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BIOCHEMICAL COMPOSITION OO THE OVARY OF MYSTUS NEMURUS (CUVIER AND VALENCIENNES) AND INFLUENCE OF ASCORBIC ACID SUPPLEMENTATION ON THE EGG AND LARVAL QUALITY

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BY

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LIST OF ABBREVIATIONS

AA	Ascorbic Acid
AABA	Alpha Amino Butyric Acid
AAPP	Ascorbyl-2-Polyphosphate
Ala	Alanine
Arg	Arginine
Asp	Aspartic Acid
$CHCl_3$	Chloroform
CP	Crude Protein
Cys	Cysteine
EAA	Essential Amino Acids
EtOH	Ethanol
FAME	Fatty Acid Methyl Ester
FSI	Fat Somatic Index
GC	Gas Chromatography
Glu	Glutamic Acid
Gly	Glycine
GnRH-A	Gonadotropin Releasing Hormone Analogue
GSI	Gonad Somatic Index
GV	Germinal Vesicle
GVB	Germinal Vesicle Breakdown
GVM	Germinal Vesicle Migration
His	Histidine
HPLC	High Performance Liquid Chromatography

HSI . . . . Hepatosomatic Index

Ile . . . . Isoleucine

KCl . . . . Potassium Chloride

Leu . . . . Leucine

Lys . . . . Lysine

Meth . . . . Methionine

MSA . . . . 4n-Methanesulfonic Acid

NaOH . . . . Sodium Hydroxide

OI . . . . Ovarian Index

Phe . . . . Phenylalanine

Pro . . . . Proline

PUFA . . . . Polyunsaturated Fatty Acids

Ser . . . . Serine

TCA . . . . Trichloroacetic acid

Thr . . . . Threonine

Tryp . . . . Tryptophan

Tyr . . . . Tyrosine

Val . . . . Valine

YG . . . . Yolk Globule

Abstract of the thesis submitted to the Senate of the Universiti Pertanian Malaysia in fulfilment of the requirement for the degree of Master of Science

BIOCHEMICAL COMPOSITION OF THE OVARY OF *MYSTUS NEMURUS* (Cuvier & Valenciennes) AND INFLUENCE OF ASCORBIC ACID SUPPLEMENTATION ON EGG AND LARVAL QUALITY

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Selected biochemical compositions of the ovaries such as proximate composition, amino acid, fatty acid and ascorbic acid were determined and their role in developing eggs and larvae were evaluated. The ovaries contained substantial amounts of protein, lipid, and ascorbic acid. The protein comprised of high levels of both essential and non essential amino acids, while, the lipid consisted of higher proportions of saturated and monounsaturated fatty acids, and lower proportion of polyunsaturated fatty acid (PUFA), respectively. These biochemical components did not show any significant ($p>0.05$) changes at different gonad somatic index levels. Although histology revealed that ovaries demonstrated asynchronous oocyte development, it appeared that there is continuous accumulation of nutrients into the oocyte until final maturation stage. In developing

eggs and larvae, marked reduction in protein, lipid, essential amino acid, saturated fatty acid and ascorbic acid contents indicated endogenous utilization. Quantitative decrease of these constituents suggested that more nutrients were utilized during embryonic development.

Supplementation of ascorbyl-2-polyphosphate in the broodstock diet demonstrated that the dosage and duration of feeding both affected the accumulated ascorbic acid in the eggs and their quality. Egg quality increased significantly ($p<0.05$) from broodstocks fed higher dosage of ascorbyl-2-polyphosphate based on fertilization rate, hatching rate, survival rate and percentage of abnormal larvae.

The results of this study indicated that the egg composition and its quality were affected by the biochemical composition of the ovary. While it is directly regulated by the broodstock nutrition it can nevertheless be considered as an important element for optimum reproductive efficiency that may reduce factors limiting aquaculture production.

Abstrak tesis yang dikemukakan kepada Senat Universiti Pertanian Malaysia sebagai memenuhi syarat keperluan untuk ijazah Master Sains

KOMPOSISI BIOKIMIA OVARI *MYSTUS NEMURUS* (Cuvier & Valenciennes) DAN KESAN PEMBERIAN ASID ASKORBIK KE ATAS KUALITI TELUR DAN LARVA

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Komposisi biokimia ovari seperti komposisi proksimat, asid amino, asid lemak dan asid askorbik telah ditentukan dan peranannya di dalam perkembangan telur dan larva telah dinilai. Ovari ini mengandungi banyak protein, lipid, dan asid askorbik. Protein tersebut mengandungi kedua-dua asid amino perlu dan tidak perlu pada paras yang tinggi, sementara lipid mengandungi lebih banyak bahagian asid lemak tepu dan asid lemak mono tak tepu dan kurang asid lemak poli tak tepu (PUFA). Komponen biokimia ini tidak menunjukkan sebarang perbezaan bererti ($p>0.05$) pada paras indeks gonad somatik yang berlainan. Walaupun kajian histologi menunjukkan bahawa ovari mengalami perkembangan oosit tidak serentak, namum jelas kelihatan terdapatnya pengumpulan berterusan nutrien di dalam oosit sehingga ke peringkat akhir kematangan. Di dalam telur dan larva yang

sedang berkembang terdapat pengurangan yang ketara pada kandungan protein, lipid, asid amino perlu, asid lemak tepu dan asid askorbik yang menunjukkan terdapatnya penggunaan endogenus. Pengurangan kuantitatif komponen-komponen ini, mencadangkan bahawa lebih banyak nutrien telah digunakan semasa perkembangan embrionik.

Penambahan askorbil-2-polifosfat dalam diet induk menunjukkan bahawa dos dan jangkamasa pemberian makanan kedua-duanya memberi kesan ke atas pengumpulan asid askorbik di dalam telur dan terhadap kualitinya. Kualiti telur meningkat dengan ketara ($p<0.05$) dari induk yang telah diberi makanan yang mengandungi dos askorbil-2-polifosfat yang tinggi dan ini adalah berdasarkan pada kadar persenyawaan, kadar penetasan, kadar kemandirian dan peratus larva yang abnormal.

Keputusan-keputusan daripada kajian ini menunjukkan bahawa komposisi telur dan kualitinya adalah dipengaruhi oleh komposisi biokimia ovari. Sehubungan dengan itu, sementara ia dikawal secara langsung oleh nilai pemakanan induk, ia juga boleh disifatkan sebagai elemen yang penting untuk kecekapan pembiakan yang optimum dan mungkin mengurangkan faktor-faktor penghad dalam pengeluaran akuakultur.

CHAPTER I

GENERAL INTRODUCTION

Egg quality in general reflects the egg production capability of the broodstock together with the survival of the egg and growth rates of fry. Egg quality is basically influence by the conditions under which the broodstock are maintained, their husbandry, types of diet and genetic make-up (Bromage *et al.*, 1992). Thus, the parent condition apparently regulates the physical and chemical dimensions of the egg as well as the subsequent progeny survival (Springate *et al.*, 1984).

In fish, reproduction is triggered by external cues such as photoperiod, temperature, feeding and social factors. These stimulate the multifaceted reproductive hormonal centres that induce the maternal production of vitellogenin and deposition of yolk into the oocyte (Peter, 1983). Vitellogenin constitutes the carrier molecule for various classes of compounds and ample amounts of nutrition being accumulated by the developing oocyte for proper assembly (Wallace, 1985). Incorrect composition of the circulating vitellogenin due to unbalanced diet can cause a negative effect on vitellogenic and oocyte maturation process (Cerda *et al.*, 1994).

It is well known that nutrition influences reproduction by affecting fecundity and egg size, egg hatchability and fry viability as well as its biochemical composition (Eskelinen, 1989). The quality and quantity of broodstock diet is important for spawning and egg quality (Rainuzzo, 1993; Watanabe *et al.*, 1984). Positive correlation of broodstock nutrition on reproduction and egg quality have been found in red seabram (Watanabe *et al.*, 1991), Atlantic salmon (Eskelinen, 1989) and rainbow trout and carp (Sato *et al.*, 1987). Likewise, food restriction generally reduces total fecundity and may delay oocyte maturation process and decrease the proportion of maturing fish (Horwood *et al.*, 1989; Kjesbu, 1988; Springate and Bromage, 1985).

Mobilization of nutrients during gonadal development was reviewed by Lie and Mangor-Jensen (1993). Nutrient distribution seems to become intense in the ovaries during oocyte maturation process. For instance, changes in the plasma constituents of the maturing turbot has been related to the transport of nutrients from various tissues to the gonads (Lie and Mangor-Jensen, 1993). There was a shift in metabolism and redistribution of nutrient reservoir during gonad maturation.

Fecundity represents the true reproductive capability of the broodstock (Bromage *et al.*, 1992). Fecundity is expressed in terms of the number of eggs released at spawning and the total volume of water-hardened eggs

(Bromage *et al.*, 1992). It is deemed to be related to the size of the broodstock as well as the size of the egg. Changes in fecundity can be achieved through modification of the rate of recruitment of pre-vitellogenic oocytes and cortical alveoli into vitellogenesis (Bromage *et al.*, 1992), but knowledge regarding the dynamics of these processes is limited.

There are a number of egg characteristics that are considered essential in measurement of egg quality. Physiological processes that occur from fertilization until hatching is a complex mechanism and regarded as universal criteria of egg quality. After fertilization, activation process by enzyme reaction cause hardening of the egg chorion that serves as the egg's ability to sustain mechanical resistance (Kjorsvick *et al.*, 1990). Deviation in chorion integrity and morphological malformation precede failure in hatching.

Egg size is known to be correlated to the larval size. Larger larvae tend to survive longer without food than those hatched from smaller eggs (Springate *et al.*, 1984). This may however does not give long term advantage as far as growth and survival of the larvae are concerned (Blaxter, 1988). Springate and Bromage (1985) suggest that size-dependent survival rates might be a reflection of differences in stage of ripeness of the egg and it is not a basis of a lesser quality than the larger eggs.