{abc}	470.5±50.1^{bcd}	64.45±5.57^{ab}	44.63±7.1^{abcde}	89.1±16.45^{bcd}
time1_T9	1.19±0.58^{c}	106±5.6^{fgh}	369.75±21.4^{abc}	493.25±114^{bcd}	79.55±8.78^{a}	72.05±5.27^{ab}	94.75±9.28^{ab}
time2_T0	3.9±1.21^{abc}	155±10.95^{abcde}	295.75±16^{bc}	272.25±42.2^{def}	48.23±5.64^{bc}	32.6±11.9^{bcde}	54.4±8.4^{cdeg}
time2_T1	3.33±1.45^{abc}	153.8±18.8^{abcdef}	317±26.47^{abc}	297.5±50.7^{cdef}	53.93±8.72^{bc}	36.6±17.3^{abcde}	57.8±4.4^{bcdeg}
time2_T2	2.83±0.7^{abc}	145.2±17.3^{abcdefg}	330±30.4^{abc}	289 ±22.1^{cdef}	61.53±4.58^{ab}	38.9±13.5^{abcde}	66.5±7.2^{bcdeg}
time2_T3	2.44±1.1^{abc}	117.7±3.8^{cdefgh}	351.25±14.6^{abc}	348±69.3^{bcdef}	61.8±2.89^{ab}	61.03±11.1^{abc}	73±15.6^{bcdeg}
time2_T4	3.71±1.97^{abc}	140.2±6.98^{abcdefgh}	304.5±37^{abc}	332.75±36^{bcdef}	56.35±10.55^{bc}	53.3±16.3^{abcd}	61.5±4.8^{bcdeg}
time2_T5	2.39±1.7^{abc}	151.2±10.7^{abcdefg}	331.5±53.8^{abc}	307.5±23.9^{cdef}	60±3.1^{ab}	42.4±8.4^{abcde}	69.5±13.8^{bcdeg}
time2_T6	1.69±0.38^{c}	118.75±7.9^{cdefgh}	420.75±20.5^{a}	392.5±15.1^{bcdef}	68.35±3.24^{ab}	66.88±4.7^{ab}	78.48±3^{bcde}
time2_T7	3.5±1.22^{abc}	149.5±10.7^{abcdefg}	305.5±22.1^{abc}	331.8±34.9^{bcdef}	59.45±2.16^{ab}	49.5±12.9^{abcde}	66.4±6.95^{bcdeg}
time2_T8	2.04±1.71^{c}	140.5±11.4^{abcdefgh}	321.75±24.8^{abc}	354.5±67.9^{bcdef}	64.03±6.03^{ab}	32.9±14.5^{bcde}	59.5±10.2^{bcdeg}
time2_T9	1.48±0.41^{c}	130.25±18^{bcdefgh}	418.25±57.7^{a}	518.3±112^{bc}	67.8±3.8^{ab}	67.4±12.2^{ab}	86.08±9.7^{bcde}
time3_T0	5.98±0.44^{a}	180±16.77^{a}	255.8±39.5^{c}	191±21.37^{f}	38.21±1.84^{c}	11.4±7.37^{e}	38.26±4.26^{g}
time3_T1	5.96±0.11^{a}	174.8±8.2^{ab}	256.2±33.5^{c}	252±37.7^{def}	47.67±4.65^{bc}	18.4±8.39^{de}	50.4±7.55^{eg}
time3_T2	4.03±0.65^{abc}	148±8.5^{abcdefg}	292.6±47.7^{bc}	274±25.6^{def}	56.98±6.92^{bc}	22.2±8.4^{cde}	54.78±5.1^{cdeg}
time3_T3	3.1±0.69^{abc}	142.6±7.4^{abcdefg}	329±17.8^{abc}	374.6±35^{bcdef}	60.2±4.55^{ab}	45.48±8^{abcde}	70±4.7^{bcdeg}
time3_T4	5.94±0.9^{ab}	159±14.3^{abcd}	286±30.56^{bc}	205.6±22.4^{ef}	52.7±5.26^{bc}	19.78±8.2^{de}	45.12±6.9^{g}
time3_T5	3.88±0.68^{abc}	166.4±10.1^{abc}	351±16.7^{abc}	324±30.3^{bcdef}	59.12±4.51^{ab}	33.02±12^{bcde}	64.8±6.1^{bcdeg}
time3_T6	3.55±0.58^{abc}	118±10.9^{cdefgh}	363±40.87^{abc}	380±59.4^{bcdef}	62.72±7.93^{ab}	53.62±14^{abcd}	78.32±13.9^{bcde}
time3_T7	4.49±0.9^{abc}	177.6±12.55^{ab}	279.4±24.8^{bc}	255.8±37.6^{def}	59.42±5.38^{ab}	19.02±8.16^{de}	51.14±7.5^{bcdeg}
time3_T8	4.06±1.2^{abc}	159.6±8.68^{abcd}	326.2±34.2^{abc}	342±38^{bcdef}	62.28±5.13^{ab}	46.2±14.5^{abcde}	68.5±7.6^{bcdeg}
time3_T9	1.86±0.64^{c}	144±19.7^{abcdefg}	379.2±36.4^{ab}	349.6±23.5^{bcdef}	62.52±7.3^{ab}	57.4±17.14^{abcd}	76.3±8.3^{bcde}

*The lack of common letters indicates significant differences between treatments (P<0.05)

The abbreviations used in Diagrams (1) and (2) and Tables (2), (3) and (4) are as follows: T1=7.5 min, T2=15 min, T3=30 min CORT=Cortisol, GLU=Glucose, CHOL=Cholesterol, TRY=Triglyceride, HDL=High-density Lipoprotein, LDL=Low-density Lipoprotein, VLDL=Very Low-density Lipoprotein.

P≤0.05). The lowest level of cortisol was observed in fish fed experimental diets containing highest level of CLA and 4-2% SBL, respectively. Significant differences in cortisol level were observed between treatment 9 containing 3% CLA and 4% SBL with control group and with treatments 1, 4 and 7 containing lowest levels of CLA (Table 2, P≤0.05).

Higher stress period resulted in a significant increase in blood glucose level (P≤0.05) (Fig. 1). In addition, blood glucose in fish under 7.5 min stress showed significant differences between treatments 6, 8 and 9 with the control group exposed to 30 min stress

($P \leq 0.05$) (Table 3). Comparing the effect of different treatments on the levels of Cholesterol, triglyceride and serum lipoproteins (Table 2) showed an increase in these parameters with increasing the amount of dietary CLA ($P \leq 0.05$). Comparison of the means revealed significant differences between the levels of triglyceride, VLDL and HDL in fish exposed to 30 and 15 min hypoxic stress compared to those under 7.5 min stress ($P \leq 0.05$). Considering the combined effect of feeding treatments and duration of stress on the serum triglyceride level (Table 3), significant differences were observed between the fish of treatment 6 and 9 under 7.5 and 15 minutes compared to the control group ($P \leq 0.05$), but no significant difference was observed at 30 min stress among the treatments. Cholesterol level was significantly higher in treatment 6 and 9 stressed for 15 min and treatment 9 stressed for 30 min compared to the control fish ($P \leq 0.05$) (Table 3). LDL and HDL were significantly higher in treatments received highest levels of CLA and SBL stressed for 7.5 and 30 min compared to control. Similarly, VLDL also was significantly higher in treatments 6 and 9 containing highest amount of CLA and SBL stressed for 30 min in comparison to control ($P \leq 0.05$, Table 3).

Discussions

One of the indicators of stress is cortisol (hydrocortisone) which is secreted via the renal tissue as a result of the release of Adrenocorticotropic hormone (ACTH) (Pierson et al., 2004; Ramsay et al., 2006; Martinez-Porchasi et al., 2009). Based on the results, the lowest levels of cortisol in all stress phases was observed in fish fed experimental diets with the highest levels of CLA (3, 6 and 9 treatments), indicating the effect of dietary CLA on reducing stress. CLA will increase the beta-fat oxidation rate in the body of experimental fish (Leaver et al., 2006) as seen in the current study. Since fat catabolism generates less respiratory fraction than carbohydrates and proteins, therefore in exchange for taking a unit of fat in the body, small amounts of CO_2 is released (McDonald et al., 1979) and this reduces the need of experimental fish to absorb dissolved oxygen and increases the ability of the fish to tolerate hypoxia stress i.e. CLA increases the production of phospholipids such as DHA which affect resistance to hypoxic stress (Berg et al., 2004, Makol et al., 2009). The treatments 3, 6 and 9 have the highest ratio of CLA to lecithin. Considering lecithin's role as an emulsifier in the transmission of intestinal fatty acids (Atar et al., 2009; Azarm et al., 2012), lecithin also indirectly effects increase of stress resistance in the fish due to increased intestinal absorption of CLA. Lecithin as a source of phospholipids such as phosphatidylcholine can have an indirect impact on generation, transmission and absorption of EPA and DHA (Tago, 1999; Liu et al., 2002), that increase fish resistance to hypoxic stress.

Increased blood glucose level with increasing the duration of hypoxia stress was evident from the first to third phase. By increasing stress time, increase of both blood glucose and cortisol level was observed. Pickering et al. (1989) and Martinez (2009) pointed out the regulatory relationship between cortisol and blood glucose levels through the process of gluconeogenesis i.e. cortisol increases blood glucose level (Hyperglycemia) and stimulates gluconeo-genesis of proteins and lipids. After applying stress, cortisol ranges from 40 to 200 ng/ml (Pickering et al., 1989) and in some species it can exceed 1000 ng/ml (Barton et al., 1998). According to Pickering et al. (1989) and Tocher et al. (2008), the cortisol secretion is a hormonal response of the body of the fish under stress that increases the resistance of the aquatic body to stress conditions i.e. the level of plasma cortisol and changes in carbohydrate metabolism, such as glucose can be used as a general indicator of stress. Our results were in line with the findings of Pickering et al. (1989) and Martinez et al. (2009). The lowest amount of blood glucose was observed in treatments of 3, 6 and 9. The reason for the significant difference in the level of glucose in treatments 3, 6 and 9 (treatments that received the highest levels of CLA) is the physiological effect of CLA on reducing the oxygen demand of the fish.

The undeniable role of CLA in lipid metabolism (Leaver et al., 2006) and the reduction of oxygen

demand during hypoxia stress (Berge et al., 2004), as well as the role that lecithin plays in the absorption of CLA and the production, absorption and transfer of phospholipids, such as DHA and EPA (Kanazawa 1999; Liu et al., 2002) can be reasons for interpreting the synergistic effects of these two compounds in reducing stress and subsequently reducing cortisol and glucose levels in fish fed diets of 3, 6 and 9. Furthermore, these are reasons for explaining the effects of treatments on changes in blood glucose levels. CLA also increases polyunsaturated fatty acids (PUFAs) by increasing the enzyme gene transcription rate of aceyl coenzyme A oxidase. Therefore, in both cases, CLA provides a substrate for the pathway of gluconeogenesis. Given the fact that the experimental diets 3, 6 and 9 had the highest levels of CLA, it was expected that the highest levels of glucose would be obtained for fish fed these diets, but the results showed the reverse. As CLA increases the activity of carnitine palaeomethyl transferase 1 and beta-oxidation rate, the consumption of free fatty acids, which are substrates for the process of gluconeogenesis, increases and its result can be the reduction of blood glucose concentration in fish fed diets containing CLA.

In addition to increasing the activity of carnitine palaeomethyl transferase 1 and increasing the beta-oxidation of fatty acids in the aquatic body, CLA decreases the activity of Δ 9-desaturase (Pariza et al., 2001; Berg et al., 2004; Leaver et al., 2006). Lipoprotein lipase is an enzyme which hydrolyzes the core of chylomicrons and VLDL and changes them to Di-glycerides and Mono-glycerides and finally free fatty acids. It seems that the effect of CLA on serum lipoprotein levels occurs by inhibition of lipoprotein lipase (LPL) (Pariza et al., 2001, Luo et al., 2011). An increase in the rate of gluconeogenesis requires substrates such as fatty acids (Pickering et al., 1989) which can be a feed-back to increase blood lipoproteins.

An increase in the rate of gluconeogenesis requires providing substrates such as fatty acids (Pickering et al., 1989), the consumption of which can be a feed-back to produce blood lipoproteins. Lecithin plays an interactive role in the absorption of cholesterol (ADM Specialty Ingredient, 2003). Cholesterol is a lipoprotein precursor and, the lecithin has an emulsifier role, therefore, lecithin increases fat absorption rate and forms chylomicrons, increasing in the concentration of lipoproteins. Based on the results, levels higher than 2% lecithin and 3% CLA increased lipoprotein accumulation rate and by increasing the stress time from 7.5 to 30 min, the blood lipoprotein levels decreased. This process showed that with increasing stress time, the consumption of fatty acids (as gluconeogenesis substrate) increased and as a result cholesterol, triglyceride and lipoprotein concentrations are decreased.

As conclusion, it can be noted that the greatest synergistic effect of lecithin and CLA on increasing rainbow trout resistance was observed against oxygen deficit stress when CLA was 3% and lecithin was 2, 3 and 4% in the diet. Therefore, it is recommended that in intensive culturing systems which are under hypoxia stress, the above-mentioned levels of these two supplements are used in formulation of rainbow trout feed in growing phase. Regarding the influence of 3% CLA and 2, 3 and 4% lecithin on levels of serum cholesterol, triglyceride and lipoproteins, it is suggested that these levels be periodically used in formulating diets for rainbow trout fed fat-rich diets.

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