EFFECT OF PLANT PIGMENTS ON BROODSTOCK, EGG QUALITY AND GROWTH OF RAINBOW TROUT

YADOLLAH MEHRABI

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YADOLLAH MEHRABI

Thesis submitted to the School of Graduate Studies, Universiti Putra Malaysia, in fulfillment of the Requirements for the Degree of Doctor of Philosophy

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DEDICATION

To my wife Farkhondeh and my children, Vahid, Navid and Yasaman and my parents and my brother Khani Mehrabi, who gave me supports and understanding during my study.

Abstract of thesis submitted to the senate of University Putra Malaysia in fulfilment of the requirements for the Degree of Doctor of Philosophy

EFFECT OF PLANT PIGMENTS ON BROODSTOCK, EGG QUALITY AND GROWTH OF RAINBOW TROUT

By

YADOLLAH MEHRABI

June 2006

Chairman: Associate Professor Che Roos Saad

Faculty: Agriculture

Four experiments were conducted to determine the effects of plant pigments (carotenoids) on rainbow trout (*Oncorhynchus mykiss*) broodstock, egg quality, growth, FCR, SGR, survival and carotenoids retention in the flesh, skin, egg of female and male broodstocks. Six experimental diets with different sources of plant pigments containing alfalfa, clover, carrot, tomato, acorn fruit and corn gluten meal and two other diets consisting of commercial feed + 50ppm artificial astaxanthin for broodstock and commercial feed +100ppm for fingerling and juvenile were examined. In the first experiment the effects of plant pigments on broodstock were studied. In this experiment, each replication contained 10 females and 5 males broodstock aged about four years old with 1240 $\pm$ 10g lives weight. There were 8 treatments namely control (A) which contained commercial feed and treatment B (A+5%alfalfa meal), treatment C (A+5% clover meal), treatment D (A+5% carrot meal), treatment E (A+5% acorn fruit meal), treatment F (A+5% tomato meal),

treatment G (A+5% corn gluten) and treatment H (A+50ppm artificial astaxanthin) for broodstock and (A+100ppm astaxanthin) for fingerling. Duration of this experiment was six months. Results indicated that higher amount of carotenoids deposited in female broodstock as compared to the male broodstock. The amount of different carotenoids deposited in their tissues also varies. For example, females fed with diet H (artificial astaxanthin) retained astaxanthin 32.87 mg/kg and 25.57mg/kg of canthaxanthin in their flesh, while fish fed diet B (alfalfa meal) retained 43.15mg/kg α-carotene and 38.2mg/kg β-carotene in their flesh. Lycopene was retained the most in fish fed diet F (41.75mg/kg). The female broodstock also retained higher amount of carotenoids in eggs and skin and significantly ($P<0.05$) different than the control treatment. Similarly, the same results were observed in the flesh, skin and testis of the male broodstocks. Plant pigments had no adverse effect on mortality of broodstock and is significantly different ($P<0.05$) with the control treatment. Plant pigments also had no negative effect on all stripped fish, but instead they increased the relative fecundity and production of green egg. Fish fed diet F (tomatoes) had the highest relative fecundity of 451.4g/fish and 45991 green egg were produced while the control treatment had only a fecundity of 332.7g/fish and 28997 of green eggs produced and was significantly different ($P<0.05$) with other treatments. It was shown that plant pigments also increased egg fertilization, survival and reduced mortality in different stages of egg development. Similarly fish fed diet containing tomatoes had the highest fertilized and eyed eggs and hatched into larvae. Plant pigments increased the survival of fish fingerlings that similarly pigments were deposited in the flesh of the fingerlings. Fish fed diet

containing artificial astaxanthin had 93.6% survival compared to control and other treatments and was significantly different ($P<0.05$). Similar results in juvenile were also observed, which showed that plant pigments would increase survival and retention of carotenoids in their flesh. The juvenile fed diet containing tomato had the highest total length (24.2 cm), survival (92.3%), SGR (1.7) and FCR (1.1) and were significantly different ($P<0.05$) to control. It can be concluded that plant pigments have significant positive effects on health, survival, FCR, SGR, development of egg, and pigment retention in the flesh, skin and gonads. Additionally, carotenoids were shown to protect the fishes against most diseases because they have important roles in respiration, membrane permeability, light absorption and immune system.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia untuk memenuhi syarat mendapatkan ijazah Doktor Falsafah

KESAN PIGMEN TUMBUHAN KE ATAS INDUK, KUALITI TELUR DAN PERTUMBUHAN IKAN RAINBOW TROUT

Oleh

YADOLLAH MEHRABI

Jun 2006

Pengerusi: Professor Madya Che Roos Saad

Fakulti: Pertanian

Empat eksperimen telah dijalankan untuk menentukan kesan pigmen-pigmen tumbuhan (karotenoid) ke atas induk ikan (*Oncorhynchus mykiss*), kualiti telur, pertumbuhan, kadar pertukaran makanan (KPM), kadar pertumbuhan spesifik (KPS), kadar hidup, dan pengekalan karotenoid dalam isi, kulit untuk ikan induk jantan dan betina serta telur bagi ikan betina. Lapan jenis diet, satu setiap rawatan, disediakan. Ini termasuk enam diet mengandungi sumber pigmen-pigmen tumbuhan yang berbeza iaitu alfalfa, clover, lobak merah, tomato, buah acorn dan gluten jagung dan dua jenis diet yang mengandungi diet komersil + 50ppm astaxanthin tiruan untuk induk ikan dan diet komersil +100ppm astaxanthin tiruan untuk ikan jejari ikan dan juvana disediakan. Setiap eksperimen yang dijalankan telah diulang sebanyak 4 kali. Ikan diberi makan dalam 32 kolam pembelaan konkrit secara rawak. Eksperimen yang pertama telah mengkaji kesan-kesan pigmen tumbuhan ke atas induk ikan. Dalam kajian ini setiap replikasi menerima sebanyak 10 ekor

induk ikan betina dan 5 ekor induk ikan jantan berumur dalam lingkungan 4 tahun dengan berat basah sebanyak 1240 $\pm$ 10g. Terdapat 8 kumpulan rawatan iaitu kawalan (A) mengandungi diet komersil, rawatan B (A+5% diet alfalfa), rawatan C (A+5 % diet clover), rawatan D (A+5% diet lobak merah), rawatan E (A+5% diet buah acorn), rawatan F (A+5% diet tomato), rawatan G (A+5% gluten jagung) dan rawatan H (A+50 ppm astaxanthin tiruan) untuk ikan induk dan (A+100 ppm astaxanthin) untuk ikan saiz jejari dan juvana. Jangkamasa kajian adalah selama 6 bulan. Hasil kajian menunjukkan amaun keratenoid yang dikekalkan dalam induk ikan betina adalah lebih tinggi berbanding amaun yang dikekalkan dalam induk jantan. Amaun jenis keratenoid yang dikekalkan dalam ikan juga berbeda. Contohnya, induk betina yang diberi makan diet H (astaxanthin tiruan) mengekalkan kandungan sebanyak 32.87mg/kg astaxanthin dan 25.57mg/kg canthaxanthin yang tertinggi dalam isi, manakala ikan yang diberi makan diet B (alfalfa mil) mengekalkan kandungan alfa karoten sebanyak 43.15mg/kg dan beta karoten sebanyak 38.2mg/kg dalam isi. Sementara itu, lycopen dikekalkan terbanyak dalam ikan yang diberi makan diet F (41.75mg/kg). Induk betina juga mengekalkan amaun keratenoid terbanyak dalam telur dan kulit berbanding dengan induk yang menerima rawatan kawalan ($P<0.05$). Keputusan yang sama juga dapat dilihat pada isi, kulit dan testis bagi induk ikan jantan. Pigmen-pigmen tumbuhan tidak mempunyai kesan negatif ke atas keupayaan untuk hidup dan perbezaan ini adalah signifikan ($P < 0.05$) berbanding kumpulan kawalan. Pigmen-pigmen tumbuhan juga tidak mempunyai kesan negatif ke atas semua ikan yang dilurut, tetapi meningkatkan fekunditi relatif dan penghasilan telur hijau. Ikan yang diberi

makan diet F (tomato) mempunyai fekunditi relatif yang paling tinggi iaitu sebanyak 451.4g/ikan dan sebanyak 45991 telur hijau telah dihasilkan sementara kumpulan kawalan hanya mempunyai fekunditi relatif sebanyak 332.7g/ikan dan sebanyak 28997 telur hijau dihasilkan. Didapati, perbezaan ini adalah signifikan dengan kumpulan-kumpulan rawatan yanglain ($P < 0.05$). Didapati pigmen-pigmen tumbuhan juga meningkatkan pensenyawaan telur, kemandirian yang tinggi dalam tahap perkembangan telur yang berbeda. Begitu itu juga bagi ikan yang diberi makan diet mengandungi tomato juga mempunyai jumlah telur ikan yang disenyawakan dan telur yang bermata (eyed eggs) yang paling banyak dan menetas kepada larva. Pigmen-pigmen tumbuhan juga dikenalpasti dapat meningkatkan kemandirian dan kadar penyerapan pigmen-pigmen tumbuhan ke dalam isi bagi kumpulan ikan jejari. Ikan yang diberi makan diet mengandungi astaxanthin tiruan pula mempunyai 93.6% kemandirian berbanding ikan dalam kumpulan kawalan dan rawatan yang lain. Perbezaan ini adalah signifikan ($P < 0.05$). Hasil yang sama juga dapat dilihat dalam ikan juvana dimana pigmen-pigmen tumbuhan meningkatkan kemandirian dan pengekalan karotenoid dalam badan mereka. Kumpulan juvana yang diberi makan diet mengandungi tomato mempunyai saiz ikan paling panjang (24.2 cm), kemandirian yang paling tinggi (92.3%), KPS (1.7) dan KPM (1.1) dan mempunyai perbedaan yang bererti ($P< 0.05$) berbanding dengan kumpulan ikan yang mendapat rawatan kawalan. Adalah disimpulkan bahawa pigmen tumbuhan mempunyai kesan yang signifikan ke atas kesihatan, kemandirian, KPM, KPS, perkembangan telur dan pengekalan pigmen dalam isi, kulit dan gonad ikan. Tambahan pula, karotenoid telah diketahui dapat melindungi ikan daripada

kebanyakan jenis penyakit kerana fungsi pentingnya ke atas respirasi, kadar serapan membran, penyerapan cahaya dan sistem imun badan.

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I certify that an Examination Committee has met on 2nd June 2006 to conduct the final Examination of Yadollah Mehrabi on his Doctor of Philosophy thesis entitled "Effects of plant pigments on broodstock, egg quality and growth of rainbow trout (*Oncorhynchus mykiss*)" in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that candidate be awarded the relevant degree. Members of the Examination Committee are as follows:

Sharr Azni b. Harmin, PhD
Associate Professor
Faculty of Agriculture
Universiti Putra Malaysia
(Chairman)

Zainal Aznam Mohd Jelan, PhD
Professor
Faculty of Agriculture
Universiti Putra Malaysia
(Internal Examiner)

Annie Christianus, PhD
Lecturer
Faculty of Agriculture
Universiti Putra Malaysia
(Internal Examiner)

Roshada Hashim, PhD
Professor
School of Biological Sciences
Universiti Sains Malaysia
(External Examiner)

_____
HASANAH MOHD. GHAZALI, PhD
Professor/Deputy Dean
School of Graduate Studies
Universiti Putra Malaysia

Date:

This thesis submitted to the senate of Universiti Putra Malaysia and has been accepted as fulfillment of the requirement for degree of Doctor of Philosophy. The members Supervisory Committee are as follows:

Che Roos Saad, PhD
Associate Professor
Faculty of Agriculture
Universiti Putra Malaysia
(Chairman)

Abdul Razak Alimon, PhD
Professor
Faculty of Agriculture
Universiti Putra Malaysia
(Member)

Hishamuddin Omar, PhD
Lecturer
Faculty of Science
Universiti Putra Malaysia
(Member)

Homayon Mahmoudzadeh, PhD
Professor
Faculty of Veterinary
Tehran University
(Member**)**

AINI IDERIS, PhD
Professor/Dean
School of Graduate Studies
Universiti Putra Malaysia

Date:

DECLARATION

I hereby declare that the thesis is based on my original work except for quotations and citations, which have been duly acknowledged. I also declare that it has not been previously or currently submitted for other degree at UPM or other institution.

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YADOLLAH MEHRABI

Date:

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LIST OF ABBREVIATIONS

α-Car	α-carotene
β-Car	β-carotene
Ast	Astaxanthin
Ave	Average
ANOVA	Analyses of variance
BW	Body weight
CP	Crude protein
CF	Crude fiber
Ca	Calcium
^{0}C	Degree Celsius
CCL	Carotenoid caring lipoprotein
Can	Canthaxanthin
DNMRT	Duncan's New Multiple Range Test
Diam	Diameter
DM	Dry Matter
DF	Degree of freedom
EE	Ether Extract
FCR	Feed Conversion Ratio
G	Gram
GLM	General Linear Model
HDL	High Density Lipoprotein
Kg	Kilogram
Kcal	Kilo calorie
L	Liter
LDL	Low Density Lipoprotein
ln	Natural Logarithm
Lyc	Lycopene

MS222	Three cain metan solfonate
mm	Millimeter
mg	Milligram
Mort	Mortality
ml	Milliliter
NFE	Nitrogen Free Extract
NO	Number
NRC	National Research Council
Oct.	October
O2	Oxygen
PE	Pellet
PPM	Parts Per Million
P	Phosphorous
pH	Hydrogen ion concentration
RCBD	Randomized Complete Block Design
SGR	Specific Growth Rate
Se	Standard error
SD	Standard deviation
SAS	Statistical analytical system
SEM	Standard error of mean
Sep	September
SP	Shortcut Pellet
Tot.Car	Total carotenoids
UV	Ultra Violet
V	Volume
VHDL	Various High Density Lipoprotein
W	Weight
W0	Initial Weight
W1	Final Weight