

**UTILIZATION OF FERMENTED FAETHER MEAL
REPLACEMENT OF FISH MEAL IN FISH FEED**



**A THESIS SUBMITTED IN PARTICAL FULFILLMENT
OF THE REQUIREMENTS FOR
THE DEGREE OF MASTER OF SCIENCE
(APPROPRIATE TECHNOLOGY FOR RESOURCES AND
ENVIRONMENTAL DEVELOPMENT)
FACULTY OF GRADUATE STUDIES
MAHIDOL UNIVERSITY
2005**

**ISBN 974-04-5967-6
COPYRIGHT OF MAHIDOL UNIVERSITY**

Thesis
Entitled

**UTILIZATION OF FERMENTED FAETHER MEAL
REPLACEMENT OF FISH MEAL IN FISH FEED**



E. Rakyutithamkul
.....
Mr.Ek Rakyutithamkul
Candidate

[Signature]
.....
Asst. Prof.Chumlong Arunlertaree,
Ph.D.
Major-Advisor

P.smb
.....
Lect. Pisamai Somsueb,
M.Sc.
Co-Advisor

A. Limsakoon
.....
Lect. Suntharaporn Limsakoon
M.Sc.
Co-Advisor

Rassmidara Hoonsawat
.....
Assoc. Prof. Rassmidara Hoonsawat
Ph.D.
Dean
Faculty of Graduate Studies

[Signature]
.....
Asst. Prof. Chumlong Arunlertaree, Ph.D.
Acting Chair
Master of Science Programme in
Appropriate Technology for Resources
and Environmental Development
Faculty of Environmental and Resource
Studies

Thesis
Entitled

**UTILIZATION OF FERMENTED FAETHER MEAL
REPLACEMENT OF FISH MEAL IN FISH FEED**

was submitted to the Faculty of Graduate Studies, Mahidol University
for the degree of Master of Science (Appropriate technology for resource development)

on
April 18, 2005

E. Rakyuttithamkul

Mr. Ek Rakyutithamkul
Candidate



Asst. Prof. Chumlong Arunlertaree,
Ph.D.
Chair

P. Somb

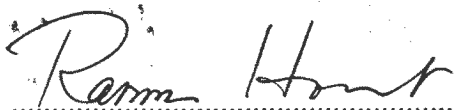
Lect. Pisamai Somsueb,
M.Sc.
Member

SAKSIT

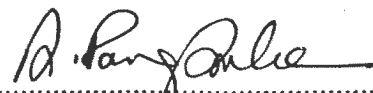
Lect. Saksit Vibulsuk
M.Sc.
Member



Lect. Suntharaporn Limsakoon
M.Sc.
Member



Assoc. Prof. Rassmidara Hoonsawat
Ph.D.
Dean
Faculty of Graduate Studies
Mahidol University



Assoc. Prof. Antchat Pongsomlee, Ph.D.
Dean
Faculty of Environmental and Resource
Studies
Mahidol University

ACKNOWLEDGEMENT

This thesis was completed successfully with kindness and suggestion of advisor, Asst.Prof.Dr.Chumlong Arunlertaree who always give me kindness and suggestion. I would like to express my gratitude to him for everything especially reviewing and making my thesis to be complete.

Grateful appreciation to co-advisor Pisamai Somsurb for giving me the laboratory test, experimental farm and fish fees calculation, Any problems I met between times at proceeding thesis would be cleared by her advice.

Grateful appreciation to co-advisor Suntharaporn Limsalul for her advice of experiment and her supervision on experiment planning.

Grateful appreciation to co-advisor Saksit Vibulsul for his kindness and supervision on how to deploy my thesis in commercial fish farming.

I also would like to thank to my friends, Mr. Pornthep Nirmpitak and Mr.Pichit Promprasri, for their help on blood collection and water quality analysis.

Ek Rakyuttithamkul

UTILIZATION OF FERMENTED FEATHER MEAL FOR REPLACEMENT OF FISHMEAL IN FISH FEED

EK RAKYUTTITHAMKUL 4337329 ENAT/M

M.Sc.(APPROPRIATE TECHNOLOGY FOR RESOURCES AND ENVIRONMENTAL DEVELOPMENT)

THESIS ADVISORS: CHUMLONG ARUNLERTAREE, Ph.D., PISAMAI SOMSUEB, M.Sc., SUNTHARAPORN LIMSAKON, M.Sc.

ABSTRACT

The effects of partially replacing fish meal, in Nile Tilapia (*Oreochromis nilotica*) and Walking Catfish (*Clarias* sp.), diets with fermented feather meal on growth and feed efficiency was studied. Five isonitrogenous and isocaloric diets were formulated. Diet 1 contained 100% fish meal, diet 2, 3, 4 and 5 contained 25%, 50%, 75%, and 100% of fermented feather meal replacement of fish meal in feed formula, the control diet contained 0% fish meal.

The results showed that mean final weight of tilapias fed with diet formulas 1, 2 and 3 were not significantly different ($P>0.05$). Feed conversion ratios were not significant among tilapias fed with diet formulas 1 and 2. Protein efficiency rates were not significant ($P>0.05$) among tilapias fed with diet formulas 1, 2 and 3. Apparent net protein retention of tilapias fed with diet formulas 1, 3, 4 and 5 was lower ($P<0.05$) than formula 2. Feed consumption, protein intake, and survival rate were not significantly different ($P>0.05$) among treatments. Meanwhile mean final weight of walking catfishes fed with diet formulas 1, 2 and 3 was not significantly difference ($P>0.05$). Feed consumption was not significant among walking catfishes fed with diet formulas 1, 2, 3 and 4. Feed conversion ratios were not significant ($P>0.05$) among walking catfishes fed with diet formulas 1, 2 and 3. Protein efficiency rates were not significant ($P>0.05$) among walking catfishes fed with diet formulas 1, 2, 3 and 4. Apparent net protein retention of walking catfishes fed with diet formulas 1, 2 and 3 was not significantly different ($P>0.05$). Protein intakes were not significant ($P>0.05$) among walking catfishes fed with diet formulas 1, 2, 3 and 4, and survival rate was not significantly different ($P>0.05$) among treatments.

This study suggests that the percentage of replacement fish meal with fermented feather meal in Nile tilapia and walking catfish at 25% had a close growth and feed efficiency to fish meal feed and reduced diet cost by about 0.71 and 0.81 baht/kg., respectively. The 50% replacement fish meal by fermented feather meal was the most effective for reducing the cost of diet, while the growth and feed efficiency were not significantly different ($P>0.05$) from fish fed with the control diet.

KEY WORDS: FEATHER MEAL / FERMENTATION / FISH FEED/TILAPIA/WALKING CAT FISH

100 P.ISBN 974-04-5967-6

การใช้ขี้ไก่ป่นหมักทดแทนปลาป่นในอาหารปลา (UTILIZATION OF FERMENTED FEATHER MEAL FOR REPLACEMENT OF FISHMEAL IN FISH FEED)

เอก รักษ์อุทิศธรรมกุล 4337329 ENAT/M

วท.ม. (เทคโนโลยีที่เหมาะสมเพื่อการพัฒนาทรัพยากรและสิ่งแวดล้อม)

คณะกรรมการควบคุมวิทยานิพนธ์: ผศ. จำลอง อรุณเลิศอารีย์ Ph.D., พิศมัย สมสืบ M.Sc.,สุนทรภรณ์ ลิ้มสกุล M.Sc.

บทคัดย่อ

การใช้ขี้ไก่ป่นหมักทดแทนปลาป่นในสูตรอาหารสำหรับปลานิล (*Oreochromis nilotica*) และปลาอุก ลูกผสม (*Clarius macrocephalus* x *Clarias gariepinus*) ต่อผลการเจริญเติบโตและการใช้ประโยชน์จากอาหารของปลา โดยมีโปรตีนและพลังงานสุทธิในอาหารเท่ากัน (30% และ 400 kcal/100 g diet) อาหารสูตรที่ 1 ใช้ปลาป่น 100% เพื่อเป็นอาหารชุดควบคุม สูตรที่ 2, 3, 4 และ 5 ใช้ขี้ไก่ป่นหมักแทนปลาป่น 25%, 50%, 75% และ 100% ตามลำดับ

ผลการวิจัยพบว่า ปลานิลที่เลี้ยงด้วยอาหารสูตรที่ 1 มีน้ำหนักเมื่อสิ้นสุดการทดลองไม่แตกต่าง ($P>0.05$) จากสูตรที่ 2 และ 3 อัตราแลกเปลี่ยนปลาป่นที่เลี้ยงด้วยอาหารสูตรที่ 1 ไม่แตกต่าง ($P>0.05$) จากสูตรที่ 2 ประสิทธิภาพการใช้โปรตีนปลาป่นที่เลี้ยงด้วยอาหารสูตรที่ 1 ไม่แตกต่าง ($P>0.05$) จากสูตรที่ 2 และ 3 โปรตีนที่เพิ่มขึ้นในตัวปลานิลที่เลี้ยงด้วยอาหารสูตรที่ 1, 3, 4 และ 5 ต่ำกว่าปลานิลที่เลี้ยงด้วยอาหารสูตรที่ 2 ($P<0.05$) ปริมาณอาหารที่กิน, ปริมาณโปรตีนที่กิน และ อัตรารอด ไม่มีความแตกต่างกันระหว่างกลุ่มทดลอง ($P>0.05$) และผลการทดลองในปลาอุกพบว่า ปลาอุกที่เลี้ยงด้วยอาหารสูตรที่ 1 มีน้ำหนักเมื่อสิ้นสุดการทดลองไม่แตกต่าง ($P>0.05$) จากสูตรที่ 2 และ 3 ปริมาณอาหารที่กินของปลาที่เลี้ยงด้วยอาหารสูตรที่ 1, 2, 3 และ 4 ไม่แตกต่างกัน ($P>0.05$) อัตราแลกเปลี่ยนปลาอุกที่เลี้ยงด้วยอาหารสูตรที่ 1 ไม่แตกต่าง ($P>0.05$) จากสูตรที่ 2 และ 3 ประสิทธิภาพการใช้โปรตีนปลาอุกที่เลี้ยงด้วยอาหารสูตรที่ 1, 2 และ 3 ไม่แตกต่างกัน ($P>0.05$) โปรตีนที่เพิ่มขึ้นในตัวปลาอุกที่เลี้ยงด้วยอาหารสูตรที่ 1, 2 และ 3 ไม่แตกต่างกัน ($P>0.05$) ปริมาณ ปริมาณโปรตีนที่กินปลาอุกที่เลี้ยงด้วยอาหารสูตรที่ 1, 2, 3 และ 4 ไม่แตกต่างกัน ($P>0.05$) อัตรารอด ไม่มีความแตกต่างกันระหว่างกลุ่มทดลอง ($P>0.05$)

จากการทดลองพบว่า การใช้ขี้ไก่ป่นหมัก 25% แทนที่ปลาป่นในสูตรอาหารปลานิลและปลาอุกมีอัตราการเจริญเติบโต และประสิทธิภาพการใช้อาหารใกล้เคียงกับกลุ่มควบคุมมากที่สุดและจะลดต้นทุนในการผลิตอาหารทดลองได้ 0.71 และ 0.81 บาท/ กิโลกรัม ตามลำดับ และ การใช้ขี้ไก่ป่นหมัก 50% สามารถลดต้นทุนค่าอาหารปลาได้มากที่สุดโดยไม่ทำให้อัตราการเจริญเติบโต และประสิทธิภาพการใช้อาหารแตกต่างจากกลุ่มควบคุม

100 หน้า .ISBN 974-04-5967-6

CONTENTS

	Page
ACKNOWLEDGEMENTS	iii
ABSTRACT	iv
LIST OF TABLES	viii
LIST OF FIGURES	x
CHAPTER	
I INTRODUCTION	
1.1 Stage of problem.....	1
1.2 Conceptual Framework.....	4
1.3 Research Objectives.....	5
1.4 Hypothesis.....	5
1.5 Boundary.....	5
1.6 Expected Results.....	5
1.7 Research Variables.....	5
II LITERATURE REVIEW	
2.1 Fish feed.....	6
2.2 Protein requirements.....	13
2.3 Feather meal.....	15
2.4 Fermentation.....	18
2.5 Traditional cultured freshwater fish.....	19
2.6 Relevant research.....	21
III MATERIAL AND METHODS	
3.1 Experimental planning.....	26
3.2 Experimental procedure.....	28
3.3 Data collection and analysis method.....	34
IV RESULTS	
4.1 Quality of experimental diets.....	36

CONTENTS(CONT.)

	Page
4.2 Growth and feed efficiency.....	38
4.3 Water quality in the experiment.....	54
4.4 Cost of experimental diets.....	56
V DISCUSSION	
5.1 Quality of experimental diet.....	58
5.2 Growth and feed efficiency.....	59
5.3 Water quality.....	67
VI CONCLUSION	
6.1 Conclusion.....	68
6.2 Recommendation.....	69
BIBLIOGRAPHY	70
APPENDIX A	75
APPENDIX B	76
APPENDIX C	83
BIOGRAPHY	99

LIST OF TABLES

	Page
Table 1.1 The percentage of Indispensable amino acid in protein source materials.....	2
Table 2.1 The standard of proximate composition of commercial for registering aqua feed produce in Thailand	10
Table 2.2 The sample of herbivorous fish feed formulate.....	11
Table 2.3 The sample of nile tilapia feed formulate.....	11
Table 2.4 The sample of carnivorous fish feed formulate.....	12
Table 2.5 The sample of walking catfish feed formulate.....	12
Table 2.6 The amino acid requirement of fishes.....	14
Table 3.1 Experimental diet formula of nile tilapia.....	27
Table 3.2 Experimental diet formula of walking cat fish.....	28
Table 4.1 Proximate composition of nile tilapia experimental diet with different ratio fermented feather meal replacement of fish meal.....	36
Table 4.2 Proximate composition of walking catfish experimental diet with different ratio fermented feather meal replacement of fish meal.....	37
Table 4.3 Average weight of nile tilapia that fed with different diet every 2 weeks throughout the experiment.....	38
Table 4.4 Growth and feed efficiency of nile tilapia fed with different diets during 12 weeks.....	42
Table 4.5 The chemical compositions of nile tilapia fed with different diets.....	43
Table 4.6 Level of triiodotyronine (T3) and thyroxin hormone (T4) in blood of nile tilapia.....	44
Table 4.7 Average weight of walking catfish that fed with different diet every 2 weeks throughout the experimental.....	46
Table 4.8 Growth and feed efficiency of walking catfish fed with different diets during 8 weeks.....	50

LIST OF TABLE (CONT.)

	Page
Table 4.9 The chemical compositions of walking catfish fed with different diets during 8 weeks.....	51
Table 4.10 Level of triiodotyronine (T3) and thyroxin hormone (T4) in blood of walking catfish.....	52
Table 4.11 Water quality in the experimental of nile tilapia fed with different diets during 12 weeks.....	54
Table 4.12 Water quality in the experimental of walking catfish fed with different diets during 8 weeks.....	55
Table 4.13 Cost of nile tilapia experimental diets.....	56
Table 4.14 Cost of walking catfish experimental diets.....	57

LIST OF FIGURES

	Page
Figure 2-1 Broiler feather by-product processing.....	16
Figure 3-1 Raw materials were mixed in a meat grinder.....	29
Figure 3-2 Moist feed mixture were passed through a meat grinder.....	30
Figure 3-3 Feed were dried in open air.....	30
Figure 3-4 Experimental fish culture.....	31
Figure 3-5 Weighing of experimental fishes.....	32
Figure 4-1 The average weight every 2 weeks of Nile tilapia fed with different diets.....	39
Figure 4-2 Nile tilapia fed with diet formula 1.....	45
Figure 4-3 Nile tilapia fed with diet formula 2.....	45
Figure 4-4 Nile tilapia fed with diet formula 3.....	45
Figure 4-5 Nile tilapia fed with diet formula 4.....	45
Figure 4-6 Nile tilapia fed with diet formula 5.....	45
Figure 4-7 The average weight every 2 weeks of walking catfish fed with different diets.....	47
Figure 4-8 Walking catfish fed with diet formula 1.....	53
Figure 4-9 Walking catfish fed with diet formula 2.....	53
Figure 4-10 Walking catfish fed with diet formula 3.....	53
Figure 4-11 Walking catfish fed with diet formula 4.....	53
Figure 4-12 Walking catfish fed with diet formula 5.....	53
Figure 4-13 Close flow through system in experimental tank and filter pond.....	55

CHAPTER I

INTRODUCTION

1.1 Stage of problem

In artificial fish feed manufacturing, the cost for feed has been highly depended on fish meal as major protein source that using in artificial fish feed production because of the suitable protein quality, amino acid profile that closely matched fish requirement and palatability of fish feed. In present day, the increasing demand and uncertain availability of fish meal was interested for the nutritionist to studying alternative source of protein for supply dietary protein in artificial fish feeds.

Feather meal are the by-product from poultry production and slaughter house. The constituent of feather are composed of a complex protein (keratin), which can be broken down by hydrolysis to make them digestible and the digestibility of feather meal is directly affected by cooking time and pressure. Feather meal is rich in indispensable amino acid such as cystine, threonine and arginine and have high level of Pepsin Digestible Protein 75-87% (Wilder *et al.*, (1976) cited by Sinchermsiri *et al.*, 1988) the amino acid balance of feather meal was closely like fish meal and soybean meal as presented in table 1.1

Table 1.1 The percentage of Indispensable amino acid in protein source materials

Indispensable amino acid ¹	Percentage in protein			
	Fish meal ¹	Soybean meal ¹	Feather ¹	Feather meal ²
Arginine	5.6	7.5	7.3	7.5
Histidine	2.6	2.5	0.6	0.6
Isoleucine	4.4	4.7	6.4	4.1
Leucine	7.4	7.7	8.5	7.3
Lysine	7.3	6.2	1.6	2.2
Methionine (+ Cystine)	3.9	2.5	0.5	6.8
Phenylalanine (+ Tyrosine)	7.2	8.6	5.5	8.0
Threonine	4.9	4.3	4.7	4.4
Tryptophan	1.2	1.3	0.7	0.7
Valine	5.3	4.8	8.9	5.9

Note : ¹Ogino, 1980 cited by Sarnwatanakul and Bamrongtum , 2000

²Sinchermsiri *et al*, 1988

Feather meal was interested in using in animal feed such as chicken, swine, goat, sheep and cow. The utilization of feather meal better by ruminant animals and can be improved when the feather supplemented with urea, but the utilization of feather meal in fish were found that high percentage of using feather meal in fish feed were not succeeded any more, because of protein of feather meal was keratin that had sulfide bonding from 2 molecules of cysteine. The sulfide bonding was hardly digested by monogastric animal. However, Koops *et al.*(1982)(cited by Fowler, 1990) had formulated diet containing 14-15% feather meal for ranbow trout with good success and Fowler (1990) reported that the feather meal could replaced 15% of fish meal in diet without different growth and feed efficiency of Chinook salmon.

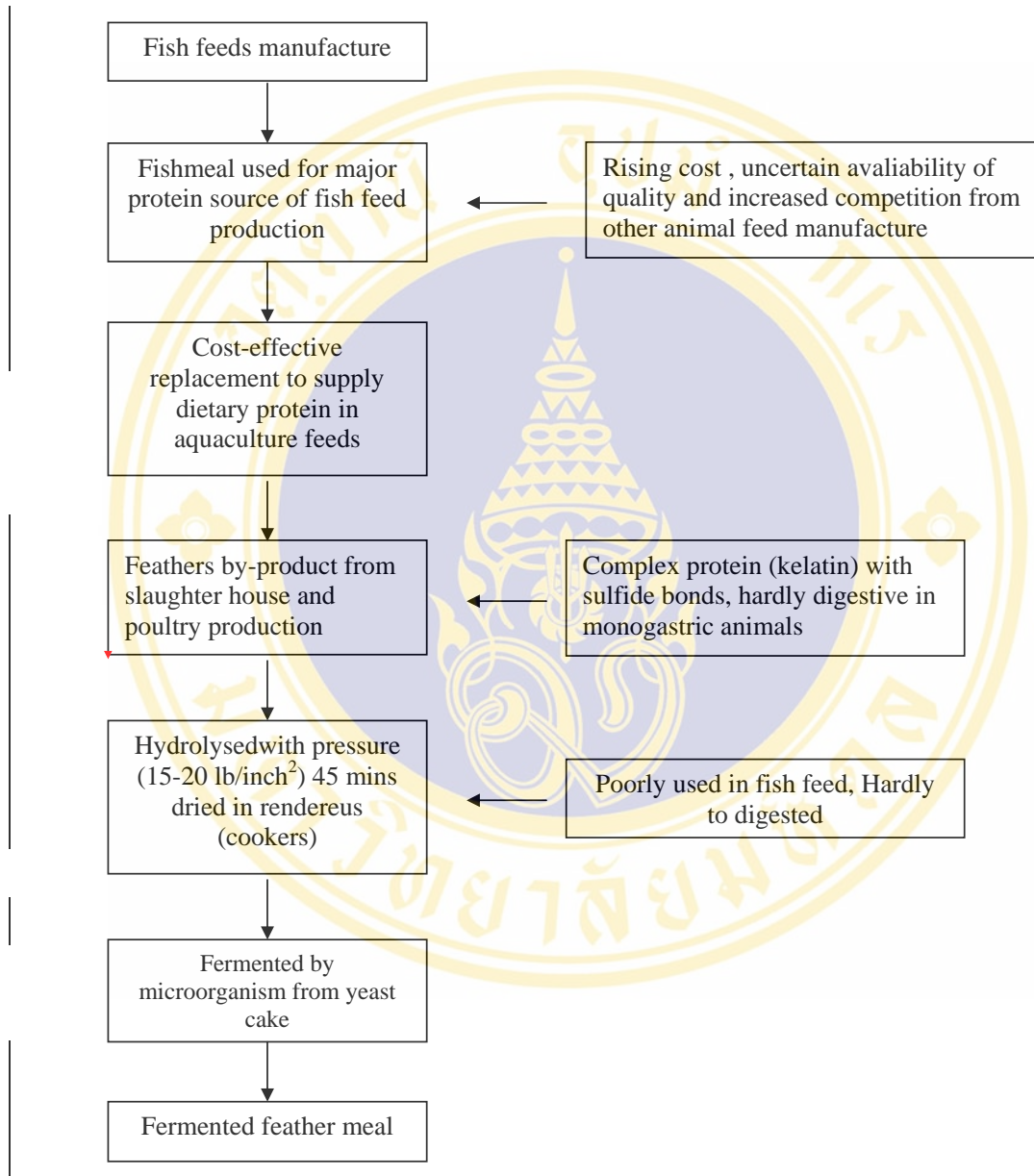
The improvement protein quality by fermentation with microorganism was studied in by-product from caned fish, shrimp production and non-edible part of fish

(head and visceral) for using substitute fish meal in artificial fish feed. Generally, in natural, there are some of keratinophilic microorganisms in soil and animal skins such as bacteria: *Bacillus*, *Streptomyces* and fungi: *Moccus*, *Rhizopus* and *Aspergillus*, which active in room temperature and optimum conditions (moisture more than 20%).

From the above reasons, the fermentation of feather meal are possible to improve the protein quality in feather meal and more able to used for partial or total replacement of fish meal in artificial fish feed as major protein source.



1.2 Conceptual Framework



1.3 Research Objectives

Study the results of replacement fish meal with fermented feather meal in the herbivorous and carnivorous fish diets on growth, feed efficiency and fish diets quality.

1.4 Hypothesis

Fermented feather meal can replace fishmeal in herbivore and canivor juvenile fish diets.

1.5 Boundary

1.5.1 Ferment feather meal with microorganism from yeast cake for 48 hours and cassava powder were added for microorganism's energy source.

1.5.2 Experimental diets were formulated to be iso-nitrogenous and iso-caloric, each diet were contained 30% protein and gross energy 400 kcal/ 100g.diet.

1.5.3 Used juvenile Nile tilapia (*Oreochromis nilotica*) initial body weight of 120±5 g. for representative of herbivorous fishes and used juvenile hybrid Walking fish (*Clarius macrocephalus* x *Clarias gariepinus*) initial body weight of 75±5 g. for representative of carnivorous fishes.

1.6 Expected Results

Fermented feather meal were replaced for fish meal as a protein source at the optimum level in juvenile fish diets.

1.7 Research Variables

1.7.1 Free variable

- Percentage of replacement fish meal with fermented feather meal

1.7.2 Dependent variable

- Feed quality
- Growth and feed efficiency

CHAPTER II

LITERATURE REVIEW

2.1 Artificial fish feed

Artificial fish feed has been material that used as natural food in fish culture. Most of fish feed were composed of more than 2 raw materials that have the objective to increasing fish products, reduced time of fish culture and reduced the trouble of feed management.

2.1.1 Raw material using as protein source in fish feed

The objective of choosing raw material for fish feed must be considered the available nutrition of material and price. (Feed Quality Control and Development Division, 2541) The feed nutrition that fish need is showing as below.

1. Protein and ten essential amino acids
2. Essential fatty acids, phospholipids and sterol
3. Energy from protein lipid and carbohydrate
4. Lipid-soluble vitamins and water-soluble vitamins
5. Minerals

Normally the nutrition requirement can be supplied by suitable material. Each of material has different nutrient and digestibility. Raw material that usually used in feed production was categorized to 5 types as following.

2.1.1.1 *Raw material used as protein source*

The materials that are able to use as protein source in fish feed should have crude protein more than 20% by weight (Feed Quality Control and Development Division, 2541).

Dietary protein source material were divided into 2 sources as following.

1. Animal proteins such as fish meal, trash fish, blood meal, bone meal, shrimp meal, squid meal, mixed poultry by-product meal and dairy by-product
2. Plant proteins such as soybean, peanut, cotton seed, coconut, sesame, corn gluten

2.1.1.2 *Material using as energy source*

The material that be able to use as energy source in fish feed is either powder or fat, which should have more than 60-80% carbohydrate (by weight). This material will perform as binding agent (binder). Most of them are seed and product from cereals such as broken rice, corn, ground rice, wheat, millet, cassava, plam seed meal, etc.

2.1.1.3 *Material using as lipid source*

This material contains the essential fatty acid, lipid-soluble vitamin and it can stimulate palatability of feed. This material were categorized to 2 types. First, animal lipids, which are favorited for using in artificial fish feed such as fish oil, squid oil and lard. Second are lipids from plant such as soybean oil, corn oil, palm oil, peanut oil and sunflower oil.

2.1.1.4 *Material using as vitamin and mineral source*

Most of vitamin and mineral that usually used in feed production are chemical component and in mixing method, sometimes those vitamins may not mix with other component throughout if there is small number of vitamins. Therefore, vitamin always mixed with some component such as soybean, calcium, grounded husk and grounded limestone. That compound called premix, which makes all components mix more all through.

2.1.1.5 *Material using as complementary*

This material is used for mixing with feed for other reasons not for additional nutrition. There are 3 types of this material.

1. Starch or Binder

The starch or binder makes feed more consistent to maintain its figure in water so that fish has more time to consume. Most of starch using in fish feed manufacturing are protein starch (gluten, gellatin) carbohydrate-starch (corn starch, wheat, rice, molasses) and synthetic-starch (licnosulphonate, cassava rubber, caragenan, aquabide).

2. Flavouring material

Flavouring materials were used for making feed more attractive taste and smell. Most of the traditional flavouring materials are animal such as shrimp flesh, mollus flesh, fish flesh and other by-product such as fish oil, squid oil, ground squid visceral, ground shrimp head and shrimp meal.

3. Oxidisation and fungi protecting material

Oxidisation and fungi protecting material are used for preserve fish feed quality from rancid smell and fungi contaminate. The material that usually fill in fish feed are chemical agent such as Butylated Hydroxy Toluene (B.H.T.) and Butylated Hydroxy Anisol (B.H.A.) 0.2% by weight for protected rancid smell and Propionic acid 0.3% by weight for protected fungi.

2.1.2 Fish feed production

The fish feed production compose of 4 processes which are decreasing size, mixing material, compressing and drying

1. Decreasing size

For all big size of feeds should be grinded to have size less than 0.5 ml. (Hastings and Higgs.1980 cited by Chuapoehuk, 2542). This method may help the starch material better binding and better digestible.

2. Feed mixing

Feed mixing is process, which makes all components mix all throughout, first vitamins and minerals were mixed with other material that makes vitamins and minerals mix more throughout such alpha starch, cassava powder and ground husk. Feed mixing process usually use feed mixing machine which can use for wet and dry materials, in case that the material are too dry 30-40% by weight of water were added, mixed until makes all components all through.

3. Feed compressing process.

This process was changed form of feed, soft and ground to pellet by pressure, heat and moisture in feed. Moisture and heat were compressed raw material hold on together, moisture in feed was lubricated feed components and heat from compressed was changed plant material to be the sticky component and holding other materials.

4. Feed drying process

Drying process is made feed have long time to keep by roasted or air-dried to eliminate moisture.

2.1.3 Fish feed formulation

Feed formulation should be considered nutrition requirement of fishes such as protein, lipid, carbohydrate, vitamin and mineral. Most of fish feed formulation is widely divided into 2 types of fish: herbivorous fish and carnivorous fish with different protein quality. Generally, feed formulation are compounded with more over 2 raw materials for correct nutrition requirement of fishes (Feed Quality Control and Development Division, Department of Fisheries, 2541)

Department of Fisheries has the standard for registering aquatic feed for controlled market-sell aquatic feed by considering nutrition requirement of aquatic animal.

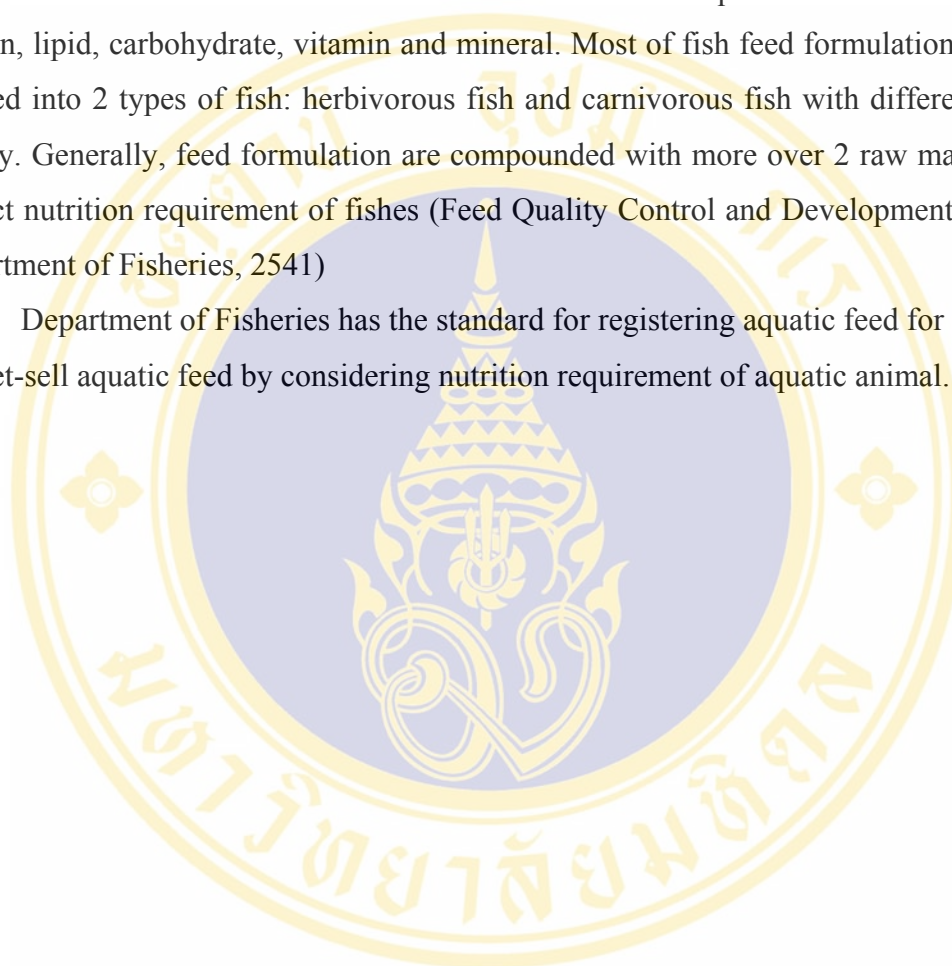


Table 2.1 The standard of proximate composition of commercial for registering aqua feed produce in Thailand

For	Characteristic	Size	Protein (%) More than	Lipid (%) more than	Refuse (%) lower than	Moisture (%) lower than
1. Walking catfish						
1.1 Fry walking catfish 1-2 week olds or length <3 cm.	Powder	Passed through 500 micron sieve >80%	35	5	6	12
1.2 Fingerling walking catfish 2 week olds or length 3-5 cm.	pellet	Diameter ≤ 2.5 mm.	35	4	6	12
1.3 Grow-out walking catfish 1-3 month olds or length >5 cm.	pellet	Diameter ≤ 4.3 mm.	30	4	6	12
1.4 Finisher walking catfish >3 month olds	pellet	No limited	28	3	8	12
2. Intensive herbivorous fishes						
2.1 Fingerling herbivorous fishes such as Nile tilapia 3-150 g.	pellet	No limited	28	3	8	12
2.2 Grow-out herbivorous fishes such as Nile tilapia 150-250 g.	pellet	No limited	25	3	8	12
2.3 Finisher herbivorous fishes such as Nile tilapia >250 g.	pellet	No limited	20	3	8	12

Feed Quality Control and Development Division Department of Fisheries has been formulated fishes feed by considering nutrition requirement of fish as following.

Table 2.2 The sample of herbivorous fish feed formulate

Raw material (% by weight)	Formula 1	Formula 2	Formula 3	Formula 4
Fish meal	25.0	10.0	-	12.0
Soybean	-	-	50.0	23.0
Peanut	-	20.0	-	-
Sesame	-	35.0	-	-
Rice bran	10.0	34.0	46.2	40.0
Ground bone	-	-	3.5	-
Broken rice	62.5	-	-	20.0
Horse leucaena leaf	-	-	-	4.0
Salt	-	0.3	0.2	-
Di-calsiumphosphate	-	0.5	-	-
Vitamins and Minerals	+	+	+	+

Note +: with the additional followed the suggestion on container

From :Feed Quality Control and Development, 2541

Table 2.3 The sample of nile tilapia feed formulate

Raw materials(% by weight)	Formula 1	Formula 2	Formula 3	Formula 4
Fish meal	12.0	5.0	-	8.0
Soybean	43.0	45.5	11.6	28.8
Ground broken rice	39.0	-	-	-
Corn or broken rice	-	30.0	-	59.4
Corn gluten	-	-	11.6	-
Vegetable or fish oils	1.9	-	-	-
Rice bran	-	17.0	-	-
Rape seed	-	-	19.8	-
Corn starch	-	-	15.0	-
Corn oil	-	-	6.0	-
Wheat bran	-	-	25.0	-
Alpha starch	2.0	2.0	-	2.0
Di-calsiumphosphate	2.0	1.0	-	1.5
Vitamins and Minerals	+	-	+	+

Note +: with the additional followed the suggestion on container

From :Feed Quality Control and Development, 2541

Table 2.4 The sample of carnivorous fish feed formulate

Raw material (% by weight)	Formula 1	Formula 2	Formula 3	Formula 4
Trace fish	50.0	-	50.0	49.5
Fish meal	7.0	20.0	17.5	10.0
Soybean	11.0	7.0	17.5	19.0
Peanut	-	-	17.0	-
Rice bran	-	-	5.0	-
Wheat bran	29.5	30.5	-	15.5
Wheat starch	-	-	2.5	-
Water	-	40.0	-	-
Fish's liver oil	-	-	-	3.0
Squid	-	-	-	1.0
Vitamins and Minerals	+	-	+	+

Note +: with the additional followed the suggestion on container

From :Feed Quality Control and Development, 2541

Table 2.5 The sample of walking catfish feed formulate

Raw materials,% by weight)	Formula 1	Formula 2	Formula 3	Formula 4
Fish meal	28.0	20.0	18.0	55.0
Soybean meal	23.5	-	12.0	12.0
Rice bran	10.0	-	36.0	10.0
Coconut meal	3	20.0	12.0	-
Peanut meal	-	10.0	-	-
Ground corn	28.5	-	-	12.0
Cassava starch	-	30.0	12.0	-
Broken rice	10.0	-	-	-
Ground horse leucaena leaf	-	-	9.0	-
Ami-ami	-	20.0	-	-
Di-calsiumphosphate	-	-	-	0.5
Vitamins and Minerals	+	-	+	+

Note +: with the additional followed the suggestion on container

From :Feed Quality Control and Development, 2541

2.2 Protein requirements

The growth in term of increasing of weight and size are indicated by increase of structural tissue, muscle and bone (Watanabe (1988) ,cited by Lalu, 2003). Quality of diet for growth are sufficient amount in nutrient and able to be digested and absorbed for providing enery and substrate for growth of aquatic animals. The parameters are used to determine the value of feed for providing the necessary energy for growth include feed efficcency rate, protein efficcency rate and net protein utilization. Protein is main essential nutrient for maintaining life and promoting organs building for growth and health.

Protein is the principle constituent of the tissues and organs of the aquatic animal bodies and also of other nitrogen compounds such as, nucleic acid, enzymes, hormones, vitamins and minerals, continuous supply is required in the food for growth and repair of decomposed tissue protein. The optimal dietary protein level is also affected by the digestibility of the dietary protein and the nature of the non-protein energy source in the diets (De Silva and Anderson, 1995 cited by Lalu, 2003) .

Protein is a complex molecular consists of carbon (50-55%), hydrogen (5-7%) and oxygen (20-25%) in common with the lipid and carbohydrate (Chuapoehuk, 2542). Quality of protein in diets are different by the quality and quantity of animo acid and other bioavailability. The high quality protein for animal are refer to useable protein in feed that consistent with the amino acid requirement in animal (Vorrajanra and Sittikripong, 2539).

The amino acid requirement in fish is 10 indispensable amino acids such as argenine, histidine, isoleucine, methionine, phenylalanine, threonine, tryptophan and valine and 9 dispensable amino acids such as alanine, sapatatic acid, cystine, glutamic acid, hydroxyproline, proline, sarine and tyrosine (Chuapoehuk, 2542).

The demand of dietary protein is higher during the larval and fingerling stages (<10cm.) because it is believed that the protein and evergy requirements on a unit mass basis are higher in the early stages of life and the highest on relative weight gain (specific growth) is achieved in the fingerling stage. It is important to ensure the full growth potential is realized during this stage of development for all culture systems. The highest protein requirements in the initial feeding of fry and decrease as fish increase (De Silva and Anderson, 1995 cited by Lalu, 2003).

2.2.1 Effects of fish growth by amino acids

Balance of amino acids are effected to protein quality especially (Vorrajanra and Sittikripong, 2539), but amino acids requirement are depended on type, size and age. Ogino (1980) (cited by Vuttiapanchai, 2537) reported that amino acid balance of fish could estimated by analyzed and calculated from the whole amino acid in promptly killed fish following the principle that the whole amino acid of fish are fish requirement according to table 2.6

Table 2.6 The amino acid requirement of fishes

Indispensable amino acid	Essential Amino Acid (EAA) (% amino in protein)			
	Carp ¹	Canel catfish ²	Japaness eel ²	Chinook salmon ²
Arginine	4.3	4.3	3.9	6.0
Histidine	2.1	1.5	1.9	1.8
Isoleucine	2.5	2.6	3.6	2.2
Leucine	4.3	3.5	4.8	3.9
Lysine	5.7	5.0	4.8	5.0
Methionine (+ Cystine)	4.1	2.3	2.1	4.0
Phenylalanine (+Tyrosine)	6.3	5.0	2.9	4.1
Threonine	3.9	2.2	3.6	2.2
Tryptophan	0.8	0.5	1.0	0.5
Valine	2.9	3.0	3.6	3.3

Note: ¹Ogino, 1980 cited by Sarmwatanakul and Bamrongtum 2000

²Vuttiapanchai, 2537

The imamino acid balance in feed are effected growth and feed consumption , because of the low level of in dispensable amino acids in blood are reduced other amino acid and are excreted that interfered protein synthesis in liver (Srisakul and Ronnachai, 2539).

2.2.2 Relation of protein and energy sources in fish feed

Carbohydrate and lipid are used as source of energy and oil-soluble vitamin, hold the feed materials together, and reduce the rate at which a feed will dissolve in water. A good binder will minimize wastage of feed. The presence of some carbohydrates and lipid may also enhance the texture and palatability of feeds formulation. Dietary energy sources are important for normal growth, development, that was obtained from carbohydrate, lipid and protein. 1 gram of carbohydrate, lipid and protein was carried 2 kcal., 8kcal. and 4 kcal. respectively. Under the condition where energy intake is inadequate, dietary protein are used as an energy source. Cowey (1978) cited by Das *et al.*, (1984) observed that unless sufficient dietary protein is wasteful and stresses the animal while excess energy means more fatty fish. Thus optimum protein to energy (P/E) ratio in diet is very important to maintain fish quality and to reduce dietary cost.

2.3 Feather meal

Feather meal are the by-product from poultry processing industry, may be utilized for fish feed. (Tsang *et al.*, 1963 cited by Sinchermsiri *et al.*, 1992) An average composition is 81% protein, lipid 3.45%, fiber 1.4%, calcium 0.57% and phosphorus 0.31%. Sinchermsiri *et al.*, 1992 reported that feather meal produce in Thailand have estimate digestible dry matter 84.60-90.53%, digestible protein 27.83-64.75%, and have nearly indispensable amino acid to soy bean meal and fish meal according to table 1.1

2.3.1 Feather meal production process

Protein in feathers is complex protein, which broken down by hydrolysis to make them digestible. Most of production have similar principle, after collection from processing plant then washed with water, after that wet-cooked for hydrolysis under pressure and then dried, after that ground (Pongpiajun, 2539). Different process resulted to the quality of feather meal.

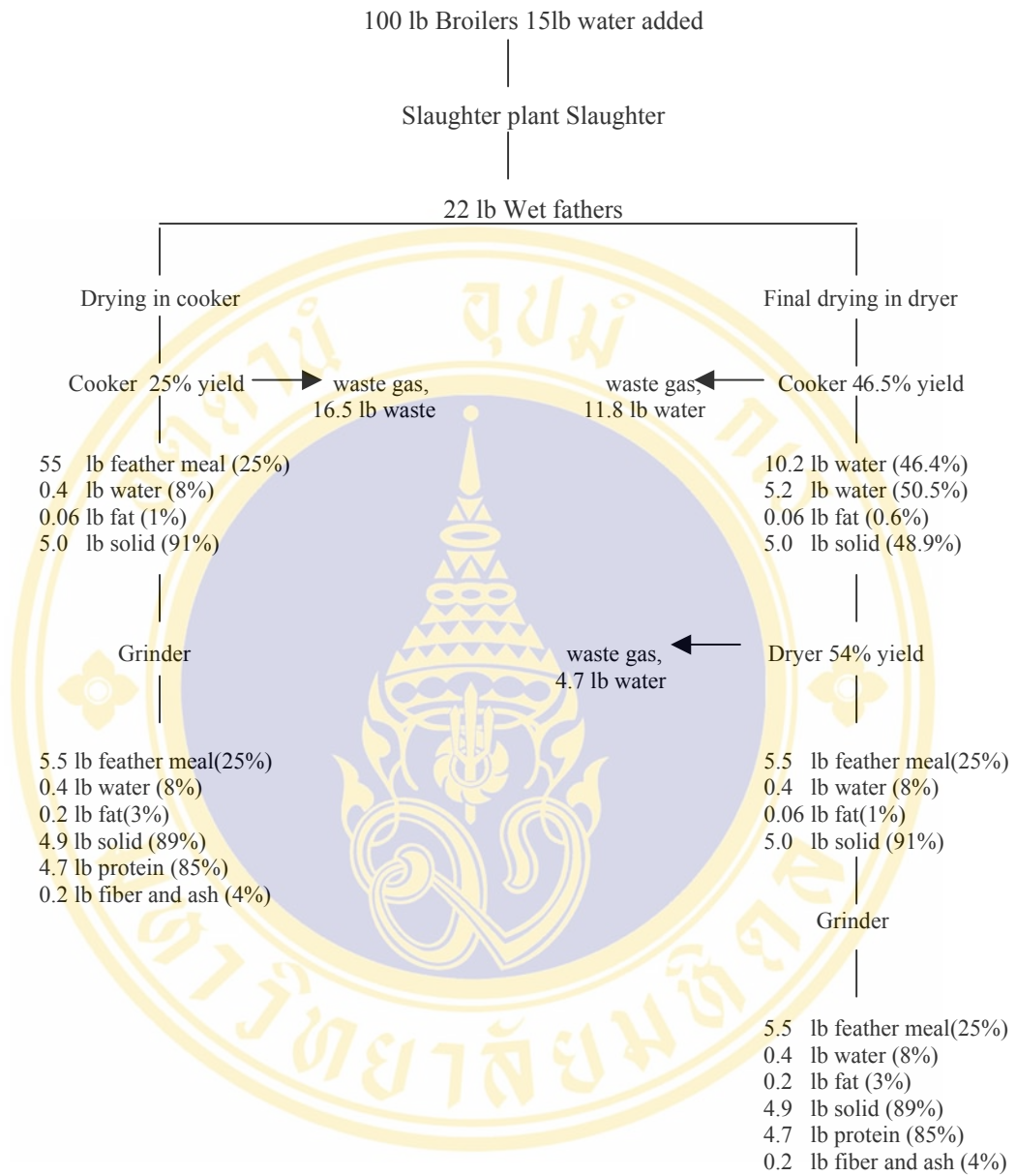


Figure 2-1 Broiler feather by-product processing (Lortscher *et al.*,1957)

Dried feathers before steamed at pressure 35 pound / inch² for 1 hour. The feather are then dried and then ground. Feather meal from the process, in animal feed, have better growth than other processes (Sullivan and Stephenson, 1957 cited by Pongpiajun, 2539)

Steamed at pressure 50 pound / inch² 30-40 minutes, dried and ground (Tsang *et al.*, 1963 cited by Pongpiajun, 2539)

Steamed at pressure 30 pound / inch² at 134⁰C for 2-5 hours, dried by oven at 60⁰C after that ground (McCasland and Richardsom, cited by Pongpiajun, 2539)

Steamed feather at pressure 40-50 pound / inch², stired 1 minute every 2 minutes for 30-60 minutes, dried by oven and ground. Feather meal from this process has better quality than feather meal that steamed at pressure 50 pound / inch². Staired feather, while dried by oven resulting in higher net protein and higher lysine, methionine and histidine (Moriss and Balloun, 1973. cited by Pongpiajun, 2539)

2.3.2 Feather meal nutrition for using in animal feed

Feather meal were composed of 80% complex protein (kelatin), which have a lot of sulfide bound from cysteine resulting in hard digested by fish (Yaowapak and Wongkaew, 1996) but from pepsin enzyme digestion found that feather meal have > 75% digestable protein and 60% total protein in feather meal.

Quantity of digestable protein from feather meal, soybean meal, uria and cotton seed meal have no significant difference (Punsri, 1987; Punsri *et al.*, 1987; and Aderibigbe and Church, 1983 (cited by Punsri P, 1994).

A suitable partial feather meal replacement fish meal in animal feeds not effected, but using for only one protein source in feed must be supplemented with some amino acid (Punsri, 1994, Yaowapak and Wongkaew, 1996).

The imamino acid balance of feather meal for using in fish feed, which compare with fish meal some indispensable amino acid can replaced by dispensable such as methionine (indispensable amino acid) that are composed of sulfer as cystine (dispensable amino acid). In fishes, 2 parts of cystine can replace 1 part of methionine also have no effected in growth (Setasitti *et al.*, 2527)

2.4 Fermentation

Fermentation is chemical changing from metabolism of microorganism in complex organic compound; products of metabolism are deffered by microorganism reaction (Jarernjiratrakul, 2539).

2.4.1 Chemical changing protein compound during fermentation

Protein compounds are hydrolyzed by enzyme from self-digestion and microorganism to be poly-peptide, peptide or amino acid under sufficiently oxygen condition before used as nitrogen source. Unsufficiently oxygen condition will produced ammonia, amine, indose, skatal, fatty acid and sulfer compounds that resulting in rank smell (Jarernjiratrakul, 2539).

Protein hydrolyzation found in metabolism of fungi such as *Aspergillus*, *Rhizopus* and bacteria such as *Clostridium*, *Bacillus*, *Pseudomonas* and *Proteus* (Kungsuwan *et al.*, 1996).

2.4.2 Fermentation in feathers

Feathers under optimum condition (room temperature and moisture more than 20%) was digested by Keratinophilic microorganism such as *Thrichophyton* sp., *Aspergillus* sp., *Rhizopus* sp. and certain bacteria such as *Bacillus* sp. *Kellatiaophilic* and Certain bacteria generally found in soil, animal skin (Michale, 2001).

2.5 Traditional cultured freshwater fish

In Thailand, the traditional freshwater fishes cultured are divided by characteristic into 2 groups as following.

1. Herbivorous fishes such as Nile tilapia, Snakeskin guarami, Striped catfish, Mud carp and Giant guarami.
2. Carnivorous fishes such as Walking catfish, Snakehead, Sea bass, Spotted featherback and Sand goby.

2.5.1 Nile tilapia (*Oreochromis nilotica*)

The Nile tilapia is one of the genus *Oreochromis* of the family Cichlidae that is widely distributed in Africa region from Sudan, Jordan, tilapia live in freshwater such as streams, lakes, ponds and canals. General characteristics as follow: Body rounded, depressed, length from snout to tail was 3 times of high, 4 rows small scale on operculum, upper and lower lips were same level. Dorsal and anal fins with many spines and soft rays, pectoral fin rounded. Color either were brown / green with 9-10 stripes, white spots and bar on fin (Department of Fisheries, 2545).

The protein requirement of Nile tilapia were differed by age, fry tilapia (weight <1 g.) require 40-50% protein in diet, sub-adult tilapia (weight 1-10 g.) require 28-35% protein in diet, adult tilapia (weight >30 g.) require 28-30% protein in diet. In present days, tilapia pond culture were divided into 4 types (Chuapohuk, 2542).

One generation culture same size of fry tilapia. In 6-12 months the fish reach the market size, all of fishes were harvested.

1. Several generation cultures in same pond. Only market size tilapia were harvested.
2. Polyculture with other fish such as striped catfish, common silver barb and other carnivorous fishes that for eliminate unwanted tilapia fry such as snakehead fish, spotted featherback fish.
3. Monosexual tilapia culture, result of tilapia mature at early age and reproduce proficially, the presence of both sexes in the culture system often causes overpopulation at the latter part of the production season. Being the mouth

brooder, female tilapia normally grow much more slowly than male once they become sexually mature.

Mono culture tilapia in pond should have size ranged from 0.5-3.0 rais, and should have several ponds for continuous harvesting. Before using pond should eliminated pond weed and other carnivorous animals such as fishes, amphibian, reptile. 3-5 cm. tilapia fry were stocked in pond at 1-3 fish/m.² The diets should be fed 5% by-wight of fish and there are some suggestion to filled other cabohydrate feed such as broken rice (fed 5% by-wight of fish) and weed such as duck weed, water hyasinth and natural fertilizer such as manure for produce the natural feed (phytoplankton, insect larva). The product from this culture were mean weight 500 g. and were harvested at 5-6 months of culture (Department of Fisheries, 2545).

2.5.2 Walking catfish (*Clarias* sp.)

The walking catfish is one of the genus *Clarias* of the family clariidae that is widely distribution in Southern Asia region to South Eastern Asia region from India Mianmar Veitnam Thailand and Malaysia, walking fish live in freshwater such as lakes, ponds and irrigation ditches .General characteristic as follow: Body elongate, ell like, scaleless, 8 barbels around terminal mouth, upper lip longer than lower lip, with abolescent organ in skull. Dorsal and anal fins with many soft rays, pectoral fin rounded with 1 spine. Color either were brown / grey with or without white spots on body and fin (Department of Fisheries, 2545).

Traditional walking catfish (*Clarias* family) that was culture in Thailand are as followed:

1. *Clarias macrocephalus* Gunther
2. *Clarias batrachus* Linnaeus
3. *Clarias garipeynus*
4. Hybrid of *Clarias garipeynus* x *Clarias macrocephalus*

The protein requirement of walking catfish were differed by age, fry walking catfish (length 2-4 cm.) require 35-40% protein in diet, walking catfish (length 5-6 cm.) require 25-30% protein in diet, adult (breeding-mature) walking catfish require 28-32% protein in diet (Chuapoehuk, 2542).

In present days, walking catfish could be culture in concrete tank and pond. The water level of culture in pond was depth ranged 1.2-1.5 m. In concrete tank walking

catfish fry were stocked in pond at 50-70 fish/m², and in pond should eliminated pond weed and other carnivorous animals such as fishes, amphibian, reptile. Walking catfish fry (2-3cm.) were stocked in pond at 40-100 fish/m². The wet-diets should be fed 3-7% by-wight of fish and then partial turn to artificial feed and there are some suggestion to added trashfish or other animal parts. The product from this culture were mean weight 300-400 g. and totally product are 5-12 ton/rai (Department of fisheries, 2545).

2.6 Relevant research

Punsri (1991) reported that the digestibility and growth performance of wether sheep averaging 30 kg. fed with a 3 experimental diets; feather meal (HFM), soybean meal (SBM) and urea diet (U). Crude protein digestibility was significantly less for HFM (76.85%) diet than those for SBM (84.92%) and U (88.75%) diets ($P < 0.05$). Crude fiber digestibility in HFM (65.06%) diet was significantly greater than SBM (58.02%) and U (40.76%) diets ($P < 0.05$). Nitrogen retention and biological value were significantly higher for animal fed on HFM diet comparing with SBM and U diets ($P < 0.05$). The results indicated that feather meal was more achieved as a nitrogen source for sheep production.

Punsri (1991) reported that feather meal from different processes; common steamed feather meal (CHFM), autoclaved 45 minutes feather meal (HFM-45) and autoclaved 90 minutes feather meal (HFM-90) in native goat diet. Urea was supposed as a control diet. Crude protein digestibility was significantly less for CHFM than those with HFM-45, HFM-90 and U diets ($P < 0.05$). Average daily gain per day was significantly higher for HFM-45 and HFM-90 than those with CHFM and U diets. It was evidenced that the processing method could be effective protein utilization in ruminants. In addition HFM-45 was considerably greater utilized as a protein source for ruminants comparing with the other processing methods of feather meal.

Langar and Metailler (1989) studied on the replacement of fish meal by blood meal, hydrolyzed feather meal and meat meal in sea bass (*Dicentrarchus labrax*) diets. The results were presented of experienments: specific growht rate, feed conversion, protein efficiency ration, and protein utilization coefficient were used to estimate the food

quality. Findings showed meat meal at a level allowing 30% of dietary crude protein in complement of fish meal to be promising for cultured sea bass diets.

Fowler (1990) studied on the usage feather meal in juvenile fall chinook salmon (*Oncorhynchus tshawytscha*) diets, feather meal was substituted at levels of 0, 5 and 15% for portions of the fish meal in the diet (protein 45% and DE 3,880kcal/kg.diet). Weight gains and condition factors of the fish did not differ at the end of the experiment. Gill ATPase activity began increasing after 16 weeks of feeding and was highest in the fish fed the diet with 15% feather meal. Plasma thyroxine levels peaked before the increase in ATPase; fish fed diets with no feather meal maintained higher levels after peaking than did the other two diet groups. Regulation of plasma sodium levels after a seawater challenge was best in May. Starvation for 1-3 weeks adversely affected ATPase, thyroxine, plasma sodium regulation, and condition factor.

Shearer and Hardy (1987) studied on combination feather meal with whole Pacific hake (*Merluccius productus*) and hake filler scrap were ground, heated to accelerate enzymatic hydrolysis, and acidified to prevent microbial degradation. Half of the fillet scrap was screened to reduce the bone content of the product. After 10 d of storage, the liquefied products were combined with feather meal and dried. The dried products replaced fish meal in practical diets. Significant reductions in plasma phosphorus and whole-body and vertebral calcium, phosphorus, and magnesium concentrations were found in rainbow trout fed the diet containing the deboned fillet scrap, while fish fed a phosphorus-supplemented diet had levels similar to fish fed the diets containing whole fish. Plasma alkaline phosphatase activity was unaffected by diet. Rainbow trout fed a diet containing unscreened fillet scrap were similar in all respects to fish fed a diet containing whole fish.

Hardy *et al.*, (1984) studied on the nutrition of co-dried fish silage in rainbow trout (*Salmo gairdneri*) dry diet. The feed ingredients containing fish silage and liquided fish made from ground, whole Pacific whiting and co-dried in vacuum dryer with mixture of soybean meal and feather meal to facilitate drying were prepared. An additional batch of fish silage was co-dried with the other dry ingredients in diet formulation that was used, Abernathy diet S8-1. fish meal, made by vacuum drying Pacific whiting, was used in the control diet. Co-dried fish meal, made by the co-drying Pacific whiting with a soybean meal –feather meal mixture . Fish meal was entirely replaced by the co-dried products in

the experimental diets, which were fed to rainbow trout (*Salmo gairdneri*) for 32 weeks. The best growth and food conversion value were obtained by feeding the fish meal diet or diet in which the fish meal was replaced with co-dried liquefied fish. No significant differences in the final weight were found between trout fed diets containing co-dried fish meal or co-dried fish silage (fish products were 25% of the diets), but these fish were significantly smaller than fish fed the fish meal control or the co-dried liquefied fish diets. Reducing the fish silage to 12.5% or increasing to 50% further reduced weight gains in the trout. Food conversion values, protein efficiency ratio and net protein utilization value generally followed the same trends between diets as did the final weight values. Apparent digestibility coefficients for the co-dried products were lower than for the fish meal, possibly because they obtained soybean meal-feather meal mixtures. Organoleptic properties of the fish were not affected by diet.

Wiesmann *et al.*, (1988) studied on water pollution with phosphorus of dietary origin. The analysis of water entering and leaving nine commercial trout (*Salmo gairdneri*) farms at three times during summer showed that concentrations of orthophosphate in the water may increase substantially during passage through the farm. Growth rates and conversion ratios were not impaired concentrations of semi-purified trout diets were reduced from about 10 to about 4 g per kg dry matter. It may thus be practical to limit P concentrations of trout feed to less than 10 g/kg. Economically attractive performance and comparatively little pollution with phosphorus may result from a feed containing 30% low-ash fish meal, 20% hydrolysed feather meal, 37% hydrolysed wheat and 11% fat.

Wiesmann (1986) studied on combination feather meal with different wheat and corn cereals as feed for rainbow trout (*Salmo gairdneri*). Corn starch substituted by casein, hydrolyzed feather meal or hydrolyzed wheat was found to increase the growth in case of controlled ad libitum feeding. With increased casein ratios, trout reached 300 g market size within approximately equals 120 days, instead of 178 days with the corn starch diet. With increasing raw protein content of the feed the energy utilization also increased. Contrarily, the protein conversion reached a maximum at 35% raw protein content.

Hilge (1984) studied on different protein sources in diets. Four freshwater fish species (*Cyprinus carpio*, *Silurus glanis*, *Ictalurus punctatus*, and *Tinca tinca*) were used for experiments on feeding, growth, food utilization and body composition. Two series of

9 rations each with similar, graduated raw protein and raw fat levels, but of different protein sources served as experimental feeds. The optimum protein level for carp and the European catfish is between 40 and 50%, while the raw fat level is near 13-14% for carp, but below 10% for the catfish. The latter exhibits the same or even better growth when 50% of a fish meal – feather meal – poultry by product meal mixture is replaced by field bean/corn gluten meal or by whey 25-28 degree C are the most favourable range for growth of the European catfish. In the American and the European catfish the edible part is about 50% of the total body weight.

Viola and Zohar (1984) studied on different protein levels in diet in Nile tilapia protein levels of 25%, 30%, 35% were tested in three series of pond trials with hybrid tilapia (*Oreochromis*). Growth rates and feed conversions were not significantly different in the first series. In the two later series growth rates were increased significantly for the 30% ratio. The 35% ratio excelled significantly in summer only. Body fat was found to increase toward autumn and also with the size of fish. Poultry meal, “Proteen” and cottonseed meal as replacements for 10% fishmeal sustained growth at an equal rate. Feather meal at this level of replacement reduced growth by 7%. Urea reduced growth proportionately to its rate of inclusion and apparently was utilized very little, if at all.

Markert *et al.*, (1984) studied on different protein sources in dry diets (less than or equal to 10% moisture) rather than Oregon moist pellets (OMP; 30% moisture) for coho salmon culture in B.C. hatcheries, juvenile coho salmon *Oncorhynchus kisutch* of about 2.4 g initial wet weight were fed one of two West Vancouver Laboratory dry diets (WV diets) or OMP at one of two ration levels for 301 days. The WV diets contained 25 (WV1) or 50 (WV2) % of dietary protein from a mixture of poultry-by-product meal and feather meal by replacement of herring meal. All diets had similar protein and metabolizable energy contents. It was concluded that there was high potential for using dry diets in coho salmon culture provided that the available levels and balance of nutrients in dry diets were similar to those in OMP and good diet manufacturing practices were employed.

Schulz *et al.*, (1982) studied the substitution with the combination of blood-feather meal in rainbow trout (*Salmo gairdneri*). The rainbow trout were given feed combinations where casein-gelatine-protein (75:25) of a standard diet was gradually replaced by blood-feathermeal-protein (50:50) (1/3, 2/3 and 3/3 substitution). All groups of rainbow trout reached nearly two to three time increase of the initial body weight of 23 g. Using the

standard diet a feed efficiency of 0.86 (PER=2.33) on an average was reached with the feed mixture containing 100 percent blood meal-feather meal-protein a feed efficiency of 1.62 (PER=1.24) was attained. No pathological changes of any organs were detected. The quite impressive efficiency rates of blood meal and feather meal providing 1/3 or 2/3 of the total protein in the standard diet (feed efficiency 0.89 and 1.03; PER 2.25 and 1.94) show the suitability of these products of animal origin as components for rainbow trout diets.

Kikuchi *et al.*, (1994)(cited by Somserb; Boonyaratpalin, 2001) reported that the replacement percentage of fish meal by 0, 12, 25, 37 and 50% feather meal with addition amino acid tryptophan 0.3%, metionine 0.5%, lysine 1.0% and histidine 0.3% resulted hematocrit, glucose and calcium were not significantly different from control.

Somsueb; Boonyaratpalin (2001) reported that walking catfish (*Clarias* sp.) fed with diet containing 0-5% feather meal had better ($P < 0.01$) weight gain than fish fed with diet containing 7.5-10% feather meal. The amount of feed consumed by fish fed with diet containing 7.5-10% feather meal diet was significantly lower than fish fed with diet containing 0-5% feather meal ($p < 0.05$). Protein consumed, feed conversion ratio and protein efficiency ratio were better in fish fed with diet containing 0-5% feather meal than fish fed with diet containing 7.5-10% feather meal ($P < 0.01$). Apparent net protein retention in fish fed with diet containing 0-5% feather meal were higher than fish fed with diet containing 7.5-10% feather meal ($P < 0.05$). Protein digestibility of feather meal by hybrid catfish was 79.38%. The survival rate and body composition of fish were not significantly different ($P > 0.05$) in all treatments. This study suggested that the maximum dietary feather meal level for hybrid clarias is 5%

CHAPTER III

MATERIALS AND METHODS

The study on replacement of fermented feather meal in for fish meal in fish were investigated on fish feed production process, fish culture, feed quality, growth and feed efficiency. The research was conducted at experimental unit of Feed Quality Control and Development Division Department of Fisheries at Pranakornsri Ayutthaya province. Experimental result was analysed at Laboratory of Feed Quality Control and Development Division, Department of Fisheries. Blood samples were sent to Blood Research Unit of Ramathibodi hospital for analysed Tyroxin hormone and Triiodotyronine (T4, T3).

3.1 Experimental planing

The experimental was divide into 2 types of fish, each fish had 5 treatments. Each treatment was feed with different diets that were formulated from pratrical ingredients with 0, 25, 50, 75 and 100% of fermented feather meal replaced fish meal. The diets were isotrogenous (30%) and isocalorics (400kcal GE/100g. diet). Experimental fishes were fed with experimental diets in 120 liters glass aquarium with close flow though systems with aeration, and then experimental diets and fishes sample were collected for composition analyzed. The formula of experimental diets were shown in table 3.1.

Table 3.1 Experimental diet formula of Nile tilapia

Raw material	Diet formula and percentage of fermented feather meal replacement of fish meal				
	Formula 1. (0%)	Formula 2. (25%)	Formula 3. (50%)	Formula 4. (75%)	Formula 5. (100%)
Fish meal	25	18.75	12.5	6.25	-
Feather meal	-	4.4	8.8	13.3	17.7
Soybean meal	30	30	30	30	30
Ground rice broken	15	15	15	15	15
Cassava powder	10	10	10	10	10
Alpha starch	6	6	6	6	6
Vegetable oil	1.3	1.3	1.3	1.3	1.3
Fish oil	1.0	1.4	1.9	2.3	2.7
Lard	-	0.4	0.6	0.9	1.6
Di-calcium phosphate	1.53	1.43	1.63	1.96	2.24
Vitamins & minerals	2	2	2	2	2
Ground husk	7.67	8.82	9.77	10.49	10.96
Yeast cake	0.5	0.5	0.5	0.5	0.5

Note: Five diets were formulated to contain 30% crude protein and Gross energy 400kcal/100g.diet

Table 3.2 Experimental diet formula of walking cat fish

Raw material	Diet formula and percentage of fermented feather meal replacement of fish meal				
	Formula 1. (0%)	Formula 2. (25%)	Formula 3. (50%)	Formula 4. (75%)	Formula 5. (100%)
Fish meal	25	18.75	12.5	6.25	-
Feather meal	-	4.4	8.8	13.3	17.7
Soybean meal	30	30	30	30	30
Ground rice broken	15	15	15	15	15
Cassava powder	10	10	10	10	10
Alpha starch	6	6	6	6	6
Vegetable oil	1.3	1.3	1.3	1.3	1.3
Fish oil	1.0	1.4	1.9	2.3	2.7
Lard	-	0.4	0.6	0.9	1.6
Di-calcium phosphate	0.92	0.78	1.11	1.34	1.63
Vitamins & minerals	2	2	2	2	2
Ground husk	8.28	9.47	10.29	11.11	11.57
Yeast cake	0.5	0.5	0.5	0.5	0.5

Note: Five diets were formulated to contain 30% crude protein and gross energy 400kcal/100g.diet

3.2 Experimental procedure

The study was divided into 4 parts.

3.2.1 Production of experimental diet that using fermented feather meal, and fish meal for protein source.

3.2.2 Experimental fish culture

3.2.3 Nutrition analysis of experimental diets.

3.2.4 Nutrition analysis of experimental fish was fed with experimental diets.

3.2.1 Production of experimental diet that using fermented feather meal, and fish meal for protein source.

Preparing fermented feather meal and mixing other raw material as followed

1. Ferment feather meal with yeast cake and cassava powder ($x\%:0.5\%:10\%$ (x = percentage of feather meal in feed formula)) and then add 30% water (by weight), staired every 4 hours for air transfer, fermented for 48 hours.

2. Formulate 5 experimental diets that contained 30% protein and 400 kcal GE/100 g. diet.

Formula 1 100% fish meal : 0% fermented feather meal as feed control

Formula 2 75% fish meal : 25% fermented feather meal

Formula 3 50% fish meal : 50% fermented feather meal

Formula 4 25% fish meal : 75% fermented feather meal

Formula 5 0% fish meal :100% fermented feather meal

Mixed raw materials in table 3.1 and 3.2 in homogenous mixture, and then adding oil. Subsequently, water around 30% was added to dry mixture and blened until it becomes dough like paste.

3. Moist feed mixture were passed through a meat grinder with die size of 3 mm. and dried in open air then broken into small crumble. Dried pellet diets were stored in airtight plastic bags at $-4\text{ }^{\circ}\text{C}$



Figure 3-1 Raw materials were mixed in a meat grinder



Figure 3-2 Moist feed mixture were passed through a meat grinder



Figure 3-3 Feed were dried in open air

3.2.2 Experimental fish culture

1. Nile tilapia (hormonal treated) received from private company with average weight 120 ± 5 gram were weaned to experimental diets about 7 days period before the start of the feeding trial.

2. 20 fishes were randomly sampled, stocked individual in 120 liters glass aquarium with close flow through systems and aeration. Fish was fed 2 times a day at 9.00 and 16.00 until stop eating. Leftover diets were collected by siphonation, dried and weighed and then minus from eaten diet weight every 2 weeks.

3. Fishes were counted and weighed every 2 weeks. Diets were not fed on morning period of weighing day.

4. Observation of water quality in the tank was conducted. Temperature was measured every day at 9.00 where as dissolved oxygen (DO) by titration method, pH by pH meter, Alkalinity by titration method, Hardness by EDTA titration method, Dissolved Ammonia by Indophel Blue Colorimetry method.

5. At the end of experiment, 3 fishes of each treatment were randomly sampled for analyses carcass composition and collected 3 ml. blood for analyse level of thyroxin hormone (T4) and triiodotyronine (T3) in blood, which were presented of thyroid activity of fish and assume that thyroid activity of fish were caused from obtain low quality dietary protein.

Repeat the experiment by changing nile tilapia to hybrid walking catfish (*Clarias macrocephalus* x *Clarias gariepinus*) with initial mean weight 70 ± 5 g.



Figure 3-4 Experimental fish culture



Figure 3-5 Weighing of experimental fishes

3.2.3 Nutrition analysis of experimental diets.

Diets were randomly sampled, dried, blended in size that passed 200 micron mesh sieve Subsequently homogenize and then stored in airtight containers at 18 °C until analyzed. (Setasitti, 2527).(Appendix B)

1. Crude Protein by Kjeldahl Nitrogen.
2. Crude Lipid by Ether Extraction
3. Total Ash by Muffle Furnace Combustion
4. Crude Fiber by Weede Method
5. Carbohydrate by NFE equation

$$\text{NFE} = 100 - (\% \text{ moisture} - \% \text{ protein} + \% \text{ lipid} \% \text{ ash} 1\% \text{ fiber})$$

6. Gross Energy as follows.

$$\text{GE} = (\% \text{ NFE} \times 4.11) + (\% \text{ protein} \times 5.64) + (\% \text{ lipid} \times 9.44)$$

3.2.4 Nutrition analysis of experimental fish was fed with experimental diets.

Fish growth and feed utilization of this experiment were evaluated every two weeks and at the end of all experiments as following (Tinnungwatana and Viputhanumas ., 2000, Jantrarotai *et al.*, 1996, Somsueb and Boonyaratpalin , 2001 and Chuapoehuk, 2542)

1. Percentage of weight gain (%)

$$= \frac{(\text{Mean final fish weight} - \text{Mean initial fish weight}) \times 100}{\text{Mean initial fish weight}}$$

2. Daily mean weight gain or average daily gain (g/ind/day)

$$= (\text{Mean final fish weight} - \text{Mean initial fish weight}) / \text{culture period}(\text{day})$$

3. Survival rate (%) = (Final number of fish / Initial number of fish) × 100

4. Feed conversion ratio (FCR) = Total feed consumed / Increase mass fish

5. Protein efficiency ratio (PER)

$$= \text{Increasing weight in mass fish} / \text{Protein intake in diet}$$

6. Apparent net protein retention (ANRP) = $\{[(W_1 \times \%P_1) - (W_0 \times \%P_2)] / P\} \times 100$

$$W_1 = \text{Mean final fish weight (g.)}$$

$$W_0 = \text{Mean initial fish weight (g.)}$$

$$P = \text{Protein intake in diet}$$

$$P_1 = \text{Final percentage of protein in fish}$$

$$P_2 = \text{Initial percentage of protein in fish}$$

7. Protein intake = Quantity of diet consumption × % Protein in diet

8. Carcass composition of fishes at the beginning and the end of experiment were observed on crude protein, crude lipid and crude fiber content as following (Appendix B)

1. Crude Protein by Kjeldahl Nitrogen.

2. Crude Lipid by Ether Extraction

3. Crude Fiber by Weede Method

9. The level of thyroxin hormone (T4) and triiodotyronine (T3) in blood of fishes were evaluated at the end of all experiments by collect blood from the caudal vein of three fishes from each treatment around 3 ml. and sent to Blood Research Unit of

Ramathibodi Hospital for analysed Thyroxin hormone and Triiodotyronine (T4, T3) to search for unusual thyroid activity, which effected to growth and metabolism. Thyroid activity were depressed by low protein and low calories diets (Higg and Eales, 1979 cited by Fowler, 1990) and Plasma T4 is affected by type of dietary lipid (Leatherland *et al.*, 1977 cited by Fowler, 1990).

3.3 Data collections and analytical methods.

Experimental data were collected and assigned in ANOVA (Analysis of Valiance) with 5 treatments and 3 replications in each treatment. The DMRT (Duncan's Multiple range Test) were used to determine the differences between the treatment means. The alphabetical mutations a, b, c, d, e, f) were used to mark the differences at significant level of an alpha 0.05. All data were analyzed by using a SPSS program.

CHAPTER IV

RESULTS

The study of utilization fermented feather meal replacement of fish meal for protein source in fish feed was conducted for 12 weeks in Nile tilapia (*Oreochromis nilotica*) and for 8 weeks in walking catfish (*Clarias* sp.). This experiment was studied for evaluated the effect of totally and partially replacement fermented feather meal of fish meal in fish feed on fish feed quality, growth and feed utilization. The results of this study were presented as following.

- 4.1 Quality of experimental diets.
- 4.2 Growth and feed efficiency
- 4.3 Water quality in the experimental
- 4.4 Cost of experimental diets

4.1 Quality of experimental diets.

4.1.1 Physical characteristic of experimental diets

All diets had same characters that cylindrical 3 mm. diameter, 5 – 10 mm. length, light- brown, breakable by fingers, suddenly sink in aquarium and stand in water about 5 – 8 minuets.

4.1.2 Composition of experimental diet

1. *Tilapia experimental diet*

From analyses proximate composition of experimental diets containing fermented feather meal substituted for fish meal in nile tilapia was presented in Table 4.1

Table 4.1 Proximate composition of nile tilapia experimental diet with different ratio fermented feather meal replacement of fish meal

Proximate analyses	Formula 1	Formula 2	Formula 3	Formula 4	Formula 5	Commercial feed (30% protein)
Protein (%)	29.51	30.38	29.05	29.73	28.48	30
Lipid (%)	3.48	4.27	4.64	4.81	5.77	≥ 4
Fiber (%)	5.91	5.99	6.42	6.65	6.94	≤ 6
Ash (%)	10.44	9.10	7.73	6.71	5.35	-
Moisture (%)	15.10	12.94	15.18	14.19	16.15	≤ 12
Gross Energy (kcal/100g.diet)	345	365	359	368	368	-

According to Table 4.1 the contents of crude protein in the experimental diet 1, 2, 3, 4 and 5 were 29.51%, 30.38%, 29.05%, 29.73% and 28.48% respectively. The crude lipid in the experimental diet 1, 2, 3, 4 and 5 were 3.48%, 4.27%, 4.64%, 4.81% and 5.77% respectively. The fiber in the experimental diet 1, 2, 3, 4 and 5 were 5.91%, 5.99%, 6.42%, 6.65% and 6.94% respectively. The gross energy of the experimental diet 1, 2, 3, 4 and 5 were 345, 365, 355, 368 and 368 kcal/100 g. diet respectively.

2. Walking cat fish experimental diet

From analyses proximate composition of experimental diets containing fermented feather meal substituted for fish meal in Nile tilapia was presented in Table 4.2

Table 4.2 Proximate composition of walking catfish experimental diet with different ratio fermented feather meal replacement of fish meal

Proximate analyses	Formula 1	Formula 2	Formula 3	Formula 4	Formula 5	Commercial feed (30% protein)
Protein (%)	31.17	30.40	31.35	31.36	31.18	30
Lipid (%)	2.86	4.70	5.18	5.59	6.23	≥ 4
Fiber (%)	6.51	5.80	6.35	6.89	6.92	≤ 6
Ash (%)	11.38	9.41	8.65	7.35	6.29	-
Moisture (%)	9.61	11.44	9.78	8.61	8.32	≤ 12
Gross Energy (kcal/100g.diet)	365	373	385	395	403	-

According to Table 4.2 the contents of crude protein in the experimental diet 1, 2, 3, 4 and 5 were 31.17%, 30.40%, 31.35%, 31.36% and 31.18% respectively. The crude lipid in the experimental diet 1, 2, 3, 4 and 5 were 2.86%, 4.70%, 5.18%, 5.59% and 6.23% respectively. The fiber in the experimental diet 1, 2, 3, 4 and 5 were 6.51%, 9.41%, 8.65%, 7.35% and 6.29% respectively. The gross energy of the experimental diet 1, 2, 3, 4 and 5 were 365, 373, 385, 395 and 403 kcal/100 g. diet respectively.

4.2 Growth and feed efficiency

4.2.1 Nile tilapia

1) Average weight of nile tilapia throughout the experimental

At the beginning, average initial weight ranged from 122.81 to 123.68 g. The significantly difference of average weight was begun at week 10th of the experiment. The fish fed with diet1 and 2 were significantly higher than other experiment ($P<0.05$) and at the end of the experimental period (week 12th), the average final weight ranged from 192.08 to 224.08 g. The fish fed with diet 1, 2 and 3 were significantly higher than other experimental ($P<0.05$). The average weight of nile tilapia during the experimental period of 12 weeks was presented in table 4.3 and figure 4-1.

Table 4.3 Average weight of nile tilapia that fed with different diet every 2 weeks throughout the experiment

Time (week)	Average weight (g.)				
	Fishmeal 100%+ Fermented feather meal 0%	Fishmeal 75%+ Fermented feather meal 25%	Fishmeal 50%+ Fermented feather meal 50%	Fishmeal 75%+ Fermented feather meal 25%	Fishmeal 0%+ Fermented feather meal 100%
Initial weight	123.08 ^a ±1.67	122.81 ^a ±1.43	123.50 ^a ±1.03	123.68 ^a ±0.57	123.03 ^a ±0.26
2	150.13 ^a ±4.35	151.81 ^a ±2.02	150.96 ^a ±3.16	149.62 ^a ±6.43	144.27 ^a ±4.30
4	155.36 ^b ±3.37	155.30 ^b ±2.51	152.82 ^b ±3.85	152.21 ^b ±0.22	144.29 ^a ±3.18
6	173.73 ^a ±11.11	175.55 ^a ±6.77	170.11 ^a ±14.66	163.21 ^a ±7.49	159.28 ^a ±4.52
8	182.79 ^a ±12.53	187.31 ^a ±10.21	181.28 ^a ±15.55	170.02 ^a ±6.36	168.23 ^a ±3.14
10	202.14 ^{bc} ±13.21	204.90 ^c ±9.42	194.09 ^{abc} ±14.99	184.12 ^{ab} ±4.16	180.77 ^a ±3.27
12	224.08 ^a ±12.64	222.35 ^a ±10.98	206.97 ^{ab} ±14.53	195.97 ^b ±4.10	192.08 ^b ±3.07

Note : Mean with different subscript (a,b,c,d) in the same row were significantly different ($P<0.05$)

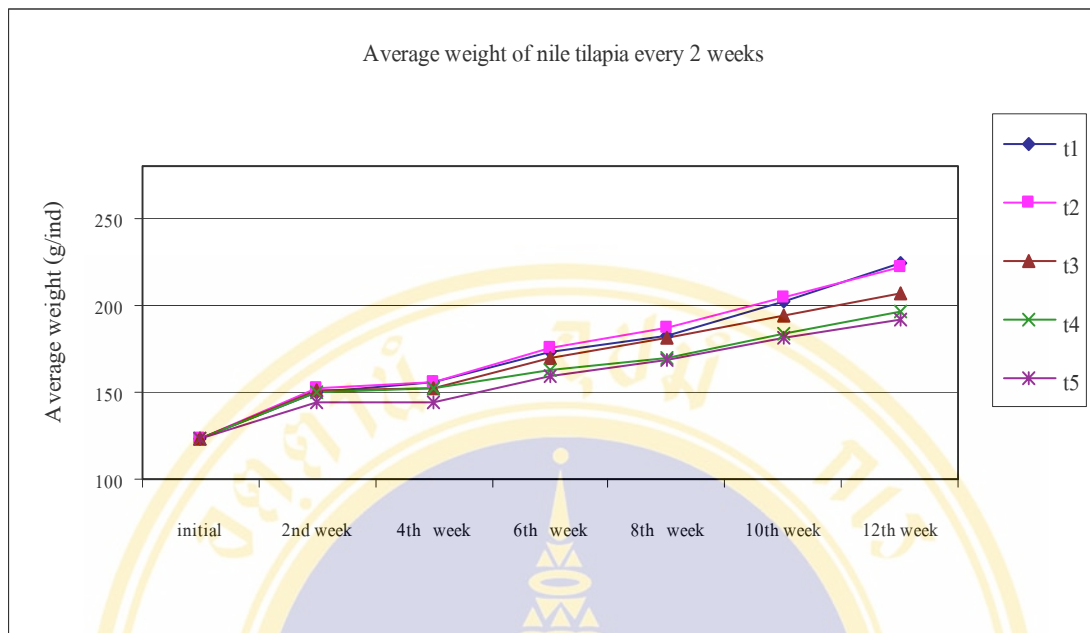


Figure 4-1 The average weight every 2 weeks of Nile tilapia fed with different diets

- t1 : tilapia fed with Fish meal 100% + Fermented feather meal 0% in diet
- t2 : tilapia fed with Fish meal 75% + Fermented feather meal 25% in diet
- t3 : tilapia fed with Fish meal 50% + Fermented feather meal 50% in diet
- t4 : tilapia fed with Fish meal 25% + Fermented feather meal 75% in diet
- t5 : tilapia fed with Fish meal 0% + Fermented feather meal 100% in diet

2) Percentage of weight gain

Percentage of weight gain in fish fed with diet containing 0, 25, 50, 75 and 100% fermented feather meal were significantly difference ($P < 0.05$) among treatment groups. According to table 4.4 the results showed that percentage of weight gain was highest on fish fed with diet formula 1 ($105.88\% \pm 8.35$) and lowest on diet formula 5 ($72.97\% \pm 2.13$). There were not significantly difference ($P > 0.05$) between fish fed with diet formula 1 (105.88 ± 8.35), formula 2 ($101.22\% \pm 10.45$) and formula 3 ($86.27\% \pm 8.55$) and also no significantly difference ($P > 0.05$) between fish fed with diet formula 3 ($86.27\% \pm 8.55$), formula 4 ($75.12\% \pm 6.78$) and formula 5 ($72.97\% \pm 2.13$).

3) Average daily weight gain

Average daily weight gain in fish feds with diet containing 0, 25, 50, 75 and 100% fermented feather meal were significantly difference ($P < 0.05$) among treatment groups. According to table 4.4 the results showed that daily weight gain was highest on fish fed with diet formula 1 ($1.20 \text{ g} \pm 0.15$) and lowest on diet formula 5 ($0.82 \text{ g} \pm 0.03$). There were not significantly difference ($P > 0.05$) between fish fed with diet formula 1 (1.20

g. \pm 0.15), formula 2 (1.19 g. \pm 0.13) and formula 3 (0.99 g. \pm 0.16) and also no significantly difference ($P>0.05$) between fish fed with diet formula 3 (0.99 g. \pm 0.16), formula 4 (0.86 g. \pm 0.04) and formula 5 (0.82 g. \pm 0.03).

4) *Survival rate*

Survival rate showed the same value (100%) on all treatment groups.

5) *Feed conversion ratio*

Feed conversion ratio in fish feds with diet containing 0, 25, 50, 75 and 100% fermented feather meal were significantly difference ($P<0.05$) among treatment groups. According to table 4.4 the results showed that feed conversion ratio was lowest on fish fed with diet formula 1 (1.93 \pm 0.20) and highest on diet formula 5 (2.93 \pm 0.19). There were not significantly difference ($P>0.05$) between fish fed with diet formula 1 (1.9 \pm 0.20), and formula 2 (1.78 \pm 0.13) and also no significantly difference ($P>0.05$) between fish fed with diet formula 3 (2.32 \pm 0.36) and formula 4 (2.54 \pm 0.08) and significantly difference ($P<0.05$) from formula 5 (2.93 \pm 0.19).

6) *Protein efficiency ratio*

Protein efficiency ratio in fish fed with diet containing 0, 25, 50, 75 and 100% fermented feather meal were significantly difference ($P<0.05$) among treatment groups. According to table 4.4 the results showed that protein efficiency ratio was highest on fish fed with diet formula 1 (1.77 \pm 0.17) and lowest on diet formula 5 (1.26 \pm 0.08). There were not significantly difference ($P>0.05$) between fish fed with diet formula 1 (1.77 \pm 0.17) and formula 2 (1.85 \pm 0.13) and also no significantly difference ($P>0.05$) between fish fed with diet formula 1 (1.77 \pm 0.17) and formula 3 (1.51 \pm 0.20), and also no significantly difference ($P>0.05$) between fish fed with diet formula 3 (1.51 \pm 0.20), formula 4 (1.32 \pm 0.04) and formula 5 (1.26 \pm 0.08).

7) *Apparent net protein retention*

Apparent net protein retention in fish feds with diet containing 0, 25, 50, 75 and 100% fermented feather meal were significantly difference ($P<0.05$) among treatment groups. According to table 4.4 the results showed that apparent net protein retention was highest on fish fed with diet formula 2 (31.86 \pm 1.13) and lowest on diet formula 4 (24.19 \pm 2.57). There were not significantly difference ($P>0.05$) between fish fed with diet formula 2 (31.86 \pm 1.13) and formula 5 (28.62 \pm 1.20) and also no significantly difference ($P>0.05$) between fish fed with diet formula 1 (27.73 \pm 1.69), formula 3 (27.64 \pm 2.67) and

formula 5 (28.62 ± 1.20) and also no significantly difference ($P > 0.05$) between fish fed with diet formula 1 (27.73 ± 1.69), formula 3 (27.64 ± 2.67), formula 4 (53.25 ± 1.61) and formula 5 (28.62 ± 1.20).

8) *Protein intake*

Protein intake in fish fed with diet containing 0, 25, 50, 75 and 100% fermented feather meal were not significantly difference ($P > 0.05$) among treatment groups. According to table 4.4 the results showed that protein intake was highest on diet formula 1 (57.00 ± 2.64) and lowest on fish fed with diet formula 4 (53.25 ± 1.61).

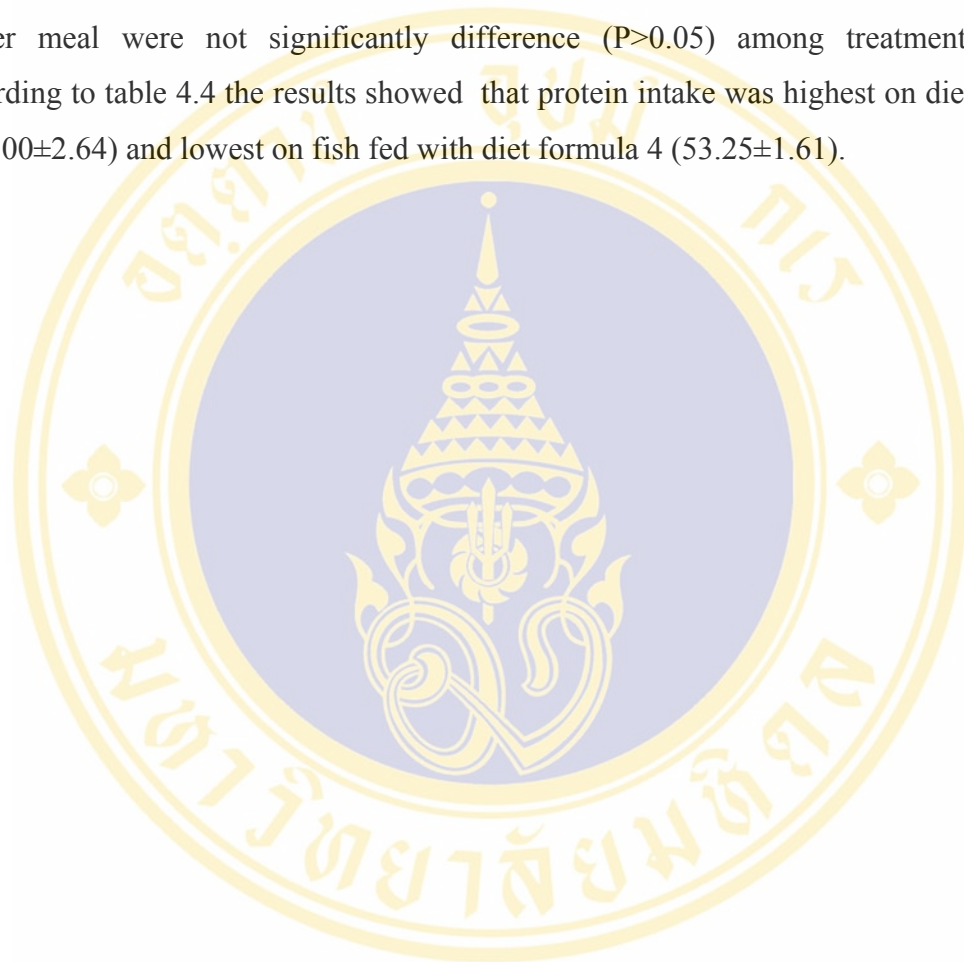


Table 4.4 Growth and feed efficiency of Nile tilapia fed with different diets during 12 weeks

	Fermented feather meal substituted for fish meal in Nile tilapia diets				
	Formula 1. (0%)	Formula 2. (25%)	Formula 3. (50%)	Formula 4. (75%)	Formula 5. (100%)
Average initial weight (g.)	123.08±1.67 ^a	122.81±1.43 ^a	123.50±1.03 ^a	123.68±0.57 ^a	123.03±0.38 ^a
Average final weight (g.)	224.08±12.64 ^b	222.35±10.98 ^b	206.97±14.53 ^{ab}	195.97±4.10 ^a	192.08±3.07 ^a
Survival rate (%)	100±0 ^a	100±0 ^a	100±0 ^a	100±0 ^a	100±0 ^a
Percentage of weight gain (%)	105.88±8.35 ^b	101.22±10.45 ^b	86.27±8.55 ^{ab}	75.12±6.78 ^a	72.97±2.13 ^a
Average daily weight gain (g./ind/day)	1.20±0.15 ^b	1.19±0.13 ^b	0.99±0.16 ^{ab}	0.86±0.04 ^a	0.82±0.03 ^a
Feed consumption (g./ind)	193.15±5.91 ^a	198.38±13.02 ^a	195.88±22.55 ^a	181.59±13.91 ^a	183.43±8.42 ^a
Feed conversion ratio	1.93±0.20 ^a	1.78±0.13 ^a	2.32±0.36 ^b	2.54±0.08 ^b	2.93±0.19 ^c
Protein efficiency ratio	1.77±0.17 ^{bc}	1.85±0.13 ^c	1.51±0.20 ^{ab}	1.32±0.04 ^a	1.26±0.08 ^a
Apparent net protein retention (%)	27.73±1.69 ^{ab}	31.86±1.13 ^c	27.64±2.67 ^{ab}	24.19±2.57 ^a	28.62±1.20 ^{abc}
Protein intake (g./ind)	57.00±2.64 ^a	54.70±1.93 ^a	55.13±0.72 ^a	53.25±1.61 ^a	54.96±4.93 ^a

Note: Mean with different superscript (a,b,c,d) in the same row were significantly different ($P < 0.05$)

9) Carcass composition of fish

The proximate compositions of experimental fish at the beginning and the end of experiment were presented in table 4.5. The carcass compositions of initial fish was observed on crude protein, crude lipid and fiber content (61.41%, 19.52% and 1.23% respectively). At the end of experimental, the carcass composition of fish fed diet fomula 1, 2, 3, 4 and 5 were composed of crude protein content (68.67%, 70.21%, 70.99%, 69.48% and 75.12% respectively), crude lipid content (17.21%, 19.71%, 19.68%, 17.78% and 10.92% respectively) and fiber content (1.26%, 1.13%, 0.98%, 0.96% and 1.35% respectively)

Table 4.5 The chemical compositions of nile tilapia fed with different diets

Composition	Initial fish	Fermented feather meal substitution for fish meal in nile tilapia feed				
		Formula 1. (0%)	Formula 2. (25%)	Formula 3. (50%)	Formula 4. (75%)	Formula 5. (100%)
Protein(g/ind.)	75.83±0.40 ^a	84.52±1.15 ^b	86.22±1.00 ^c	87.18±0.73 ^c	85.93±0.39 ^c	92.42±0.28 ^d
Protein (%)	61.40	68.67	70.21	70.59	69.48	75.12
Lipid (g/ind.)	23.77±0.13 ^d	21.18±0.29 ^b	24.21±0.28 ^c	24.30±0.20 ^c	21.99±0.10 ^c	13.44±0.04 ^a
Lipid (%)	19.25	17.21	19.71	19.68	17.78	10.92
Fiber(g/ind.)	1.52±0.01 ^d	1.55±0.02 ^e	1.39±0.02 ^c	1.21±0.01 ^b	1.19±0.01 ^a	1.66±0.01 ^f
Fiber (%)	1.23	1.26	1.13	0.98	0.96	1.35

Note: Mean with different supscript (a,b,c,d) in the same row were significantly different (P<0.05)

10) Level of thyroxin hormone (T4) and triiodotyronine (T3) in tilapia

The level of thyroxin hormone (T4) and triiodotyronine (T3) in blood of fishes at the end of experiment, they were presented in table 4.6. The fish fed diet formula 1, 2, 3, 4 and 5 were had level of triiodotyronine (T3) in blood 0.23, 1.07, 1.89, 1.26 and 1.52 $\mu\text{g/g}$. fish respectively, and level of thyroxin hormone (T4) in blood 4.47, 5.86, 7.75, 5.11 and 5.21 $\mu\text{g/g}$. fish respectively. Both of thyroxine hormone were tend to increased by increasing of fermented feather meal.

Table 4.6 Level of triiodotyronine (T3) and thyroxin hormone (T4) in blood of Nile tilapia

Thyroxin	Fermented feather meal substituted for fish meal in Nile tilapia diets				
	Formula 1. (0%)	Formula 2. (25%)	Formula 3. (50%)	Formula 4. (75%)	Formula 5. (100%)
Total T3 ($\mu\text{g/g}$. fish)	0.23 \pm 0.019 ^a	1.07 \pm 0.05 ^b	1.89 \pm 0.13 ^c	1.26 \pm 0.03 ^d	1.52 \pm 0.03 ^c
Total T4 ($\mu\text{g/g}$. fish)	4.47 \pm 0.25 ^a	5.86 \pm 0.28 ^c	7.75 \pm 0.55 ^d	5.11 \pm 0.11 ^b	5.21 \pm 0.09 ^b

Note: Mean with different subscript (a,b,c,d) in the same row were significantly different ($P < 0.05$)



Figure 4-2 Nile tilapia fed with diet formula 1



Figure 4-3 Nile tilapia fed with diet formula 2



Figure 4-4 Nile tilapia fed with diet formula 3



Figure 4-5 Nile tilapia fed with diet formula 4



Figure 4-6 Nile tilapia fed with diet formula 5

4.2.2 Walking catfish

1) Average weight of walking catfish throughout the experimental

At the beginning, average initial weight ranged from 73.73 to 74.09 g. The significantly difference of average weight was begun at week 2nd of the experiment. The fish fed with diet1, 2 and 3 were significantly higher than other experiment ($P<0.05$) and at the end of the experimental period (week 8th), the average final weight ranged from 96.56 to 132.67 g. The fish fed with diet1 and 2 were significantly higher than other experiment ($P<0.05$). The average weight of walking catfish during the experimental period of 8 weeks was presented in table 4.7 and figure 4-7.

Table 4.7 Average weight of walking catfish that fed with different diet every 2 weeks throughout the experimental

Time (week)	Average weight				
	Fishmeal100%+ Fermented feather meal 0%	Fishmeal 75%+ Fermented feather meal 25%	Fishmeal 50%+ Fermented feather meal 50%	Fishmeal 75%+ Fermented feather meal 25%	Fishmeal 0%+ Fermented feather meal 100%
Initial weight	74.04 ±0.26 ^a	74.09±0.18 ^a	73.98 ± 0.30 ^a	73.73± 0.46 ^a	74.09±0.26 ^a
2	80.64±1.33 ^b	82.25±1.28 ^b	81.89±1.79 ^b	79.55±1.59 ^{ab}	77.41±1.50 ^a
4	89.62±3.64 ^b	91.69±1.58 ^b	90.96±3.71 ^b	86.67±1.60 ^{ab}	81.95±1.31 ^a
6	106.39±5.92 ^b	108.71±3.83 ^b	107.02±8.54 ^b	101.05±2.11 ^b	89.76±2.84 ^a
8	132.58±6.65 ^c	132.67±6.91 ^c	125.72±10.65 ^{bc}	114.43±1.79 ^b	96.56±6.81 ^a

Note: Mean with different superscript (a,b,c,d) in the same row were significantly different ($P<0.05$)

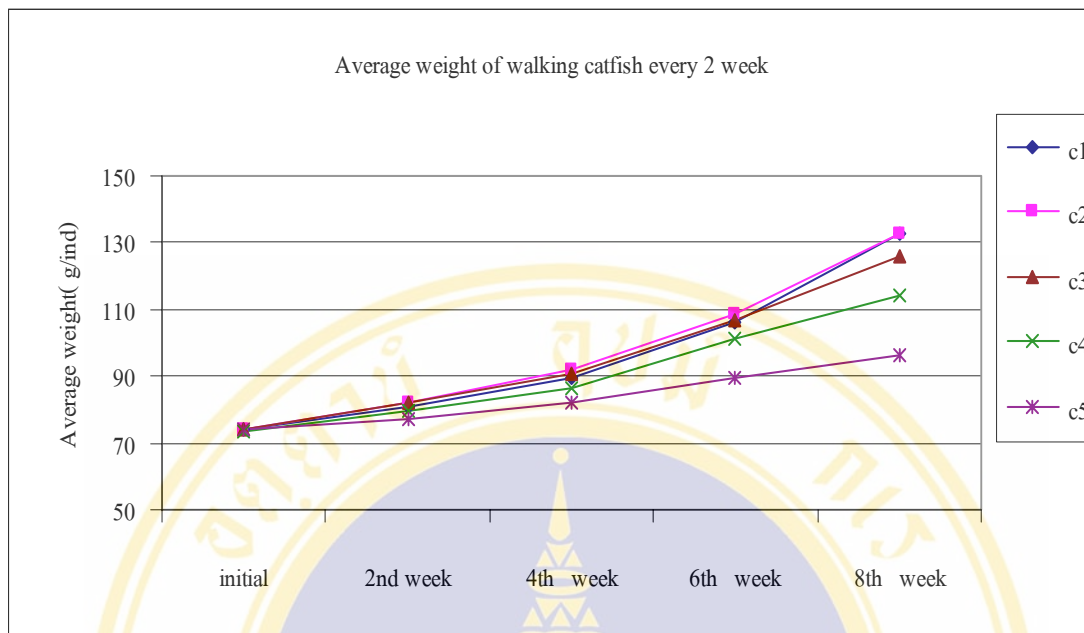


Figure 4-7 The average weight every 2 weeks of walking catfish fed with different diets

- c1 : walking catfish fed with Fish meal 100% + Fermented feather meal 0% in diet
- c2 : walking catfish fed with Fish meal 75% + Fermented feather meal 25% in diet
- c3 : walking catfish fed with Fish meal 50% + Fermented feather meal 50% in diet
- c4 : walking catfish fed with Fish meal 25% + Fermented feather meal 75% in diet
- c5 : walking catfish fed with Fish meal 0% + Fermented feather meal 100% in diet

2) Percentage of weight gain

Percentage of weight gain in fish fed with diet containing 0, 25, 50, 75 and 100% fermented feather meal were significantly difference ($P < 0.05$) among treatment groups. According to table 4.8 the results showed that percentage of weight gain was highest on fish fed with diet formula 1 ($79.08\% \pm 9.54$) and lowest on diet formula 5 ($30.13\% \pm 8.48$). There were not significantly difference ($P > 0.05$) between fish fed with diet formula 1 ($79.08\% \pm 9.54$), formula 2 ($79.07\% \pm 9.00$) and formula 3 ($69.97\% \pm 15.08$) and also no significantly difference ($P > 0.05$) between fish fed with diet formula 3 ($69.97\% \pm 15.08$) and formula 4 ($55.21\% \pm 2.63$) and also no significantly difference ($P > 0.05$) between fish fed with diet formula 4 ($55.21\% \pm 2.63$) and formula 5 ($30.13\% \pm 8.48$).

3) *Average daily weight gain*

Average daily weight gain in fish fed with diet containing 0, 25, 50, 75 and 100% fermented feather meal were significantly difference ($P < 0.05$) among treatment groups. According to table 4.8 the results showed that daily weight gain was highest on fish fed with diet formula 1 and 2 ($1.05 \text{ g} \pm 0.12$) and lowest on diet formula 5 ($0.40 \text{ g} \pm 0.11$). There were not significantly difference ($P > 0.05$) between fish fed with diet formula 1 (1.05 ± 0.12), formula 2 (1.05 ± 0.12) and formula 3 (0.92 ± 0.20) and also no significantly difference ($P > 0.05$) between fish fed with diet formula 3 (0.92 ± 0.20), formula 4 (0.73 ± 0.03) and also no significantly difference ($P > 0.05$) between fish fed with diet formula 4 (0.73 ± 0.03) and formula 5 (0.40 ± 0.11).

4) *Survival rate*

Survival rate showed the same value (100%) on all treatment groups.

5) *Feed conversion ratio*

Feed conversion ratio in fish fed with diet containing 0, 25, 50, 75 and 100% fermented feather meal were significantly difference ($P < 0.05$) among treatment groups. According to table 4.8 the results showed that feed conversion ratio was lowest on fish fed with diet formula 2 (2.62 ± 0.30) and highest on diet formula 5 (4.03 ± 0.32). There were not significantly difference ($P > 0.05$) between fish fed with diet formula 1 (2.77 ± 0.33), formula 2 (2.62 ± 0.30) and formula 3 (2.97 ± 0.62) and also no significantly difference ($P > 0.05$) between fish fed with diet formula 3 (2.97 ± 0.62), formula 4 (3.55 ± 0.19) and significantly difference ($P < 0.05$) from formula 5 (4.03 ± 0.32).

6) *Protein efficiency ratio*

Protein efficiency ratio in fish fed with diet containing 0, 25, 50, 75 and 100% fermented feather meal were significantly difference ($P < 0.05$) among treatment groups. According to table 4.8 the results showed that protein efficiency ratio was highest on fish fed with diet formula 2 (1.26 ± 0.15) and lowest on diet formula 5 (0.79 ± 0.01). There were not significantly difference ($P > 0.05$) between fish fed with diet formula 1 (1.15 ± 0.12), formula 2 (1.26 ± 0.15) and formula 3 (1.11 ± 0.24) also no significantly difference ($P > 0.05$) between fish fed with diet formula 3 (1.11 ± 0.24) and formula 4 (0.91 ± 0.04) and significantly difference ($P < 0.05$) from fish fed with diet formula 5 (0.79 ± 0.01).

7) *Apparent net protein retention*

Apparent net protein retention in fish fed with diet containing 0, 25, 50, 75 and 100% fermented feather meal were significantly difference ($P < 0.05$) among treatment groups. According to table 4.8 the results showed that apparent net protein retention was highest on fish fed with diet formula 2 (29.89 ± 2.89) and lowest on diet formula 5 (14.82 ± 1.20). There were not significantly difference ($P > 0.05$) between fish fed with diet formula 1 (28.65 ± 2.56), formula 2 (29.89 ± 2.89) and formula 3 (25.43 ± 4.51) and also no significantly difference ($P > 0.05$) between fish fed with diet formula 4 (18.12 ± 6.21) and formula 5 (14.28 ± 1.20). The apparent net protein retention was reduced by increasing fermented feather meal

8) *Protein intake*

Protein intake in fish fed with diet containing 0, 25, 50, 75 and 100% fermented feather meal were significantly difference ($P < 0.05$) among treatment groups. According to table 4.8 the results showed that protein intake was highest on diet formula 1 (50.88 ± 0.48) and lowest on fish fed with diet formula 5 (28.14 ± 8.26). There were not significantly difference ($P > 0.05$) between fish fed with diet formula 1 (50.88 ± 0.48), formula 2 (46.32 ± 0.07), formula 3 (46.75 ± 0.29) and formula 4 (45.23 ± 0.41) and significantly difference ($P < 0.05$) from fish fed with diet formula 5 (28.14 ± 8.26). The protein intake was reduced by increasing fermented feather meal..

Table 4.8 Growth and feed efficiency of walking catfish fed with different diets during 8 weeks

	Fermented feather meal substituted for fish meal in walking catfish diets				
	Formula 1. (0%)	Formula 2. (25%)	Formula 3. (50%)	Formula 4. (75%)	Formula 5. (100%)
Average initial weight (g.)	74.04±0.26 ^a	74.09±0.18 ^a	73.98±0.30 ^a	73.73±0.45 ^a	74.09±0.26 ^a
Average final weight (g.)	132.58±6.65 ^c	132.67±6.91 ^c	125.72±10.65 ^{bc}	114.43±1.79 ^b	96.56±6.81 ^a
Percentage of weight gain (%)	79.08±9.54 ^c	79.07±9.00 ^c	69.97±15.08 ^{bc}	55.21±2.63 ^{ab}	30.13±8.48 ^a
Average daily weight gain (g./ind/day)	1.05±0.12 ^c	1.05±0.12 ^c	0.92±0.20 ^{bc}	0.73±0.03 ^b	0.40±0.11 ^a
Survival rate (%)	100±0 ^a	100±0 ^a	100±0 ^a	100±0 ^a	100±0 ^a
Feed consumption (g./ind)	160.79±1.53 ^b	152.37±0.22 ^b	149.13±0.93 ^b	144.23±1.30 ^b	90.25±26.50 ^a
Feed conversion ratio	2.77±0.33 ^a	2.62±0.30 ^a	2.97±0.62 ^{ab}	3.55±0.19 ^{bc}	4.03±0.32 ^c
Protein efficiency ratio	1.15±0.12 ^{bc}	1.26±0.15 ^c	1.11±0.24 ^{bc}	0.91±0.04 ^{ab}	0.79±0.01 ^a
Apparent net protein retention (%)	28.65±2.56 ^b	29.86±2.89 ^b	25.43±4.51 ^b	18.12±6.21 ^a	14.82±1.20 ^a
Protein intake (g./ind)	50.88±0.48 ^b	46.32±0.07 ^b	46.75±0.29 ^b	45.23±0.41 ^b	28.14±8.26 ^a

Note: Mean with different superscript (a,b,c,d) in the same row were significantly different (P<0.05)

9) Carcass composition of fish

The proximate compositions of experimental fish at the beginning and the end of experiment were presented in table 4.9. The carcass compositions of initial fish was observed on crude protein, crude lipid and fiber content (81.00%, 7.25% and 2.01% respectively). At the end of experiment, the carcass composition of fish fed diet fomula 1, 2, 3, 4 and 5 were composed of crude protein content (84.71%, 84.14%, 82.12%, 86.30% and 82.13% respectively), crude lipid content (4.33%, 4.79%, 4.48%, 2.80% and 4.96% respectively) and fiber content (1.63%, 1.70%, 1.28%, 1.19% and 1.13% respectively)

Table 4.9 The chemical compositions of walking catfish fed with different diets during 8 weeks

Composition	Initial fish	Fermented feather meal substitution for fish meal in walking catfish feed				
		Formula 1. (0%)	Formula 2. (25%)	Formula 3. (50%)	Formula 4. (75%)	Formula 5. (100%)
Protein (g/ind.)	59.83±0.23 ^a	62.73±0.22 ^c	62.34±0.15 ^c	60.75±0.25 ^b	63.63±0.40 ^d	60.85±0.22 ^b
Protein (%)	81.00	84.71	84.14	82.12	86.30	82.13
Lipid (g/ind.)	5.36±0.02 ^f	3.21±0.01 ^b	3.55±0.01 ^d	3.31±0.01 ^c	2.06±0.01 ^a	3.67±0.01 ^e
Lipid (%)	7.25	4.33	4.79	4.48	2.80	4.96
Fiber (g/ind.)	1.48±0.01 ^f	1.21±0.06 ^d	1.26±0.00 ^e	0.95±0.00 ^c	0.88±0.01 ^b	0.84±0.00 ^a
Fiber (%)	2.01	1.63	1.70	1.28	1.19	1.13

Note: Mean with different supscript (a,b,c,d) in the same row were significantly different (P<0.05)

10) Level of triiodotyronine (T3) and thyroxin hormone (T4) of walking catfish

The level of thyroxin hormone (T4) and triiodotyronine (T3) in blood of fishes at the end of experiment were presented in table 4.10. The fish fed diet fomula 1, 2, 3, 4 and 5 were had level of triiodotyronine (T3) in blood 1.93, 1.72, 2.69, 1.96 and 2.21 $\mu\text{g/g}$. fish respectively, and level of thyroxin hormone (T4) in blood 7.56, 9.82, 9.59, 8.74 and 10.4 $\mu\text{g/g}$. respectively. Both of tyroxine hormone were trend to increased by increasing of fermented feather meal.

Table 4.10 Level of triiodotyronine (T3) and thyroxin hormone (T4) in blood of walking catfish

Thyroxin	Fermented feather meal substituted for fish meal in walking catfish diets				
	Formula 1. (0%)	Formula 2. (25%)	Formula 3. (50%)	Formula 4. (75%)	Formula 5. (100%)
Total T3 ($\mu\text{g/g}$.fish)	1.93 \pm 0.10 ^a	1.72 \pm 0.09 ^a	2.69 \pm 0.23 ^c	1.96 \pm 0.03 ^a	2.21 \pm 0.14 ^b
Total T4 ($\mu\text{g/g}$.fish)	7.56 \pm 0.39 ^a	9.82 \pm 0.51 ^b	9.59 \pm 0.80 ^{bc}	8.74 \pm 0.14 ^c	10.4 \pm 0.68 ^b

Note: Mean with different supscript (a,b,c,d) in the same row were significantly different ($P < 0.05$)



Figure 4-8 Walking catfish fed with diet formula 1



Figure 4-9 Walking catfish fed with diet formula 2



Figure 4-10 Walking catfish fed with diet formula 3



Figure 4-11 Walking catfish fed with diet formula 4



Figure 4-12 Walking catfish fed with diet formula 5

4.3 Water quality in the experiment

4.3.1 Water quality in experiment of Nile tilapia

During the experimental period, the water temperature were monitored everyday. The average temperature ranged from 24.06-24.63 °C. Dissolved oxygen, pH, Hardness, Alkalinity and Ammonia concentrations of water were biweekly monitored. Dissolved oxygen, pH, Hardness, Alkalinity and Ammonia concentrations of water ranged from 5.27-5.68 ppm., 7.79-7.88, 132.50-133.75 ppm., 368.75-390.25 ppm. and 0.56-0.79 ppm. respectively, as presented in table 4.11.

Table 4.11 Water quality in the experiment of Nile tilapia fed with different diets during 12 weeks

Treatment	Mean					
	Temperature (°C)	Dissolved oxygen (ppm.)	pH	Hardness (ppm.)	Alkalinity (ppm.)	Ammonia (ppm.)
Diet 1	24.12±0.76	5.58± 0.25	7.83±0.07	132.50±1.00	368.75±18.93	0.67±0.32
Diet 2	24.21±1.24	5.38± 0.46	7.79±0.32	133.50±1.91	386.25±19.62	0.76±0.20
Diet 3	24.06±0.97	5.68± 0.43	7.88±0.20	133.00±1.63	382.50±17.82	0.56±0.24
Diet 4	24.17±0.53	5.45± 0.42	7.81±0.26	133.75±1.50	386.25±26.85	0.75±0.27
Diet 5	24.63±1.61	5.27± 0.34	7.84±0.17	132.50±1.91	390.25±30.89	0.79±0.16

4.3.2 Water quality in the experiment of walking catfish

During the experimental period, the water temperature were monitored everyday. The average temperature ranged from 24.04-24.45 °C. Dissolved oxygen, pH, Hardness, Alkalinity and Ammonia concentration of water were biweekly monitored. Dissolved oxygen, pH, Hardness, Alkalinity and Ammonia concentration of water ranged from 5.30-5.70 ppm., 7.67-8.03 , 132.33-134.67 ppm., 357.67-424.33 ppm. and 0.24-0.48 ppm. respectively, as presented in table 4.12.

Table 4.12 Water quality in the experiment of walking catfish fed with different diets during 8 weeks

Treatment	Mean					
	Temperature (°C)	Dissolved oxygen (ppm.)	pH	Hardness (ppm.)	Alkalinity (ppm.)	Ammonia (ppm.)
Diet 1	24.42±0.70	5.53± 0.21	7.67±0.11	132.33±0.58	357.67±18.15	0.48±0.04
Diet 2	24.07±0.55	5.30± 0.10	7.73±0.27	134.00±1.00	389.00±17.58	0.24±0.08
Diet 3	24.45±1.08	5.70± 0.17	7.68±0.27	132.33±1.53	392.67±12.50	0.35±0.28
Diet 4	24.04±0.79	5.53± 0.21	7.70±0.14	134.67±0.58	392.33±8.74	0.26±0.01
Diet 5	24.36±0.48	5.37± 0.06	8.03±0.14	134.33±0.58	424.33±26.63	0.24±0.07

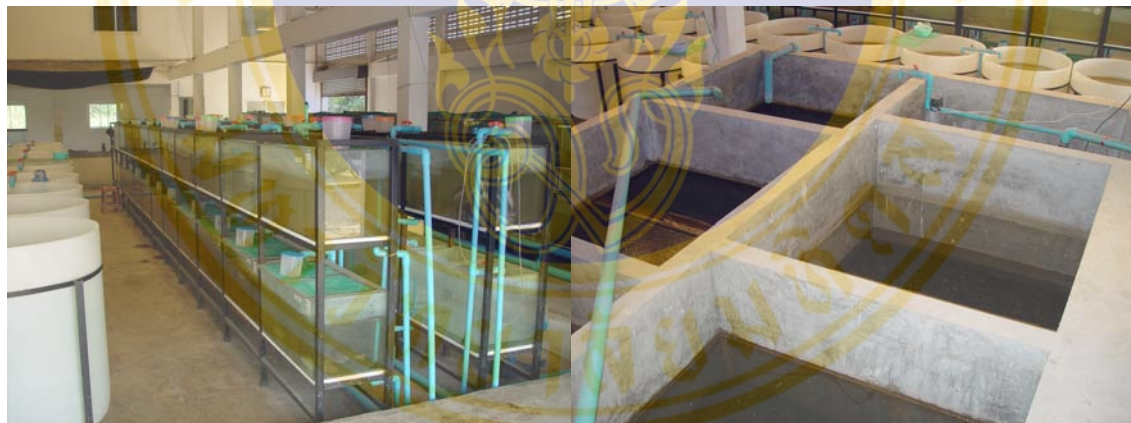


Figure 4-13 Close flow through system in experimental tank and filter pond

4.4 Cost of experimental diets

4.4.1 Nile tilapia experimental diets

Raw material using for experimental diets were studied, totally raw material cost of 1-5 feed formula ranged from 17.25-14.79 baht /kg. Replacement of fermented feather meal 25, 50, 75 and 100% of fish meal were reduced cost about 0.71, 1.31, 1.97 and 2.46 baht/kg. respectively.

In addition, when compare with commercial feed (containing 30% protein (22 baht/kg.)) were reduced cost about 2.82, 5.46, 6.06, 6.72 and 7.21 baht/kg. respectively.

Table 4.13 Cost of nile tilapia experimental diets

Raw material	Price (baht/kg.)	Diet formula				
		1	2	3	4	5
Fish meal	22	0.25	0.1875	0.125	0.0625	-
Feather meal	7	-	0.044	0.088	0.133	0.177
Soilbean meal	14	0.30	0.30	0.30	0.30	0.30
Ground rice brokens	8	0.15	0.15	0.15	0.15	0.15
Cassava powder	4	0.10	0.10	0.10	0.10	0.10
Alpha starch	25	0.06	0.06	0.06	0.06	0.06
Vegetable oil	32	0.013	0.013	0.013	0.013	0.013
Fish oil	40	0.01	0.014	0.019	0.023	0.027
Lard	40	-	0.004	0.006	0.009	0.016
Di-calsium phosphate	40	0.0153	0.0143	0.0163	0.0196	0.0224
Vitamins & minerals	80	0.02	0.02	0.02	0.02	0.02
Ground husk	4	0.0767	0.0882	0.0977	0.1049	0.1096
Yeast cake	600	0.005	0.005	0.005	0.005	0.005
Total (baht/kg.)	-	17.25	16.54	15.94	15.28	14.79

4.4.2 Walking cat fish experimental diets

Raw material using for experimental diets were studied, totally raw material cost of 1-5 feed formula ranged from 19.18-16.59 baht /kg.. Replacement of fermented feather meal 25, 50, 75 and 100% of fish meal were reduced cost about 0.81, 1.38, 2.09 and 2.59 baht/kg. respectively.

In addition, when compare with commercial feed (containing 30% protein (22 baht/kg.)) were reduced cost about 2.82, 3.62, 4.20, 4.91 and 5.41 baht/kg. respectively.

Table 4.14 Cost of walking catfish experimental diets

Raw material	Price (baht/kg.)	Diet formula				
		1	2	3	4	5
Fish meal	22	0.25	0.1875	0.125	0.0625	-
Feather meal	7	-	0.044	0.088	0.133	0.177
Soilbean meal	14	0.30	0.30	0.30	0.30	0.30
Ground rice brokens	8	0.15	0.15	0.15	0.15	0.15
Cassava powder	4	0.10	0.10	0.10	0.10	0.10
Alpha starch	25	0.06	0.06	0.06	0.06	0.06
Vegetable oil	32	0.013	0.013	0.013	0.013	0.013
Fish oil	40	0.01	0.014	0.019	0.023	0.027
Lard	40	-	0.004	0.006	0.009	0.016
Di-calsium phosphate	40	0.0092	0.0078	0.0111	0.0134	0.0163
Vitamins & minerals	80	0.02	0.02	0.02	0.02	0.02
Ground husk	4	0.0828	0.0947	0.1029	0.1111	0.1157
Yeast cake	600	0.005	0.005	0.005	0.005	0.005
Total (baht/kg.)	-	19.18	18.38	17.80	17.09	16.59

CHAPTER V

DISCUSSION

The study on replacement of fishmeal with fermented feathermeal was conducted on Nile tilapia and walking catfish with initial weight 120 ± 5 g. and 70 ± 5 g. respectively. The experimental fishes were fed with experimental diets for 84 days and 56 days in flow through system with aeration. The objectives of the study were determined on increasing the percentage of fermented feather meal in diets would enable Nile tilapia and walking catfish to grow equally while enable the amount of fish meal in the diets to be reduced, this study was investigated on

- 5.1 Quality of experimental diets
- 5.2 Growth and feed efficiency
- 5.3 Water quality

5.1 Quality of experimental diets

Nile tilapia experimental diets, according to the table 4.1 proximate compositions of experimental diets were contained protein ranged from 28.48 to 30.38. Gross energy ranged from 345 to 368 kcal/100 g.diet.

Walking catfish experimental diets, according to the table 4.2 proximate compositions of experimental diets were contained protein ranged from 30.40 to 31.36. Gross energy ranged from 365 to 403 kcal/100 g.diet.

The results of diet for 2 fishes analysis showed that the high percentage replacement of fermented feather meal in diet were not significant different ($P > 0.05$) between treatment and higher than the standard for registering of commercial herbivorous aquatic feed (weight 3-150 g. require 28% protein in diet) (Table 2.1) and protein requirement of juvenile Nile tilapia (weight > 30 g. require 28-30% protein in diet) (Chuapohuk, 2542) and commercial walking catfish feed (1.5-3 month olds require 28% protein in diet) (Table 2.1) and protein requirement of juvenile walking catfish (require 25-30% protein in diet) (Chuapohuk, 2542) but in this study the fishes that fed with diets containing high percentage ($\geq 75\%$) of fermented feather meal were presented lower ($P < 0.05$) growth and

feed utilization than fishes fed with diets containing low percentage ($\leq 50\%$). The result showed that most effective using fermented feather meal were used as partial replacement for fish meal ($\leq 50\%$) or used for combination with other material such as fish meal, blood meal and poultry by-product meal (non edible parts + blood).

5.2 Growth and feed efficiency

5.2.1 Nile Tilapia

The results that obtained from the experiments showed that the growth responses of fish fed with contained 0, 25, 50, 75 and 100% fermented feather meal in term of percentage of weight gain, daily weight gain, survival rate and also feed efficiency such as feed consumption, feed conversion ratio, protein efficiency ratio and apparent net protein retention were reduced by increasing of fermented feather meal in diets. The tilapia fed with diet containing 0% fermented feather meal showed the best growth rate and feed efficiency and were not significantly different ($P > 0.05$) from the Nile tilapia that fed with diet containing 25% and 50% fermented feather meal but significantly different ($P < 0.05$) from diet containing 75% and 100% fermented feather meal. This study showed better result (ADG = 1.19 g./ind/ day in fish fed with diet 2) from the study of Boonyaratpalin and Kultangvatthana on red tilapia (*Oreochromis nilotica*) with diet 40% dietary protein that had average daily weight gain 0.5 g./ind/day and agree with the study of Somsueb and Boonyaratpalin (2001) on hybrid catfish (*Clarias* sp.) reported that hybrid catfish fed with diet that replace more than 35% of fish meal by feather meal had low final weight gain.

Once the percentage of fermented feather meal in the diet was decreased, the fish growth was increased. That was resulted from the quality of protein from fermented feather meal were lower than protein from fish meal. However, in this study showed that the feather meal fermentation had improved with the percentage of replacement for fish meal that more than the studied in market size hybrids tilapia (*Oreochromis*) Viola & Zahar, (1984) showed that fish fed with 7.5% feather meal replaced fish meal was reduced growth by 7% from fish fed with 7.5% bacteria's protein (proteen), poultry meal and control diets. Fowler (1990) reported that juvenile fall chinook salmon (*Oncorhynchus tshawytscha*) fed with diets that replaced 15% of fish meal by feather

meal, at the end of experiment weight gain did not differ from fed with diets that replaced 0, 5% of fish meal by feather meal

Feed conversion ratio in fish fed diets containing 0% and 25% fermented feather meal was significantly ($P < 0.05$) lowest and increased slightly by increasing fermented feather meal level in diets. These results agreed with the studied on sub-adult tilapia (*Oreochromis nilotica*) and Japanese flounder (*Paralichthys olivaceus*) (Kikuchi *et.al.*, 1994; Tacon *et.al.*; 1983 cited by Somsueb and Boonyaratpalin, 2001) reported that the feed conversion ratio was increased by increasing percentage of feather meal replacement of fish meal in fish feed.

The feed consumption of fish fed with diet containing 0% fermented feather meal was highest but not *significantly* different from other, which resulted on not significantly different ($P < 0.05$) protein intake. Anyway, the growth rate of the fish was higher when fed containing more fish meal than fermented feather meal and this result did not related to the palatability of diets because the increasing of fermented feather meal in the diets did not decrease the feed consumption. That means the rank smell of feather meal was reduced by fermentation.

Protein efficiency ratio (PER) of fish fed with diet containing 25 % fermented feather meal was highest but not significantly different ($P > 0.05$) from fish fed with diet containing 0% fermented feather meal but significantly different ($P < 0.05$) from fish fed with diet containing 50%, 75% and 100% fermented feather meal, protein efficiency ratio were decreased slightly when the fermented feather meal increased in diets. The result presented that the amino acid balance of fermented feather meal was not suitable for fish requirement when compared with fish meal. This experiment was agreed with the study of Henrichfreise, (1989) on rainbow trout (*Salmo gairdneri*) that the utilization of feather meal in rainbow trout was limited because of amino acid deficiencies, but additional feeding of lysine, methionine and histidine was necessary and supplementary feeding of case in (300g) caused a digestibility of nearly 100% and agree with and the study of Tacon *et al.*, (1983) cited by Somsueb and Boonyaratpalin (2001) on sub-adult Nile tilapia reported that the 30% feather meal replacement of fish meal with addition L-methionine or L-histidine or L-lysine or all 3 amino acid together have better protein efficiency ratio than without addition.

The apparent net protein retention were highest on fish fed with diet containing 25% fermented feather meal replacement and were not significantly different ($P>0.05$) from diet containing 0% replaced fish meal by fermented feather meal but higher than ($P<0.05$) diet containing 50%, 75% and 100% replaced fish meal fermented feathermeal. The different apparent net protein retention perhaps were caused from different quality of protein that fish obtained from diet because the value of protein intake of fish in all treatment were not significantly different ($P>0.05$) that meant on the fermentation of feather meal process perhaps protein in feather meal were digested to small peptide and free amino acid, which effected to slowly rate of amino acid absorption in intestines (Hardy *et al.*, (1984)).

The initial fish carcass trended to had lower protein than those of the fish at the end of the experiment. The percentage of protein in experimental fish carcass was related to the percentage of fat content in carcass that lowest when the fermented feather meal in diets increased up to 100%, but the lipid content of the diet containing 100% fermented feather meal was more than those diets. These indicated that body lipid was affected by fermented feather meal. These results agreed with the study on Japanese flounder (*Paralichthys olivaceus*), (Kikuchi *et al.*, 1994 cited by Somserb and Boonyaratpalin, 2001) reported that body lipid content were reduced by increasing feather meal in the diets.

The level of thyroxin hormone (T4) and triiodotyronine (T3) in blood of fishes at the end of experiment appeared that both T4 and T3 level of fish fed with diet containing 0% fermented father meal were tended to be lower than in Nile tilapia fed with diet containing fermented feather meal replacement of fish meal diet and the lipid in fish carcass were tended to be decreased by increasing of level of T3 and T4 in blood. In this study there was disagree with the study of Fowler (1990) on juvenile fall chinook salmon (*Oncorhynchus tshawytscha*) reported that mean plasma T4 concentrations for all groups of fish were low during the first periods showed no significantly different but at the last period of the experiment, fish fed with diet containing 0% of feather meal replacement fish meal had significantly higher plasma T4 than other (5% and 15% replacement). In addition, the thyroid activity was depressed by low protein and low calories diet depresses (Higgs and Eales, 1979 cited by Fowler, 1990) and Plasma T4 is affected by type of dietary lipid (Leatherland *et al.*, 1977 cited by Fowler, 1990).

5.2.2 Walking cat fish

The results of the experiments showed that the growth responses of fish fed with diet contained 0, 25, 50, 75 and 100% fermented feather meal in term of percentage of weight gain, daily weight gain, survival rate and also feed efficiency such as feed consumption, feed conversion ratio, protein efficiency ratio and apparent net protein retention were reduced by increasing of fermented feather meal in diets. The walking catfish fed with diet containing 25% fermented feather meal showed the best growth rate and feed efficiency but were not significantly different ($P>0.05$) from walking catfish fed with diet containing 0 and 50% fermented feather meal.

The average final weight, percentage of weight gain and average daily gain of fish fed with diet containing 25% fermented feather meal was highest but not significantly different ($P>0.05$) growth rate of the fish fed with diet with fermented feather meal were higher when fed containing 75% and 100% fermented feather meal. The results were similar tend with the study of Hardy *et al.*, (1984) on rainbow trout (*Salmo gairdneri*) fed with the combination with 33.8% fermented feather meal with fish silage (white pacific liquid-fish) had better growth rate than control (fish meal) and this study had better average daily gain than the study of Jantharotai (2539) on hybrid catfish (*Clarias macrocephalus* x *C. gariepinus*) fed with artificial feed contained 30% protein. In addition to the result of this study had better average daily gain than the study of Kosutarak (1999) on hybrid catfish (*Clarias* sp.) that fed with 25% chicken head silaged with 0 and 1.3% fish oli supplemented in diet reported that average daily gain were 0.2 and 0.23 g/ind/day, respectively and the study of Boonyaratparin *et al.*, (1982) on walking catfish (*Clarias batrachus*) fed with diet containing fermented trash fish with 5% salt (30% protein) reported that average daily gain was 0.92 g./ind/day, which lower than this study and agree with the study of Somsueb and Boonyaratpalin (2001) on hybrid catfish (*Clarias* sp.) reported that hybrid catfish fed with diet that replace more than 35% of fish meal by feather meal had low final weight gain.

The protein intake of fish fed with diet containing 100% were lower than others ($P<0.05$) that were caused from feed consumption of fish fed with diet containing 100% were lower than others ($P<0.05$). This result related to the palatability of diets because the increasing of fermented feather meal in the diets were tended to decrease the feed

consumption, perhaps the rank smell of diet that containing 100% fermented feather meal were effect to the palatability.

The fish growth was increased when percentage of fermented feather meal in the diet was decreased. That was caused of the amino acid balance of fermented feather meal were not suitable for fish when compared with fish meal, high percentage using of fermented feather meal in fish feed were reduced percentage of weight gain and daily wetght gain. However, in these experiment showed that the feather meal fermenteation had improved with the percentage of replacement for fish meal that better than the study of Somsueb and Boonyaratpalin (2001) on walking catfish (*Clarias* sp.) fed with diet containing 0-5% feather meal had better ($P < 0.01$) weight gain than fish fed with det containing 35% feather meal replaced fish meal and the study of Viola & Zahar (1984) on market size hybrids tilapia (*Oreochromis*) fed with 7.5% feather meal replaced fish meal was reduced growth by 7% from fish fed with 7.5% bacteria's protein (proteen), poultry meal and control diets.

Feed conversion ratio in fish fed diets containing 0% fermented feather meal was significantly lowest ($P < 0.05$) among the treatment but were not significantly different ($P > 0.05$) from 25%, 50% fermented feather meal replacement of fish meal but significantly higher ($P < 0.05$) than 75% and 100% fermented feather meal replacement of fish meal. Feed conversion ratio in diet that had fermented feather meal increased slightly by increasing fermented feather meal level in diets. The results were showed similary tend as the study of Hardy *et al.*, (1984) on rainbow trout (*Salmo gairdneri*) fed with the combination with 33.8% fermented feather meal with fish silage (white pacific liquid-fish) had better feed conversion ratio than control (fishmeal) and this study had better result than the study of Somsueb and Boonyaratpalin (2001) on walking catfish (*Clarias* sp.) reported that the highest percentage of fish meal replacement by feather meal were 35%, which the feed conversion ratio were not significantly different ($P > 0.05$) and agreed with the study of Kikuchi *et al.*, (1994) (cited by Somsueb and Boonyaratpalin, 2001) on Japanese flounder (*Paralichthys olivaceus*) reported that feed conversiom ratio were reduced by increasing percentage of feather meal in fish feed.

Protein efficiency ratio (PER) of fish fed with diet containing 25 %fermented feather meal was highest but not significantly different ($P > 0.05$) from fish fed with diet containing 0% and 50% fermented feather meal but significantly different ($P < 0.05$) from

fish fed with diet containing 75% and 100% fermented feather meal, the diet that had fermented feather meal protein efficiency ratio were decreased slightly when the fermented feather meal increased in diets. The result presented that the amino acid balance of fermented feather meal was not suitable for fish requirement when compared with fish meal. These experiment was agreed with the study of Henrichfreise, (1989) on rainbow trout (*Salmo gairdneri*) that the utilization of feather meal in rainbow trout was limited because of amino acid deficiencies, but additional feeding of lysine, methionine and histidine was necessary and supplementary feeding of casein (300g) caused a digestibility of nearly 100% and agree with and the study of Tacon *et al.*, (1983) cited by Somsueb and Boonyaratpalin (2001) on sub-adult Nile tilapia reported that the 30% feather meal replacement of fish meal with addition L-methionine or L-histidine or L-lysine or all 3 amino acid together have better protein efficiency ratio than without addition and agreed with the study of Somsueb and Boonyaratpalin (2001) that increased feather meal in fish diet were decreased protein efficiency ratio.

The apparent net protein retention were highest on fish fed with diet containing 25% fermented feather meal replacement and were not significantly different ($P>0.05$) from diet containing 0% and 50% replaced fish meal by fermented feathermeal but higher than ($P<0.05$) diet containing 75% and 100% replaced fish meal fermented feathermeal. The different apparent net protein retention perhaps were caused from different quality of protein that fish obtained from diet because the value of protein intake of fish fed with diet containing 0-75% replacement of fish meal by fermented feather meal were not significantly different ($P>0.05$) that meant on the fermentation of feather meal perhaps protein in feather meal were digested to small peptide and free amino acid, which effected to slowly rate of amino acid absorption in intestines (Hardy *et al.*, (1984)). The result agree with the study of Somsueb and Boonyaratpalin (2001) that increased feather meal in fish diet were decreased apparent net protein retention.

The initial fish carcass had little different protein composition from those of the fish at the end of the experiment. The percentage of protein in experimental fish carcasses were not significantly different ($P>0.05$). These indicated that body lipid was not affected by fermented feather meal. These results agreed with the study of Hilge (1984) on European catfish (*Silurus glanis*) that the raw fat level of European catfish fed with diet containing 50% of a fish meal – feather meal – poultry by product meal mixture replaced

by field bean/corn gluten meal or by whey was below than 10% by weight and disagreed with the study of Kikuchi *et al.*, (1994)(cited by Somsueb and Boonyaratpalin, 2001) on Japanese flounder that body lipid content were reduced by increasing feather meal in the diets.

The level of thyroxin hormone (T4) and triiodotyronine (T3) in blood of fishes at the end of experiment appears that the level of T3 in blood were not clearly related to the percentage of replacement but the level of T4 in blood of fish fed with diet containing 0% fermented feather meal were tended to be lower than in fish fed with diet containing fermented feather meal replacement of fish meal diet and the lipid in fish carcass were tended to be decreased by increasing of level of T3 and T4 in blood. In this study there was disagree with the study of Fowler (1990) on juvenile fall chinook salmon (*Oncorhynchus tshawytscha*) reported that mean plasma T4 concentrations for all groups of fish were low during the first periods showed no significantly different but at the last period of the experiment, fish fed with diet containing 0% of feather meal replacement fish meal had significantly higher plasma T4 than other (5% and 15% replacement). In addition, the thyroid activity was depressed by low protein and low calories diet depresses (Higgs and Eales, 1979 cited by Fowler, 1990) and Plasma T4 is affected by type of dietary lipid (Leatherland *et al.*, 1977 cited by Fowler, 1990).

5.2.3 Comparison of nile tilapia and walking catfish experimental result

Growth rate of nile tilapia and walking catfish were increased when fed containing more fish meal than fermented feather meal, but the fishes fed with 25 and 50% fermented feather meal replacement of fish meal in diets and the fishes fed with only fish meal in diet were not significantly different ($P > 0.05$) in these study, had better results than the study of Viola & Zahar (1984) on market size hybrids tilapia (*Oreochromis*) that 7.5% feather meal replaced fish meal was reduced growth by 7% from fish fed with control diet and better than the study of Somsueb and Boonyaratpalin (2001) on walking catfish (*Clarias* sp) that highest percentage of replacement fishmeal by feather meal were 5% and the study of Fowler (1990) on juvenile fall chinook salmon (*Oncorhynchus tshawytscha*) that the highest percentage of replacement was 15% of fish meal by feather meal that at the end of experiment weight gain did not differ from fed with diets that replaced 0, 5% of fish meal by feather meal

The feed consumption of Nile tilapia showed that there were not related to the palatability of diets, but walking catfish related to the palatability of diets because the increasing of fermented feather meal in the diets were tended to decrease the feed consumption, but there were not significantly different ($P > 0.05$). Any way, although that means the rank smell of feather meal was reduced by fermentation, the results from Nile tilapia were more effective than walking catfish. Perhaps, the different palatability of diet were varied by characteristic of fishes, in natural the walking catfish are nocturnal and had sensitively label that were used for finding diet (Chuapoehek, 2542) smell receptor of walking catfish probably more efficiency than Nile tilapia

The feed conversion ratio were increased by increasing of fermented feather meal. The feed conversion ratio of walking catfish were higher than Nile tilapia that probably caused from the digestive of herbivorous fish (about 5-8 times of total length in Nile tilapia) were longer than carnivorous fish (about 2 times of total length in walking catfish) (Chuapoehek, 2542) that was made Nile tilapia had more digestive and absorptive area than walking catfish, which similar to the utilization of feather meal in ruminant animal (cattle) had better result than monogastric animal (poultry and swine) (Church, 1983 cited by Punsri, 1991). Protein efficiency ratios of Nile tilapia and walking catfish were reduced by increasing of fermented feather meal. The value of apparent net protein retention of Nile tilapia and walking cat fish had similar tend that were reducing by increasing of fermented feather meal. The different protein efficiency ratio and apparent net protein retention, when considered the protein intake, there were caused from the utilization of available protein in feather meal by Nile tilapia and walking catfish was lower than fish meal. The result indicated that protein quality of feather meal were unsuitable for using in high percentage and protein quality were decreased by increasing of feather meal (Somsueb and Boonyaratpalin, 2001).

For cost of diet related reason, the 50% fermented feather meal replaced fish meal in experimental diet was the most effective because there were not significantly different ($P > 0.05$) from control diet and diet that containing 25% fermented feather meal replaced fish meal. In addition the cost of diet was reduced more than 25% fermented feather meal replaced fish meal. Although the growth and feed efficiency were tended to be lower than 0% (control diet) and 25% fermented feather meal replaced fish meal in experimental diet

and control diet (100% fish meal), prolong culture period would be recommended but must be considered about expenses of farm maintenance prolongation.

For growth and feed efficiency, 25% of fermented feather meal replacement for fish meal in Nile tilapia and walking catfish diet was suitable because there had closely growth and feed efficiency to fish fed with diet containing only fish meal (control).

In this study, the 25% replacement of fish meal by fermented feather meal could reduced Nile tilapia and walking catfish experimental diet cost 0.71 and 0.81 baht/kg. respectively (compare with control diet) and when compared with commercial feed (containing 30% protein (22 baht/kg.)) were reduced cost about 5.46 and 3.62 baht/kg. respectively.

The 50% replacement of fish meal by fermented feather meal could reduced Nile tilapia and walking catfish experimental diet cost 1.31 and 1.38 baht/kg. respectively (compare with control diet) and when compare with commercial feed (containing 30% protein (22 baht/kg.)) were reduced cost about 6.06 and 4.20 baht/kg. respectively.

5.3 Water quality

Water temperature in culture tank, generally did not greater fluctuation in the morning and after noon. The minimum temperature was 23°C and maximum temperature was 26°C. The Dissolved oxygen were ranged from 5.27-5.68 mg/l., pH were ranged from 7.79-7.88, Hardness were ranged from 132.50-133.75 mg/l., Alkalinity were ranged from 368.75-390.25 mg/l and Ammonia concentration of water were ranged from 0.56-0.79 mg/l

Anyway, in this study the water quality were all within the acceptable tolerance or desirable ranges for Nile tilapia and walking catfish culture.

CHAPTER VI

CONCLUSION AND RECOMENDATION

The study on replacement of fish meal with different percentage of fermented feather meal was conducted on Nile tilapia and walking cat fish. Based on the results obtained from this study, it can be concluded as follows:

6.1 Conclusion

1. Quality of experimental diets

Nile tilapia experimental diets were contained protein ranged from 28.48 to 30.38, gross energy ranged from 345 to 368 kcal/100g.diet and walking catfish experimental diets were contained protein ranged from 30.40 to 31.36 gross energy ranged from 365 to 403 kcal/100g.diet.

2. Growth and feed efficiency

Growth and feed efficiency of Nile tilapia and walking catfish, that fed with 0, 25 and 50% fermented feather meal in diet were not significantly different ($P < 0.05$) and fish fed with 0 and 25% fermented feather meal in diet seem to get performance closely together.

3. Cost of experimental diets

The cost of 1-5 Nile tilapia feed formula ranged from 17.25-14.79 baht /kg.. The replacement of fermented feather meal 25, 50, 75 and 100% of fish meal were reduced cost about 0.71, 1.31, 1.97 and 2.46 baht/kg., respectively and when compared with commercial feed (containing 30% protein (22 baht/kg.)) were reduced cost about 2.82, 5.46, 6.06, 6.72 and 7.21 baht/kg., respectively.

The cost of 1-5 walking catfish feed formula ranged from 19.18-16.59 baht/kg. Replacement of fermented feather meal 25, 50, 75 and 100% of fish meal were reduced cost about 0.81, 1.38, 2.09 and 2.59 baht/kg., respectively and compared with commercial feed (containing 30% protein (22 baht/kg.)) were reduced cost about 2.82, 3.62, 4.20, 4.91 and 5.41 baht/kg., respectively.

6.2 Recommendation

6.2.1 Recommendation in this study

1. In feather meal fermentation process should be chosen more effective aeration method than stirred for increase the digestive of microorganism, reduce time for fermentation and reduce anaerobic fermentation that was produced stink smell component such as ammonia.

2. The experimental fish should be fed in a little amount of diets and must be scatter all through tank, when diet was eaten step by step added little amount of diets. Especially in Nile tilapia usually grabbed diets and sucked in the mouth, and then disgorged out later that was caused of incorrect feed consumption

3. The results of this study some factor of 50 and 75 percentage replacement fish meal with feather meal are overlapped, the regression would be recommended to searching for maximum percentage of replacement fish meal by fermented feather meal.

6.2.2 Recommendation in next study

1. The machine using for aeration are interesting to use for increase the digestive of microorganism, reduce time for fermentation and reduce anaerobic fermentation.

2. Finding method for reduce rank smell of feather meal, which caused of decreasing palatability of feather diet or using feather meal as combined with effective attract material such as tuna oil, flavouring material.

3. Repeat the experiment at 25-50 percentage of replacement of fish meal by fermented feather meal in pond and concrete tank with suitable density (at traditional culture density) for finding the most effective ratio of replacement and closely results to traditional fish culture.

BIBLIOGRAPHY

- กรมประมง.คู่มือฝึกอบรมการเลี้ยงสัตว์น้ำ โครงการฟื้นฟูอาชีพเกษตรกรหลังพื้กชำระหนี้ด้านการประมง. กระทรวงเกษตรและสหกรณ์; 2545
- กองควบคุมและพัฒนาอาหารสัตว์น้ำ. การทำอาหารสัตว์น้ำสำเร็จรูปแบบพื้นบ้าน.กรมประมง; 2541
- คณะประมง มหาวิทยาลัยเกษตรศาสตร์. โครงการพัฒนาศักยภาพของผู้เพาะเลี้ยงสัตว์น้ำเพื่อลดต้นทุนและเพิ่มผลผลิตภายใต้ โครงการเพื่อบรรเทาผลกระทบทางสังคมเนื่องจากวิกฤตการณ์ทางเศรษฐกิจ คณะประมง มหาวิทยาลัยเกษตรศาสตร์; 2542.
- ประเสริฐ สีตะสิทธิ์ มะลิบุญรัตน์ผลิน และ นันทิยา อุ่นประเสริฐ.อาหารปลา.สถาบันประมงน้ำจืดแห่งชาติ กองประมงน้ำจืด กรมประมง;2527.
- พันทิพา พงษ์เพ็ญจันทร์.หลักการอาหารสัตว์เล่ม 2 หลักโภชนศาสตร์และการประยุกต์.ภาควิชาสัตวศาสตร์ คณะเกษตรศาสตร์ มหาวิทยาลัยเชียงใหม่; 2539.
- วิลาวัณย์ เจริญจิระตระกูล.จุลินทรีย์ที่มีความสำคัญในด้านอาหาร.คณะวิทยาศาสตร์ มหาวิทยาลัยสงขลานครินทร์.กรุงเทพฯ:สำนักพิมพ์โอเดียนสโตร์;2539
- วีรพงศ์ วุฒิพันธุ์ชัย.อาหารปลา.กรุงเทพฯ.ภาควิชาวาริชศาสตร์ คณะวิทยาศาสตร์ มหาวิทยาลัยบูรพา: สำนักพิมพ์โอเดียนสโตร์; 2537.
- เวียง เชื้อโพธิ์หัก.โภชนศาสตร์สัตว์น้ำและการให้อาหารสัตว์น้ำ.ภาควิชาเพาะเลี้ยงสัตว์น้ำ คณะประมง มหาวิทยาลัยเกษตรศาสตร์.กรุงเทพฯ: สำนักพิมพ์มหาวิทยาลัยเกษตรศาสตร์; 2542.
- ศรีสกุล วรจันทร์ และ รัชชัย สีทธิไกรพงษ์.โภชนศาสตร์สัตว์ .ภาควิชาเทคโนโลยีการผลิตสัตว์ คณะเทคโนโลยีการเกษตร สถาบันเทคโนโลยีพระจอมเกล้าเจ้าคุณทหารลาดกระบัง.กรุงเทพฯ: สำนักพิมพ์โอเดียนสโตร์; 2539.
- Boonyaratpalin M., Unprasert N., and Palmalio E. Fermented Fish Feed. Technical Paper No.8. National Inland Fisheries Institute, Department of Fisheries; 1982 (in Thai)
- Boonyaratpalin M. and Kultungwattana W. Using Soybean Meal as Substitute for Fish Meal in Red Tilapia nilotica diet. Technical Paper. National Inland Fisheries Institute, Department of Fisheries; 1987 (in Thai)
- Boonyaratpalin M., Suraneeranat P. and Tumpibal T. Replacement of Fish Meal with Various Type of Soybean Products in Seabass Diet. Technical Paper No.19/1996. National Institute of Coastal Aquaculture, Department of Fisheries; 1996 (in Thai)
- Das KM., Mohanty SN. and Sarkar S.Optimum dietary protein to energy ratio for *Labeo rohata* Fingerling. Central Institute of Freshwater Aquature Kausalyaganga; 1984

- Fowler LG. Feather meal as a dietary protein sources during parr-smolt transformation in Fall Chinook salmon diets. *Aquaculture*, vol. 89, no 3-4, pp. 301-314; 1990
- Hardy W., Shearer D. and John Spinelli. The nutrition of co-dried fish silage in rainbow trout (*Salmo gairdneri*) dry diet. *Aquaculture*, vol. 38, pp.35-44; 1984
- Henrichfreise B. Valutin of available grain and hydrolyzed feather meal for the feeding of rain bow trout(*Salmo gairdneri* Rich); 1989
- Hiilge V. Development of formulated feed for warm water fish aquaculture. VEROEFF.INST.KUEST.-BINNENFISCH . HAMB;no.86; 1984
- Jantrarotai W., Viputhanumas T. and Somsueb P. Partially Replacing Fish Meal with Corn Gluten Meal Improve Growth and Flesh Coloration of Hybrid *Clarias* catfish(*Clarias macrocephalus* X *Clarias gariepius*). Technical Paper No.178.National Inland Fisheries Institute; 1996 (in Thai)
- Kosutarak P. Use of Chicken-head Silaged for Diet of Hybrid *Clarias* Catfish (*Clarias macrocephalus* x *Clarias gariepinus*). Technical Paper No.1/1999. Feed Quality Control and Development Division, Department of Fisheries; 1999 (in Thai)
- Kungsuwan A., Ittipong B., Junkrajang S., Kriatkangwanklai P., Rao M. and Stephen W. Protein from continuous fermentation of shrimp by-product. Technician paper No.10/1996 Aquatic Industrial Institute, Department of Fisheries; 1996 (in Thai)
- Langar H. and Metailler R. Use of some protein from animal origin for European sea bass (*Dicentrarchus labrax* L.) feeding. *Aquaculture Europe'89*. Short communications and abstracts of review paper; 1989
- Lalu Mayadi. Nitrogen budget in sea bass (*Lates calcarifer* Bloch) culture with different level protein diets:Thesis for the Master Degree of Science (Aquature).Bangkok.Graduate School.Kasetsart University; 2003
- Lim C., Beames RM., Eales JG., Prendergast AF., Mcleese JM., Shearer KD. and Higg DA. Nutritive values of low and high fibre canola meals for shrimp (*Penaeus vannamai*). *Aquaculture Nutrition*; 1997
- Lortscher LL., Sacher GF., Wilkelmy D. and Filbert RB. Processing poultry by-products in poultry slaughter plants. U.S. Department of Agriculture Marketing Research Department; 1957

- Markkert JR., Higgs DA., Mac Quarrie D., McBride JR., Dosanjh BS., Van Tine J. and Reinhardt R. Evaluation of the potential for using dry rather than semi-moist food for culturing coho salmon in British Columbia hatchery facilities .2.Quensam Hatchery; 1984
- Michael J. Waites, Neil L. Morgan, John S. Rockey and Gary Higton .Industrial Microbiology:An Introduction. School of Appiled Science.London; 2001
- Norachan C., Tabphiwan P., Jintasathaporn O. and Mahasawat S. Substitution of dried Golden Apple Snail mail for fish Meal in Tilapia (*Oreochromis nilotica*, Linn.) diets. Faculty of Fisheries. Kasetsart University; 2003(in Thai)
- Punsri P. Effect of Processing Methods of Feather Meal on Protein Utilization by Goat. Sakon Nakhorn Agricultural Research and Training Center; 1991 (in Thai)
- Punsri P. Utilization of Nitrogen from Feather Meal, Soybean Meal and Urea for Sheep. Sakon Nakhorn Agricultural Research and Training Center; 1991 (in Thai)
- Sarmwatanakul A. and Bamrongtum B. Aquarium Fish Nutrition. Extention paper No. 1 /2000. Ornament Fish Research and Public Aquarium; 2000 (in Thai)
- Schulz D., Hartfael W. and Greuel E. Unusual Products Animal Origin in Feeding of Rainbow Trout (*Salmo gairdnerii* R). Use of Blood Meal and Feather Meal in a Purified Diet. Z.TIERPHYSIOL. TIERERNAEHR. FUTTERMITTELKD ; vol 47; 1982
- Sherer KD. and Hardy RW. Phosphorus deficiency in rainbow trout fed a det containing deboned fillet scraps.Prog FISH-CULT. ;vol 49; 1987
- Sinchermiri D., Harnbunchon A. and Boaban R. A Study on Native Value of Hydrolysed Feather Meal Producted in Thailand. Kasetsart University; The proceeding of 42th Kasetsart Univercity Annual Conference. Faculty of Agriculture; 1988 (in Thai)
- Somsueb P. and Boonyaratpalin M. Use of Feather meal in Hybrid *Clarias* catfish Feed (*Clarias macrocephalus* X *Clarias gariepius*). Technical Paper No.5/2001. Feed Quality Control and Development Division, Department of Fisheries; 2001 (in Thai)
- Tinnungwatana W. and Viputhanumas T. Feeding of Thai Silver Barb, *Burbodes gonionotus* (Bleeker) with Brewery Activated Sludge. Technical paper No.12/2000. Phetchaburi Inland Fisheries Division, Department of Fisheries; 2000 (in Thai)

- Viola S. and Zohar G. Nutrition studies with market size hybrids of tilapia (*Oreochromis* sp.) in intensive culture. Protein level and source; 1984
- Viola S., Arieli Y. and Zohar G. Animal-Protein-Free Feed for Hybrid Tilapia (*Oreochromis nilotica* x *Oreochromis aureus*) in Intensive Culture. Aquaculture; vol 75 ; 1988
- Wiesmann D. Investigations on the utilization of cereals and hydrolyzed feather meal in a culture of rainbow trout (*Salmo gairdneri* Rich) ; 1986
- Wiesmann D., Scheid H. and Pfeffer E. Water pollution with phosphorus of dietary origin by intensively fed rainbow trout (*Salmo gairdneri* Rich). Aquaculture; vol 69 ; 1988
- Wimol J. The Estimation of Economic Protein Requirement of Hybrid *Clarias* Catfish (*Clarias macrocephalus* x *Clarias gariepinus*) Technical Paper No.165. National Inland Fisheries Institute; 1995 (in Thai)
- Yaowapak C. and Wongkaew S. Quality of Thai Fish Meal and Perusai Developing Hydrolyzed Feather Meal Protein. Department of Animal Production Technology. Faculty of Agricultural Technology. King Mongkut's Institute of Technology Lardkrabang; 1996 (in Thai)



APPENDIX (A)

การสร้างสูตรอาหารทดลอง

การคำนวณสูตรอาหารที่ใช้ในการทดลองครั้งนี้จะใช้วิธีการคำนวณโดยการแทนที่ (Substitution method) จากสูตรอาหารที่มีปลาป่นในสูตรอาหาร 25% ระดับปริมาณโปรตีน 30% และพลังงาน 400 kcal/ 100g.อาหาร จากนั้นมีการคำนวณหาปริมาณการแทนที่ปลาป่น (โปรตีน 60%) ด้วยขนไก่ป่น (โปรตีน 85%) ในอัตราส่วน 25%, 50%, 75% และ 100% ตามลำดับ (Faculty of Fisheries, 1999)

Raw material (Protein (%))	Raw material (% in diet)					Protein from diet (% in diet)				
	For.1	For.2	For. 3	For. 4	For. 5	For. 1	For. 2	For. 3	For. 4	For. 5
Fish meal (60%)	25	18.75	12.5	6.25	-	15.00	11.25	7.50	3.75	-
Feather meal (85%)	-	4.4	8.8	13.3	17.7	-	3.75	7.50	11.25	15.00

แต่เนื่องจากไขมันและแคลเซียมในขนไก่ป่นต่ำกว่าที่ได้รับจากปลาป่นจึงจำเป็นต้องมีการปรับให้มีพลังงานเท่ากันทุกสูตรอาหารด้วยน้ำมันปลา น้ำมันหมู และไดแคลเซียมฟอสเฟต จากนั้นจะปรับให้อาหารมีปริมาณเท่ากันโดยการเติมเกลือบด จะได้สูตรอาหารสำหรับการทดลอง

Raw material	Diet formula and percentage of fermented feather meal replacement of fish meal				
	Formula 1. (0%)	Formula 2. (25%)	Formula 3. (50%)	Formula 4. (75%)	Formula 5. (100%)
Fish meal	25	18.75	12.5	6.25	-
Feather meal	-	4.4	8.8	13.3	17.7
Soybean meal	30	30	30	30	30
Ground rice broken	15	15	15	15	15
Cassava powder	10	10	10	10	10
Alpha starch	6	6	6	6	6
Vegetable oil	1.3	1.3	1.3	1.3	1.3
Fish oil	1.0	1.4	1.9	2.3	2.7
Lard	-	0.4	0.6	0.9	1.6
Di-calsium phosphate	0.92	0.78	1.11	1.34	1.63
Vitamins & minerals	2	2	2	2	2
Ground husk	8.28	9.47	10.29	11.11	11.57
Yeast cake	0.5	0.5	0.5	0.5	0.5

APPENDIX (B)

วิธีวิเคราะห์คุณค่าทางโภชนาการอาหารและปลา

โปรตีน(Protien)

โปรตีนในอาหารสัตว์จะมีองค์ประกอบของไนโตรเจนเป็นสัดส่วนที่แน่นอน โดยปกติ โปรตีนจะมีปริมาณของไนโตรเจนอยู่ประมาณ 16% ดังนั้นเมื่อวิเคราะห์หาค่าของ ไนโตรเจน แล้วคูณด้วย 6.25 จะได้ค่า Percentage ของโปรตีนของตัวอย่างอาหาร

สารเคมีที่ใช้

1. Sel-dahl pack (เป็นชื่อของ Premixed Catalysts ซึ่งประกอบด้วย 9.7 gm K_2SO_4 , 0.19 gm $Cu SeO_3$, H_2O , 0.11 gm permice) หรืออาจใช้ Catalyst ซึ่งประกอบด้วย 100 กรัม ของ K_2SO_4 10 กรัม ของ $CuSeO_4 \cdot 5H_2O$ และ 1 กรัมของ Se เป็นส่วนผสมก็ได้
2. Concentrated H_2SO_4 ใช้ Oxidized โปรตีนให้ไนโตรเจนอยู่ในรูปของ $(NH_4)_2SO_4$
3. Sat. NaOH ละลาย 580 กรัมของ NaOH ในน้ำ 1 ลิตร
4. Boric acid 4% ละลาย 40 กรัม boric acid ในน้ำกลั่นและทำเป็น 1 ลิตร
5. Indicator ละลาย 0.5 กรัม Bromocresal Green และ 0.1 กรัม Methyl red ใน 100 ลบ.ซม. ของ 95% Ethanal
6. Standardized 0.1 N HCl
7. Boiling chips หรือ Glass beads

วิธีการ

1. ชั่งตัวอย่างใส่กระดาษกรอง แล้วใส่ใน Kjeldahl flask ขนาด 800 ml. (ถ้าปริมาณโปรตีนในตัวอย่างมีมากกว่า 20% ชั่งตัวอย่างประมาณ 0.7 กรัม อย่างละเอียด ถ้าคาดว่าตัวอย่างมีปริมาณโปรตีนต่ำกว่า 20% ชั่งตัวอย่างประมาณ 1.4 กรัม) และเตรียม Blank โดยใส่เฉพาะ Filter paper และใส่ Boiling chips
2. ใส่ Sel-dahl catalyst 10 กรัม และ 25 ลบ.ซม. ของ H_2SO_4 concentrate ใน flask ทุกอัน
3. ย่อยด้วยความร้อนจนสีดำใน flask เปลี่ยนเป็นสีใส คอยหมุนขวดให้ส่วนที่เหลือถูกย่อยจนในหมด และย่อยต่อไปอีก 1.5 ชั่วโมง หลังจากย่อยจนใสแล้ว

4. ตั้ง flask ทิ้งไว้ให้เย็น เติมน้ำ 300 ลบ.ซม. อย่างช้าๆ โดยเอียงคอขวดของ flask เขย่าให้ผสมกัน ตั้งทิ้งให้เย็นในอุณหภูมิห้อง
5. ค่อยๆ เท NaOH Saturated 100 ลบ.ซม. อย่างระมัดระวัง ค่อยๆ ทำให้ไหลตามคอขวดพร้อมกับแตะส่วนของหลอดเก็บ distiller พร้อมเขย่าให้ผสมกัน
6. เก็บ distillate 150 ลบ.ซม. ใน 25 ลบ.ซม. 4% boric acid และเติม 4 หยดของ indicator
7. Titrate ด้วย 0.1 N HCl จนถึง end point

การคำนวณ

$$\% \text{ไนโตรเจน} = \frac{(S-B)(14.007)(n)}{\text{gm ของตัวอย่าง} \times 100} \times 100$$

โดยที่

S = ml HCL ที่ใช้ titrate ตัวอย่าง

B = ml HCL ที่ใช้ titrate กับ Blank

n = Normally ของ HCl

จากนั้นจะได้เปอร์เซ็นต์โปรตีนโดย

$$\% \text{ crude protein} = \% \text{ไนโตรเจน} \times 6.25$$

สำหรับในกรณีตัวอย่างสด ใช้ตัวอย่างประมาณ 2 กรัม และใช้ H_2SO_4 เพิ่มเป็น 40 ลบ.ซม.

ไขมัน(Lipid)

การวิเคราะห์ปริมาณไขมันในอาหารจะใช้วิธี Ether Extraction

สารเคมีและเครื่องมือที่ใช้

1. Petroleum Sperate
2. เตาอบที่สามารถอบที่ 80 °C.
3. Poursous extraction thimbles
4. Extraction beaker
5. Extraction tubes
6. Recovery tubes
7. Goldfish extractor

วิธีการ

1. อบ extraction beakers และ thimbles ใน oven เป็นเวลา 1 ชั่วโมง แล้วทำให้เย็นใน dessicator
2. ชั่ง beaker และ thimbles
3. ชั่งตัวอย่างประมาณ 2 กรัมอย่างละเอียดใส่ใน thimble
4. ใส่ thimble และตัวอย่างลงใน extraction tube แล้วใส่ใน goldfish extractor
5. ใส่ Petroleum ether ประมาณ 35-40 ml. ลงใน extraction beaker แล้วใส่ในเครื่อง Goldfish extractor
6. เปิดน้ำและไฟให้พอเหมาะที่ Petroleum ether จะระเหยเป็นไอ และ condense กลับลงมาอีกได้ ให้ Petroleum ether สกัดไขมันออกจากตัวอย่างเป็นเวลา 4 ชั่วโมงแล้วปิดไฟให้ petroleum ether หยุดการสกัดผ่านตัวอย่าง
7. เอา Beaker และ Extraction tube ออก เปลี่ยนเอ Recovery tube ใส่แทน เปิดไฟให้ Petroleum จาก Beaker กลับ Condense ไปอยู่ใน Recovery tube
8. เอา Petroleum ออกประมาณให้เหลืออยู่ 3/8 ที่มีอยู่แล้วเอา Beaker และ Recovery tube ออกเก็บ Petroleum ether ไว้ใช้ใหม่
9. ปลอ่ยให้ Petroleum ether ที่เหลืออยู่ระเหยออก แล้วเอา Beaker ใส่ใน Oven เพื่อทำให้แห้งเป็นเวลา 1 ชั่วโมง
10. เก็บ Beaker ให้เย็นใน Dessicator
11. ชั่งน้ำหนักของ Beaker อีกครั้ง คำนวณหา % ไขมัน

วิธีการคำนวณ

$$\% \text{ ไขมัน} = \frac{(\text{น้ำหนัก Beaker} + \text{ไขมัน}) - \text{น้ำหนักของ Beaker}}{\text{น้ำหนักของตัวอย่าง}} \times 100$$



เส้นใย (Crude Fiber)

สารเคมี และเครื่องมือที่ใช้

1. Residue จากการหาไขมัน ถ้าตัวอย่างมีไขมันต่ำกว่า 1 % ไม่ต้องสกัดไขมันออกก่อนได้
2. Sulfuric Acid Solⁿ 1.25 กรัม/100 ลบ.ซม. หรือ 0.255 N.
3. Sodium hydroxide solution 1.25 gm./100 ml. หรือ 0.312 N.
4. Ethyl Alcohol 95%
5. Digestion Flask
6. Condenser ช่วยให้ปริมาตรของสารละลายคงที่ตลอดการย่อย
7. Funnel
8. Ashless filter paper

วิธีการ

1. นำตัวอย่างที่สกัดไขมันออกแล้ว (รู้น้ำหนักอย่างละเอียด) มาใส่ลงใน digestion flask เติมสารละลายกรดที่กำลังเดือดอยู่ 200 ml. ลงไปต่อ Condenser กับขวด ส่วนผสมกรดเดือดภายใน 1 นาทีแล้วต้มต่อไป 30 นาที หมุนขวดไปมาทุกๆ นาที เพื่อให้ตัวอย่างถูกกับกรดทั่วถึงกันดี
2. กรองส่วนผสมในขวดทันทีด้วย Ashless filter paper ล้างส่วนที่ค้างอยู่ บนกระดาษกรองด้วยน้ำเดือดจนหมดกรด
3. เตรียมขวดใส่สารละลายด่างที่เดือดไว้ให้พร้อม นำส่วนที่เหลือบนกระดาษกรองใส่ลงใน Digestion flask แล้วเติมสารละลายต่างๆ 200 ลบ.ซม. ลงไป ใช้สารละลายนี้ล้างตัวอย่างบนกระดาษกรองให้หมด แล้วต้ม 30 นาทีเขย่าเป็นครั้งคราว
4. กรองด้วยกระดาษกรองแผ่นเดิมแล้วล้างตัวอย่างถ่ายน้ำร้อนจน Filtrate ปราศจากด่างแล้วล้างด้วย Alcohol 95% นำตัวอย่างที่เหลืออยู่บนกระดาษกรองไปอบที่ 100 เซลเซียส แล้วหักค่าของกระดาษกรองออกได้เป็นน้ำหนักของตัวอย่างที่ไม่ละลายในกรดและด่างหรือเท่ากับ Dried Weight ของ Fiber
5. นำไปเผาเพื่อหาค่าของเถ้าจะได้น้ำหนักของเถ้า
6. คำนวณค่าของ Crude Fiber จาก Dried Weight ของ Fiber

$$\text{Weight of ash} = \text{Crude fiber}$$

$$\% \text{ Crude fiber} = \frac{\text{Crude fiber}}{\text{น้ำหนักของตัวอย่างแห้ง}} \times 100$$

เถ้า (Ash)

เถ้าเป็นส่วนหนึ่งของ Inorganic ในอาหารและส่วนผสมของอาหาร เป็นส่วนที่เหลืออยู่เมื่อเผาไหม้เอาน้ำและส่วนของ organic ออกไปแล้ว

วิธีการ

1. อบCrucible และฝาที่ 100 องศาเซลเซียส ใน Oven เป็นเวลา 1 ชั่วโมงทำให้เย็นใน Desicator และชั่งอย่างละเอียด
2. ชั่งตัวอย่างใส่ใน Crusible ปริมาณ 2 กรัม อย่างละเอียด (อาจใช้ตัวอย่างที่หาความชื้น แล้วมาทำเป็นเถ้าต่อได้)
3. วางCrusible บน Hot plate และเติม 0.2 ลบ.ซม. ของ Concentrate Nitric acid ภายใต้ Hood และเปิดไฟของ Hot plate ให้สูงขึ้น และตั้งทิ้งไว้ 45 นาที และใช้ฝาเปิดเผยอไว้เล็กน้อย
4. ย้ายไปใส่ใน Muffle Furnace เป็นเวลา 2 ชั่วโมงที่ 600 องศาเซลเซียส
5. ทำให้เย็นลงใน Desicator และชั่งน้ำหนักตัวอย่างที่เหลือใน Crusible

การคำนวณ

$$\% \text{ เถ้า} = \frac{\text{น้ำหนักเถ้า}}{\text{น้ำหนักของตัวอย่างแห้ง}} \times 100$$

เถ้าที่ได้เก็บไว้วิเคราะห์หา Ca และ Phosphorus

ความชื้น (moisture)

วิธีการ

1. อบ Crucible หรือขวดชั่งพร้อมฝาที่ 100 องศาเซลเซียส ใน Oven นาน 30 นาที และ เก็บให้เย็นใน Desiccator และชั่งน้ำหนัก Crucible หรือขวดชั่งโดยไม่วางฝา
2. ใส่ตัวอย่างลงไปใน Crucible หรือขวดชั่งประมาณ 2 กรัม
3. ทำให้ตัวอย่างแห้งโดยใช้ Oven 100 องศาเซลเซียส และให้เปิดฝาแย้มๆไว้
4. ตัวอย่างแห้ง เช่น อาหารปลา ก็ทำให้แห้งโดยใช้เวลา 6 ชั่วโมง
5. ตัวอย่างสด (เปียก) เช่น เนื้อเยื่อปลา ฯลฯ ใช้เวลา 24 ชั่วโมง ในการทำให้แห้งหรือจนกว่าน้ำหนักของตัวอย่างจะคงที่
6. หลังจากทำให้แห้งแล้ว เก็บให้เย็นใน Dessicator และชั่งน้ำหนักอีกครั้งโดยไม่ต้องวาง ฝาครอบ

วิธีการคำนวณ

$$\% \text{ Dry Matter} = \frac{\text{น.น. ของตัวอย่างแห้ง}}{\text{น.น. ตัวอย่างเริ่มต้น}} \times 100$$

APPENDIX (C)

Table A-1 Data analysis (ANOVA) of average weight of Nile tilapia every 2 weeks throughout the experiment

		Sum of Squares	Df	Mean Square	F	Sig.
Initial	Between Groups	1.538	4	.384	.302	.870
	Within Groups	12.708	10	1.271		
	Total	14.245	14			
Wk 2	Between Groups	105.238	4	26.309	1.417	.298
	Within Groups	185.687	10	18.569		
	Total	290.924	14			
Wk 4	Between Groups	247.205	4	61.801	7.247	.005
	Within Groups	85.279	10	8.528		
	Total	332.484	14			
Wk 6	Between Groups	577.546	4	144.387	1.566	.257
	Within Groups	921.791	10	92.179		
	Total	1499.337	14			
Wk 8	Between Groups	838.289	4	209.572	1.894	.188
	Within Groups	1106.572	10	110.657		
	Total	1944.861	14			
Wk 10	Between Groups	1364.191	4	341.048	3.306	.057
	Within Groups	1031.694	10	103.169		
	Total	2395.885	14			
Wk 12	Between Groups	2590.185	4	647.546	6.253	.009
	Within Groups	1035.638	10	103.564		
	Total	3625.823	14			

Table A-2 Data analysis (Duncan) of initial average weight of Nile tilapia

Diet	N	Subset for alpha = .05
		1
2	3	122.806000
5	3	123.031833
1	3	123.078667
3	3	123.495167
4	3	123.678333
Sig.		.402

Means for groups in homogeneous subsets are displayed.

a Uses Harmonic Mean Sample Size = 3.000.

Table A-3 Data analysis (Duncan) of average weight of Nile tilapia at week 2nd

Diet	N	Subset for alpha = .05	
		1	
5	3	144.273833	
4	3	149.615167	
1	3	150.134167	
3	3	150.955000	
2	3	151.809833	
Sig.			.077

Means for groups in homogeneous subsets are displayed.

a Uses Harmonic Mean Sample Size = 3.000.

Table A-4 Data analysis (Duncan) of average weight of Nile tilapia at week 4th

Diet	N	Subset for alpha = .05	
		1	2
5	3	144.289000	
4	3		152.214833
3	3		152.816333
2	3		155.304500
1	3		155.363000
Sig.		1.000	.246

Means for groups in homogeneous subsets are displayed.

a Uses Harmonic Mean Sample Size = 3.000.

Table A-4 Data analysis (Duncan) of average weight of Nile tilapia at week 6th

Diet	N	Subset for alpha = .05	
		1	
5	3	159.282000	
4	3	163.210667	
3	3	170.107833	
1	3	173.729167	
2	3	175.550833	
Sig.			.086

Means for groups in homogeneous subsets are displayed.

a Uses Harmonic Mean Sample Size = 3.000.

Table A-5 Data analysis (Duncan) of average weight of Nile tilapia at week 8th

diet	N	Subset for alpha = .05	
		1	
5	3	168.226833	
4	3	170.023667	
3	3	181.284000	
1	3	182.788333	
2	3	187.306333	
Sig.			.069

Means for groups in homogeneous subsets are displayed.

a Uses Harmonic Mean Sample Size = 3.000.

Table A-6 Data analysis (Duncan) of average weight of Nile tilapia at week 10th

diet	N	Subset for alpha = .05		
		1	2	3
5	3	180.765833		
4	3	184.120000	184.120000	
3	3	194.091000	194.091000	194.091000
1	3		202.139000	202.139000
2	3			204.903667
Sig.		.156	.064	.242

Means for groups in homogeneous subsets are displayed.

a Uses Harmonic Mean Sample Size = 3.000.

Table A-7 Data analysis (Duncan) of average weight of Nile tilapia at week 12th

diet	N	Subset for alpha = .05	
		1	2
5	3	192.082333	
4	3	195.966500	
3	3	206.968667	206.968667
2	3		222.350000
1	3		224.082667
Sig.		.118	.077

Means for groups in homogeneous subsets are displayed.

a Uses Harmonic Mean Sample Size = 3.000.

Table A-8 Data analysis (ANOVA) of feed consumption of Nile tilapia

		Sum of Squares	df	Mean Square	F	Sig.
Feed consumption	Between Groups	387.641	4	96.910	1.593	.250
	Within Groups	608.376	10	60.838		
	Total	996.017	14			

Table A-9 Data analysis (Duncan) of feed consumption of Nile tilapia

Diet	N	Subset for alpha = .05	
		1	
4	3	181.594700	
5	3	183.426350	
1	3	193.147050	
3	3	195.876633	
2	3	198.382083	
Sig.			.121

Means for groups in homogeneous subsets are displayed.

a Uses Harmonic Mean Sample Size = 3.000.

Table A-10 Data analysis (ANOVA) of growth and feed efficiency of Nile tilapia

		Sum of Squares	Df	Mean Square	F	Sig.
Percentage of weight gain	Between Groups	1790.412	4	447.603	6.950	.006
	Within Groups	644.015	10	64.402		
	Total	2434.427	14			
Survival rate	Between Groups	.000	4	.000	.	.
	Within Groups	.000	10	.000		
	Total	.000	14			
FCR	Between Groups	1.228	4	.307	8.980	.002
	Within Groups	.342	10	.034		
	Total	1.570	14			
PER	Between Groups	.450	4	.112	7.768	.004
	Within Groups	.145	10	.014		
	Total	.594	14			
ANPR	Between Groups	114.584	4	28.646	9.107	.002
	Within Groups	31.456	10	3.146		
	Total	146.040	14			
Protein intake	Between Groups	114.365	4	28.591	1.693	.227
	Within Groups	168.861	10	16.886		
	Total	283.226	14			
ADG	Between Groups	.377	4	.094	6.693	.007
	Within Groups	.141	10	.014		
	Total	.517	14			

Table A-11 Data analysis (Duncan) of percentage weight gain of Nile tilapia

Diet	N	Subset for alpha = .05	
		1	2
5	3	72.971691	
4	3	75.120588	
3	3	86.272414	86.272414
2	3		101.215378
1	3		105.876872
Sig.		.127	.060

Means for groups in homogeneous subsets are displayed.

a Uses Harmonic Mean Sample Size = 3.000.

Table A-11 Data analysis (Duncan) of feed conversion ratio of Nile tilapia

Diet	N	Subset for alpha = .05		
		1	2	3
2	3	1.779199		
1	3	1.929401		
3	3		2.319264	
4	3		2.537406	
5	3			2.931920
Sig.		.652	.084	.274

Means for groups in homogeneous subsets are displayed.

a Uses Harmonic Mean Sample Size = 3.000.

Table A-12 Data analysis (Duncan) of protein efficiency ratio of Nile tilapia

Diet	N	Subset for alpha = .05		
		1	2	3
5	3	1.264917		
4	3	1.315149		
3	3	1.511462	1.511462	
1	3		1.768781	1.768781
2	3			1.848486
Sig.		.214	.086	.251

Means for groups in homogeneous subsets are displayed.

a Uses Harmonic Mean Sample Size = 3.000.

Table A-13 Data analysis (Duncan) of apparent net protein retention of Nile tilapia

Diet	N	Subset for alpha = .05		
		1	2	3
4	3	24.193333		
3	3	27.643333	27.643333	
1	3	27.736667	27.736667	
5	3	28.620000	28.620000	28.620000
2	3			31.863333
Sig.		.964	.087	.223

Means for groups in homogeneous subsets are displayed.

a Uses Harmonic Mean Sample Size = 3.000.

Table A-14 Data analysis (Duncan) of protein intake of Nile tilapia

Diet	N	Subset for alpha = .05	
		1	
4	3	53.251879	
2	3	54.704867	
5	3	54.962685	
3	3	55.132248	
1	3	56.999298	
Sig.			.053

Means for groups in homogeneous subsets are displayed.

a Uses Harmonic Mean Sample Size = 3.000.

Table A-15 Data analysis (Duncan) of average daily weight gain of Nile tilapia

Diet	N	Subset for alpha = .05	
		1	2
5	3	.822067	
4	3	.860600	
3	3	.993733	.993733
2	3		1.185067
1	3		1.202433
Sig.		.121	.066

Means for groups in homogeneous subsets are displayed.

a Uses Harmonic Mean Sample Size = 3.000.

Table A-16 Data analysis (ANOVA) of average weight of walking catfish every 2 week though out the experiment

		Sum of Squares	Df	Mean Square	F	Sig.
Initial	Between Groups	.275	4	.069	.729	.592
	Within Groups	.944	10	.094		
	Total	1.220	14			
Wk 2	Between Groups	46.039	4	11.510	5.047	.017
	Within Groups	22.805	10	2.280		
	Total	68.843	14			
Wk 4	Between Groups	189.724	4	47.431	6.101	.009
	Within Groups	77.748	10	7.775		
	Total	267.472	14			
Wk 6	Between Groups	715.019	4	178.755	6.619	.007
	Within Groups	270.047	10	27.005		
	Total	985.067	14			
Wk 8	Between Groups	2793.929	4	698.482	13.692	.000
	Within Groups	510.142	10	51.014		
	Total	3304.070	14			

Table A-17 Data analysis (Duncan) of initial average weight of walking catfish

Diet	N	Subset for alpha = .05	
		1	
4	3	73.726000	
3	3	73.979000	
1	3	74.044500	
2	3	74.085667	
5	3	74.089833	
Sig.			.211

Means for groups in homogeneous subsets are displayed.

a Uses Harmonic Mean Sample Size = 3.000.

Table A-18 Data analysis (Duncan) of average weight of walking catfish at week 2nd

Diet	N	Subset for alpha = .05	
		1	2
5	3	77.411500	
4	3	79.551500	79.551500
1	3		80.642000
3	3		81.887333
2	3		82.254000
Sig.		.113	.068

Means for groups in homogeneous subsets are displayed.

a Uses Harmonic Mean Sample Size = 3.000.

Table A-19 Data analysis (Duncan) of average weight of walking catfish at week 4th

Diet	N	Subset for alpha = .05	
		1	2
5	3	81.948333	
4	3	86.667333	86.667333
1	3		89.624167
3	3		90.958000
2	3		91.689500
Sig.		.065	.067

Means for groups in homogeneous subsets are displayed.

a Uses Harmonic Mean Sample Size = 3.000.

Table A-20 Data analysis (Duncan) of average weight of walking catfish at week 6th

Diet	N	Subset for alpha = .05	
		1	2
5	3	89.763500	
4	3		101.053667
1	3		106.391000
3	3		107.019167
2	3		108.705167
Sig.		1.000	.123

Means for groups in homogeneous subsets are displayed.

a Uses Harmonic Mean Sample Size = 3.000.

Table A-21 Data analysis (Duncan) of average weight of walking catfish at week 8th

Diet	N	Subset for alpha = .05		
		1	2	3
5	3	96.556833		
4	3		114.428833	
3	3		125.715000	125.715000
1	3			132.580167
2	3			132.670000
Sig.		1.000	.082	.281

Means for groups in homogeneous subsets are displayed.

a Uses Harmonic Mean Sample Size = 3.000.

Table A-22 Data analysis (ANOVA) of feed consumption of walking catfish

		Sum of Squares	Df	Mean Square	F	Sig.
Feed consumption	Between Groups	496.815	4	124.204	36.292	.000
	Within Groups	34.224	10	3.422		
	Total	531.039	14			

Table A-22 Data analysis (Duncan) of feed consumption of walking catfish

Diet	N	Subset for alpha = .05			
		1	2	3	4
5	3	90.248067			
4	3		144.233333		
3	3		149.127500	149.127500	
2	3			152.371000	
1	3				160.793833
Sig.		.145	.128	.057	1.000

Means for groups in homogeneous subsets are displayed.

a Uses Harmonic Mean Sample Size = 3.000.

Table A-23 Data analysis (ANOVA) of growth and feed efficiency of walking catfish

		Sum of Squares	Df	Mean Square	F	Sig.
Percentage of weight gain	Between Groups	5117.686	4	1279.421	13.381	.001
	Within Groups	956.176	10	95.618		
	Total	6073.862	14			
Survival rate	Between Groups	.000	4	.000		
	Within Groups	.000	10	.000		
	Total	.000	14			
FCR	Between Groups	4.158	4	1.039	8.464	.003
	Within Groups	1.228	10	.123		
	Total	5.386	14			
PER	Between Groups	.435	4	.109	5.628	.012
	Within Groups	.193	10	.019		
	Total	.628	14			
ANPR	Between Groups	525.014	4	131.254	8.727	.003
	Within Groups	150.400	10	15.040		
	Total	675.414	14			
Protein intake	Between Groups	935.617	4	233.904	17.007	.000
	Within Groups	137.538	10	13.754		
	Total	1073.155	14			
ADG	Between Groups	.895	4	.224	13.654	.000
	Within Groups	.164	10	.016		
	Total	1.059	14			

Table A-24 Data analysis (Duncan) of percentage weight gain of walking catfish

Diet	N	Subset for alpha = .05		
		1	2	3
5	3	30.127658		
4	3	55.212400	55.212400	
3	3		69.973133	69.973133
2	3			79.065667
1	3			79.075000
Sig.		.647	.094	.302

Means for groups in homogeneous subsets are displayed.

a Uses Harmonic Mean Sample Size = 3.000.

Table A-25 Data analysis (Duncan) of feed conversion ratio of walking catfish

diet	N	Subset for alpha = .05		
		1	2	3
2	3	2.623733		
1	3	2.773100		
3	3	2.968917	2.968917	
4	3		3.549217	3.549217
5	3			4.034697
Sig.		.276	.070	.121

Means for groups in homogeneous subsets are displayed.

a Uses Harmonic Mean Sample Size = 3.000.

Table A-26 Data analysis (Duncan) of protein efficiency ratio of walking catfish

Diet	N	Subset for alpha = .05		
		1	2	3
5	3	.794935		
4	3	.906800	.906800	
3	3		1.106833	1.106833
1	3		1.150100	1.150100
2	3			1.264867
Sig.		.347	.067	.213

Means for groups in homogeneous subsets are displayed.

a Uses Harmonic Mean Sample Size = 3.000.

Table A-27 Data analysis (Duncan) of apparent net protein retention of walking catfish

Diet	N	Subset for alpha = .05	
		1	2
5	3	14.816667	
4	3	18.116667	
3	3		25.433333
1	3		28.653333
2	3		29.856667
Sig.		.322	.212

Means for groups in homogeneous subsets are displayed.

a Uses Harmonic Mean Sample Size = 3.000.

Table A-28 Data analysis (Duncan) of protein intake of walking catfish

Diet	N	Subset for alpha = .05	
		1	2
5	3	28.139042	
4	3		45.231600
2	3		46.320800
3	3		46.751467
1	3		50.875133
Sig.		1.000	.113

Means for groups in homogeneous subsets are displayed.

a Uses Harmonic Mean Sample Size = 3.000.

Table A-29 Data analysis (Duncan) of average daily weight gain of walking catfish

Diet	N	Subset for alpha = .05		
		1	2	3
5	3	.398800		
4	3		.726833	
3	3		.923867	.923867
1	3			1.045300
2	3			1.046167
Sig.		1.000	.089	.290

Means for groups in homogeneous subsets are displayed.

a Uses Harmonic Mean Sample Size = 3.000.

Table A-30 Data analysis (ANOVA) of chemical composition of Nile tilapia carcass

		Sum of Squares	Df	Mean Square	F	Sig.
Protein	Between Groups	437.165	5	87.433	161.607	.000
	Within Groups	6.492	12	.541		
	Total	443.657	17			
Lipid	Between Groups	257.206	5	51.441	1338.534	.000
	Within Groups	.461	12	.038		
	Total	257.667	17			
Fiber	Between Groups	.552	5	.110	715.964	.000
	Within Groups	.002	12	.000		
	Total	.554	17			

Table A-30 Data analysis (Duncan) of crude protein composition of Nile tilapia carcass

Diet	N	Subset for alpha = .05			
		1	2	3	4
Initial	3	75.831968			
1	3		84.518120		
4	3			85.931706	
2	3			86.222093	
3	3			87.175238	
5	3				92.421513
Sig.		1.000	1.000	.072	1.000

Means for groups in homogeneous subsets are displayed.

a Uses Harmonic Mean Sample Size = 3.000.

Table A-31 Data analysis (Duncan) of crude lipid composition of Nile tilapia carcass

Diet	N	Subset for alpha = .05				
		1	2	3	4	5
5	3	13.435076				
1	3		21.181839			
4	3			21.990008		
Initial	3				23.774680	
2	3					24.205063
3	3					24.303849
Sig.		1.000	1.000	1.000	1.000	.549

Means for groups in homogeneous subsets are displayed. a Uses Harmonic Mean Sample Size = 3.000.

Table A-32 Data analysis (Duncan) of crude fiber composition of Nile tilapia carcass

FOOD	N	Subset for alpha = .05					
		1	2	3	4	5	6
4	3	1.187312					
3	3		1.210253				
2	3			1.387708			
Initial	3				1.519109		
1	3					1.550791	
5	3						1.660930
Sig.		1.000	1.000	1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a Uses Harmonic Mean Sample Size = 3.000.

Table A-33 Data analysis (ANOVA) of chemical composition of walking catfish carcass

		Sum of Squares	df	Mean Square	F	Sig.
Protein	Between Groups	30.823	5	6.165	94.936	.000
	Within Groups	.779	12	.065		
	Total	31.602	17			
Lipid	Between Groups	16.957	5	3.391	17820.101	.000
	Within Groups	.002	12	.000		
	Total	16.959	17			
Fiber	Between Groups	.981	5	.196	10380.254	.000
	Within Groups	.000	12	.000		
	Total	.981	17			

Table A-34 Data analysis (Duncan) of crude protein composition of walking catfish carcass

Diet	N	Subset for alpha = .05			
		1	2	3	4
initial	3	59.830515			
3	3		60.751555		
5	3		60.849980		
2	3			62.335680	
1	3			62.723096	
4	3				63.625538
Sig.		1.000	.645	.087	1.000

Means for groups in homogeneous subsets are displayed.

a Uses Harmonic Mean Sample Size = 3.000.

Table A-35 Data analysis (Duncan) of crude lipid composition of walking catfish carcass

Diet	N	Subset for alpha = .05					
		1	2	3	4	5	6
4	3	2.064328					
1	3		3.206127				
3	3			3.314259			
2	3				3.548703		
5	3					3.674856	
initial	3						5.355200
Sig.		1.000	1.000	1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a Uses Harmonic Mean Sample Size = 3.000.

Table A-36 Data analysis (Duncan) of crude fiber composition of walking catfish carcass

Diet	N	Subset for alpha = .05					
		1	2	3	4	5	6
5	3	.837215					
4	3		.877339				
3	3			.946931			
1	3				1.206925		
2	3					1.259456	
Initial	3						1.484683
Sig.		1.000	1.000	1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed. a Uses Harmonic Mean Sample Size = 3.000

BIOGRAPHY

NAME	Mr. Ek Rakyuttithamkul
DATE OF BIRTH	21 Novenber 1976
PLACE OF BIRTH	Prachinburee, Thailand
INSTITUTIONS ATTENDED	Kasetsart University, 1994-1999 Bachelor of Science (Fisheries) Mahidol University, 2000-2005 Master of Science (Appropriate technology for resources and environmental development)
HOME-ADDRESS	201/542 Vibhavadi 64 Rd. Laksi Bangkok, 10210 Tel. 091209101