sl.no. 09

ORIGINAL ARTICLE

WILEY Aquaculture Research

Larval rearing of hilsa shad, *Tenualosa ilisha* (Hamilton 1822)

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Funding information
National Agricultural Science Fund, ICAR, Grant/Award Number: NFBSFARA/WQ-3021

Abstract

Hilsa, *Tenualosa ilisha* has received much attention for culture due to decline of the natural population. Lack of knowledge on larval rearing is the bottleneck for its culture. This study was aimed at developing larval rearing protocols for hilsa shad. Hilsa larvae (4 days old, 4.76 ± 0.06 mm/0.49 ± 0.01 mg) were stocked in fibreglass-reinforced plastic tanks (1.7 m^3 water volume) at 300, 600 and 1,200 nos/m^3 in triplicates in three experimental systems viz., E-I (circular, 0.567 m water depth), E-II (circular, 0.962 m water depth) and E-III (rectangular, 0.567 m water depth) and reared for 46 days. The larvae were supplied with *Chlorella vulgaris*, *Brachionus calyciflorus*, mixed phytoplankton and mixed zooplankton during 4–50, 6–25, 8–50 and 26–50 days of their age respectively. In each system, higher ($p < 0.05$) fry survival at 300 nos/m^3 than in higher densities indicates density dependent stress. Circular tanks showed higher survival (13.3%–61.31%) than in rectangular tanks (6.88%–27.26%) in each stocking density, indicating the importance of tank shape for rearing. Water depth affected fry survival in circular tanks (E-I and E-II) at 300 nos/m^3; at 0.962 m depth, survival was higher (61.31%, $p < 0.05$) than that of 0.567 m depth (49.93%). Good fry survival was achieved through feeding the larvae initially with *Chlorella* followed by co-feeding with *Brachionus*, mixed phytoplankton and zooplankton and rearing in circular tanks at 300 nos/m^3 densities at 1 m depth. This first-ever larval rearing protocol is useful for mass production of fry to support hilsa aquaculture in future.

KEYWORDS
fry survival, stocking density, tank design, zooplankton culture

Animal Nutrition and Feed Technology (2020) 20: 525-533
DOI: 10.5958/0974-181X.2020.00046.3

sl.no. 09

Short Communication

Protein Requirement of *Ompok bimaculatus* (Bloch, 1794) Larvae

B.N. Paul*, A. Das, R.N. Mandal, P. Singh, S. Adhikari, K. Ghosh[1], D. Chowdhury[1], P.P. Chakrabarti and S.S. Giri[2]

Regional Research Centre ICAR-Central Institute of Freshwater Aquaculture, Kolkata-700118, India

(Received: January 01, 2020)

ABSTRACT

Paul, B.N., Das, A., Mandal, R.N., Singh, P., Adhikari, S., Ghosh, K., Chowdhury, D., Chakrabarti, P.P. and Giri, S.S. 2020. Protein requirement of Ompok bimaculatus *(Bloch, 1794) larvae. Animal Nutrition and Feed Technology, 20: 525-533.*

A 22 d experiment was carried out to study the protein requirement of *Ompok bimaculatus* larvae (weight 0.11±0.01 g; length 20.49±0.70 mm). Three different formulated feeds were prepared with graded levels of crude protein i.e., with low (35% CP; LP), medium (40% CP; MP) and high (45% CP; HP) crude protein levels. The water quality parameters were optimum during the whole experimental duration. The survival rate was more than 70%. The fish grew to 0.34±0.06, 0.91±0.15 and 0.36±0.07 g, respectively in LP, MP and HP groups. The final weight was significantly ($P<0.01$) higher in MP having 40% CP in the diet. Both the net weight gain and specific growth rate were significantly ($P<0.05$) higher in MP having 40% CP in the diet as compared to LP and HP. The FCR was also significantly ($P<0.05$) lower in MP having 40% protein in the diet when compared with LP and HP. However, the protein efficiency ratio was similar ($P>0.05$) among the three treatment groups. Further, it was also revealed that the activities of enzymes in the digestive tract namely, α-amylase, lipase and pepsin were significantly ($P<0.05$) higher in MP diet having 40% crude protein. The present experiment, thus, revealed that 40% CP was sufficient for the optimum growth and survival of *O. bimaculatus* larvae.

Keywords: Amylase, Growth, Lipase, *Ompok*, Pepsin

INTRODUCTION

Ompok bimaculatus (Bloch, 1794) popularly known as the 'butter catfish' are found in lakes, rivers, canals, beels, swamps, floodplains and ponds, etc. and are distributed in India, Bangladesh, Borneo, Java, Sri Lanka, Myanmar, Pakistan, Thailand, Cambodia and Vietnam, etc. (Jayaram, 1977). It is a non-air breathing fish belonging

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Acta Ichthyologica et Piscatoria 51(3), 2021, 289–298 | DOI 10.3897/aiep.51.67079

PENSOFT.

ACTA ICHTHYOLOGICA ET PISCATORIA

Effect of dietary lipid levels on growth, body composition, and enzyme activities of larvae of butter catfish, *Ompok bimaculatus* (Actinopterygii: Siluriformes: Siluridae)

Baidya Nath PAUL[1], Debnarayan CHOWDHURY[2], Arabinda DAS[1], Rathindra Nath MANDAL[1], Puja SINGH[1], Subhendu ADHIKARI[1], Partha Pratim CHAKRABARTI[1], Shiba Sankar GIRI[3], Koushik GHOSH[2]

sl.no 09

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Academic editor: Jolanta Kiełpińska ♦ **Received** 14 October 2020 ♦ **Accepted** 6 March 2021 ♦ **Published** 13 September 2021

Citation: Paul BN, Chowdhury D, Das A, Mandal RN, Singh P, Adhikari S, Chakrabarti PP, Giri SS, Ghosh K (2021) Effect of dietary lipid levels on growth, body composition, and enzyme activities of larvae of butter catfish, *Ompok bimaculatus* (Actinopterygii: Siluriformes: Siluridae). Acta Ichthyologica et Piscatoria 51(3): 289–298. https://doi.org/10.3897/aiep.51.67079

Abstract

The Indian butter catfish, *Ompok bimaculatus* (Bloch, 1794), is a high-value catfish that has gained immense consumer preference in South-East Asia. However, information on the nutritional requirements of this species is scanty. Hence, an experiment was conducted to evaluate the effects of varying dietary lipid levels on growth, body composition, and activities of digestive and metabolic enzymes in larvae. Three isonitrogenous (40% crude protein) diets were formulated by supplementing fish and vegetable oil (1:1) at 4.5% (D1), 7% (D2), and 9.5% (D3) levels (containing crude lipid 5.7%, 8.0%, and 10.45%, respectively in diets D1–D3) to a fish meal- and oilcake-based formulated diet. Experimental diets were fed to butter catfish larvae (0.15 ± 0.01 g) in triplicate groups for a period of 42 days. Proximate compositions of the experimental diets, as well as fish carcass, were analyzed using standard procedures (AOAC 2005). Digestive and metabolic enzyme activities were analyzed at the completion of the experiment by standard methodology. Butter catfish larvae fed the diet D2 (8% crude lipid) resulted in the best performance in terms of weight gain (final weight 1.40 ± 0.07 g), net weight gain (1.31 ± 0.06 g), specific growth rate (5.50 ± 0.05% · day^{-1}), and protein efficiency ratio (2.39 ± 0.17). The highest lipid deposition (2.90 ± 0.12%) in the carcass was also recorded in fish reared on diet D2. The final weight, net weight

sl. no. 09

Dr. Debidas Mondal

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No.R-Ph.D./Regn. / SC/Zoo/172 Dated: 04.08 , 2017

To:
Shri/Sm. Arijit Chakraborty
C/o Late DebKumar Chakraborty
385. Merry Park P.O. L.D.T. - Hooghly.
Pin - 712103

Sub: Grant of Registration as a candidate for Ph.D.degree in Zoology
with effect from 18.08.2015.

Sir/Madam,

I am to inform you that the Vice-Chancellor's in his orders dated 19.07.2017 permitted you to get yourself registered as a candidate for Ph.D. degree, mentioned above, the title of your thesis being, "ARTIFICIAL PROPAGATION AND NURSERY REARING OF INDIAN SHAD, TENUALOSA ILISHA IN FRESHWATER CULTURE SYSTEM"

Subject to fulfillment of the requirements set forth in the University Ordinances relating to Doctoral Degrees and such terms and conditions as may be laid down by the appropriate authorities of the University from time to time.

You will now be required to deposit the Ph.D. Registration fee of Rs. 2000/- along with part-time research fee of Rs. 4000/- (Total Rs. 6000/-) for enrolment of your name in the Register of candidates for Ph.D. degree, positively within a month from the date of issue of this letter, failing which your case will not be considered for Registration as a Ph.D. candidate.

In this connection you are requested to note that ---

a) You will be required to get yourself registered as a student of this University on migration after completing all the necessary formalities prescribed in this behalf, unless you are already a registered student of this University.

b) On enrolment, you will be required to deliver one seminar talk before submission of the thesis pertaining to the project of your research you have undertaken within the period of your research work and before submission of the thesis.

c) You will have to published at least one research paper related to your research work in a referred journal / peer reviewed journal / journal having ISSN or in a book having ISBN number before submission of the thesis and produce evidence for the same in the form of acceptance letter or the reprint at the time of submission of your thesis.

d) You have been permitted to do research work under Dr. Koushik Ghosh, Dept of Zoology, B.U. & Dr. D. N. Chattopadhyay, Principal Scientist, ICAR A-5 (Phase-III), Santalpara, Kalyani-741235 as your Supervisor / Joint Supervisors.

e) You will have to submit your thesis within six years from the date of your registration for Ph.D. degree mentioned above, but not earlier than 18.08.2017 in the prescribed manner along with the fee of Rs. 4000/- or as may be fixed by the Executive Council from time to time towards submission of thesis.

f) You will be required to undergo a preliminary test within one year of your enrolment, to be taken by the Supervisor(s) on subject(s) connected with the area of your research as well as on your linguistic equipment as may be prescribed by the Supervisor(s) . Further continuance of your research work will depend on satisfactory result at the test. In the event of unsatisfactory result, the appropriate authority of the University may allow you to appear at a second test within six months of such decision, but not before six months from the date of the first test. If the result of the second test is also found not to be satisfactory, the registration may be cancelled.

After satisfactory result at the test, you will have to get yourself in touch with your Supervisor(s) at least once in every two months in course of research work (applicable in the case of part-time researcher(s) and produce a certificate from the Supervisor(s) about your continuous research and satisfactory progress, from time to time.

g) All the requisite fees should be deposited in case at the University Cash Counter and the relevant copy of the Cash Receipt should be submitted to the ***Ph.D. Unit*** of the Registrar's Department.

h) In your case , ***four/five copies*** of the thesis along with a C***.D. in PDF format*** (containing the Synopsis and the Thesis) be submitted and one copy be retained by you as a reference copy.

i) At the time of submission of thesis, a certificate in the prescribed form furnished by your Supervisor(s) will have to be pasted on all the copies of the thesis.

j) **The registration granted under this letter will remain valid for six years from the date of registration.** In the event of failure of submission of the thesis within the stipulated period, re-registration may be sought for and the same may be granted after observing all the formalities required in this behalf and on the receipt of the prescribed fee(s).

k) The registration granted herein may be cancelled by the concerned authority/ body of the University in the event of failure of the candidate to fulfill any of the prescribed requirements at any stage.

l) Residential requirements should be fulfilled and maintained (applicable in the case of part-time researchers).

m) Progress, Attendance and Good Conduct Reports of the Supervisor(s) in respect of the candidate should be submitted regularly every three months during the research.

n) Application forms for University Registration/Restoration of University Registration Number and Inward Migration are available at the University Sales Counter.

o) You will be required to submit six typed copies of Synopsis/Abstract of the thesis (not exceeding ten pages) along with the certificate mentioned in Clause(l) above and a certificate of delivering Seminar talk(s) and the Clearance Certificate from the Librarian of the Central Library, Burdwan University at the time of submission of thesis.

Yours faithfully,

Sd/-

Jt.Registrar

No. R/Ph.D./Regn. /sc/zoo/172/11(4) Dated: 04.08.2017

Copy forwarded for information to:

1) The Head of the Department of Zoology B.U.

2) Supervisor(s) of the candidate: i) Dr. Koushik Ghosh, Dept of Zoology B.U.

ii) Dr. D.N. Chattopadhyay, PI Scientist, ICAR Kalyani

3) The Secretary, Faculty Council for P.G. Studies in Science B.U.

4) The Finance Officer, B.U.

Jt. Registrar

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ORIGINAL ARTICLE

sl.no. 09

WILEY Aquaculture Research

Larval rearing of hilsa shad, *Tenualosa ilisha* (Hamilton 1822)

Debnarayan Chattopadhyay[1] | Arijit Chakraborty[1] | Pratyush Kumar Ray[1] | Rathindranath Mandal[2] | Surajit Kangsa Banik[3] | Vettath Raghavan Suresh[3] | Koushik Ghosh[4]

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Funding information
National Agricultural Science Fund, ICAR, Grant/Award Number: NFBSFARA/WQ-3021

Abstract

Hilsa, *Tenualosa ilisha* has received much attention for culture due to decline of the natural population. Lack of knowledge on larval rearing is the bottleneck for its culture. This study was aimed at developing larval rearing protocols for hilsa shad. Hilsa larvae (4 days old, 4.76 ± 0.06 mm/0.49 ± 0.01 mg) were stocked in fibreglass-reinforced plastic tanks (1.7 m^3 water volume) at 300, 600 and 1,200 nos/m^3 in triplicates in three experimental systems viz., E-I (circular, 0.567 m water depth), E-II (circular, 0.962 m water depth) and E-III (rectangular, 0.567 m water depth) and reared for 46 days. The larvae were supplied with *Chlorella vulgaris*, *Brachionus calyciflorus*, mixed phytoplankton and mixed zooplankton during 4–50, 6–25, 8–50 and 26–50 days of their age respectively. In each system, higher ($p < 0.05$) fry survival at 300 nos/m^3 than in higher densities indicates density dependent stress. Circular tanks showed higher survival (13.3%–61.31%) than in rectangular tanks (6.88%–27.26%) in each stocking density, indicating the importance of tank shape for rearing. Water depth affected fry survival in circular tanks (E-I and E-II) at 300 nos/m^3; at 0.962 m depth, survival was higher (61.31%, $p < 0.05$) than that of 0.567 m depth (49.93%). Good fry survival was achieved through feeding the larvae initially with *Chlorella* followed by co-feeding with *Brachionus*, mixed phytoplankton and zooplankton and rearing in circular tanks at 300 nos/m^3 densities at 1 m depth. This first-ever larval rearing protocol is useful for mass production of fry to support hilsa aquaculture in future.

KEYWORDS
fry survival, stocking density, tank design, zooplankton culture

1 | INTRODUCTION

Hilsa shad, *Tenualosa ilisha* is a high value food fish with rich in *n*-3 polyunsaturated fatty acids, eicosapentaenoic acid and docosahexaenoic acid (Mohanty et al., 2012). The species is widely distributed in Bay of Bengal, Indian Ocean, Persian Gulf and Arabian Sea and is also found in coastal areas, estuaries and freshwater rivers of India, Bangladesh, Pakistan, Indonesia, Sumatra, Myanmar, Malaysia, Kuwait, Qatar, Oman, Thailand, Saudi Arabia, United Arab Emirates, Iraq, Iran, Sri Lanka and Vietnam, where it contributes to the most important commercial fishery (Freyhof, 2014). In India, the fish migrate from Bay of Bengal to the Hooghly river for breeding (Jones & Menon, 1951). Recently, the availability of hilsa has been drastically declining with a consequent increase in demand and price, which often reaches $ 22 per kg (US) depending on the size and freshness. Therefore, the urgent need is culture of the species in confined water systems. The main bottlenecks for hilsa culture are insufficient fry for stocking, lack of knowledge on larval rearing and fry production and lack of ability to consistently produce a steady

sl.no. 09

Institute Project of ICAR-CIFA-reg

2 messages

Baidya Paul <bnpaulcifa@gmail.com> 13 July 2017 at 07:02
To: Koushik Ghosh <kghoshbu@gmail.com>

To
Dr. Koushik Ghosh
Assistant Professor (Stage –III)
Aquaculture Laboratory, Department of Zoology

(DST-FIST & UGC-SAP-DRS Sponsored)

The University of Burdwan

Golapbag, Burdwan – 713 104, West Ben

Dear Sir,

Your name has been proposed and accepted as a Co-PI in the Annual Institute Research Council Meeting held during 3-5th May, 2017 meeting at ICAR-CIFA, Bhubaneswar to execute the Institute funded project entitled " **Development of Larval diet for *Ompok bimaculatus*, a high- valued fish of regional importance".** I may therefore, request you to act as a Co-PI in the said project. Your consent in this collaborative effort and participation in the project will be highly appreciated.

Regards

Dr.Baidya Nath Paul
PI of the Project
Principal Scientist
Regional Research Centre
ICAR-Central Institute of Freshwater Aquaculture (www.cifa.in)
P.O.Rahara. Kolkata-700118
+91 33 25683023(Work)
Mob:+91 9432334390

3 attachments

RPP of larval feed project.docx
47K

RPP signature pages.pdf
559K

proceedings of the 31st Annual IRC meeting of ICAR-CIFA (1).pdf
3445K

Koushik Ghosh <kghoshbu@gmail.com> 13 July 2017 at 08:35
To: Baidya Paul <bnpaulcifa@gmail.com>

Received.
Thanks & best regards,

sl.no. 10

J. Botan. Soc. Bengal 75(2) : 1-16 (2021), ISSN 0971-2976

University of Calcutta, Kolkata 700 019, India

FULL LENGTH ARTICLE

Megaspores of heterosporous lycopsid affinity from the late Permian of Chhattisgarh, Central India and their evolutionary significance

Subhankar Pramanik[1], J.P. Keshri[2], Ratan Kar[1] and Amit K. Ghosh[1]*

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Received : Accepted : Published :

Dispersed fossil megaspores of heterosporous lycopsids have been recorded for the first time from the late Permian (Raniganj Formation) sediments exposed along the left bank of Banki River, near the village Chaki in Chhattisgarh, India. The outcrop has been palynologically dated based on miospore assemblage of typical late Permian affinity. The assemblage is represented by 7 species of trilete megaspores belonging to the genera *Noniasporites, Maiturisporites, Pantiella, Bokarosporites, Biharisporites, Hughesisporites* and a newly instituted genus of monolete megaspore i.e., *Monoletosporites* gen. nov. For the first time, the genera *Maiturisporites, Pantiella* and *Hughesisporites* have been recorded from the late Permian sediments; which were known earlier only from the Triassic sediments of Peninsular India. This indicates that the opportunistic species which successfully radiated during the Mesozoic had started colonizing themselves in the late Permian. A comparative study reveals that the megaspores possess affinity with the modern day lycopsids.

Key-words: Fossil megaspores, affinity, Raniganj Formation, late Permian, Peninsular India.

INTRODUCTION

The sexual life cycle of plants was a progressive evolutionary innovation that is vividly documented in the fossil records (Chaloner, 1967, 1970; Stewart, 1983). The evolutionary innovations (Niklas *et al*., 1980) in the reproductive biology of vascular plants have been thoroughly characterized by several workers namely Chaloner (1967), Stewart and Rothwell (1993) and Bateman and DiMichele (1994). In early land plants, these innovations in sexual life cycle can be categorized into homospory, incipient heterospory and advanced heterospory i.e., reduction in the number of functional megaspores to one, including gymnospermous reproduction. These newly induced innovations impart significant selective advantages over the less specialized grades (Chaloner, 1967; Niklas *et al*., 1980; Chaloner and Pettitt, 1987). The conjectural selective advantages are widely believed to play a major role in heterosporous plants that became dominant over homosporous pteridophytes and thereby the widespread dominance of flowering plants over the rest of the plant groups (Niklas *et al*., 1983; Stewart, 1983; Chaloner and

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sl.no. 10

Burdigalian to Early Serravallian Diatom Biostratigraphy from Havelock Island, Northern Indian Ocean

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Abstract—In the Ritchie's Archipelago of the Andaman group of islands (Northern Indian Ocean), Havelock Island is the largest one. The present study on diatoms has been carried out on three outcrops of Havelock Island situated in Kalapathar, South Point and Laccam Point localities. Samples collected from the three Neogene outcrops yielded poor to moderately preserved diatom valves. Two lithological units are exposed in this island, i.e., Inglis and Long formations. The diatoms have been recovered from the Inglis Formation and the diatom assemblages are represented by the marker diatom species, viz., *Actinocyclus ingens, Annellus californicus*, *Araniscus lewisianus*, *Cestodiscus peplum*, *Craspedodiscus coscinodiscus*, *Denticulopsis simonsenii*, and *Rossiella paleacea* that indicate the age as Burdigalian to early Serravallian.

Keywords: biostratigraphy, diatoms, late early to middle Miocene, Ritchie's Archipelago, Andaman and Nicobar Islands

DOI: 10.1134/S0869593821020064

INTRODUCTION

The Ritchie's Archipelago in the Andaman group consists of several islands, namely, Havelock, Neil, Henry Lawrence, John Lawrence, Peel, Wilson, Outram, Hugh Rose, Northern Button etc. including some islets. Sporadic works on fossil diatoms from Neogene sediments have been carried out from some of these islands though Neogene deposits are widely distributed in the Ritchie's Archipelago (Sharma and Srinivasan, 2007). Pioneering work from the Ritchie's Archipelago was done by Jacob and Shrivastava (1952). From the Miocene of Colebrook and other islands of the Ritchie's Archipelago, they reported the occurrence of diatoms along with radiolarians and silicoflagellates. Fifteen diatom taxa were reported by Mathur (1973) from the early Miocene of Havelock Island. Subsequently, seven diatom taxa were listed from the early Pliocene of Neil Island (Singh and Vimal, 1973). Thirty taxa of fossil diatoms belonging to 13 genera were recorded from the same sequence of Neil Island (Singh et al., 1978). Later on, Singh (1979) reported some additional diatom taxa. From the Outram Island, Mathur (1981) described 10 species of diatoms and correlated them to the middle Miocene *Craspedodiscus coscinodiscus* Zone of Bukry and Foster (1973). Further, Mathur (1985) identified 18 genera of diatoms with one new species *Liostephania ovalis* from the middle Miocene of Nicholson Island. After a decade, Singh and Sharma (1996) worked on the diatoms of Neil East Coast Section of Neil Island and recognised ten diatom zones. Chakraborty and Ghosh (2016) studied the late Miocene sediments (Tortonian) of the Neil Island and reported 82 planktonic and benthic diatoms. Recently, Chakraborty et al. (2019) have identified 22 taxa belonging to 17 genera of diatoms from Havelock Island. They are assigned to the late early to early middle Miocene based on multiple microfossils.

It may be mentioned here that planktonic diatoms are useful tool for biostratigraphic interpretation, dating and correlation of Neogene marine sediments (Barron, 1985a) and consequently the planktonic diatoms are frequently used in biostratigraphic studies (Barron, 1992). In view of this, the present study has been undertaken to make more precise age determination based on the study of diatoms from three outcrops exposed at Havelock Island, to identify the palaeoecological zones based on diatoms, and to decipher the diversity patterns of diatom taxa recovered from three different outcrops of Havelock Island.

MATERIALS AND METHODS

Andaman and Nicobar group of islands consists of two major groups, i.e., Andaman group and Nicobar group (Fig. 1a). Amongst these, the Andaman group

Item no: 8

Dr. Debidas Mondal

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****

No.R-Ph.D./Regn. /Sc/Botany/ Dated:

To:
Shri/Sm...Ruma Bhadra
Central National Herbarium , AJC Bose Indian Garden,
Shibpur, Howrah-711103

Sub: Granting Registration under the supervision of Dr. P.V. Prasanna, Scientist-F(BSI),Deccan Regional Centre ,Hyderabad as **Supervisor** and Dr. Saikat Naskar, Dept. of Botany, B.U. as **Co-Supervisor**.

Sir/Madam,

This has a reference to our earlier letter no. R-Ph.D./Regn./Sc/Bot/598 Dated 16.01.2020.

I am to inform you that the Joint Meeting (2022-2023)of the Faculty Councils for P.G. studies in Science &Arts, Commerce, Law etc . at its meeting held on 09.06.2022,resolved that you have been permitted to do research work under Dr. P.V. Prasanna, Scientist-F(BSI), Deccan Regional Centre ,Hyderabad as **Supervisor** and Dr. Saikat Naskar, Dept. of Botany, B.U. as **Co-Supervisor**.

Other terms and Conditions will remain same.

Yours faithfully,

Sd/-

Joint Registrar

No.R-Ph.D./Regn. /Sc/Botany/ 86/1(4) Dated: 08-07-2022

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PCP
PLANT & CELL PHYSIOLOGY

Exposure to Low UV-B Dose Induces DNA Double-Strand Breaks Mediated Onset of Endoreduplication in *Vigna radiata* (L.) R. Wilczek Seedlings

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Multiple lines of evidence indicate that solar UV-B light acts as an important environmental signal in plants, regulating various cellular and metabolic activities, gene expression, growth and development. Here, we show that low levels of UV-B (4.0 kJ m^{-2}) significantly influence plant response during early seedling development in the tropical legume crop *Vigna radiata* (L.) R. Wilczek. Exposure to low doses of UV-B showed relatively less growth inhibition yet remarkably enhanced lateral root formation in seedlings. Both low and high (8.0 kJ m^{-2}) doses of UV-B treatment induced DNA double-strand breaks and activated the SOG1-related ATM-ATR-mediated DNA damage response pathway. These effects led to G2-M-phase arrest with a compromised expression of the key cell cycle regulators, including CDKB1;1, CDKB2;1 and CYCB1;1, respectively. However, along with these effects, imbibitional exposure of seeds to a low UV-B dose resulted in enhanced accumulation of FZR1/CCS52A, E2Fa and WEE1 kinase and prominent induction of endoreduplication in 7-day-old seedlings. Low dose of UV-B mediated phenotypical responses, while the onset of endoreduplication appeared to be regulated at least in part via UV-B induced reactive oxygen species accumulation. Transcriptome analyses further revealed a network of co-regulated genes associated with DNA repair, cell cycle regulation and oxidative stress response pathways that are activated upon exposure to low doses of UV-B.

Keywords: DNA double-strand breaks • DPI • Endoreduplication • ROS • UV-B • *Vigna radiata*

Introduction

Plants, being sessile in nature and with their obligatory dependence on sunlight for photosynthesis, cannot escape the damaging effects of solar UV-B light. Therefore, they have developed a sophisticated and highly regulated balance between optimal light capture and UV-B protection. Some plant species escape UV-B exposure by limiting their life span to the season or places where they perceive only low levels of UV-B, while others, including crops, grow during the summer months and experience a high incidence of solar UV-B light (Ulm and Jenkins 2015). In tropical climates, plants receive sunlight for longer duration, and the effects of UV-B radiation are greater under such conditions. Early studies in tropical crops, including *Oryza sativa* (Teramura and Sullivan 1994), *Vigna mungo* (Fukumoto and Mazza 2000), *Vigna radiata* (Amudha et al. 2005), *Glycine max* (Guruprasad et al. 2008) and *Triticum aestivum* (Kataria and Guruprasad 2014) have shown compromised growth and yield in response to exposure to ambient UV-B light. More recent studies in cucumber (*Cucumis sativus* L. cv. 'Hi Jack') have correlated the growth retardation effect with the regulatory mechanism associated with the acclimation processes of UV radiation (Qian et al. 2021). Other studies mainly in *Arabidopsis* and some other species indicated that two major mechanisms of UV-B mediated responses, including the accumulation of UV-B absorbing compounds and the DNA damage response, actually vary among various plant species. These factors eventually generate variations in UV-B response in plants (Hidema et al. 2007, Xu and Sullivan 2010). However, the variations in UV-B response in nonmodel crops and other field plant communities remain largely unexplored.

UV-B represents an energy-rich intrinsic component of solar radiation. It affects plant growth and development through diverse physiological and metabolic processes (Jenkins 2009). At high fluence rate, UV-B causes damage to the photosynthetic components (Correia et al. 1998), DNA (Schmitz-Hoerner and Weissenböck 2003), proteins and membranes (Bornman and Teramura 1993). The UV-B-induced photodimers (Taylor 2006), primarily cyclobutane pyrimidine dimers (CPDs) and pyrimidine-6,4-pyrimidinone dimers (6,4PPs) (Gill et al. 2015), create distortions in the DNA double-helical structure and eventually block transcription and replication (Britt 2004, Manova and Gruszka 2015). Furthermore, the inefficient repair

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ORIGINAL ARTICLE

The Negative Impact of Prolonged Desiccation on the Recovery of *Selaginella bryopteris*: Insights Into Autophagy and Cellular Protection Strategies

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Keywords: autophagy | cell death | cellular protection | desiccation tolerance | metabolomics | protein homeostasis | ROS | *Selaginella bryopteris* | transcriptome | WGCNA

ABSTRACT

Desiccation tolerance is a complex biological phenomenon that allows certain plants to survive extreme dehydration and revive upon rehydration. Although significant progress has been made in understanding the physiological and molecular mechanisms involved in desiccation tolerance, recovery mechanisms after prolonged desiccation periods are enigmatic. Combining physiological, biochemical, transcriptomic and metabolomic approaches, we investigated the role of prolonged desiccation on recovery of *Selaginella bryopteris*. Prolonged desiccation causes a decline in the antioxidant system, leading to accumulation of ROS that hinder recovery by inducing cellular damage. Transcriptome and WGCNA analysis revealed the significance of protective proteins, alternative respiration and protein homeostasis in cellular protection and recovery after short and long-term desiccation. Metabolomic analysis exhibited an increased accumulation of antioxidant compounds, which can be substituted for antioxidant enzymes to maintain cellular protection during prolonged desiccation. The significant role of autophagy and autophagic components was evaluated by H_2O_2 treatment and phylogenetic analysis of ATG4 and ATG8, which unveiled their substantial role in desiccation tolerance and remarkable conservation of the autophagy-related genes across plant species. Our data demonstrated that prolonged desiccation leads to ROS-induced cell death by extensive autophagy due to enormous loss of protective proteins, antioxidant enzymes and energy resources during desiccation.

1 | Introduction

Drought, a major threat to global food security, is considered a central environmental challenge for crop growth and productivity (Zhu 2002; Lobell, Schlenker, and Costa-Roberts 2011). Extreme drought damages plants by desiccating cells. Seeds and spores of most plants can withstand desiccation (Bewley 1979), while the vegetative tissues of most plants lack this feature (Dinakar and Bartels 2013). However, a limited group of plants, termed desiccation tolerant plants (hereafter DT plants), possess exceptional survival potential in the vegetative tissues against desiccation. DT plants are also known as 'resurrection plants' because of their ability to revive from a complete water loss or an air-dry state, upon re-watering. DT plants can survive dehydration by

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Nonautonomous matter wave bright solitons in a quasi-1D Bose-Einstein condensate system with contact repulsion and dipole-dipole attraction

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ABSTRACT

We investigate the existence and propagation properties of envelope solitons of an extended nonlinear Schr ödinger equation with the time-modulated dispersion, quadratic-cubic nonlinearities and linear gain or loss, which govern the nonlinear wave propagation in a nonautonomous quasi-1D Bose-Einstein condensate system with contact repulsion and dipole-dipole attraction. A novel class of nonautonomous bright soliton solutions on continuous-wave background is identified for the first time. It is shown that these localized structures possess interesting features that differ from the usual bright solitons. A rich variety of evolution behaviors, which include snakelike and periodic oscillating bright soliton dynamics, is revealed. The constraints of the system parameters to form these nonlinear localized structures are also suggested.

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1. Introduction

The study of nonautonomous soliton propagation through nonlinear optical media has been one of the considerable recent interests [1–5]. This should not be surprising because the existence of these nonlinear waves have been demonstrated experimentally in a variety of settings, including nonlinear optics, fluid dynamics, condensed matter physics and plasma physics [5]. In general, the wave dynamics in some of the above mentioned areas and many other nonlinear mathematical-physics fields is governed by the well known nonlinear Schrödinger equation [6]. In the setting of Bose-Einstein condensates (BECs), such NLS equation is usually called the Gross-Pitaevskii (GP) equation [7].

To describe the wave propagation behaviors in realistic systems, one uses the variable-coefficient NLS equation and its variants. Noting that most of dynamical models are inhomogeneous in reality because of fluctuations in environmental environment and nonuniform medium, then the governing envelope wave equation should be included the spatially and/or

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Advanced asymmetric supercapacitor with $NiCo_2O_4$ nanoparticles and nanowires electrodes: A comparative morphological hierarchy

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ABSTRACT

In the present work, hydrothermal and wet chemical methods are adopted to fabricate $NiCo_2O_4$ nanowires (NiCo-NW) and $NiCo_2O_4$ nanoparticles (NiCo-NP) respectively. Owing to the mesoporous nature of these subunits, fast and convenient electron-ion transport and redox reaction, NiCo-NW achieves excellent electrochemical performance. Structure and microstructural characterizations of these samples are carried out by analyzing X-ray diffraction data employing the Rietveld method of structure refinement method and analyzing HRTEM, FESEM images and FTIR spectra. The low dimensional NiCo-NP is found to provide superior electrochemical performance than the NiCo-NW (~13 nm) due to its smaller particle size (~9 nm). This porous structure effectively helps in better transport of ions in the electrolyte. It manifests high specific capacitance 1066.03 F g^{-1} and enormous areal capacitance up to 5.96 F cm^{-2} whereas NiCo-NW exhibits specific capacitance up to 880.72 F g^{-1} and high areal capacitance of 4.93 F cm^{-2}. An asymmetric supercapacitor (ASC) has been fabricated with NiCo-NP and activated carbon as positive and negative electrodes respectively in 1 M Na_2SO_4 electrolyte medium. This device offers maximum specific energy 59.56 Wh Kg^{-1} and maximum power density 3403 W kg^{-1} with a high energy density of 4.197 Wh Kg^{-1} and shows excellent cyclic stability.

1. Introduction

In recent years, enormous attention has been drawn to develop novel materials and devices for the new renewable and sustainable energy sources with high efficiency, high reliability and high energy density. The supercapacitor has been used massively in last few decades as a green energy storage device combining the features of the conventional capacitor (high power density, long cycling life) and rechargeable batteries (high energy density) [1–8]. Based on the charge storage mechanism supercapacitors are of two types: (i) electric double-layer capacitor(EDLC), and (ii) pseudocapacitors. For EDLCs electric energy is stored by separation of charge in Helmholtz double-layer and for pseudocapacitor storage of electric energy is achieved by a faradaic redox reaction with charge transfer [8–10]. Various carbonaceous materials like activated carbon, CNT, graphene are being used as electrode materials for EDLCs for their higher surface area with a porous surface and electrically intercalated networks. EDLCs show high power density, better cycle life than pseudocapacitor but possess very low specific capacitance. However, due to fast multi electro-redox reaction, pseudocapacitors possess higher specific capacitance, higher energy density than observed in EDLCs [11,12], but it leads to deficient cycle stability because of redox reaction like a battery.

The primary focus of the present work is to improve cell voltage and energy density by developing an ASC device in which (EDLC) electrode has been used as the negative electrode and redox-active transition metal oxides as a positive electrode. The maximum operating voltage in the cell system can be reached by using different potential windows of the two-electrode system. Primarily, activated carbon has been used as the negative electrode and transition metal oxides as a positive electrode. So, the main focus of ASC is to develop better metal oxides for advanced positive electrode [3,13].

Various metal oxides and hydroxides with their variable valence states had been widely used for electrode materials in pseudocapacitors [14,15]. Attempts had been made to prepare inexpensive metal oxides like Co_3O_4 [16,17], NiO [10,18], MnO_2 [19], V_2O_5 [20], Fe_2O_3 [21] for high theoretical capacitance and low toxicity. Both Ni and Co-based materials were considered to be the most admirable

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Enhanced electrochemical properties of Co_3O_4 with morphological hierarchy for energy storage application: A comparative study with different electrolytes

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ABSTRACT

A facile hydrothermal route synthesizes Co_3O_4 nanocrystals with urchin spine-like morphology. Structure and microstructural characterizations of the sample are carried out. Electrochemical properties have been explored in the presence of different electrolytes. In order to find out the best electrolyte, three electrolytes (Na_2SO_4, NaOH and Na_2SO_4 with Hq) of fixed concentration (1 M) are used to record the cyclic voltammetry data. In the presence of Na_2SO_4 as an electrolyte, specific capacitance becomes 218 F g^{-1}, possibly because of low ionic conductivity of SO_4^{2-}, higher charge transfer resistance. When NaOH and Na_2SO_4 (with Hq) are used as electrolytes, high specific capacitances of 1720 F g^{-1} and 2433 F g^{-1} respectively are obtained due to extra pseudocapacitive effect of redox reaction. It is worth noting that the semicircle diameter in the EIS plot is highest for Na_2SO_4 and lowest for Na_2SO_4 (with Hq) electrolyte. The R_{ct} value depends on the type of electrode and the interaction between electrolyte ions with the electrode.

1. Introduction

Nowadays, one of the primary focuses of the scientific community is to harvest new sustainable energy materials to cope up with the continuous changes in the global climate. The demand for energy, however, is increasing day by day. It becomes very urgent for a scientist to develop new renewable energy sources with high power and better efficiency. It is now well known that supercapacitors have emerged as an alternative energy storage device with better efficiency than a rechargeable battery [1,2]. Supercapacitors exhibit higher energy efficiency, excellent reversibility, higher energy density than a conventional capacitor. Generally, supercapacitors can be classified into three types based on the charge storage mechanism: (i) electrical double-layer capacitor (EDLC), (ii) pseudocapacitors, and (iii) hybrid system. The energy storage mechanism in the electrochemical capacitor is of two types: faradaic and non-faradaic. The non-faradaic reaction arises in the EDLC due to ion adsorption at the electrode/electrolyte [3]. Various carbonaceous materials such as activated carbon, carbon nanotube (CNT), graphene oxide belong to the EDLCs. Such carbonaceous materials possess a large surface area with a porous surface with the interlaced network [4]. However, EDLCs cannot fulfill the requirement for the peak power assistance in the vehicle since EDLC offers low energy density. Instead, the faradaic pseudocapacitors are based on the fast reversible redox reaction within electroactive materials on the electrode, and its energy density is at least one order of magnitude higher than EDLCs [3,4].

In contrast, various inexpensive transition metal oxides such as Co_3O_4 [1,5–8], NiO [4,9], MnO_2 [10], and Fe_3O_4 [11], $NiCo_2O_4$ [12] are mainly used as electrode materials for pseudocapacitors. They provide enhanced electrochemical performance over EDLCs because of their higher specific capacitance generating from rapid and productive redox reaction. Finding cheap material with superior pseudocapacitive performance has thus attracted enormous attention. Among all these transition metal oxides, Co_3O_4 has been studied extensively for its supercapacitor application due to its high surface area, easily tunable surface area, multiple oxidation states and tunable structural properties. The Co_3O_4 is a p-type direct optical bandgap semiconductor that shows the high theoretical capacity, excellent corrosion stability and can act as

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Article

Ultrastable Asymmetric Supercapacitor Device with Chemically Derived and Mechanically Activated $NiCo_2O_4$

Mahasweta Chatterjee, Adwaita Kundu, Sachindranath Das, and Swapan Kumar Pradhan*

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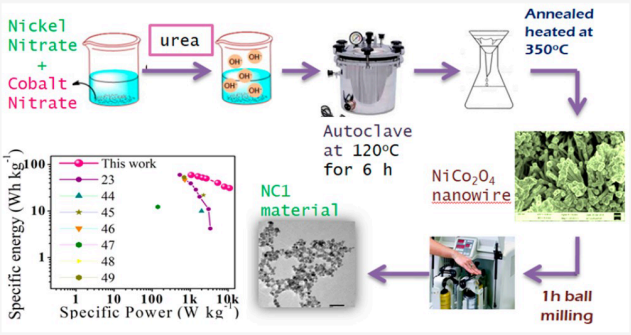
ABSTRACT: We report the effect of mechanical alloying on the chemically synthesized $NiCo_2O_4$ nanowire for better electrochemical performance. The nickel cobaltite nanowires (NC) were successfully synthesized via the hydrothermal method without any surfactant. Then they were milled for 1 h (NC1) and 2 h (NC2) to boost the electrochemical performance. The structural and microstructural parameters, shape, size, and morphology of these samples are revealed by X-ray diffraction (XRD), Fourier transform infrared spectroscopy (FTIR) and transmission electron microscopy (TEM) techniques. The Brunauer–Emmett–Teller (BET) characterization and Barrett–Joyner–Halenda (BJH) model reveal that the NC1 sample offers the highest specific surface area among all three samples with its one-dimensional mesoporous structure (pore diameter, ~7 nm). The NC1 sample displays an excellent specific capacitance and rate capability (1234 F g^{-1} at a scan rate of 2 mV s^{-1}). However, upon further milling (2 h) the electrochemical performance of the sample decays rapidly due to an increase in particle size and reduction in specific surface area. A remarkable specific capacity of 1196 F g^{-1} is achieved in the 1 h milled sample at the lowest current density of 12 A g^{-1}, and at 40 A g^{-1} and 129.2 F g^{-1} specific capacitance can be retained. We further demonstrate an asymmetric device based on the NC1 sample as a positive electrode, which produces an excellent energy density of 59.221 Wh kg^{-1} at a power density of 1065.4 W kg^{-1}. The assembled device can attain an outstanding power density of 10.992 kW kg^{-1} at an enormous high current density of 13.33 A g^{-1} and demonstrates an excellent cyclic performance of 91.7% retention after 5000 cycles.



INTRODUCTION

Due to the rapid growth of portable energy storage systems, mobile systems, and other electronic gadgets, the main interest of scientists in these fields is to develop advanced new generation high energy and power density devices.[1−3] Various transparent energy storage systems are used in commercial and industrial areas. A supercapacitor can be recognized as an efficient, clean energy storage candidate due to its excellent cycle life, high power density, and better cycle stability. Typically, the charge storage mechanism of a supercapacitor is of two types: one is the capacitive type and the other is the pseudocapacitive type. Generally, the charge storage process of the capacitive type is an electric double-layer capacitor that relies on electrostatic charge storage separation of ions at the electron electrolyte interface.[4,5]

In contrast, in a pseudocapacitor, capacitance is produced by a fast multielectron faradaic surface redox reaction. The capacitance performance is much better than the electric double-layer capacitor (EDLC), especially in energy density. Several transition metal oxides (NiO, $NiCo_2O_4$, $CoFe_2O_4$, MnO_2, and Co_3O_4) and sulfides are vastly used and studied as positive electrodes for their different pseudocapacitive nature.[6−10] The binary oxides manifest extraordinary electrochemical performance than a single metal oxide because of their redox reaction between valence states, large electrode–electrolyte contact surfaces, and many defects, which improves pseudocapacitance as well as the energy density of the material.[11−13] The crucial parameters which regulate the electrochemical performance are the porosity, particle size, specific surface area, oxygen vacancy, and surface defects. Scientists these days try to incorporate an optimized amount of oxygen vacancy and surface defects to balance the electrochemical performance of the material in a well-mannered way.[14−16] Since metal oxide with a higher oxygen vacancy ensures a higher CV current and higher positive potential, forming an oxygen vacancy becomes one of the main choices for getting higher electrochemical performance by an easy and economical technique. In metal oxides or ceramics with

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Superior photocatalytic performance and photo disinfection of bacteria of solvothermally synthesized mesoporous La-doped CeO_2 under simulated visible light irradiation for wastewater treatment

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ABSTRACT

A simple, cost-effective, and facile solvothermal approach has been adopted to synthesize mesoporous CeO_2 nanostructures with varying La-doping (2, 4, and 6 mol%) concentrations. Photocatalytic and antibacterial performances are investigated against the inactivation of *Escherichia coli* and *Bacillus licheniformis* bacteria cells. Structural and microstructural characterizations of La-doped CeO_2 nanostructures are performed by analyzing X-ray diffraction (XRD) data employing the Rietveld refinement method, scanning electron (SEM) and transmission electron microscopy (TEM) images, Brunauer–Emmett–Teller (BET), energy-dispersive X-ray (EDX), and X-ray photoelectron spectroscopy (XPS) spectra. Among three doped samples, the 4 mol% La-doped CeO_2 (LCe4) has exhibited high oxygen and Ce^{3+} concentrations, high microstrain, small crystallite size, and lowest band gap energy, as are revealed by the analysis of XPS, UV–VIS absorption spectra, photoluminescence (PL) spectra, and Rietveld refinement result. The LCe4 sample with the highest number of oxygen vacancies and high surface area shows superior photocatalytic activity (~95% Rhodamin B (RhB) degradation in 130 min, ~70% Methylene Blue (MB) degradation within 30 min, and ~95% phenol degradation in 180 min under solar radiation). It shows a striking photo-disinfection effect and enhanced antibacterial activity (almost identical to a pure drug) against gram-positive and gram-negative bacteria under visible light irradiation. This novel disinfection and catalytic property of the LCe4 sample is attributed to the mesoporous structure of materials and surface activity, which lowers the electron-hole recombination rate and transports more photogenerated electrons and holes. The nanostructured mesoporous LCe4 material has been used as an effective visible light-activated photocatalyst and photo disinfection for treating wastewater containing organic dyes and gram-negative and gram-positive bacteria.

1. Introduction

Water pollution from mixing hazardous materials and heavy metals has become a serious global issue. Because of water pollution, various water-born diseases become vulnerable to humanity in most developing countries due to the lack of adequate purifier systems like UV radiation and chlorofication, particularly in rural areas. Thus, the availability of purified drinking water becomes a critical issue for the increasing population. Photocatalytic degradation of pollutants is a facile green chemical, sustainable and cost-effective method to remove contaminants from wastewater containing organic dyes [1–3].

CeO_2 is considered one of the most abundant rare earth oxides frequently used in electrochemical cells, energy storage and optical devices, photocatalysis, and as a biomaterial. CeO_2 is an n-type semiconductor material with various chemical and physical properties, like pollutant elimination with non-toxicity [5–7]. The main feature of CeO_2 is the transformation of the Ce^{4+} to Ce^{3+} valence state, which causes oxygen vacancies and a high stoichiometry deviation, consequentially increasing visible light absorbance [4–8]. Various reports on CeO_2 as a photocatalyst with different morphologies, like nanocube, nanowire, and nanodisc, using different templates are available. The present study intends to develop CeO_2 nanomaterials with an optimum mesoporous structure and

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Research Paper

Mn-doped $NiWO_4$ quantum dots with superior electrochemical and conductivity performance for energy storage application

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Supercapacitor

ABSTRACT

Monoclinic amorphous $Ni_{1-x}Mn_xWO_4$ (x = 0.00, 0.02) compounds have been successfully synthesized by hydrothermal technique for achieving better capacitive and conductive performances. Different characterization techniques like X-ray diffraction (XRD), Fourier transform infrared spectroscopy (FTIR), ultraviolet-visible (UV–Vis) and photoluminescence (PL) spectroscopy have been employed to investigate their structural, microstructural, and optical properties. Mn-ion incorporation in the $NiWO_4$ lattice reduces the particle size of the sample to ~4.5 nm, compared to the pure undoped $NiWO_4$ sample (~18 nm), confirmed from the transmission electron microscopy image and Brunauer–Emmett–Teller analyses (BET). Tauc plot of $Ni_{0.98}Mn_{0.02}WO_4$ sample exhibits a significant increase in bandgap energy, compared to pure undoped $NiWO_4$ sample due to the quantum confinement effect. The electrochemical performance of electrodes made with these materials has been revealed by cyclic voltammetry (CV), galvanostatic charge-discharge (GCD) properties and electrochemical impedance spectroscopy (EIS). Moreover, the addition of 2 % Mn in $NiWO_4$ causes an increase in specific surface area (117.390 m^2/g) due to the reduced particle size of the material, resulting in excellent specific capacitance of 463 F g^{-1} at 0.5 A g^{-1} current density. The detailed charge storage mechanism for the improvement of conductivity and electrochemical performance of the Mn-doped $NiWO_4$ has been revealed in different studies. An asymmetric supercapacitor device (ASC) has been fabricated using Mn-doped $NiWO_4$ electrode material as positive electrode. The device shows superior cyclic stability upto 5000 cycles, can retain 88.4 % of its initial value.

1. Introduction

Electrochemical storage devices such as supercapacitors, fuel cells, and Li-ion batteries are more sustainable clean energies to deal with the global warming issues [1–3]. Among all three renewable energy sources, a supercapacitor is more promising than Li-ion batteries due to its fast charging, longer recyclability, better power density, and easy maintenance. Supercapacitors are classified into two categories, (i) electric double-layer capacitors (EDLC) and (ii) pseudocapacitors [2–5]. Researchers are continuously trying to improve the energy density of supercapacitors without hampering their power density and cycle life. Pseudocapacitor materials store more energy than an electric double-layer capacitor.

For this reason, various binary and ternary metal hybrid oxides with different morphologies were synthesized for supercapacitor applications [6,7]. However, some drawbacks of using metal oxides in electrochemical applications include poor conductivity, low energy density, and poor cycle stability [8,9]. It has been revealed from recent works that the electrochemical properties of some complex oxides (such as $NiCo_2O_4$ and $MnCo_2O_4$) are superior to single oxides like NiO, MnO_2, and Co_3O_4 because of multiple oxidation states of different metal cations [10–12]. The $NiWO_4$ compound is an attractive material in the electrochemical field because of its high electrical conductivity of $\sim 10^{-7}$–10^{-3} S cm^{-1} [13–15], which is higher than NiO (10^{-13} S cm^{-1}), and $CoWO_4$ compounds [16]. It was reported that the incorporated W atoms had improved the electrical conductivity and electrochemical activity of the compound [17]. Recent reports on core-shell heterostructures with multi-component, such as $MnCo_2O_4/NiWO_4$, Ni.Co.Zn oxide/$NiWO_4$, and $NiWO_4/NiCO_2O_4$ grown on nickel foam showed enormous high electrochemical performance than the $NiWO_4$ lattice

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SL. NO. 14

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Institute Project of ICAR-CIFA-reg

2 messages

Baidya Paul <bnpaulcifa@gmail.com> 13 July 2017 at 07:02
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To
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(DST-FIST & UGC-SAP-DRS Sponsored)

The University of Burdwan

Golapbag, Burdwan – 713 104, West Ben

Dear Sir,

Your name has been proposed and accepted as a Co-PI in the Annual Institute Research Council Meeting held during 3-5th May, 2017 meeting at ICAR-CIFA, Bhubaneswar to execute the Institute funded project entitled " **Development of Larval diet for *Ompok bimaculatus*, a high- valued fish of regional importance".** I may therefore, request you to act as a Co-PI in the said project. Your consent in this collaborative effort and participation in the project will be highly appreciated.

Regards

Dr.Baidya Nath Paul
PI of the Project
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3445K

Koushik Ghosh <kghoshbu@gmail.com> 13 July 2017 at 08:35
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Received.
Thanks & best regards,

K. Ghosh
[Quoted text hidden]

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Short Communication

Protein Requirement of *Ompok bimaculatus* (Bloch, 1794) Larvae

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ABSTRACT

Paul, B.N., Das, A., Mandal, R.N., Singh, P., Adhikari, S., Ghosh, K., Chowdhury, D., Chakrabarti, P.P. and Giri, S.S. 2020. Protein requirement of Ompok bimaculatus *(Bloch, 1794) larvae. Animal Nutrition and Feed Technology, 20: 525-533.*

A 22 d experiment was carried out to study the protein requirement of *Ompok bimaculatus* larvae (weight 0.11±0.01 g; length 20.49±0.70 mm). Three different formulated feeds were prepared with graded levels of crude protein i.e., with low (35% CP; LP), medium (40% CP; MP) and high (45% CP; HP) crude protein levels. The water quality parameters were optimum during the whole experimental duration. The survival rate was more than 70%. The fish grew to 0.34±0.06, 0.91±0.15 and 0.36±0.07 g, respectively in LP, MP and HP groups. The final weight was significantly ($P<0.01$) higher in MP having 40% CP in the diet. Both the net weight gain and specific growth rate were significantly ($P<0.05$) higher in MP having 40% CP in the diet as compared to LP and HP. The FCR was also significantly ($P<0.05$) lower in MP having 40% protein in the diet when compared with LP and HP. However, the protein efficiency ratio was similar ($P>0.05$) among the three treatment groups. Further, it was also revealed that the activities of enzymes in the digestive tract namely, α-amylase, lipase and pepsin were significantly ($P<0.05$) higher in MP diet having 40% crude protein. The present experiment, thus. revealed that 40% CP was sufficient for the optimum growth and survival of *O. bimaculatus* larvae.

Keywords: Amylase, Growth, Lipase, *Ompok*, Pepsin

INTRODUCTION

Ompok bimaculatus (Bloch, 1794) popularly known as the 'butter catfish' are found in lakes, rivers, canals, beels, swamps, floodplains and ponds, etc. and are distributed in India, Bangladesh, Borneo, Java, Sri Lanka, Myanmar, Pakistan, Thailand, Cambodia and Vietnam, etc. (Jayaram, 1977). It is a non-air breathing fish belonging

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to the family of *Siluridae* with the highest growth amongst the three species under the genus Ompok namely *O. paba, O. Pabo* and *O. bimaculatus*. These fish are commonly known as 'pabda'. Recently, the fish is gaining importance as a promising aquaculture candidate owing to its good taste, balanced nutrient profile, soft bony structure and high market value especially in the entire East and North East India (Banik *et al.,* 2012). Banik *et al.* (2011) reported that the butter catfish is considered as an important candidate for the diversification of freshwater Indian aquaculture. The wild population of *O. bimaculatus* has sharply declined due to anthropogenic activities, ecological changes and indiscriminate fishing. Thus, the species has been categorized under near-threatened category by the International Union for the Conservation of Nature and Natural Resources (IUCN) Red List and faces a risk of extinction in nature (Chakrabarti *et al.*, 2009; Lakra *et al.,* 2010; IUCN Red List, 2014). Considering high demand, price and IUCN status, the species has been prioritized for diversification of aquaculture as well as for conservation and restocking programs (Debnath *et al.*, 2016). This fish has a high commercial value and preferred in Eastern and North-Eastern India. *O. bimaculatus* was also declared as the State Fish of Tripura in the year 2007. The fish is endowed with all essential nutrients and rich in vitamin A and PUFA (Paul *et al.,* 2018). Hence, it is of utmost importance to save the fish from extinction through the development of suitable cultural technique.

Nutritional quality of starter diets and first feeding regimes greatly influenced the success of larval rearing. Santiago *et al.* (1991) reported that fry production and survival can be enhanced by feeding the broodstock with supplemental diets. The ability of fish to metabolize a diet depends on the availability of appropriate digestive enzymes, which mediate specific degradation pathways modulating both the physical and chemical nature of foods (Deng *et al.*, 2010). Fish digestive enzymes emphasizing the mechanisms and best use of nutrients are of unquestionable importance as a background for the optimization of fish feeding procedures (Suarez *et al.*, 1995). The analysis of digestive enzymes provides information on fish nutritional physiology and on their ability to take advantage of the different nutritional fractions of the feed (Odedeyi and Fagbenro, 2010). Thus knowledge of digestive enzymes of fish has important practical implications for their nutrition. The present work was aimed at the development of larval feed with appropriate protein content for *O. bimaculatus* so that the mortality will be reduced and more stocking material would be available for successful grow out of *O. bimaculatus*.

MATERIALS AND METHODS

Experimental diets

Fish meal, groundnut cake, soybean meal, wheat flour, vitamin-mineral mix and oil were used as feed ingredients in different proportions for preparation of three experimental feeds (Table 1). The finely powdered ingredients were weighed separately and after thorough mixing with water, fortified with vitamin and mineral mixtures. Three different formulated feeds were prepared to have different levels of protein

viz., low protein (LP; 35% CP), medium protein (MP; 40% CP) and high protein (HP; 45% CP). The powdered ingredients including vitamin and mineral mixture were thoroughly mixed and stored in –20°C.

Table 1. Ingredients and proximate composition of experimental diets

Particulars	Diets†		
	LP	MP	HP
Ingredient composition (%)			
Fish meal	44	53	65
Groundnut cake	15	15	15
Soyabean meal	10	10	5
Wheat flour	19	10	03
Carboxy methyl cellulose	2	2	2
Vitamin-mineral mixture#	5	5	5
Veg oil	5	5	5
Proximate composition (% DM basis)			
Dry Matter	93.02±0.93	92.39±0.22	92.34±1.33
Crude Protein	35.33±2.25	40.69±0.55	45.23±0.17
Crude lipid	9.11±0.47	9.49±0.31	9.61±0.31
Total ash	15.40±0.39	16.35±0.09	16.37±0.39

†Diets with low (35% CP; LP), medium (40% CP; MP) and high (45% CP; HP) protein levels.

‡Vitamin-mineral premix contains: Vitamin A (as acetate) 5000 IU, cholecalciferol 1000 IU, thiamine monocitrate 10 mg, riboflavin 10 mg, pyridoxine hydrochloride 5 mg, cyanocobalamine 15 μg, nicotinamide 75 mg, calcium pantothenate 10 mg, ascorbic acid 150 mg, a-tocopheryl acetate 25 mg, biotin 5 mg, folic acid 5 mg, menadione 100 mg, choline chloride 50 mg, PABA 5 mg, myoinositol 10 mg, calcium lactate 0.125 mg, magnesium oxide 60 mg, dried ferrous sulphate 30 mg, manganese sulphate 2 mg, copper sulphate 2 mg, zinc sulphate 2 mg, sodium molybdate 0.25 mg, sodium borate 0.80 mg, potassium iodate 20 mg, dicalcium phosphate 0.10g, cobalt chloride 20 mg (Paul *et al.*, 1997)

Fish maintenance and feeding

The growth experiment was carried out in ICAR-Central Institute of Freshwater Aquaculture. The duration of the study was 22 d. The larvae of *O. bimaculatus* (weight 0.11±0.01 g; length 20.49±0.70 mm) were stocked in glass jar tanks of 30 m^3 capacity with 30 larvae in each tank. Each dietary treatment consisted of three replicates of uniform-sized glass tanks.

Water quality parameters such as temperature, pH and dissolved oxygen were recorded at fortnightly intervals as per the method of APHA (2005). The feed was offered *ad libitum* during the morning (9.00 am.) and evening (4.00 pm). After the experiment, individual BW of fish was recorded. The net weight gain (NWG), protein efficiency ratio (PER), feed conversion ratio (FCR), specific growth rate (SGR) and per cent survivability was measured as per Castell and Tiews (1980). The proximate composition of feed was analyzed as per AOAC (1990).

Enzyme analysis

The fish were starved for 24 h before dissection to clean the digestive tract. A 10% homogenate of the digestive tract was prepared with chilled phosphate buffer saline (PBS, pH 7.4). The homogenates were centrifuged at 12,500 $\times g$ for 30 min at 4°C and supernatants were used as enzyme extracts. Metabolic enzymes were prepared by 10% homogenates fish tissue in 0.25 M ice-cold sucrose solution (pH 7.4). The α-amylase activity was estimated as per Bernfeld (1955). The total protease activity was measured as per Walter (1984). The activity of lipase was measured as per Bier (1955). Pepsin activity was measured as per Anson (1938). The activities of trypsin and chymotrypsin were measured as per Erlanger (1961).

The activities of alanine transaminase (ALT) and aspartate transaminase (AST) were assayed as per Reitman and Frankel (1957). Total protein of enzyme supernatant was assayed as per Lowry *et al.* (1951).

Quantification of DNA-RNA in fish tissue

Larval tissues (100 mg) were extracted in 1% sarcosine (sodium N-lauroylsarcosine) in Tris-EDTA buffer (pH 8.0). After centrifugation, the supernatant of homogenate samples was washed with phenol-chloroform-isoamyl alcohol (49.5:49.5:1; v/v) followed by further washing with isoamyl alcohol-chloroform (1:24; v/v) for purification. Purified supernatants were treated with nucleases. The DNA and RNA contents of the tissues were estimated according to the procedures of Burton (1956) and Marham (1955), respectively.

Statistical analysis

All the data generated from the experiment were statistically assayed by one-way analysis of variance (ANOVA) as per Snedecor and Cochran (1994). The differences between the means of treatments were examined using the least significance difference (LSD).

RESULTS

The feed formulations and proximate composition of different feeds are shown in Table 1. The protein content of the three feeds was 35.33 ± 2.55, 40.69 ± 0.55 and 45.23 ± 0.17 per cent, respectively, in the LP, MP and HP groups. Because of the graded increase in protein levels, the total ash contents of diets increased linearly from 15.40 ± 0.39 to 16.37 ± 0.39, which could be attributed to the presence of higher levels of fish meal as the major feed ingredient. Water quality parameters during the study were: temperature, 28-30°C, pH 7.4-7.8, dissolved oxygen 5.0-5.8 mg/L and total alkalinity 235-240 mg $CaCO_3$/L. All the water quality parameters were also within the acceptable range as reported earlier (Paul *et al.,* 2000). Dissolved oxygen plays a vital role in the rearing of larvae because larvae require an optimum level of oxygen for sustaining their physiological condition. Paul and Giri (2016) reported that suitable temperature required for optimum growth of catfish is 30°C.

Table 2 represents the growth performance data of *Ompok* larvae fed with different levels of protein. The feed was offered twice in a day as per the findings of Paul *et al.* (2014) who studied that a feeding frequency of up to two times is optimum for catfish *O. pabda*. Initially, the larvae were fed with natural food which was followed by feeding of the formulated feed. The data on the initial BW, net weight gain, specific growth rate, feed conversion ratio, protein efficiency ratio and survivability are shown in Table 2. Butter catfish larvae fed diet MP containing 40% CP had the highest weight gain, which was significantly ($P<0.01$) different from the other two dietary protein levels. The net weight gain was significantly ($P<0.01$) higher in MP. The SGR was significantly ($P<0.01$) higher in MP in comparison to LP and HP groups. The values of final weight, NWG and SGR were significantly ($P<0.05$) higher in MP compared to those of LP and HP groups. The FCR value was significantly ($P>0.05$) lower for fish fed diet MP vis-a-vis other groups. Lowest FCR in MP indicated that a lower amount of feed was needed to produce one unit of fish biomass; lower the FCR, better is the efficiency of the feed utilization. The PER, RNA, DNA, RNA/DNA ratio and survival (%) did not differ significantly among the dietary treatments.

Amylase activity of fish fed the LP differed significantly ($P<0.05$) from those on the MP and HP diet (Table 3). Lipase and pepsin activities were significantly ($P<0.05$) higher on MP diet which was also accompanied by significantly ($P<0.05$) lower trypsin and chymotrypsin activities. The ALT activity changed positively according to the increase in CP content of the diets and, therefore, was significantly ($P<0.05$) higher on HP diet. The AST activity, on the other hand, was significantly ($P<0.05$) lower in MP.

Table 2. Growth of *O. bimaulatus* larvae fed with different levels of dietary protein.

Particulars	Dietary groups[†]		
	LP	MP	HP
Initial weight (g)	0.11±0.004	0.106 ±0.008	0.106±0.008
Final weight (g)	0.34^{a}±0.06	0.91^{b}±0.15	0.36^{a}±0.07
NWG (g/22d)	0.23^{a}±0.05	0.81^{b}±0.15	0.26^{a}±0.06
SGR (%/d)	4.54^{a}±0.60	7.97^{b}±0.76	5.30^{a}±0.53
FCR	3.62^{b}±0.51	1.93^{a}±0.43	3.68^{b}±0.35
PER	0.82±0.13	1.42±0.33	0.62±0.05
Survivability	73.50±2.02	71.00±1.00	59.00±4.70
DNA (μg/mg)	30.59±0.30	34.31±0.43	31.04±0.54
RNA (μg/mg)	34.86±0.41	45.16±0.36	35.65±0.31
RNA/DNA	1.14±0.07	1.32±0.04	1.15±0.04

[†]Diets with low (35% CP; LP), medium (40% CP; MP) and high (45% CP; HP) protein levels.

[ab]Means with different superscripts in a row differ significantly ($P<0.01$).

Table 3. Enzyme contents of digestive tract of *O. bimaculatus* larvae fed with different levels of dietary protein

Particulars	Initial	Dietary groups[†]		
		LP	MP	HP
Amylase[‡]	17.4[a]±0.33	23.87[b]±0.17	25.36[c]±0.34	25.46[c]±0.47
Total protease[§]	1.76[d]±0.03	0.67[a]±0.02	0.81[b]±0.01	0.73[c]±0.01
Lipase[#]	0.40[a]±0.01	0.57[b]±0.02	0.69[d]±0.03	0.61[c]±0.02
Pepsin[¥]	0.38[a] ±0.01	2.53[b]±0.02	2.67[c]±0.01	2.49[b]±0.01
Trypsin[¶]	0.89[c]±0.02	0.20[a]±0.01	0.18[a]±0.02	0.26[b]±0.03
Chymotrypsin[¶]	0.72[c]±0.02	0.29[b]±0.02	0.19[a]±0.01	0.28[b]±0.03
Alanine transaminase[•]	3.14[a]±0.03	3.44[b]±0.02	3.51[c]±0.02	4.25[d]±0.02
Aspartate transaminase[£]	3.24[a]±0.02	4.61[c]±0.03	4.38[b]±0.01	5.12[d]±0.02

[†]Diets with low (35% CP; LP), medium (40% CP; MP) and high (45% CP; HP) protein levels.

[‡]mg maltose liberated/mg protein/h.

[§]μg of tyrosine liberated/mg protein/min.

[#]μM of fatty acid liberated/mg protein/min.

[¶]mmol of 4-nitroaniline liberated/mg protein/min.

[•]μM of pyruvate formed/mg protein/min.

[£]μM of oxaloacetate formed/mg protein/min.

[abc]Means with different superscripts in a row differ significantly ($P<0.05$).

DISCUSSION

The optimum dietary protein requirement for *O. bimaculatus* larvae as observed in the present study appears to be 40% CP, promoting the highest weight gain. The study suggests that 40% CP in a diet would support better growth and survivability of the butter catfish larvae during early development. Our results are in agreement with some of the previous reports depicting 40% protein level optimal for growth and efficient feed utilization in juvenile red- and white-coloured fancy carp *Cyprinus carpio* var. Koi (Choi *et al.*, 2015). However, the finding of our experiment on protein requirement was somewhat higher than the earlier CP levels documented for the maximum growth of other catfishes viz., 35% CP level in the diet of *Horabargus brachysoma* fingerlings (Giri *et al.*, 2011); 30% CP for *O. bimaculatus* (Debnath *et al.,* 2018); 35% CP level in the diet of *O. bimaculatus* fingerlings (Biswas *et al.,* 2019) and 33.2% CP level in the diet of *O. pabda* fry (Paul *et al.,* 2012). On the contrary, still higher levels of CP requirements have also been suggested, e.g., 41-43% CP for grass carp fry (Dabrowski, 1977) and 47% CP for *Catla catla* fry (Singh *et al.*, 1988). Giri *et al.* (2011) reported that the total protein requirement for optimum growth in catfish varies from 25 to 50% of the diet. Protein requirement for younger catfish is higher than the adult ones (Paul and Giri, 2016). However, our study of 40% CP for *O. bimaculatus* was similar to the protein requirement of catfish as reported earlier (BIS, 2014). A reduced growth was recorded at high-protein diets i.e., at 45% CP in the diet. Many authors reported similar findings (Ye *et al.,* 2016).

In the present study, digestive enzymes such as amylase, protease, lipase, pepsin, trypsin, chymotrypsin were estimated in Ompok and the value was similar to the earlier observation by Parra *et al.* (2007). There were no significant ($P > 0.05$) changes observed in α-amylase activity in MP and HP groups but pepsin activity was significantly higher with the feeding of the MP diet. Trypsin and chymotrypsin activities were higher in LP and MP groups suggesting that MP group fishes were more carnivores in nature. Lemieux *et al.* (1999) observed that trypsin is a proteolytic enzyme, it also takes part in other pancreatic zymogens and also limit the growth rate of *Gadus morhua*. Different parts of the digestive tract such as the pancreas, pyloric cecum and liver of different teleosts have shown different activities of amylase (Deng *et al.,* 2010). Sabapathy and Teo (1993) reported a lower pepsin activity in herbivorous fish. Digestive enzymes might contribute towards efficient digestion of the dietary components, which could be reflected through the growth of the fishes. Thus, increased growth in fish associated with enhanced activities of the digestive enzymes might be indicative of improved nutrient utilization in fish as reported earlier (Mandal and Ghosh, 2018). The activities ALT and AST are indicators of liver function with the high activities being the indicator of poor liver function and the present study also revealed normal levels of ALT and AST.

DNA is a prerequisite for RNA synthesis, which in turn is a requirement for protein synthesis (Mitra and Mukhopadhyay, 2002). RNA/DNA ratio is the indicator of the growth of fish (Dey *et al.,* 2018) and also considered as a reliable indicator of protein synthesis (Gangadhar *et al.*, 1997). However, no variation was observed in the above parameters in the present study.

Overall, the present experiment revealed that 40% crude protein is sufficient for the optimum growth and survival of *O. bimaculatus* larvae.

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REFERENCES

AOAC, 1990. *Official Method of Analysis*, 15th ed. Association of Official Analytical Chemists, Washington, DC, USA.

APHA, 2005. *Standard Methods for Examination of Water and Wastewater*, 21st ed. American Public Health Association, Washington, DC, USA.

Anson, M.L. 1938. The estimation of pepsin, trypsin, papain and cathepsin with haemoglobin. *Journal of General Physiology*, 22: 79-89.

Banik, S., Goswami, P. and Malla, S. 2011. Ex-situ studies of captive breeding of *Ompok bimaculatus* (Bloch, 1794) in Tripura. *Journal of Advanced Laboratory Research Biology*, 2: 133-137.

Banik, S., Goswami, P. and Malla, S. 2012. Studies on breeding physiology of *Ompok bimaculatus* (Bloch, 1794) in Tripura. *Uttar Pradesh Journal of Zoology*, 32: 67-72.

Bernfeld, P. 1955. Amylase (alpha) and (beta). In: *Methods in Enzymology*, Vol 1 (Eds. S.P. Colowick and N.O. Kaplan), Academic Press, New York, pp. 149-150.

Bier, M. 1955. Lipases. In: *Methods in Enzymology*, Vol 1 (Eds. S.P. Colowick and N.O. Kaplan), Academic Press, New York, pp. 627-642.

BIS. 2014. IS 16150: Part 2 2014 *Fish Feed Specifications*. *Part 2. Catfish Feed*. Bureau of Indian Standards, New Delhi, India.

Biswas, P., Jena, A.K., Patel, A.B. and Pandey, P.K. 2019. Dietary protein requirement of Indian Butter catfish, *Ompok bimaculatus* (Bloch) fingerlings. *Journal of Applied Aquaculture*, 32: 1-17.

Castell, J.D. and Tiews, K. 1980. *Report of the EIFAC, IUNS and ICES Working Group on Standardization of Methodology in Fish Nutrition Research*. EIFAC/T36, European Inland Fisheries Advisory / Food and Agriculture Organisation, Hamburg, Germany, p. 24.

Chakrabarti, P.P., Chakraborty, N.M. and Mondal, S.C. 2009. Breeding and seed production of butter catfish, *Ompok pabda* (Siluridae) at Kalyani centre of CIFA, India. *Aquaculture Asia,* 14: 33-25.

Choi, J., Aminikhoei, Z., Kim, Y-O. and Lee, S.M. 2015. Effect of dietary protein and lipid levels on growth and body composition of juvenile Fancy Carp, *Cyprinus carpio* var. Koi. *International Journal of Biological, Biomolecular, Agricultural, Food and Biotechnological Engineering*, 9: 31-37.

Dabrowski, K. 1977. Protein requirements of grass carp fry (*Ctenopharyngodon idella* Val.). *Aquaculture,* 12: 63-73.

Debnath, C., Dube, K., Saharan, N., Tiwari, V.K., Datta, M., Sahoo, L., Yadav, G.S. and Das, P. 2016. Growth and production of endangered Indian Butter catfish, *Ompok bimaculatus* (Bloch) at different stocking densities in earthen ponds. *Aquaculture Research*, 47: 3265-3275.

Deng, J., Mai, K., Al, Q., Zhang, W., Tan, B., Xu, W. and Liufu, Z. 2010. Alternative protein sources in diets for Japanese flounder *Paralichthys olivaceus* (Temminck and Schlegel): II. Effects on nutrient digestibility and digestive enzyme activity. *Aquaculture Research*, 41: 861-870.

Dey, A., Ghosh, K. and Hazra, N. 2018. Effects of probiotics-encapsulated live feed on growth and survival of juvenile *Clarias batrachus* (Linnaeus, 1758) after differential exposure to pathogenic bacteria. *SAARC Journal of Agriculture*, 16: 105-113.

Erlanger, B.F., Kokowsky, N. and Cohen, W. 1961. The preparation and properties of two chromogenic substances of trypsin. *Archives of Biochemistry and Biophysics,* 95: 271-278.

Gangadhara, B., Nandeesha, M.C., Varghese, T.J. and Keshavanth, P. 1997. Effect of varying protein and lipid levels on the growth of rohu, *Labeo rohita*. *Asian Fisheries Sciences*, 10: 139-147.

Giri, S.S., Sahoo, S.K., Paul, B.N., Mohanty, S.N. and Sahu, A.K. 2011. Effect of dietary protein levels on growth, feed utilization and carcass composition of endangered bagrid catfish *Horabagrus brachysoma* (Gunther 1864) fingerlings. *Aquaculture Nutrition*, 17: 332-337.

IUCN, 2014. *IUCN Red List of Threatened Species*. Version, 2014.3. www.iucnredlist.org

Jayaram, K.C. 1977. Aid to identification of siluroid fishes of India, Burma, Sri Lanka, Pakistan and Bangladesh. I. Bagridae. *Records of Zoological Survey of India, Miscellaneous Publications, Occasional Paper*, 8: 1-41.

Lakra, W.S., Sarkar, U.K., Gopalakrishnan, A. and Pandian, A.K. 2010. *Threatened Freshwater Fishes of India*. National Bureau of Fish Genetic Resources, Lucknow, India, pp. 1-20.

Lemieux, H., Blier, P. and Dutil, J.D. 1999. Do digestive enzymes set a physiological limit on growth rate and food conversion efficiency in the Atlantic cod (*Gadus morhua*)? *Fish Physiology and Biochemistry*, 20: 293-303.

Lowry, O.H., Ronebrough, N.J., Farr, A.L. and Randell, R.J. 1951. Protein measurement with Folin phenol reagent. *Journal of Biological Chemistry,* 193: 265-276.

Mandal, S. and Ghosh, K. 2018. Utilization of fermented Pistia leaves in the diet of Rohu, *Labeo rohita* (Hamilton): Effects on growth, digestibility and whole body composition. *Waste and Biomass Valorization*, 10: 3331-3342.

Marham, R. 1955. Nucleic acids, their components and related compounds. In: *Modern Methods of Plant Analysis,* Vol. 4 (Eds. K. Paech and M.V. Tracey), Springer-Verlag, Berlin. Germany, pp. 264-350.

Mitra, G. and Mukhopadhyay, P.K. 2002. Growth, nutrient utilization and tissue biochemical changes in rohu, *Labeo rohita* fed with natural and prepared diets. *Journal of Applied Aquaculture*, 12: 65–78.

Odedeyi, D.O. and Fagbenro, O.A. 2010. Feeding habits and digestive enzymes in the gut of *Mormyrus rume* (Valenciennes 18460 (*Osteichthtes, Mormyridae*). *Tropical Zoology*, 23: 75-89.

Parra, A.M., Rosas, A., Lazo, J.P. and Viana, M.T. 2007. Partial characterization of the digestive enzymes of Pacific bluefin tuna *Thunnus orientalis* under culture conditions. *Fish Physiology and Biochemistry*, 33: 223-231.

Paul, B.N., Adhkari, S. and Ayyappan, S. 2000. Effect of feeding frequency on the growth of *Labeo rohita* juvenile and on water quality in flow-through system. *Indian Journal of Animal Nutrition*, 17: 64-66.

Paul, B.N. and Giri, S.S. 2016. Carp and Catfish. In: *Animal Feeding - Concepts and Practices* (Eds. M.P.S. Bakshi and M. Wadhwa). Satish Serial Publishing House, New Delhi, India, pp. 377-403.

Paul, B.N., Bhowmick, S., Chanda, S., Sridhar, N. and Giri, S.S. 2018. Nutrient profile of five freshwater fish species. *SAARC Journal of Agriculture*, 16: 25-41.

Paul, B.N., Das, S., Datta, A.K., Giri, S.S. and Mohanty, S.N. 2012. Effect of dietary protein levels on growth of *Ompok pabda* (Siluridae) Fry. *Animal Nutrition and Feed Technology*, 12: 241-246.

Paul, B.N., Nandi, S., Sarkar, S. and Mukhopadhyay, P.K. 1997. Effects of feeding unconventional animal protein sources on the nitrogen metabolism in *Labeo rohita* (Hamilton). *Israeli Journal of Aquaculture-Bamidgeh*, 49: 183-192.

Reitman, S. and Frankel, S. 1957. A colorimetric method for the determination of serum glutamic α-oxaloacetic and glutamic pyruvic transaminases. *American Journal of Clinical Pathology*, 28: 56-63.

Sabapathy, U. And Teo, L.H. 1993. A quantitative study of some digestive enzymes in the rabbit fish, *Siganus canaliculatus* and the sea bass, *Lates calcarifer*. *Journal of Fish Biology*, 42: 595-602.

Santiago, C.B., Camacho, A.S. and Laron, M.A. 1991. Growth and reproductive performance of bighead carp (*Aristichthys nobilis*) reared with or without feeding in floating cages. *Aquaculture*, 96: 109-117.

Singh, B.N. and Bhanot K.K. 1988. Protein requirement of the fry of *Catla catla* (Ham.). In: *Proceedings of the First Indian Fisheries Forum* (Ed. M.M. Joseph), Asian Fisheries Society, Indian Branch, Mangalore, India, pp. 77-78.

Snedecor, G.W. and Cochran, W.G. 1994. *Statistical Methods,* 8th ed. Oxford and IBH Publishing Co. Pvt. Ltd., New Delhi, India.

Suarez, M.D., Hidalgo, M.C., Garcia, M., Sanz, A. and Higuera, M. 1995. Influence of the relative proportions of the energy yielding nutrients on the liver intermediary metabolism of the European eel. *Comparative Biochemistry and Physiology Part A: Physiology*, 111: 421-428.

Walter, H.E. 1984. *Methods of Enzymatic Analysis*. Verlag Chemie, Weinheim, Germany.

Ye, C., Wu, Y., Sun, Z. and A. Wang. 2016. Dietary protein requirement of juvenile obscure puffer, *Takifugu obscurus*. *Aquaculture Research,* 48: 2064-2073.

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PENSOFT

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Effect of dietary lipid levels on growth, body composition, and enzyme activities of larvae of butter catfish, *Ompok bimaculatus* (Actinopterygii: Siluriformes: Siluridae)

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Abstract

The Indian butter catfish, *Ompok bimaculatus* (Bloch, 1794), is a high-value catfish that has gained immense consumer preference in South-East Asia. However, information on the nutritional requirements of this species is scanty. Hence, an experiment was conducted to evaluate the effects of varying dietary lipid levels on growth, body composition, and activities of digestive and metabolic enzymes in larvae. Three isonitrogenous (40% crude protein) diets were formulated by supplementing fish and vegetable oil (1:1) at 4.5% (D1), 7% (D2), and 9.5% (D3) levels (containing crude lipid 5.7%, 8.0%, and 10.45%, respectively in diets D1–D3) to a fish meal- and oilcake-based formulated diet. Experimental diets were fed to butter catfish larvae (0.15 ± 0.01 g) in triplicate groups for a period of 42 days. Proximate compositions of the experimental diets, as well as fish carcass, were analyzed using standard procedures (AOAC 2005). Digestive and metabolic enzyme activities were analyzed at the completion of the experiment by standard methodology. Butter catfish larvae fed the diet D2 (8% crude lipid) resulted in the best performance in terms of weight gain (final weight 1.40 ± 0.07 g), net weight gain (1.31 ± 0.06 g), specific growth rate (5.50 ± 0.05% · day^{-1}), and protein efficiency ratio (2.39 ± 0.17). The highest lipid deposition (2.90 ± 0.12%) in the carcass was also recorded in fish reared on diet D2. The final weight, net weight gain, protein efficiency ratio, and specific growth rate were significantly ($P < 0.05$) higher in D2 having 8% lipid. Moisture and lipid contents of the whole body were significantly ($P < 0.05$) higher in larvae fed diet D2. Amylase activity in fish significantly ($P < 0.05$) decreased with increasing dietary lipid levels. The maximum alkaline protease, pepsin, and lipase activities were noticed in the larvae fed diet D2. Progressive decrease in liver glucose-6-phosphate dehydrogenase activities and significant increase ($P < 0.05$) in the activities of neoglucogenic enzymes (glucose-6-phosphatase and fructose-1,6-bis phosphatase) were noticed with an increase in dietary lipid levels. Significantly lower ($P < 0.05$) activities of LDH, ALT, and AST were recorded in the group fed diet D2. Results of the study indicated that 8% crude lipid in the diet could assure optimum growth and survival of butter catfish larvae during early development. An appraisal on growth, body composition, and digestive as well as metabolic function in the butter catfish larvae recorded in the study might provide some important information to consider application of formulated diets for the larviculture of *Ompok bimaculatus*.

Keywords

lipid, larvae, *Ompok bimaculatus*, growth, lipase, metabolic enzyme

Introduction

The Indian butter catfish, *Ompok bimaculatus* (Bloch, 1794), is indigenous to the South East Asian countries (Giri et al. 2019) and has recently gained immense importance because of its good taste, high lipoprotein, low fat, soft bony structure, and competitive prices (Rawat et al. 2018). It is an excellent source of ω-3 and ω-6 fatty acids, vitamins, minerals, protein, and fat (Paul et al. 2018). The wild population of *O. bimaculatus* has sharply declined due to ecological changes and indiscriminate fishing. Thus, the species has been categorized under the "near threatened" category by the IUCN Red List and faces a risk of extinction in nature (Lakra et al. 2010; IUCN 2014). Considering high demand, price, and IUCN status, the species has been prioritized for diversification of aquaculture as well as for conservation and restocking programs (Debnath et al. 2016). Although its aquaculture potential has been realized, the species has not yet received adequate attention due to insufficient information on larval rearing and culture technology.

The successful culture of any fish species largely depends on the accessibility of nutritionally balanced practical diets. Although species of the genus *Ompok* have been generally recognized as carnivorous to omnivorous, reports on nutritional requirements of the species are scanty (Chakrabarti et al. 2012). Therefore, no commercially formulated diet has yet been available for this species. Since captive breeding of *O. bimaculatus* has been established (Raizada et al. 2013), it is necessary to develop larval diets to ensure growth and survivability of the species during the stages of early development, which is essential for reliable and regular supply of the fish for widespread commercial production. A previous study conducted on *O. bimaculatus* larvae determined a required level of 40% crude protein in the diets for this species (Paul et al. 2020).

However, dietary protein requirements are known to be affected by the amount of non-protein energy sources in the diet (NRC 2011). When non-protein energy is insufficient, a part of dietary protein may be catabolized to supply energy affecting the growth of the organism. Therefore, supplementation of energy-yielding nutrients, mainly lipid has been suggested as a strategy to improve protein utilization in fish (Sankian et al. 2017). Supplementation of lipid rather than carbohydrate as a source of non-protein energy is generally more effective for enhancing dietary energy level as lipid is an energy-dense nutrient that is readily metabolized by fish, particularly the carnivorous one (NRC 1993). Further, all-round development and well being of fish are known to be greatly influenced by dietary lipids that are not only important as an energy source but also for the supply of essential fatty acids as well as carrier of fat-soluble vitamins (Glencross 2009). Moreover, the incorporation of a proper amount of lipid seems to be important as lipid level determines the palatability of the diet (Boonyaratpalin 1991). Therefore, the presently reported study was conducted to determine the effects of dietary lipid levels on the growth, survivability, body composition, and activities of digestive as well as metabolic enzymes in butter catfish larvae.

Vegetable and fish oils are rich in different fatty acids, which were recognized as effective for diverse freshwater fish species (Paul et al. 2011). Hence, in the presently reported study, a practical diet was fortified with a combination of vegetable and fish oils (1:1) to have the desired lipid levels in the diets. The nutrient utilization and digestive physiology in fish are indicated by the activity of digestive and metabolic enzymes that ultimately affect the growth and development of fish (Chen and Zhang 2004; Wei et al. 2010). Therefore, the presently reported study considered an appraisal of digestive enzymes and some key metabolic enzymes to evaluate the effects of formulated diets with varying lipid levels. The results of the study could be helpful to provide some important information for feed formulation of *O. bimaculatus* larvae.

Materials and methods

Experimental diets

Three experimental diets were formulated by incorporating equal proportions of fish oil (cod liver oil) and vegetable oil (sunflower oil) (1:1) at 4.5% (D1), 7% (D2), and 9.5% (D3) levels to a basal mixture of fish meal (**FM**), soybean meal (**SBM**), and groundnut oil cake (**GNOC**). After analysis of lipid content of the feed, it was noticed to contain 5.7%, 8.0%, and 10.5% crude lipids, respectively. The amount of lipid sources used was adjusted at the expense of wheat flour. A vitamin–mineral premix was added to the diets as per Paul et al. (1997). Dietary ingredients were finely powdered, sieved to obtain uniform particle size (<400 μm in diameter), mixed thoroughly, and fortified with a calculated amount of vitamin-mineral premix and oil sources. The prepared powered feeds were stored in a freezer at –20°C until use.

Experimental fish and feeding trial

The experiment was conducted at the Regional Research Centre of ICAR-Central Institute of Freshwater Aquaculture, Rahara, Kolkata. Farm-raised larvae of the butter catfish were collected from ICAR-Central Institute of Freshwater Aquaculture, Kalyani Field Station, and acclimatized to the laboratory condition for one week in fiber-reinforced plastic (**FRP**) tanks with the provision of continuous aeration. During this period the larvae were fed a basal formulated diet and natural food (mixed zooplankton and chopped tubifex). After acclimatization, the larvae (mean weight 0.15 ± 0.02 g; length 22.65 ± 1.70 mm; 14 days old) were randomly distributed in 9 FRP tanks at a stocking density of 50 fish per tank. Thus, there were three replicates for each dietary group. The experiment was conducted in 150 L FRP tanks, each

containing 50 L of water, with continuous aeration and water exchange at every 5 days interval. The powdered feed mixtures were made to soft dough with distilled water and the fish were fed ad libitum to apparent satiation twice daily, at 10.00 and 16.00 h, for 42 days. Feed consumption and mortality in each tank were recorded separately, and the survival rate was calculated. During the experimental period, water quality parameters were monitored on weekly basis following the standard methods of the American Public Health Association (APHA 2005) and noticed to vary within the acceptable range (temperature 28–30°C; pH 6.8–7.7, dissolved oxygen 6.8–7.4 mg · L^{-1}, total alkalinity 230–240 mg · L^{-1}, ammonia 0.26–0.64 mg · L^{-1}, nitrite 0.001–0.003 mg · L^{-1}, nitrate 0.002–0.074 mg · L^{-1}).

Proximate composition of experimental diets and fish carcass

Proximate compositions of the experimental diets, as well as fish carcass, were analyzed using standard procedures portrayed by the Association of Official Analytical Chemists (AOAC 2005). Moisture content was determined by oven drying (initially at 100 ± 5°C for 30 min, thereafter at 60°C); crude protein (Nitrogen × 6.25), by a semi-automatic digestion system together with micro Kjeldahl distillation Unit (KelPlus-Elite Ex, Pelican Equipments, Chennai, India); crude lipid (ether extract; petroleum ether, 60–80°C), by a Soxhlet apparatus (Socsplus, Pelican Equipments, Chennai, India); and ash, by combustion at 550°C in a muffle furnace. Nitrogen-free extract (**NFE**) was calculated by subtracting the sum of values for crude protein, crude lipid, ash, crude fiber, and moisture from 100 (Maynard et al. 1979). The gross energy of the diets was measured with a bomb calorimeter (Lab-X, Kolkata, India). Proximate analyses of the fish carcass (whole body) were done on wet weight basis.

Growth parameters

At the end of the feeding trial fish were collected from each tank, weighed, and analyzed for calculating the growth parameters. Net weight gain [%], specific growth rate (**SGR** [% · day^{-1}]), feed conversion ratio (**FCR**), protein efficiency ratio (**PER**), apparent net protein utilization (**ANPU**), and survivability [%] were calculated following standard methods described by Castell and Tiews (1980). The daily growth coefficient (**DGC**) was calculated as per Cowey (1992).

Estimation of digestive enzymes

Digestive enzymes (amylase, alkaline protease, pepsin, and lipase) of fish from each experimental set were estimated at the termination of the experiment. For each replicate, digestive tracts of 20 experimental fish from each tank were dissected out, washed thoroughly with chilled distilled water, taken on an ice-cooled Petri plate, and weighed. A 10% homogenate with chilled 0.1 (M) phosphate buffer (pH 7) was prepared and centrifuged at 10 000 rpm (10 min, 4°C). The ensuing supernatant was used as the enzyme extract to appraise the activities of the digestive enzymes. The protein content of the extract was estimated after Lowry et al. (1951) using bovine serum albumin (**BSA**) as standard. Amylase (α-amylase) activity was determined using dinitro salicylic acid (**DNSA**) reagent following Bernfeld (1955). Amylase activity (unit) was expressed as mg maltose liberated mg^{-1} protein h^{-1}. Alkaline protease activity was estimated using Hammerstein casein substrate according to Walter (1984). One unit of enzyme activity was defined as µg of tyrosine liberated mg^{-1} protein h^{-1}. Pepsin activity was resolved after Anson (1938) with minor modifications, using 2% hemoglobin as a substrate. The specific activity was expressed as µg of tyrosine liberated mg^{-1} protein min^{-1}. Lipase activity was determined with the olive oil substrate following Bier (1955). Lipase activity was expressed as µ mole of fatty acid liberated mg^{-1} protein h^{-1}.

Estimation of metabolic enzymes

Following the collection of the digestive tracts, hepatic tissues were removed, collected separately and a 10% homogenate was made in sucrose solution (0.25 M, pH 7.4). Remains of the cell along with nuclei were removed by centrifugation (1000 g, 30 min, 4°C), and the supernatants were further centrifuged (10 000 g, 15 min, 4°C) to get the mitochondrial pellets (Biswas et al. 2006). The resultant supernatant was again centrifuged (12 500 g, 1 h, 4°C) and the cytosolic fraction thus obtained was used as the crude enzyme extract for other metabolic enzyme assays. The mitochondrial pellet was treated with triton X-100 (0.1%), washed with PBS (0.1 M, pH 7.4) and the supernatant was used as crude extracts for mitochondrial metabolic enzyme assays. The tissue fractions were kept at –20°C until use. The soluble protein content of the crude enzyme extracts was determined following Lowry et al. (1951).

Hexokinase (HK) activity was measured by the reduction of NADP to produce NADPH according to Tranulis et al. (1996). Enzyme activity was expressed as µM of NADPH formed mg^{-1} protein h^{-1}. Pyruvate kinase (PK) activity was assayed after Driedzic and Almeida-Val (1996) with minor modification. Enzyme activity was presented as µmole of pyruvate converted to NADH mg^{-1} protein min^{-1}. Glucose-6-phosphatase (G6P) and fructose-1, 6-bis phosphatase (FBP) activities were measured by estimating the amount of phosphorus released from the substrates, glucose-6-phosphate (Marjorie 1964) and fructose-di-phosphate (Freeland and Harper 1959), respectively. Release of phosphorus by both the enzymes was estimated after Fiske and Subbarow (1925), and activities were expressed as µg of phosphorus released mg^{-1} protein min^{-1}.

Glucose-6-phosphate dehydrogenase (G6PD) activity was analyzed using glucose-6-phosphate (substrate) and NADP following Kornberg and Horecker (1955). Enzyme activity was expressed as µM of NADPH formed mg^{-1} protein h^{-1}. NADP-malic enzyme (NADP-ME) activity was determined using L-malic acid as substrate (Hsu and Lardy 1967, modified by Murphy and Walker 1974). Enzyme activity was presented as µM of NADPH formed mg^{-1} protein h^{-1}. Lipid peroxidation (LPO) activity was measured according to Okhawa et al. (1979). Enzyme activity was expressed as thiobarbituric acid reactive substance (TBARS) formed mg^{-1} protein min^{-1}.

Alanine transaminase (ALT) activity was determined using α-ketoglutarate and DL-Alanine as substrates (Reitman and Frankel 1957). ALT activity was expressed as µM of pyruvate formed mg^{-1} protein min^{-1}. Likewise, Aspartate transaminase (AST) activity was measured with the substrate solution containing α-ketoglutarate and DL-aspartic acid (Reitman and Frankel 1957). AST activity was expressed as µM of oxaloacetate formed mg^{-1} protein min^{-1}. Glutamate dehydrogenase (GDH) activity of the crude mitochondrial enzyme extract was measured using sodium glutamate and tetrazolium salt (Lee and Lardy 1965). Enzyme activity was expressed as µM of formazan formed mg^{-1} protein h^{-1}.

Statistical analysis

The data were analyzed by one-way analysis of variance (ANOVA) as per Snedecor and Cochran (1994) to calculate the effect of dietary lipid level on growth performance and activities of the digestive as well as metabolic enzyme of fish and the least significance (LSD) was used for comparison of the mean values. Data are presented as treatment mean ± standard error of the mean (SE).

Results

The ingredients and proximate composition of the experimental diets are presented in Table 1. Experimental diets were isoproteinous (crude protein ≈ 40%). However, supplementation of fish oil and vegetable oil has led to varying crude lipid levels in the diets (D1–D3) as 5.7%, 8.0%, and 10.45%, respectively. All experimental diets were readily accepted by the *O. bimaculatus* larvae.

The growth performance of *O. bimaculatus* larvae fed varying levels of dietary lipid for 42 days is depicted in Table 2. The growth of the larvae was significantly ($P < 0.05$) affected by the dietary crude lipid levels. The net weight gain (%) of the larvae showed an increasing trend with increasing levels of the dietary lipid up to 8% and thereafter decreased. Butter catfish larvae fed diet D2 containing 8% crude lipid had the highest weight gain, which was significantly different ($P < 0.05$) from other dietary lipid levels. The highest values of PER and ANPU were recorded in fish fed diet D2. The value of FCR was the lowest for fish fed diet D2, however, didn't differ significantly between the diets D1 and D3. Butter catfish larvae in all treatment groups survived well (more than 80%) during the experimental period, and there were no significant differences among the groups.

Proximate carcass compositions of the butter catfish larvae fed experimental diets are presented in Table 3.

Table 1. Feed formulation and proximate composition (% DM Basis) of the experimental diets.

Parameter	Experimental diet		
	D1	D2	D3
Fish meal	53.00	53.0	53.00
Groundnut oil cake	15.00	15.0	15.00
Soybean meal	10.00	10.0	10.00
Wheat flour	10.50	8.0	5.50
Carboxy methyl cellulose	2.00	2.0	2.00
Fish:Veg. oil (1:1)	4.50	7.0	9.50
Vitamin-mineral mix*	5.00	5.0	5.00
Proximate composition [% DM basis]			
Dry matter	92.85 ± 0.06	92.37 ± 0.23	92.06 ± 0.05
Crude protein	40.46 ± 0.06	40.18 ± 0.49	40.61 ± 0.83
Crude lipid	5.70 ± 0.20	8.00 ± 0.25	10.45 ± 0.45
Total Ash	14.40 ± 0.30	15.40 ± 0.20	16.50 ± 0.30
Nitrogen free extracts	29.50 ± 0.37	27.61 ± 2.05	21.80 ± 0.28
Crude protein:crude fat	7:1	5:1	4:1
Energy [kJ g^{-1}]	13.85 ± 0.02	14.09 ± 0.08	14.39 ± 0.08

*Vitamin-mineral premix contains: Vitamin A (as acetate) 5000 I.U., cholecalciferol 1000 I.U., thiamine monocitrate 10 mg, riboflavin 10 mg, pyridoxine hydrochloride 5 mg, cyanocobalamine 15 µg, nicotinamide 75 mg, calcium pantothenate 10 mg, ascorbic acid 150 mg, α-tocopheryl acetate 25 mg, biotin 5 mg, folic acid 5 mg, menadione 100 mg, choline chloride 50 mg, PABA 5 mg, myoinositol 10 mg, calcium lactate 0.125, magnesium oxide 60 mg, dried ferrous sulphate 30 mg, manganese sulphate 2 mg, copper sulphate 2 mg, zinc sulphate 2 mg, sodium molybdate 0.25 mg, sodium bortate 0.80 mg, potassium iodate 20 mg, bicalcium phosphate 0.10 g, cobalt chloride 20 mg (Paul et al. 1997).

Table 2. Growth performance in *Ompok bimaculatus* larvae fed with graded levels of lipid.

Parameter	Experimental diet		
	D1	D2	D3
Initial weight [g]	0.15 ± 0.02	0.14 ± 0.01	0.15 ± 0.01
Final weight [g]	1.10 ± 0.12^{a}	1.40 ± 0.07^{b}	1.06 ± 0.03^{a}
Net weight gain	0.95 ± 0.12^{a}	1.31 ± 0.06^{b}	0.91 ± 0.03^{a}
Specific growth rate [%]	4.73 ± 0.35^{a}	5.50 ± 0.05^{b}	4.66 ± 0.22^{a}
Daily growth coefficient	0.73 ± 0.02^{a}	1.003 ± 0.05^{b}	0.76 ± 0.09^{a}
Survivability	83.85 ± 6.15	83.85 ± 6.15	79.55 ± 5.46
Number of dead fish	25	25	31
Feed conversion ratio	1.86 ± 0.10^{b}	1.39 ± 0.05^{a}	1.74 ± 0.07^{b}
Protein efficiency ratio	1.31 ± 0.09^{a}	2.39 ± 0.17^{b}	1.30 ± 0.08^{a}
Apparent net protein utilization	16.09 ± 0.92^{a}	23.19 ± 1.10^{b}	17.18 ± 0.82^{a}

Data are presented as mean ± standard error of the mean; mean values with different superscripts in a row differ significantly ($P < 0.05$).

Table 3. Carcass composition [g · 100 g^{-1}] of *O. bimaculatus* larvae fed different levels of lipid.

Constituent [g · 100 g^{-1}]	Experimental diet		
	D1	D2	D3
Moisture	79.37 ± 0.09^{a}	80.93 ± 0.22^{b}	79.80 ± 0.17^{a}
Crude protein	13.93 ± 0.09	14.40 ± 0.21	14.03 ± 0.08
Crude lipid	2.50 ± 0.06^{a}	2.90 ± 0.12^{b}	2.77 ± 0.07^{b}
Ash	1.70 ± 0.06	1.97 ± 0.09	1.80 ± 0.06

Data are presented as mean ± standard error of the mean; mean values with different superscripts in a row differ significantly ($P < 0.05$).

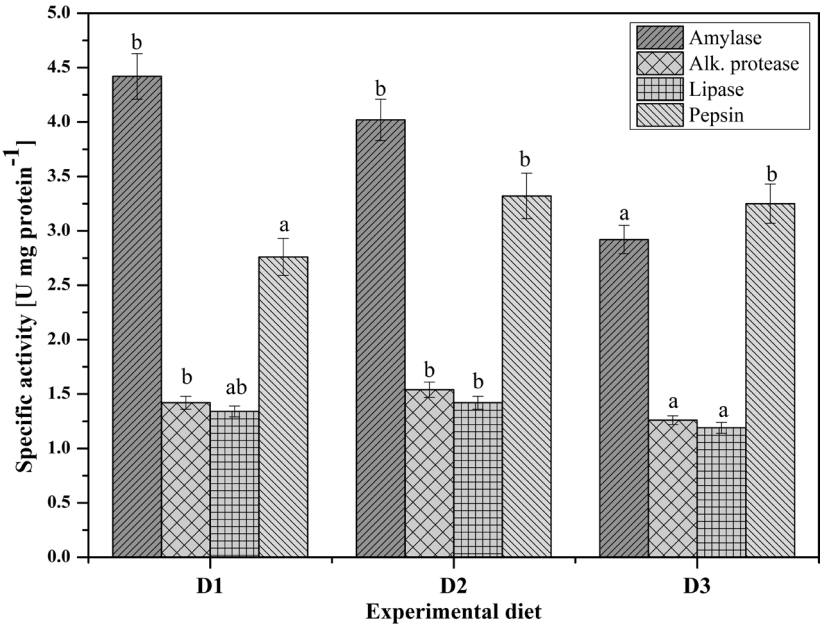

Figure 1. Specific activity of digestive enzymes of *Ompok bimaculatus* larvae fed varying levels of crude lipid. Mean values (± SE) with no common superscript letters are significantly different ($P < 0.05$).

Moisture and lipid contents of the whole body were significantly ($P < 0.05$) affected by the dietary lipid levels, being the highest in larvae fed diet D2 containing 8% crude lipid. However, varying dietary lipid had no significant effect on crude protein and ash contents of *O. bimaculatus* larvae at the tested lipid levels.

Digestive enzymes i.e., amylase, alkaline protease, lipase, and pepsin of the butter catfish larvae fed diets with varied lipid levels are given in Fig. 1. Overall, digestive enzymes were significantly ($P < 0.05$) affected by the dietary lipid levels. Amylase activity in *O. bimaculatus* larvae significantly ($P < 0.05$) decreased with increasing dietary lipid levels. The highest alkaline protease and lipase activities were noticed in the butter catfish larvae fed diet D2 consisting of 8% crude lipid, while it was not significantly ($P < 0.05$) different from the group fed diet D1. The highest pepsin activity was also documented in the fish fed diet D2, although it did not differ significantly from the larvae that received diet D3 with 10.45% dietary lipid.

Activities of the hepatic enzymes involved in the intermediary metabolism of carbohydrate, protein, and lipid are depicted in Table 4. Varying dietary lipid levels led to significant differences ($P < 0.05$) in the activities of PK, G6P, FBP, G6PD, LDH, ALT, and AST in *O. bimaculatus* larvae. While no significant differences were detected in the activities of HK, NADP-ME, GDH, and LPO, the activities of PK and two major neoglucogenic enzymes, G6P and FBP, significantly increased ($P < 0.05$) with the increase in the dietary lipid levels. The activity of G6PD, a key enzyme of lipogenesis, revealed a significant decrease ($P < 0.05$) with an increase in the dietary lipid level from 5.7% (D1) to 8% (D2). Further, significantly lower ($P < 0.05$) activities of LDH, ALT, and AST were recorded in *O. bimaculatus* larvae fed diet D2 with 8% dietary lipid.

Table 4. Specific activity [U mg protein^{-1}] of metabolic enzymes of *Ompok bimaculatus* larvae fed varying levels of lipid.

Enzyme	Experimental diet		
	D1	D2	D3
Hexokinase	9.82 ± 0.35	10.27 ± 0.47	10.54 ± 0.51
Pyruvate kinase	5.6 ± 0.24^{a}	6.2 ± 0.27^{b}	6.4 ± 0.29^{b}
Lactate dehydrogenase	0.845 ± 0.03^{c}	0.507 ± 0.02^{a}	0.690 ± 0.03^{b}
Malate dehydrogenase	2.35 ± 0.11	2.24 ± 0.09	2.20 ± 0.11
Glucose 6 phosphatase	4.05 ± 0.13^{a}	4.38 ± 0.17^{ab}	4.62 ± 0.22^{b}
Fructose 1,6 bis phosphatase	3.10 ± 0.11^{a}	3.42 ± 0.14^{ab}	3.72 ± 0.15^{b}
Alanine aminotransferase	3.88 ± 0.16^{b}	3.55 ± 0.09^{a}	3.78 ± 0.12^{b}
Aspartate aminotransferase	6.55 ± 0.17^{b}	6.15 ± 0.12^{a}	6.45 ± 0.14^{b}
Glutamate dehydrogenase	5.12 ± 0.20	5.20 ± 0.23	5.28 ± 0.27
Glucose-6-phosphate dehydrogenase	32.6 ± 0.81^{b}	27.5 ± 0.76^{a}	26.4 ± 0.72^{a}
Lipid peroxidation	0.92 ± 0.06	0.96 ± 0.004	1.02 ± 0.006

Data are presented as mean ± standard error of the mean; mean values with different superscripts in a row differ significantly ($P < 0.05$).

Discussion

During the experimental rearing of the *O. bimaculatus* larvae, water temperature varied within a narrow range (28–30°C) that was considered suitable since a temperature of around 30°C was suggested as optimum for the growth of catfish (Paul and Giri 2016). Other water quality parameters were also within the acceptable range as recommended elsewhere (Paul et al. 2000; Debnath et al. 2016). Apart from environmental factors, rearing of early larval stages under captive condition depends mostly on the availability of suitable diets that are readily acceptable and consists of nutrients at the required level to support growth and well being of the fish. Different larval stages of fish may have specific nutritional requirements (Malla and Banik 2015). Digestive systems of fish larvae are immature and therefore they depend on live food organisms to a great extent for the supply of exogenous

enzymes. Generally, fish larvae do not prefer artificial diets, even if larviculture with formulated diets is essential for large-scale production of any species. The limited success of the dry formulated diets in larval rearing might be attributed to insufficient feed intake, imbalanced protein (non-protein energy sources), impaired digestive, as well as metabolic functions (Lee et al. 2002).

In the presently reported study, formulated diets were readily accepted by the 14 day old *O. bimaculatus* larvae. The study suggests that 8% lipid in a diet with 40% crude protein might support the growth and survivability of the butter catfish larvae during early development. The required lipid level detected in the presently reported study was close to the suggested lipid levels documented for other catfishes. For example, 6.5% and 7% optimum dietary lipid requirements were reported for *Ompok pabda* (Hamilton, 1822) fry (Paul et al. 2011) and *Mystus montanus* (Jerdon, 1849) (see Raj et al. 2007), respectively. Among carps, 6.5% lipid in the diets of *Ctenopharyngodon idella* (Valenciennes, 1844) (see Jin et al. 2013) and 7% lipid for the juveniles of common carp, *Cyprinus carpio* Linnaeus, 1758 (see Choi et al. 2015) supported maximum growth. On the contrary, elevated lipid requirements have also been suggested. For example, lipid levels of 10% for larvae of magur, *Clarias batrachus* (Linnaeus, 1758) (see BIS 2014b) and 17% for far eastern catfish, *Silurus asotus* Linnaeus, 1758 (see Kim et al. 2012) were reported. Therefore, the majority of the preceding studies suggested varying lipid requirement levels between 6% and 10% in diverse fish species, with few exceptions. Hence, the presently reported study considered this narrow level of variation for evaluation of the lipid levels. The ability of the fish to use lipid as a source of energy was noticed to vary among diverse fish species (Jauncey (1982)). Thus, different fish species at different life stages might require different dietary lipid levels and it needs to be evaluated separately for individual fish species. Our results were in agreement with some of the previous reports depicting 8% lipid requirement as optimal for a minor carp, *Barbonymus gonionotus* (Bleeker, 1849) (see Paul et al. 2010) and fingerlings of rohu, *Labeo rohita* (see Mishra and Samantaray 2004). BIS (2014a) also suggested an 8% crude lipid requirement for carp spawn and fry.

The presently reported study revealed that an increase in the dietary lipid level from 5.7% to 8% was associated with maximum growth and increased SGR [% · day−1] of the butter catfish larvae. Similarly, the lowest FCR and the maximum PER and ANPU values were recorded in the larvae fed diets with 8% crude lipid (D2). Our result was in compliance with the preceding reports indicating that increase in the dietary lipid up to a certain level might aid in efficient protein utilization that results in improved growth of the fish (Jauncey 1982; Kim et al. 2012). Similar results were recorded for the stinging catfish, *Heteropneustes fossilis* (Bloch, 1794) (see Akand et al. 1991) and rohu, *L. rohita* (see Mishra and Samantaray 2004). In contrast to these observations, high dietary lipid might cause to reduce fish growth, as documented for gibel carp, *Carassius gibelio* (Bloch, 1782); and Chinese long snout catfish, *Leiocassis longirostris* Günther, 1864 (see Pei et al. 2004). In the presently reported study, the group of larvae fed diet D3 with 10.45% crude lipid was associated with poor growth, which was in agreement with Pei et al (2004). When the non-protein energy source in the diet becomes insufficient or inaccessible, the protein is used as a source of energy instead of growth (Mohanta et al. 2009). In the presently reported study, the groups reared with diets D1 (5.7% lipid) and D3 (10.45% lipid) portrayed relatively poor growth that might be indicative of poor utilization of the non-protein energy source (Wang et al. 2018). Further, in the presently reported study, around 80% survivability of the butter catfish larvae was achieved with the formulated diets during the feeding trial. Previously, 52.18% and 45.82% survivability of the *O. bimaculatus* larvae with egg custards and compound feed was documented by Malla and Banik (2015), which was relatively lower than the presently reported findings. Improved survivability accomplished in the presently reported study could be due to improved feed utilization by the larvae.

An increase in dietary lipid levels seems to be an important consideration for the food fishes as it might have a significant effect on the carcass quality (Cowey 1993). There might be a positive correlation between lipid levels in the diets and carcass lipid deposition (Cowey 1993), which was in harmony with the presently reported study. Similar observations have been recorded in several species, e.g., rockfish, *Sebastes schlegelii* Hilgendorf, 1880 (see Lee et al. 2002); Eurasian perch; *Perca fluviatilis* Linnaeus, 1758 (see Mathis et al. 2003); cobia, *Rachycentron canadum* (Linnaeus, 1766) (see Craig et al. 2006); and grouper, *Epinephelus malabaricus* (Bloch et Schneider, 1801) (see Williams 2007). On the contrary, Paul et al. (2011) could not find any difference in carcass lipid in another species of butter catfish, *O. pabda* by feeding different levels of lipid. In the presently reported study, carcass protein content was not significantly affected by the dietary lipid levels, which was consistent with previous reports on the juveniles of pike perch, *Sander lucioperca* (Linnaeus, 1758) (see Schulz et al. 2008) and cobia (Webb et al. 2010). Overall, the carcass composition of the *O. bimaculatus* larvae detected in the presently reported study was similar to the previous report by Debnath and Sahoo (2013).

Although the ontogeny of the digestive enzymes during the early development of the *O. bimaculatus* has been documented by some authors (Pradhan et al. 2013; Chowdhury et al. 2019), to the authors' knowledge, there is no information on the diet-related changes in the digestive enzymes in the butter catfish. Adaptations of the digestive system in different species exhibit close association with their diet (Fernandez et al. 2001). Thus, changes in digestive enzyme activity could be correlated with the biochemical composition of food and feeding behavior of fish (Kuzmina 1996). In the presently reported study, amylase activity in *O. bimaculatus* larvae was noticed to be significantly decreased with elevated dietary

lipid levels. Previously, amylase activity in gilthead sea bream, *Sparus aurata* Linnaeus, 1758, was noticed to be influenced by dietary lipid levels (Fountoulaki et al. 2005). While, maximum activities of the alkaline protease, pepsin, and lipase were recorded with the group that was fed 8% lipid (D2) and achieved the highest growth. Digestive enzymes might contribute towards efficient digestion of the dietary components, which could be reflected through the growth of the fishes. Thus, increased growth in fish (fed 8% dietary lipid) associated with enhanced digestive enzyme activities might be indicative of better nutrient utilization in fish as stated elsewhere (Mandal and Ghosh 2018).

The presently reported study appraised activities of some major metabolic enzymes to evaluate the effects of the varying dietary lipid levels. Activities of the amino acid catabolizing enzymes were influenced by the dietary lipid levels. The fish liver is the hotspot for transdeamination with ALT and AST as the major enzymes (Enes et al. 2006; Kumar et al. 2008). A decrease in the activities of ALT, AST, and LDH might suggest reduced protein catabolism in fish fed diet D2 with 8% lipid. LDH is the enzyme of the glycolytic pathway that mediates the bidirectional conversion of pyruvate to lactate. A hike in LDH activity could be noticed under stress (Chatterjee et al. 2006). Thus, reduced LDH activity in the fish reared on D2 might indicate no or negligible stress on the experimental fish. In the presently reported study, increased activities of the gluconeogenic enzymes (G6P and FBP) coincided with an increase in the dietary lipid levels. Gluconeogenesis is a major pathway for glucose homeostasis, where glucose is produced from non-carbohydrate precursors (e.g., amino acid, lactate, glycerols). Increased activity of the neoglucogenic enzymes associated with decreased activity of digestive amylase might be indicative of the production of glucose by gluconeogenesis to meet the energy demand in this carnivorous species. No significant variation was noticed in the activity of the major glycolytic enzyme, HK. G6PD is the key enzyme catalyzing the first step of the HMP-shunt (pentose phosphate pathway) that generates NADPH for lipogenesis and stress management (Pandolfi et al. 1995). In the presently reported study, the activity of the lipogenic enzyme (G6PD) was inhibited by an increase in the dietary lipid, which was similar to the observations recorded in juveniles of Senegalese sole, *Solea senegalensis* Kaup, 1858 (see Dias et al. 2004; Guerreiro et al. 2012). Further, NADP-ME, GDH, and LPO activities were more or less unaffected by the dietary lipid levels. NADP-ME is responsible for NADP-dependent oxidative decarboxylation (malate to pyruvate and carbon dioxide) with the generation of NADPH that may be utilized for lipid biosynthesis, while GDH had been considered as a sensitive indicator of stress (Susan et al. 2010). Therefore, the results of the presently reported study might suggest that increased dietary lipid levels are neither required to augment lipid biosynthesis by the fish nor to induced stress on the experimental fish.

Conclusion

Results of the presently reported study indicated that 8% crude lipid in the diet with 40% crude protein might assure optimum growth and survival of *Ompok bimaculatus* larvae during early development. An appraisal on growth, body composition, and digestive as well as metabolic function in the butter catfish larvae recorded in the study might provide some important information to consider the application of formulated diets for the larviculture of *Ompok bimaculatus*.

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References

Akand AM, Hasan MR, Habib MAB (1991) Utilization of carbohydrate and lipid as dietary energy sources by stinging catfish. Pp. 93–100. In: De Silva S (Ed.) Fish nutrition research in Asia, Proceedings of the Fourth Asian Fish Nutrition Workshop, Asian Fisheries Society, Manila, the Philippines.

Anson ML (1938) The estimation of pepsin, trypsin, papain and cathepsin with haemoglobin. Journal of General Physiology 22(1): 79–89. https://doi.org/10.1085/jgp.22.1.79

AOAC (2005) Official methods of analysis of AOAC International (18th edn). Association of Official Analytical Chemists, Gaithersburg, MD, USA.

APHA (2005) Standard methods for examination of water and wastewater (21th edn). American Public Health Association/American Water Works Association/Water Environment Federation, Washington DC, USA.

Bernfeld P (1955) Amylase (α) and (β). Pp. 149–150. In: Colowick SP, Kaplan NO (Eds) Methods in enzymology, Vol. 1. Academic Press, New York, NJ, USA.

Bier M (1955) Lipases. Pp. 627–642. In: Colowick SP, Kaplan NO (Eds) Methods in enzymology, Academic Press, New York, NJ, USA. https://doi.org/10.1016/0076-6879(55)01111-7

BIS (2014a) IS 16150: Fish Feed Specification Part 1: Carp feed. Bureau of Indian Standards, New Delhi, India. https://bis.gov.in

BIS (2014b) IS 16150: Fish Feed Specifications Part 2: Catfish feed. Bureau of Indian Standards, New Delhi, India. https://bis.gov.in

Biswas A, Pramanik M, Kundu S, Roy S, De J, Ray AK (2006) Alterations in enzyme activities in vital organs of triploid female catfish *Heteropneustes fossilis* (Bloch). Fish Physiology and Biochemistry 32(1): 73–81. https://doi.org/10.1007/s10695-006-7657-0

Boonyaratpalin M (1991) Nutritional studies on sea bass (*Lates calcarifer*). Pp. 33–44. In: De Silva SS (Ed.) Fish nutrition research in Asia. Asian Fisheries Society Publication No: 5. Manila, the Philippines.

Castell JD, Tiews K (1980) Report of the EIFAC, IUNS and ICES Working Group on Standardization of Methodology in Fish Nutrition Research. P. 24. EIFAC/T36, EIFA/FAO.

Chakrabarti PP, Mondal SC, Chattopadhaya DN, Mandal RN, Paul BN (2012) Pabda-Seed production and culture. Central Institute of Freshwater Aquaculture, Bhubaneswar, India.

Chatterjee N, Pal AK, Das T, Manush SM, Sarma K, Venkateshwarlu G, Mukherjee SC (2006) Secondary stress response in Indian major carps *Labeo rohita* (Ham), *Catla catla* (Ham) and *Cirrhinus mrigala* (Ham) fry to increasing packing densities. Aquaculture Research 37(5): 472–476. https://doi.org/10.1111/j.1365-2109.2006.01469.x

Chen MY, Zhang XM (2004) [Recent advances in digestive physiology of marine fish larvae-juvenile]. Marine Fisheries Research 25(3): 81–88. [In Chinese]

Choi J, Aminikhoei Z, Kim YO, Lee SM (2015) Effects of dietary protein and lipid levels on growth and body composition of juvenile Fancy carp (*Cyprinus carpio*. Var.) Koi. International Journal of Biological, Biomolecular, agricultural. Food and Biotechnological Engineering 9(1): 31–37.

Chowdhury D, Paul BN, Ghosh K (2019) Profiles of digestive and metabolic enzymes in butter catfish, *Ompok bimaculatus* (Bloch, 1794) during early development. Golden Jubilee International Conference on "Trends in Zoology", 3–4 January 2019, Burdwan, India, 90.

Cowey CB (1992) Nutrition: Estimating requirements of rainbow trout. Aquaculture 100(1–3): 177–189. https://doi.org/10.1016/0044-8486(92)90370-Z

Cowey CB (1993) Some effects of nutrition on flesh quality of cultured fish. P. 227. In: Kaushik SJ, Luquet P (Eds) Fish nutrition in practice. INRA, Paris, France.

Craig SR, Schwarz MH, McLean E (2006) Juvenile cobia (*Rachycentron canadum*) can utilize a wide range of protein and lipid levels without impacts on production characteristics. Aquaculture 261(1): 384–391. https://doi.org/10.1016/j.aquaculture.2006.08.004

Debnath C, Sahoo L (2013) Body composition of *Ompok bimaculatus* (Bloch, 1794) from Tripura waters with respect to body size, condition factor and sex. Fishery Technology 50(4): 354–356.

Debnath C, Dube K, Saharan N, Tiwari VK, Datta M, Sahoo L, Yadav GS, Das P (2016) Growth and production of endangered Indian butter catfish, *Ompok bimaculatus* (Bloch) at different stocking densities in earthen ponds. Aquaculture Research 47(10): 3265–3275. https://doi.org/10.1111/are.12780

Dias J, Rueda-Jasso R, Panserat S, da Conceicao LE, Gomes EF, Dinis MT (2004) Effect of dietary carbohydrate to lipid ratios on growth, lipid deposition and metabolic hepatic enzymes in juvenile Senegalese sole (*Solea senegalensis*, Kaup). Aquaculture Research 35(12): 1122–1130. https://doi.org/10.1111/j.1365-2109.2004.01135.x

Driedzic RW, Almeida-Val VMF (1996) Enzymes of cardiac energy metabolism in Amazonian teleosts and fresh-water stingray (*Potamotrygon hystrix*). Journal of Exploratory Zoology 274(6): 327–333. https://doi.org/10.1002/(SICI)1097-010X(19960415)274:6%3C327::AID-JEZ1%3E3.0.CO;2-Q

Enes P, Panserat S, Kaushik S, Oliva-Teles A (2006) Effect of normal and waxy maize starch on growth, food utilization and hepatic glucose metabolism in European sea bass (*Dicentrarchus labrax*) juveniles. Comparative Biochemistry and Physiology Part A 143(1): 89–96. https://doi.org/10.1016/j.cbpa.2005.10.027

Fernandez I, Moyano FJ, Diaz M, Martinez T (2001) Characterization of α-amylase activity in five species of Mediterranean sparid fishes (Sparidae, Teleotei). Journal of Experimental Marine Biology and Ecology 262(1): 1–12. https://doi.org/10.1016/S0022-0981(01)00228-3

Fiske CH, Subbarow Y (1925) The colorimetric determination of phosphorus. Journal of Biological Chemistry 66(2): 375–400. https://doi.org/10.1016/S0021-9258(18)84756-1

Fountoulaki E, Alexis MN, Nengas I, Venou B (2005) Effect of diet composition on nutrient digestibility and digestive enzyme levels of gilthead sea bream (*Sparus aurata* L.). Aquaculture Research 6(13): 1243–1251. https://doi.org/10.1111/j.1365-2109.2005.01232.x

Freeland RA, Harper AL (1959) The study of metabolic pathway by means of adaptation. Journal of Biological Chemistry 234(6): 1350–1354. https://doi.org/10.1016/S0021-9258(18)70010-0

Giri SS, Bokhtiar SM, Sahoo SK, Paul BN, Mohanty SS (2019) Aquaculture of commercially important finfishes in south Asia. SAARC Agriculture Centre, SAARC, Dhaka, Bangladesh.

Glencross BD (2009) Exploring the nutritional demand for essential fatty acids by aquaculture species. Reviews in Aquaculture 1(2): 71–124. https://doi.org/10.1111/j.1753-5131.2009.01006.x

Guerreiro I, Peres H, Castro-Cunha M, Oliva-Teles A (2012) Effect of temperature and dietary protein/lipid ratio on growth performance and nutrient utilization of juvenile Senegalese sole (*Solea senegalensis*). Aquaculture Nutrition 18(1): 98–106. https://doi.org/10.1111/j.1365-2095.2011.00884.x

Hsu RY, Lardy HA (1967) Pigeon liver malic enzyme II isolation, characterization and some properties. Journal of Biological Chemistry 242(3): 520–526. https://doi.org/10.1016/S0021-9258(18)96304-0

IUCN (2014) IUCN Red List of Threatened Species. Version, 2014.3. http://www.iucnredlist.org

Jauncey K (1982) The effects of varying protein level on growth, food conversion, protein utilization and body composition of juvenile tilapias (*Sarotherodon mossambicus*). Aquaculture 27(1): 43–54. https://doi.org/10.1016/0044-8486(82)90108-9

Jin Y, Tian LX, Zeng SL, Xie SW, Yang HJ, Liang GY, Liu YJ (2013) Dietary lipid requirement on non-specific immune responses in juvenile grass carp (*Ctenopharyngodon idella*). Fish and Shellfish Immunology 34(5): 1202–1208. https://doi.org/10.1016/j.fsi.2013.01.008

Kim KD, Lim SG, Kang YJ, Kim KW, Son MH (2012) Effects of dietary protein and lipid levels on growth and body composition of juvenile far eastern catfish *Silurus asotus*. Asian-Australasian Journal of Animal Sciences 25(3): 369–374. https://doi.org/10.5713/ajas.2011.11089

Kornberg A, Horecker BL (1955) Glucose-6-phosphate dehydrogenase. Pp. 323–333. In: Colowick SP, Kaplan NO (Eds) Methods in enzymology. Academic Press, New York, NJ, USA. https://doi.org/10.1016/0076-6879(55)01046-X

Kumar V, Sahu NP, Pal AK, Kumar S, Gupta SK (2008) Gelatinized to non-gelatinized starch ratio in the diet of *Labeo rohita*: Effect on digestive and metabolic response and on growth. Journal of Animal Physiology and Animal Nutrition 92(4): 492–501. https://doi.org/10.1111/j.1439-0396.2007.00739.x

Kuzmina VV (1996) Digestive enzymes as an indicator of feeding ecology of wild fish. Pp. 9–13. In: MacKinlay D, Shearer K (Eds) Feeding Ecology and Nutrition in Fish Symposium Proceedings, American Fisheries Society, Vancouver, BC, Canada.

Lakra WS, Sarkar UK, Gopalakrishnan A, Pandian AK (2010) Threatened freshwater fishes of India. National Bureau of Fish Genetic Resources, Lucknow, India.

Lee YL, Lardy MA (1965) Influence of thyroid hormones on L α-glycerophosphate dehydrogenases in various organs of rat. Journal of Biological Chemistry 240(3): 1427–1430. https://doi.org/10.1016/S0021-9258(18)97593-9

Lee SM, Jeon IG, Lee JY (2002) Effects of digestible protein and lipid levels in practical diets on growth, protein utilization and body composition of juvenile rockfish (*Sebastes schlegeli*). Aquaculture 211(1): 227–239. https://doi.org/10.1016/S0044-8486(01)00880-8

Lowry OH, Ronebrough NJ, Farr AL, Randall RJ (1951) Protein measurement with Folin phenol reagent. Journal of Biological Chemistry 193(3): 265–276. https://doi.org/10.1016/S0021-9258(19)52451-6

Malla S, Banik S (2015) Reproductive biology of an endangered catfish, *Ompok bimaculatus* (Bloch, 1794) in the lotic waterbodies of Tripura, North-East India. International Journal of Fisheries and Aquatic Studies 2(4): 251–260.

Mandal S, Ghosh K (2018) Utilization of fermented pistia leaves in the diet of rohu, *Labeo rohita* (Hamilton): Effects on growth, digestibility and whole body composition. Waste and Biomass Valorization 10(11): 3331–3342. https://doi.org/10.1007/s12649-018-0336-4

Marjorie AS (1964) Methods in enzymology Vol II. P. 541. In: Colowick SP, Kaplan NO (Eds) Academic Press, New York, NJ, USA.

Mathis N, Feidt C, Brun-Bellut J (2003) Influence of protein/energy ratio on carcass quality during the growing period of Eurasian perch (*Perca fluviatilis*). Aquaculture 217(1): 453–464. https://doi.org/10.1016/S0044-8486(02)00122-9

Maynard L, Loosil J, Hintz H, Warner R (1979) Animal Nutrition (7th edn). McGraw-Hill, New York, NJ, USA.

Mishra K, Samantaray K (2004) Interacting effects of dietary lipids and temperature on growth, body composition and fatty acid profile of rohu, *Labeo rohita* (Hamilton). Aquaculture Nutrition 10(6): 359–369. https://doi.org/10.1111/j.1365-2095.2004.00311.x

Mohanta KN, Mohanty SN, Jena J, Sahu NP (2009) A dietary energy level of 14.6 MJ kg^{-1} and protein to energy ratio of 20.2 g MJ kg^{-1} results in best growth performance and nutrient accretion in silver barb *Puntius gonionotus* fingerling. Aquaculture Nutrition 15(6): 627–637. https://doi.org/10.1111/j.1365-2095.2008.00632.x

Murphy G, Walker DG (1974) Enzyme synthesis in the regulation of hepatic 'malic' enzyme activity. Biochemical Journal 144(1): 149–160. https://doi.org/10.1042/bj1440149

NRC (1993) Nutrient requirements of fish. National Research Council (NRC). National Academy Press. Washington, DC, USA.

NRC (2011) Nutrient requirements of fish. National Research Council (NRC). National Academy Press. Washington, DC, USA.

Okhawa H, Ohishi N, Yagi K (1979) Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Analytical Biochemistry 95(2): 351–358. https://doi.org/10.1016/0003-2697(79)90738-3

Pandolfi PP, Sonati F, Rivi R, Manson P, Grosveld F, Luzzatto L (1995) Targeted disruption of the house keeping genes encoding Glucose 6-phosphate dehydrogenase (G6PD):G6PD is dispensable for pentose synthesis but essential defense against oxidative stress. EMBO Journal 14(21): 5209–5215. https://doi.org/10.1002/j.1460-2075.1995.tb00205.x

Paul BN, Adhkar iS, Ayyappan S (2000) Effect of feeding frequency on the growth of *Labeo rohita* juvenile and on water quality in Flow-through system. Indian Journal of Animal Nutrition 17(1): 64–66.

Paul BN, Giri SS (2016) Carp and Catfish. Pp. 337–403. In: Bakshi MPS, Wadhwa M (Eds) Animal feeding-concepts and practices. Satish Serial Publishing House, Azadpur, Delhi, India.

Paul BN, Nandi S, Sarkar S, Mukhopadhyay PK (1997) Effects of feeding unconventional animal protein sources on the nitrogen metabolism in *Labeo rohita* (Hamilton). Israeli Journal of Aquaculture – Bamidgeh 49(4): 183–192.

Paul BN, Giri SS, Sarkar S, Mohanty SN, Sarangi N (2010) Effect of varying protein and lipid levels on the growth of medium carp, *Puntius gonionotus*. Animal Nutrition and Feed Technology 10(1): 81–86.

Paul BN, Datta AK, Das S, Chattopadhyay DN, Giri SS, Mohanty SN (2011) Effect of dietary lipid levels on growth and carcass composition of *Ompok pabda* (*Siluridae*) fry. Indian Journal of Animal Nutrition 28(2): 198–202.

Paul BN, Bhowmick S, Chanda S, Sridhar N, Giri SS (2018) Nutrient profile of five freshwater fish species. SAARC Journal of Agriculture 16(2): 25–41. https://doi.org/10.3329/sja.v16i2.40256

Paul BN, Das A, Bhowmick S, Mandal RN, Singh P, Adhikari S, Chowdhury D, Ghosh K, Chakrabarti PP, Giri SS (2020) Protein requirement of *Ompok bimaculatus* (1794) Larvae. Animal Nutrition and Feed Technology 20(3): 525–533. https://doi.org/10.5958/0974-181X.2020.00046.3

Pei Z, Xie S, Lei W, Zhu X, Yang Y (2004) Comparative study on the effect of dietary lipid level on growth and feed utilization for gibel carp (*Carassius auratusgibelio*) and Chinese longsnout catfish (*Leiocassis longirostris* Gunther). Aquaculture Nutrition 10(4): 209–216. https://doi.org/10.1111/j.1365-2095.2004.00291.x

Pradhan PK, Jena JK, Mitra G, Sood N, Gisbert E (2013) Ontogeny of the digestive enzymes in butter catfish *Ompok bimaculatus* (Bloch) larvae. Aquaculture 372–375: 62–69. https://doi.org/10.1016/j.aquaculture.2012.10.024

Raizada S, Lal KK, Sarkar UK, Varshney PK, Sahu V, Yadav KC, Jena JK (2013) Captive breeding and embryonic development of butter catfish (*Ompok bimaculatus*, Bloch 1794), a threatened fish of Indian sub-continent in North India. Proceedings of the National Academy of Sciences. India. Section B, Biological Sciences 83(3): 333–339. https://doi.org/10.1007/s40011-013-0156-z

Raj AJA, Haniffa MA, Seetharaman S, Appelbaum S (2007) Effect of dietary lipid levels on survival and growth of the threatened freshwater catfish *Mystus montanus*. Ege Journal of Fisheries and Aquatic Sciences 24(1–2): 51–54.

Rawat P, Biswas P, Patel B, Saha H (2018) Effect of dietary incorporation of chemo-attractants on growth and survival during seed rearing of *Ompok bimaculatus* (Bloch). Turkish Journal of Fisheries and Aquatic Sciences 18(3): 491–499. https://doi.org/10.4194/1303-2712-v18_3_15

Reitman S, Frankel S (1957) A colorimetric method for the determination of serum glutamic α-oxaloacetic and glutamic pyruvic transaminases. American Journal of Clinical Pathology 28(1): 56–63. https://doi.org/10.1093/ajcp/28.1.56

Sankian Z, Khosravi S, Kim YO, Lee SM (2017) Effect of dietary protein and lipid level on growth, feed utilization and muscle composition in golden mandarin fish *Siniperca scherzeri*. Fisheries and Aquatic Sciences 20(1): 1–7. https://doi.org/10.1186/s41240-017-0053-0

Schulz C, Huber M, Ogunji J, Rennert B (2008) Effects of varying dietary protein to lipid ratios on growth performance and body composition of juvenile pike perch (*Sander lucioperca*). Aquaculture Nutrition 14(2): 166–173. https://doi.org/10.1111/j.1365-2095.2007.00516.x

Snedecor GW, Cochran WG (1994) Statistical methods (8th edn). Oxford and IBH Publishing Co. Pvt. Ltd., New Delhi, India.

Susan TA, Sobha K, Veeraiah K, Tilak KS (2010) Studies on biochemical changes in the tissues of *Labeo rohita* and *Cirrhinus mrigala* exposed to fenvalerate technical grade. Journal of Toxicology and Environmental Health Sciences 2(5): 53–62.

Tranulis MA, Dregni O, Christophersen B, Krogdahl A, Borrebaek B (1996) A glucokinase-like enzyme in the liver of Atlantic salmon (*Salmo salar*). Comparative Biochemistry and Physiology. Part B, Biochemistry and Molecular Biology 114(1): 35–39. https://doi.org/10.1016/0305-0491(95)02119-1

Walter HE (1984) Methods of enzymatic analysis. Verlag Chemie, Weinheim, Germany.

Wang C, Hu G, Sun P, Gu W, Wang B, Xu Q, Liu H (2018) Effects of dietary protein at two lipid levels on growth, gonadal development, body composition and liver metabolic enzymes of brown trout (*Salmo trutta fario*) broodstock. Aquaculture Nutrition 24(5): 1587–1598. https://doi.org/10.1111/anu.12795

Webb Jr KA, Rawlinson LT, Holt GJ (2010) Effects of dietary starches and the protein to energy ratio on growth and feed efficiency of juvenile cobia, *Rachycentron canadum*. Aquaculture Nutrition 16(5): 447–456. https://doi.org/10.1111/j.1365-2095.2009.00672.x

Wei L, Xiu-Mei Z, Li-Bo W (2010) Digestive enzyme and alkaline phosphatise activities during the early stages of *Silurus soldatovi* development. Zoological Research 31(6): 627–632.

Williams KC (2007) Optimum dietary protein and lipid specifications for juvenile Malabar grouper (*Epinephelus malabaricus*). Aquaculture 267(1): 129–138. https://doi.org/10.1016/j.aquaculture.2007.03.007

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Optimization of dietary protein and lipid levels for butter catfish, (*Ompok bimaculatus*) (Bloch, 1794) fingerlings: An appraisal on growth, body composition, digestive enzymes, and metabolic function

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ABSTRACT

A completely randomized 3 × 3 factorial feeding trial was conducted to evaluate the effects of dietary protein and lipid levels on growth, feed utilization, body composition, and digestive as well as metabolic enzymes of the butter catfish (*Ompok bimaculatus*) fingerlings. Fish (average weight 0.66 ± 0.12 g; 30 d) were fed nine experimental diets containing three protein levels (35%, 40%, 45%) and three lipid levels (5%, 10%, 15%) in triplicate groups for a period of 90 days. The formulated diets were named P35L05, P35L10, P35L15, P40L05, P40L10, P40L15, P45L05, P45L10, and P45L15 (P = Protein; L = Lipid) respectively. The results indicated no protein-sparing effect of lipid in *O. bimaculatus* fingerlings beyond 35% dietary protein. Overall, diets P40L5 and P35L10 had significantly ($P < 0.05$) higher SGR (% day^{-1}) and lower FCR than other experimental groups. The highest weight gain (10.89 ± 0.07 g) and carcass protein (16.71 ± 0.2%) were recorded in fish fed diet P40L05. Increasing dietary lipid levels brought about an increase in carcass lipid in fish. Activities of α-amylase and acid-protease were decreased (except at 5% lipid), whereas alkaline-protease was increased with increasing dietary protein. An increase in pyruvate kinase (PK, glycolytic enzyme) activity and decrease in glucose-6-phosphate dehydrogenase (G6PD, lipogenic enzyme) activity were noticed with increasing dietary lipid content. A decrease in PK and increase in G6PD activities were observed with increasing dietary protein levels. Activities of the gluconeogenic enzymes (glucose-6-phosphatase, fructose-1,6-phosphatase) increased significantly ($P < 0.05$) with increasing dietary protein and lipid levels. Amino acid catabolizing enzymes, alanine transaminase and aspartate transaminase, were slightly increased with increasing dietary protein levels. Considering growth, body composition, metabolic function, and survivability (>95%), the diet containing 40% crude protein with 5% lipid might be suggested as optimum for *O. bimaculatus* fingerlings under the tested conditions.

KEYWORDS

Body composition; digestive enzymes; feed efficiency; metabolic enzymes; *Ompok bimaculatus*

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Introduction

The butter catfish, *Ompok bimaculatus* (Bloch, 1794), although indigenous to India, are also distributed in rivers, lakes, floodplains, and wetlands of other South East Asian countries, e.g., Bangladesh, Borneo, Java, Sumatra, Laos, Sri Lanka, Nepal, Malaya, Myanmar, Pakistan, Thailand, Cambodia, and Vietnam (Giri et al. 2019). The species has immense consumer preference and economic importance due to its excellent taste, balanced nutrient profile, soft bony structure, and high market price, especially in the east and north east of India as well as Bangladesh (Banik, Goswami, and Malla 2012; Paul et al. 2020a). It has been recognized as an excellent source of ω-3 and ω-6 fatty acids, lipoproteins, vitamins, and minerals together with easily digestible low fat content (Paul et al. 2020b). The wild population of *O. bimaculatus* has intensely declined due to anthropogenic activities like indiscriminate fishing and habitat degradation leading to ecological changes (Paul et al. 2020a). Considering restricted distribution and reduced abundance, the species has been categorized under near threatened category by the International Union for the Conservation of Nature and Natural Resources (IUCN) red list experiencing a risk of extinction in nature (IUCN 2014; Lakra et al. 2010). Because of high demand, competitive prices, and IUCN status, the species has been prioritized as a candidate for diversification of aquaculture and *in situ* conservation along with restocking through ranching programs (Debnath et al. 2016; Paul et al. 2020a). However, even though the aquaculture potential of *O. bimaculatus* has been recognized of late, the species has not yet attracted adequate attention for captive rearing, and thus information on the nutritional requirements of this species at different life stages is scarce (Biswas et al. 2020, 2019; Chakrabarti et al. 2012; Paul et al. 2021, 2020a).

Standardization of breeding protocol and feeding strategy accompanied by nutritionally balanced formulated diets are the prerequisites to establishing captive culture conditions for any fish species. Captive breeding of *O. bimaculatus* has already been established (Banik, Goswami, and Malla 2012; Raizada et al. 2013). Other studies conducted very recently indicated dietary requirements of 40% crude protein (Paul et al. 2020a) and 8% crude lipid (Paul et al. 2021) to assure optimum growth and survival of the *O. bimaculatus* larvae during early development. Requirement of around 35% dietary protein was suggested for the fingerlings of this species (Biswas et al. 2020). However, to the authors' knowledge, studies on dietary requirements of the major nutrients for the fingerlings of *O. bimaculatus* have not yet been reported. Thus, it was considered reasonable to appraise the nutritional requirements of *O. bimaculatus* fingerlings to ensure regular and reliable supply of the fingerlings for commercial production and conservation issues.

Protein is the most important and often an expensive dietary component; determination of its requirement levels is fundamental to formulating high-quality as well as cost-effective diets (Wang et al. 2013). Adequate levels of good-quality protein in fish feed is necessary to support high growth at the younger stages. If added in excess, the surplus protein would be converted to energy through catabolism of amino acids, and that should be avoided as it would increase the feed cost and nitrogenous waste output (National Research Council 2011; Wu and Gatlin 2014). Therefore, it is important to improve protein utilization for synthesis of body protein and important biomolecules rather than for energy purposes (Lee, Jeon, and Lee 2002). Dietary protein requirements in fish could be affected by the amount of nonprotein energy sources in the diet. Elevated lipid or carbohydrate levels in the diet could spare protein without negatively affecting growth and feed utilization efficiency in fish (De Silva, Gunasekera, and Shim 1991; Wang et al. 2013). Species under the genus *Ompok* have been generally recognized as carnivorous to omnivorous and prefer natural food items like zooplankton and tubifex (Malla and Banik 2015). Similarly, the carnivorous to omnivorous feeding aptitude of *O. bimaculatus* has been established (for review, see Gupta 2015). In carnivorous fish species, lipid is generally preferred as a nonprotein energy source since lipid is an energy-dense nutrient that is readily metabolized, and carbohydrate is less available in their natural food (Lee, Jeon, and Lee 2002). In addition, dietary lipids serve as the source of essential fatty acids and a carrier of fat-soluble vitamins. However, excessive lipid in the diet could disrupt the health and growth of fish because of abnormal lipid deposition in the body, and thus careful considerations must be given regarding protein-to-lipid ratios in formulated diets (Lee, Jeon, and Lee 2002; Shapawi et al. 2014; Wang et al. 2013). Thus, the present study was conducted to optimize dietary protein and lipid levels for formulation of practical diets for butter catfish fingerlings. Moreover, the present study evaluated the protein-sparing potential of lipid and appraised whether an interaction of protein and lipid existed for growth performance, body composition, and enzyme activities in *O. bimaculatus* fingerlings.

As requirement levels for protein and lipid may vary in different species and at different life stages, designing species-specific and age-specific diets can improve performance of the fish under commercial aquaculture. Optimization of the dietary protein:lipid ratio and their interaction has been studied in diverse fish species (Guerreiro et al. 2012; Kim and Lee 2009; Sagada et al. 2017; Wang et al. 2013). However, no commercial formulated diet has yet been available for the fingerlings of *O. bimaculatus*. Considering different protein and lipid levels studied for diverse fish species and reports on the nutrient requirements of the *O. bimaculatus* larvae, in the present study, practical diets were formulated with three protein (35%, 40%, 45%) and three lipid levels (5%, 10%, 15%) in a 3 × 3 factorial design to optimize the dietary protein and lipid

levels. Therefore, the present study considered an appraisal of digestive enzymes along with some key metabolic enzymes to evaluate the effects of formulated diets with varying protein and lipid levels. The results of the study might be helpful in making some important information available for formulation of diets for *O. bimaculatus* fingerlings.

Material and methods

Fish and culture conditions

Butter catfish (*O. bimaculatus*) fry and fingerlings were raised in the wet laboratory of the Department of Zoology, The University of Burdwan. Fish were handled and the experiment was performed following the approved guidelines of the Institutional Ethical Committee. Prior to the experiment, the fish were acclimated to the experimental conditions for one week in fiber-reinforced plastic (FRP) tanks (150 L) with continuous aeration; during this period fish were fed a formulated diet containing 40% crude protein and 8% lipid. After acclimatization, the fingerlings (average weight 0.66 ± 0.12 g; average length 4.8 ± 0.20 cm; 30 d old) were randomly distributed in 27 FRP tanks (nine experimental sets in triplicates) at a stocking density of 100 fish per tank. The fish were manually fed to apparent satiation twice a day at 10:00 and 16:00 hours, and it was ensured that no feed was leftover. Feed consumption and mortality in each tank were recorded separately, and survivability (%) was calculated. Seasoned groundwater was used for rearing of the experimental fish. The experiment was conducted for 90 days with continuous aeration and daily exchange of 25% water to ensure good water quality. During the experimental period, water quality parameters (temperature, pH, and dissolved oxygen) were monitored on a regular basis using a portable multiparameter analyzer (Orion Star™ A329) that utilizes the standard methods of American Public Health Association (American Public Health Association [APHA] 2012). Total alkalinity was determined by titration of the water sample with H_2SO_4 (0.1 N) and expressed as $CaCO_3$ equivalent (APHA 2012). Water quality parameters were noticed to vary within a narrow range: temperature 28°C–30°C, pH 6.8–7.7, dissolved oxygen 6.8–7.4 mg/L, and total alkalinity 230–240 mg/L.

Experimental design and diets

The experiment had a completely randomized 3 × 3 factorial design with triplicate groups. Nine experimental diets were formulated consisting of three protein levels (35%, 40%, 45%) and three lipid levels (5%, 10%, 15%). The formulated diets were named P35L05, P35L10, P35L15, P40L05, P40L10, P40L15, P45L05, P45L10, and P45L15 (P = Protein, L = Lipid) respectively.

Proximate compositions of the dietary ingredients were determined and diets were formulated using WinFeed software (Version 2.8). A blending of soybean meal (SBM), groundnut oil cake (GNOC), and casein along with fish meal (FM) at varied levels served as the protein sources; a combination of vegetable and fish oils (1:1) at different inclusion levels met the requirement of desired lipid levels in the diets. Crude protein (CP) and crude lipid (CL) levels of the major ingredients were: SBM (CP 45%, CL 2.5%), GNOC (CP 39%, CL 8%), and FM (CP 55%, CL 8%). All dry ingredients were finely powdered with a laboratory grinder, sieved to obtain uniform particle size (<400 μm, diameter), and mixed thoroughly with the required amount of vitamin-mineral premix (Supradyn, Piramal Enterprises Ltd., Mumbai, India) and oil sources. Carboxymethyl-cellulose (1%) was added as a binder, and the mixture was made to stiff dough with an appropriate amount of lukewarm water. The dough was pelleted (1 mm in diameter) with an electrically operated pelletizer and dried initially in the sun (6 h) and further in a hot air oven at 60°C (96 h). The dried pellets were crumbled, packed in airtight plastic bags, and stored in a refrigerator at 4°C until use. Formulation and proximate composition of the experimental diets are depicted in Table 1.

Proximate compositions of diets

Analyses of proximate compositions of the experimental diets (dry weight) were carried out following the procedures of the Association of Official Analytical Chemists (Association of Official Analytical Chemists [AOAC] 2005). Dry weight was determined by oven drying (initially at 100 ± 5°C for 30 min, thereafter at 60°C) until constant weight. Crude protein (N × 6.25) was determined by a semi-automatic Kjeldahl system (KjelTRON, Tulin Equipments, Chennai, India); crude lipid by ether extraction (petroleum ether, 60 to 80°C) using a Soxhlet apparatus (Socsplus, Pelican Equipments, Chennai, India); crude fiber as loss on ignition of dried fat-free residues after digestion with 1.25% H_2SO_4 and 1.25% NaOH using a Fibraplus system (Pelican Equipments, Chennai, India); and ash by combustion at 550°C in a muffle furnace. Nitrogen-free extract (NFE) was determined by subtracting the sum of values for crude protein, crude lipid, crude fiber, ash, and moisture from 100 (Maynard et al. 1979). Gross energy of the experimental diets was measured with a bomb calorimeter (Lab-X, Kolkata, India).

Growth parameters

After termination of the experiment, 20 fish were randomly picked from each tank, weighed, and analyzed to evaluate the growth parameters. Live weight gain (%), specific growth rate (SGR; % day^{-1}), protein efficiency ratio (PER), feed conversion ratio (FCR), apparent net protein utilization (ANPU %), and

Table 1. Ingredient composition and proximate composition of the experimental diets on a dry matter (%) basis.

	Diets								
Ingredients (g/100 g)	P35L05	P35L10	P35L15	P40L05	P40L10	P40L15	P45L05	P45L10	P45L15
Fishmeal	18	18	18	29	29	29	40	40	40
Wheat flour	37	32	27	26	22	17	16	11	6
Casein	10	10	10	10	10	10	10	10	10
Soybean meal	15	15	15	15	15	15	15	15	15
Ground nut oil cake	15	15	15	15	15	15	15	15	15
Fish oil + veg. oil (1:1)	2	7	12	2	6	11	1	6	11
Vitamin + mineral*	2	2	2	2	2	2	2	2	2
Carboxy methyl cellulose	1	1	1	1	1	1	1	1	1
Proximate composition (% dry matter)									
Dry matter	93.02 ± 1.51	92.87 ± 1.45	92.67 ± 1.46	93.12 ± 1.32	92.31 ± 1.40	92.05 ± 1.37	91.91 ± 1.38	91.72 ± 1.35	91.52 ± 1.47
Protein	35.42 ± 4.1	35.11 ± 4.0	34.9 ± 3.7	40.76 ± 3.8	40.22 ± 3.4	40.09 ± 3.1	45.58 ± 2.1	45.24 ± 2.7	44.82 ± 2.9
Lipid	5.15 ± 0.24	10.21 ± 0.17	15.40 ± 0.19	5.0.±0.25	9.87 ± 0.17	14.85 ± 0.12	5.19 ± 0.22	10.09 ± 0.16	14.92 ± 0.14
Ash	10.50 ± 0.74	10.15 ± 0.56	9.81 ± 0.62	10.02 ± 0.47	9.78 ± 0.68	9.56 ± 0.87	9.67 ± 0.54	9.45 ± 0.69	9.25 ± 0.78
Crude fiber	22.78 ± 2.14	21.54 ± 2.12	20.78 ± 2.14	20.52 ± 1.97	19.52 ± 2.15	18.98 ± 1.78	17.98 ± 1.69	17.74 ± 1.75	16.95 ± 1.85
NFE	26.15 ± 2.84	22.99 ± 2.69	19.12 ± 1.78	23.63 ± 1.67	20.61 ± 1.48	16.52 ± 1.47	21.58 ± 1.65	17.48 ± 1.47	14.06 ± 1.54
KJ/g	18.86	20.02	21.22	19.28	20.35	21.49	19.69	20.79	21.87
P:E ratio (mg/KJ)	18.78	17.54	16.45	21.14	19.76	18.66	23.15	21.76	20.49

*Vitamin+mineral contains Vitamin A I.P.(as acetate) 10,000 I.U, Cholecalciferol I.P.(Vitamin D3) 1000 I.U., Thiamine mononitrate I.P. 10 mg, Riboflavine I.P. 10 mg, Pyridoxine Hydrochloride I.P. 3 mg, Cyanocobalmin I.P. 15 mcg, Nicotinamide I.P. 100 mg, Calcium Pantothenate I.P. 16.30 mg, Ascorbic Acid I.P. 150 mg, α Tocopheryl Acetate I.P. 25 mg, Biotin U.S.P. 0.25 mg, Tribasic Calcium Phosphate I.P. 129 mg, Magnesium Oxide Light I.P. 60 mg, Dried Ferrous Sulfate I.P. 32.04 mg, Manganese Sulfate Monohydrate B.P. 2.03 mg, Total Phosphorus in the preparation 25.80 mg, Copper Sulfate Pentahydrate B.P. 3.39 mg, Zinc Sulfate I.P. 2.20 mg, Sodium Molybdate Dihydrate B.P. 0.25 mg, Sodium Borate B.P. 0.88 mg.

survivability (%) were determined following standard methods outlined by Steffens (1989), a detailed description of which was depicted in Ghosh and Mondal (2015).

Digestive enzymes

Activities of the digestive enzymes (α-amylase, alkaline protease, pepsin, and lipase) in fish were determined at the initiation and completion of the experiment. Digestive tracts of 20 fish from each experimental tank were taken out, cleaned properly with chilled distilled water, kept on an ice-cooled Petri plate, and weighed. Pooled samples collected from each tank were used for a replicate; thus there were three replicates for each experimental set. A 10% homogenate was prepared with chilled phosphate buffer (0.1 M, pH 7.4) and centrifuged at 10,000 rpm (10 min, 4°C). The resulting supernatant was used as the enzyme extract for estimation of digestive enzymes. Protein content of the supernatant was analyzed using bovine serum albumin as a standard (Lowry et al. 1951). Amylase activity was determined after Bernfeld (1955) using dinitro salicylic acid (DNSA) reagent, and unit activity (U) was expressed as mg maltose liberated mg^{-1} protein hr^{-1}. Alkaline protease activity was measured following Walter (1984), using Hammerstein casein substrate and presented as μg of tyrosine liberated mg^{-1} protein hr^{-1} (U). Acid protease (pepsin) activity was determined using hemoglobin (2%) substrate according to Anson (1938) with minor alterations as described in Worthington (1991) and expressed as μg of tyrosine liberated mg^{-1} protein min^{-1} (U). Lipase activity was measured after Bier (1955) using olive oil substrate and documented as μ mole of fatty acid liberated mg^{-1} protein hr^{-1} (U).

Metabolic enzymes

For determination of metabolic enzymes, hepatic tissues were collected and a 10% homogenate (in 0.25 M sucrose, pH 7.4) was prepared. Following removal of cellular debris by centrifugation (1,000 g, 30 min, 4°C), the supernatant was further centrifuged at 10,000 g (15 min, 4°C) to obtain mitochondrial pellets (Biswas et al. 2006). The supernatant was centrifuged at 12,500 g (1 h, 4°C), and the resultant fraction was used as the source of cytosolic metabolic enzymes. Although the mitochondrial pellet was further processed with triton X-100 (0.1%) and repeatedly washed (10,000 *g*, 30 min, 4°C) with PBS (0.1 M, pH 7.4), the supernatant thus obtained was used as the source of mitochondrial metabolic enzymes. The tissue fractions were stored in −20°C until use.

Among carbohydrate metabolizing enzymes, hexokinase (HK) was determined after Tranulis et al. (1996) through the reduction of NADP to generate NADPH. HK activity was presented as μM of NADPH formed mg^{-1} protein

h^{-1} (U). Pyruvate kinase (PK) was measured following Driedzic and Almeida-Val (1996) with minor alteration. PK activity was expressed as μmole of pyruvate converted to NADH mg^{-1} protein min^{-1} (U). Gluconeogenic enzymes, glucose-6-phosphatase (G6P), and fructose-1,6-bis phosphatase (FBP) were determined through the amount of phosphorus (Pi) released from glucose-6-phosphate (Marjorie 1964) or fructose-di-phosphate (Freeland and Harper 1959) as substrates respectively. Release of Pi was measured following Fiske and Subbarow (1925). G6P and FBP activities were expressed as μg of phosphorus released mg^{-1} protein min^{-1} (U).

Among lipogenic enzymes, glucose-6-phosphate dehydrogenase (G6PD) was determined using glucose-6-phosphate as the substrate and NADP (Kornberg and Horecker 1955). G6PD activity was expressed as μM of NADPH formed mg^{-1} protein hr^{-1} (U). NADP-malic enzyme (NADP-ME) activity was measured using L-malic acid as the substrate following Hsu and Lardy (1967) with alterations proposed by Murphy and Walker (1974). NADP-ME activity was expressed as μM of NADPH formed mg^{-1} protein h^{-1} (U).

Amino acid catabolizing enzymes, alanine transaminase (ALT) and aspartate transaminase (AST), were measured using α-ketoglutarate and DL-Alanine or DL-Aspartic acid substrates respectively (Reitman and Frankel 1957). ALT activity was presented as μM of pyruvate formed mg^{-1} protein min^{-1} (U); AST activity was expressed as μM of oxaloacetate formed mg^{-1} protein min^{-1} (U). Mitochondrial glutamate dehydrogenase (GDH) activity was determined after Lee and Lardy (1965) using sodium glutamate and tetrazolium salt. GDH activity was expressed as μM of formazan formed mg^{-1} protein h^{-1} (U).

Compositions of fish carcass

Analyses of proximate compositions of the fish carcass (wet weight) were carried out by standard procedures of the Association of Official Analytical Chemists (AOAC 2005) as described in a previous section.

Statistical analysis

Data were presented as mean ± standard error (SE). Data were analyzed by one-way (dietary treatments) and two-way (dietary protein and lipid levels) analysis of variance (ANOVA) following Zar (2010). Multiple comparisons were made by Tukey's post hoc HSD test to analyze significant differences ($P < 0.05$) between the means of experimental groups.

Results

Data pertaining to growth performance, feed utilization, and survivability of *O. bimaculatus* fingerlings fed diets with different protein and lipid levels for 90 days are presented in Table 2. Results of the one-way ANOVA indicated that net weight gain, SGR (% per day), FCR, and protein utilization were significantly ($P < 0.05$) influenced by varying levels of dietary protein and lipid. The highest weight gain was recorded in fish fed diet P40L5. Overall, diets with 45% crude protein produced fish with lower SGR, PER, and ANPU. Increasing lipid levels from 5% to 15% resulted in a decrease in growth and protein utilization in fish fed diets with 40% or 45% crude protein. Survivability rates in butter catfish fingerlings were >90% except the groups fed diets P45L10 and P45L15. Analysis by two-way ANOVA revealed that SGR, FCR, PER, ANPU, and survivability were significantly affected by the dietary protein and lipid levels ($P < 0.05$). Further, except for survivability, there were significant interaction between the dietary protein and lipid levels on the growth and feed utilization parameters (e.g., SGR, FCR, PER, and ANPU) of *O. bimaculatus* fingerlings.

Activities of the digestive enzymes are depicted in Table 3. Overall, one-way ANOVA indicated that activities of α-amylase and acid protease were decreased (except at 5% lipid, up to 40% CP), whereas alkaline protease was increased with increasing levels of dietary protein. The maximum α-amylase and acid protease activities were noticed in the fish fed diet P35L10, though it was not significantly different from the groups fed diets P35L15 and P40L05. Significantly ($P < 0.05$) higher alkaline protease activity was recorded with the fish fed diets with 45% crude protein irrespective of tested lipid levels, when compared to other protein levels. Lipase activity increased significantly with increasing dietary lipid from 5 to 10 at the 35% CP level. Although the maximum lipase activity was noticed in the fish fed diet P45L05, that didn't differ significantly ($P < 0.05$) with the other groups receiving 40% or 45% crude protein in the diets. Thus, at higher CP levels, lipase activity didn't differ significantly with increasing lipid levels. According to two-way ANOVA, activities of the tested digestive enzymes were significantly affected ($P < 0.05$) by dietary protein and lipid levels and their interaction.

Some of the key hepatic enzymes concerned with intermediary metabolism of protein, lipid, and carbohydrate are portrayed in Table 4. One-way ANOVA showed that varying dietary protein and lipid levels led to significant differences in the activities of PK, G6P, FBP, G6PD, ALT, and AST in *O. bimaculatus* fingerlings; differences were not significant for the activities of HK, ME, and GDH. Activity of the glycolytic enzyme PK significantly decreased with increasing dietary protein levels but increased by increasing lipid levels at the same protein level. Activities of two major neoglucogenic enzymes, G6P and FBP, significantly increased with increases in the dietary

Table 2. Growth performance and feed utilization efficiency of *Ompok bimaculatus* fingerlings fed varying dietary protein and lipid levels for 90 days.

Experimental diets	Initial (g)	Final Weight (g)	Live weight gain	FCR$^{\alpha}$	SGR$^{\beta}$	PER$^{\gamma}$	ANPU$^{\Sigma}$	Survivability
P35/L05	0.652 ± 0.12	10.05 ± 0.06^{ab}	9.40 ± 0.08^{ab}	1.46 ± 0.01^{b}	2.77 ± 0.02^{b}	1.95 ± 0.02 ^{f}	32.06 ± 0.39^{c}	96 ± 0.57^{b}
P35/L10	0.661 ± 0.11	11.08 ± 0.06^{e}	10.43 ± 0.10^{e}	1.37 ± 0.01^{a}	3.00 ± 0.04 ^{cd}	2.09 ± 0.01 ^{g}	35.00 ± 0.6^{d}	93 ± 1.52^{b}
P35/L15	0.656 ± 0.12	10.43 ± 0.07^{bcd}	9.78 ± 0.09^{bcd}	1.57 ± 0.01^{c}	2.75 ± 0.02^{b}	1.82 ± 0.02^{d}	30.26 ± 0.48^{c}	92 ± 1.15^{b}
P40/L05	0.657 ± 0.13	11.54 ± 0.08^{f}	10.89 ± 0.07 ^{f}	1.34 ± 0.01^{a}	3.08 ± 0.04^{d}	1.86 ± 0.02^{e}	31.44 ± 0.20^{c}	95 ± 1.15^{b}
P40/L10	0.672 ± 0.16	10.86 ± 0.07^{e}	10.21 ± 0.09^{e}	1.51 ± 0.01^{bc}	2.91 ± 0.03^{c}	1.65 ± 0.01^{c}	27.66 ± 0.40^{b}	93 ± 0.57^{b}
P40/L15	0.681 ± 0.19	10.42 ± 0.10^{bcd}	9.77 ± 0.06^{bcd}	1.63 ± 0.02 ^{cd}	2.71 ± 0.03^{b}	1.53 ± 0.01^{b}	25.40 ± 0.44^{b}	90 ± 2.30^{ab}
P45/L05	0.654 ± 0.12	9.99 ± 0.06^{a}	9.34 ± 0.06^{a}	1.67 ± 0.02^{d}	2.41 ± 0.02^{a}	1.33 ± 0.02^{a}	21.56 ± 0.52^{a}	91 ± 2.08^{ab}
P45/L10	0.665 ± 0.14	10.08 ± 0.08^{ac}	9.43 ± 0.07^{ac}	1.71 ± 0.03^{de}	2.37 ± 0.02^{a}	1.30 ± 0.02^{a}	20.92 ± 0.57^{a}	88 ± 1.52^{ab}
P45/L15	0.654 ± 0.11	10.28 ± 0.10^{ad}	9.63 ± 0.06^{ad}	1.76 ± 0.02^{e}	2.3 ± 0.02^{a}	1.27 ± 0.02^{a}	20.14 ± 0.37^{a}	83 ± 3.00^{a}
Means of main effect								
P35	–	10.52	9.87	1.46^{A}	2.84^{B}	1.94^{B}	32.44^{B}	93.66
P40	–	10.94	10.29	1.49^{A}	2.90^{B}	1.82^{B}	28.16^{B}	92.66
P45	–	10.12	9.46	1.71^{B}	2.36^{A}	1.29^{A}	20.87^{A}	87.33
L5	–	10.53	9.88	1.49	2.75	1.84	28.35	94.00
L10	–	10.67	10.02	1.53	2.76	1.67	27.86	91.33
L15	–	10.38	9.72	1.65	2.59	1.54	25.26	88.33
Two-way ANOVA (P value)								
Protein	–	0.001	0.001	P < 0.001	0.005	P < 0.001	P < 0.001	0.003
Lipid	–	P < 0.001	P < 0.001	P < 0.001	P < 0.001	P < 0.001	P < 0.001	P < 0.001
Interaction	–	P < 0.001	P < 0.001	P < 0.001	0.032	P < 0.001	P < 0.001	0.744

Note. Data are presented as Mean ± SE of three determinations (n = 3). Means with different superscripts in a column differ significantly (P < 0.05).
$^{\alpha}$Feed conversion ratio, $^{\beta}$Specific growth rate, $^{\gamma}$Protein efficiency ratio, $^{\Sigma}$Apparent net protein utilization.

Table 3. Activities of digestive enzymes in *Ompok bimaculatus* fingerlings fed varying dietary protein and lipid levels for 90 days.

Experimental diets	Amylase	Alkaline protease	Lipase	Pepsin
P35/L05	10.86 ± 0.08^{c}	1.63 ± 0.03^{a}	1.37 ± 0.02^{a}	3.35 ± 0.06^{b}
P35/L10	12.25 ± 0.10^{e}	1.68 ± 0.01^{ab}	1.61 ± 0.03^{bc}	3.65 ± 0.07^{b}
P35/L15	11.96 ± 0.12^{de}	1.74 ± 0.03^{ac}	1.56 ± 0.03^{b}	3.42 ± 0.06^{b}
P40/L05	11.86 ± 0.11^{de}	1.82 ± 0.02^{bcd}	1.72 ± 0.03^{bd}	3.55 ± 0.07^{b}
P40/L10	11.5 ± 0.12^{d}	1.94 ± 0.04^{de}	1.81 ± 0.04^{d}	2.89 ± 0.05^{a}
P40/L15	9.65 ± 0.12^{b}	1.96 ± 0.03^{df}	1.78 ± 0.03^{d}	2.92 ± 0.05^{a}
P45/L05	10.52 ± 0.07^{c}	2.12 ± 0.04^{fg}	1.86 ± 0.04^{d}	2.75 ± 0.05^{a}
P45/L10	9.95 ± 0.15^{b}	2.16 ± 0.04^{g}	1.78 ± 0.04^{cd}	2.64 ± 0.06^{a}
P45/L15	8.77 ± 0.11^{a}	2.06 ± 0.04^{efg}	1.71 ± 0.03^{bd}	2.61 ± 0.05^{a}
Means of main effect				
P35	11.69	1.68^{A}	1.51	3.47^{B}
P40	11.00	1.91^{B}	1.77	3.36^{AB}
P45	9.74	2.11^{C}	1.78	3.13^{A}
L05	11.08	1.86	1.65	3.45
L10	11.23	1.93	1.73	3.37
L15	10.12	1.92	1.68	3.14
Two-way ANOVA (P value)				
Protein	$P < 0.001$	0.024	0.019	$P < 0.001$
Lipid	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$
Interaction	$P < 0.001$	0.027	$P < 0.001$	$P < 0.001$

Note. Data are presented as Mean ± SE of three determinations ($n = 3$). Means with different superscripts in a column differ significantly ($P < 0.05$).

protein and lipid levels. Activity of the lipogenic enzyme, G6PD, revealed a significant decrease with an increase in the dietary lipid levels and increased with an increase in the dietary protein levels. Further, activities of amino acid catabolizing enzymes, ALT and AST, revealed a marginal increase with an increase in the dietary protein levels for all of the tested lipid levels.

Based on one-way ANOVA, whole body lipid and protein were significantly affected by varying levels of dietary proteins and lipids, although moisture and ash contents remained statistically neutral (Table 5). The highest crude protein in the carcass was recorded in the group P40L05, although it did not differ significantly ($P < 0.05$) with the groups P35L10 and P40L10. Further, an increase in dietary lipid levels at both 40% and 45% crude protein resulted in a decrease in carcass protein deposition in fish. The maximum crude lipid in the carcass was noticed in fish fed diet P40L15, which was not significantly different ($P < 0.05$) to the fish fed diets P35L10, P40L05, and P40L10. An increase in dietary lipid levels brought about an increase in carcass lipid contents in fish at the same protein level, although the difference was significant with only 35% crude protein. The two-way ANOVA indicated that dietary protein and lipid levels and their interaction had a significant influence on carcass protein and lipid of *O. bimaculatus* fingerlings. However, there was no significant interaction between dietary protein and lipid levels on carcass ash and moisture contents.

Table 4. Hepatic metabolic enzymes of *Ompok bimaculatus* fingerlings fed varying dietary protein and lipid levels for 90 days.

	Glycolytic enzyme		Gluconeogenic enzyme	
Experimental Diets	Hexokinase	Pyruvate Kinase	Glucose 6 Phosphatase	Fructose 1,6 Phosphatase
P35/L05	10.43 ± 0.27	7.25 ± 0.06^{d}	4.15 ± 0.03^{a}	2.87 ± 0.05^{a}
P35/L10	10.56 ± 0.27	8.67 ± 0.08 ^{f}	4.27 ± 0.05^{ab}	3.15 ± 0.06^{ab}
P35/L15	10.72 ± 0.27	9.89 ± 0.12 ^{g}	4.38 ± 0.03^{bc}	3.35 ± 0.06^{bc}
P40/L05	11.23 ± 0.26	6.01 ± 0.06^{b}	4.42 ± 0.04^{bcd}	3.67 ± 0.07 ^{cd}
P40/L10	11.52 ± 0.27	6.82 ± 0.06^{c}	4.57 ± 0.06^{cde}	3.75 ± 0.07^{de}
P40/L15	10.89 ± 0.25	7.65 ± 0.05^{e}	4.61 ± 0.05^{def}	3.96 ± 0.07^{de}
P45/L05	11.67 ± 0.28	5.35 ± 0.05^{a}	4.82 ± 0.05 ^{f}	4.03 ± 0.07^{ef}
P45/L10	10.96 ± 0.29	6.21 ± 0.05^{bd}	5.21 ± 0.04 ^{g}	4.27 ± 0.08 ^{fg}
P45/L15	10.76 ± 0.27	6.96 ± 0.06 ^{cd}	5.37 ± 0.04 ^{g}	4.42 ± 0.07 ^{g}
Means of main effect				
P35	10.57	8.6	4.27^{A}	3.12^{A}
P40	11.21	6.83	4.53^{A}	3.79^{B}
P45	11.13	6.17	5.13^{B}	4.24^{B}
L05	11.11	6.2	4.46	3.52
L10	11.01	7.23	4.68	3.72
L15	10.79	8.17	4.78	3.91
Two-way ANOVA (P value)				
Protein	0.871	P < 0.001	P < 0.001	P < 0.001
Lipid	0.368	P < 0.001	P < 0.001	P < 0.001
Interaction	0.494	P < 0.001	0.005	0.555

(*Continued*)

Table 4. (Continued).

	Lipogenic enzymes		Amino acid catabolizing enzymes		
Experimental diets	Glucose 6 phosphate dehydrogenase	Malic enzyme	Alanine aminotransferase	Aspartate aminotransferase	Glutamate dehydrogenase
P35/L05	29.72 ± 0.26^{b}	2.52 ± 0.14	3.65 ± 0.06^{ab}	6.10 ± 0.06^{b}	5.60 ± 0.10
P35/L10	26.42 ± 0.41^{a}	2.47 ± 0.15	3.54 ± 0.04^{a}	5.96 ± 0.06^{ab}	5.55 ± 0.06
P35/L15	24.34 ± 0.55^{a}	2.38 ± 0.17	3.56 ± 0.05^{a}	5.62 ± 0.07^{a}	5.53 ± 0.08
P40/L05	34.54 ± 0.48^{d}	2.75 ± 015	3.80 ± 0.09^{ad}	6.55 ± 0.09^{b}	5.65 ± 0.08
P40/L10	33.17 ± 0.34^{cd}	2.68 ± 0.13	3.65 ± 0.06^{a}	6.25 ± 0.07^{ab}	5.62 ± 0.09
P40/L15	31.65 ± 0.34^{bc}	2.62 ± 0.14	3.71 ± 0.03^{ac}	6.17 ± 0.10^{ab}	5.56 ± 0.08
P45/L05	39.67 ± 0.80^{e}	2.95 ± 0.13	4.06 ± 0.05^{de}	6.97 ± 0.06^{c}	5.70 ± 0.05
P45/L10	37.43 ± 0.82^{e}	2.81 ± 0.10	3.92 ± 0.08^{bcde}	6.80 ± 0.10^{c}	5.65 ± 0.07
P45/L15	34.27 ± 0.49^{cd}	2.75 ± 0.12	3.78 ± 0.04^{ae}	6.83 ± 0.08^{c}	5.62 ± 0.05
Means of main effect					
P35	26.82^{A}	2.46^{A}	3.58^{A}	5.89^{A}	5.56
P40	33.12^{B}	2.68^{B}	3.72^{AB}	6.32^{A}	5.61
P45	37.12^{B}	2.84^{B}	3.92^{B}	6.87^{B}	5.65
L05	34.64	2.74	3.84	6.54	5.65
L10	32.34	2.65	3.7	6.34	5.6
L15	30.08	2.58	3.71	6.21	5.57
Two-way ANOVA (P value)					
Protein	$P < 0.001$	0.395	0.011	$P < 0.001$	0.473
Lipid	$P < 0.001$	0.011	$P < 0.001$	$P < 0.001$	0.342
Interaction	0.112	0.997	0.395	0.115	0.999

Note. Data are presented as Mean ± SE of three determinations ($n = 3$). Means with different superscripts in a column differ significantly ($P < 0.05$).

Table 5. Whole body carcass composition (%) of *Ompok bimaculatus* fingerlings fed varying dietary protein and lipid levels for 90 days.

Experimental diets	Moisture	Crude protein	Crude lipid	Crude ash
P35/L05	79.75 ± 2.19	16.21 ± 0.05^{b}	2.5 ± 0.02^{a}	1.78 ± 0.02
P35/L10	79.54 ± 1.25	16.61 ± 0.04^{d}	2.71 ± 0.02^{b}	1.81 ± 0.02
P35/L15	78.67 ± 1.57	16.42 ± 0.03^{c}	2.89 ± 0.03^{e}	1.83 ± 0.01
P40/L05	78.82 ± 2.54	16.71 ± 0.02^{d}	2.82 ± 0.02 ^{cd}	1.86 ± 0.03
P40/L10	79.27 ± 2.28	16.52 ± 0.03 ^{cd}	2.85 ± 0.04^{de}	1.76 ± 0.01
P40/L15	79.06 ± 1.42	16.40 ± 0.04^{c}	2.91 ± 0.01^{e}	1.74 ± 0.02
P45/L05	79.98 ± 2.25	16.04 ± 0.02^{ab}	2.74 ± 0.01^{bc}	1.84 ± 0.01
P45/L10	78.57 ± 2.05	15.95 ± 0.03^{a}	2.76 ± 0.01^{bd}	1.76 ± 0.01
P45/L15	78.86 ± 2.78	15.87 ± 0.04^{a}	2.82 ± 0.02 ^{cd}	1.76 ± 0.03
Means of main effect				
P35	79.32	16.41^{B}	2.70	1.80
P40	79.05	16.54^{B}	2.86	1.78
P45	79.13	15.95^{A}	2.77	1.78
L05	79.51	16.32	2.68	1.82
L10	79.12	16.36	2.77	1.77
L15	78.86	16.23	2.87	1.77
Two-way ANOVA (P value)				
Protein	0.897	$P < 0.001$	$P < 0.001$	0.388
Lipid	0.913	$P < 0.001$	$P < 0.001$	0.387
Interaction	0.951	$P < 0.001$	$P < 0.001$	0.434

Note. Data are presented as Mean ± SE of three determinations ($n = 3$). Means with different superscripts in a column differ significantly ($P < 0.05$).

Discussion

Nutritional requirements are the reflection of growth and physiology in fish, as dietary nutrients are utilized for energy metabolism, tissue restoration, and growth (Wang et al. 2013). The present study aimed at determining the optimal combination of dietary protein and lipid levels to support growth, feed utilization, and metabolic functions in *Ompok bimaculatus* fingerlings under captive conditions. The use of lipid in the diets has to be critically evaluated as excessive lipid may not only interfere with pellet quality and shelf life of the diets but also growth and feed utilization in fish (Li et al. 2010). Thus, an appropriate lipid level in fish diets is of great importance. On the other hand, dietary protein is the major factor affecting growth of fish along with feed cost (Lovell 1989). In general, increasing the dietary protein level could be linked with improved fish production, especially for carnivorous fish (Lee, Jeon, and Lee 2002). Dietary lipid levels might have considerable influence on the effect of protein on the growth and normal well-being of fish. To the best of our knowledge, this is the first report on the effects of varying dietary protein and lipid levels on growth, feed utilization, body composition, and metabolic enzymes of *O. bimaculatus* fingerlings. In the presently reported study, the maximum weight gain and SGR were noticed in *O. bimaculatus* fingerlings fed the diet P40L05 with 40% crude protein and 5% lipid. However, SGR and FCR did not vary significantly from the fish fed diet with 35% crude protein and 10% lipid (P35L10). Therefore, improved growth and feed utilization efficiency associated with increasing lipid content of the diet from 5% to 10% at

the 35% dietary protein level might indicate the protein-sparing effect of dietary lipid in *O. bimaculatus* fingerlings, as suggested elsewhere (Sagada et al. 2017). Such protein-sparing effect was also noticed in several fish species, including bagrid catfish (*Pseudobagrus fulvidraco*) (Kim and Lee 2005), blunt snout bream (*Megalobrama amblycephala*) (Li et al. 2010), Asian catfish (*Pangasius hypophthalmus*) (Liu et al. 2011), juvenile northern snakehead fish (*Channa argus*) (Sagada et al. 2017), and Juvenile Yellow Drum (*Nibea albiflora*) (Wang et al. 2018).

In the present report, an increase of crude protein from 35% (with 10% lipid) to 45% did not cause significant improvement in fish growth and feed utilization efficiency. This could be an indication that the minimal amino acid requirement was met at 35% protein and beyond which excess protein might be extravagantly converted to energy and nitrogenous excreta. Similar observations were documented in previous reports on diverse fish species (Sagada et al. 2017; Tu et al. 2015), including fingerlings of *O. bimaculatus* (Biswas et al. 2020). Further, SGR tended to decrease and FCR tended to increase with increasing the lipid levels from 5% to 15% in the diets with higher levels of protein (40% and 45%). This might indicate that elevating the dietary lipid levels from 5% to 15% did not induce protein-sparing action in *O. bimaculatus* fingerlings fed diets with ≥ 40% dietary protein. In accordance to our report, Wang et al. (2013) indicated that golden pompano (*Trachinotus ovatus*) reared in net pens attained higher weight gain (%) at the dietary lipid level of 6.5% than at 12.5% when fed with varying dietary protein levels (33%–50%). Similarly, juveniles of ayu (*Plecoglossus altivelis*) (Lee, Jeon, and Lee 2002) and flounder (*Paralichthy solivaceus*) (Lee and Kim 2005) exhibited faster growth at the dietary crude lipid levels of 6.0%–6.5% than at 13%–19%. Thus, as in the presently reported study, previous works also confirmed that protein-sparing action could not be sustained beyond certain levels of dietary protein and lipid (Sagada et al. 2017). Furthermore, the present study recorded a marginal improvement in weight gain (%) with increasing the protein level from 35% (diet P35L10, 10% lipid) to 40% (diet P40L05, 5% lipid). Incorporation of 40% crude protein for *O. bimaculatus* was in agreement with the protein requirement of catfish, as reported previously (BIS 2014).

Growth is extremely influenced by digestive and absorptive physiology, which affects utilization of the ingested nutrients in an organism (Sagada et al. 2017). The capability of fish to efficiently utilize a given diet could be apprehended by the activity of digestive enzymes and their responsiveness toward diverse dietary compositions (Pérez-Jiménez et al. 2009). Thus, adaptations of the digestive enzymes to feeding and related metabolic alterations might be used as a clue for formulation of nutritionally effective diets (Lundstedt, Melo, and Moraes 2004). However, to the

authors' knowledge, diet-related changes in the activity of digestive enzymes in the butter catfish fingerlings appears to be mostly unexplored and warrants more insight for better understanding of the relationship between digestive as well as metabolic enzymes and growth performance. In the presently reported study, increasing dietary protein and lipid levels were inversely proportional to dietary carbohydrate content and associated with a decrease in α-amylase activity. Our study was in accordance with the results obtained in hybrid catfish (*Clarias batrachus* × *C. gariepinus*) fed diets with high protein levels (Giri et al. 2003) and contradicted with African catfish *C. gariepinus* (Ali and Jauncey 2004). The present study noticed to enhance alkaline protease activity with increasing dietary protein levels, which was in agreement with the preceding reports on hybrid catfish (*Clarias batrachus* × *C. gariepinus*) (Giri et al. 2003), spotted sorubim (*Pseudoplatystoma corruscans*) (Lundstedt, Melo, and Moraes 2004), silver barb (*Puntius gonionotus*) (Mohanta et al. 2008), and rice field eel (*Monopterus albus*) (Ma et al. 2014). Thus, it could be suggested that dietary protein levels might ascertain protease secretion and augment proteolytic activities in the digestive tract (Huang, Zhao, and Luo 2013). Although results on the acid protease activity recorded in the present study did not comply with this hypothesis, it was similar to the observation of Santos et al. (2020) depicting an increase in the CP level from 36% to 42% associated with a decrease in acid protease activity. On the contrary, protease activity was not significantly affected by dietary protein levels in gibel carp (*Carassius auratus gibelio*) (Ye et al. 2015) and juvenile northern snakehead fish (*C. argus*) (Sagada et al. 2017). Several studies reported a positive correlation between intestinal lipase activity and dietary lipid levels (Sagada et al. 2017; Wang et al. 2018). However, in the present study, lipase activity didn't differ significantly with increasing lipid contents at higher CP levels, supporting our observation that there was no protein-sparing effect of lipid in juvenile *O. bimaculatus* at ≥ 40% dietary protein level.

The present study evaluated activities of some key metabolic enzymes to appraise consequences of the varying dietary protein-lipid levels in juvenile *O. bimaculatus*. Neoglucogenesis is an important pathway for glucose homeostasis, where glucose is synthesized from noncarbohydrate precursors (e.g., amino acid, glycerol, lactate). The present study recorded increased activities of the neoglucogenic enzymes (G6P and FBP) with an increase in both dietary protein and lipid levels. Enhanced activity of the neoglucogenic enzymes noticed in this study linked with reduced activity of digestive amylase might indicate production of glucose by neoglucogenesis so as to accomplish the energy demand in *O. bimaculatus*, which is supposed to be a carnivorous fish (Paul et al. 2021). The major glycolytic enzyme, HK, was not significantly affected by the varying dietary protein and lipid levels in the present study.

However, activity of another glycolytic enzyme PK decreased and neoglucogenic enzyme FBP increased with an increase in the dietary protein, which was in accordance with the observation made by Wang et al. (2018) in brown trout (*Salmo trutta fario*).

The lipogenic enzyme, G6PD, catalyzes the first step of the HMP-shunt (pentose phosphate pathway) generating NADPH for lipid biosynthesis and stress management (Pandolfi et al. 1995). The present study noticed around 10 times higher activity of G6PD than that of ME with an increase in dietary protein levels, suggesting that NADPH production for lipogenesis is mainly obtained from the pentose-phosphate pathway (Sá, Pousão-Ferreira, and Oliva-Teles 2007). Further, activity of the G6PD was inhibited in the present study by an increase in the dietary lipid, which was similar to the observations recorded in brown trout (*Salmo trutta fario*) (Wang et al. 2018) and Senegalese sole (*Solea senegalensis*) (Guerreiro et al. 2012). In contradiction, Sá, Pousão-Ferreira, and Oliva-Teles (2006) couldn't detect a significant effect of dietary protein or lipid levels on G6PD activity in White sea bream (*Diplodus sargus*). ME is associated with NADP-dependent oxidative decarboxylation (malate to pyruvate and carbon dioxide) generating NADPH that is likely to be utilized for lipid biosynthesis; GDH is considered a sensitive stress indicator (Paul et al. 2021; Susan et al. 2010). ME and GDH activities were more or less unaffected by the dietary protein and lipid levels, which was consistent with the findings of Wang et al. (2018) in brown trout. Consequently, results of the present study might suggest that increasing dietary lipid at the same protein level could neither improve lipogenesis nor induce stress in *O. bimaculatus* fingerlings.

ALT and AST are the main transaminases in fish liver (Sá, Pousão-Ferreira, and Oliva-Teles 2007). In the present study, activities of the amino acid-catabolizing (ALT and AST) enzymes were influenced by the dietary protein and lipid levels. The activities of ALT and AST were proportional to the increasing dietary protein levels, which was similar to the observations made by Sá, Pousão-Ferreira, and Oliva-Teles (2007) in White Sea bream and Wang et al. (2018) in brown trout. Increased ALT and AST activities might be indicative of increased protein catabolism at high dietary protein levels corroborated with the increased activities of alkaline proteases. Further, increased dietary lipid at the same protein level was associated with a decrease in the activities of both ALT and AST, suggesting use of lipid as an energy source along with reduced catabolism of protein. Similar observations were reported in other species, e.g., *Sparus aurata* (Fernández et al. 2007) and *Salmo trutta fario* (Wang et al. 2018).

Increasing dietary lipid levels might have a significant effect on the carcass quality of fish (Cowey 1993). The presently reported study noticed an increased accumulation of body lipid with an increase in the dietary

lipid contents at each protein level. Similar effects were also detected in numerous species, e.g., rockfish (*Sebastes schlegelii*) (Lee, Jeon, and Lee 2002), Eurasian perch (*Perca fluviatilis*) (Mathis, Feidt, and Brun-Bellut 2003), bagrid catfish (*P. fulvidraco*) (Kim and Lee 2005), cobia (*Rachycentron canadum*) (Craig, Schwarz, and McLean 2006), Malabar grouper (*Epinephelus malabaricus*) (Williams 2007), brown-marbled grouper (*Epinephelus fuscoguttatus*) (Shapawi et al. 2014), and juveniles of snakehead fish (*Channa argus*) (Sagada et al. 2017). In contrast, Paul et al. (2011) could not detect any difference in carcass lipid in another species of butter catfish (*O. pabda*) by feeding different levels of lipid. In the present study, the diets containing the maximum protein level were not associated with the highest whole body protein in *O. bimaculatus* fingerlings, which was comparable with the other findings (Rahimnejad et al. 2015; Tuan and Williams 2007). On the contrary, several studies suggested a significant increase in the body protein content with the increase in the dietary protein level (Chen et al. 2010; Wang et al. 2016). Further, whole body moisture and ash contents of *O. bimaculatus* fry in the present study were not affected by the dietary treatments. In accordance, varying dietary protein and lipid levels did not have any effect on whole body ash content of the grouper (*E. fuscoguttatus*) (Shapawi et al. 2014). Although numerous studies indicated a decrease in whole body moisture content with increasing dietary protein (Sagada et al. 2017; Wang et al. 2016) and lipid levels (Lim et al. 2009; Luo et al. 2005; Sagada et al. 2017).

Conclusion

Considering the highest weight gain (%) and SGR (% per day), the lowest FCR, and 95% survivability achieved with the diet P40L05 together with the carnivorous nature of the species, it may be concluded that 40% crude protein with 5% lipid was optimum for the growth and feed utilization of *O. bimaculatus* fingerlings. In addition, since no protein-sparing effect of lipid in juvenile *O. bimaculatus* was noticed beyond the 35% dietary protein level, excessive dietary lipid should be avoided for economic diet formulation as well as health concerns (Craig, Schwarz, and McLean 2006). Variations in some key intermediary metabolic enzymes owing to alterations in the dietary components recorded in the present study might suggest high metabolic adaptability of this species to the varying dietary protein and lipid levels. Moreover, information generated on growth, body composition, and digestive as well as metabolic function of the butter catfish fingerlings might aid in the formulation of practical diets for captive rearing of the highly valued catfish *O. bimaculatus*.

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References

Ali, M. Z., and K. Jauncey. 2004. Optimal dietary carbohydrate to lipid ratio in African catfish *Clarias gariepinus* (Burchell 1822). *Aquaculture International* 12 (2):169–80. doi:10.1023/B:AQUI.0000032065.28059.5b.

American Public Health Association (APHA). 2012. *Standard methods for the examination of water and wastewater*. 22nd. Washington D.C: American Public Health Association/ American Water Works Association.

Anson, M. L. 1938. The estimation of Pepsin, trypsin, papain and cathepsin with haemoglobin. *Journal of General Physiology* 22 (1):79–89. doi:10.1085/jgp.22.1.79.

Association of Official Analytical Chemists (AOAC). 2005. *Official methods of analysis*. 18th. USA: Association of Official Analytical Chemists.

Banik, S., P. Goswami, and S. Malla. 2012. Studies on breeding physiology of *Ompok bimaculatus* (Bloch, 1794) in Tripura. *Uttar Pradesh Journal of Zoology* 32:6772.6772.

Bernfeld, P. 1955. Amylase (α) and (β). In *Methods in enzymology*, (ed.). S. P. Colowick and N. O. Kaplan, Vol. 1, 149–50. New York: Academic Press.

Bier, M. 1955. Lipases. In *Methods in enzymology*, (ed.). S. P. Colowick and N. O. Kaplan, Vol. 1, 627–42. New York: Academic Press.

BIS. 2014. IS 16150: Fish feed specifcations part 2: Catfsh feed. *Bureau of Indian Standards*, New Delhi, India. https://bis.gov.in

Biswas, A., M. Pramanik, S. Kundu, S. Roy, J. De, and A. K. Ray. 2006. Alterations in enzyme activities in vital organs of triploid female catfish *Heteropneustes fossilis* (Bloch). *Fish Physiology and Biochemistry* 32 (1):73–81. doi:10.1007/s10695-006-7657-0.

Biswas, P., P. Rawat, A. B. Patel, and A. K. Jena. 2019. Dietary supplementation of L-tryptophan: Effect on growth and survival of Pabda, *Ompok bimaculatus* (Bloch) fry. *Journal of Applied Aquaculture* 31 (4):322–36. doi:10.1080/10454438.2018.1545721.

Biswas, P., A. K. Jena, A. B. Patel, and P. K. Pandey. 2020. Dietary protein requirement of Indian Butter catfish, *Ompok bimaculatus* (Bloch) fingerlings. *Journal of Applied Aquaculture* 32 (2):107–23. doi:10.1080/10454438.2019.1633984.

Chakrabarti, P. P., S. C. Mondal, D. N. Chattopadhaya, R. N. Mandal, and B. N. Paul. 2012. *Pabda-Seed production & culture*. Bhubaneswar, India: CIFA.

Chen, J. M., J. Y. Ye, Q. Pan, B. Q. Shen, and Y. H. Wang. 2010. Effect of dietary protein levels on growth performance and whole body composition of summerling and winterling spotted barbel (*Hemibarbus maculates* Bleeker). *Aquaculture Nutrition* 16 (4):412–18. doi:10.1111/j.1365-2095.2009.00680.x.

Cowey, C. B. 1993. Some effects of nutrition on flesh quality of cultured fish. In *Fish Nutrition in Practice*, ed. S. J. Kaushik and P. Luquet, 227. Paris: INRA.

Craig, S. R., M. H. Schwarz, and E. McLean. 2006. Juvenile cobia (*Rachycentron canadum*) can utilize a wide range of protein and lipid levels without impacts on production characteristics. *Aquaculture* 261 (1):384–91. doi:10.1016/j.aquaculture.2006.08.004.

De Silva, S. S., R. M. Gunasekera, and K. F. Shim. 1991. Interactions of varying dietary protein and lipid levels in young red tilapia: Evidence of protein sparing. *Aquaculture* 95 (3–4):305–18. doi:10.1016/0044-8486(91)90096-P.

Debnath, C., K. Dube, N. Saharan, V. K. Tiwari, M. Datta, L. Sahoo, G. S. Yadav, and P. Das. 2016. Growth and production of endangered Indian Butter catfish, *Ompok bimaculatus* (Bloch) at different stocking densities in earthen ponds. *Aquaculture Research* 47 (10):3265–75. doi:10.1111/are.12780.

Driedzic, R. W., and V. M. F. Almeida-Val. 1996. Enzymes of cardiac energy metabolism in amazonian teleosts and fresh-water stingray (*Potamotrygon hystrix*). *Journal of Experimental Zoology* 274 (6):327–33. doi:10.1002/(SICI)1097-010X(19960415)274:6<327::AID-JEZ1>3.0.CO;2-Q.

Fernández, F., A. G. Miquel, M. Córdoba, M. Varas, I. Metón, A. Caseras, and I. V. Baanante. 2007. Effects of diets with distinct protein-to-carbohydrate ratios on nutrient digestibility, growth performance, body composition and liver intermediary enzyme activities in gilthead sea bream (*Sparus aurata* L.) fingerlings. *Journal of Experimental Marine Biology and Ecology* 343 (1):1–10. doi:10.1016/j.jembe.2006.10.057.

Fiske, C. H., and Y. Subbarow. 1925. The colorimetric determination of phosphorus. *Journal of Biological Chemistry* 66 (2):375–400. doi:10.1016/S0021-9258(18)84756-1.

Freeland, R. A., and A. L. Harper. 1959. The study of metabolic pathway by means of adaptation. *Journal of Biological Chemistry* 234 (6):135054.135054.

Ghosh, K., and S. Mondal. 2015. Nutritional evaluation of groundnut oil cake in formulated diets for rohu, *Labeo rohita* (Hamilton) fingerlings after solid state fermentation with a tannase producing yeast, *Pichia kudriavzevii* (GU939629) isolated from fish gut. *Aquaculture Reports* 2:82–90. doi:10.1016/j.aqrep.2015.08.006.

Giri, S. S., S. K. Sahoo, A. K. Sahu, and P. K. Meher. 2003. Effect of dietary protein level on growth, survival, feed utilization and body composition of hybrid clarias catfish (*Clarias batrachus* × *Clarias gariepinus*). *Animal Feed Science and Technology* 104 (1–4):169–78. doi:10.1016/S0377-8401(02)00295-X.

Giri, S. S., S. M. Bokhtiar, S. K. Sahoo, B. N. Paul, and S. S. Mohanty. 2019. *Aquaculture of Commercially Important Finfishes in South Asia*. Dhaka, Bangladesh: SAARC Agriculture Centre SAARC.

Guerreiro, I., H. Peres, M. Castro-Cunha, and A. Oliva-Teles. 2012. Effect of temperature and dietary protein/lipid ratio on growth performance and nutrient utilization of juvenile Senegalese sole (*Solea senegalensis*). *Aquaculture Nutrition* 18 (1):98–106. doi:10.1111/j.1365-2095.2011.00884.x.

Gupta, S. 2015. An overview on feeding habit, reproductive biology and induced breeding of *Ompok bimaculatus* (Bloch, 1794). *European Journal of Biological Sciences* 7 (4):147–53. doi:10.5829/idosi.ejbs.2015.7.04.95163.

Hsu, R. Y., and H. A. Lardy. 1967. Pigeon Liver malic enzyme II Isolation, characterization and some properties. *Journal of Biological Chemistry* 242 (3):520–26. doi:10.1016/S0021-9258(18)96304-0.

Huang, J. F., Z. G. Zhao, and L. Luo. 2013. Effects of water temperature and dietary protein level on intestinal digestive enzyme activities of juvenile Songpu Mirro carp (*Cyprinus specularis Songpu*). *Chinese Journal of Animal Nutrition* 25:651–60.

IUCN. 2014. IUCN red list of threatened species. Version. Accessed 3, 2014. www.iucnredlist.org

Kim, L. O., and S. M. Lee. 2005. Effects of the dietary protein and lipid levels on growth and body composition of bagrid catfish, *Pseudobagrus fulvidraco*. *Aquaculture* 243 (1–4):323–29. doi:10.1016/j.aquaculture.2004.11.003.

Kim, S. S., and K. J. Lee. 2009. Dietary protein requirement of juvenile tiger puffer (*Takifugu rubripes*). *Aquaculture* 287 (1–2):219–22. doi:10.1016/j.aquaculture.2008.10.021.

Kornberg, A., and B. L. Horecker. 1955. Glucose-6-phosphate dehydrogenase. In *Methods in enzymology*, ed. S. P. Colowick and N. O. Kaplan, 323–33. New York, USA: Academic Press.

Lakra, W. S., U. K. Sarkar, A. Gopalakrishnan, and A. K. Pandian. 2010. *Threatened freshwater fishes of India*. Lucknow, India: NBFGR.

Lee, Y. L., and M. A. Lardy. 1965. Influence of thyroid hormones on L α-glycerophosphate dehydrogenases in various organs of rat. *Journal of Biological Chemistry* 240 (3):1427–36. doi:10.1016/S0021-9258(18)97593-9.

Lee, S. M., I. G. Jeon, and J. Y. Lee. 2002. Effects of digestible protein and lipid levels in practical diets on growth, protein utilization and body composition of juvenile rockfish (*Sebastes schlegeli*). *Aquaculture* 211 (1):227–39. doi:10.1016/S0044-8486(01)00880-8.

Lee, S. M., and K. D. Kim. 2005. Effect of various levels of lipid exchanged with dextrin at different protein level in diet on growth and body composition of juvenile flounder *Paralichthys olivaceus*. *Aquaculture Nutrition* 11 (6):435–42. doi:10.1111/j.1365-2095.2005.00372.x.

Li, X. F., W. B. Liu, Y. Y. Jiang, H. Zhu, and X. P. Ge. 2010. Effects of dietary protein and lipid levels in practical diets on growth performance and body composition of blunt snout bream (*Megalobrama amblycephala*) fingerlings. *Aquaculture* 303 (1–4):65–70. doi:10.1016/j.aquaculture.2010.03.014.

Lim, C., M. Yildirim-Aksoy, M. H. Li, T. L. Welker, and P. H. Klesius. 2009. Influence of dietary levels of lipid and vitamin E on growth and resistance of Nile tilapia to Streptococcus *iniae* challenge. *Aquaculture* 298 (1):76–82. doi:10.1016/j.aquaculture.2009.09.025.

Liu, X., K. Liu, G. Yao, X. Feng, and S. Guo. 2011. Effects of different dietary protein levels on growth and muscle nutrient components of hybrid F1 from *Channa maculates* (♀) × *Channa argus* (♂). *Acta Agriculturae Jiangxi* 23 (4):144–47. In Chinese with English abstract.

Lovell, R. T. 1989. *Nutrition and feeding of fish*. New York: Van Nostrand Reinhold. doi:10.1007/978-1-4757-1174-5.

Lowry, O. H., N. J. Ronebrough, A. L. Farr, and R. J. Randell. 1951. Protein measurement with Folin phenol reagent. *Journal of Biological Chemistry* 193 (3):265–76. doi:10.1016/S0021-9258(19)52451-6.

Lundstedt, L. M., J. F. B. Melo, and G. Moraes. 2004. Digestive enzymes and metabolic profile of *Pseudoplatystoma corruscans* (Teleostei: Siluriformes) in response to diet composition. *Comparative Biochemistry and Physiology Part B* 137 (3):331–39. doi:10.1016/j.cbpc.2003.12.003.

Luo, Z. H. I., Y. J. Liu, K. S. Mai, L. X. Tian, D. H. Liu, X. Y. Tan, and H. Z. Lin. 2005. Effect of dietary lipid level on growth performance, feed utilization and body composition of grouper Epinephelus coioides juveniles fed isonitrogenous diets in floating netcages. *Aquaculture International* 13 (3):257–69. doi:10.1007/s10499-004-2478-6.

Ma, X., Y. Hu, X. Q. Wang, Q. H. Ai, Z. G. He, F. X. Feng, and X. Y. Lu. 2014. Effects of practical dietary protein to lipid levels on growth, digestive enzyme activities and body composition of juvenile rice field eel (*Monopterus albus*). *Aquaculture International* 22 (2):749–60. doi:10.1007/s10499-013-9703-0.

Malla, S., and S. Banik. 2015. Reproductive biology of an endangered catfish, *Ompok bimaculatus* (Bloch, 1794) in the lotic waterbodies of Tripura, North-East India. *International Journal of Fisheries and Aquatic Studies* 2 (4):251–60.

Marjorie, A. S. 1964. Glucose-6-phosphatase from liver. In *Methods in enzymology*, (ed.). S. P. Colowick and N. O. Kaplan, Vol. II, 541–543. New York: Academic Press Inc.

Mathis, N., C. Feidt, and J. Brun-Bellut. 2003. Influence of protein/energy ratio on carcass quality during the growing period of Eurasian perch (*Perca fluviatilis*). *Aquaculture* 217 (1–4):453–64. doi:10.1016/S0044-8486(02)00122-9.

Maynard, L., J. Loosil, H. Hintz, and R. Warner. 1979. *Animal Nutrition*. 7th ed. New York, USA: McGraw: McGrawHill.

Mohanta, K. N., S. N. Mohanty, J. K. Jena, and N. P. Sahu. 2008. Protein requirement of silver barb, *Puntius gonionotus* fingerlings. *Aquaculture Nutrition* 14 (2):143–52. doi:10.1111/j.1365-2095.2007.00514.x.

Murphy, G., and D. G. Walker. 1974. Enzyme synthesis in the regulation of hepatic 'Malic' enzyme activity. *Biochemical Journal* 144 (1):149–60. doi:10.1042/bj1440149.

National Research Council. 2011. *Nutrient requirements of fish*. Washington, DC, USA: National Academy Press.

Pandolfi, P. P., F. Sonati, R. Rivi, P. Manson, F. Grosveld, and L. Luzzatto. 1995. Targeted disruption of the house keeping genes encoding Glucose 6-phosphate dehydrogenase (G6PD):G6PD is dispensable for pentose synthesis but essential defense against oxidative stress. *EMBO Journal* 14 (21):5209–15. doi:10.1002/j.1460-2075.1995.tb00205.x.

Paul, B. N., A. K. Datta, S. Das, D. N. Chattopadhyay, S. S. Giri, and S. N. Mohanty. 2011. Effect of dietary lipid levels on growth and carcass composition of *Ompok pabda* (*Siluridae*) fry. *Indian Journal of Animal Nutrition* 28 (2):198–202.

Paul, B. N., A. Das, S. Bhowmick, R. N. Mandal, P. Singh, S. Adhikari, D. Chowdhury, K. Ghosh, P. P. Chakrabarti, and S. S. Giri. 2020a. Protein requirement of *Ompok bimaculatus* (1794) larvae. *Animal Nutrition and Feed Technology* 20 (3):525–33. doi:10.5958/0974-181X.2020.00046.3.

Paul, B. N., A. Das, R. N. Mandal, P. Singh, S. Adhikari, P. P. Chakrabarti, K. Ghosh, and S. S. Giri. 2020b. Proximate and fatty acid profile of brood fish, egg and larvae of *Ompok bimaculatus*. *Indian Journal of Animal Health* 59 (2):205–09. doi:10.36062/ijah.59.2.2020.205-209.

Paul, B. N., D. Chowdhury, A. Das, R. N. Mandal, P. Singh, S. Adhikari, P. P. Chakrabarti, S. S. Giri, and K. Ghosh. 2021. Effect of dietary lipid levels on growth, body composition, and enzyme activities of larvae of butter catfsh, *Ompok bimaculatus* (Actinopterygii: Siluriformes: Siluridae). *Acta Ichthyologica et Piscatoria* 51 (3):289–98. doi:10.3897/aiep.51.67079.

Pérez-Jiménez, A., G. Cardenete, A. E. Morales, A. G. Alcazar, E. Abellan, and M. C. Hidalgo. 2009. Digestive enzymatic profile of Dentex dentex and response to different dietary formulations. *Comparative Biochemistry and Physiology Part A* 154 (1):157–64. doi:10.1016/j.cbpa.2009.05.126.

Rahimnejad, S., I. C. Bang, J. Y. Park, A. Sade, J. Choi, and S. M. Lee. 2015. Effects of dietary protein and lipid levels on growth performance, feed utilization and body composition of juvenile hybrid grouper, *Epinephelus fuscoguttatus* × *E. lanceolatus*. *Aquaculture* 446:283–89. doi:10.1016/j.aquaculture.2015.05.019.

Raizada, S., K. K. Lal, U. K. Sarkar, P. K. Varshney, V. Sahu, K. C. Yadav, and J. K. Jena. 2013. Captive breeding and embryonic development of butter catfish (*Ompok bimaculatus*, Bloch 1794), a threatened fish of Indian sub-continent in North India. *Proceedings of the National Academy of Sciences. India Section B* 83 (3):333–39. doi:10.1007/s40011-013-0156-z.

Reitman, S., and S. Frankel. 1957. A colorimetric method for the determination of serum glutamic α-oxaloacetic and glutamic pyruvic transaminases. *American Journal of Clinical Pathology* 28 (1):56–63. doi:10.1093/ajcp/28.1.56.

Sá, R., P. Pousão-Ferreira, and A. Oliva-Teles. 2006. Effect of dietary protein and lipid level on growth and feed utilization of White sea bream (*Diplodus sargus*) juveniles. *Aquaculture Nutrition* 12 (4):310–21. doi:10.1111/j.1365-2095.2006.00434.x.

Sá, R., P. Pousão-Ferreira, and A. Oliva-Teles. 2007. Growth performance and metabolic utilization of diets with different protein: Carbohydrate ratios by white sea bream (*Diplodus sargus*, L.) juveniles. *Aquaculture Research* 38 (1):100–05. doi:10.1111/j.1365-2109.2006.01629.x.

Sagada, G., J. Chen, B. Shen, A. Huang, L. Sun, J. Jiang, and C. Jin. 2017. Optimizing protein and lipid levels in practical diet for juvenile northern snakehead fish (*Channa argus*). *Animal Nutrition* 3 (2):156–63. doi:10.1016/j.aninu.2017.03.003.

Santos, W. M., L. S. Costa, J. F. López-Olmeda, N. C. S. Costa, F. A. C. Santos, C. G. Oliveira, H. O. Guilherme, R. N. Bahiense, R. K. Luz, and P. A. P. Ribeiro. 2020. Dietary protein modulates digestive enzyme activities and gene expression in red tilapia juveniles. *Animal* 14 (9):1802–10. doi:10.1017/S1751731120000543.

Shapawi, R., I. Ebi, A. S. K. Yong, and W. K. Ng. 2014. Optimizing the growth performance of brown- marbled grouper, *Epinephelus fuscoguttatus* (Forskal), by varying the proportion of dietary protein and lipid levels. *Animal Feed Science and Technology* 191:98–105. doi:10.1016/j.anifeedsci.2014.01.020.

Steffens, W. 1989. *Principles of fish nutrition*. Chichester, England: Ellis Horwood Ltd.

Susan, T. A., K. Sobha, K. Veeraiah, and K. S. Tilak. 2010. Studies on biochemical changes in the tissues of *Labeo rohita* and *Cirrhinus mrigala* exposed to fenvalerate technical grade. *Journal of Toxicology and Environmental Health Sciences* 2 (5):53–62. doi:10.5897/JTEHS.9000064.

Tranulis, M. A., O. Dregni, B. Christophersen, A. Krogdahl, and B. Borrebaek. 1996. A glucokinase-like enzyme in the liver of atlantic salmon (*Salmo salar*). Comp. *Comparative Biochemistry and Physiology Part B* 114 (1):35–39. doi:10.1016/0305-0491(95)02119-1.

Tu, Y., S. Xie, D. Hana, Y. Yang, J. Jin, and H. Liua. 2015. Growth performance, digestive enzyme, transaminase and GH-IGF-I axis gene responsiveness to different dietary protein levels in brood stock allogenogynetic gibel carp (*Carassius auratusgibelio*) CAS III. *Aquaculture* 446:290–97. doi:10.1016/j.aquaculture.2015.05.003.

Tuan, L. A., and K. C. Williams. 2007. Optimum dietary protein and lipid specifications for juvenile malabar grouper (*Epinephelus malabaricus*). *Aquaculture* 267 (1–4):129–38. doi:10.1016/j.aquaculture.2007.03.007.

Walter, H. E. 1984. *Methods of enzymatic analysis*. Weinheim: Verlag Chemie.

Wang, Y. Y., G. J. Ma, Y. Shi, D. S. Liu, J. X. Guo, Y. H. Yang, and C. D. Chen. 2013. Effects of dietary protein and lipid levels on growth, feed utilization and body composition in *Pseudobagrus ussuriensis* fingerlings. *Aquaculture Nutrition* 19 (3):390–98. doi:10.1111/j.1365-2095.2012.00972.x.

Wang, J., Y. Jiang, X. Li, T. Han, Y. Yang, S. Hu, and Y. Min. 2016. Dietary protein requirement of juvenile red spotted grouper (*Epinephelus akaara*). *Aquaculture* 450:289–94. doi:10.1016/j.aquaculture.2015.08.007.

Wang, C., G. Hu, P. Sun, W. Gu, B. Wang, Q. Xu, and H. Liu. 2018. Effects of dietary protein at two lipid levels on growth, gonadal development, body composition and liver metabolic enzymes of brown trout (*Salmo trutta fario*) broodstock. *Aquaculture Nutrition* 24 (5):1587–98. doi:10.1111/anu.12795.

Williams, K. C. 2007. Optimum dietary protein and lipid specifications for juvenile malabar grouper (*Epinephelus malabaricus*). *Aquaculture* 267 (1):129–38. doi:10.1016/j.aquaculture.2007.03.007.

Worthington, C. C. 1991. *Worthigton Enzyme Manual Related Biochemical*. 3th. Freehold, NJ: Worthington Biochemical Corporation.

Wu, X. Y., and D. M. Gatlin III. 2014. Effects of altering dietary protein content in morning and evening feedings on growth and ammonia excretion of red drum (*Sciaenops ocellatus*). *Aquaculture* 434:33–37. doi:10.1016/j.aquaculture.2014.07.019.

Ye, W., D. Han, X. Zhu, Y. Yang, J. Jin, and S. Xie. 2015. Comparative studies on dietary protein requirements of juvenile and on-growing gibel carp (*Carassius auratus gibelio*) based on fishmeal-free diets. *Aquaculture Nutrition* 21 (3):286–99. doi:10.1111/anu.12156.

Zar, J. H. 2010. *Biostatistical analysis*. 5th ed. New Delhi, India: Pearson Education.

SL. NO. 14

DEVELOPMENT OF LARVAL DIET FOR *Ompok bimaculatus* A HIGH VALUE FISH OF REGIONAL IMPORTANCE

FINAL PROJECT REPORT

DURATION: 01/04/2017 - 31/03/2020

ICAR-Central Institute of Freshwater Aquaculture
(ISO 9001:2015 Certified Institute)
(Indian Council of Agricultural Research)
RRC,Rahara, 700118,West Bengal

ANNEXURE -VI

INDIAN COUNCIL OF AGRICULTURAL RESEARCH

CHECKLIST FOR SUBMISSION OF FINAL RESEARCH PROJECT REPORT (RPP-III)

(For Guidelines Refer ANNEXURE – XI (F))

1. Institute Project Code: I-95-G

2.Investigators as approved in RPP-I, If any change attach IRC proceedings:

Principal Investigator	CC-PI	Co-PI
Dr. B.N.Paul		Dr. S Adhikar Dr.P.P.Chakrabarti Dr. N Mandal Mr. A Das Dr. K Ghosh (Burdwan University)

3.Any change in objectives and activities No
(If yes, attach IRC proceedings)

	Date of Start & Date of Completion (Actual). If any extension granted enclose IRC proceedings		Yes	
	Whether all objectives met		Yes	
	All activities completed		Yes	
	Salient achievements/major recommendations included		Yes	
	Annual Progress Reports (RPP-II) submitted	1st Year	Yes	
		2nd Year	Yes	
		3rd Year	Yes	
		nth year		No
	Reprint of each of publication attached		Yes	

	Action for further pursuit of obtained results indicated	Yes	No
	Report presented in Divisional seminar (enclose proceedings & action taken report)	Yes	No
	Report presented in Institute seminar (enclose proceedings & action taken report)	Yes	
	IRC number in which the project was adopted	IRC No: I-95-G	
	Any other Information		

4.Signature:

Project Leader	Co-PI	Co-PI	Co-PI	Co-PI
(Dr. B.N. Paul)	(P.P. Chakrabarti)	(Dr. S. Adhikari)	(Dr. R.N. Mandal)	(Mr. A. Das)

Co-PI

(Dr. K. Ghosh)

HOD/PD/I/c.

ANNEXURE – VII

INDIAN COUNCIL OF AGRICULTURAL RESEARCH
FINAL RESEARCH PROJECT REPORT (RPP- III)
(For Guidelines Refer ANNEXURE – XI(G))

1. Institute Project Code : I-95-G
2. Project Title: **Development of Larval diet for *Ompok bimaculatus*, a high- valued fish of regional importance**
3. Key Words: *Ompok bimaculatus,*Ontogeny, larvae, egg, enzyme,larval diet and nutrient composition
4. (a) Name of the Lead Institute : ICAR-Central Institute of Freshwater Aquaculture,
5. (b) Name of Division/ Regional Center/ Section: Regional Research Center, ICAR-CIFA, Rahara

   (a) Name of the Collaborating Institute(s): University of Burdwan

   (b) Name of Division/ Regional Center/ Section of Collaborating Institute(s)

   Dept. of Zoology, Burdwan Rajbati, Raiganj, Burdwan, West Bengal 713104
6. Project Team(Name(s) and designation of PI, CC-PI and all project Co-PIs, with time spent)

Sl. No.	Name, designation and institute	Status in the project (PI/CC-PI/ Co-PI)	Time to be spent (%)	Work components assigned to individual scientist
1	Dr. B.N.Paul	PI	50	Planning, Nutrient analysis, evaluation of larval feed, data analysis and reporting
2.	Dr.P.P.Chakrabarti	Co-PI	25	Brood stock development of high value species
2	Dr. S Adhikari	Co- PI	25	Water quality parameters and reporting.
3	Dr. R.N Mandal	Co-PI	25	Production of Live food organisms ,data analysis and reporting

4	Mr. A Das	Co-PI	25	Production of larvae, Evaluation of larval feed, data analysis and reporting
Collaborating University: University of Burdwan, West Bengal				
5.	Dr. K. Ghosh	Co-PI	25	Ontogeny study of larvae and enzyme analysis.

7. Priority Area: Aquaculture Diversification.

8. Project Duration: Date of Start – **01.04.2017**

Date of Completion – **31.03.2020**

9. a. **Objectives**

1. To study nutrient composition of egg and different stages of larvae

2. To study the ontogeny of larval development.

3. To formulate larval feed and evaluation

b**. Practical utility**

Ompok bimaculatus, popularly known as the 'butter catfish', is a freshwater teleost species native to the South-East Asia. It is piscivorous as well as carnivorous fish. Due to its air-breathing nature, it could become a very good candidate species for stocking small, shallow seasonal ponds and tanks which are oxygen-deficient and could grow to required marketable size in just 6months. Over the years, the natural occurrence of this fish species has been depleted due to some anthropogenic activities and hence it has been listed under Near Threatened (NT) category of IUCN Red List (2010). This fish has a high commercial value and preferred in Eastern and North-Eastern India. *O. bimaculatus* was also declared as the State Fish of Tripura in the year 2007.The captive breeding of *O. bimaculatus* has opened a new road map for successful aquaculture of the species. During its metamorphosis, larval rearing is a big challenge. The larval stage is a critical stage in fish life cycle that necessitates an appropriate exogenous nutrition once the embryonic yolk is used up. Research has been carried out over the few decades to reduce the period over which live pray must be used, using better understanding of larval behavior and physiology and

improvement in microparticle formulation. The success of larval rearing is greatly influenced by first feeding regimes and the nutritional quality of starter diets. Thus to domesticate the species larval rearing is a bottleneck, especially the larval feed. Thus the present proposal is aimed at development of larval feed for *O. bimaculatus* so that the mortality will be reduced and more stocking material would be available for successful growout of *O. bimaculatus.*

Keeping in view of the above concept an attempt has been taken up to domesticate *Ompok bimaculatus* in terms of its understanding of larval nutrition and feed development.

Collaborating University: University of Burdwan

10. Final Report on the Project (materials and methods used, results and discussion, objective wise achievements and conclusions)

1. **Material** : *Ompok bimaculatus*

2. **Techniques/Methodology** :

3. The larval feed development for the *O. bimaculatus* is critical as the species requires exogenous nutrition once the embryonic yolk is exhausted. The development of exogenous larval feed is very essential at this stage. The study of ontogeny of larvae will give a guideline ragarding the enzyme status and metamorphosis of its digestive system. Co-feeding strategy to be evolved

for proper feeding of the larvae with natural and formulated feed. Further the exogenous formulated feed will be developed for the larvae.

4. Sample Analysis:

5. The samples were collected as per the method of Sankar (2010).The nutrient analysis of brood fish, egg and larvae and feed samples were done as per AOAC (2005). Water quality was studied as per APHA (2005).

6. Fatty acid analysis:

7. Extraction of pooled samples for fatty acid analysis was done as per Folch *et al*. (1957). Preparation of Fatty acid Methyl Esters (FAME) was done as per Metcalfe *et al.* (1966). The prepared samples were quantified by injecting into Gas Chromatograph (GC) (Perkin Elmer; CLARUS 480). GC operating software 'Total Chrom' was used and identification of individual Fatty Acid was done by comparison of retention time to those of standards (SUPELCO, Cat.No. 47885-U). Data were subjected to statistical analysis as per Snedecor and Cochran (1994) and the least significance difference (LSD) was used for comparison of the mean values.

Gas Chromatograph (GC) (Perkin Elmer; CLARUS 480).

8.

9. Enzyme assay: Digestive enzymes was assayed as per Reitman and Frankel (1957).

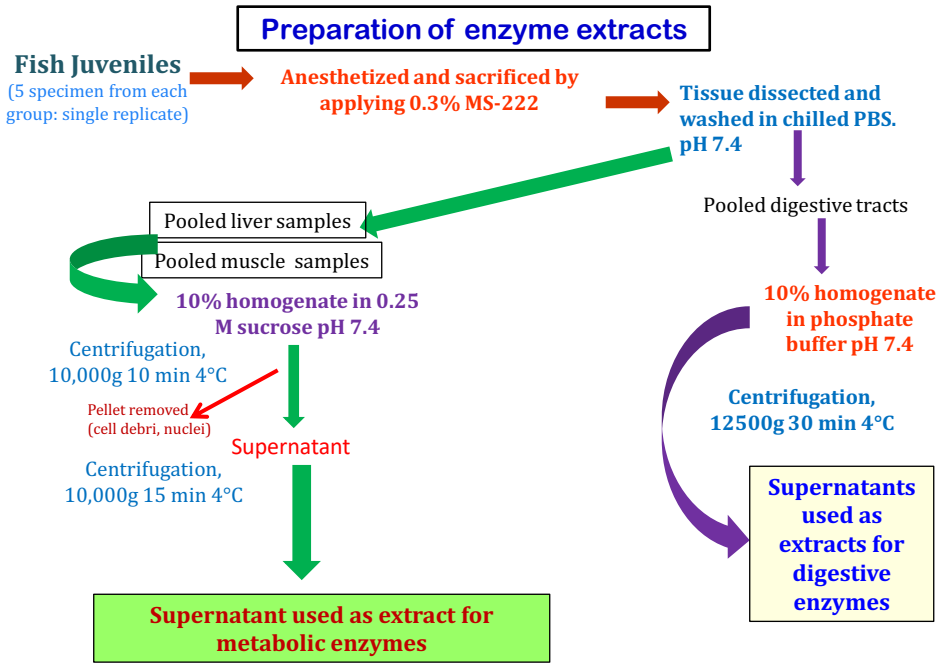
1. Protease, amylase and lipase activities was measured after Walter (1984), Bernfeld (1955) and Bier (1955), respectively
2. Estimation of trypsin and chymotrypsin activity according to Erlanger et al. (1961)
3. Apart from digestive enzymes, the following metabolic enzymes were studied as key enzymes of the major metabolic pathways:

Glucose 6 phosphate dehydrogenase (De Moss, 1955), cytosolic NADP malate dehydrogenase (Hsu and Lardy, 1967), mitochondrial NAD malate dehydrogenase (Englard and Siegel, 1969), alanine

transaminase (Reitman and Frankel,1957), aspartate transaminase (Reitman and Frankel, 1957), glucose-6-phosphatase (Marjoric, 1964), fructose- 1,6-bisphosphatase (Freeland and Harper, 1959) and hexokinase (Tranulis et al., 1996)

4. Changes in the profile of proteolytic enzymes through ontogeny was studied through separation and characterization of proteases by substrate SDS-PAGE after Garcıa-Carreno et al. (1993).

Average live weight gain (%), specific growth rate (SGR: % day−1), feed conversion ratio (FCR), protein efficiency ratio (PER) and apparent net protein utilization (ANPU%) were worked out as per (Steffens, 1989).



7

Objective1. To study nutrient composition of egg and different stages of larvae

Nutrient composition Egg, Brood fish and Larvae

The proximate and fatty acid composition of *Ompok bimaculatus* (pabda) brood fish, egg and larvae was assayed to have an insight of the nutrient metamorphosis during early life of the species and vis-à-vis parental nutrient composition. The collected brood fish, eggs and larvae were analyzed for proximate and fatty acid composition.

The brood fish and its egg were collected from ICAR-Central Institute of Freshwater Aquaculture, Kalyani field station. Soon after hatching the *O. bimaculatus* larvae were also collected from Kalyani Field Station. The collected samples of brood fish, egg and larvae of ompok were iced immediately and sent for analysis. Sampling procedure and sample preparation was done as per the standard methodology of Sankar *et al.* (2010).

The proximate composition of Pabda brood, egg and larvae are presented in Table 1. The data revealed that the crude protein, fat and ash content of brood fish, egg and larvae were 14.4±0.07, 25.90±0.20, 12.23±0.88; 1.06±0.07, 0.64±0.15, 0.42±0.04 and 2.33±0.05, 1.61±0.10 and 1.79±0.04 (%) respectively.. The studies revealed that the crude protein content was significantly ($P<0.01$) higher in pabda egg however moisture, crude lipid and total ash (%) was significantly ($P>0.01$) lower pabda egg. The result of the study also concluded that the moisture, crude lipid and total ash (%) was significantly ($P<0.01$) higher in pabda brood.

Table 1: Proximate composition of Brood pabda, egg and larvae

Particulars	Brood Pabda	Pabda Egg	Pabda Larvae
Moisture	80.09 ± 0.64^{b}	61.84 ± 1.40^{a}	82.02 ± 0.62^{b}
Crude Protein	14.44 ± 0.07^{a}	25.90 ± 0.20^{b}	12.23 ± 0.88^{a}
Crude Fat	1.06 ± 0.07^{b}	0.64 ± 0.15^{a}	1.61 ± 0.10^{a}
Total Ash	2.33 ± 0.05^{b}	1.61 ± 0.10^{a}	1.79 ± 0.04^{a}

Data presented as Mean± S.E. Superscripts ^{ab} in a row differs significantly ($P<0.01$)

Table 2: Fatty acid (% of total fatty acid) composition of Pabda brood, egg and larvae

Particulars	Pabda brood	Pabda Egg	Pabda Larvae
Butyric Acid	0.02 ± 0.01^{a}	0.01 ± 0.001^{a}	2.08 ± 0.05^{b}
Myristic acid (C14:0)	0.12 ± 0.01^{a}	0.22 ± 0.03^{a}	4.03 ± 0.57^{b}
Palmitic acid (C16:0)	87.81 ± 1.53^{b}	66.15 ± 0.77^{a}	22.52 ± 0.64^{a}
Arachidic acid (C20:0)	0.2 ± 0.03^{a}	0.05 ± 0.001^{a}	2.31 ± 0.12^{b}
Others	0.33 ± 0.02^{a}	9.59 ± 0.19^{b}	17.85 ± 0.52^{c}
ΣSFA	88.48 ± 0.66^{c}	76.02 ± 0.98^{b}	48.6 ± 0.66^{a}
Pentadecanoic acid (C15:1)	0.62 ± 0.05^{b}	0.4 ± 0.001^{a}	1.29 ± 0.015^{c}
Oleic acid (C18:1n9c)	0.61 ± 0.001^{a}	12.08 ± 0.09^{c}	4.29 ± 0.08^{b}
Elaidic acid (C18:1n9t)	6.03 ± 0.02^{a}	ND	11.02 ± 0.1^{b}
Others	0.41 ± 0.02^{a}	1.43 ± 0.07^{b}	1.42 ± 0.02^{b}
ΣMUFA	7.67 ± 0.12^{a}	13.91 ± 0.24^{b}	18.02 ± 0.02^{c}
Linolelaidic acid (C18:2n6t)	0.02 ± 0.01^{a}	0.06 ± 0.01^{a}	7.03 ± 0.08^{b}
Linoleic acid (C18:2n6c)	1.89 ± 0.09^{b}	4.25 ± 0.09^{c}	0.47 ± 0.01^{a}
ϒ-Linolenic acid (C18:3n6)	0.05 ± 0.01^{a}	0.43 ± 0.02^{b}	0.56 ± 0.02^{c}
α-Linolenic acid (C18:3n3)	0.1 ± 0.01^{a}	0.21 ± 0.01^{b}	1.58 ± 0.045^{a}
Eicosadienoic acid (C20:2)	0.28 ± 0.006^{a}	0.51 ± 0.01^{b}	1.4 ± 0.01^{c}
Eicosatrienoic acid (C20:3n6)	0.64 ± 0.02^{a}	1.68 ± 0.02^{b}	11.17 ± 0.06^{c}
Eicosatrienoic acid (C20: 3n3)	ND	0.05 ± 0.01^{a}	0.32 ± 0.02^{b}
Arachidonic acid (C20:4n6)	ND	0.07±0.01	ND
EPA (C20:5n3)	0.73 ± 0.03^{b}	0.23 ± 0.01^{a}	2.71 ± 0.01^{c}
DHA(C22:6n3)	0.8 ± 0.02^{a}	2.56 ± 0.04^{b}	6.87 ± 0.03^{c}
ΣPUFA	4.52 ± 0.03^{a}	10.05 ± 0.02^{b}	33.35 ± 0.40^{c}
ω 3	1.63 ± 0.06^{a}	3.05 ± 0.04^{b}	11.5 ± 0.02^{c}
ω 6	2.6 ± 0.07^{a}	6.49 ± 0.08^{b}	19.23 ± 0.01^{c}

Data presented as Mean± S.E. Superscripts ^{abc} in a row differs significantly ($P<0.05$)

ND: Not detected

Haul of pabda brood fish at Kalyani Field Station

Brood fish (*O. bimaculatus*)

***O.bimaculatus* brood fish**

The fatty acid profile obtained by GC analysis are presented in Table 2. The fatty acid composition revealed that the saturated fatty acid (SFA) content (% of total fatty acid) of brood pabda, eggs and larvae were 88.48%; 76.04% and 48.60%. Among the MUFA, oleic acid and elaidic acid was significantly ($P<0.05$) higher in pabda larvae. The poly unsaturated fatty acid (PUFA) content (% of total fatty acid) of brood pabda, eggs and larvae were 4.5%; 10.05% and 33.35% respectively in brood fish, egg and larva. The EPA and DHA contents were 2.71% and 6.87% in larve; which was significantly ($P<0.05$) higher in comparison to brood fish (EPA-0.73% and DHA-0.80%) and egg (EPA-0.23% and DHA-2.56%). Among PUFA, total ω-3 content was 1.63, 3.05 and 11.5 (%) respectively in pabda brood, egg and larvae

The studies regarding nutritional characterization of brood fish, eggs and larvae of *Ompok bimaculatus* revealed that crude protein content was higher in egg whereas crude fat and ash content was higher in brood fish. However, PUFA, EPA and DHA content was higher in larvae.

Objective 2. To study the ontogeny of larval development.

The Ontogeny of *Ompok bimaculatus* was studied with the hatched larvae at ICAR-CIFA, Kalyani field station.he ontogeny and enzyme study was done at Burdwan University, which is the collaborating University in the project.The digestive tract of *O. bimaculatus* comprised of a straight tube dorsally attached to the yolk sac. Table 3. Describes the ontogeny development of *O.bimaculattus* larvae. It was observed that at 2 day post hatching (dph) mouth opened, teeth were visible and exogenous feeding started. The yolk sac disappeared within 3 dph. In course of the study, *O. bimaculatus* larvae were fed *Artemia* nauplii from 2 dph mixed zooplankton and chopped Tubifex during 4–7 dph, and only chopped Tubifex from 7 dph onwards.

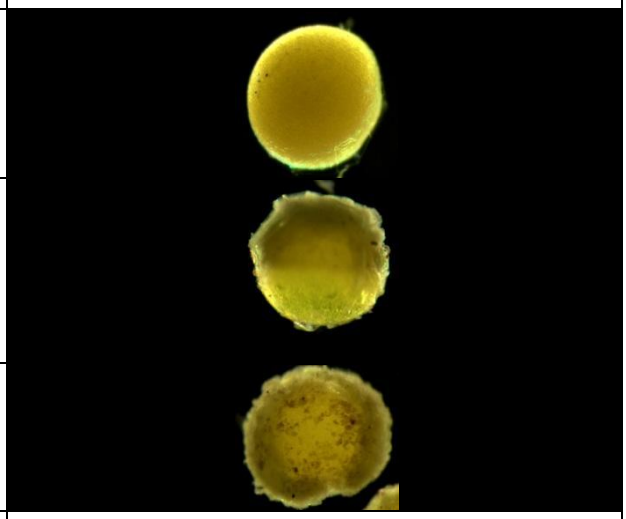
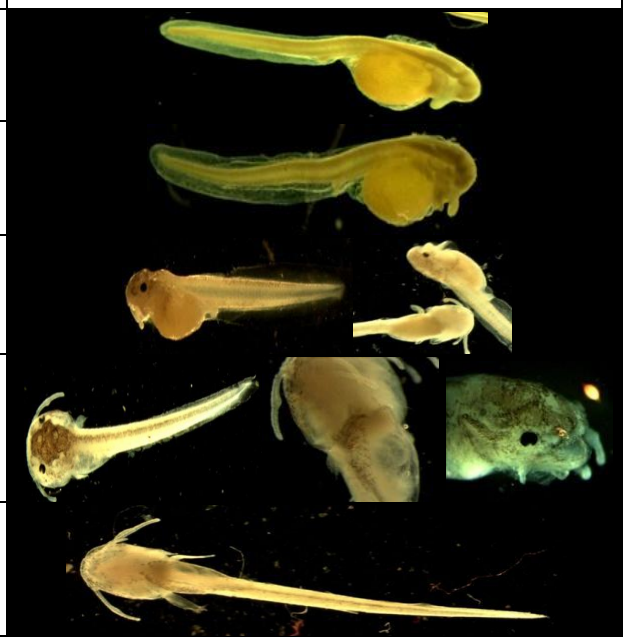
Catching of hatched out *O.bimaculatus* larvae

Collection of *O. bimaculatus* larvae

Collection of *O. bimaculatus* larvae

Collection of *O. bimaculatus* larvae

Table 3. Ontogeny: study of morphology

Time	Observation	Photograph(s)
3 hour post fertilization (HPF)	Gastrulation starts	
5:30 HPF	Dorsal (prospective head) and ventral (prospective tail) ends cover half of the egg; embryonic shield observed; ectoderm expands through epiboly	
8 HPF	Head and tail buds appear close together and cover entire yolk	
11 HPF	First movement starts near anal region; movement proceeds through tail region and tail detaches from yolk; eye spot becomes prominent.	
14 HPF	Embryo rotates circularly and outer layer of egg expands; anterior portion of head detaches from yolk	
15:30 HPF	Tail becomes visible outside the egg case	
17 HPF	Hatching starts and larva emerges from egg case; larval weight 0.5 mg, length 0.3 cm	
6 hour post hatching (HPH)	Anal opening becomes prominent and head becomes condensed	
1 day post hatching (DPH)	Larva with yolk sac and small barbells; weight 0.9 mg, length 0.5cm	
2 DPH	Yolk sac almost absorbed and mouth opens, teeth become visible; weight 1.20 mg, length 0.5-0.6 cm	
3 DPH	Weight 1.45 mg, length 0.8cm	

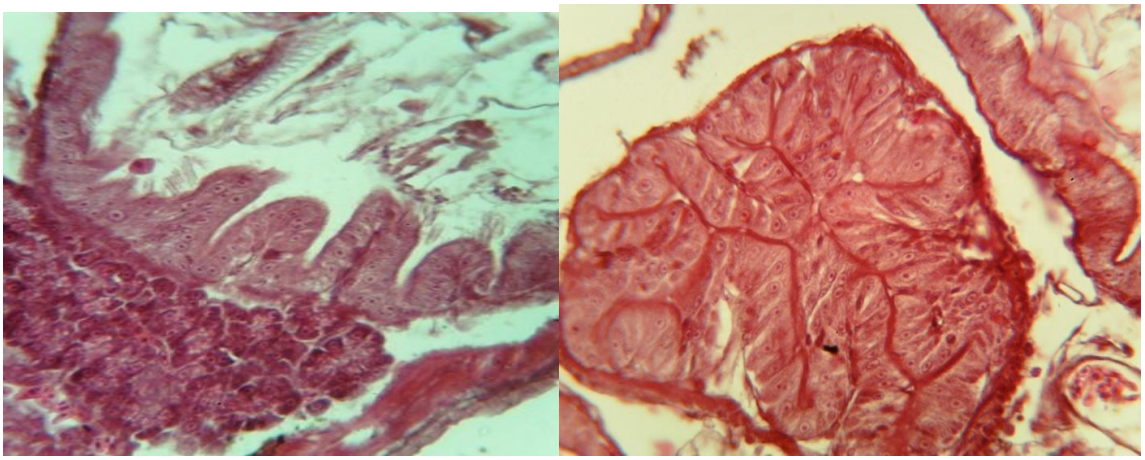
4 DPH	Fin rays become prominent; weight 3.4 mg, length 1 cm	
8 DPH	Intestine coiled and stomach divided; weight 62.6 mg, length 1.4 cm	
12 DPH	Weight 92.4 mg, length 1.7 cm, barbell 0.3 cm, gape of mouth 0.2 cm	
16 DPH	Weight 131.1 mg, length 2.4 cm, barbell 0.6 cm, gape of mouth 0.3 cm	
20 DPH	Weight 141.4 mg, length 3.2 cm, barbell 0.8 cm, gape of mouth 0.4 cm	
24 DPH	Weight 214.4 mg, length 4.5cm, barbell 1.0 cm, gape of mouth 0.6 cm	

Sample collection at Kalyani Field station

Sample preparation for enzyme analysis

Enzyme assay at the University of Burdwan

Figure 1. Ontogeny: histological study



A **B**

Fig: (A) Section of intestinal villi with food particles and (B) section of non glandular stomach at 7 day post hatching

Table 4. Digestive enzymes activities of *O. bimaculatus* from day 4 to day 24.

Day post hatching	Amylase	Total Protease	Trypsin	Chymotrypsin	Pepsin	Lipase
4	12.73±0.62	2.19±0.15	0.45±0.02	0.26±0.02	0.09±0.08	1.89±0.02
8	14.56±0.73	1.40±0.20	0.60±0.02	0.48±0.02	0.26±0.01	2.17±0.11
12	19.53±0.78	3.31±0.14	1.29±0.04	0.87±0.03	0.34±0.02	8.0±0.12
16	13.76±0.63	1.59±0.21	1.01±0.04	0.67±0.02	0.57±0.02	4.84±0.08
20	9.98±0.42	1.80±0.12	1.38±0.04	0.72±0.03	0.74±0.02	5.16±0.06
24	15.78±0.75	2.43±0.08	1.58±0.05	0.92±0.06	1.19±0.53	5.92±0.06

1. Trypsin and chymotrypsin activities (U) = 1µmol of 4-nitroaniline liberated min^{-1} mg^{-1} protein
2. Lipase activity (U) = 1µmol of free fatty acid liberated min^{-1} mg^{-1} protein
3. α-amylase activity (U) = µg maltose liberated min^{-1} mg^{-1} protein
4. Protease activity (U) = µg tyrosine liberated min^{-1} mg^{-1} protein
5. Pepsin activity (U) = µg tyrosine liberated min^{-1} mg^{-1} protein

Figure 2. Protein enzymes activity of *O. bimaculatus* larvae

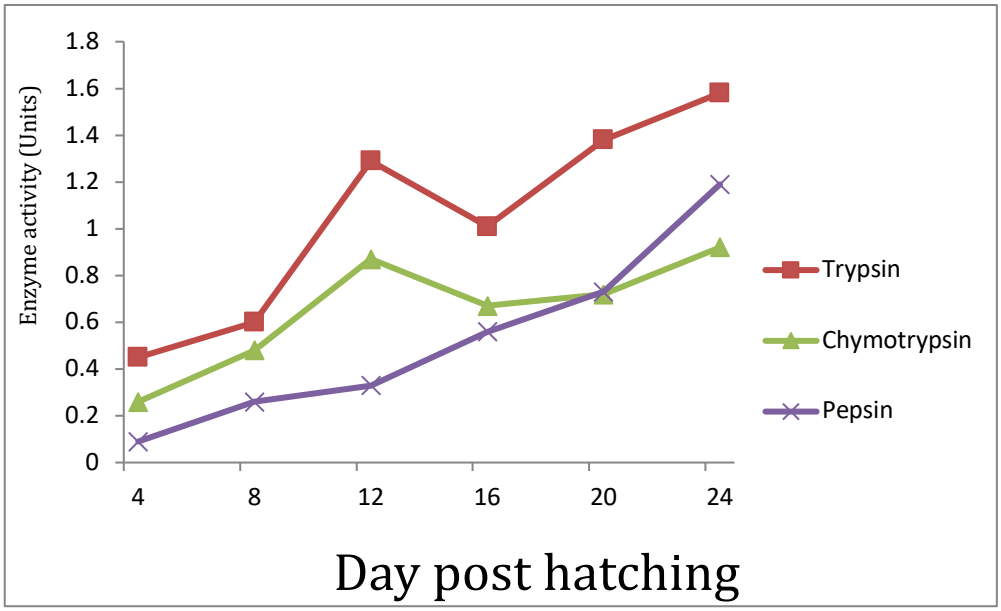


Figure 3. Digestive enzymes activity of *O. bimaculatus* larvae

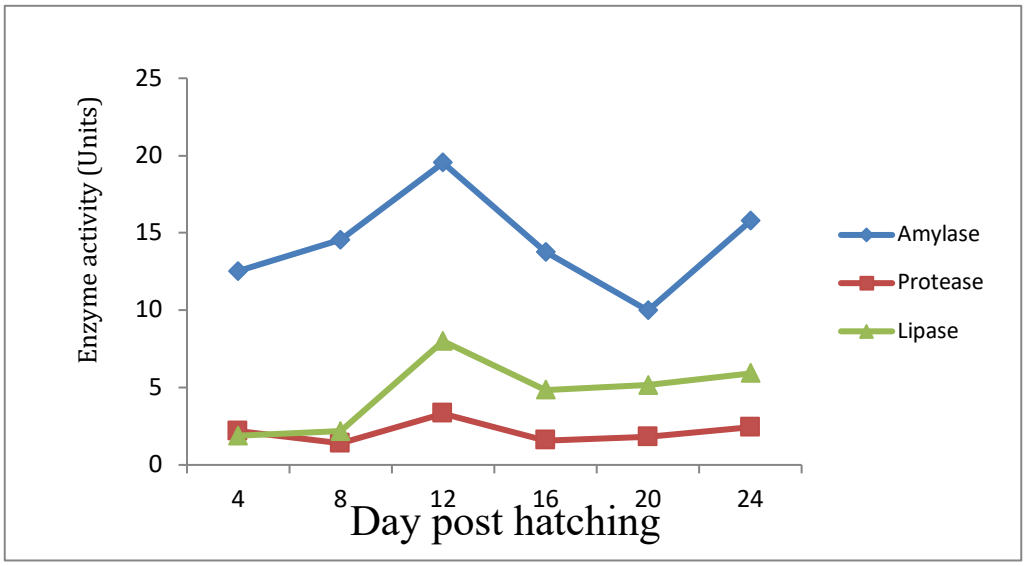


Figure 2 & 3 and table 4 describes the protein and digestives enzymes activity of *O. bimaculatus* larvae from day 4 to 24. Ther is gradual increase in the activity of the acid protease (pepsin) was noticed after formation of stomach, i.e. 8th day onward. Activities of amylase, lipase, trypsin and chymotrypsin were also detected in course of development post hatching

The activity of digestive enzymes indicated that enzymes involved in the digestion of proteins, lipids and carbohydrates were present in *O. bimaculatus* larvae since hatching (Figure 4 & 5), except pepsin. Coiling of intestine and presence of stomach were noticed by 8 dph, which was marked with the appearance of pepsin. Activities of the amylase, trypsin and chymotrypsin were increased initially after the onset of exotrophic phase, and decreased thereafter during 12 to 20 dph corresponding to the increase of pepsin. Thus, a change in the digestive physiology was indicated by progressive shift in the activity from alkaline (trypsin and chymotrypsin) to acid (pepsin) proteases.

Figure 4. Digestive Enzymes activities of *O. bimaculatus* larvae

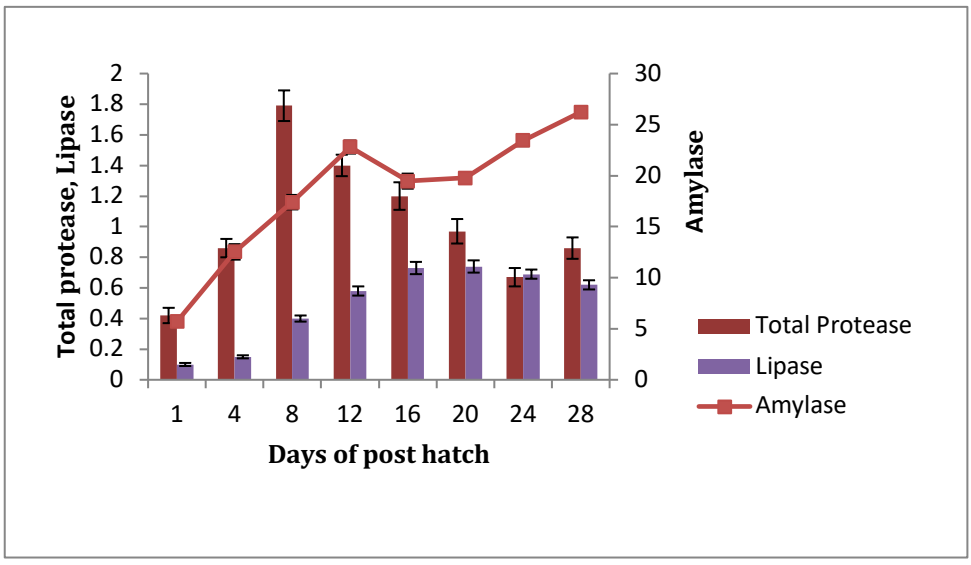


Figure 5. Protein enzymes activities of larvae

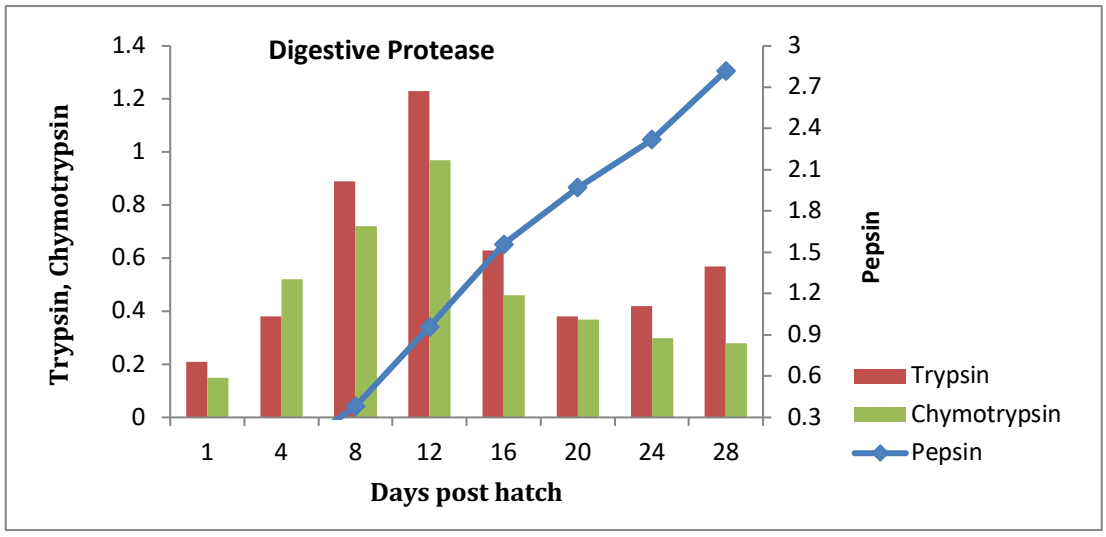


Figure 6. Carbohydrate metabolism enzymes of *O. bimaculatus* larvae

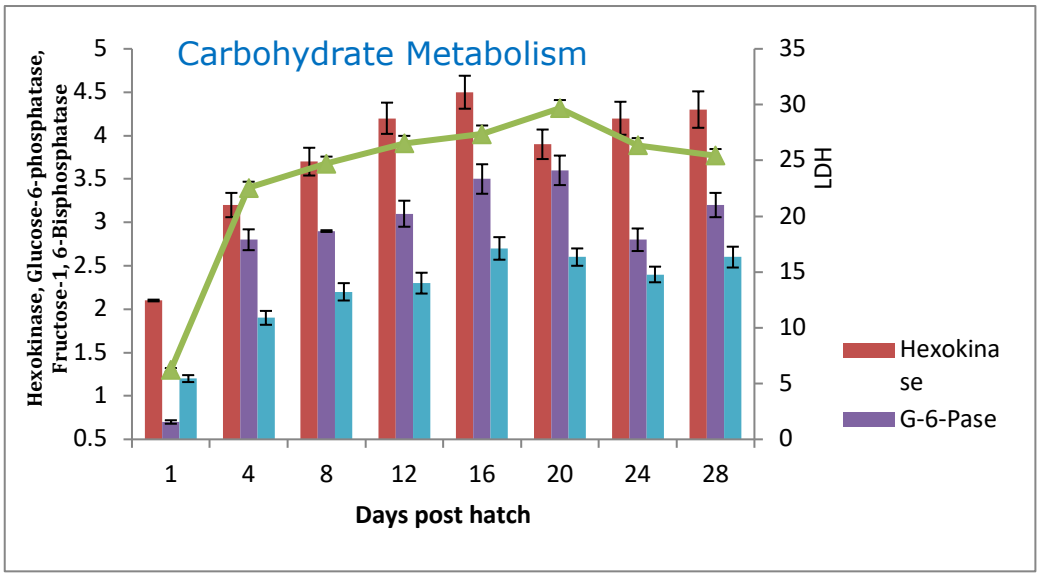


Figure 7. Amino Acid metabolism enzymes of larvae

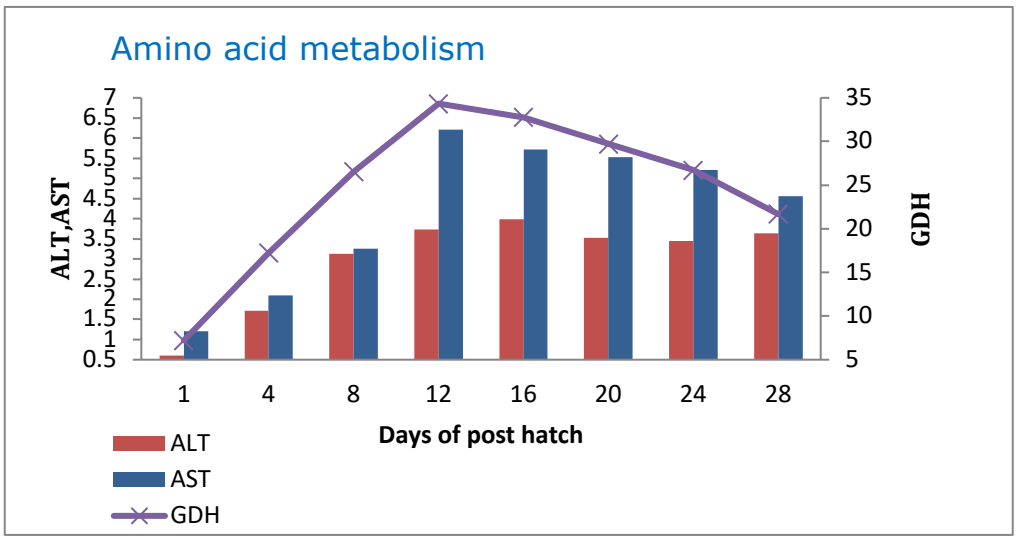


Figure 8. Carbohydrate Metabolic Enzymes of *O. bimaculatus* larvae

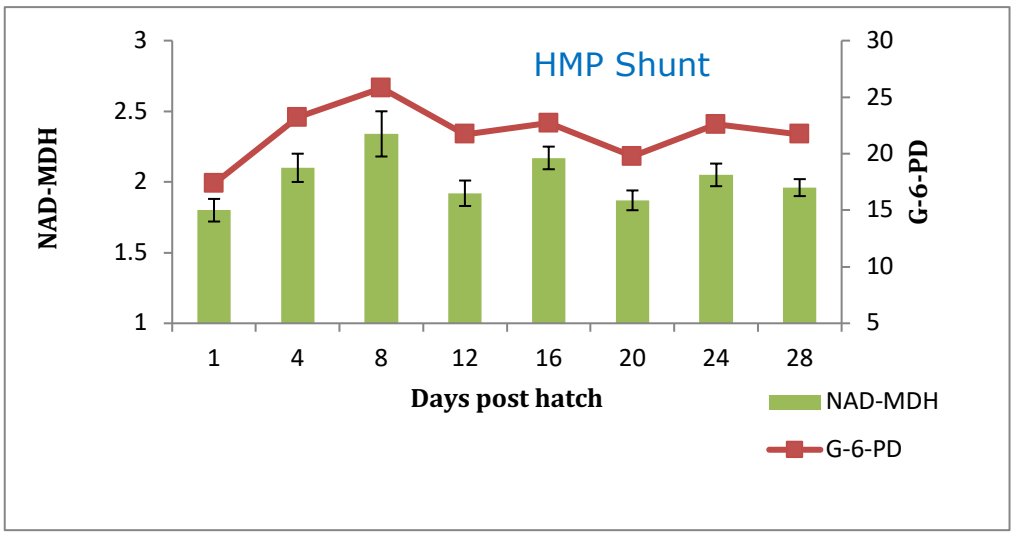


Figure 6, 7 and 8 shows the metabolic enzymes activities of *O. bimaculatus* larvae. The increase in Lactate dehydrogenase (LDH) activity from 4 to 20 dph was noticed, that could be indicative of stress. During this phase, increase in the activities of neoglucogenic enzymes (Glucose-6-phosphatase, Fructose-1, 6-bisphosphatase) was also noticed. However, a decreasing trend of LDH was apparent with increase in the Hexokinase

activity. Further, augmented Mitochondrial NAD-glutamate dehydrogenase activity coupled with increased Aspartate transaminase (and Alanine transaminase) at 12 dph was noticed signifying protein catabolism during this phase. Reduced neo glucogenic pathway at 4-8 dph was associated with high Glucose-6-phosphate dehydrogenase and cytosolic NADP-malate dehydrogenase activities that might indicate stress management and lipogenesis in this species during early development.

Activities of the enzymes indicated ontogenetic shift during the period of 8 to 20 dph, which could be associated with changes in food, gut morphology and shift in the activity pattern of digestive and metabolic enzymes. Overall, enzyme activities remained more or less stable beyond 20 dph.

Objective 3. To formulate larval feed and evaluation

Experiment No. 1: Feed evaluation with different levels of protein:

An experiment was conducted for 22 days initiated to study the protein requirement of *O. bimaculatus* larvae. The initial weight and length of *O. bimaculatus* larvae were 0.11±0.01 g and 20.49±0.70 mm. In the present experiment 3 formulated feeds were prepared with different graded levels of protein viz., Feed 1 (35%), Feed 2 (40%) and Feed 3 (45%). Fish meal, ground nut cake, soybean meal, wheat flour, vitamin and mineral mix and oil were used as feed ingredients in different proportions for preparation of experimental feed.

The feed formulations and proximate compositions of different feeds are presented in Table 5. The protein content of different feeds was 35.33±2.55, 40.69±0.55 and 45.23±0.17 respectively in Feed 1-3. Different proportions of fish meal, ground nut cake, soyabean meal, wheat flour etc were used to prepare the feed. Because of the graded increase in protein levels, the total ash contents of diets increased linearly from 15.40±0.39 to 16.37±0.39, which could be attributed to the presence of higher levels of fish meal as a major feed ingredient.

Water quality parameters were recorded as 28-30^{0}C, 7.4-7.8, 5.0-5.8 mg/l and 235-240mg/l $CaCO_3$ for temperature, pH, dissolved oxygen and total alkalinity respectively. Dissolved oxygen play a vital role in rearing of larvae because larvae require optimum level of oxygen for sustaining their physiological condition

The growth performance of *Ompok* larvae feed with different levels of Protein (Table no 6). Initially the larvae were fed with natural food and their formulated feed. The initial body weight, net weight gain, specific growth rate, feed conversion ratio, protein efficiency ratio and survivability are shown in Table 6. The initial weight of larvae were 0.11±0.004, 0.106±0.004 and 0.106±0.004 (g) in different feed Treatments (Feed 1-3) respectively while the average final weights after 22 days of experimental period was 0.34±0.06, 0.91±0.15 and 0.36±0.07 (g) in the respective treatments. The net weight gain were 0.23±0.05, 0.81±0.15 and 0.26±0.06 (g)respectively in Feed 1-3, which showed that the highest gain in weight of the larvae was 0.81±0.15 (g) in treatment Feed 2 followed by Feed 1 and Feed 3 . The net weight gain was significantly ($P<0.01$) higher in Feed 2 (Table 6 and Fig 9). The survival (%) was 73.0±2.02, 71.0±1.0 and 59.0±4.7 respectively in Feed 1-3. Significantly higher specific growth rate (SGR) was observed in Feed 2 and followed by Feed 1 and Feed 3. The final weight, net weight gain and

specific growth rate was significantly (P<0.05) higher in Feed 2 compared to those of Feed 1 and Feed 3. The value of feed conversion ratio (FCR) was significantly (P>0.05) lower in Feed 2 while protein efficiency ratio (PER) was non-significant among all the Feed treatments. Lowest FCR in Feed 2 indicated that lower amount of feed needed to produce one unit of fish biomass. So lower the FCR is, better the efficiency of the feed is and such type of feed in the present experiment was Feed 2.

Table 5. Ingredients and proximate composition (%DM basis) of experimental diets

Particulars	Feed 1	Feed 2	Feed 3
Fish Meal	44	53	65
Ground nut cake	15	15	15
Soyabean Meal	10	10	5
Wheat flour	19	10	03
CMC	2	2	2
Vit. & min. Mixture	5	5	5
Veg oil	5	5	5
Proximate Composition (% DM basis)			
Dry Matter	93.02±0.93	92.39±0.22	92.34±1.33
Crude Protein	35.33±2.25	40.69±0.55	45.23±0.17
Crude lipid	9.11±0.47	9.49±0.31	9.61±0.31
Total ash	15.40±0.39	16.35±0.09	16.37±0.39

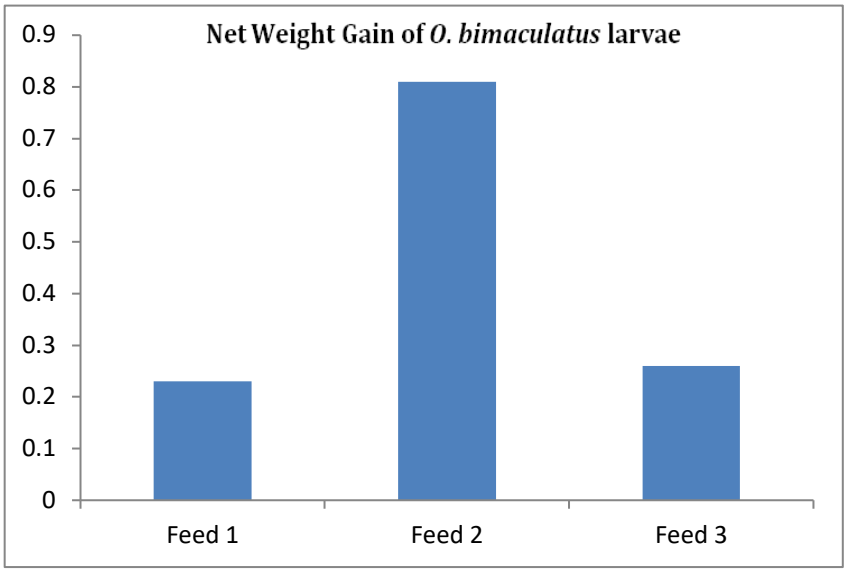
Data are presented as Mean± S.E. CMC- Carboxy methyl cellulose.

Table 6. Growth of *O.bimaulatus* larvae fed with different levels of Protein.

Particulars	Feed 1	Feed 2	Feed 3
Initial weight(g)	0.11±0.004	0.106 ±0.008	0.106±0.008
Final weight(g)	0.34 ± 0.06^{a}	0.91 ± 0.15^{b}	0.36 ± 0.07^{ab}
NWG(g/22d)	0.23 ± 0.05^{a}	0.81 ± 0.15^{b}	0.26 ± 0.06^{a}
SGR(%/d)	4.54 ± 0.60^{a}	7.97 ± 0.76^{b}	5.30 ± 0.53^{a}
FCR	3.62 ± 0.51^{b}	1.93 ± 0.43^{a}	3.68 ± 0.35^{b}
PER	0.82±0.13	1.42±0.33	0.62±0.05
Survivability	73.5±2.02	71.0±1.0	59.0±4.7
DNA(µg/mg)	30.59±0.30	34.31±0.43	31.04±0.54
RNA(µg/mg)	34.86±0.41	45.16±0.36	35.65±0.31
RNA/DNA	1.14±0.07	1.32±0.04	1.15±0.04

Data are presented as Mean± S.E. ^{a,b} Means with different superscripts in a row differ significantly ($P<0.01$)

Figure. 9. Net weight gain of *O.bimaculatus* fed with different levels of Protein



Sampling of *O.bimaculatus* larvae

Weight measurement

Length measurement

Table 7. Enzyme contents of *O. bimaculatus* larvae fed with different levels of Protein

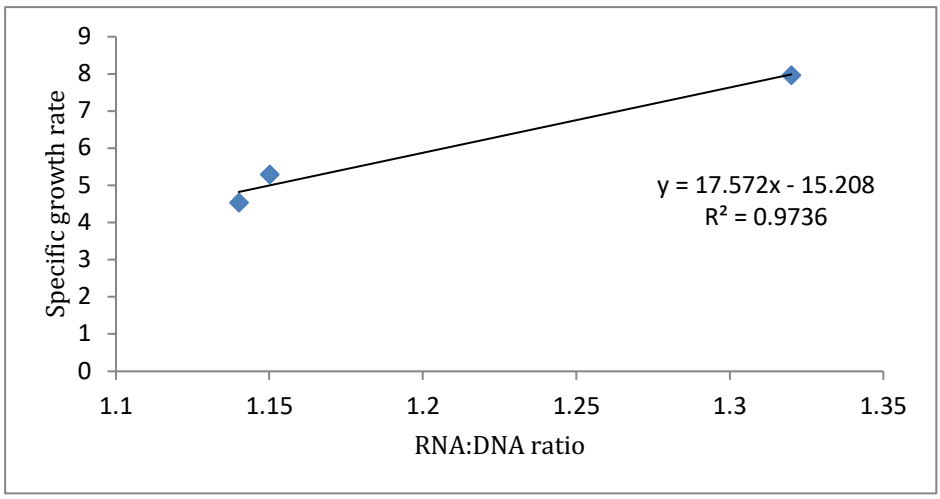
Particulars	Initial	Feed 1	Feed 2	Feed 3
Amylase*	17.4±0.33^{a}	23.87±0.17^{b}	25.36±0.34^{c}	25.46±0.47^{c}
Total Protease$^{¥}$	1.76±0.03^{d}	0.67±0.02^{a}	0.81±0.01^{b}	0.73±0.01^{c}
Lipase$^{\#}$	0.4±0.01^{a}	0.57±0.02^{b}	0.69±0.03^{d}	0.61±0.02^{c}
Pepsin$^{¥}$	0.38±0.01^{a}	2.53±0.02^{b}	2.67±0.01^{c}	2.49±0.01^{b}
Trypsin¶	0.89±0.02^{d}	0.20±0.01^{a}	0.18±0.02^{a}	0.26±0.03^{c}
Chymotrypsin¶	0.72±0.02^{c}	0.29±0.02^{b}	0.19±0.01^{a}	0.28±0.03^{b}
ALT(Alanine transaminase) $^{€}$	3.14±0.03^{a}	3.44±0.02^{b}	3.51±0.02^{c}	4.25±0.02^{d}
AST(Aspartate transaminase) ^{ƨ}	3.24±0.02^{a}	4.61±0.03^{c}	4.38±0.01^{b}	5.12±0.02^{d}

Data are presented as Mean± S.E. ^{a,b} Means with different superscripts in a row differ significantly (P<0.05)

* mg maltose liberated mg^{-1} protein h$^{-1,}$ $^{¥}$µg of tyrosine liberated mg^{-1} protein min^{-1}, $^{\#}$ µM of fatty acid liberated mg^{-1} protein min^{-1}, ¶1µmol of 4-nitroaniline liberated min^{-1} mg^{-1} protein Enzyme activity units mg^{-1} protein min^{-1}, $^{€}$ µM of pyruvate formed mg^{-1} protein min^{-1}, ^{ƨ} µM of oxaloacetate formed mg^{-1} protein min^{-1}

Perusal of table 7 reveals that Amylase activity of feed 1 was significantly differed from feed 2 and feed 3 diet. Lipase and pepsin activity was significantly (P<0.05) higher in feed 2. Trypsin and Chymotrypsin activity significantly (P>0.05) lower in feed 2. ALT and AST activities changes were significantly (P<0.05) higher in Feed 3 but both AST and ALT activities were significantly (P>0.05) lower in feed 2.

Figure 10. Relation between SGR and RNA-DNA ratio in *O. bimaculatus* Larvae



The DNA, RNA and RNA/DNA ratio of larvae was non significant among the fed treatments. . The Relationship between SGR and RNA/DNA ratio in *O. bimaculatus* has depicted that RNA/DNA ratio was highly correlated with Feed 2 having 40% protein vis-à-vis other Groups(Figure 10). The present experiment revealed that 40% crude protein was sufficient for the optimum growth and survival of *O. bimaculatus* larvae and it also revealed that the amylase, lipase and pepsin enzymes was significantly ($P<0.05$) higher in Feed 2 having 40% protein.

Experiment No. 3: Feed evaluation with Lipid requirement for *Ompok bimaculatus* larvae

An experiment was conducted 42 days to study the lipid requirement of *O. bimaculatus* larvae. The initial weight and length of *O. bimaculatus* larvae were 0.15±0.003 g and 22.65±1.70 mm. In the present experiment 3 formulated feeds were prepared having different lipid levels viz., Feed 1 (4.5%), Feed 2 (7.0%) and Feed 3 (9.5%).

The Proximate composition and feed formulation is presented in Table 8. The feed ingredients viz., fish meal, ground nut cake, soyabean meal, wheat flour, fish and vegetable oil (1:1) and vitamin and mineral mixture were used in different proportions for preparation of feed. Perusal of table 8 reflects that the protein content of different feeds were 40.46±0.06, 40.18±0.49 and 40.61±0.83 (%) respectively in Feeds1-3. The analysed lipid content of different feeds were 5.7±0.2, 8.0±0.25 and 10.45±0.45 respectively in Feed treatments 1-3.

Experimental work at wet Laboratory of RRC, Rahara

Table 8: Feed formulation and proximate composition (%DM Basis)

Particulars	Feed 1	Feed 2	Feed 3
FM	53	53	53
GNOC	15	15	15
SBM	10	10	10
Wheat flour	10.50	8.0	5.50
CMC	2.0	2.0	2.0
Fish :Veg oil (1:1)	4.5	7.0	9.5
Vit-Min mix	5	5	5
Proximate composition (%DM Basis)			
Dry matter	92.85±0.06	92.37±0.23	92.06±0.05
Crude protein	40.46±0.06	40.18±0.49	40.61±0.83
Crude lipid	5.7±0.2	8.0±0.25	10.45±0.45
Total Ash	14.4±0.3	15.4±0.2	16.5±0.3

Fig. 11: Net weight gain of *O. bimaculatus* larvae fed with different lipid levels

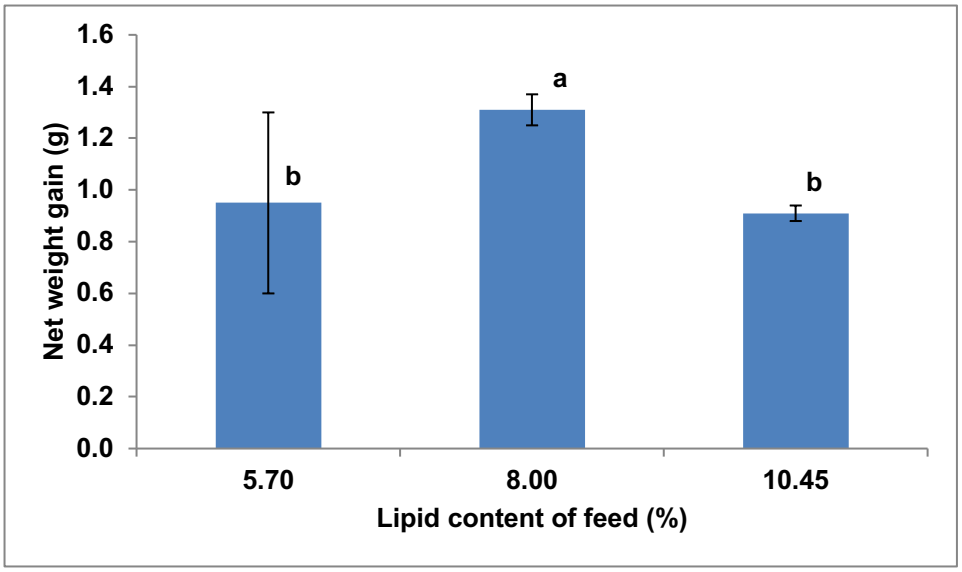


Table 9: Growth Performance of *O. bimaculatus* larvae fed with different level of lipids

Particulars	Feed 1	Feed 2	Feed 3
Initial Weight (g)	0.15±0.003	0.14±0.01	0.15±0.01
Final Weight (g)	1.10±0.12^{a}	1.40±0.07^{b}	1.06±0.03^{a}
Net weight gain (g/42d)	0.95±0.12^{a}	1.31±0.06^{b}	0.91±0.03^{a}
Specific Growth Rate (%/day)	4.73±0.35^{a}	5.50±0.05^{b}	4.66±0.22^{a}
DGC	0.73±0.02^{a}	1.003±0.05^{b}	0.76±0.09^{a}
Survivability (%)	83.85±6.15	83.85±6.15	79.55±5.46
FCR	1.86±0.10^{b}	1.39±0.05^{a}	1.74±0.07^{b}
PER	1.31±0.09^{a}	2.39±0.17^{b}	1.30±0.08^{a}

Data are presented as Mean± S.E. ^{a,b} Means with different superscripts in a row differ significantly (P<0.01)

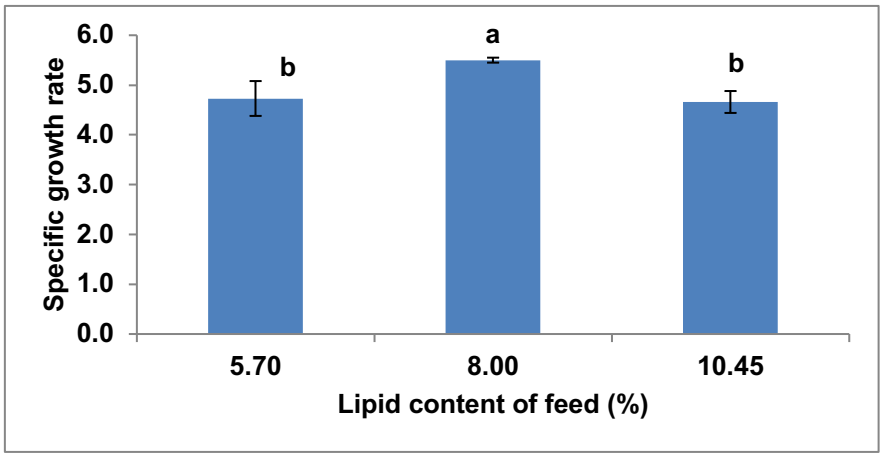
Sampling of *O. bimaculatus* larvae

The water quality parameters were: temp 28-30^{0}C, pH 6.8-7.7, dissolved oxygen 6.8-74 mg/l and total alkalinity 230-240 mg/l. Perusal of Table 9 shows the growth performance of *O. bimaculatus* larvae reared with different lipid levels. The final weight of fish were 1.10±0.12, 1.40±0.07 and 1.06±0.03 g respectively in Feed 1-3. The final weight was significantly (P<0.01) higher in Feed 2 having 8 % lipid. The net weight gain were 0.95±0.12, 1.31±0.06 and 0.91±0.03 (g) respectively in feed treatments Feed 1-3 (Figure 11). The specific growth rate (SGR) were 4.73±0.35, 5.50±0.05 and 4.66±0.22 respectively in Feed 1-3. The net weight gain, DGC and specific growth rate was significantly (P<0.05) higher in Feed 2 having 8% lipid. The Survivability

(%) were 83.85±6.15, 83.85±6.15 and 79.55±5.46 respectively in Feeds 1-3. The feed conversion ratio (FCR) were1.86±0.10, 1.39±0.05 and 1.74±0.07 in Feed treatments 1-3. He FCR was significantly (P< 0.05) lower in Feed 2 group.

***O.bimaculatus* larvae after 42 days of experiment**

Fig. 12: Specific Growth rate of *O. bimaculatus* larvae reared with different lipid levels



The protein efficiency ratio (PER) were 1.31±0.09, 2.39±0.17 and 1.30±0.08 respectively in Feed 1-3. The protein efficiency ratio was significantly (P<0.05) higher in Feed 2 (Figure 12).

Recording of length of pabda larvae Recording of weight of pabda larvae

Table 10 Carcass composition (% as such) of *O.bimaculatus* larvae fed different level of lipid

Particulars	Feed 1	Feed 2	Feed 3
Moisture	79.37±0.09^{a}	80.93±0.22^{b}	79.80±0.17^{a}
Crude Protein	13.93±0.09	14.40±0.21	14.03±0.08
Fat	2.50±0.06^{a}	2.90±0.12^{b}	2.77±0.0^{b}
Ash	1.70±0.06	1.97±0.09	1.80±0.06

Data are presented as Mean± S.E. ^{a,b} Means with different superscripts in a row differ significantly (P<0.01)

The carcass composition of *O. bimaculatus* larvae are presented in table 10. Perusal of table 10 reflects that the moisture and fat content carcass tissue of pabda larvae differed significantly (P<0.05) among the feed treatments. The moisture and fat content was significantly higher in Feed 2, and it did not differ significantly with Feed 3. Protein and ash content did not differ significantly among the feed treatments.

Table 11 Digestive enzyme activity of *O.bimaculatus* larvae

Particulars	Feed 1	Feed 2	Feed 3
Amylase$^{\alpha}$	4.42^{b}±0.21	4.02^{b}±0.19	2.92^{a}±0.13
Protease$^{\beta}$	1.42^{b}±0.06	1.54^{b}±0.07	1.26^{a}±0.04
Lipase$^{\pi}$	1.34^{b}±0.05	1.42^{b}±0.06	1.19^{a}±0.07

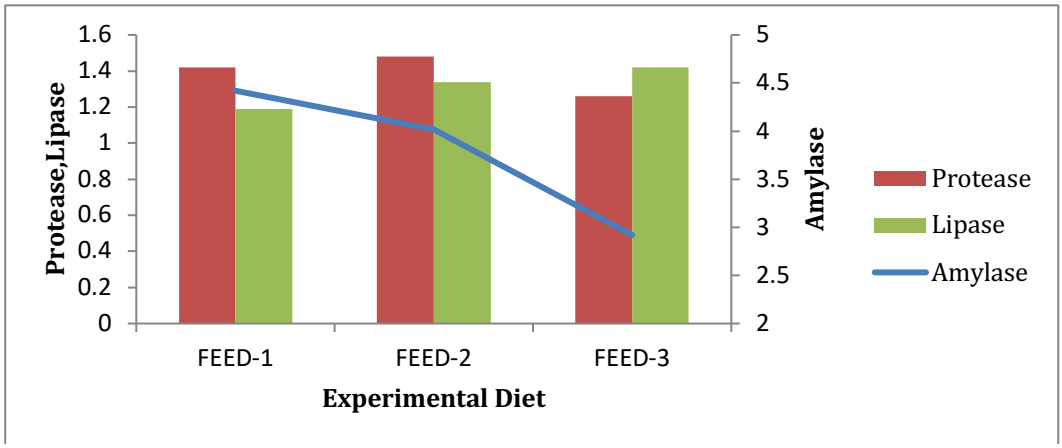
Data are presented as Mean± S.E. ^{a,b} Means with different superscripts in a row differ significantly (P<0.05)

$^{\alpha}$Amylase activity (U) = µg maltose liberated min^{-1} mg^{-1} protein

$^{\beta}$Protease activity (U) = µg tyrosine liberated min^{-1} mg^{-1} protein

$^{\pi}$Lipase activity (U) = 1µmol of free fatty acid liberated min^{-1} mg^{-1} protein

Fig. 13: Digestive enzyme activities of *O. bimaculatus* larvae fed different levels of lipids



The activities of digestive enzymes in *O. bimaculatus* were assayed by feeding different levels of lipids as given in Table 11. The activities of digestive enzymes in pabda larvae revealed that amylase, Protease and lipase activity was significantly ($P<0.05$) higher in F_2 and it did not differ significantly with F_1.

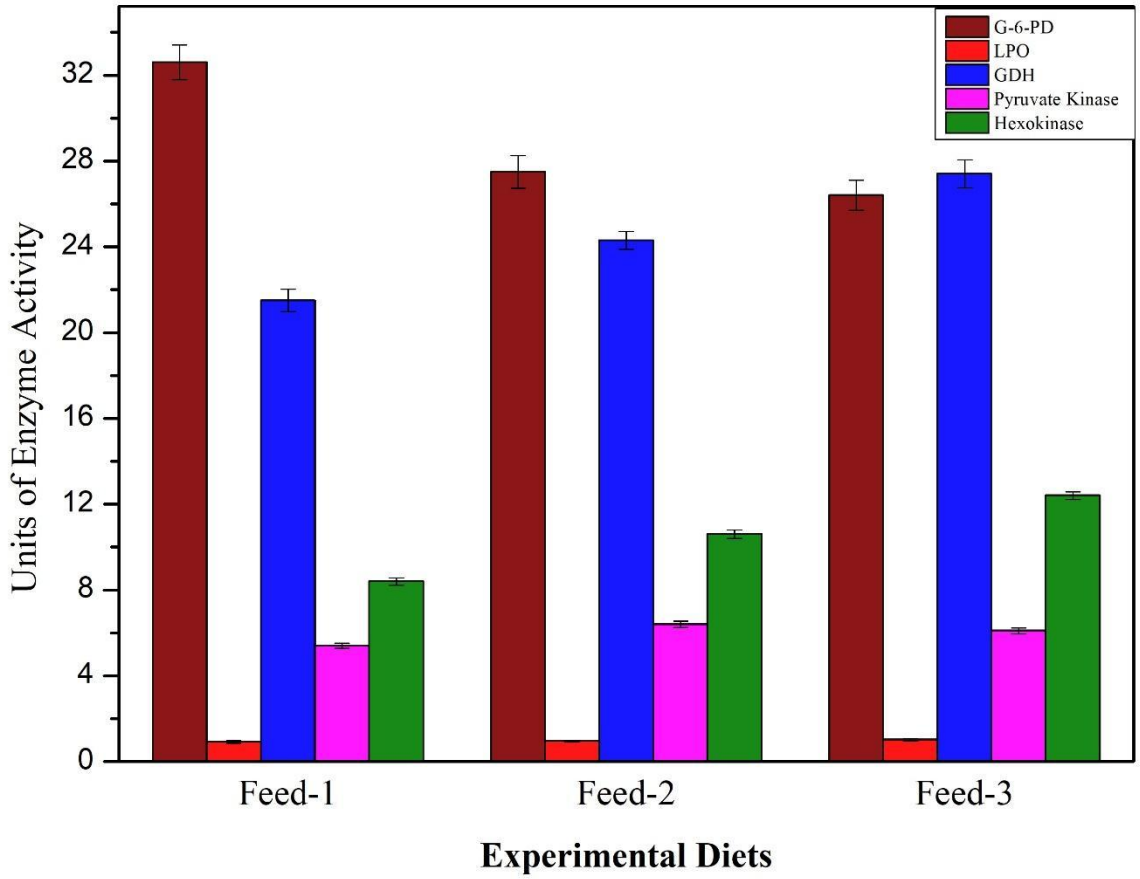
Table 12 Metabolic enzymes of *O.bimaculatus* larvae fed with different levels of lipid

Particulars	Feed 1	Feed 2	Feed 3
Glucose 6 Phosphate Dehydrogenase$^{\alpha}$	$32.6^{a}\pm0.81$	$27.5^{b}\pm0.76$	$26.4^{b}\pm0.7$
Glutamate Dehydrogenase$^{\beta}$	$21.5^{a}\pm0.53$	$24.3^{b}\pm0.42$	$27.4^{c}\pm0.65$
Pyruvate kinase$^{\mu}$	$5.4^{a}\pm0.14$	$6.4^{b}\pm0.14$	$6.1^{b}\pm0.14$
Hexokinase$^{\epsilon}$	$8.4^{a}\pm0.17$	$10.6^{b}\pm0.19$	$12.4^{c}\pm0.17$
Lipid Peroxidation$^{\phi}$	0.92±0.06	0.96±0.04	1.02±0.06

Data are presented as Mean± S.E. ^{a,b} Means with different superscripts in a row differ significantly ($P<0.05$)

$^{\alpha}$G-6-PD(U)& NAD-MDH(U) = µM of NADPH formed mg protein^{-1} h^{-1}
$^{\beta}$GDH(U)= µM of Fomazan formed mg protein^{-1} h^{-1}
$^{\mu}$Pyruvate kinase^{+}µM of NAD formed mg protein^{-1} h^{-1}
$^{\epsilon}$Hexokinase(U)= µM of NAD formed mg protein^{-1} h^{-1}
$^{\phi}$Lipid Peroxidation (U)= µM thiobarbituric acid reactive substance (TBARS) formed mg protein^{-1} min^{-1}

Figure 14. Metabolic enzymes of *O.bimaculatus* larvae fed with different levels of lipid



The metabolic enzymes activity is presented on table 12. Glutamate dehydrogenase and Hexokinase enzyme was significantly ($P<0.05$) higher in larvae fed feed-3. Glucose 6 phosphatase dehydrogeanse and pyruvate kinase was significantly ($P<0.05$) higher in F_2 and it did not differ significantly with F_3. He lipid peroxidation enzyme did not differ significantly among the feed treatments. The present experiment reveals that 8% lipid is sufficient for the optimum growth and survival of *O. bimaculatus* larvae. The present experiment reveals that 8% lipid is sufficient for the optimum growth and survival of *O. bimaculatus* larvae.

Experiment no. 4: Feed evaluation with different feed additives for *O. bimaculatus*

The study aimed at evaluation of animal protein (on wet basis) supplemented to dry formulated feed of the butter catfish, *Ompok bimaculatus* (Bloch) juveniles. Hatchlings were produced by induced breeding of mature *O. bimaculatus* using a hormone analogue (synthetic GnRH) at the Department of Zoology, University of Burdwan, Golapbagh, Burdwan, West Bengal. Juveniles were raised in the laboratory and 30 day old juveniles (0.44±0.02 g) were stocked in five groups in triplicate for 50 days. A formulated basal diet (40% crude protein) was fed to the control group (D1), whereas diets supplemented with (10%, wet weight) blood meals (D2), chicken viscera (D3), egg albumin (D4) and mussel meat (D5) were fed to the experimental groups for 90 days.

Five experimental diets were prepared along with Fish meal, Soya bean meal, Ground nut cake, wheat flour, veg. oil & Vitamin and mineral mixture with 40 % protein. Feeds were prepared, sterilized and stored at -20^0 C and fresh protein sources viz., blood meal,chicken viscera, egg albumin and mussel meat were mixed daily before feeding. The experimental fish were fed twice daily at 09:00h and 15:00h at a fixed feeding rate. Water quality was maintained in all all experimental tanks by daily renewal of 30% of their total volume.

The feed ingredient composition and proximate composition is presented in Table 13. In diet D-2, D-3, D-4 and D-5; animal protein component (10% w/w) viz., Blood Meal (D-2), Chicken Viscera (D-3), Egg albumin (D-4) and Mussel meat (D-5) were supplemented in the control diet. The protein content of different feeds were 40.14±0.55, 40.89±0.41, 41.64±0.67, 41.24±0.54 and 41.56±0.72 (%) respectively in diets D-1 to D-5.

The growth performance and feed utilization efficiencies of *O. bimaculatus* juveniles fed with different animal protein supplements is presented in Table 14 and Figure 15. The final weight were 4.81±0.07, 4.65±0.06, 5.36±0.05, 5.85±0.06 and 5.46±0.04 (g) respectively in diets D-1 to D-5.

Table 13. Ingredient and Proximate composition (% DM) of different Experimental diets

Particulars	D-1	D-2	D-3	D-4	D-5
Fish Meal	53	53	53	53	53
Soybean Meal	15	15	15	15	15
Groundnut cake	10	10	10	10	10
Wheat Flour	10	10	10	10	10
Sunflower oil	5	5	5	5	5
Agrimin (Vit. & Min. Mix.)	5	5	5	5	5
Carboxy methyl cellulose	2	2	2	2	2
Animal protein supplement (10% w/w)	-	Blood Meal	Chicken Viscera	Egg albumin	Mussel Meat
Proximate composition					
Dry Matter	92.39±0.32	92.86±0.41	93.94±0.56	92.95±0.53	93.62±0.74
Crude Protein	40.14±0.55	40.89±0.41	41.64±0.67	41.24±0.54	41.56±0.72
Crude Lipid	9.48±0.31	9.67±0.24	9.64±0.35	9.51±0.47	9.78±0.36
Ash	16.35±0.09	16.45±0.12	16.76±0.04	16.48±0.07	16.53±0.36

The final weight was significantly ($P<0.05$) higher in Diet-4 vis-à-vis other diets. The FCR and SGR were 1.63±0.08, 1.69±0.10, 1.51±0.07, 1.32±0.06 and 1.38±0.06; 2.65±0.06, 2.62±0.05, 2.80±0.4, 2.87±0.07 and 2.78±0.05 respectively in Diets D-1 to D-5. The FCR was significantly ($P<0.05$) lower in D-4 and SGR was significantly higher in D-4.. The ANPU were 24.20±0.45, 22.43±0.35, 26.53±0.52, 33.76±0.47 and 28.28±0.6 respectively in D-1 to D-5. The ANPU was significantly ($P<0.05$) higher in D-4 group.

The diet D4 exhibited better performance in terms of growth, feed conversion ratio, specific growth rate, protein efficiency ratio and apparent net protein utilization. The mortality(%) was significantly lower in D-4 and D-2.

Table14. Growth performances and feed utilization efficiencies in *Ompok bimaculatus*

Particulars	D-1	D-2	D-3	D-4	D-5
Initial weight(g)	0.44±0.20				
Final weight (g)	4.81±0.07^{a}	4.65±0.06^{b}	5.36±0.05^{c}	5.85±0.06^{d}	5.46±0.04^{c}
FCR	1.63±0.08^{b}	1.69±0.10^{b}	1.51±0.07^{a}	1.32±0.06^{a}	1.38±0.06^{a}
SGR	2.65±0.06^{a}	2.62±0.05^{a}	2.80±0.4^{b}	2.87±0.07^{b}	2.78±0.05^{b}
Mortality (%)	17.14±0.71^{b}	14.29±0.10^{a}	18.21±0.71^{b}	14.29±0.12^{a}	21.42±0.95^{c}
ANPU	24.20±0.45^{a}	22.43±0.35^{a}	26.53±0.52^{b}	33.76±0.47^{d}	28.28±0.61^{c}
PER	1.52±0.03^{b}	1.37±0.03^{a}	1.53±0.04^{b}	1.84±0.04^{d}	1.68±0.04^{c}

Data are Means ± Standard error (n=3). Means with different superscript in a row are significant ($P < 0.05$)

The PER were 1.52±0.03, 1.37±0.03, 1.53±0.04, 1.84±0.04 and 1.68±0.04 respectively in diets D-1 to D-5. The PER was significantly ($P<0.05$) higher in D-4 group.

Table 15. Carcass composition of *O.bimaculatus* juveniles feed with different Protein supplements (w/w)

Particulars	D-1	D-2	D-3	D-4	D-5
Moisture	77.37±0.57^{a}	77.17±0.54^{a}	77.99±0.59^{a}	78.23±0.52^{b}	77.93±0.53^{a}
Protein	14.84 ±0.22^{a}	14.98±0.24 ^{a}	15.49±0.26^{b}	16.19±0.27^{c}	15.79±0.28^{b}
Lipid	2.47±0.12 ^{a}	2.46±0.90 ^{a}	2.55±0.14^{b}	2.51±0.10 ^{a}	2.88±0.13^{b}

Data are Mean ± S.E. Means with different superscript in a row are significant ($P < 0.05$)

The carcass composition of juvenile *O. bimaculatus* are presented in Table 15. The moisture and protein content were significantly ($P<0.05$) higher in D-4. However, the lipid content was significantly ($P<0.05$) higher in D-3and D-5. The experimental diet D4 exhibited significantly ($P<0.05$) higher growth, feed conversion ratio, specific growth rate and carcass protein deposition.

Table 16. Activities of enzymes of *Ompok bimaculatus* fed with different feed additives

Enzymes	D-1	D-2	D-3	D-4	D-5
Amylase	11.40 ± 0.52^{a}	16.59 ± 0.62^{b}	17.60 ± 0.54^{b}	16.22 ± 0.53^{b}	12.77 ± 0.54^{a}
Protease	1.13 ± 0.07^{c}	1.32 ± 0.04^{c}	0.79 ± 0.05^{b}	0.55 ± 0.04^{a}	0.54 ± 0.06^{a}
Lipase	1.82 ± 0.04^{a}	1.70 ± 0.04^{a}	2.24 ± 0.05^{c}	2.35 ± 0.06^{c}	2.01 ± 0.04^{b}
Pepsin	3.25 ± 0.06^{a}	3.35 ± 0.07^{a}	3.89 ± 0.09^{b}	4.35 ± 0.12^{c}	4.0 ± 0.10^{b}
Trypsin	0.57 ± 0.04^{c}	0.56 ± 0.06^{c}	0.43 ± 0.05^{b}	0.37 ± 0.05^{a}	0.36 ± 0.04^{a}
Chymotrypsin	0.43 ± 0.05^{b}	0.54 ± 0.06^{b}	0.33 ± 0.04^{a}	0.27 ± 0.05^{a}	0.23 ± 0.05^{a}
Alanine transaminase (ALT)	1.65 ± 0.09^{c}	1.75 ± 0.12^{c}	1.62 ± 0.08^{b}	1.36 ± 0.08^{a}	1.67 ± 0.09^{b}
Asparate transaminase (AST)	5.77 ± 0.12^{b}	6.03 ± 0.14^{c}	5.73 ± 0.10^{b}	5.49 ± 0.13^{a}	5.78 ± 0.08^{b}

Data are Mean ± S.E. Means with different superscript in a row are significant ($P < 0.05$)

Trypsin and chymotrypsin activities (U) = 1µmol of 4-nitroaniline liberated min^{-1} mg^{-1} protein

Lipase activity (U) = µmol of free fatty acid liberated min^{-1} mg^{-1} protein

α-amylase activity (U) = µg maltose liberated min^{-1} mg^{-1} protein

Protease activity (U) = µg tyrosine liberated min^{-1} mg^{-1} protein

Pepsin activity (U) = µg tyrosine liberated min^{-1} mg^{-1} protein

ALT & AST (U) = nmol sodium pyruvate released min^{-1} mg^{-1} protein

The enzymes activities of *Ompok bimaculatus* fed with different feed additives are presented in Table 16 and figure 16 & 17. Activities of amylase and lipase were significantly higher in groups D3 and D4, respectively, as compared to the other groups. Diet D4 sustained the highest pepsin activity (4.35±0.12 U); however, activities of the alkaline proteases (Trypsin, Chymotrypsin) were reduced in fish fed diets D4 and D5. Significantly lower aspartare aminotransferase (AST) and alanine amniotransferase (ALT) activities in the liver were recorded in *O.bimaculatu*s juveniles fed Diets D4 and D5, respectively.

Figure15.GrowthPerformanceof*O.bimaculatus*

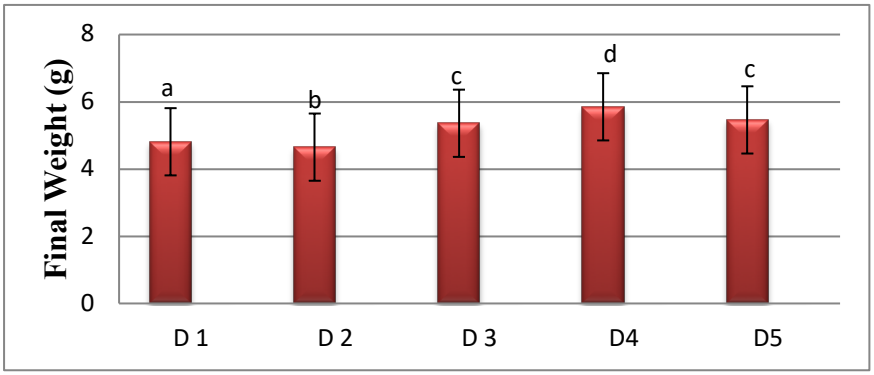


Fig 16. Activities of Protease, Lipase and Amylase in *O. bimaculatus* fed with different Feed additives

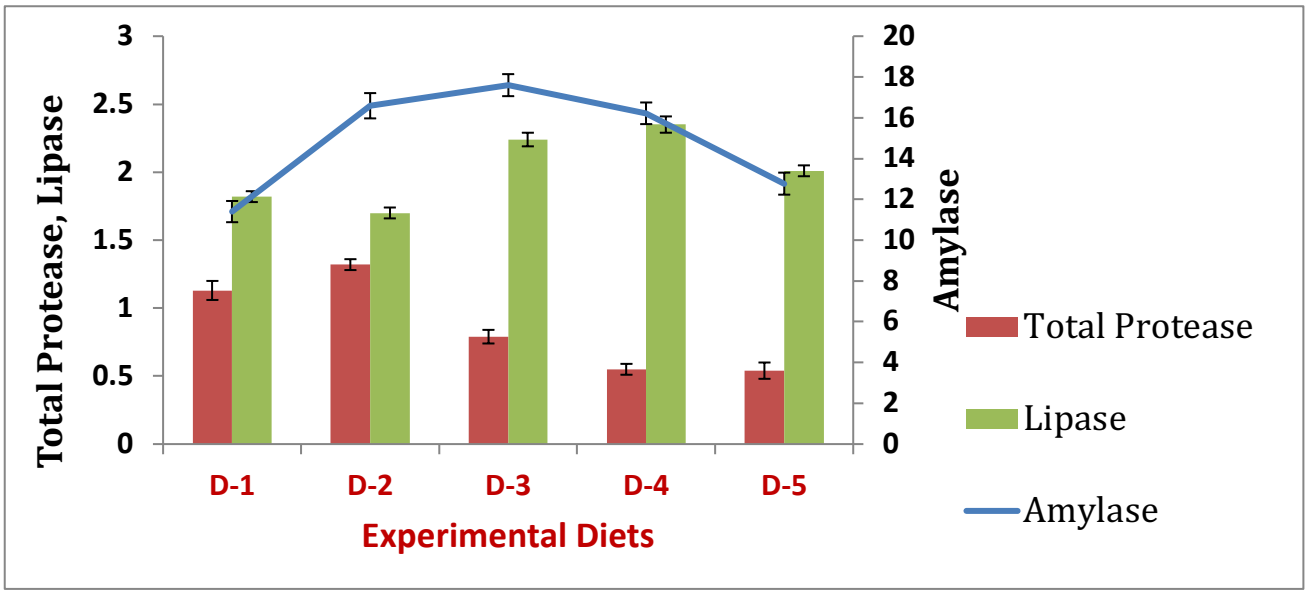
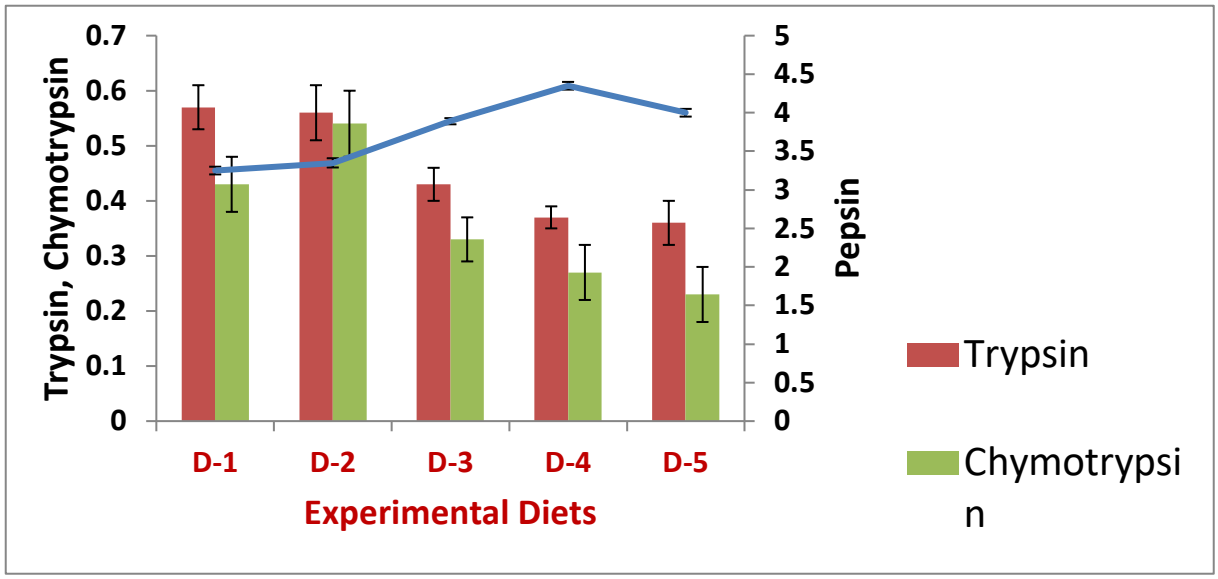


Fig 17. Activity of protein enzymes of *Ompok bimaculatus* fed with different Feed additives



Considering growth performance and survivability of the juveniles, the study suggests that supplementation of egg albumin might be practiced to make the *O. bimaculatus* juveniles adaptive to the formulated diets. The present experiment suggests that a formulated feed having 40% crude protein along with 10% of egg albumin is a better feeding strategy when compared with blood meal, chicken viscera and mussel meat. The study suggests that supplementation of egg albumin might be effective to make the *O. bimaculatus* larvae and juveniles adaptive to the formulated diets for its domestication in freshwater ecosystem..

11. Financial Implications (in Lakhs)

11.1 Expenditure on

(a) Manpower: 33.57 lakhs

(b) Research/Recurring Contingencies: 8.50 lakhs

(c) Non-Recurring Cost (Including cost of equipment): Nil

(d) Any Other Expenditure Incurred: Nil

11.2 Total Expenditure:

Item	Year (1)	Year (2)	Year (3)	Total (Lakhs)
Grand Total	14.19	14.19	13.69	42.07

12. Cumulative Output

a. Special attainments/innovations

1. The proximate and fatty acid composition of *Ompok bimaculatus* (pabda) brood fish, egg and larvae was assayed to have an insight of the nutrient metamorphosis during early life of the species and vis-a-vis parental nutrient composition. The studies regarding nutritional characterization of brood fish, eggs and larvae of *Ompok bimaculatus* revealed that crude protein content was higher in egg whereas crude fat and ash content was higher in pabda brood fish. However, PUFA, EPA and DHA content was higher in larvae.

2. Ontogeny Study of *O. bimaculatus* larvae suggests that the digestive tract of larvae comprised a straight tube dorsally attached to the yolk sac. At 2 dph, mouth opened, teeth were visible and exogenous feeding started. The yolk sac disappeared within 3 dph. Coiling of intestine and presence of stomach were noticed by 8 dph, which was marked with the appearance of pepsin. Activities of the enzymes indicated ontogenetic shift during the period of 8 to 20 dph, which could be associated with changes in food, gut morphology and shift in the activity pattern of digestive and metabolic enzymes. Overall, enzyme activities remained more or less stable beyond 20 dph.

3. A experiment was conducted to study the protein requirement of *O. bimaculatus* larvae 22 for days . The experimental data revealed that 40% crude protein was sufficient for the optimum growth and survival of *O. bimaculatus* larvae and it also recorded that the amylase, lipase and pepsin enzymes was significantly ($P<0.05$) higher in Feed 2 having 40% protein.

4. An experiment was conducted for 42 days to study the lipid requirement of *O. bimaculatus* larvae and it was observed that 8% lipid is sufficient for the optimum growth and survival of *O. bimaculatus* larvae. The activities of digestive enzymes in *O. bimaculatus* were assayed by feeding different levels of lipids. The activities of digestive enzymes in pabda larvae revealed that lipase activity was significantly ($P<0.05$) higher in Feed 2 having 8% lipid.

5. The experiment evaluation of supplementation of different protein sources (w/w basis) suggests that a feed having 40% crude protein along with 10% of egg albumin is a better feeding strategy when compared with blood meal, chicken viscera and mussel meat. The study suggests that supplementation of egg albumin might be effective to make the *O. bimaculatus* larvae and juveniles adaptive to the formulated diets.

b. List of Publications (one copy each to be submitted if not already submitted)

i. Research papers ; 2 nos

Paul, B .N., A. Das, A., R.N. Mandal,R.N., Singh,P., Adhikari, S., Ghosh, K.,Chowdhury,D., Chakrabarti,P.P. and Giri,S.S. (2020) Protein requirement of *Ompok bimaculatus* (Bloch, 1794) larvae. Animal Nutrition and Feed technology (**Communicated)**.

Paul, B .N., A. Das, A., R.N. Mandal,R.N., Singh,P., Adhikari, S., Chakrabarti,P.P. and Giri,S.S. (2020) Proximate and Fatty acid composition of brood fish, egg and larvae of *O.bimaculatus*. Indian Journal of animal Nutrition (**Communicated)**.

i. Reports/Manuals ; 3 training manuals

ii. Working and Concept Papers

iii. Popular articles

iv. Books/Book Chapters: 1

Paul,B.N. and Giri, .S. (2018). Nutrition and Feeding of catfish larvae. S.K.Sahoo, R.Kumar, P.K.Tiwari, B.R.Pillai, S.S.Giri. (Eds.) 2018 Training Manual on Mass Breeding and culture Techniques of Catfishes. SAARC Agriculture Centre, Dakha, Bangladesh pp 73-82.

v. Extension Bulletins

b. Intellectual Property Generation

(Patents - filed/obtained; Copyrights- filed/obtained; Designs- filed/obtained; Registration details of variety/germplasm/accession if any)

c. Presentation in Workshop/Seminars/Symposia/Conferences

(Relevant to the project in which Scientists have participated)

- Paul, B.N**.,** Das, A., Bhowmick, S., Mandal, R.N., S., Singh, Adhikari, S., Ghosh, K., Chowdhury, D. and Chakrabarti, P.P. (2019). Protein Requirement of *Ompok bimaculatus* Larvae. Golden Jubilee International Conference on "Trends in Zoology". January, 03-04, 2019 held at The University of Burdwan, Golapbag, Burdwan, India. Pp 88.

- Chowdhury, D., Paul, B.N. and Ghosh, K. (2019). Profiles of Digestive and Metabolic Enzymes in Butter Catfish, *Ompok bimaculatus* (Bloch, 1794) during early development. Golden Jubilee International Conference on "Trends in Zoology". January, 03-04, 2019 held at The University of Burdwan, Golapbag, Burdwan, India. Pp 90.

- Paul, B.N., Das, A., Mandal, R.N., Singh, P. And Adhikari, S. 2019. Proximate and Fatty acid composition of brood fish, egg and larvae of *Ompok bimaculatus*. Fourth PAF Congress on "Increasing Aquaculture Production in India through Synergetic Approach between Multinational Industries, Domestic Entrepreneurs and Aquaculturists" held at ICAR-CIFA, Bhubaneswar during 15-17 November, 2019. Pp. 27.

- Chowdhury, D., Paul, B.N. and Ghosh, K. 2019. Evaluation of Formulated Diets for Survival, Growth, Digestive Enzymes and Metabolic Functions in Butter Catfish*,* *Ompok bimaculatus* (Bloch) juveniles. International Conference on Animal Nutrition on "Nutritional Strategies for Improving Farm Profitability and Clean Animal Production" held at Biswa Bangla Convention Centre, Kolkata, 17-19 December, 2019.pp 392.

d. Details of technology developed

(Crop-based; Animal-based, including vaccines; Biological – biofertilizer, biopesticide, etc; IT based – database, software; Any other – please specify)

e. Trainings/demonstrations organized

- Organised a Training programme on Applications and Practices of fish feed was organised at RRC, CIFA, Rahara during 23-28 Oct.,2017
- Organised a Training programme on Application of Farm Made Feed for Sustainable Aquaculture Production was organised at RRC, CIFA, Rahara during 03-07 Dec.,2018
- Organised a Training programme on Fish Nutrition and Feeding of Fish at RRC, CIFA, Rahara during 17-21 June ,2019
- Organised a Training programme on 'Captive breeding and seed production of indigenous catfishes with emphasis on *Ompok* and *Mystus* Species' at Kalyani Field Station, RRC Rahara during 30.07.2019 to 03.08.2019.

f. Training received

➢ Mr. Arabinda Das, Scientist received a training on 'Experimental design and statistical data analysis' during January 3-16, 2019 at ICAR-IASRI, New Delhi.

g. Any other relevant information

13. (a) Extent of achievement of objectives and outputs earmarked as per RPP-I

Objective wise	Activity	Envisaged output of monitorable target(s)	Output achieved	Extent of Achievement (%)
1. To study nutrient composition of egg and different stages of larvae	1. Brood stock development of *O.bimaculatus* 2. Production of egg and larvae of *O.bimaculatus* 3. Nutrient composition of egg and larva	Production of Larvae through good brood stock development and production of larvae for experimental work. To know the nutrient profile of brood pabda, egg and larvae.	Sufficient larvae were produced for the experimental work. The nutrient composition data revealed that the crude protein content of brood fish, egg and larvae were 14.4±0.07, 25.90±0.20, 12.23±0.88 (%) respectively and crude protein content was significantly higher in pabda egg.	100%

	4. Collection of Literature, Planning and design of experiment		The fatty acid composition revealed that the saturated fatty acid (SFA) content (% of total fatty acid) of brood pabda, eggs and larvae were 88.48%; 76.04% and 48.60%. The poly unsaturated fatty acid (PUFA) content of brood pabda, eggs and larvae were 4.5%; 10.05% and 33.35% respectively. The EPA and DHA contents were 2.71% and 6.87% in larvae; which was significantly higher in brood fish and egg. It may be summarized crude protein content was higher in egg whereas crude fat and ash content was higher in brood fish. However, PUFA, EPA and DHA content was higher in larvae.	
	Ontogeny and enzyme study	Ontogeny of *O. bimaculatus* was worked out.he yolk sac disappears within 3 days.the overall enzyme activities remained stable after 20days of post hatching.	Ontogeny Study of *O. bimaculatus* larvae: The digestive tract of larvae comprised a straight tube dorsally attached to the yolk sac. At 2 dph, mouth opened, teeth were visible and exogenous feeding started. The yolk sac disappeared within 3 dph. Coiling of intestine and presence of stomach were noticed by 8 dph, which was marked with	100%

			the appearance of pepsin. Activities of the enzymes indicated ontogenetic shift during the period of 8 to 20 dph, which could be associated with changes in food, gut morphology and shift in the activity pattern of digestive and metabolic enzymes. Overall, enzyme activities remained more or less stable beyond 20 dph.	
3.To formulate larval feed and evaluation	1. Feed formulation and Feeding of larvae of *O. bimaculatus* with live and exogenous feed 2. Production of Live food organisms. 3. Monitoring of Water Quality parameters 4. Data analysis and preparation of final report	Development of larval feed for *O.bimaculatus* larvae. A larval feed was developed with 40% protein and 8% lipid. Supplementation of 10% egg albumen (w/w) with the feed should be the feeding protocol for the species to domesticate them in the Aquaculture production system.	(i) **Protein requirement of larvae** An experiment was conducted to study the protein requirement of *O. bimaculatus* larvae for 22 days. During the experiment the water quality parameters were monitored from time to time. The experimental data revealed that 40% crude protein was sufficient for the optimum growth and survival of *O. bimaculatus* larvae and it also recorded that the amylase, lipase and pepsin enzymes was significantly ($P<0.05$) higher in Feed 2 having 40% protein. (ii) **Lipid requirement study of *O. bimaculatus* larvae.** An experiment was conducted 42 days to	100%

			study the lipid requirement of *O. bimaculatus* larvae and it was observed that 8% lipid is sufficient for the optimum growth and survival of pabda larvae. The activities of digestive enzymes in the species were assayed by feeding different levels of lipids. The activities of digestive enzymes in pabda larvae revealed that lipase activity was significantly ($P<0.05$) higher in Feed 3 having 8% lipid. **(iii)Supplementation of live protein supplements** The experimental data suggests that a formulated feed having 40% crude protein along with 10% of egg albumin is a better feeding strategy when compared with blood meal, chicken viscera and mussel meat. Considering growth performance and survivability of the juveniles, the study suggests that supplementation of egg albumin might be practiced to make the *O. bimaculatus* juveniles adaptive to the formulated diets.	

(b) Reasons of shortfall, if any: Nil

14. Efforts made for commercialization/technology transfer

The experimental results obtained are disseminated with the fish famers, fishery extension officers, students, feed manufactures and entrepreneurs in various training programmes organised at RRC, Rahara and its field station at Kalyani, CIFA HQs, farmers field and KVKs. In those training programmes delivered lectures on larval Nutrition, ontogeny of the *O.bimaculatus* larvae and emphasized about the use of precise nutrients(quality fish meal) and fish oil required to prepare the feed for the species.

15. (a) How the output is proposed to be utilized?

The output is proposed to be utilized at farmers field through KVKs, stake holders, feed manufacturers and other fisheries developmental agencies.

(b) How it will help in knowledge creation

The knowledge of ontogeny Study of *O. bimaculatus* larvae was known and it helped to develop the larval feed. The digestive tract of larvae comprised a straight tube dorsally attached to the yolk sac. At 2 dph, mouth opened, teeth were visible and exogenous feeding started. The yolk sac disappeared within 3 dph. Coiling of intestine and presence of stomach were noticed by 8 dph, which was marked with the appearance of pepsin. Activities of the enzymes indicated ontogenetic shift during the period of 8 to 20 dph, which could be associated with changes in food, gut morphology and shift in the activity pattern of digestive and metabolic enzymes. Overall, enzyme activities remained more or less stable beyond 20 dph. The nutrient requirement of larvae was worked out viz., Protein and Lipid required by the species are 40 and 8 (%) respectively. The findings were presented in National and International Seminars and data will also be published in peer reviewed journals.

16. Expected benefits and economic impact(if any)

The feed and feeding protocol of *O. bimaculatus* has been worked out. The larval feed having 40 % protein and 8 % lipid has been developed. As there was no larval feed of *O. bimucalatus*, thus this feed will cater the needs of the sector and help in increasing the butter catfish production in the region.

17. Future line of research work/other identifiable problems

Nutragenomics study on the nutrients of larval feed of butter catfish *O. bimaculatus* would be a line for future research work.

18. Details on the research data (registers and records) generated out of the project deposited with the institute for future use: The register and data generated are maintained and submitted to the Institute in the form of RPF-II and III and Annual Report.

19. Signature of PI, CC-PI(s), all Co-PIs

Project Leader	Co-PI	Co-PI	Co-PI	Co-PI
(Dr. B.N. Paul)	(P.P. Chakrabarti)	(Dr. S. Adhikari)	(Dr. R.N. Mandal)	(Mr. A. Das)

Co-PI

(Dr. K. Ghosh)

20. Signature of Head of Division

21. Observations of PME Cell based on Evaluation of Research Project after Completion

22. Signature (with comments if any along with rating of the project in the scale of 1 to 10 on the overall quality of the work) of JD (R)/ Director

ANNEXURE - VIII

INDIAN COUNCIL OF AGRICULTURAL RESEARCH

(For Guidelines Refer ANNEXURE – XI (H))

PROFORMA FOR RESEARCH PERFORMANCE EVALUATION OF INDIVIDUAL SCIENTIST

23. Institute Project Code * I-95-G

1. Evaluation by PI on the contribution of the team in the project including self

Sl. No.	Name	Status in the project (PI/CC-PI/Co-PI)	*Rating in the scale of 1 to 10
1.	Dr. B.N.Paul	PI	10
2.	Dr.S.Adhikari	Co-PI	10
3.	Dr.P.P.Chakrabarti	Co-PI	10
4.	Dr. R.N Mandal	Co-PI	10
5.	Mr. A. Das	Co-PI	10
6.	Dr. K.Ghosh (Burdwan University)	Co-PI	10

2. Signature of PI

* Individual scientists participating in the project would be assessed for their performance through an appraisal system in a scale of 1 to 10 for each of the following attributes:

No.	Criteria	Marks
1.	Percentage of the assigned activity completed	**40**
2.	Quality of the completed activity	**10**
3.	Authenticity/reliability of the data generated	**10**
4.	Enthusiasm and sincerity to work	**10**
5.	Inferences made	**10**
6.	Collaboration and cooperation demonstrated in performing the task at hand	**10**
7.	Amenability to scientific/academic/laboratory discipline	**10**
	l Score	**100**

ANNEXURE - IX

INDIAN COUNCIL OF AGRICULTURAL RESEARCH

(For Guidelines Refer ANNEXURE – XI (I))

PROFORMA FOR EVALUATION OF A RESEARCH PROJECT AFTER COMPLETION BY PI

10. Institute Project Code

11. Evaluation research project after completion by PI

	Criteria	**Methodology**	**Marks (output)**	**Evaluation by PI**
	Achievements against approved and stipulated outputs under project	**Qualitative and quantitative assessment of objectives and stipulated outputs under the project will be carried out**	**75**	
		a) Activity Input /Projected Output/ Output Achieved	35	**35**
		b) Extent to which standard design methodology, experimental designs, test procedures, analytical methods followed	10	**10**
		c) Does the data justify the conclusions?	05	**05**
		d) Innovativeness and creating of new knowledge	10	**10**
		e) Additional outputs over those stipulated under the project	05	**05**
		f) Creation of linkages for commercialization of technology developed under the project	05	**05**
		g) Is scientific input commensurate to output (manpower, Financial input and time duration)?	05	**05**
	Publication/ awards	A essment will be done in respect of: Research papers; Reports/Manuals; Working and Concept Papers; Books/Book Chapters/Bulletins. Quality of publication (s) and Awards /Scientific recognitions received llowing publications were made during the project work: Research papers-2 (communicated to peer reviewed journals), Book chapter -1, Training Manual-3 nos. and Conference/Seminar presentations 4. (International -3 and National level -1)	**10**	**10**

	Additional facilities created	Facilities created in terms of laboratory. Research set-up, instrumentation, etc. during the project. Fatty acid analysis facility was created in the Laboratory with preparation of Fatty Acid Methyl Ester and analysis in gas Chromatography. institute collaboration was developed with University of Burdwan where Dr. Koushik Ghosh was the collaborator. Ontogeny study protocol of *O.bimaculatus* was developed along with study of enzymes during early stage of larvae in collaboration with University of Burdwan. Feeding protocol *O.bimaculatus* was worked out in the project.	**05**	**05**
	Human Resource Development (Scientific and Technical)	Scientist trained in different areas Arabinda Das, Scientist trained on a Programme 'Experimental design and statistical data analysis' during January 3-16, 2019 at ICAR-IASRI , New Delhi. B.N.Paul, attended two International and one National Conferences, Dr. R.N.Mandal attended one International conference. B.N.Paul and Mr. A.Das attended one National Conference. All the Scientist participated and presented paper in the above seminars during technical discussions.	**05**	**05**
	Revenue generated under the project/ avenues created for revenue generation	Resources and revenues generated different training programmes were conducted 1.Organised a Training programme on Applications and Practices of fish feed was organised at RRC, CIFA, Rahara during 23-28 Oct.,2017 2.Organised a Training programme on Application of Farm Made Feed for Sustainable Aquaculture Production was organised at RRC, CIFA, Rahara during 03-07 Dec.,2018 3.Organised a Training programme on Fish Nutrition and Feeding of Fish at RRC, CIFA, Rahara during 17-21 June ,2019 Revenue generated from the training programme were 2.04 Lakhs	**05**	**05**

	Product/Process/Technology/ IPR / commercial value of the technology developed	Details to be provided on a) Products: The larval feed was developed for *O.bimaculatus* larvae. b) Process: Preparation of larval feed process was developed with different quality fish meal and fish oils alonf with other feed ingredients. c) Technology: Feed and feeding protocol was developed for the *O.bimaculatus* larvae d) IPR e) Registration of the varieties	**10**	**06**
	Quality of available documents of the project duly authenticated	Research Project Files, Data, Reports etc. records are available with the Centre	**05**	**05**
Total Marks			**115**	**111**
	Time lines of execution of the project	Marks will be deducted if extension sought over the approved project duration beyond recorded and officially granted extension with recorded reasons		
		Up to 5%		
		Up to 10%		
		Up to 30 %		
		Beyond 30 %		
Score: Score obtained to be counted out of 100 to compensate for activities not relevant to the project				

However, looking into the requirements of different research institutes and disciplines, IRC may modify the indicators, their weights and total scores. The time gap for assessment of different indicators may also be decided by IRC

12. Signature of PI

भाकृअनुप
ICAR

ICAR-Central Institute of Freshwater Aquaculture
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(Indian Council of Agricultural Research)
RRC,Rahara, 700118,West Bengal

CIFA