# EFFECT OF USING HUMIC ACID AS A PREBIOTIC WITH DIFFERENT PROTEIN LEVELS IN FISH DIETS ON WATER QUALITY AND PRODUCTIVE PERFORMANCE OF TILAPIA AND COMMON CARP UNDER EGYPTIAN CONDITIONS

# HOSSAM AHMED MOHAMMED MOUNES

B.Sc. Agric. Sc. (Animal Production), Ain Shams University, 2000 M.Sc. Agric. Sc. (Animal Nutrition), Ain Shams University, 2006

A thesis submitted in partial fulfillment

of

the requirements for the degree of

# DOCTOR OF PHILOSOPHY

in
Agricultural Sciences
(Animal Nutrition)

Department of Animal Production Faculty of Agriculture Ain Shams University

2015

# **Approval Sheet**

# EFFECT OF USING HUMIC ACID AS A PREBIOTIC WITH DIFFERENT PROTEIN LEVELS IN FISH DIETS ON WATER QUALITY AND PRODUCTIVE PERFORMANCE OF TILAPIA AND COMMON CARP UNDER EGYPTIAN CONDITIONS

By

# HOSSAM AHMED MOHAMMED MOUNES

B.Sc. Agric. Sc. (Animal Production), Ain Shams University, 2000 M.Sc. Agric. Sc. (Animal Nutrition), Ain Shams University, 2006

This thesis for Ph.D. degree has been approved by:
Dr. Ashraf Yossef Ibrahim El- Dakr
Prof. of Fish Nutrition, Faculty of Fisheries, Suez University
Dr. Mohamed Fathy Osman
Prof. Emeritus of Fish Nutrition, Faculty of Agriculture, Ain Shams
University
Dr. Hamdy Mohammed Mohammed Khattab
Prof. Emeritus of Animal Nutrition, Faculty of Agriculture, Air
Shams University

Date of Examination: 14 / 5 / 2015

# EFFECT OF USING HUMIC ACID AS A PREBIOTIC WITH DIFFERENT PROTEIN LEVELS IN FISH DIETS ON WATER QUALITY AND PRODUCTIVE PERFORMANCE OF TILAPIA AND COMMON CARP UNDER EGYPTIAN CONDITIONS

By

## HOSSAM AHMED MOHAMMED MOUNES

B.Sc. Agric. Sc. (Animal Production), Ain Shams University, 2000 M.Sc. Agric. Sc. (Animal Nutrition), Ain Shams University, 2006

# **Under the supervision of:**

## Dr. Hamdy Mohammad Mohammad Khattab

Prof. Emeritus of Animal Nutrition, Department of Animal Production, Faculty of Agriculture, Ain Shams University (Principal supervisor)

### Dr. Tarek Aboelmakarem Ali Mohammad Mohammad

Lecturer of Fish Nutrition, Department of Animal Production, Faculty of Agriculture, Ain Shams University

### Dr. Zienab Attia Nagdi

Prof. Emeritus of Limnology, Department of Limnology, Central Laboratory for Aquaculture Research (CLAR), Agricultural Research Center

### **ACKNOWLEDGMENT**

First and foremost, all praise to Allah; the Magnificent, the merciful, without whose bless and guidance this work would never have been started nor completed.

The author would like to express his sincere grateful and appreciation to the supervisor of the present work, Dr. Hamdy Mohammed Mohammed Khattab Professor Emeritus of Animal Nutrition, Animal Production Department, Faculty of Agriculture, Ain Shams University, for proposing the point of research, for his kind care during the progress and finishing of this work.

My sincere thanks to Dr. Zienab Attia Nagdi, Professor Emeritus of Limonology, Department of Limnology, Central Laboratory for Aquaculture Research (CLAR), Agricultural Research Center, for her supervision and great help in the practical work and provision of facilities.

Deepest thanks are also extended to Dr. Tarek Aboelmakarem Ali Mohammad Mohammad, Lecturer of Fish Nutrition, Department of Animal Production, Faculty of Agriculture, Ain Shams University for his valuable guidance.

### **ABSTRACT**

Hossam Ahmed Mohammed Mounes. Effect of Using Humic Acid as a Prebiotic With Different Protein Levels in Fish Diets on Water Quality and Productive Performances of Tilapia and Common Carp Under Egyptian Conditions. Unpublished ph. D. Thesis, Animal Production Department, Faculty of Agriculture, Ain Shams University, 2015

The present work was conducted in Central Laboratory For Aquaculture Research, (CLAR) Abassa, Abu-Hammad, Sharkia, Egypt, to investigate the effect of addition humic acid (Humabol) with three different protein levels (25, 22.5 and 20 % CP) on water quality and growth performance of Tilapia (*Oreochromis niloticus*) and Common Carp (*Cyprinus carpio*).

Thirty six concrete ponds of 2 meter as a diameter x 1.2 m as depth, respectively, represents eighteen treatments (two replicates / each) in three experiments, with a (3.77 m<sup>3</sup> total volume) with 3 fish / m<sup>3</sup>. The experimental treatments were as follow: Tilapia were fed diets contained 0.2 % Humic acid with 25 or 22.5 or 20 % protein ( $T_1$ ,  $T_2$  and  $T_3$ ), respectively, Common carp were fed diets contained 0.2 % Humic acid with 25 or 22.5 or 20 % protein (T<sub>4</sub>, T<sub>5</sub> and T<sub>6</sub>), respectively, represent the first experiment. Tilapia were fed diets without humic acid contained 25 or 22.5 or 20 % protein (T<sub>7</sub>, T<sub>8</sub> and T<sub>9</sub>), respectively, Common carp were fed diets without humic acid contained 25 or 22.5 or 20 % protein  $(T_{10}, T_{11})$  and  $T_{12}$ , respectively, represent the second experiment. Tilapia and common carp in the same pond were fed diets contained 0.2 % Humic acid with 25 or 22.5 or 20 % protein  $(T_{13}, T_{14})$  and  $T_{15}$ , respectively, Tilapia and common carp in the same pond were fed diets without humic acid contained 25 or 22.5 or 20 % protein (T<sub>16</sub>, T<sub>17</sub> and  $T_{18}$ ), respectively, represent the third experiment. The experimental ponds were supplied with well water. Water exchange rate was 100 % of the total pond area / 14 days. The first experiment lasted 105 days from the  $1^{st}$  of July to the  $15^{th}$  of October 2013 ( $T_1$  to  $T_6$ ), the second and the third experiments lasted 105 days from the  $1^{st}$  of July to the  $15^{th}$  of October 2014 ( $T_7$  to  $T_{18}$ ).

Results showed that there were no significant differences between treatments with and without humic in temperature degrees, dissolved oxygen, pH, Secchi disk, orthophosphate and chlorophyll (a), while there were increased in humic acid treatments in NH<sub>3</sub>, NO<sub>3</sub>, NO<sub>2</sub>, total alkalinity and total hardness.

There were significant differences (P < 0.05) in  $NO_3$ ,  $NO_2$ , total alkalinity, total hardness and orthophosphate due to fish species.

Protein level did not effect significantly on temperature, dissolved oxygen, secchi disk, NH<sub>3</sub>, NO<sub>3</sub>, total alkalinity, total hardness and chlorophyll (a). However, pH and NO<sub>2</sub> were significantly increased gradually as the level of protein increased.

Humic acid significantly decreased iron, zinc and cadmium, while it significantly (P < 0.05) increased in lead, and had no significant effect on manganese and copper.

Fish species had a significant (P < 0.05) effect on iron, zinc, manganese copper, cadmium and lead.

Protein level effect significantly on iron, zinc, copper, cadmium and lead. However, manganese had no significant effect due to level of protein.

The highest value of chlorophyceae and cyanophyceae (313336 and 11655.5) were observed in humic acid treatments with significant differences (P < 0.05), while the highest values of bacillariophyceae and euglenophyceae (1546 and 2328) were detected in treatments without humic acid.

Fish species had a significant (P < 0.05) effect on phytoplankton during the experiment.

Protein level had significant effect on phytoplankton, 25 % protein level recorded the highest chlorophyceae (279892), while 20 % protein

level recorded the highest cyanophyceae, bacillariophyceae and euglenophyceae values (9210, 1008 and 1903, respectively).

Average of body weight, RGR, SGR, moisture % and fat % and survival rate in humic acid treatments was higher (64.7 g, 18.46 g / g, 1.11 % / day, 71.6, 20.4 % and 81.25 %, respectively) than those without humic acid (57.1 g, 15.1 g / g and 0.98 % / day, 71.1, 19.5 % and 48.5 %, respectively) with significant effects. While humic acid didn't significantly affect fish body length, daily gain or condition factor.

The highest body weight, body length and daily gain, FCR, ash % and survival rate were (89.4 g, 17.46 cm, 0.59 g / day and 3.4, 14.3 % and 80.5 %, respectively) for tilapia, while carp showed the lowest body weight, body length and daily gain, ash % and survival rate were (35.4 g, 12.82 cm, 0.41 g / day, 2.1 and 10.9 % and 49.25 %, respectively).

The highest body weight, body length, daily gain and condition factor, head weight value and its percentage and viscera weight and its percentage and survival rate were (70.03 g, 15.73 cm. 0.55 g / day, 1.65 g / cm<sup>3</sup>, 27.1 g / fish, 29.8 %, 9.5 g / fish and 10.8 % and 67.13 %, respectively) were shown for the group fed 25 % protein, while the lowest body weight and body length head weight value and its percentage and viscera weight and its percentage and survival rate (63.31 g and 15.23 cm, 23 g / fish, 27.4 %, 7.9 g / fish, 9.7 % and 62.38 %) were recorded for fish fed 22.5 % protein, the lowest value of daily gain was found in treatment 20 % protein level (0.48 g / day).

Carp recorded the highest overall mean value of RGR, SGR, PER, PPV, moisture % and fat % (25.49 g/g, 1.52 % / day, 0.57, 33.9, 72.6 % and 21.7 %, respectively) whereas the lowest RGR and SGR, condition factor, PER, PPV, Fe, Mn, Cu and Cd were detected in tilapia (10.73 g/g, 0.61 % / day, 1.42 g/cm³, 0.35, 21.5, 0.19, 0.002, 0.003 and 0.0007 mg/g, respectively). The highest value of condition factor and protein % was detected in tilapia + carp (1.66 g/cm³ and 63.1 %).

Fish group fed 22.5 % protein level recorded the highest significant (P < 0.05) RGR, SGR and FCR, Fe, Zn, Cu and Pb values (17.32 g / g and

1.07~% / day, 2.8, 0.22~mg / g, 0.0.07~mg / g, 0.003~mg / g and 0.00009~mg / g, respectively); while the 25~% protein level noticed the lowest RGR and SGR, FCR, PER and PPV, Zn, Mn, Cd and Pb values (15.99 g / g and 1~% / day, 2.7, 0.39, 23.2, 0.0.05~mg / g, 0.002~mg / g, 0.001~mg / g and 0.00006~mg / g, respectively). Moreover, the 20~% protein level had significant best values of both PER and PPV (0.5~and~30.1, respectively).

Values of FCR, PER and PPV for treatments with or without humic acid were (2.7, 0.46, 27.1 and 2.8, 0.46 27.9, respectively).

The values of head weight and its percentage to the body weight were significant (P < 0.05) higher (25.8 g / fish and 30.3 %) recorded in treatments without humic acid than those with humic acid (23.5 g / fish and 26.6 %). Viscera weight and viscera percentage values recorded in treatments with humic acid (9.3 g / fish and 10.6 %) while the lowest one (7.5 g / fish and 9.4 %) was found in treatments without humic acid.

**Keywords:** Humic acid, Protein levels, Tilapia, *Oreochromis niloticus* and Common Carp, *Cyprinus carpio*.

# **CONTENTS**

	Page
LIST OF TABLES	VII
LIST OF FIGURES	X
LIST OF SYMBOLS OF SCIENTIFIC TERMS AND	XIII
ABBREVATION	
1. INTRODUCTION	1
2. REVIEW OF LITERATURE	4
2.1. Factors affecting water quality in aquaculture	4
2.1.1. Abiotic factors	4
2.1.1.1. Physical parameters of water	4
2.1.1.1. Dissolved oxygen (DO)	4
2.1.1.1.2. Water temperature (°C)	5
2.1.1.2. Chemical parameters of water	5
2.1.1.2.1. Concentration of hydrogen ion (pH)	5
2.1.1.2.2. Turbidity	6
2.1.1.2.3. Ammonia (NH <sub>3</sub> )	7
2.1.1.2.4. Nitrite (NO <sub>2</sub> )	7
2.1.1.2.5. Nitrate (NO <sub>3</sub> )	8
2.1.1.2.6. Total hardness	8
2.1.1.2.7. Total alkalinity	9
2.1.1.2.8. Total phosphorus	9
2.1.1.2.9. Soluble phosphorus	10
2.1.1.2.10. Heavy metals	11
2.1.1.2.10.1. Iron (Fe)	11
2.1.1.2.10.2. Zinc (Zn)	12
2.1.1.2.10.3. Copper (Cu)	13
2.1.1.2.10.4. Cadmium (Cd)	14
2.1.1.2.10.5. Lead (Pb)	15
2.1.2. Biotic factors	16
2.1.2.1. Phytoplankton	17

	Page
2.2. Fish	19
2.2.1. Tilapia fish	20
2.2.1.1. Feeding habits	21
2.2.2. Carp	21
2.2.2.1. Describe of carp	21
2.2.2. Habitat and biology	22
2.3. Importance of protein in fish diets	23
2.3.1. Protein requirements of fish	24
2.3.1.1. Protein requirements of tilapia	25
2.3.1.2. Protein requirements of carp	25
2.4. Prebiotics	26
Definition of prebiotics	26
2.4.1. Fulvic Acid	27
2.4.2. Humates	28
2.4.2.1. Safety of humates	28
2.4.2.2. The humic acid	29
2.4.2.2.1. Benefits of humic acid application in aquaculture are	30
3. MATERIALS AND METHODS	32
3.1. The experimental humic acid	32
3.2. Experimental design	32
3.3. Sampling of fish	36
3.4. Fish performance measurements	36
3.5. Carcass characteristics	36
3.6. Chemical analysis of diets and fish	37
3.6.1. Preparation of fish samples	37
3.6.2. Analytical methods	37
3.7. Water analysis	37
3.7.1. Physico-chemical analysis of water	38
3.7.1.1. Physical parameters	38
3.7.1.1.1. Water temperature (°C) and dissolved oxygen (DO)	38
3.7.1.1.2. Water transparency (cm)	38

	Page
3.7.1.2. Chemical analyses methods	38
3.7.1.2.1. Hydrogen ion concentration (pH)	38
3.7.1.2.2. Total ammonia (NH $_4$ $^+$ + NH $_3$ ) and un-ionized	20
ammonia (NH <sub>3</sub> -N)	38
3.7.1.2.3. Nitrite-nitrogen (NO <sub>2</sub> -N)	39
3.7.1.2.4. Nitrate-nitrogen (NO <sub>3</sub> -N)	39
3.7.1.2.5. Total alkalinity (mg / l)	39
3.7.1.2.6. Total hardness (mg / l)	40
3.7.1.2.7. Dissolved orthophosphate (mg / l)	40
3.7.1.2.8. Heavy metal in water samples	41
3.8. Biological Analyses	41
3.8.1. Chlorophyll "a"	41
3.8.2. Phytoplankton	42
3.9. Statistical analysis	42
3.9.1. Model for water quality	42
3.9.2. Model for fish performance	43
4. RESULTS AND DISCUSSION	45
4.1. Effect of treatments on some physico-chemical parameters of water	45
4.1.1. Effect of humic acid on water quality parameters	45
4.1.2. Effect of fish species on water quality	48
4.1.3. Effect of protein level on water quality	51
4.1.4. Effect of the individual treatments (18 treatments)	54
4.1.4.1. Water temperature °C	54
4.1.4.2. Dissolved oxygen (DO)	56
4.1.4.3. Concentration of hydrogen ion (pH)	56
4.1.4.4. Secchi disc (SD) visibility	56
4.1.4.5. Ammonia (NH <sub>3</sub> )	57
4.1.4.6. Nitrate (NO <sub>3</sub> )	57
4.1.4.7. Nitrite (NO <sub>2</sub> )	58
4.4.8. Total alkalinity (T. Alk)	58
4.4.9. Total hardness (TH)	59

	Page
4.4.10. Orthophosphate (OP)	59
4.4.11. Chlorophyll (a)	59
4.1.5. Effect on heavy metals	60
4.1.5.1. Effect of humic acid on heavy metals	60
4.1.5.2. Effect of fish species on heavy metals	62
4.1.5.3. Effect of protein level on heavy metals	63
4.1.5.4. Effect of the 18 treatments	65
4.1.5.4.2. Zinc (Zn)	65
4.1.5.4.3. Manganese (Mn)	66
4.1.5.4.4. Copper (Cu)	67
4.1.5.4.5. Cadmium (Cd)	67
4.1.5.4.6. Lead (Pb)	67
4.1.6. Phytoplankton	68
4.1.6.1. Effect of humic acid on phytoplankton	68
4.1.6.2. Effect of fish species on phytoplankton	69
4.1.6.3. Effect of protein level on phytoplankton	71
4.1.6.4. Effect of the 18 treatments	73
4.2. Growth parameters	74
4.2.1. Body weight	74
4.2.1.1. Effect of humic acid on body weight	74
4.2.1.2. Effect of fish species on body weight	75
4.2.1.3. Effect of protein level on body weight	76
4.2.1.4. Effect of the 18 treatments	77
4.2.2. Body length	79
4.2.2.1. Effect of humic acid on body length	79
4.2.2.2. Effect of fish species on body length	80
4.2.2.3. Effect of protein level on fish body length	81
4.2.2.4. Effect of the 18 treatments	82
4.2.3. Daily gain	84
4.2.3.1. Effect of humic acid on daily gain	84
4.2.3.2. Effect of fish species on daily gain	85

	Page
4.2.3.3. Effect of protein level on daily gain	86
4.2.3.4. Effect of the 18 treatments	87
4.2.4. Relative growth rate	88
4.2.4.1. Effect of humic acid on relative growth rate	88
4.2.4.2. Effect of fish species on relative growth rate	89
4.2.4.3. Effect of protein level on relative growth rate	91
4.2.4.4. Effect of the 18 treatments	92
4.2.5. Specific growth rate	93
4.2.5.1. Effect of humic acid on specific growth rate	93
4.2.5.2. Effect of fish species on specific growth rate	94
4.2.5.3. Effect of protein level on specific growth rate	95
4.2.5.4. Effect of the 18 treatments	97
4.2.6. Condition factor	98
4.2.6.1. Effect of humic acid on condition factor	98
4.2.6.2. Effect of fish species on condition factor	99
4.2.6.3. Effect of protein level on condition factor (K)	100
4.2.6.4. Effect of the 18 treatments	101
4.2.7. FCR, PER and PPV	103
4.2.7.1. Effect of humic acid on FCR, PER and PPV	103
4.2.7.2. Effect of fish species on FCR, PER and PPV	104
4.2.7.3. Effect of protein level on FCR, PER and PPV	106
4.2.7.4. Effect of the 18 treatments	108
4.2.7.5. Protein efficiency ratio	110
4.2.7.6. Protein productive value	110
4.2.8. Carcass traits	110
4.2.8.1. Effect of humic acid on carcass traits	110
4.2.8.2. Effect of fish species on carcass traits	111
4.2.8.3. Effect of protein level on carcass traits	112
4.2.8.4. Effect of the 18 treatments	113
4.2.9. Chemical composition of the whole fish	115
4.2.9.1. Effect of humic acid on chemical composition of the whole fish	115

	Page
4.2.9.2. Effect of fish species on chemical composition of the whole fish	116
4.2.9.3. Effect of protein level on chemical composition of the whole fish	118
4.2.9.4. Effect of the 18 treatments	119
4.2.10. Heavy metals in fish	121
4.2.10.1. Effect of humic acid on heavy metals in fish	121
4.2.10.2. Effect of fish species on heavy metals in fish	123
4.2.10.3. Effect of protein level on heavy metals in fish	126
4.2.10.3. Effect of the 18 treatments	128
4.2.10.3.1. Iron (Fe)	128
4.2.10.3.2. Zinc (Zn)	130
4.2.10.3.3. Manganese (Mn)	130
4.2.10.3.4. Copper (Cu)	130
4.2.10.3.5. Cadmium (Cd)	131
4.2.10.3.6. Lead (Pb)	131
4.2.11. Survival rate	131
4.2.11.1. Effect of humic acid on survival rate	131
4.2.11.2. Effect of fish species on survival rate	132
4.2.11.3. Effect of protein level on survival rate	133
4.2.11.4. Effect of the 18 treatments	133
5. SUMMARY AND CONCLUSIONS	135
6. REFERENCES	146
7. ARABIC SUMMARY	180

# LIST OF TABLES

	Page
1. The experimental design of the experiments	33
2. Rations formulation and chemical analysis (% on dry matter	35
basis) used during the experiment	
3. Summary of the experimental methodology	36
4. Percentage of unionized ammonia in aqueous solutions at	39
different pH values and temperatures	39
5. Effect of humic acid on water quality parameters	46
6. Effect of fish species on water quality	48
7. Effect of protein level on water quality	52
8. Water quality parameters of the experimental treatments	55
9. Effect of humic acid on heavy metals	61
10. Effect of fish species on heavy metals	62
11. Effect of protein level on heavy metals	64
12. Concentrations of heavy metals in the experimental	66
treatments	00
13. Effect of humic acid on phytoplankton (organism / L x 10 <sup>6</sup> )	68
14. Effect of fish species on phytoplankton (organism / L x 10 <sup>6</sup> )	70
15. Effect of protein level on phytoplankton (organism / L x 10 <sup>6</sup> )	71
16. The overall means of phytoplankton (organism / L x $10^6$ )	73
divisions in water samples collected throughout the experiment.	73
17. Effect of humic acid on body weight during the experiment	74
18. Effect of fish species on body weight (g) during the experiment	75
19. Effect of protein level on body weight (g) during the experiment	76
20. Effect of treatments on live body weight (g) during 14	70
weeks of the experiment	78
21. Effect of humic acid on body length (cm) during the experiment	79
22. Effect of fish species on body length (cm) during the experiment	80
23. Effect of protein level on body length (cm) during the experiment	82
24. Effect of treatments on body length (cm) during the experiment	83