TURKISH
JOURNAL OF AQUATIC SCIENCES

RESEARCH ARTICLE/ARAŞTIRMA MAKALESİ ISSN: 2149-9659 E-ISSN: 2528-9462

EVALUATION OF MORINGA OLEIFERA LEAVES AND THEIR AQUEOUS EXTRACT IN IMPROVING GROWTH, IMMUNITY AND MITIGATING EFFECT OF STRESS ON COMMON CARP (*Cyprinus Carpio*) FINGERLINGS

Fatma KHALİL[1] ORCID ID: 0000-0002-8231-6644, **Fatma M.M. KORNİ**[2] ORCID ID: 0000-0002-1236-400X

[1]Department of Management and Development of Animal and Poultry Wealth, Faculty of Veterinary Medicine, Beni-Suef University, Beni-Suef-Egypt
[2]Department of Fish Diseases and Management, Faculty of Veterinary Medicine, Beni-Suef University, Beni-Suef-Egypt

ARTICLE INFO

Received: 24.04.2017

Accepted: 01.07.2017

Published online: 16.07.2017

Khalil and Korni 32(3): 170-177 (2017)

doi: 10.18864/TJAS201715

Corresponding author: Fatma M.M. KORNİ, Department of Fish Diseases and Management, Faculty of Veterinary Medicine, Beni-Suef University, Beni-Suef 62511, Egypt

E-mail: naglaamostafa1@yahoo.com

Keywords:
Cyprinus carpio,
Moringa oleifera leaves,
Aqueous extract,
Stress,
Cortisol,
Behavior

Abstract

This study was designed to evaluate dietary *Moringa oleifera* leaves (MOLs) and their aqueous extract in enhancing the growth rate and immunity and decreasing the acute stress response in common carp (*Cyprinus carpio*) fingerlings. A total of 180 fish were divided into three groups for feeding on diet1 (d1) with no additives (control), diet2 (d2) containing 10 g of MOLs/kg feed, and diet3 (d3) containing 20 mL of MOL aqueous extract/kg feed for 60 days. At the end of the feeding period, the specific growth rate (SGR) was calculated, and serum was obtained for biochemical analysis. In addition, 6 fish from each group were subjected to confinement stress for 20 min. Thereafter, locomotor activity, opercular movement, and plasma and water cortisol levels were measured. The d2 and d3 groups showed an increase in SGR and levels of total protein, globulin, and lysozyme, in addition to a decrease in the locomotor activity and opercular movement than d1 (control) group. MOLs and their aqueous extract had an improving effect on growth and immunity and mitigated the adverse effects of stressors in *C. carpio* fingerlings. Moreover, MOL aqueous extract induced a more marked effect on growth performance and stress resistance than that by MOLs.

INTRODUCTION

World aquaculture is developing fast to overcome the increase in fish demand (Reverter et al., 2014). This development leads to intensification of fish culture, potentiating of stressors and thus impairs immunity, which increase diseases incidence. In aquaculture, fish may suffer from various stressors such as overcrowding, confinement, periodic handling, poor water quality and malnutrition (Quesada et al., 2013) causing adverse effects on growth, immunosuppression, disease outbreaks, mortality and high economic losses (Barton, 2002).

Stress stimulates the hypothalamo-pituitary-adrenal axis (HPA-axis) to increase blood cortisol and locomotor activity (Carrasco and Van de Kar, 2003). Hence, fish behavioral and physiological responses are usually correlated with stress conditions (Koolhaas et al., 1999). Behavioral response is the first alarm to stress (Ursin and Eriksen, 2004). Moreover, locomotor activities are sensitive indicators to many stressors, thus it is commonly used for behavioral analysis (Huntingford et al., 2006). In addition, gills are important targets for cortisol in fish (Wendelaar Bonga, 1997). Therefore, opercular movement reflects stress level posed on fish (Martins et al., 2011).

Cortisol is the main corticosteriod in teleost fish and its plasma concentrations rise greatly during stress (Mommsen et al., 1999). Fish release steroid hormones into the water in measurable amount (Scott and Sorensen, 1994). Also, stressed common carp (*Cyprinus carpio*, *C. carpio*), excreted cortisol into the water (Scott et al., 2001). Hence, cortisol can be measured in fish plasma (Kittilsen et al., 2009) and water samples (Ruane and Komen, 2003). Since, fish plasma and water cortisol levels are related (Ruane and Komen, 2003), water cortisol may be used as a valuable tool for measuring stress in aquaculture without sampling or disturbing fish.

Nutritional manipulations are considered a useful way for stress mitigation in fish (Kanazawa, 1997). Dietary supplementation with medicinal plants is used in fish aquaculture to improve immunity and resisting stressors (Reverter et al., 2014).

Among these plants, *Moringa oleifera (Moringaceae*); which is a highly valued plant and it distributed in tropical and subtropical countries. It has a wide range of medicinal uses with high nutritional value, the so called "miracle tree". *Moringa oleifera* leaves (MOLs) are rich in minerals and provide a good source of protein, vitamins, β -carotene, amino acids and phenolics (Ramachandran et al., 2014).

Dietary supplementation of MOLs increased growth performance (Abou-Elezz et al., 2012). In addition, using MOLs extract overcame NaCl stress in bean seeds (Rady a et al., 2013).

Literature lacks information about the application of aqueous extract of MOLs in aquaculture as most of its uses are confined to in vitro applications. Therefore, the present work focuses on evaluating the effect of dietary MOLs and their aqueous extract on growth, immunity and mitigation of confinement stress in *C. carpio* fingerlings.

MATERIAL AND METHOD

Fish Collection and Maintenance

A total of 180 apparently healthy *C. carpio* fingerlings with average body weight 7±2.0 g were collected from Abo-Saleh fish hatchery, Beni-Suef, Egypt. They were transferred in plastic bags containing oxygenated water to the wet laboratory of Fish Department, Faculty of Veterinary Medicine, Beni-Suef University, Egypt. Fish was kept in three fiberglass tanks of 400 l capacity for each, supplied with chlorine-free tap water and continuous aeration. The fingerlings were acclimatized for 14 days in the experimental fiberglass tanks and were fed 5% body weight pelleted commercial fish diet (Brsiek factory, Egypt, Table 1) during acclimation.

After acclimatization fish were redistributed into glass aquaria of 70x25x40 cm for running the experiments. Water quality parameters were measured twice a week during acclimatization and throughout the experimental periods. These parameters include measuring dissolved oxygen using DO meter (India), temperature using water thermometer (UK), measuring pH using PH indicator paper (USA), as well as measuring ammonia, nitrite and nitrate using commercial test kits (Aquamerck; Merck, Darmstadt, Germany).

Table 1. Composition of the commercial pelleted fish diet (Brsiek factory, Egypt).

Composition of ingredients (25% crude protein)	The percentage (%)
Fish meal	6
Soya bean meal	36
Rice polish	22
Yellow corn	34
Mono-calcium phosphate	1
Common salt	0.5
Premix	0.5

Source of *Moringa oleifera* Leaves (MOLs)

MOLs were obtained from local market. The specimen was examined and identified by a botanist from Botany Department, Faculty of Sciences, Beni-Suef University. The obtained leaves were washed several times with distilled water and air dried in shaded area. The dried leaves were grinded into fine powder using mixer and stored in sterilized glass containers at room temperature for use.

Preparation of Aqueous MOLs Extract

The extract of aqueous MOLs was carried out according to the method suggested by Fatope et al. (1993) with minor modification. Twenty five g powder of grinded leaves was mixed with 250 ml hot (98°C) distilled water and stayed for 24 h. The extract was filtered using a muslin cloth and then re-filtered using filter paper. The extracts were labeled and preserved in the refrigerator at 4°C and used within 1 week.

Diet Preparation

The pelleted commercial fish diet (Brsiek factory, Egypt, Table 1) was ground into fine powder by using mortar, then, the MOLs and its aqueous form were mixed separately with the fine powder. Three fish diets were prepared, including, diet1 (d1) with no additives (control), diet2 (d2) containing 10 g of MOLs/kg feed and the third diet (d 3) containing 20 ml of MOLs aqueous extract/kg feed. Afterwards, each fish diet contents were mixed with distilled water until obtaining a homogenous mixture. The mixture was passed through a hand minced-meat processing machine, producing extruded strings, which were dried at room temperature for 24 h and then broken down to small pellets.

Experimental Design

Growth performance

After acclimatization of 180 fingerlings, they were divided into three groups (20 fish/each) with three replicates for feeding on d1, d2 and d3 for 60 days. Throughout the experimental period the fingerlings were fed 5% of body weight with its specific diet once a day at 10 am.

Growth measurements including individual weight and length of the fingerlings were recorded and the cumulative fish weights per group were weighed weekly to adjust the new required feed amount. At the end of experimental period, specific growth rate (SGR) of fingerlings fed with different diets was calculated according to (Ricker, 1979) using the following formula:

SGR=[logarithm (final weight)–logarithm (initial weight)/t (time interval in days)]×100

Blood collection and biochemical analysis

At the end of feeding period (60 days), 30 fish from each experimental group (10 fish from each replicate group) were anaesthetized with tricaine methane sulfonate (MS222, Sigma-Aldrich Chemical Co. Egypt). Blood was collected from caudal veins without anticoagulant for serum separation. The collected serum was stored at -20 °C for estimation of total protein using the method of Bradford (1976); albumin concentration (Doumas et al., 1971). Additionally, serum lysozyme activity was measured based on the lysis of *Micrococcus lysodeikticus* according to the method of Ellis (1990).

Measuring behavior and cortisol level in water and plasma after exposure to confinement stress

Sixty days after feeding of the tested diets, six fish from each group were individually confined for 20 min in the aquarium shown in Figure 1. This aquarium was designated to restrict fish swimming and provided adequate aeration for avoiding hypoxia stress. The behavior of fish was videotaped using digital video camera (SONY, Japan). After that, opercular movement number/min (at 10^{th}, 15^{th} and 20^{th} min of confinement) was counted. Furthermore, duration of fish activity/min (body movement from side to side) was calculated.

For water cortisol level analysis, whole water in each confinement area was collected immediately after ending of stress period (20 min). The collected water was thoroughly mixed and only 10 ml water was kept frozen (-20°C) for analysis of cortisol level.

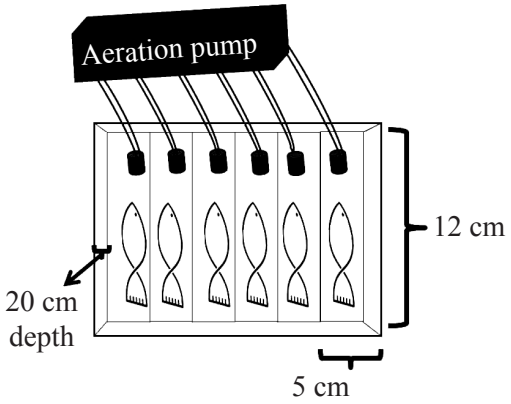

Figure 1. Aquarium used for confinement stress test; Fiber glass aquarium (12cm width×30cm length ×20 cm depth), equally subdivided into 6 equal units (5cm width×12 cm length ×20cm depth). Each unit was supplied with aeration.

Table 2. Effect of dietary MOLs and their aqueous extract on growth and specific growth rate (SGR) of *C. carpio* fingerlings.

Parameter	Diet 1 (control)	Diet 2 (*M. oleifera* leaves)	Diet 3 (*M. oleifera* aqueous)
Total initial weight (g)	139.99±3.87	143.5±2.36	144.09±2.65
Total final weight (g)	211.33±4.7	233.5±4.7*	248.96±2.3**
SGR (%/day)	0.686	0.811	0.911

*p=0.02, considered significant in comparison with control group.
**p=0.002, considered very significant in comparison with control group.

Table 3. Serum parameters of fish groups feed on MOLs and their aqueous extract.

Items	Diet 1 (control)	Diet 2 (*Moringa oleifera* leaves)	Diet 3 (aqueous extract)
Total protein (g/dL)	4.6±0.11	6.3±0.2ˆˆ	7.86±0.14ˆˆˆ
Albumin (g/dL)	1.66±0.12	1.1 ±0.05	0.7±0.1
Globulin (g/dL)	2.9±0.08	5.2±0.2 **	7.16±0.03***
A/G ratio	0.57±0.2	0.19±0.003	0.08±0.008
Lysozyme (ug/mL)	111.1±0.3	121.2±0.4˜˜˜	132.7±1.1˜˜˜

Total protein: ˆˆ very significant (p=0.004) - ˆˆˆ extremely significant (p=0.0001).
Globulin: * very significant (p=0.001) -*** extremely significant (p=0.0001).
Lysozyme: ˜˜˜ extremely significant (p=0.0001).

For plasma cortisol measurement, the fish were anaesthetized using tricaine methane sulfonate (MS222; Sigma-Aldrich Chemical Co. Egypt), then, the blood samples were collected from caudal vein of each fish. The collected blood sample was placed into cooled plastic tubes containing 3 mg Na2EDTA, mixed and centrifuged at 3000 rpm; 4 ºC for 5 min. The collected plasma was stored at -20 ºC for further analyses.

Moreover, cortisol levels in the collected plasma and water samples were estimated by Cortisol ELISA kit® (Calbiotech, catalog No. CO103S, Canada) following the manufacture instructions. Results calculation was carried out by automatic ELISA reader (SUNRISE®; Tecan, Austria) according to Schlaghecke et al. (1992).

Statistical Analyses

Statistical analyses were done using all data one way ANOVA (post hoc test; Dunnetstest) Advanced Models 16.0 software (SPSS, Tokyo, Japan). P<0.05 was considered as statistically significant.

Ethics

The present experiment was approved by the BSU-IACUC (Beni-Suef Institutional Animal Care and Use Committee).

RESULTS AND DISCUSSION

Growth Performance

Significant differences were observed in final weight in groups fed MOLs and its aqueous extract (P=0.02 & 0.002 respectively) when comparing with control group (Table 2). Whereas, the total initial weight of fish group fed control diet (d1), *M. oleifera* leaves (d2) and *M. oleifera* aqueous (d3) was 139.99 ±3.87, 143.5±2.36 and 144.09±2.65 respectively, while, the total final weight of fish group fed control diet (d1), *M. oleifera* leaves (d2) and *M. oleifera* aqueous (d3) was 211.33±4.7, 233.5±4.7and 248.96±2.3 respectively. Additionally, the SGR was 0.686, 0.811 and 0.911 in fish group fed control diet (d1), *M. oleifera* leaves (d2) and *M. oleifera* aqueous (d3) respectively.

Effect of MOLs and Their Aqueous Extract on Biochemical Parameters of *C. carpio* Fingerlings

Values of total serum protein, albumin, globulin and albumin/globulin ratio are shown in Table 3. Total serum protein and globulin levels were significantly increased in group of fish fed *M. oleifera* leaves (d2), (p=0.001& 0.001 respectively) and their values were 6.3±0.2 and 5.2±0.2 respectively, while, they were extremely significant (p=0.0001) in group fed *M. oleifera* aqueous (d3) with values of 7.86±0.14 and 7.16±0.03 respectively. Addi-

Table 4. Effect of dietary MOLs and their aqueous extract on water and plasma cortisol level of of C. carpio fingerlings in response to confinement stress

Cortisol (ng/mL)	Diet 1 (control)	Diet 2 (*Moringa oleifera* leaves)	Diet 3 (aqueous extract)
Water	24.24±2.7	10.26±2.2*	12.36±0.39*
Plasma	431.2±22.3	288 ±25.8*	229.7±34.9*

*Significant at $p<0.05$

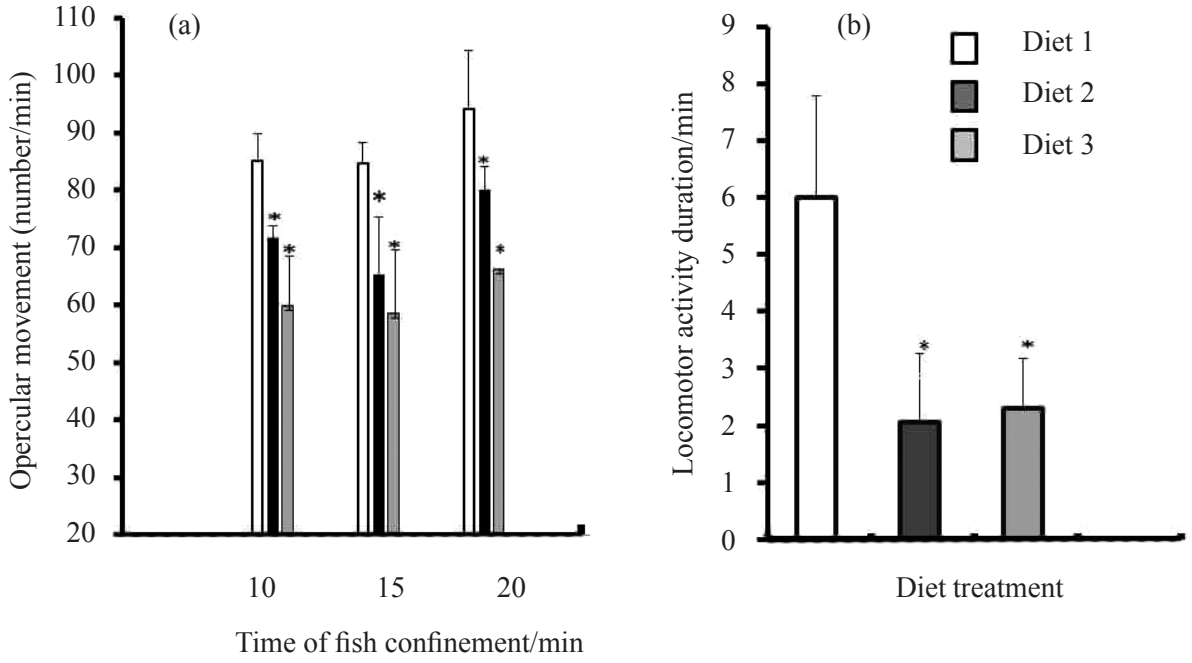

Figure 2. a, b. Effect of MOLs and their aqueous extract on (a) opercular movement (number/min) and (b) locomotor activity duration/min of *C. carpio* fingerlings in response to confinement stress. Diet 1 (control), diet 2(10 mg of MOLs/kg feed and diet 3 (20 mL of MOLs aqueous extract/Kg feed. Data described as means±SE, n=6, at $p<0.05$.

tionally, there was significant increase (p=0.0001) of lysozyme in all experimental groups compared to control group (d1), (Table 3).

Mitigation of Stress by Supplementation of MOLs and Their Aqueous Extract in the Diet of *C. carpio* Fingerlings

Water and plasma cortisol level

The results of cortisol in plasma and water as an indicator for stress response to confinement showed that there was a significant reduction in cortisol level of group fed *M. oleifera* feed (d2), (in water; p=0.001 and in plasma; p=0.007) and *M. oleifera* aqueous (d3), (in water; p=0.001 and in plasma; p= 0.039) in comparison with control group (d1), (Table 4).

Behavioral response to stress

Stress confinement posed less effect on fish fed *M. oleifera* feed (d2) and *M. oleifera* aqueous (d3) compared to control group (d1) (Figure 2). Figure 2a showed that opercula movement number/min was significantly low in d2 (at 10th min; p=0.001, at 15th min; p=0.001 and at 20th min; p=0.001) and d3 (at 10th min; p=0.001, at 15th min; p=0.003 and at 20th min; p=0,037) than d1 fed fish. Similarly, fish fed d2 (p=0.001) and d3 (p=0.001) spent significant shorter activity duration/min than that of d1 (Figure 2b).

In this study, dietary incorporation of MOLs and their aqueous extract increased final weight and SGR of *C. carpio* fingerlings when fed the experimental diets for a period of 60 days. These results are in agreement with those of Makkar and Becker, (1996) and Soliva et al., (2005). They reported that MOLs are of high protein supplement for ruminants which is potentially available for digestion due to a high proportion of pepsin soluble nitrogen (82-91%) and low proportion (1-2%) of acid detergent insoluble protein. Moreover, Abou-Elezz et al., (2012) and Yuangsoi and Masumoto

(2012) proved that replacement of soybean meal protein by MOLs meal in carps led to increase protein digestibility, fish growth and feed conversion ratio with no harmful effects on fish health. In this study, MOLs aqueous extract induced higher growth rate than MOLs, as the aqueous form reduced the anti-nutritional factors particularly saponins and tannins by 93% and 100% respectively (Makkar and Becker, 1999).

These findings indicated that both MOLs and their aqueous extract increased serum total protein, globulin and lysozyme of *C. carpio* fingerlings, with a more prominent effect of aqueous extract. There is a dearth of information on the use of MOLs and their aqueous extract as immune stimulant or their effect on serum biochemical parameters in fish. These findings are in agreement with Soumitra et al., (2004) but disagreed with Emmanuel et al., (2014) who proved that using of MOLs in rabbit ration giving normal values of total protein and globulin, which might be due to different species and doses.

Data revealed that MOLs and their aqueous extract supplementation in diet of *C. carpio* fingerlings presented a clear suppression in water and plasma cortisol elevation after exposing to confinement. Similarly, Prabsattroo et al., (2015) recorded that MOLs extract decreased plasma cortisol level in rats.

These results indicated that measuring cortisol level in the water may be a useful tool in estimating the stress of fish under confinement conditions without disturbing fish for blood sampling. Ruane and Komen (2003) supported these findings.

Data demonstrated the decrease in locomotor activity and opercular movement of fish fed diets containing MOLs in response to confinement stress. Guhal, (2004) reported that MOLs extract decreased motor activity of rats. The observed behavioral alteration was accompanied with a prominent decrease in water and plasma cortisol level. Similar correlation between cortisol level and fish behavior was reported by Øverli et al., (1999). In addition, Øverli et al., (2002) recorded that cortisol has time- and context-dependent effects on behavior in teleost fish. Hence, cortisol may be not the only regulator to behavioral response of fish to stress.

MOLs were reported to have multi-target sites (Sutalangka et al., 2013). MOLs have antioxidant (Sreelatha and Padma, 2009), vasodilation (Dangi et al., 2002) and monoamine modulation effects such as dopamine, norepinephrine and serotonin (Ganguly and Guha, 2008). In early reports, reduction of serotonin was found to inhibit neuroendocrine and behavioral stress-responses in fish (Winberg et al., 1997). In addition, Das and Guha, (2007) assumed that locomotor behavior of rats may be increased by high level of serotonin. Therefore, we hypothesize that MOLs decreased behavior and cortisol level of *C. carpio* in response to confinement stress mediated by multi-target sites including modulation of neurotransmitters function. Further studies are required for correlation of effect of MOLs on brain monoamines, cortisol and behavior of fish.

The deleterious effects of confinement stress are known to affect many aspects of the fish's physiology including immune competence and growth rates (Barton 1997). Thus, mitigation effect of this stress using MOLs and its aqueous form is very important in the field of aquaculture for improving fish immunity and increase resistance of fish to adverse environmental conditions and subsequently preventing diseases initiation.

CONCLUSIONS

The incorporation of MOLs and their aqueous extract in the diet of *C. carpio* fingerlings for 60 days may be useful for improving growth, immunity and stress effect mitigation in aquaculture. In addition, MOLs aqueous extract induced more marked effect on fish performance and stress resistance than MOLs.

Acknowledgements

We would like to thank manager of Abo-Saleh fish hatchery, Beni-Suef, Egypt for providing fish samples.

REFERENCES

Abou-Elezz, F.M.K., Sarmiento-Franco, L., Santos-Ricalde, R., Solorio-Sanchez, J. (2012). Apparent digestibility of Rhode Island Red hen diets containing *Leucaena leucocephala* and *Moringa oleifera* leaf meals. *Tropical and Subtropical Agroecosystem*s, 15, 199-206.

Barton, B.C. (2002). Stress in fishes: responses with particular reference to changes in circulating corticosteroids. *Integrative and Comparative Biology*, 42(3), 517-525.

Barton, B.A., (1997). Stress in finfish: past, present and future - a historical perspective. In: Iwama, G.K., Pickering, A.D., Sumpter, J.P.,

Schreck, C.B. (Eds.), Fish Stress and Health in Aquaculture. Society for Experimental Biology Seminar Series, vol. 62. Cambridge University Press, Cambridge, U.K., pp. 1-33.

Bradford, M.M. (1976). A rapid and sensitive method for the quantification of microgram, quantities of protein. *Analytic Biochemistry Journal*, 72(2), 248.

Carrasco, G.A., & Van de Kar, L.D. (2003). Neuroendocrine pharmacology of stress. *European Journal of Pharmacology*, 463(3), 235-272.

Dangi, S.Y., Jolly, C.I., Narayanan, S. (2002). Antihypertensive activity of the total alkaloids from the leaves of *Moringa oleifera*. *Pharmaceutical Biology*, 40(2), 144-148.

Das, S., & Guha D. (2008). CNS depressive role of aqueous extract of *Spinacia oleracea* L. leaves in adult male albino rats. *Indian Journal of Experimental Biology*, 46 (3), 185-190.

Doumas, B.T., Watson, W.A., Biggs, H.G. (1971). Albumin standards and the measurement of serum albumin with bromocersol green. *Clinical Chemistry*, 31(1), 87-96.

Ellis, A. E. (1990). Lysozyme Assays. In: Techniques in Fish Immunology, Stolen, J. S., T. C. Fletcher, D. P. Anderson and W. B. van Muiswinke l (Eds.). 1, SOS Publications, New Jersey, USA, pp. 101-103.

Emmanuel, O.E., Olujide, A.S., Kafayat, M. S., Oluwaseyi, M. O., Temi-tope, T. L. (2014). Haematological and serum biochemical responses of rabbit does to crude *Moringa oleifera* leaf extract at gestation and lactation. *Tropical Animal Health and Production*, 47(4), 637-642.

Fatope, M. O., Ibrahim, H., Takeda, Y. (1993). Screening of higher plants reputed as pesticides using the Brine Shrimp Lethality Assay. *Pharmaceutical Biology*, 31, 240-254.

Ganguly, R., & Guha, D. (2008). Alteration of brain monoamines & EEG wave pattern in rat model of Alzheimer's disease and protection by Moringaoleifera. *Indian Journal of Medical Research,* 128(6), 744-751.

Guhal, D. 2004. Role of 5-hydroxytryptamine in Moringaoleifera induced potentiation of pentobarbitone hypnosis in albino rats. *Indian Journal of Experimental Biology*, 42, 632-635.

Huntingford, F.A., Adams, C., Braithwaite, V.A., Kadri, S., Pottinger, T.G., Sandøe, P., Turnbull, J.F. (2006). Current issues in fish welfare. *Journal of Fish Biology*, 68(1), 332-372.

Kanazawa, A., (1997). Effects of docosahexaenoic acid and phospholipids on stress tolerance of fish. *Aquaculture*, 155(1), 129-134.

Kittilsen, S., Ellis, T., Schjolden, J., Braastad, B. O. Øverli, Ø. (2009). Determining stress-responsiveness in family groups of Atlantic salmon (Salmosalar) using non-invasive measures. *Aquaculture*, 298(2),146-152.

Koolhaas, J.M., Korte, S.M., De Boer, S.F., Van Der Vegt, B.J., Van Reenen, C.G., Hopster, H., Blokhuis, H. J. (1999). Coping styles in animals: current status in behavior and stress-physiology. *Neuroscience and Biobehavioral Reviews*, 23(7), 925-935.

Makkar, H.P.S., & Becker, K. (1996). Nutritional value and antinutritional components of whole and ethanol extracted *Moringa oleifera* leaves. *Animal Feed Science and Technology*, 63(4), 211-228.

Makkar, H.P.S., Becker, K. (1999). Plant toxins and detoxification methods to improve feed quality of tropical seeds. Review. *Asian -Australian Journal Animal Science*, 12(3), 467-480.

Martins, C.I.M. , Leonor, G., Chris, N., Børge, D., Maria, T.S., Walter Z., Marilyn, B., Ewa, K., Jean-Charles, M., Toby, Carter., Sònia, R.P., Tore, K. (2011). Behavioural indicators of welfare in farmed fish. F*ish Physiolology and Biochemistry*, 38, 17-41.

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(3), 211-268.

Radya, M. M., Varma, C.B., Howladar, M. S. (2013). Common bean *(Phaseolus vulgaris* L.) seedlings overcome NaCl stress as a result of presoaking in *Moringa oleifera* leaf extract. Scientia Horticulturae, 162, 63-70.

Øverli, Ø., Pottinger, T.G., Carrick , T.R., Øverli, E., Winberg, S. (2002). Differences in behavior between rainbow trout selected for high- and low-stress responsiveness. *Journal of Experimental Biology*, 205, 391-395.

Øverli, Ø., Olsen, R.E., Løvik, F., Ringø, E. (1999). Dominance hierarchies in Arctic charr, Salvelinusalpinus L.: differential cortisol profiles of dominant and subordinate individuals after handling stress. *Aquaculture Research,* 30(4), 259-264.

Prabsattroo, T., Jintanaporn, W., Sitthichai, I., Pichet, S., Opass, S., Wipawee, T., Supaporn, M. (2015). *Moringa oleifera* extract enhances sexual performance in stressed rats. J*ournal of Zhejiang University Science B,* 16(1), 179-190.

Quesada, S.P., Paschoal, J.A.R., Reyes, F.G.R., (2013). Considerations on the aquaculture development and on the use of veterinary drugs: special issue for fluoroquinolones-a review. *Food Science,* 78(9), 1321-1333.

Ramachandran, C., Nivatha, S., Lavanya, K., Usha, A. (2014). *Moringa oleifera*: a plant with multiple medicinal uses and food preservative. *International Journal of Food and Nutritional Sciences,* 3, 69-72.

Ruane, N.M., & Komen, H. (2003). Measuring cortisol in the water as an indicator of stress caused by increasing loading density in common carp (*Cyprinus carpio*). *Aquaculture,* 218(4), 685-693.

Reverter, M., Bontemps, N., Lecchini, D., Banaigs, B., Sasal, P. (2014). Use of plant extracts in fish aquaculture as an alternative to chemotherapy: current status and futureperspectives. *Aquaculture*, 433, 50-61.

Ricker, W.E. (1979). Growth rate and models. In: Fish Physiology, Hore, W.S. and Brett, P.J. (Eds.), Academic Press, New York, USA; 677-743.

Schlaghecke, R., Kornely, E., Reinhard, T.h. S., Ridderskamp, P. (1992). The Effect of Long-Term Glucocorticoid Therapy on Pituitary–Adrenal Responses to Exogenous Corticotropin-Releasing Hormone. *New England Journal of Medicine*, 326, 30.

Scott, A.P., & Sorensen, P.W., (1994). Time course of release of pheromonally active gonadal steroids and their conjugates by ovulatory gold fish. *General and Comparative Endocrinology*, 96(2), 309-323.

Scott, A.P., Pinillos, M., Ellis, T., (2001). Why measure steroids in fish plasma when you can measure them in water. In: Goos, H.J.T., Rastogi, R.K., Vaudry, H., Pierantoni, R. (Eds.), Perspective in Comparative Endocrinology: Unity and Diversity. Monduzzi Editore, Bologna. pp. 1291-1295.

Soliva, C.R., Kreuzera, M., Foidlb, N., Foidlb, G., Machm¨ullera, A., Hessa, H.D. (2005). Feeding value of whole and extracted *Moringa oleifera* leaves for ruminants and their effects on ruminal fermentation in vitro. *Animal Feed Science Technology*, 118(2), 47-62.

Soumitra, M., Indranil C., Malay P., Dilip, R., Syed, S.I. (2004). Structural studies of an immuno-enhancing polysaccharide isolated from mature pods (fruits) of *Moringa oleifera* (sajina). *Medicinal Chemistry Research*, 13(6), 390-400.

Sreelatha, S., & Padma, P.R., (2009). Antioxidant activity and total phenolic content of Moringa oleifera leaves in two stages of maturity. *Plant Foods and Human Nutrition*, 64(4), 303-311.

Sutalangka, C., Wattanathorn, L., Muchimapura, S., Thukham-mee, W. (2013). *Moringa oleifera* mitigates memory impairment and neurodegeneration in animal model of age-related dementia. *Oxidative Medicine and Cellular Longevity*. 1-9.

Ursin, H., Eriksen, H. R. (2004). The Cognitive Activation Theory of Stress. Psychoneuroendocrinology, 29, 567-92.

Wendelaar Bonga, S.E. (1997). The Stress Response in Fish. *Physiological Reviews,* 77(3), 592-625.

Winberg, S., Nilsson, A., Hylland, P., Söderstöm, V., Nilsson, G.E. (1997). Serotonin as a regulator of hypothalamic-pituitary-interrenal activity in teleost fish. *Neuroscience letters*, 230(2), 113-116.

Yuangsoi, B., & Masumoto, T. (2012). Replacing moringa leaf (*Moringa oleifera*) partially by protein replacement in soybean meal of fancy carp (*Cyprinus carpio*). S*ongklanakarin Journal of Science and Technology*, 34(5), 479-485.