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Dietary clenbuterol modifies the expression of genes involved in the regulation of lipid metabolism and growth in the liver, skeletal muscle, and adipose tissue of Nile tilapia (*Oreochromis niloticus*)

Radi A. Mohamed[a], Zizy I. Elbialy[b], Amira S. Abd El Latif[c], Mustafa Shukry[d], Doaa H. Assar[e,*], Asmaa M. El Nokrashy[a], Ahmed Elsheshtawy[b], Mahmoud A.O. Dawood[f,*], Bilal Ahamad Paray[g], Hien Van Doan[h,i,*], Ali H. El-Far[j]

[a] Department of Aquaculture, Faculty of Aquatic and Fisheries Sciences, Kafrelsheikh University, Egypt
[b] Department of Fish Processing and Biotechnology, Faculty of Aquatic and Fisheries Sciences, Kafrelsheikh University, Egypt
[c] Department Pharmacology, Faculty of Veterinary Medicine, Kafrelsheikh University, Egypt
[d] Department of Physiology, Faculty of Veterinary Medicine, Kafrelsheikh University, Egypt
[e] Department of Clinical Pathology, Faculty of Veterinary Medicine, Kafrelsheikh University, Egypt
[f] Department of Animal Production, Faculty of Agriculture, Kafrelsheikh University, 33516, Kafr El-Sheikh, Egypt
[g] Department of Zoology, College of Science, King Saud University, PO Box 2455, Riyadh, 11451, Saudi Arabia
[h] Department of Animal and Aquatic Sciences, Faculty of Agriculture, Chiang Mai University, Chiang Mai 50200, Thailand
[i] Innoviative Agriculture Research Center, Faculty of Agriculture, Chiang Mai University, Chiang Mai 50200, Thailand
[j] Department of Biochemistry, Faculty of Veterinary Medicine, Damanhour University, Egypt

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ABSTRACT

The current study aimed to evaluate whether clenbuterol, a β_2-adrenergic agonist, supplementation in Nile tilapia (*Oreochromis niloticus*) diets can influence growth and blood parameters. Besides, assessment of adipogenic genes as fatty acid synthase (*FAS*) and lipoprotein lipase (*LPL*) which is a key enzyme in the regulation of the flux of fatty acids in liver, muscle, and adipose tissue as well as muscle growth-regulating genes as myostatin (*MYO*) in muscle and insulin-like growth factor-1 (*IGF-1*) in liver. The fish were allocated into three equal groups; control group that fed basal diet only and the other two groups fed a basal diet containing clenbuterol at two doses 5 ppm and 10 ppm/kg diet for 30 consecutive days. Results revealed that clenbuterol supplementation significantly increased body weight, decreased liver, spleen and abdominal fat weights, and decreased total circulatory cholesterol and triacylglycerol levels. Moreover, clenbuterol inhibits lipogenesis by downregulation of *FAS* gene expression by dose and time-dependent manner in the liver while enhanced lipolysis in both the liver and in the adipose tissue. Moreover, lipolysis was reduced in muscle by dose 10 ppm on day 30. Furthermore, clenbuterol presented higher gene expression of *MYO* and *IGF-1* in muscle and liver respectively by dose 5 ppm at day 15 on the other hand, these findings were reversed by day 30 compared with control. In conclusion, clenbuterol efficacy was apparent in a dose and time response pattern to boost growth and reduce fat deposition rates, indicating for the first time that clenbuterol has a profitable growth impact on Nile tilapia.

1. Introduction

Fish production has increased intensely and it is foreseen to be 140 million tons by 2050 (Dawood and Koshio, 2018; Waite et al., 2014) which reflects the increased demand for fish protein (FAO, 2014). Worldwide the aquaculture faces the challenge to optimize its production through sustainable practices including cost savings and improved growth (Dawood and Koshio, 2019; FAO, 2018). The growth performance of fish is one of the main economic characteristics which is controlled by the metabolism of energy and muscle growth (Dai et al., 2015; Dawood, 2016). Strengthening muscle development is, therefore, a good approach to improve the quality of fish (Vélez et al., 2017). The excessive fat build-up of the viscera is a common problem in fish. Therefore, the enhancement of muscle growth by reducing body fat is a

* Corresponding authors.
E-mail addresses: doaa_assar2000@yahoo.com (D.H. Assar), Mahmouddawood55@gmail.com, mahmoud.dawood@agr.kfs.edu.eg (M.A.O. Dawood), hien.d@cmu.ac.th (H.V. Doan).

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promising tool for dealing with this problem (Du et al., 2005).

Balanced aquafeed aims to improve the quality and quantities of myotomous muscle by upregulating of somatotropic genes, such as growth hormone (*GH*) and insulin-like growth factor-1 (*IGF-1*) (Li et al., 2014; Wang et al., 2001). GH/IGF axis adjusts body and bone masses together with carbohydrate and lipid metabolism that regulate somatic growth (Yakar et al., 2018).

Feed additives including β_2-adrenergic agonists (β_2-agonist) were used to improve lean carcass contents and reduce feed requirements for animals with increased muscle-to-fat ratios (Dawood et al., 2018; Johnson et al., 2014). Clenbuterol is one of β_2-agonist which works on the skeletal muscle causing skeletal fibers to hypertrophy (Burniston et al., 2007). However, data on the molecular mechanisms through which IGFs stimulate muscle growth in fish are not provided in detailed studies concerning the impact of clenbuterol supplementation in Nile tilapia (*Oreochromis niloticus*) on lipid metabolism and growth.

Therefore, in this study, we assessed the effects of clenbuterol on growth parameters, serum variables, fatty acid synthase (*FAS*), lipoprotein lipase (*LPL*), myostatin (*MYO*), and *IGF-1* expression levels in the liver, muscle, and visceral adipose tissues in Nile tilapia.

2. Material and methods

2.1. Ethics statement

This experiment was conducted according to the codes of Egyptian ethics and approved by the Committee of Animal Ethics in Kafrelsheikh University, Egypt.

2.2. Fish

Healthy Nile tilapia specimens were obtained from a private farm located in Kafrelsheikh, Egypt. The fish were collected using 0.25 cm mesh size net and placed in separate polypropylene containers before transporting to the laboratory. Fish were maintained under normal lab conditions and fed the basal diet (Table 1) during 2 weeks of acclimation. The fish were dried using a clean sterile filter papers to remove the excess water before weighing them. Fish were weighed using digital balance (ADAM equipment Co., Connecticut, USA). The length was measured as a distance from the snout to the beginning of the caudal fin using a measuring board as described by Lagler (1970). The length and weight of fish were recorded to the nearest mm and 0.1 g, respectively.

Table 1
Basal diet composition and chemical analyses (dry matter basis).

Composition	(%)	Chemical analyses	(%)
Fish meal (65%)	12	Dry matter	92.1
Soybean meal (45%)	39.5	Crude protein	30
Yellow corn	30	Crude lipid	5
Wheat bran	15.5	Ash	6.5
Sunflower oil	2.5	NFE[ϕ]	49.6
Vitamin and mineral mixture*	0.5	Gross energy (Kcal/Kg)	4326.5

* Vitamin premix contained (mg g^{-1} mixer) thiamin hydrochloride, 5 mg; riboflavin, 5 mg; calcium pantothenate, 10 mg; nicotic acid, 6.05 mg; l-ascorbyl-2-monophosphate-Mg, 3.95 mg; pyridoxine hydrochloride, 4 mg; folic acid, 1.5 mg; inositol, 200 mg; menadione, 4 mg; alpha-tocopherol acetate, 50 mg; retinyl acetate, 60 mg; biotin, 0.6 mg. All ingredients were diluted with alpha-cellulose to 1 g. Mineral premix contained (g kg^{-1} diet) calcium biphosphate, 13.58 g; calcium lactate, 32.7 g; $FeSO_4 \cdot 6H_2O$, 2.97 g; magnesium sulfate, 13.7 g; potassium phosphate dibasic, 23.98 g; sodium biphosphate, 8.72 g; sodium chloride, 4.35 g; $AlCl_3 \cdot 6H_2O$, 0.015 g; KI, 0.015 g; $CuCl_2$, 0.01 g; $MnSO_4 \cdot H_2O$, 0.08 g; $CoCl_2 \cdot 6H_2O$, 0.1 g; $ZnSO_4 \cdot 7H_2O$, 0.3 g.

[ϕ] Nitrogen-free extract.

2.3. Experimental design and fish management

Seventy-two monosex Nile tilapia (*O. niloticus*) (166.13 ± 6.1 g, initial mean weight ± standard error) fish were equally allocated into three equal groups; control group (n = 24): received basal diet only, CLE5 (n = 24) fed on basal diet supplemented with 5 ppm clenbuterol (5 mg/kg bw), and CLE10 (n = 24) fed on basal diet contain 10 ppm clenbuterol (10 mg/kg bw) for 30 constitutive days. Each group was subdivided into three equal replicates, 8 fish per each.

Fish were reared in glass aquariums (80 × 45 × 35 cm) contain 70 L water. The water was stored overnight before use to be free from chlorine. Each aquarium had a separate mechanical filter (JAD, China) to remove wastes from water and a stone for oxygen supply. Water quality parameters (total ammonia, PH, conductivity, and total dissolved salts) were checked once per week. The three experimental groups were fed extruded floated pellets (ALEKHWA® feed factory, Baltim, Kafrelsheikh, Egypt) (Table 1). Fish in all groups were fed 2 % of their body weight in a single meal at 10 am for 30 days. Clenbuterol was mixed with fish feed by mixing of 5 ppm and 10 ppm clenbuterol/kg feed with 20 ml (corn oil for all fish groups).

2.4. Growth parameters

At the end of the experiment, the final body weight, liver weight, spleen weight, and abdominal fat weight values were determined using digital balance (ADAM equipment Co., Connecticut, USA).

2.5. Sampling

Fish were anaesthetized with 0.02% benzocaine solution after which two separate blood samples were collected from the caudal vein, the first one was taken on EDTA as an anticoagulant for hematological analyses, while the second one was obtained in a plain centrifuge tube allowed to clot then centrifuged at 3000 rpm at 4 °C for 15 min for serum separation. Clear sera were collected and stored in Eppendorf tubes at −20 °C until for biochemical analyses.

Liver, muscle, and adipose tissue samples for mRNA expression have been taken from anaesthetized fish, washed by saline, and kept in −80 °C until molecular analysis.

2.6. Leukogram and biochemical analyses

Differential leukocytic counts were assessed according to the method of Weiss and Wardrop (2011). Serum samples were analyzed for total proteins (TP), albumin (Alb), alanine aminotransferase (ALT), and aspartate aminotransferase (AST) following the instructions enclosed in the manufactured kits produced by Stanbio Laboratories Incorporation (Texas, USA). Serum glucose levels were determined using kit supplied by Analyticon Biotechnologies (Lichtenfels, Germany). Serum total cholesterol and triacylglycerol concentrations were determined by kit produced by BIO-Merieux Co. (Marcy l'Etoile, France).

2.7. RNA extraction and RT-PCR

Direct-zol RNA™ Miniprep kit (Zymo Research, California, USA) was used to isolate total RNA from fish's liver, muscle, and abdominal fat samples according to the manufacturer's protocol. RNA integrity of all samples was assessed by electrophoresis on 1 % ethedium bromide-stained agarose gels and purity was determined by O.D.260 nm /O.D.280 nm ratio. 2 µg of total RNA were reverse transcribed to cDNA using first-strand cDNA kits (Bioline, London, UK) according to the manufacturer's instructions.

The produced cDNA was used as a template to determine the relative expression of the *FAS*, *LPL*, *MYO*, *IGF-1,* and *β-actin* (as internal control to calculate fold change in target genes). The primer sequences are shown in Table 2. The quantities critical threshold (Ct) of target

Table 2
Primers sequences for RT-PCR analysis.

Gene	Primer sequence (5'-3')	References
FAS	F: TGAAACTGAAGCCTTGTGTGCC	Tian et al. (2015)
	R: TCCCTGTGAGCGGAGGTGATTA	Tian et al. (2015)
LPL	F: TGCTAATGTGATTGTGGTGGAC	
	R: GCTGATTTTGTGGTTGGTAAGG	
MYO (exon 2)	F: GCATCTGTCTCAGATCGTGCT	Elkatatny et al. (2016)
	R: TGCCATCATTACAATTGTCTCCG	
IGF-1	F: TCCTGTAGCCACACCCTCTC	Costa et al. (2016)
	R: ACAGCTTTGGAAGCAGCACT	
*β-actin**	F: CCACACAGTGCCCATCTACGA	Qiang et al. (2014)
	R: CCACGCTCTGTCAGGATCTTCA	

FAS: fatty acid synthase, *LPL*: lipoprotein lipase, *MYO*: myostatin, *IGF-1*: insulin-like growth factor, *housekeeping gene.

gene were normalized with quantities (Ct) of *β-actin* using $2^{-\Delta\Delta Ct}$ method as previously described by Livak and Schmittgen (2001). All samples were performed and analyzed in triplicate.

2.8. Statistical analysis

Statistical analyses were performed using the SPSS program (IBM SPSS. 201, IBM Corp., Armonk, NY, USA) using two-way ANOVA followed by Tukey's multiple comparisons test was used to examine the statistically significant differences of clenbuterol dose and time effects on parameters measured including; growth, differential leukocytic count, and biochemical parameters as well as gene expression levels. Means with the same lower-case letter are not significantly different according to a Tukey's test ($P < 0.05$). One-way ANOVA was used to test the effect of clenbuterol on abdominal fat weight followed by Tukey's multiple comparisons test to test the significance between the different treatments. Figures have been generated using GraphPad Prism 6 (GraphPrism Software, La Jolla, CA, USA).

3. Results

3.1. Growth parameters

The results of the growth parameters are represented in Table 3. The growth parameters pointed out that clenbuterol supplementation revealed a significant increasing effect on body weight and length, while significantly reducing effects on the liver, spleen, and abdominal fat tissue weights were detected in clenbuterol treated fish compared with the control fish.

3.2. Leukocytic differential counts

Clenbuterol dietary supplementation led to non-significant changes on white blood cells differential counts as shown in Table 4 compared with the control untreated fish.

3.3. Serum biochemical parameters

As shown in Table 5, the results indicated that clenbuterol dietary supplementation did not alter the normal liver function as confirmed by the non-significant changes on serum albumin concentration. Furthermore, total proteins and globulins concentrations were observed to be increased significantly by dose depended manner at day 30 compared to control group. Moreover, clenbuterol treatment revealed a significant increase in serum ALT activities by dose 10 ppm at day 30 with a significant decline in AST activities by dose 5 ppm at day 15 compared to control group.

On the other hand, significant reductions were detected in serum total cholesterol and triacylglycerol levels at dose and time depended manner. Furthermore, glucose concentrations showed a significant reducing effect on day 15 but this effect was reversed by dose 10 ppm of clenbuterol at day 30 in clenbuterol-supplemented fish compared with the control untreated fish group.

Table 3
Effect of clenbuterol on body weight, body length, liver, spleen, and abdominal fat weights of control and clenbuterol-supplemented fish.

Items		Control	CLE5	CLE10
Initial body weight		167 ± 5.30	165.25 ± 6.50	166.08 ± 6.70
Body weight	15th day	172.5 ± 2.63^{Aa}	174.67 ± 4.96^{Aa}	175.33 ± 2.60^{Ba}
	30th day	180 ± 2.80^{Aa}	183.17 ± 2.86^{Aa}	187 ± 1.81^{Ba}
Initial body length		21.62 ± 0.16	21.36 ± 0.21	21.26 ± 0.27
Body length	15th day	21.75 ± 0.25^{Aa}	21.90 ± 0.07^{Aa}	21.98 ± 0.24^{Aa}
	30th day	21.92 ± 0.27^{Aa}	22.18 ± 0.13^{Ba}	22.73 ± 0.18^{Ba}
Initial liver weight		5.34 ± 0.45	5.22 ± 0.61	4.91 ± 0.54
Liver weight	15th day	0.25 ± 0.05^{Aa}	0.15 ± 0.01^{Ab}	0.14 ± 0.02^{Ab}
	30th day	5.46 ± 0.28^{Aa}	4.44 ± 0.53^{Bb}	4.26 ± 0.29^{Ab}
Spleen weight	15th day	0.25 ± 0.05^{Aa}	0.15 ± 0.01^{Ab}	0.14 ± 0.02^{Ab}
	30th day	0.36 ± 0.07^{Aa}	0.26 ± 0.09^{Ab}	0.21 ± 0.04^{Ab}
Abdominal fat weight		1.83 ± 0.25^{a}	0.67 ± 0.20^{b}	0.71 ± 0.06^{b}

Values are expressed as means ± standard error.
''A'' and ''B'' letters in the same column differ significantly at $P < 0.05$ for each parameter.
''a'' and ''b'' letters in the same row differ significantly at $P < 0.05$ for each period.
Data were analyzed by Two-way ANOVA followed by Tukey's multiple comparisons test.

Table 4
Effect of clenbuterol on white blood cells differential counts of control and clenbuterol-supplemented fish.

Items	Periods	Control	CLE5	CLE10
Heterophils %	15th day	5.4 ± 0.51^{Aa}	4.6 ± 0.4^{Aa}	4.8 ± 0.37^{Aa}
	30th day	4.8 ± 0.37^{Aa}	4.8 ± 0.2^{Aa}	4.2 ± 0.2^{Aa}
Lymphocytes %	15th day	93.8 ± 0.66^{Aa}	94.4 ± 0.4^{Aa}	94 ± 0.32^{Aa}
	30th day	9.6 ± 0.24^{Aa}	94.2 ± 0.2^{Aa}	94.6 ± 0.24^{Aa}
Monocytes %	15th day	0.8 ± 0.2^{Aa}	1.0 ± 0.0^{Aa}	1.2 ± 0.2^{Aa}
	30th day	0.6 ± 0.24^{Aa}	1.0 ± 0.0^{Aa}	1.2 ± 0.37^{Aa}

Values are expressed as means ± standard error.
''A'' and ''B'' letters in the same column differ significantly at $P < 0.05$ for each parameter.
''a'' and ''b'' letters in the same row differ significantly at $P < 0.05$ for each period.
Data were analyzed by Two-way ANOVA followed by Tukey's multiple comparisons test.

3.4. Relative mRNA expression levels

The findings of this study demonstrated that clenbuterol inhibits lipogenesis by downregulating mRNA expression levels of the gene encoding *FAS* in the liver by dose 10 ppm only at day 15 and by both doses at day 30 compared with the control fish group (Fig. 1A).

Clenbuterol dietary administration enhanced lipolysis by upregulating gene encoding *LPL* in the liver by dose 10 ppm only at day 15 but non-significantly changed by both doses at day 30 (Fig. 1B).

Concerning mRNA expression levels of the gene encoding *IGF-1* in the liver; clenbuterol supplementation significantly upregulated *IGF-1* at dose 10 ppm only at day 15 but its expression levels were downregulated but statistically were non-significant by both doses at day 30 compared with control fish group (Fig. 1C).

Clenbuterol supplementation, significantly upregulated gene encoding *FAS* in the muscle by dose 10 ppm only at day 15 and

Table 5
Effect of clenbuterol on serum biochemical parameters of control and clenbuterol-supplemented fish.

Items	Periods	Control	CLE5	CLE10
Total proteins (g/dl)	15th day	2.21 ± 0.02^{Ba}	2.20 ± 0.01^{Ba}	2.21 ± 0.01^{Ba}
	30th day	3.33 ± 0.09^{Ab}	3.59 ± 0.10^{Ab}	3.97 ± 0.11^{Aa}
Albumin (g/dl)	15th day	1.34 ± 0.06^{Ba}	1.46 ± 0.09^{Ba}	1.39 ± 0.07^{Ba}
	30th day	2.37 ± 0.03^{Aa}	2.38 ± 0.06^{Aa}	2.53 ± 0.13^{Aa}
Globulins (g/dl)	15th day	0.87 ± 0.06^{Ba}	0.74 ± 0.09^{Ba}	0.82 ± 0.08^{Ba}
	30th day	0.96 ± 0.09^{Ab}	1.21 ± 0.13^{Ab}	1.44 ± 0.20^{Aa}
ALT (U/L)	15th day	35.4 ± 1.63^{Ba}	35.8 ± 1.16^{Ba}	36.2 ± 1.66^{Ba}
	30th day	38.4 ± 0.68^{Ab}	39.2 ± 3.9Aa	43.8 ± 1.16^{Aa}
AST (U/L)	15th day	190.2 ± 3.06^{Aa}	188.8 ± 3.34^{Ba}	187.2 ± 3.15^{Ba}
	30th day	197.2 ± 2.06^{Aa}	204.8 ± 2.03^{Aa}	204.4 ± 2.84^{Aa}
Total cholesterol (mg/dl)	15th day	221.6 ± 0.23^{Aa}	222.6 ± 2.6^{Aa}	222.6 ± 2.71^{Aa}
	30th day	226.6 ± 2.56^{Aa}	205.4 ± 2.98^{Bb}	199.2 ± 3.38^{Bb}
Triacylglycerol (mg/dl)	15th day	247.2 ± 2.71^{Aa}	237.2 ± 1.43^{Ab}	233 ± 1.30^{Ab}
	30th day	252 ± 1.70^{Aa}	235.2 ± 4.26^{Ab}	229 ± 2.26^{Ab}
Glucose (mg/dl)	15th day	199 ± 4.49^{Aa}	190.6 ± 4.63^{Ba}	187.2 ± 2.46^{Ba}
	30th day	202 ± 2.59^{Ab}	206.8 ± 3.40^{Ab}	221.6 ± 3.37^{Aa}

Values are expressed as means ± standard error.
''A'' and ''B'' letters in the same column differ significantly at $P < 0.05$ for each parameter.
''a'' and ''b'' letters in the same row differ significantly at $P < 0.05$ for each period.
Data were analyzed by Two-way ANOVA followed by Tukey's multiple comparisons test.

upregulated at dose 5 ppm by day 30 compared with that in the control fish group (Fig. 2A). Moreover, *LPL* mRNA expression levels in muscle were non-significantly changed in clenbuterol-supplemented groups by both doses at day 15 compared with the control group while *LPL* mRNA expression levels were reduced by dose 10 ppm only at day 30 compared with the control fish group (Fig. 2B).

Regarding mRNA expression levels of the gene encoding *MYO* in muscle, clenbuterol supplementation significantly upregulated gene encoding *MYO* at dose 10 ppm only at day 15 with significant downregulation by the same dose at day 30 compared with control fish group (Fig. 2C).

While *FAS* expression levels in the fat tissues were significantly downregulated by both doses at day 15 and non-changed at both doses by day 30 (Fig. 3A). Also, clenbuterol downregulated gene encoding *LPL* by both doses at day 15, but lipolysis was increased by dose 10 ppm only at day 30 in adipose tissue compared with control fish group (Fig. 3B).

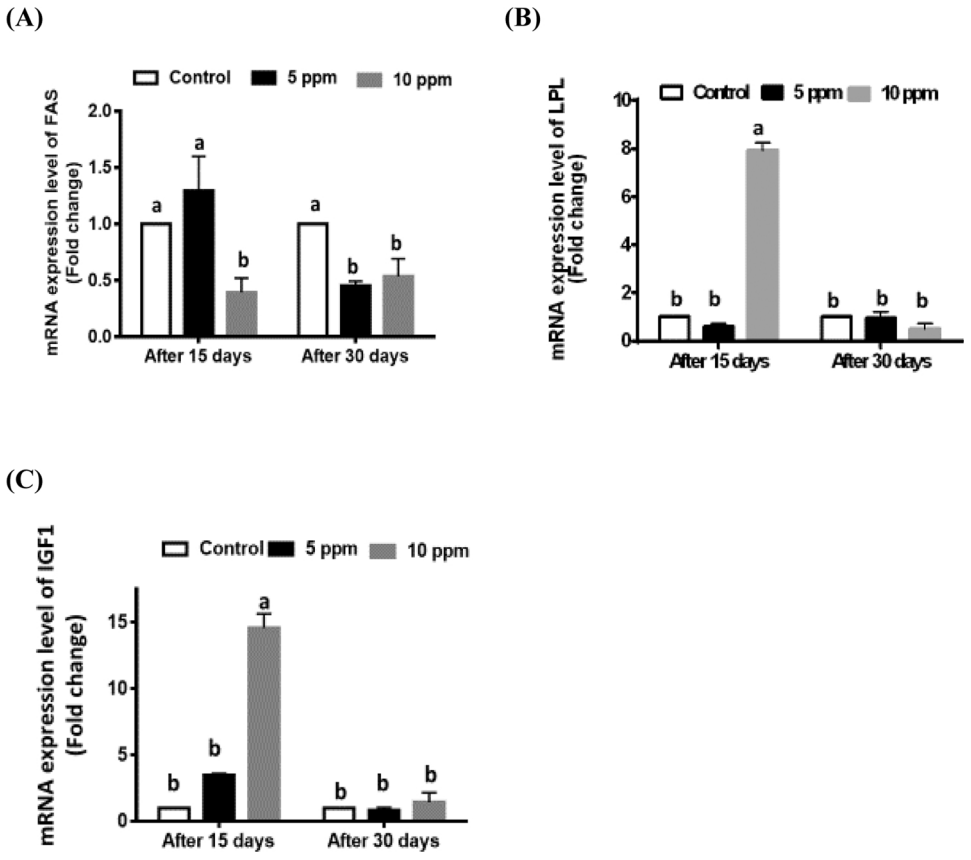

Fig. 1. Fold changes of mRNA expression levels of (A) *FAS*, (B) *LPL*, and (C) *IGF-1* in liver tissues. Different letter means significant difference effects in the same time period. Means ± SEM.

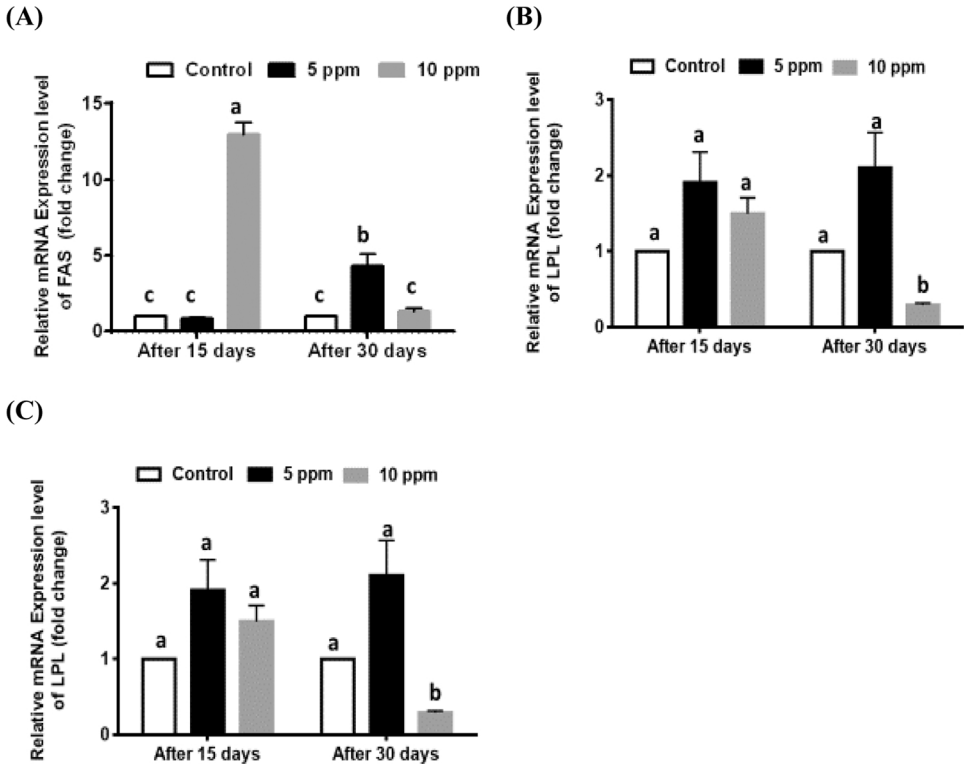

Fig. 2. Fold changes of mRNA expression levels of (A) *FAS*, (B) *LPL*, and (C) *MYO* in muscle tissues. Different letter means significant difference effects in the same time period. Means ± SEM.

4. Discussion

The current study findings clarified that clenbuterol significantly increased body weight and length at a dose and time-dependent manner, while showed significant reducing effects on the liver, spleen, and abdominal fat tissue weights in clenbuterol-supplemented fish compared with the control fish. Our findings are in agreement with that of Hamano (2002) who stated significant decreases in the abdominal fat weight pad in chicken fed with 0.25 mg/kg clenbuterol for 30 days. Also, clenbuterol by a dose of 1 mg/kg for 21 days caused decreased abdominal fat pad weights and increased weights of skeletal muscles in chickens (Rehfeldt et al., 1997). Also, clenbuterol induced 28 % reduction in body fat along with 25 % elevation in muscle mass of rats fed 2 mg/kg for 16 days (Shappell et al., 2002). Similarly, Zhao et al (2015) stated that clenbuterol induced weight gain increases in muscle and decreases adipose tissue mass.

Clenbuterol supplementation at the chosen doses and exposure time didn't alter the normal liver function as confirmed by normal hepatic synthetic function with non-significant changes on serum albumin concentration together with non-significant changes on white blood cells differential counts indicating a non-stressed condition for the clenbuterol-supplemented fish compared with the control group. Moreover, clenbuterol treatment revealed a significant increase in serum ALT activities by dose 10 ppm at day 30 with a significant decline in AST activities by dose 5 ppm at day 15 compared to the control group. This observation was supported by findings of Gojmerac et al (2002) who detected a significant increase in ALT activities and a slight decrease in AST activities in female pig after repeated administration for 28 days at dosage 10 µg/kg twice daily. In this study, we also detected reduced liver weights at a dose and time-dependent manner with decreased AST activities in the clenbuterol supplemented group by dose 5 ppm at day 15 compared to the control group.

The present work detected significantly elevated serum total proteins and globulins concentrations in clenbuterol-fed fish by dose-dependent manner at day 30 compared with the control group. This finding was comparable to previous studies on the mechanism of

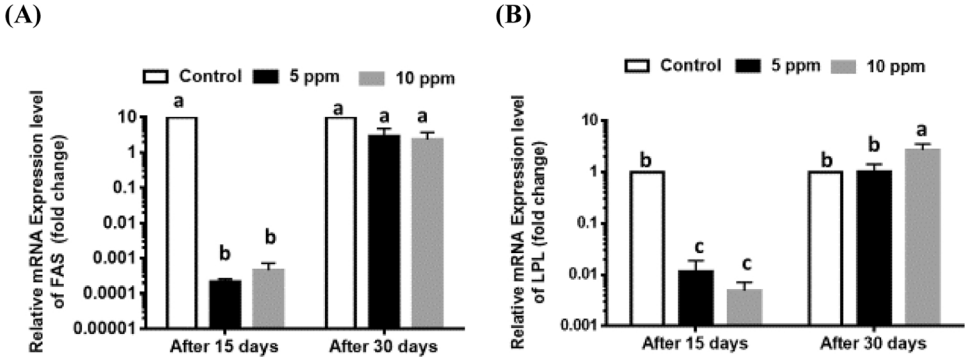

Fig. 3. Fold changes of mRNA expression levels of (A) *FAS* and (B) *LPL* in adipose tissues. Different letter means significant difference effects in the same time period. Means ± SEM.

clenbuterol on protein metabolism, which clearly concluded that clenbuterol elevated the rate of protein synthesis and reduced the rate of protein degeneration (Gojmerac et al., 2002; Ijiri et al., 2014).

Furthermore, serum total cholesterol and triacylglycerol levels were also reduced in the clenbuterol-fed fish compared with the control fish at dose and time-dependent manner which raise the explanation that clenbuterol supplementation might affect cholesterol synthesis in the liver and body fat storage in adipocytes affecting its release from adipose tissue (storage sites) to muscle (sites of utilization). Ijiri et al. (2016) stated a significant reduction in plasma cholesterol levels in chicks injected intraperitoneally by a single dose of clenbuterol (0.1 mg/kg body weight) compared with those in the control.

As supported by stimulating fatty acid oxidation in fish liver and adipose tissue, as confirmed by increased mRNA levels of *LPL* genes in liver and adipose tissue and attenuating the lipogenic activity as detected by significantly up-regulated expression levels of *FAS* in the muscle (fatty acid biosynthesis) (site of utilization) in the clenbuterol treated fish compared to the control fish. *LPL* is a key enzyme for the catalysis of triacylglycerol into glycerol and non-esterified fatty acids in a process called lipolysis (Faulconnier et al., 2001). Clenbuterol upregulated liver-*LPL* gene expression levels at 10 ppm clenbuterol by day 15 and enhanced adipose tissue-*LPL* gene expression levels by 10 ppm clenbuterol at day 30, On the other hand, the muscular-*LPL* mRNA levels were lower than those of the control group by dose 10 ppm at day 30 suggesting that clenbuterol can modulate mRNA expression levels in supplemented fish groups than control group by attenuating the lipogenic activity (downregulated *FAS* gene expression levels in liver and adipose tissue) and stimulated fatty acid oxidation in fish (enhanced *LPL* gene expression levels in liver and adipose tissue) indicating that clenbuterol induced promotion of lipid catabolism rather lipid anabolism. In agreement with our results, Kim et al (2010) reported that clenbuterol increased the rate of lipolysis and concomitantly decreased the rate of lipogenesis in adipose tissues.

Somatic growth in fish is controlled by a variety of hormones including GH/IGF-I axis (Duan et al., 2010). *IGF-I* is a mitogenic polypeptide which is the most promising candidate as a growth indicator in fish as the potential of *IGF-I* mRNA abundance is a rapid growth indicator in the Nile tilapia (Cruz et al., 2006). In the current study, expression levels of mRNAs encoding *IGF-1* have significantly increased in the liver by dose 10 ppm at day 15 of the clenbuterol-fed fish compared with the control group. Moreover, there was a significant increase in final body weight in the clenbuterol-fed fish dose and time-dependent manner compared with control fish. Therefore, it is suggested that clenbuterol supplementation may affect the weights of muscles 30 days after clenbuterol feeding compared with the control fish. This view is reinforced by the significant enhancement of the final body weight of the clenbuterol-fed fish and the gene expression of *IGF-I* in the liver. In line with our view, Ahmed et al. (2011) stated that, transgenic zebrafish that constitutively overexpressed flGHR1 increased the expression of *IGF-1*. Therefore, these fish displayed significantly higher growth rates than control fish.

Concerning mRNA expression levels of the gene encoding in *MYO* in muscle, clenbuterol supplementation significantly upregulated gene encoding *MYO* by dose 10 ppm only at day 15 with significant downregulation by the same dose at day 30 compared with control fish group. On the other hand, a significant increase in body weights by dose 10 ppm at day 30 was detected in clenbuterol treated fish compared to the control fish group. *MYO* encodes myostatin that inhibits myogenesis (McPherron and Lee, 1997). Therefore, silencing the *MYO* gene led to a dramatic weight gain and evidences of a concomitant muscle growth by either hyperplasia (recruitment of myoblasts into new fibers) or hypertrophy (incorporation of myoblasts into pre-existent fibers) in zebrafish (Acosta et al., 2005; Gao et al., 2016; Lee et al., 2009) and medaka (Chisada et al., 2011).

The inclusion of clenbuterol in livestock and poultry feeds is normally practiced around the globe. It has been reported that the residuals of clenbuterol can be accumulated in animal tissue and would damage human health by means of the food chain (Lu et al., 2017). As a result, clenbuterol had become banned drugs in the animal production industry. However, in fish, the level of muscle protein synthesis was increased by the presence of clenbuterol in the muscle of rainbow trout which make it a potential feed additives for sustainable aquaculture (Lortie et al., 2004). Although in the current study, the clenbuterol residuals in the body of tilapia were not analyzed, the obtained results show that clenbuterol probably has no harmful effects on tilapia health under the current experimental conditions. However, research studies related to this matter must be continued to determine the harmful effects of using clenbuterol in fish diets.

5. Conclusion

It seems evident that dietary clenbuterol promoted lipolysis through the downregulation of *FAS* and upregulation of *LPL* leading to the dependence of fish on lipid as a source of energy. Also, clenbuterol enhanced the body gain of fish through the upregulation of *IGF-1* and downregulated *MYO* that improved myogenesis and increased the protein synthesis in Nile tilapia muscles.

CRediT authorship contribution statement

Radi A. Mohamed: Conceptualization, Supervision. **Zizy I. Elbialy:** Conceptualization, Methodology. **Amira S. Abd El Latif:** Conceptualization, Methodology. **Mustafa Shukry:** Conceptualization, Formal analysis, Methodology. **Doaa H. Assar:** Conceptualization, Formal analysis, Investigation, Methodology, Project administration. **Asmaa M. El Nokrashy:** Formal analysis. **Ahmed Elsheshtawy:** Formal analysis. **Mahmoud A.O. Dawood:** Investigation, Supervision, Writing - original draft. **Bilal Ahamad Paray:** Funding acquisition, Writing - original draft. **Hien Van Doan:** Funding acquisition, Writing - original draft. **Ali H. El-Far:** Investigation, Writing - original draft.

Declarartion of Competing Interest

The authors declare no conflicts of interest.

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