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Using Nutraceutical to Alleviate Transportation Stress in the Nile tilapia

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ABSTRACT

To alleviate the stress of fish transportation, nutraceuticals containing one-fold and two-fold of the recommended levels of vitamin A (retinol), vitamin C (ascorbic acid), vitamin E (α-tocopherol) and zinc were added to fish during the acclimatization period (fourteen days). After one day of stocking, fish were fed boosted diets. After the acclimation period, blood parameters and immunity were assessed in addition to experimental bacterial infection. Fish were fed on basal diets for another eight weeks, and growth performance was evaluated. Erratic swimming behavior stopped feeding and aggressiveness. Fish received boosted diets restored normal behavior during the acclimatization period with a raise of mucus peroxidase activity (0.26 and 0.28 mU/Ml) and mucus lysozyme 3.14 and 3.12 (U/mL). Gene expressions of antioxidant enzymes (superoxide dismutase and catalase) and immune cytokines (immunoglobin M-2, tumor necrosis factor-alpha, interleukin-1 beta, and interleukin-10) were significantly enhanced with fish receiving boosted diets, compared to those receiving basal diet. Growth performance was assessed in the experimental fish for eight weeks after acclimatization. The initial weights were significantly higher in boosted-fish, and they also achieved higher weight gain, with a significant lower food conversion ratio (1.43 and 1.46). Based on the obtained results, boosting feed during stress condition help fish to restore normal behavior, antioxidant-immune status and enhanced growth performance.

INTRODUCTION

Live fish transportation is a stressful practice that could influence the net production and even the survival; it is routinely performed in aquaculture, and subsequently appropriate transportation protocols should be followed to minimize the stress impacts **(Taheri Mirghaed & Ghelichpour, 2019)**. To mitigate stress during transportation process, several trials were conducted using dietary probiotics **(Gomes *et al.*, 2009; da Silva *et al.*, 2022)**, anesthetics **(Ferreira *et al.*, 2021)**, glycine **(Hoseini *et al.*, 2022a)**, turmeric **(Hoseini *et al.*, 2022b)** or salt addition to transportation water

(Boaventura *et al.*, 2022). Using tranquillizers such as MS-222 and/or clover oil along with antiseptics baths at the arrival could minimize these impacts **(Park *et al.*, 2009)**; however, fish slowly and insufficiently restore feeding rate and normal metabolism **(Sampaio & Freire, 2016)**.

A high metabolic rate was recorded during the transportation of Atlantic salmon **(King, 2009)**, as well as the formation of reactive oxygen species (ROS) and other pro-oxidants that stimulate antioxidant mechanisms, such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and glutathione reductase (GR) **(Braun *et al.*, 2010)**.

Many studies confirmed that nutraceuticals supplementation could trigger the antioxidants mechanisms and thus improve the antioxidant capacity of fish tissues, counteracting the detrimental impacts of stressful conditions **(Manush *et al.*, 2005; Akhtar *et al.*, 2012; Wu *et al.*, 2017; Sherif *et al.*, 2021a, b, 2022a, b)**. Vitamins and minerals play a vital role in several metabolic processes concerning the growth, health, immune and the antioxidative status of animal. During transportation, fish are exposed to oxidative stress that nutraceuticals could mitigate. During stress, vitamin A (retinol) plays an important role in translation of pregnenolone into cortisol (stress hormone), and its deficiency declines cortisol production **(Jessani *et al.*, 2015)**. In general, vitamin E (α-tocopherol) is a lipid soluble antioxidant, and α-tocopherol (α-TOH) is the higher biological active form **(Hamre, 2011)**, protecting the adrenal cortex from ROS impacts, lowering the production of cortisol **(Wilson *et al.*, 2013)**, decreasing blood glucose (secondary stress response) **(Patrocínio-Silva *et al.*, 2016)**. A combination of vitamin C and vitamin E at a double-fold of the recommended levels was found to be able to maintain the normal physiological and biochemical status of *Macrobrachium rosenbergii* males **Manush *et al.*, 2005)** as well as *Labeo rohita* fingerlings **(Prusty *et al.*, 2011)**. Zinc is a well-known antioxidant agent, guarding metabolic processes such as growth and reproduction **(Eisler, 1993)** and protecting the tissues from oxidative stress **(Ho & Ames, 2002)**. Zinc deficiency could reduce the growth, the capacity to combat oxidative stress, and eventually mortality **(Banni *et al.*, 2011)**.

Thus, this study could provide a regime that could minimize the withdrawals attributed to the transportation process, helping fish restore their normal state of antioxidative, immunological and growth performance.

MATERIALS AND METHODS

1. Fish accommodation and experimental design

The Nile tilapia (*Oreochromis niloticus*) specimens were purchased from a freshwater fish farm at Tolompate 7 village in Kafrelsheikh Governorate and transported to the Animal Health Research Institute, (AHRI) Kafrelsheikh in two hours time. Five hundred fish individuals, with an average weight of 50 ± 4.5g b.w. were tranquillized using anesthetic agent MS-222 (tricaine methanesulfonate) at a dose of 40mg/ L of

transporting water; MS-222 is produced by Syndel, Canada. Fish were subjected to an iodine bath at the arrival in wet laboratory following the recommendations of **Sherif *et al.* (2022c)**; Betadine®, the active ingredient, is 5% of povidone-iodine and produced by the Nile Company for Pharmaceuticals, Egypt. Fish were fed on the following diets during the acclimatization fourteen days. The feed requirements were following the recommendations of **NRC (2011)** for Nile tilapia**.** In **Table 1,** the control fish were fed on the basal diet (G1), fish were fed on the basal diet and boosted with one-fold of the recommended levels of Vit A, Vit E, Vit C, and Zn (5000 IU, 100 mg, 420 mg, and 79 mg) /kg dry diet, respectively (G2), and fish were fed on the basal diet and the diet was boosted with two-fold of the recommended levels (G3).

- Fish were fed twice a day at 8:30 and 13:30 by hand and they were carefully observed till all supplied feed was consumed. On the second arrival day, Nile tilapia were weight before being stocked in each group **(Sherif *et al.*, 2019).** Fish weight after the acclimatization period (W0) and weekly weighed for a successive period of 8 weeks.

Total weight gain = Final body weight - Initial body weight

Weight gain % = Total weight gain / Initial weight ×100

FCR = Feed intake / total body weight gain

PER = Weight gain / protein intake

Table 1. List of fish feed ingredient and chemical analysis of used diet.

Ingredient	**%**	**Ingredient**	**%**
Corn	25	**MCP**	1
Soya (44%)	32	**Salt**	0.2
Fish meal	20	**Methionine**	0.05
DDGs	5	**Choline chloride**	0.05
Corn gluten	15	**Minerals premix**	0.1
Soya oil	1.5	**Vitamins premix**	0.1
Chemical composition			
Moisture	10.35	**Crude fiber**	4.22
CP	30.95	**NFE**	44.85
Ether extract	3.57	**Calcium/phosphorus**	1.09/0.52
Ash	6.06	**DE Kcal/kg**	3400.5

- DDGs = Dried Distilled Grains.
- Vitamins premix each one Kg contains: Vit A 12000IU, Vit D3 2200 IU, Vit E 10 g, Vit K3 2 g, Vit B1 1 g, Vit B2 5 g, Vit B6 1.5 g, Vit B12 0.01g, Niacin 30 g, Folic acid 1 g, Biotin 0.050 g, Vit C 250g, and Pantothenic acid 10 g; and carrier to 1000 g.
- Minerals premix: each one kg contains: Zinc 50g, Manganese 60g, Copper 4 g, Iodine 1g, iron 80g, Cobalt 0.1g, Selenium 0.1g, calcium carbonate (CaCO3) carrier to 1000g.
- NFE= Nitrogen free extract.
- DE Kcal/kg = Digestible energy was determined using the following formula based on chemical analyses of feed ingredient **NRC (2011).**

1- Serum glucose analyses

Samples for serum glucose were collected at the fish farm, on the second day of the laboratory arrival, and after fourteen days of acclimatization, then following methods described by **Trinder (1969),** the serum glucose level was determined using Spinreact® glucose test kit.

2- Non-invasive biomarkers.

3.1. Mucus analyses of the experimental fish.

For skin mucus collection, three Nile tilapias gently rubbed were inserted into polyethylene bags each containing 10 mL NaCl (50 mM) for 30 s, then the mucus was centrifuged for 10 min / 1500 rpm / 4 °C, then we discarded the supernatants and the precipitates were preserved at -96 °C to determine lysozyme activity and peroxidase activity.

-Lysozyme. The activity was evaluated according the described method of **Parry *et al.* (1965).** Briefly, the collected mucus (100 µL) from each group was placed in triplicate into 96-well plates, then we added 175 µL of *Micrococcus luteus* suspension (0.3 mg *M. luteus* dispersed in 1 mL of citrate phosphate buffer (0.1 M) at 5.8 pH), all chemicals and bacterial strain produced by Sigma-Aldrich, USA. The activities were evaluated by a microplate reader and the changes of turbidity were recorded at 540 nm every 30 s and for 10 min at 25 °C. then compared to standard and expressed as (µg/mL).

-The peroxidase activity. A 5 µL of the collected mucus was put into 96 ELIZA plates in triplicate, and the activity was determined using a microplate reader at 405 nm, the peroxidase activity was expressed in mU/Ml **(Quade and Roth 1997),** Eliza kits were purchased from Sigma-Aldrich, USA.

3.2. Food reflex of the experimental Nile tilapia.

After 24 h of Nile tilapia transportation, the stocked fish were fed on floating fish feed and observed through glass aquaria for food approach **(Samson *et al.*, 2014).**

3- Antioxidant and immune genes expression.

Total RNA was extracted using the Trizol reagent (iNtRON Biotechnology Inc., Korea from the head kidney tissues of the experimental Nile tilapia before and after 24h of transportation as well after acclimatization period (fourteen days). The complementary DNA (cDNA) was generated by means of the reverse transcription polymerase chain reaction using SensiFAST kits (Bioline, USA) following the manufacturer's instructions. The synthetized cDNA served as a template in the quantitative real-time PCR using specific primers **(Table 2)** for immunoglobin (Ig) M-2, tumor necrosis factor (TNF)-α, interleukin (IL)-1β, interleukin (IL-10), as well as superoxide dismutase enzyme (SOD) and catalase (CAT) enzyme, due to its constitutive expression, the -actin gene served as the housekeeping gene. The genes expression was estimated using the Eq. $2^{-\Delta\Delta CT}$ method **(Livak and Schmittgen, 2001).**

Table 2. Primers sequences of the studied genes.

Gene	Sequence 5-3	GenBank accession No.
IgM-2	F: CCA CTT CAA CTG CAC CCACT R: TGG TCC ACG AGA AAG TCACC	**KC677037.1**
TNF-α	F: AGG GTG ATC TGC GGG AATACT R: GGCCC AGG TAA ATG GCGTTG T	**NM_001279533.1**
IL-1β	F: TCT TCT ACA AAC GCG ACACC R: TCT GGA GCT GGA TGT TGAAG	**KF747686.1**
IL-10	F: ACC CCG TTC GCT TGCCA R: CAT CTG GTG ACA TCA CTC	**Buonocore *et al.* (2007)**
SOD	F: CAT GCC TTC GGA GAC AACAC R: ACC TTC TCG TGG ATC ACCAT	**AY491056.1**
CAT	F: AGC TCT TCA TCC AGA AACGC R: GAC GTC AGG CGT CAC ATCTT	**JF801726.1**
β-actin	F: CCA CAC AGT GCC CAT CTACGA R: CCA CGC TCT GTC AGG ATCTTCA	**EU887951.1**

F; forward, R; reverse, IgM-2; immunoglobin M-2, TNF-α; tumor necrosis factor-alpha, IL-1β; interleukin-1 beta, IL-10; interleukin-10, SOD; superoxide dismutase enzyme, CAT; catalase enzyme, β-actin; beta-actin.

4- Statistical analyses of the experimental results.

Transportation triggers stress responses and their impacts were statistically evaluated and displayed using a two-way ANOVA test as mean ± SE (standard error) using software developed by SPSS Incorporation **(SPSS, 2004).** The significance (0.05) of differences among experimental groups and feed additives was determined according to **Duncan (1955).**

RESULTS

1- On the arrival

Fish showed abnormal swimming behavioral with excessive waste discharges, no external lesions were recorded during the first day while on the second-day fish showed skin wounds and despite uniform size, some individuals had an aggressiveness attitude.

2- Glucose and non-invasive biomarkers of stress.

In **Table 3,** During the acclimatization period, Nile tilapia were fed on boosted-diets with one (G2) and two-fold (G3) of Vit A, Vit C, Vit E, and zinc requirements. Serum glucose was measured before and after transportation by 24 h the at the end of the acclimatization period, serum level was upsurge in stressed fish and ranged between 93.97 and 86.5 mg/dl, then restore normal values compared to those determined before transportation. Fish received boosted-diets have significantly higher level compared to those with basal-diet.

After feeding boosted diets for fourteen days, fish were rapidly restored normal food reflex compared to fish fed on basal diets (G1), along with enhancements of mucus peroxidase activity (0.26 and 0.28 mU/Ml) and mucus lysozyme 3.14 and 3.12 (µg/mL) in G2 and G3, respectively **(Table 3).**

Table 3. Serum glucose and non-invasive assessment of the experimental fish.

Items	**Glucose** (mg/dl)			**Food reflex**			**Mucus peroxidase activity** (mU/Ml)			**Mucus lysozyme** (µg/mL)		
	B. Stress	**P. stress**	**14 days**	**B. stress**	**P. stress**	**14 days**	**B. stress**	**P. stress**	**14 days**	**B. stress**	**P. stress**	**14 days**
G1	52.43^{Ba} ±1.26	84.23^{Aa} ±5.3	43.7^{Cb} ±1.8	ND	-	++	0.187^{Aa} ±0.02	0.09^{Ba} ±0.02	0.19^{Ab} ±0.005	2.32^{Aa} ±0.06	1.8^{Ba} ±0.21	2.53^{Ab} ±0.07
G2	50.6^{Ba} ±1.31	86.5^{Aa} ±2.9	53.4^{Ca} ±1.6	ND	-	+++	0.19^{Ba} ±0.008	0.09^{Ca} ±0.005	0.26^{Aa} ±0.02	2.32^{Ba} ±0.03	1.93^{Ca} ±0.03	3.14^{Aa} ±0.05
G3	51^{Ba} ±1.3	83.97^{Aa} ±1.05	54.4^{Ba} ±2.8	ND	-	+++	0.183^{Ba} ±0.01	0.09^{Ca} ±0.00	0.28^{Aa} ±0.01	2.26^{Ba} ±0.04	1.88^{Ca} ±0.06	3.12^{Aa} ±0.09

Notes: B.stress; 24 h before transportation, P.stress; after 24h of arrival, 14 days; the acclimatization period.

3- Antioxidative and immune status of experimental fish.

In **Table 4,** antioxidant enzymes SOD and CAT alterations were determined in response to transportation process and feeding on boosted-diets. Gene expressions of SOD and CAT in head kidney were significant higher in fish (G1) fed on basal diets after fourteen days of acclimatization 2.21and 1.07 fold change, respectively compared to those fed on boosted-diets G2 and G3 1.84 and 1.84; 0.73 and 0.76 fold change, respectively.

Table 4. Antioxidants genes expression in head kidney of the experimental fish. (fold change)

Items	SOD			CAT		
	B. Stress	P. stress	14 Days	B. stress	P. stress	14 Days
G1	1.85^{Ba} ±0.36	3.87^{Aa} ±0.2	2.21^{Ba} ±0.07	0.64^{Ca} ±0.03	1.61^{Aa} ±0.034	1.07^{Ba} ±0.09
G2	1.8^{Ba} ±0.33	4.05^{Aa} ±0.25	1.84^{Bb} ±0.4	0.61^{Ba} ±0.05	1.64^{Aa} ±0.05	0.73^{Bb} ±0.04
G3	1.81^{Ba} ±0.25	3.95^{Aa} ±0.06	1.84^{Bb} ±0.08	0.61^{Ba} ±0.1	1.58^{Aa} ±0.08	0.76^{Bb} ±0.03

Notes: B.stress; 24 h before transportation, P.stress; after 24h of arrival, 14 days; the acclimatization period. SOD; superoxide dismutase enzyme and CAT; catalase enzyme.

Overall transportation stress resulted in the raising of proinflammatory cytokines IgM-2, TNF- α, and IL-1β with significantly lower Il-10, which were significantly raised after feeding boosted diets G2 and G3 **(Table 5).** After fourteen days of acclimatization, Boosting Nile tilapia diets with Vit A, Vit C, Vit E, and zinc could promote the immune status, as gene expression of IgM-2, TNF- α, IL-1 β, and IL-10 were increased recording significantly higher values compared to fish in G1 **(Table 5).**

Table 5. Immune genes expression in head kidney of the experimental fish. (fold change)

Items	IgM-2			TNF-α			IL-1β			IL-10		
	B. Stress	P. stress	14 days	B. Stress	P. Stress	14 days	B. stress	P. stress	14 days	B. Stress	P. stress	14 days
G1	0.6^{Ba} ±0.04	1.61^{Aa} ±0.03	0.8^{Bb} ±0.03	2.56^{Ba} ±0.08	3.58^{Aa} ±0.22	2.9^{Bb} ±0.06	1.85^{Ba} ±0.03	3.19^{Aa} ±0.3	2.01^{Ba} ±0.05	3.05^{Aa} ±0.4	1.93^{Ba} ±0.03	2.58^{ABb} ±0.3
G2	0.6^{Ba} ±0.06	0.91^{Ba} ±0.02	1.37^{Aa} ±0.18	2.5^{Ba} ±0.07	3.39^{Aa} ±0.04	3.32^{Aa} ±0.13	1.88^{Ca} ±0.02	3.13^{Ba} ±0.2	3.65^{Aa} ±0.16	3.09^{Ba} ±0.4	1.93^{Ca} ±0.04	4.7^{Aa} ±0.33
G3	0.6^{Ba} ±0.05	0.95^{Ba} ±0.03	1.47^{Aa} ±0.18	2.54^{Ba} ±0.04	3.45^{Aa} ±0.03	3.39^{Aa} ±0.11	1.86^{Aa} ±0.04	3.1^{Aa} ±0.52	2.53^{Aa} ±1.3	3.2^{Ba} ±0.5	1.97^{Ca} ±0.09	4.75^{Aa} ±0.22

Notes: B.stress; 24 h before transportation, P.stress; after 24h of arrival, 14 days; the acclimatization period. IgM-2; immunoglobin M-2, TNF-α; tumor necrosis factor-alpha, IL-1β; interleukin-1 beta, IL-10; interleukin-10.

4- Growth performance

Growth performance were assessed after the acclimatizing period for about eight weeks. It was noticed that fish collected from farms had an average body weight of 50 ± 4.5 g after the acclimatization it was changed in response to feeding process. The IW of fish (G1, G2, and G3) after the acclimatization were 51, 59.97, and 59.9 g, respectively. It was noticed that the growth performance of Nile tilapia received boosted-diets was enhanced compared to those fed on basal diet **(Table 6).** Fish of G2 and G3 had high FW, DWG, TWG, WG%, PER, and FI in response to feeding diets containing one and two-fold of Vit A, Vit C, Vit E, and zinc requirements with no significant differences between each other however, both were significantly higher than fish of G1 which fed on basal-diet. While FCR was significantly lower in G2 and G3 compared with G1 1.46, 2.26, and 1.84, respectively.

Table 6. growth performance of the experimental fish.

Items	IW	FW	DWG	TWG	WG%	FCR	PER	FI
G1	51^{B} ±0.97	67.57^{B} ±1.57	0.31^{B} ±0.01	17.17^{B} ±0.67	34.05^{B} ±0.9	1.84^{A} ±0.05	1.76^{B} ±0.05	31.48^{A} ±0.5
G2	59.97^{A} ±0.83	84.42^{A} ±1.08	0.44^{A} ±0.02	24.45^{A} ±1.04	40.8^{A} ±2.02	1.46^{B} ±0.02	2.21^{B} ±0.03	35.74^{A} ±1.95
G3	59.9^{A} ±0.78	84.26^{A} ±0.93	0.435^{A} ±0.02	24.36^{A} ±1.01	40.7^{A} ±1.98	1.43^{B} ±0.03	2.26^{B} ±0.06	34.79^{A} ±1.1

Notes: IW; Initial weight, FW; Final weight, DWG; daily weight gain, TWG; total weight gain, WG%; weight gain percentage, FCR; food conversion rate, PER; protein efficiency ratio, FI; feed intake.

DISCUSSION

Experimental Nile tilapia showed abnormal swimming with an aggressiveness attitude resulting in skin wounds. During the transportation process, the stress developed from overcrowding, agitation, and scratches, the stress amplitude depending on fish species, and size, as well as the duration adversely impacted the normal physiological status, immunosuppression, impaired natural parries **(Sumpter *et al.*, 1985; Sherif *et al.*, 2022c).**

In this work, one-fold and two-fold of normal requirement vitamins (Vit A, Vit C, and Vit E) and mineral (Zn) were used to mitigate the transportation stress. They have immunostimulant properties, which protect fish from oxidative stress and maintain normal physiological functions **(Kiron, 2012),** and they were used to alleviate impacts of stressors in fish **(Naderi *et al.*, 2017).** Accordingly, it was reported that body reserve of

vitamins and mineral declined in stressful condition raising their requirements **(Küçükbay *et al.*, 2009)** for example; the tissues level of Vit C of stressed fish was significantly and rapidly declined **(Varghese *et al.*, 2021),** and its shortage lead to increase strength of the stress signs such as abnormal **(Shahkar *et al.*, 2015).**

Serum glucose level was upsurged in transported Nile tilapia ranging between 93.97 and 86.5 mg/dl after 24 h of laboratory stocking. Similarly, transportation stress induces the elevation of cortisol and glucose in the serum of tambaqui (*Colossoma macropomum*) **(Santos *et al.*, 2020),** *L. rohita* fingerlings (**Biswal *et al.*, 2021),** common carp, *Cyprinus carpio* **(Mousavi *et al.*, 2023).** It was reported that Zn has an insulin like function so it could control glucose level **(Chen *et al.*, 1998),** in addition, Zn could directly attenuate pancreatic insulin secretion **(Bēgin-Heick *et al.*, 1985).**

The transcription of antioxidants could be increased in response to stress increasing the animal capacity **(Wu *et al.*, 2017).** After acclimatization period, feeding on boosted diets, fish were significant restored normal gene expressions of SOD and CAT in head kidney compared to fish fed on basal diet, which remain high. In accordance, transportation stress triggered the gene expression of SOD and CAT in many fish species including *Alosa sapidissima* **(Zhang *et al.*, 2016),** channel catfish *Ictalurus punctatus* **(Refaey and Li, 2018)** and rainbow trout *Oncorhynchus mykiss* **(Lopez-Patino *et al.*, 2014),** common carp **(Mousavi *et al.*, 2023).**

The improve of antioxidant capacity during recovery from stressful condition is important to protect animal tissues from oxidative damages **(Hermes-Lima and Zenteno-Savın, 2002).** In this study, supplementation the fish feed of Nile tilapia with Vit A, Vit E, Vit C, and Zn enabled fish to gain normal antioxidant status. During oxidative stress, Vit C lay a crucial role as oxidase resulted in its depletion in stress *Chanos chanos* fingerlings **(Kumar *et al.*, 2017)** and striped bass **(Mehrle *et al.*, 1982).** Similarly, **Patrocínio-Silva *et al.* (2016)** found that Vit E could mitigate stress as reduction of CAT and SOD activity was observed in the experimental group. Zinc exhibits improved the antioxidant capacity of gilthead seabream (*Sparus aurata*) **(Dominguez *et al.*, 2019),** juvenile common carp (*C. carpio var. Jian*) **(Liang *et al.*, 2020)**, and Nile tilapia **(Huang *et al.*, 2015)**. While zinc-deficiency downregulated the SOD gene expression in rainbow trout **(Hidalgo *et al.*, 2002),** the activity CAT was up-regulated with dietary zinc intake in blunt snout bream *(Megalobrama amblycephala)* **(Jiang *et al.*, 2016).**

In this study, the feeds of Nile tilapia were supplemented with vitamins and minerals only during the acclimatization period. Accordingly, the timing and duration of immunostimulant administration is very important depending on many factors such as species, type of immunostimulant, temperature, and stress kind **(Sakai, 1999),** as immunostimulant effect punctual and temporary, each activity reaching its maximum at different time **(Mulero *et al.*, 1998; Ortuno *et al.*, 2000)**

The gene expression of IgM-2, TNF- α, IL-1 β, and IL-10 as well as mucus peroxidase activity and mucus lysozyme were improved in fish feed boosted-diet compared to those fed on basal diets indicating enhanced-immune status. In accordance, **Guimaraes *et al.* (2016)** observed that Vit A was significantly affected the immune responses however, no clear protective effect of stressful conditions. Also, Vit C stimulates immune responses protecting fish against stress **(Trichet, 2010).** Similarly, a combination Vit C and Vit E enhanced the immunity of *S. aurata* **(Ortuno *et al.*, 2001),** trout *O. mykiss* **(Naderi *et al.*, 2017),** and juvenile mahseer fish **(Khan *et al.*, 2017).** In accordance, genes expression of TNF- α, IL-1 β, and IL-10 in head kidney of *O. niloticus* after receiving dietary zinc oxide for four successive weeks **(Sherif *et al.*, 2022d)**.

The growth performance was improved of experimental Nile tilapia, which fed on boosted diets (Vit A, Vit C, Vit E, and zinc) during the acclimatization period, compared with those fed on basal diet. **Hu *et al.* (2006)** and **NRC (2011)** stated that the normal growth performance of Nile tilapia is affected by the dietary content of Vit A. Due to high prices of sources of Vit A (fish oil and fish meal) leading to low inclusion levels **(Lall and Parazo, 1995),** also, manufacturing the fish feed decrease the commercial Vit A to about 87% **(Gadient and Fenster, 1994).** In accordance to our results, **Guimaraes *et al.* (2016)** reported deleterious effects of Vit A feed intake, FCR, NPU and PER **(Ross *et al.*, 2000).** Under stress, depletion of Vit C concentration of body tissues and its supplementation could enhance WG, specific growth rate (SGR), and PER in treatment fish **(Varghese *et al.*, 2021),** requirement of dietary Vit C exceeds normal level for optimum growth performance in many aquatic animal species **(Lim *et al.*, 2000; NRC, 2011).** In consistency to our results, According to **Naderi *et al.* (2017)** found that Vit E significantly improved growth performance of *O. mykiss.* In accordance, a combination of both the Vit C and Vit E at a rate of double normal requirements maintains optimum physiological profile of *L. rohita* fingerlings **(Manush *et al.*, 2005; Prusty *et al.*, 2011),** Also combination could promote a normal metabolism, thus increasing weight gain, feed efficiency and SGR **(Wassef *et al.*, 2001).** Similarly, combination of (nano‑selenium, Vit C and Vit E) could increase growth performance with lower FCR in juvenile *O. mykiss* **(Harsij *et al.*, 2020).**

CONCLUSION

From previous results, Nile tilapia transportation could be attributed with oxidative stress and immunosuppression. During acclimatization period, feeding fish with boosted diets containing high levels of Vit A, Vit E, Vit C, and Zn could ameliorate such withdrawals giving the fish the advantages of rapid restore of normal physiological status and achieve higher growth performance.

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