Assessment of *Withania Somnifera* Suplemented Diet on Various Parameters of *Oreochromis Mossambicus*

DR. JASMINE ANAND
Assistant Professor and Head o f the Department of Zoology, Sree Narayana College, Punalur, Kollam, Kerala. 691305.

ABSTRACT

KEY WORDS: *Oreochromis mossambicus*, *Withania somnifera*, Growth promotion, immunomodulation

Aquaculture is an excellent, explorable avenue for a state like Kerala in India. Unplanned growth of aquaculture farms in developing countries created unsustainable environmental problems. Consequently, the emphasis of aquaculture has shifted from maximizing production to sustainable production with minimum damage to environment. *Oreochromis mossambicus* is an economically important species, due to its sturdy, fast growth, attractive size and better meat quality, this species is gaining prominence in culture fisheries. The roots of *Withania somnifera* are used to prepare medicinal Ashwagandha. Ayurvedic system of medicine recommends it for a number of human health problems. The main constituents of Ashwagandha are alkaloids and steroidal lactones, withaferine being the important alkaloid and withanolids, being the major steroidal lactones. .

The present study investigates the growth promotion and immunomodulatory potentials of *Withania somnifera* supplemented diet on *Oreochromis mossambicus*, Fish belonging to the size group 6.2 $\pm$ 0.01 cm length and 3.4 $\pm$ 0.05 gm weight were used for the experiment. Medicated feeds were prepared at 0.5% concentration. The fishes were fed twice daily at 10% body weight. The culture continued for six months. On every second and fourth week fishes were terminated for various assays such as growth parameters, biochemical, hematological and microbiological parameters. The findings are reported in the paper.

INTRODUCTION

Economic viability demands “Intensive Aquaculture systems,” which are grazing grounds for pathogenic bacteria. These susceptible- to- disease out breaks systems call for unprecedented disease management strategies. Upgrading the non-specific immune response of aquaculture candidates as fish, has become an active area of research (Awad E and Awaad A. 2017)

Kerala is bestowed with rich and variegated floristic and faunistic wealth. Ayurveda offers a hoard of immunostimulants for the well being of mankind. Fishes have an immunological set up as delicate as that of human beings. It relies heavily on non-specific immune response compared to higher vertebrates. Unlike vaccines which are pathogen specific , immune-stimulants are effective against a broad as spectrum of pathogens, The use of phytochemical as immunomodulators is still in its infancy. Much work is done in these areas with mammalian, avian, piscine and crustacean models.

The present study investigated the growth promotion and immunomodulatory potential of *Withania somnifera* on *Oreochromis mossambicus*, which were used as surrogates to large Ornamental Cichlids.

MATERIALS AND METHODS

Oreochromis mossambicus belonging to the size group 6.2 ± 0.01 cm length and 3.4 ± 0.05 gms weight were collected from M/s.Pampa Hatchery Allapuzha and transported in hyperoxygented polythene bags to our laboratory. 15 fishes were kept in each tank. The water was well aerated using standard aerators and the temperature ranged at 28.0 ± 2.11°C. Medicated feeds were prepared at 0.5% concentration. These medicated feeds were allowed to dry well in an oven at 40°C overnight then brought to room temperature 24 hours before starting the experiments. Various parameters studied include – Tissue protein (Lowry *et. al* 1951), Tissue glycogen (Sifter *et.al* (1950), Total blood Count (conventional haemocytometer method) Total Erythrocyte count and Total Leucocyte count was calculated., Haemoglobin content (Sahli's acid haematin method), ESR (Westergrons method). Serum protein (Tietz 1996), Serum glucose (Bergmayer, 1974), Serum IgM Kyle and Treib (1978) method, Dissolved oxygen (Winklers method), Total bacterial load (Kannan *et.al* 2004).

All terminations were done in duplicates (n=5) and results expressed as mean standard ± standard deviation (S.D). Dietary (test) treatment differences were calculated at specific time points using student's t-test. Differences were considered significant at p-values ≤ 0.05 .

RESULT

On administering *Withania somnifera* the growth in terms of size (cms) attained was 8.14±0.12 to 17.14±0.12 in the control and from 8.21±0.67 to 17.96±0.14 in the treated and weight gain (gms) ranged from 7.62±0.77 to 20.11±0.10 in control and from 7.01 0.11 to 19.31±0.67 in the treatments. The respiratory performance was evaluated in terms of oxygen consumed per hour per gram weight. The value in control was 0.21±0.02 to 0.72±0.13 and in treated fishes it was from 0.24±0.16 to 0.913±.0.16. The weight gain was of no significance. The size attained was marginal.

Total protein (mg%) ranged from 5.861 ±0.01 to 28.114±0.12 in the control muscle tissue and the same is treated fishes was 6.131±0.12 to 31.61±0.91. The total liver protein (mg%) in the control fishes was from 2.111±0.13 to 10.614 ± 0.71 and the same for treated ones were from 4.62±0.13 to 16.31±.71. The head kidney protein (mg%) ranged from 2.121±1.14 to 14.111±1.72 in control and from 6.11 ±0.13 to 18.81±0.61 in the treated fishes. The head kidney protein content was significantly elevated ($p \leq 0.05$) when compared to control values.

Total glycogen content (mg%) varied from 0.114 ±0.112 to 0.668±0.41 in control and from 0.121±0.13 to 0.791±0.12 in the treated fishes. The same for liver tissue ranged from 0.211 ±0.001 and 0.899±0.117 in control and from 0.211±0.11 to 0.912±0.16 in the treated. The total glycogen (mg%) in head kidney varied from 0.112±0.007 to 0.611±0.312 in the control and from 0.122 ±0.16 to 0.89±0.16 in the treated ones. The muscle glycogen did not show any variation but the liver glycogen was significantly elevated ($p \leq 0.05$) compared to controls

The bacterial load on the intestinal tissue (CFU / gram tissue) varied from $68x10^5$ to $96x10^5$ in the control and from $58x10^5$ to $31x10^5$ in the treated ones. The same for skin surface was $71x10^5$ to $94x10^5$ in the control and from

$70x10^5$ to $90 x10^5$ in the treated fishes. There was significant effect on the bacterial load at either surface when compared to the control

The TEC (cells/ mm^3) ranged from 7,40,100 to 7,40,200 in the controls and from 741000 to 790000 in the treatments. The TLC (cells. mm^3) ranged from 4900 to 5000 in the controls and from 4800 to 6200 in the treated ones. The haemoglobin content (g/dl) ranged from 3.01 to 3.67 in the controls and from 4.12 to 5.92 to the treated fishes. The ESR (mm/ hr) varied from 5.0 to 5.2 in controls and from 5.1 to 5.2 in the treated ones. The serum proteins (mg%) varied from 12.81 to 18.41 in controls and from 12.96 to 14.61 in the treatments. The serum glucose (mg%) varied from 131.0 to 149.2 in the controls and from 180.0 to 190.3 in the treatments. The serum IgM (mg/ dl) varied from 0.38 to 01.41 in the controls and from 0.62 to 0.91 in the treatments. The serum protein, glucose and IgM valued showed significant increase ($p \leq 0.05$) compared to control values. The ESR did not show significant variation.

TABLE-1 TOTAL PROTEIN CONTENT OF VARIOUS TISSUES OF *O. MOSSAMBICUS* SUPPLEMENTED WITH *WITHANIA SOMNIFERA*

Months	Muscle (mg %)		Liver (mg%)		Head Kidney (mg%)	
	2 weeks	4 weeks	2 weeks	4 weeks	2 weeks	4 weeks
July						
Control	5.861±0.01	6.130±0.12	2.111±0.13	4.492±0.31	2.121±1.14	2.023±0.01
Expt	6.131±0.12	8.161±0.13	4.62±0.13	9.13±0.16	6.11±0.13	6.96±0.21
Aug.						
Control	7.201±0.161	9.291±0.15	4.914±0.11	5.121±0.14	3.14±1.12	6.771±1.21
Expt	14.112±0.13	14.613±0.14	10.1±0.13	12.18±0.16	10.11±0.71	10.96±0.19
Sept.						
Control	10.211±0.14	11.140±0.13	5.414±0.99	6.117±0.13	6.811±1.12	7.941±1.21
Expt	16.142±0.13	16.914±0.96	12.41±0.16	13.14±0.14	14.11±0.71	17.97±0.91
Oct.						
Control	14.670±0.14	18.111±0.24	7.116±0.91	7.414±0.84	9.114±1.61	10.191±2.34
Expt	17.14±0.61	18.91±0.62	13.41±0.16	14.19±0.11	15.67±0.11	16.67±0.97
Nov						
Control	20.177±0.14	23.011±0.71	9.161±0.97	10.011±0.77	10.944±1.12	12.111±2.01
Expt	21.61±0.31	26.12±0.13	15.14±0.77	16.11±0.11	17.14±0.11	17.97±0.91
Dec						
Control	24.41±0.99	28.221±0.98	10.411±0.81	10.614±0.71	13.771±1.16	14.111±1.72
Expt	28.67±0.14	31.61±0.91	16.31±0.71	16.91±0.16	18.11±0.71	18.81±0.61

FIGURE:1 VARIATIONS AT FOURTH WEEK IN THE PROTEIN CONTENTAT VARIOUS TISSUES OF *O. MOSSAMBICUS* SUPPLEMENTED WITH *WITHANIA SOMNIFERA*

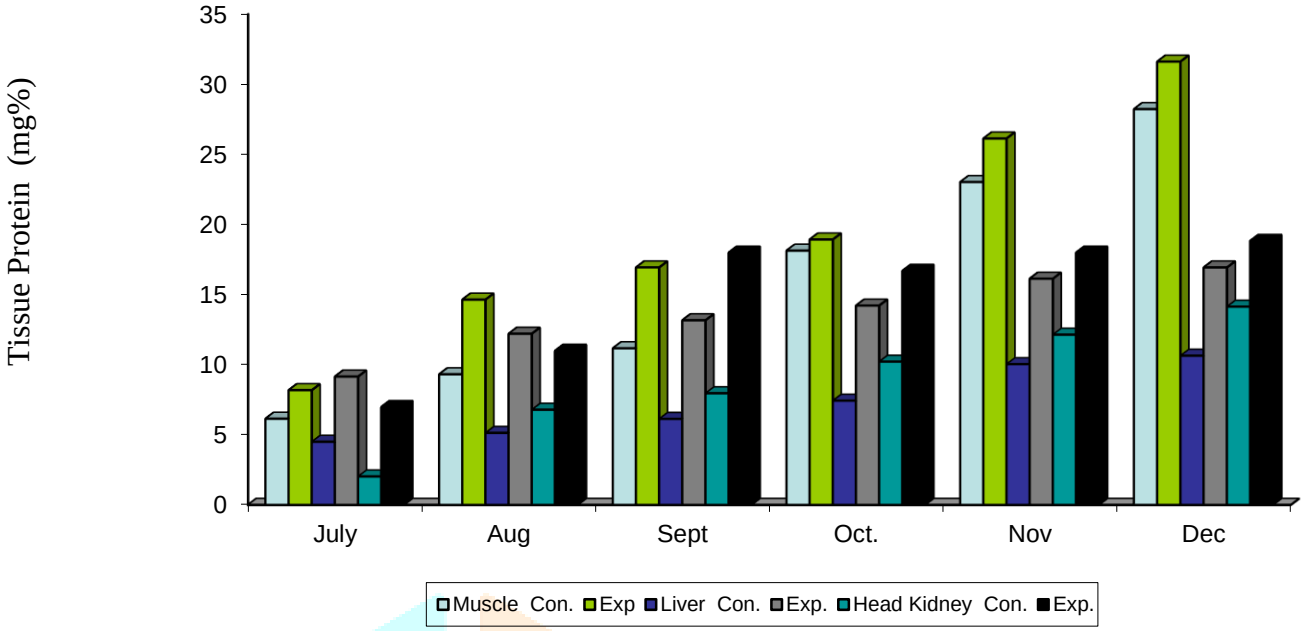


TABLE: 2 TOTAL GLYCOGEN CONTENT OF VARIOUS TISSUES OF *O. MOSSAMBICUS* SUPPLEMENTED WITH *WITHANIA SOMNIFERA*

Months	Muscle (mg %)		Liver (mg%)		Head Kidney (mg%)	
	2 weeks	4 weeks	2 weeks	4 weeks	2 weeks	4 weeks
July						
Control	0.114±0.112	0.176±0.002	0.211±0.001	0.246±0.002	0.112±0.007	0.136±0.001
Expt	0.121±0.13	0.161±0.22	0.211±0.11	0.241±0.77	0.122±0.16	0.128±0.16
Aug.						
Control	0.211±0.160	0.261±0.140	0.386±0.770	0.342±0.910	0.194±0.314	0.184±0.613
Expt	0.211±0.11	0.251±0.12	0.314±0.16	0.341±0.19	0.210±0.16	0.229±0.96
Sept.						
Control	0.341±0.110	0.399±0.112	0.512±0.771	0.569±0.270	0.199±0.410	0.214±0.113
Expt	0.391±0.11	0.411±0.77	0.416±0.11	0.491±0.16	0.311±0.16	0.314±0.11
Oct.						
Control	0.481±0.771	0.441±0.991	0.686±0.441	0.614±0.119	0.440±.0.110	0.416±0.912
Expt	0.512±0.91	0.611±0.17	0.542±0.11	0.591±0.17	0.461±0.19	0.496±0.12
Nov						
Control	0.516±0.710	0.621±0.311	0.711±0.810	0.762±0.141	0.526±0.124	0.596±0.112
Expt	0.691±0.11	0.712±0.77	0.+622±0.71	0.691±0.91	0.522±0.11	0.621±0.13
Dec.						
Control	0.691±0.311	0.668±0.44	0.811±0.002	0.899±0.117	0.631±0.112	0.611±0.312
Expt	0.771±0.11	0.791±0.12	0.871±0.21	0.912±0.16	0.714±0.12	0.890+0.16

FIGURE:2 VARIATIONS AT FOURTH WEEK IN THE GLYCOGEN CONTENT AT VARIOUS TISSUES OF *O. MOSSAMBICUS* SUPPLEMENTED WITH *WITHANIA SOMNIFERA*

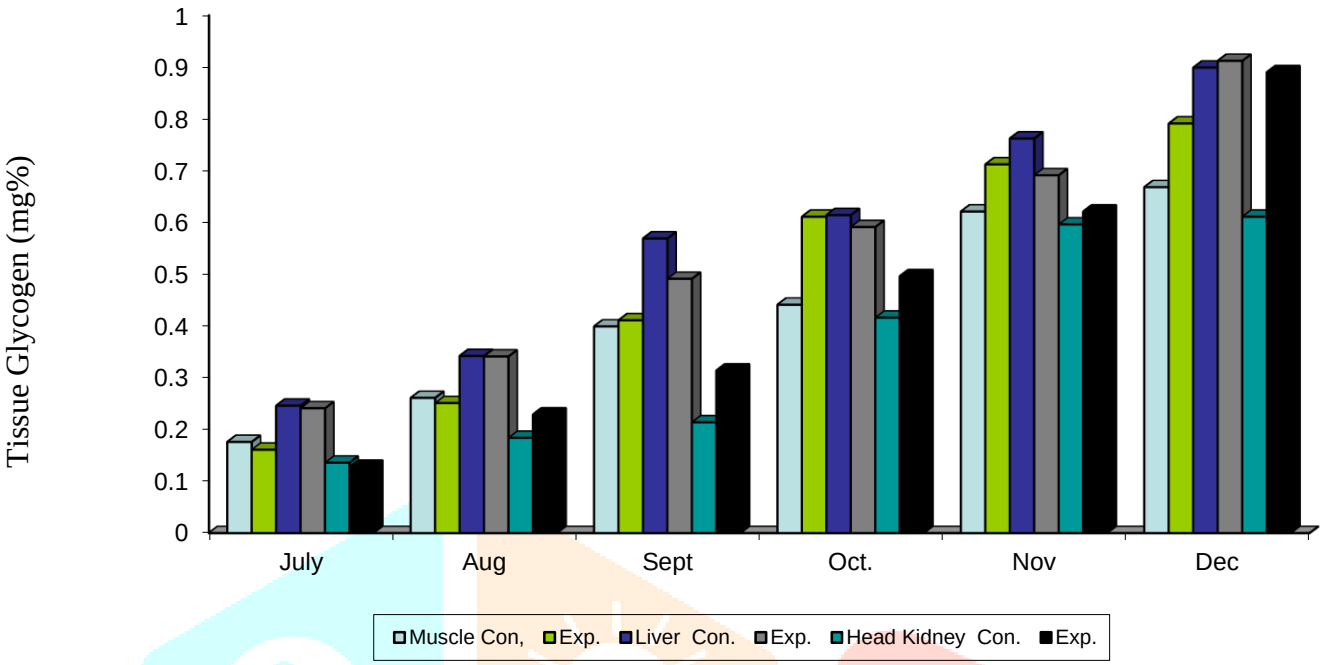


TABLE: 3 GROWTH AND RESPIRATORY PERFORMANCE OF *O. MOSSAMBICUS* SUPPLEMENTED WITH *WITHANIA SOMNIFERA*

Months	Length (cms)		Weight (gms)		0_2 Consumed / hr/ gm.wt	
	2 weeks	4 weeks	2 weeks	4 weeks	2 weeks	4 weeks
July						
Control	8.14±0.12	10.24±0.11	7.62±0.77	9.50±0.21	0.21±0.02	0.31±0.11
Expt	8.21±0.67	10.31±0.61	7.01±0.11	9.61±0.21	0.24±0.16	0.31±0.11
Aug.						
Control	11.37±0.76	11.94±0.67	9.62±0.31	9.94±0.73	0.36±0.01	0.39±0.12
Expt	11.46±0.76	11.98±0.16	9.12±0.11	10.16±0.17	0.39±0.12	0.41±0.71
Sept.						
Control	12.31±0.12	13.16±0.91	9.96±0.92	10.11±0.01	0.42±0.13	0.46±0.91
Expt	12.11±0.17	13.24±0.19	11.16±0.13	12.11±0.61	0.45±0.61	0.52±0.71
Oct.						
Control	13.94±0.91	14.16±0.19	12.69±1.13	14.192±0.16	0.49±0.21	0.52±0.61
Expt	14.62±0.71	15.14±0.11	12.34±0.16	12.96±0.11	0.621±0.11	0.726.±0.14
Nov						
Control	15.11±0.91	15.41±0.16	16.31±0.41	18.14±0.11	0.61±0.11	0.66±0.21
Expt	16.19±0.14	16.99±0.14	15.61±0.71	15.91±0.31	0.834±0.74	0.892±0.31
Dec.						
Control	16.21±0.14	17.14±0.12	19.14±0.11	20.11±0.10	0.69±1.2	0.72±0.13
Expt	17.14±0.74	17.96±0.14	18.82±0.11	19.31±0.67	0.896±0.12	0.913±0.16

FIGURE : 3 VARIATIONS AT FOURTH WEEK IN THE GROWTH AND RESPIRATORY PERFORMANCE OF *O. MOSSAMBICUS* SUPPLEMENTED WITH *WITHANIA SOMNIFERA*

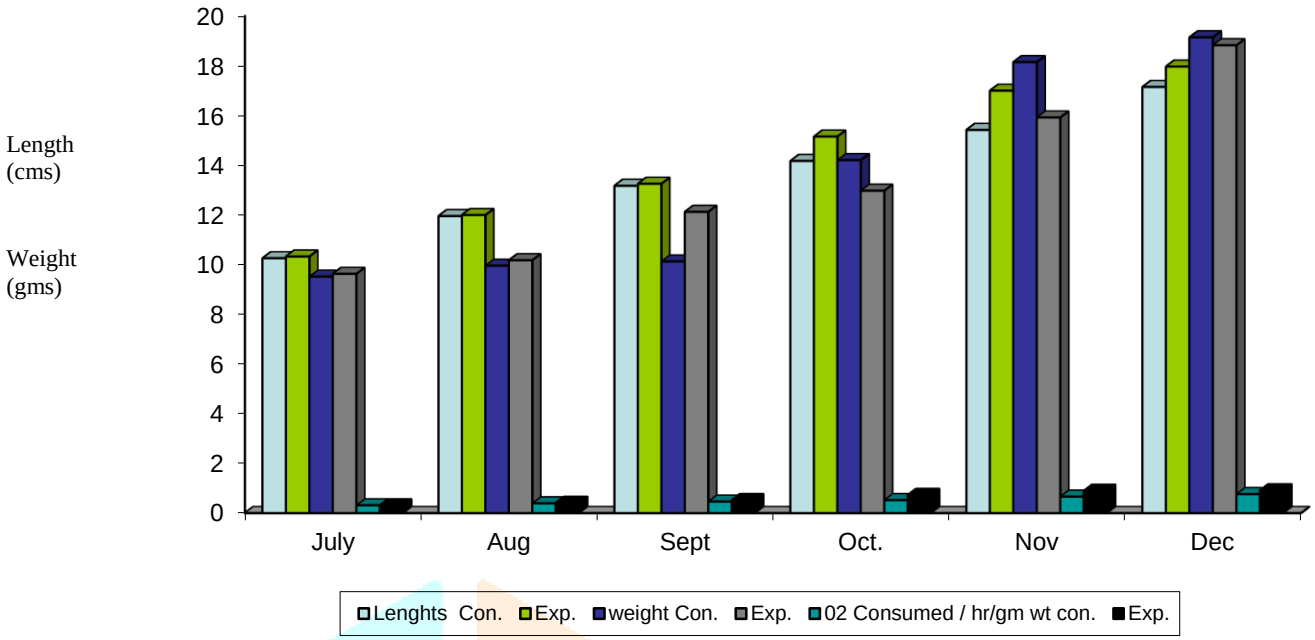
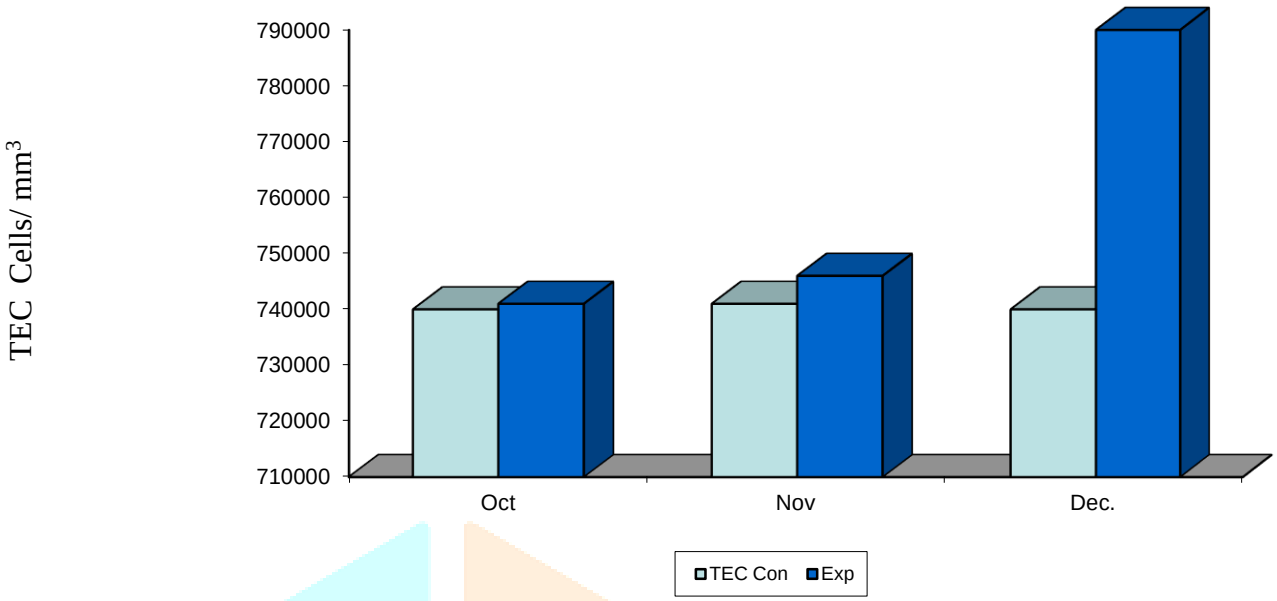


TABLE: 4 HAEMETOLOGICAL PARAMETERS OF *O. MOSSAMBICUS* SUPPLEMENTED WITH *WITHANIA SOMNIFERA*

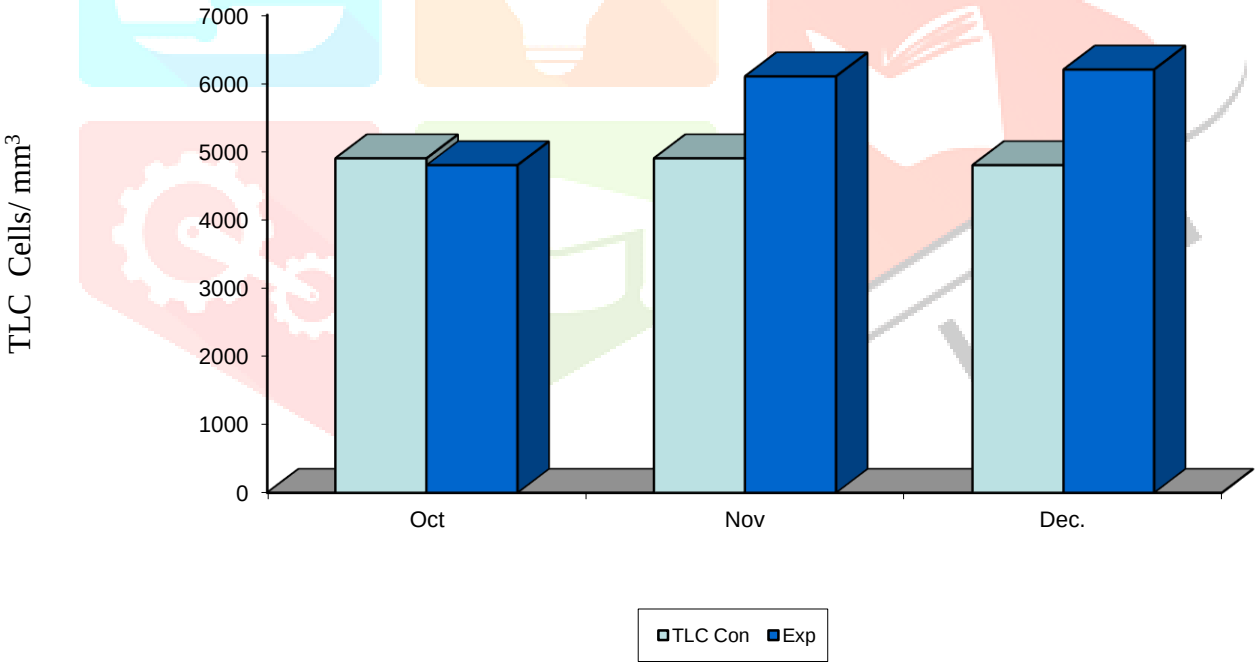
Months	TEC (cells/ mm^3)	TLC (cells/ mm^3)	Hb (g/dl)	ESR (mm/ hr)	Serum protein (mg %)	Serum Glucose (mg %)	Serum IgM (mg/ dl)
October							
Control	74000	4900	3.01	5.0	12.81	131.0	0.35
Expt	741000	4800	4.12	5.1	12.96	180.3	0.62
November							
Control	741000	4900	3.41	5.2	12.61	142.1	0.40
Expt	746000	6100	5.31	5.2	13.81	184.6	0.68
December							
Control	740000	4800	3.67	5.2	12.96	149.2	0.41
Expt	790000	6200	5.92	5.2	14.61	190.3	0.91

FIGURE: 4 VARIATIONS IN HAEMETOLOGICAL PARAMETERS OF *O. MOSSAMBICUS* SUPPLEMENTED WITH *WITHANIA SOMNIFERA*

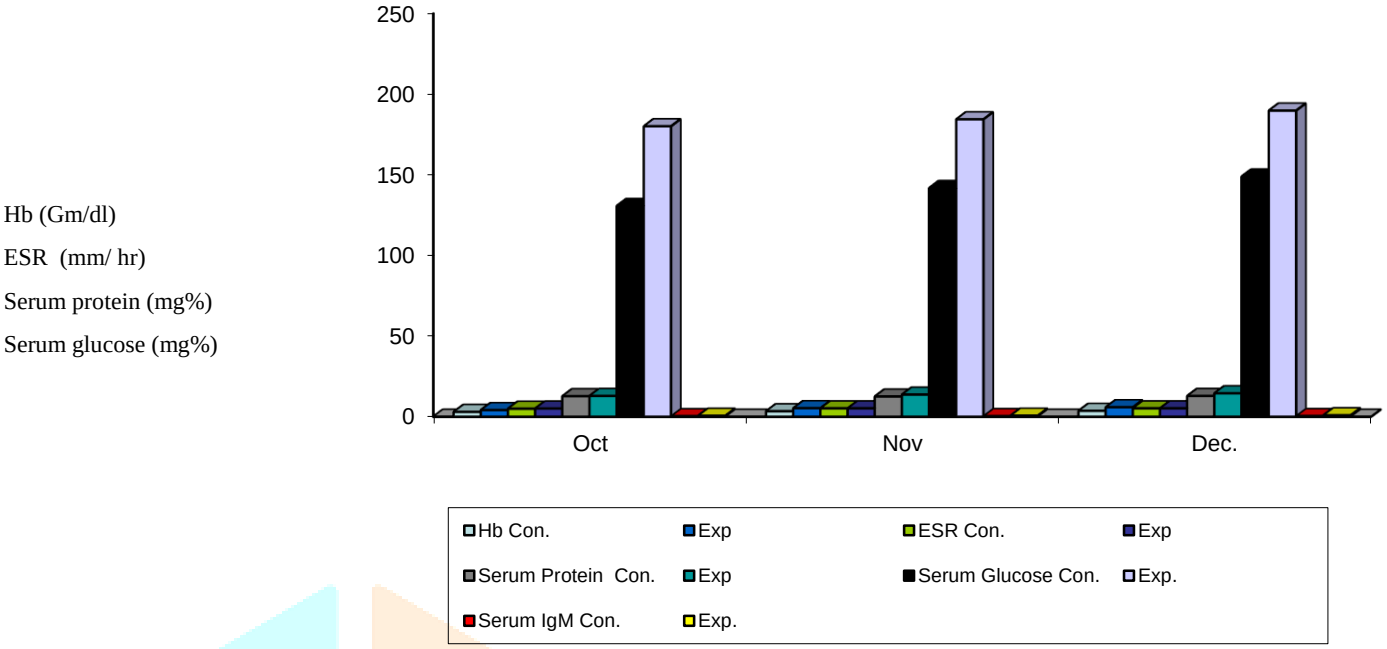
1. TOTAL ERYTHROCYTES



2. TOTAL LEUCOCYTES



3. SERUM PARAMETERS



DISCUSSION & CONCLUSION

Mukopadhya and Jena (1999) have listed out a variety of non-conventional dietary ingredients in fish feed formulation. The plant products listed expressed significant improvisation in growth and development of culture candidate. Subasinghe and Bueno (2000) have highlighted the relevance of organic farming and use of plant products in aquaculture for the third millennium. *Spirulina sp* is the much researched botanical as feed supplement for boosting non-specific immune response in mammals, birds and fishes. Cain's et al *in* 2003 reported immunomodulatory response in *O. nilotocus .L.* In vivo and in vitro immunomodulatory activities of *Trichilia glabra* aqueous leaf extract have been reported on mouse by Fabian *et. al.* (2000) . Misra *et. al.* (2006) reported the immunomodulatory effects of tulfsin on the non-specific immune system of India major carp *Labeo rohita.* Immunomodulatory potentials of *Ocimum sanctum* seed oil and its mechanism of action in rats was evaluated by Mediratta *et.al.*(2002). Pandey *et al.,(*2012) experimented immunostimulant effect of several medicinal plants on fish which is in concordance with our findings. In 2017 Awad E and Awaad A, worked on the survival, growth performance and immunological aspect of several medicinal plants on the immune status in fish.

The growth promoting and immonomodulatory efficacy of *Withania somnifera* root extracts has significantly increased when compared to the control values. From this experiment it can be clearly stated that the *Withania somnifera* is a potent immunostimulant, with a promising future in the sustainable health care management in aquaculture. For further substantiating these results, active principles need to be extracted and evaluated.

REFERENCES

1. Awad E and Awaad A. (2017) Role of medicinal plants on growth performance and immune status in fish. *Fish Shellfish Immunol*. Aug;67:40-54. doi:10.1016/j.fsi. 2017.05.034. Epub 2017 May 16.
2. Bergmayer, H.V (1974). *Methods of Enzymatic Analysis*. PP. 1196
3. Boganward, J., Anderson, A.S, Gildberg, A. (1996). The stimulatory effect of a muscle protein hydrolysate from Atlantic cod on Atlantic salmon head kidney leukocytes. *Fish and shell fish Immunology* ,6:3-16

4. Botkin, D.B., and Keller E.A.(1998). Environmental science -Earth as a living planet second edn. John Wiley and sons. The, New York.

5. Cain, K.D., Grabowski, L., Reilly, J., and Lytwyn M. (2003), Immunomodulatory effect of a bacterial derived β -1,3 glucan administered to tilapia (*Oreochromis nilotocus. L*) in a Sprirulina based diet, *Aquaculture Research.* 34, 1241-1244, Blackwell Publishing Ltd. E-mail: Kcain @uidaho.edu

6. Fabian. B., Courreges, M.C., and Coulombie, F.C, (2000). In vivo and in vitro immumodulatory activities of *Trichilia glabra* aqueous leaf extract. *Journal of Ethanopharmacology,* 69: Pg- 199-205.

7. Filotrani, L.J., (2000) "Is organic the future?".*Fish farmingi /nternational*. 27, No.ll, 36-37.

8. Lowry, O.H, Rose.B, Farr, A.L, Randall, R.J.(1981) Protein measurement with Folin phenol reagent. *J. Biol. Chem.* 193: 265-275

9. Kyle, R.A, Treib, P.R (1978) Laboratory investigation of Monoclonal Gamopathies. *Bioclinical procedures*. PP. 719-739

10. Mediratta, P.K., Sharma, K.K. and Singh, S. (2002): Evaluation of Immunomodulatory potential of *Ocimum sanctum* seed oil and its possible mechanism of action. *Journal of Ethanopharmacology*. 80 pp 15-20.

11. Misra, C.K., Das, B.K., Mukherjee, S.C., and Meher, P.K. (2006): The immunomodulatory effects of tuftsin on the non-specific immune system of Indian Major Carp *Labeo rohita*. *Fish and shell fish immunology*. 20. pg 728-738.

12. Mukopadhyay, P.K., and Jena. J.K.,(1999) "Use of non- conventional dietary ingredients in fish feed formulations". *Icthyology.*

13. Pandey Govind., Sharma Madhuri and Mandloi A.K, (2012). Immunostimulant effect of medicinal plants on fish. *International research journal of pharmacy.* vol 3 (3) Page 112. www.irjponline.com ISSN 2230 – 8407

14. Rath. S.S, Sineh, M.K. and Suryanarayan N. (2008). Food utilization efficiency. In *Antheraca mylitta* fed on *Terminalia arjuna* leaves (2008). *Int. Conf. on Biodiversity Cons. And manag*. CUSAT pg- 117.

15. Sambhu.C (2008): Role of indigenous herbs in the culture of white prawn *Fenneropeneas indicus* (M. Edwards). *Int. Conf. On Biodiversity Cons. Manag* CUSAT. Pg -94.

16. Sifter, S., Dayton, S. Naik, B., (1950). Calorimetric determination of glycogen hy Anthrone method in tissue. *Arch Biochem.* 25. PP. 191-200.

17. Singh,G.P., S.B, Zeya, N.G Ojha and N. Suryanarayan (2008), Efficacy of *Adhatoda Zeylanica* against virosis and its effect in haematological changes in Tasar Silkworm, *Antheraca mylitta D. International Conf. on Biodiversity. Coms & Manag.* CUSAT Pg- 104.

18. Subasinghe, R.P., Bueno. P.,(2000). Aquaculture in the third millennium *Tech. Proc. Of conference on Aquaculture in third millennium*. Thailand PP.239-247

19. Thomas, A.A., (1992) Phd. Thesis; Studies on influences of certain plant products with control potentials on the development and reproduction of the cotto bug, *Dydercus singulates*

20. White. K, O' Neill. B, Tzankova. Z.(2004). Will aquaculture fulfill the promise of Blue Revolution. *Seaweb aquaculture clearing report providence*, Rl: seaweb.

21. Wilier. H., Yussefi (ED). (2004). World of organic agriculture-status and emerging trends. Bonn. Germany Int. Fed. of org. Agri. movements.