



UNIVERSITI PUTRA MALAYSIA

**DIETARY PHOSPHOLIPIDS REQUIREMENT
FOR BELUGA STURGEON (*Huso huso*) JUVENILES**

MAHMOUDREZA EBRAHIMNEZHADARABI

FP 2010 26



**DIETARY PHOSPHOLIPIDS REQUIREMENT
FOR BELUGA STURGEON (*Huso huso*) JUVENILES**

MAHMOUDREZA EBRAHIMNEZHADARABI

**MASTER OF SCIENCE
UNIVERSITI PUTRA MALAYSIA**

2010



**DIETARY PHOSPHOLIPIDS REQUIREMENT
FOR BELUGA STURGEON (*Huso huso*) JUVENILES**

MAHMOUDREZA EBRAHIMNEZHADARABI

**Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia,
in Fulfilment of the Requirements for the Degree of Master of Science**

October 2010



DEDICATION

To my dear father, dear mother, my beloved wife, dear children and my family



Abstract of thesis presented to the senate of Universiti Putra Malaysia in fulfilment
of the requirement of the degree of Master Science

**DIETARY PHOSPHOLIPIDS REQUIREMENT
FOR BELUGA STURGEON (*Huso huso*) JUVENILES**

By:

MAHMOUDREZA EBRAHIMNEZHADARABI

October 2010

Chairman: Assoc. Prof. Dr. Che Roos Bin Saad

Faculty: Agriculture

A study was carried out to determine the influence of dietary phospholipids (PL) levels on growth, body composition, haematology parameters, biochemical factors and immune parameters of beluga sturgeon (*Huso huso*) juveniles. Juveniles were fed formulated diet with four varying dietary levels of PL i.e. 0 (D1), 2 (D2), 4 (D3), and 6% (D4). At the end of the experimental period (56 days), the results showed that there were significant differences ($P < 0.05$) among treatments in growth performance. Fish fed diet containing 4% phospholipids obtained the best weight gain and percentage survival ($P < 0.05$) with values of 70.3 g and 76.7% respectively. However, there was no significant difference ($P > 0.05$) was found for feed conversion ratio (FCR), specific growth rate (SGR), protein efficiency ratio (PER), and lipid efficiency ratio (LER). Significant differences ($P < 0.05$) were observed in moisture



and protein composition in fish among all treatments but however, the inclusion of phospholipids in diets had no significant effect ($P>0.05$) on the fat and ash composition in fish. Results of the experiment also showed that there was a significant difference ($P<0.05$) observed in mean corpuscular hemoglobin concentration (MCHC) (g/dl). Fish fed diet D2 (2% PL) had the highest MCHC with a value of 33.3 g/dl. There was no significant different ($P>0.05$) in other haematology parameters such as red blood cells (RBC), haematocrit (Hct), haemoglobin (Hb), mean corpuscular hemoglobin (MCH), mean corpuscular volume (MCV), and white blood cells (WBC) in treated fish . The highest RBC reading was found in fish fed diet D2 with numerical value of 1.07 (cells/l) for and the lowest value was found for fish fed diet D1 with a value of 0.86 (cells/l). Percent haematocrit readings ranged from the highest (fish fed D3) to the lowest (fish fed D1) with values of 26.33 and 25.00% respectively. Haemoglobin (Hb) ranging from the highest (fish fed D2) to the lowest (fish fed D1) with values of 8.64 and 7.67 (g/dl) respectively. Mean corpuscular hemoglobin (MCH) ranging from the highest (fish D1) to the lowest (fish fed D2) with values of 92.57 and 77.27 (pg/cell) respectively. Mean corpuscular volume (MCV) ranged from the highest (fish fed D1) lowest (fish fed D2) with values of 300.83 and 232.60 (fL/cell) respectively. White blood cells (WBC) count in fish fed with varying dietary phospholipids levels, ranged from the highest (fish fed D2) lowest (fish fed D3) with values 25866.67 and 22633.33 (cell/ μ l) respectively. The measurement of differentiated white blood cell of *Huso huso* with varying dietary phospholipids levels such as lymphocytes, monocytes, neutrophils, and eosinophils also showed that there were no significant differences ($P>0.05$) amongst the treatments. The highest percentage amount of lymphocyte and monocyte were found in fish fed diet D2 with values of 71.67% and



3.67% respectively. While the highest readings for neutrophils, and eosinophils was found in fish fed diets D1 and D4 with values of 21.33% and 8.33% respectively. The lowest amount of lymphocyte, neutrophil, monocyte, and eosinophils were shown in fish fed diets D4, D2, D3, and D1 with values of 68, 18.67, 2.33 and 6% respectively. At the end of the experimental period, there were no significant differences ($P>0.05$) were found for hepatic enzymes among treatments. The enzyme alanine aminotransferase (ALT) was highest in fish fed diet D2 while the lowest was found in fish fed diet D1 with values of 46.07 and 24.70 (IU/L) respectively. The enzyme aspartate aminotransferase (AST) was found highest in fish fed diet D3 and lowest was found in fish fed diet D2 with values 16.43 and 12.47 (IU/L) respectively. The enzyme lipase was found to be highest in fish fed diet D4 while the lowest was shown in fish fed diet D3 with values of 32.47 and 29.03 (IU/L) respectively. Among the biochemical factors, there was a significant difference ($P<0.05$) observed in blood sugar, the highest reading was found in fish fed diet D3 with amount of 83 mg/dl and the lowest amount was found in fish fed diet D2 with a value of 46 mg/dl. However, the other biochemical parameters such as cholesterol and triglyceride did not showed significant differences ($P>0.05$) among treatments. The triglyceride values ranged from the highest and lowest values were found in fish fed diets D4 and D2 with values of 349.33 and 263.00 (mg/dl) respectively. The cholesterol values ranged from the highest and lowest were found in fish fed diets D4 and D2 with values of 121.67 and 104.67 (mg/dl) respectively. Among the immunological parameters, a significant difference ($P<0.05$) was observed in the third component of complement in immune system (C3) where the highest reading was observed in fish fed diet D4 and lowest found in fish fed diet D3 with values of 7.50 and 5.83 (mg/dl) respectively. But as for the other immunological parameters which are the fourth



component of complement in immune system (C4), the alternative component of complement in immune system (CH50) and Immunoglobulin molecule consisting (IgM), showed no significant among treatments of different levels of phospholipids ($P>0.05$). The amount of C4 was highest in fish fed diet D1 and lowest was shown fish fed diet D4 with values of 4.33 and 3.10 (mg/dl) respectively. The content of CH50 was highest in fish fed diet D3 and the lowest was shown in fish fed diet D1 with values of 30.00 and 25.40 (mg/dl) respectively. The immunoglobulin molecule (IgM) ranged from the highest and lowest was shown in fish fed diets D3 and D2 with values of 4.10 and 3.10 (mg/dl) respectively. In conclusion, the addition of 4% phospholipids in the juvenile's beluga sturgeon diet can increase growth performances.



Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia
sebagai memenuhi keperluan untuk ijazah master Sains

KEPERLUAN FOSFOLIPID UNTUK JUVENIL ANAK IKAN BELUGA

(*HUSO HUSO*)

Oleh:

MAHMOUDREZA EBRAHIMNEZHADARABI

Oktober 2010

Ketua: Prof. Madya Dr. Che Roos Bin Saad, Phd

Fakulti: Pertanian

Satu kajian telah dijalankan untuk mengetahui kesan penggunaan fosfolipid dalam diet terhadap tumbesaran dan komposisi badan ikan, parameter hematologi, faktor-faktor biokimia dan juga parameter imun bagi juvenil ikan sturgeon (*Huso huso*). Juvenil ikan telah diberikan makanan berformulasi yang mengandungi empat peringkat fosfolipid yang berbeza iaitu 0 (D1), 2 (D2), 4 (D3) dan 6% (D4). Hasil daripada kajian yang telah dilakukan selama 56 hari, telah menunjukkan terdapat perbezaan yang signifikan ($P < 0.05$) bagi kadar tumbesaran antara kumpulan rawatan. Kumpulan ikan yang memakan makanan mengandungi 4% fosfolipid memberi pertambahan berat badan dan peratus kemandirian yang terbaik ($P < 0.05$) dengan nilai 70.3 dan 76.7% masing-masing. Walaubagaimana pun terdapat tiada perbezaan yang signifikan bagi nisbah pertukaran makanan (FCR), kadar pertumbuhan spesifik



(SGR), nisbah kecekapan protein (PER) dan nisbah kecekapan lemak (LER) ($P>0.05$) diakhir kajian. Kandungan kelembapan dan juga protein dalam ikan telah menunjukkan ada perbezaan yang signifikan ($P<0.05$) antara rawatan. Walaubagaimana pun terdapat tiada perbezaan yang signifikan ($P>0.05$) terhadap kandungan lemak dan abu dalam ikan. Terdapat perbezaan yang signifikan ($P<0.05$) yang telah diperhatikan pada MCHC (mean corpuscular hemoglobin concentration (g/dl)). Ikan yang diberikan makanan D2 (2% PL) mencapai MCHC yang tertinggi dengan nilai 33.3 g/dl. Tidak terdapat perbezaan yang signifikan ($P>0.05$) di dalam parameter hematologi yang lain seperti sel darah merah (RBC), hematocrit (Hct), hemoglobin (Hb), MCH (mean corpuscular hemoglobin), MCV (mean corpuscular volume), dan sel darah putih (WBC) dalam ikan yang menerima rawatan. Peratus pembacaan hematocrit berjulat daripada tertinggi (ikan yang memakan makanan D3) dan terendah (ikan yang memakan makanan D1) adalah 26.33 and 25.00% masing-masing. Bagi kandungan hemoglobin pula, berjulat dari tertinggi (ikan memakan makanan D2) hingga terendah (ikan memakan makanan D1) ialah bernilai 8.64 dan 7.67 (g/dl) masing-masing. Nilai MCH pula berjulat dari tertinggi (ikan memakan makanan D1) hingga terendah (ikan memakan makanan D2) adalah 92.57 dan 77.27 (pg/sel) masing-masing. Bagi nilai MCV pula ia berjulat dari tertinggi (ikan memakan makanan D1) hingga terendah (ikan memakan makanan D2) dengan nilai 300.83 dan 232.60 (fL/sel) masing-masing. Bagi pengiraan sel darah putih dalam ikan yang memakan makanan mengandungi tahap fosfolipid yang berbeza berjulat dari tertinggi (ikan memakan makanan D2) hingga terendah (ikan memakan makanan D3) dengan nilai 25866.67 dan 22633.33 (sel/ μ l) masing-masing. Kandungan peratus sel darah putih yang berbeza seperti limfosit dan monosit dalam ikan *Huso huso*, yang menerima makanan mengandungi kandungan fosfolipid yang



berbeza menunjukkan tidak ada perbezaan yang signifikan ($P>0.05$) diantara kumpulan rawatan. Kandungan terbanyak lymphosit dan monosit adalah dari kumpulan ikan yang menerima makanan D2 dengan nilai 71.67 dan 3.67% masing-masing. Pembacaan neutrophils dan eosinophils yang tertinggi adalah terdapat adalah dari kumpulan ikan yang menerima makanan D1 dan D4 dengan nilai 21.33 dan 8.33% masing-masing. Kandungan yang terendah bagi lymphocyte, neutrophil, monocyte, dan eosinophil adalah terdapat dalam ikan yang menerima makanan D4, D2, D3, dan D1 dengan nilai 68, 18.67, 2.33 dan 6% masing-masing. Diakhir kajian terdapat tidak ada perbezaan yang signifikan ($P>0.05$) bagi enzim hepatic antara rawatan. Bagi enzim alanine aminotransferase (ALT) adalah merupakan yang tertinggi dalam ikan yang memakan makanan D2 manakala yang terendah pula terdapat dalam ikan yang memakan makanan D1 dengan nilai 46.07 dan 24.70 (IU/L) masing-masing. Bagi enzim aspartate aminotransferase (AST) pula, amaun yang paling tinggi adalah terdapat dalam ikan yang menerima makanan D3 dan yang terendah adalah dalam ikan yang memakan makanan D2 dengan nilai 16.43 dan 12.47 (IU/L) masing-masing. Diantara faktor-faktor biokimia terdapat perbezaan yang signifikan ($P<0.05$) bagi gula dalam darah dengan nilai tertinggi dalam ikan yang memakan makanan D3 sebanyak 83 mg/dl dan amaun terendah terdapat dalam ikan yang memakan makanan D2 sebanyak 46 mg/dl. Walau bagaimana pun, tidak terdapat perbezaan yang signifikan ($P>0.05$) bagi kandungan kolesterol dan trigliserida antara rawatan. Nilai trigliserida berjulat dari nilai tertinggi dan terendah adalah terdapat bagi ikan yang memakan makanan D4 dan D2 yang bernilai 349.33 dan 263.00 (mg/dl) masing-masing. Bagi kolesterol pula, nilainya berjulat dari tertinggi hingga rendah adalah terdapat dalam ikan yang memakan makanan D4 dan D2 dengan nilai 121.67 dan 104.67 (mg/dl) masing-masing. Di antara parameter-

parameter imunologi, terdapat perbezaan yang signifikan ($P < 0.05$) pada C3 (komponen komplimen ke 3 dalam sistem imun), dimana kandungannya adalah tertinggi dalam ikan yang memakan makanan D4 dan terendah dalam ikan yang memakan makanan D3 dengan nilai 7.50 dan 5.83 (mg/dl) masing-masing. Tetapi untuk parameter imunologi yang lain seperti C4 (komponen komplimen ke 4 dalam system imun), CH50 (komponen komplimen alternatif dalam sistem imun) dan IgM tidak menunjukkan perbezaan yang signifikan ($P > 0.05$) diantara semua rawatan. Kandungan C4 adalah tertinggi dalam ikan yang memakan makanan D1 dan kandungan terendah adalah dalam ikan yang memakan makanan D4 dengan nilai 4.33 dan 3.10 (mg/dl) masing-masing. Kandungan CH50 pula adalah tertinggi dalam ikan yang memakan makanan D3 dan terendah dalam ikan yang memakan makanan D1 dengan nilai 30.00 dan 25.40 (mg/dl) masing-masing. Bagi molekul immunoglobulin pula ianya tertinggi dalam ikan yang memakan makanan D3 dan terendah bagi ikan yang memakan makanan D2 dengan nilai 4.10 dan 3.10 (mg/dl) masing-masing. Sebagai konklusi, kemasukan 4% fosfolipid dalam makanan ikan juvenile sturgeon beluga boleh meningkatkan pertumbuhan dan pembesaran ikan tersebut.

AKNOWLEDGEMENTS

“Praise be to GOD, The Lord (Cherisher and Sustainer) of the worlds

The All-Merciful, The Ever-Merciful.”

I would like to express my sincere appreciation to my supervisor and chair person of the supervisory committee, Assoc. Prof. Dr. Che Roos Bin Saad, who was the main scientific guide and his encouragements and trust lead me to finish my study. My grateful thanks to him because of friendly environment and support during my study period.

I gratefully acknowledge my supervisory committee internal members, Assoc. Prof. Dr. Sharr Azni Bin Harmin, Dr. Mustafa Kamal Abd Satar and External Member: Assoc. Prof. Dr. Abdolmohammad Abedian Kenari for their valuable contribution and guidance during this study.

I deeply thank to Universiti of Putra Malaysia and the government of Malaysia for cooperation throughout the study.

My special gratitude to, Ministry of Jihad-e-Agriculture Iran, Fisheries General Department Mazandaran Iran, Fisheries General Department Golestan Iran for support throughout the study and Tarbiat Modares University Iran for their technical support.



I certify that a Thesis Examination committee has met on 2010 to conduct the final examination of Mahmoudreza Ebrahimnezhadarabi on his thesis entitled “Dietary Phospholipids Requirement for Sturgeon (*Huso huso*) Juveniles” in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Master of Science.

Members of the Thesis Examination Committee were as follows:

Aziz Bin Arshad

Associate Professor
Faculty of Agriculture
Universiti Putra Malaysia
(Chairman)

Annie Christianus

Senior Lecturer
Faculty of Agriculture
Universiti Putra Malaysia
(Internal Examiner)

HASSAN BIN HJ MOHD DAUD

Associate Professor
Faculty of Veterinary Medicine
Universiti Putra Malaysia
(Internal Examiner)

Ahmed Jalal Khan Chowdhury

Associate Professor
Kulliyah of Science
International Islamic University Malaysia
(External Examiner)

BUJANG KIM HUAT, PhD

Professor and Deputy Dean
School of Graduate Studies
Universiti Putra Malaysia

Date:



This thesis was submitted to the Senate of University Putra Malaysia has been accepted as fulfilment of the requirement for the degree of Master of Science. The members of the Supervisory Committee were as follows:

Che Roos bin Saad, PhD

Associate Professor
Faculty of Agriculture
Universiti Putra Malaysia
(Chairman)

Mustafa Kamal Abd Satar, PhD

Lecturer
Faculty of Agriculture
Universiti Putra Malaysia
(Internal Member)

Sharr Azni bin Harmin, PhD

Associate Professor
Faculty of Agriculture
Universiti Putra Malaysia
(Internal Member)

Abdolmohammad Abediankenari, PhD

Associate Professor
Tarbiat Modares
University Iran
(External Member)

HASANAH MOHD GHAZALI, PhD

Professor and Dean
School of Graduate Studies,
Universiti Putra Malaysia

Date:



Declaration

I declare that the thesis is my original work except for quotations and citation which have been duly acknowledged. I also declare that it has not been previously, and is not or concurrently, submitted for any other degree at University Putra Malaysia or other institution.

MAHMOUDREZA EBRAHIMNEZHADARABI

Date: 8 October 2010



TABLE OF CONTENTS

	Page
DEDICATION	ii
ABSTRACT	iii
ABSTRAK	vii
ACKNOWLEDGEMENTS	xi
APPROVAL	viii
DECLARATION	x
LIST OF TABLES	xiii
LIST OF FIGURES	xiv
LIST OF ABBREVIATION	xv
CHAPTER	
1. INTRODUCTION	1
1.1. Background of study	1
1.2. Statement of problem	2
2. LITERATURE REVIEW	4
2.1. Beluga (<i>Huso huso</i>)	4
2.1.1. Acipenseridae	7
2.1.2. Acipenseridae subcategories	7
2.1.3. Acipenserinae	8
2.1.4. Scaphirhynchinae	9
2.2. Feeding Habit of <i>Huso huso</i>	11
2.2.1. Phospholipids	11
2.2.2. Phospholipids Synthesis	12
2.2.3. Phospholipids turnover and remodeling	13
2.2.4. Metabolism and Physiology Arrangements	14
2.2.5. Energy production	14
2.2.6. Importance of providing phosphorus elements, choline and inositol in feed	15
2.2.7. Requirement phospholipids on fish	16
3. MATERIALS AND METHODS	20
3.1. Location of study	20
3.2. Preparation of tanks	21
3.3. Formulation and preparation of diet	21
3.4. Feeding of fish	24
3.5. Growth measurement	24
3.6. Hematology assessment	25
3.7. Biochemical factors measurement	26
3.7.1. Determination of blood total cholesterol, triglyceride and glucose concentration	27
3.7.2. Liver enzymes measurement	28
3.8. Immune factors measurement	28



3.9. Proximate analysis	29
3.9.1. Moisture Percentage Measurement	29
3.9.2. Ash Percentage Measurement	30
3.9.3. Protein Percentage Measurement	30
3.9.4. Fat Percentage Measurement	31
3.10. Statistical data analysis	31
4. RESULTS	32
4.1. Growth indices	32
4.2. Fish proximate	39
4.3. Hematology parameters	40
4.4. Biochemical parameters	42
4.5. Hepatic Enzymes	45
4.6. Immunological Factors	45
5. DISCUSSION	48
5.1. Growth and Requirements	48
5.2. Hematology	50
5.3. Liver enzymes	51
5.4. Immune system	54
6. CONCLUSION	56
REFERENCES	58
APPENDICES	67
BIODATA OF THE STUDENT	71
LIST OF PUBLICATIONS	71

