

Ain Shams University
Faculty of Women
Arts, Science and Education
Zoology Department

Effects of Plant Protein-Based Diets Supplemented with Organic Acids on Growth and Feed Utilization of Nile Tilapia, *Oreochromis niloticus* Fingerlings

Thesis

Submitted for the partial fulfillment of the requirements for the degree of Ph.D in Zoology
Women's College for Arts, Science and Education
Ain Shams University

By Eman Youssef Mohammady Badiny M.Sc in Zoology

Under supervision

Prof. Dr. Ramadan Ahmed Mohamed Ali

Prof. of Cytogenetic and Molecular Biology Zoology Department Faculty of Women Ain Shams University

Prof. Dr. Ashraf Mohamed A-S Goda

Prof. of Fish Nutrition, Head of Aquaculture Division National Institute of Oceanography and Fisheries (NIOF)-El-Qanater El Khayria

Dr. Mohamed Shaban Mohamed Hassaan

Lecture of Fish Nutrition
National Institute of Oceanography and Fisheries
(NIOF)-El-Qanater El Khayria



Ain Shams University
Faculty of Women
Arts, Science and Education
Zoology Department

Approval sheet

Name: Eman Youssef Mohammady Badiny

Scientific Degree: Doctor Philosophy of Science (Ph. D).

Title: Effects of Plant Protein-Based Diets Supplemented with Organic Acids on Growth and Feed Utilization of Nile Tilapia, *Oreochromis niloticus* Fingerlings

Board of Scientific Supervision

Prof. Dr. Ramadan Ahmed Mohamed Ali

Prof. of Cytogenetics and Molecular Biology
Zoology Department
Faculty of Women
Ain Shams University

Prof. Dr. Ashraf Mohamed A-S Goda

Prof. of Fish Nutrition Head of Aquaculture Division National Institute of Oceanography and Fisheries (NIOF)-El-Qanater El Khayria

Dr. Mohamed Shaban Mohamed Hassaan

Lecture of Fish Nutrition

National Institute of Oceanography and Fisheries
(NIOF)-El-Qanater El Khayria

Qualification

Name : Eman Youssef Mohammady Badiny

Degree : M.Sc of Zoology-2010

Department : Zoology

Faculty : Faculty of Women for Arts, Science

and Education

University : Ain Shams

Year : 2010



تأثير اضافة الأحماض العضوية إلى العلائق النباتية على النمو وكفاءة الأستفادة من الغذاء لإصباعيات البلطى النيلي

رسالة مقدمة للحصول على درجة دكتوراه الفلسفة فى العلوم (علم الحيوان) قسم علم الحيوان كلية البنات للآداب و العلوم و التربية - جامعة عين شمس

إسم الطالبة إيمان يوسف محمدي بديني ماجستير في علم الحيوان

لجنة الإشراف

ا.د/أشرف محمد عبد السميع جودة أستاذ تغذية الأسماك ورئيس شعبة تربية الأحياء المائية المعهد القومى لعلوم البحار والمصايد القناطر الخبربة

ا.د/ رمضان أحمد محمد علي أستاذ الوراثة الخلوية والبيولوجيا الجزيئية قسم علم الحيوان كلية البنات للآداب والعلوم والتربية جامعة عين شمس

د/ محمد شعبان محمد حسان

مدرس تغذية الاسماك المعهد القومى لعلوم البحار والمصايد القناطر الخيرية



جامعة عين شمس كلية البنات للآداب و العلوم و التربية قسم علم الحيوان

أسم الطالبة : إيمان يوسف محمدي بديني

عنوان الرسالة: تأثير اضافة الأحماض العضوية إلى العلائق النباتية على النمو وكفاءة الأستفادة من الغذاء لإصباعيات البلطى النيلى.

اسم الدرجة: دكتوراه الفلسفة في العلوم (علم الحيوان)

كلية البنات للآداب و العلوم و التربية- جامعة عين شمس

لجنة الإشراف

1.د/أشرف محمد عبد السميع جودة أستاذ تغذية الأسماك ورئيس شعبة تربية الأحياء المائية المعهد القومى لعلوم البحار والمصايد القناطر الخيرية

1.د/ رمضان أحمد محمد علي أستاذ الوراثة الخلوية والبيولوجيا الجزيئية كلية البنات للأداب والعلوم والتربية جامعة عين شمس

د/ محمد شعبان محمد حسان

مدرس تغذية الاسماك المعهد القومى لعلوم البحار والمصاي القناطر الخيرية

تاريخ البحث:

الدراسات العليا:

اجيزت الرسالة بتاريخ:

موافقة مجلس الكلية:

موافقة مجلس الجامعة:

المؤهلات

الأسم : إيمان يوسف محمدي بديني

الدرجة : ماجستير في العلوم (علم الحيوان)

القسم : قسم علم الحيوان

الكلية : كلية البنات للآداب و العلوم و التربية

الجامعة : جامعة عين شمس

السنة : 2010

ACKNOWLEDGMENT

"My sincere and thanks to Allah who helped me to finish this work".

I am greatly honored to express my deep gratitude to, **Prof. Dr. Ramadan Ahmed Mohamed Ali**, professor of cytogenetic and molecular biology, zoology department, Women's College, Ain Shams University, for excellent supervision, support, meticulous advice and encouragement for the completion of this work. I always be in debt for his great help and support.

My respectful thanks to, **Prof. Dr. Ashraf Mohamed Abdelsamee Goda**, professor of fish nutrition, head of aquaculture division, National Institute of Oceanography and Fisheries, for his kind supervision and honest guidance of the present study. His research experience has added a lot to the integrity of my work. Fact I own much to him, for the valuable time he gave me.

Many thanks and gratitude is also extended to **Dr. Mohamed Shaban Mohamed Hassaan**, lecture of Fish Nutrition, National Institute of
Oceanography and Fisheries, for his keen supervision, giving me much of
his time, experience, truthful efforts and valuable remarks that helped a lot
in the final production of this work, which cannot be expressed in words.

I wish to express my sincere thanks and deepest appreciation to my grateful father, my mother and to my son Hassan for their great help and support throughout this work. Lastly, I would like to thank all my friends, National Institute of Oceanography and Fisheries, for their cooperation and help throughout this work, hoping them the best wishes in their research work.

TABLE OF CONTENTS

	Page
LIST OF TABLES	vi
LIST OF FIGURES	viii
LIST OF ABBREVIATIONS	X
ABSTRACT	xii
CHAPTER I: INTRODUCTION	1
AIM OF THE WORK	4
CHAPTER II: REVIEW OF LITERATURE	5
2.1.The need for low cost fish feeds	5
2.2. The main protein source in aquaculture	6
2.3. Alternatives protein sources in aquafeed	6
2.3.1. Soybean meal	7
2.3.2. Corn gluten	7
2.4. Genetically modified plants	
2.4.1. Safety assessment of genetically modified plants	
2.5. Limitations to the utilization of plant ingredients	10
2.5.1. Poor palatability	10
2.5.2. Poor digestibility	12
2.5.3. Anti-nutritional factors	13
2.6. Effect of replacing fishmeal by plant protein on growth performance and feed utilization	
2.7. Effect of replacing fishmeal by plant protein on nutrient digestibility	
2.8. Genetically modified plant products in fish diets	16
2.8.2. Effect of genetically modified plant on growth indices for fish	16
2.8.3. Effect of genetically modified plant on morphometric parameters for fish	17

TABLE OF CONTENTS

2.8.4. Effect of genetically modified plant on blood and physiological parameters for fish	18
2.9. Organic acid	20
2.9. 1. Effect of organic acid on growth performance of fish	21
2.9.2. Effect of organic acid on minerals availability in digestive tract of fish	23
2.10. Micronucleus test	25
2.11. DNA fragmentation assay	25
CHAPTER III: MATERIALS AND METHODS	27
3.1. Location of study	27
3.2. Experimental design	27
3.3. Fish husbandry and culture technique	27
3.4. Water quality	28
3.5. Experimental diets	28
3.5.1. Sources of dietary ingredients used in the study	28
3.5.2. Source of organic acids	29
3.5.2.1. Citric acid and formic acid	29
3.5.3. Formulation of experimental diets	29
3.6. Growth performance parameters	30
3.6.1. Live body weight and body length	30
3.6. 2. Condition factor	30
3.6. 3. Weight gain	30
3.6. 4. Specific growth rate	30
3.6. 5. Biological Parameters	31
3.7. Feed utilization parameters	31
3.7.1. Feed intake	31
3.7.2. Feed conversion ratio	31
3.7. 3. Protein efficiency ratio	31
3.7. 4. Protein productive ratio	31
3.7.5. Fat retention	32
3.7.6. Energy retention	32

TABLE OF CONTENTS

3.8. Digestibility measurements	
3.9. Blood Sampling	33
3.9. 1. Hematological Parameters	33
3.9.2. White blood cells and differential leukocytes	33
3.9.3. Blood chemistry parameters	33
3.10. Micronucleus and nuclear abnormality analysis	34
3.11. DNA fragmentation assay	34
3.12. Chemical composition	35
3.13. Statistical analysis	35
CHAPTER IV: RESULTS	39
•First experiment	39
4.1. Growth performance of Nile tilapia fed different dietary levels of conventional soybean (C-SBM) or genetically modified soybean meal (GM-SBM) supplemented with citric acid (CA)	39
4.1.1. Body weight	39
4. 1. 2. Finial body length	40
4.1. 3. Condition factor	40
4.1. 4. Weight gain	40
4.1. 5. Specific growth rate	41
4.2. Feed utilization of Nile tilapia fed different dietary levels of C-SBM or GM-SBM supplemented with CA	41
4.2.1. Feed intake	41
4.2.2. Feed conversion ratio	41
4.2.3. Protein efficiency ratio	41
4.2.4. Protein productive value	42
4.2.5. Fat retention and energy retention	42
4.3. Apparent digestibility coefficients of different dietary levels of C-SBM or GM-SBM supplemented with CA for Nile tilapia fingerlings	42
4.4. Biological parameters of Nile tilapia fed different dietary levels of C-SBM or GM-SBM supplemented with CA	43

4.5. Hematological parameters of Nile tilapia fed different dietary levels of C-SBM or GM-SBM supplemented with CA	43
4.5.1. Hematocrit and hemoglobin	43
4.5.2. White blood cells and differentiation	44
4.6. Blood chemistry parameters of Nile tilapia fed different dietary levels of C-SBM or GM-SBM supplemented with CA	44
4.6.1. Serum Total protein, albumin (A) and globulin (g)	44
4.6.2. Triglyceride	45
4.6.3 Glucose	45
4.6.4. Alanine aminotransferase and aspartate aminotransferase	45
4.7. Micronucleus and nuclear abnormality frequencies of Nile tilapia fed different dietary levels of C-SBM or GM-SBM supplemented with CA	45
4.8. DNA fragmentation of Nile tilapia fed different dietary levels of C-SBM or GM-SBM supplemented with CA	46
4.9. Chemical composition of Nile tilapia fed different dietary levels of C-SBM or GM-SBM supplemented with CA	46
•Second experiment	63
4.1. Growth performance of Nile tilapia fed different dietary levels of C-SBM or GM-SBM supplemented with formic acid (FA)	63
4.1.1. Body weight	63
4. 1.2. Final body length	63
4.1. 3. Condition factor	64
4.1. 4. Weight gain	64
4.1. 5. Specific growth rate	64
4.2. Feed utilization of Nile tilapia fed different dietary levels of C-SBM or GM-SBM supplemented with FA	65
4.2.1. Feed intake	65
4.2.2. Feed conversion ratio	
4.2.2. Feed Conversion ratio	65
4.2.3. Protein efficiency ratio	65 65

4.3. Apparent digestibility coefficients of different dietary levels of C-SBM or GM-SBM supplemented with FA for Nile tilapia fingerlings	66
4.4. Biological parameters of Nile tilapia fed different dietary levels of C-SBM or GM-SBM supplemented with FA	67
4.5. Hematological parameters of Nile tilapia fed different dietary levels of C-SBM or GM-SBM supplemented with FA	67
4.5.1. Hematocrit and hemoglobin	67
4.5.2. White blood cell and their differential	68
4.6. Blood chemistry parameters of Nile tilapia fed different dietary levels of C-SBM or GM-SBM supplemented with FA	68
4.6.1. Serum total protein, albumin and globulin	68
4.6.2. Triglyceride	69
4.6.3 Glucose	69
4.6.4. Alanine aminotransferase and aspartate aminotransferase	69
4.7. Micronucleus and nuclear abnormality frequencies of Nile tilapia fed different dietary levels of C-SBM or GM-SBM supplemented with FA	70
4.8. DNA fragmentation of Nile tilapia fed different dietary levels of C-SBM or GM-SBM supplemented with FA	70
4.9. Chemical composition of Nile tilapia fed different dietary levels of C-SBM or GM-SBM supplemented with FA	71
CHAPTER V: DISCUSSION	86
CHAPTER VI: CONCLUSIONS AND RECOMMENDATIONS	101
CHAPTER VII: SUMMERY	102
CHAPTER VIII: REFERENCES	107
ARABIC SUMMARY & ABSTRACT	

LIST OF TABLES

LIST OF TABLES

Table No.	Title	Page No.
Table (1)	Major groupings of anti-nutritional factors	13
Table (2)	Composition and proximate analysis of the experimental diets	36
Table (3)	Growth performance of Nile tilapia fed different dietary levels of conventional soybean (C-SBM) or genetically modified soybean meal (GM-SBM) supplemented with citric acid (CA)	48
Table (4)	Feed utilization of Nile tilapia fed different dietary levels of C-SBM or GM-SBM supplemented with CA	49
Table (5)	Apparent digestibility coefficients of different dietary levels of C-SBM or GM-SBM supplemented with CA for Nile tilapia fingerlings	50
Table (6)	Biological parameters of Nile tilapia fed different dietary levels of C-SBM or GM- SBM supplemented with CA	51
Table (7)	Hematological parameters and differential white blood cells of Nile tilapia fed different dietary levels of C-SBM or GM-SBM supplemented with CA	52
Table (8)	Blood chemistry parameters of Nile tilapia fed different dietary levels of C-SBM or GM-SBM supplemented with CA	53
Table (9)	Micronucleus and nuclear abnormality frequencies of Nile tilapia fed different dietary levels of C-SBM or GM-SBM supplemented with CA	54
Table (10)	DNA fragmentation of Nile tilapia fed different dietary levels of C-SBM or GM-SBM supplemented with CA	55

LIST OF TABLES

Table (11)	Chemical composition of Nile tilapia fed different dietary levels of C-SBM or GM-SBM supplemented with CA	56
Table (12)	Growth performance of Nile tilapia fed different dietary levels of C-SBM or GM-SBM supplemented with formic acid (FA)	72
Table (13)	Feed utilization of Nile tilapia fed different dietary levels of C-SBM or GM-SBM supplemented with FA	73
Table (14)	Apparent digestibility coefficients of different dietary levels of C-SBM or GM-SBM supplemented with FA for Nile tilapia fingerlings	74
Table (15)	Biological parameters of Nile tilapia fed different dietary levels of C-SBM or GM- SBM supplemented with FA	75
Table (16)	Hematological parameters and differential white blood cells of Nile tilapia fed different dietary levels of C-SBM or GM-SBM supplemented with FA	76
Table (17)	Blood chemistry parameters of Nile tilapia fed different dietary levels of C-SBM or GM- SBM supplemented with FA	77
Table (18)	Micronucleus and nuclear abnormality frequencies of Nile tilapia fed different dietary levels of C-SBM or GM-SBM supplemented with FA	78
Table (19)	DNA fragmentation of Nile tilapia fed different dietary levels of C-SBM or GM-SBM supplemented with FA	79
Table (20)	Chemical composition of Nile tilapia fed different dietary levels of C-SBM or GM-SBM supplemented with FA	80

LIST OF FIGURES

LIST OF FIGURES

Figure No.	Title	Page No.
Fig. (1)	Diagram summarizes the experimental design for citric acid experiment	37
Fig. (2)	Diagram summarizes the experimental design for formic acid experiment	38
Fig. (3)	Body weight of fish fed conventional soybean supplemented with or without citric acid (CA, 10 g/kg diet)	57
Fig. (4)	Body weight of fish fed genetically modified soybean supplemented with or without citric acid (10 g/kg diet)	57
Fig. (5)	Weight gain (g) of Nile tilapia fed experimental diets supplemented with or without citric acid	58
Fig. (6)	Specific growth rate (%) of Nile tilapia fed experimental diets supplemented with or without citric acid	58
Fig. (7)	Length of intestine (cm) of Nile tilapia fed experimental diets supplemented with or without citric acid	59
Fig. (8)	Triglyceride (mg/dl) of Nile tilapia fed experimental diets supplemented with or without citric acid	59
Fig. (9)	Glucose (mg/gl) of Nile tilapia fed experimental diets supplemented with or without citric acid	60
Fig. (10)	ALT (U/L) of Nile tilapia fed experimental diets supplemented with or without citric acid	60
Fig. (11)	AST (U/L) of Nile tilapia fed experimental diets supplemented with or without citric acid	61