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Interactive effects of dietary protein and lipid on growth of Indian climbing perch, *Anabas testudineus* fry

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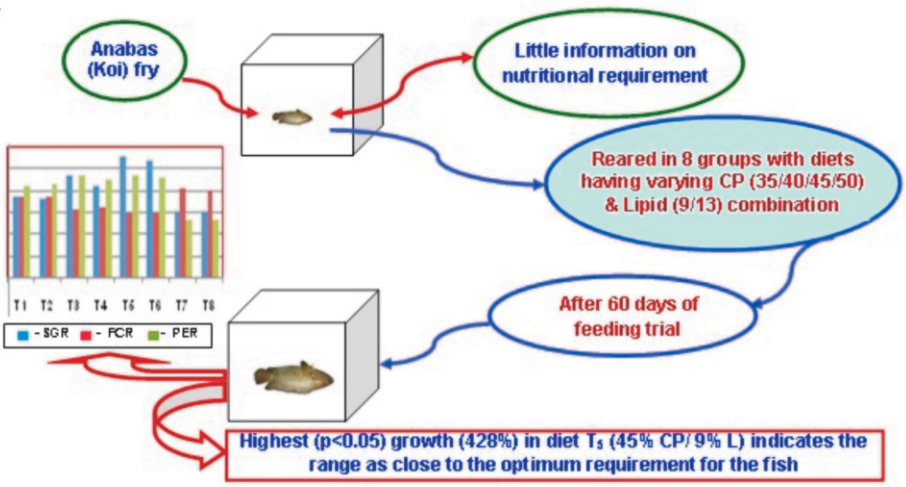
Abstract

Aim : To assess the optimum protein and lipid requirement of *Anabas testudineus* at its fry stage, based on the growth performance, feed efficiency and nutrient gain efficiency..

Methodology : Eight experimental diets were prepared with four protein (35, 40, 45 and 50 %) and two lipid (9 and 13%) levels. The diets were designated as D1 (35 P/9L), D2 (35 P/13 L), D3 (40 P/9 L), D4 (40 P/13 L), D5 (45 P/9 L), D6 (45 P/13 L), D7 (50 P/9 L) and D8 (50 P/13 L). Each of the experimental diets was fed to triplicate groups of 10 fishes with an average individual body weight of 1.23 g in 24 glass aquaria. The diets were fed at about 4 % body weight per day in two equal rations for a period of 60 days.

Results : Significantly ($p<0.05$) higher weight gain (428 %), specific growth rate (2.77 % day^{-1}), feed efficiency ratio (0.69) and protein efficiency ratio (2.34) and lower ($p > 0.05$) food conversion ratio (1.45) was observed in fish fed with diet D5 (45% protein and 9% lipid). The nutrient retention parameters like protein retention, protein productive value, lipid productive value and apparent net protein utilization were also significantly ($p < 0.05$) high in the treatment group D-5 fed with diet containing 45% protein and 9% lipid. Significantly ($P<0.05$) higher whole body protein content was observed in the treatment group D5 (45% protein / 9% lipid) along with D4 and D6.

Interpretation: The present findings on optimum protein and lipid requirement of 45% and 9%, respectively will help in formulating the cost-effective practical diets for *Anabas testudineus* fry, which is essentially required for quality seed production and grow out culture of this species in a commercial scale.



Key words: *Anabas testudineus*, Aquaculture, Indian climbing perch, Koi, Lipid, Protein

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Introduction

Indian aquaculture has made significant strides during the last few decades. Presently, it ranks second in world inland fish production next only to China. However, in terms of quantity the total aquaculture production from India remains far less from that of China (FAO, 2016). To keep the pace of growth in aquaculture production of the country, which is at present mainly contributed by pond based carp culture, the recent research activities emphasize on diversification of species and the water bodies (Sundaray *et al*., 2017).The climbing perch, *Anabas testudineus* (Bloch), locally known as Kavai or Koi, endemic to Indian water bodies, is one such potential candidate fish that can bring derelict water bodies under aquaculture and can contribute significantly to the national aquaculture production (Dash *et al*., 2019). They are air-breathing fishes and hardy in nature and can tolerate unfavorable environmental conditions with less oxygen that is usually prevalent in derelict waters bodies (Kumar *et al*., 2013). *Anabas testudineus* has a good economic value and consumer preference due to its high nutritive and therapeutic role. It's breeding and seed production methodology has already been standardized (Mandal *et al*., 2016), still, culture of this fish is not popularized due to non-availability of appropriate feed of this species for different life stages.

Formulation of appropriate feed in a cost-effective manner has paramount importance in undertaking commercial culture of a new candidate species (Jena *et al*., 2012). Information on nutritional requirements of major dietary components, such as protein and lipid, is a prerequisite for the formulation of an inexpensive and balanced diet for any fish. While excess protein content in the diet is utilized for energy production, leading to an increase in nitrogenous waste as ammonia excretion, besides increasing feed cost; inadequate dietary protein level in the diet results in poor growth and survival of fish (Li *et al*., 2010). Besides other factors, the amount of protein to be included in a fish diet is influenced by the quantity of non-protein energy sources present in the diet, since they have the potential to spare protein for growth (Kathane *et al*., 2017). Adequate quantity of lipid in the diet not only serves as an important source of non-protein energy for fish (Kaushik *et al.,* 1989), but also serves as an agent for sparing protein for growth (De Silva and Anderson, 1994; Mishra and Samantaray, 2004). At the same time, higher lipid content in the diet is also undesirable, as it may lead to reduced growth and development of fatty fish with undesirable flavor in the flesh and finished product (Hanley, 1991). Therefore, appropriate combination of dietary protein and lipid level serves as an important prerequisite in formulation of cost-effective feed for any fish. In this context, different levels of protein and lipids were used in the present study to formulate experimental diets, so as to assess the optimum dietary protein and lipid combination that can result in better growth and nutrient retention of *Anabas testudineus* at fry stage.

Materials and Methods

Experimental diets : Eight experimental diets were formulated in this study using four protein levels (35, 40, 45 and 50%) and two lipid levels (9 and 13%) based on the findings of Hossain *et al.* (2012) for protein level and Ali *et al*. (2012) for the lipid level. The digestible energy levels of formulated diets were in the range of 4.00 and 4.40 kcal g^{-1}. Laboratory-made fish meal by dry rendering method (Balachandran, 2012) was used as the major source of protein and Cod liver oil either single or with vegetable oil, was used as the source of lipid and α–cellulose was used as filler. Other ingredients like groundnut oil cake, rice bran, wheat flour etc. (Table 1) were procured from local market; oven-dried for 24 hr at 60 ºC and then finely powdered using a mixer grinder, sieved through a fine-meshed screen (0.5 mm dia) and stored in air tight jars until used for preparation of feed. Test diets were prepared as per the methods described by Kamei *et al.* (2018). Briefly, the required ingredients, as per Table 1, were precisely weighed and mixed thoroughly using a kitchen blender. Lukewarm water was then added to it and mixed properly, so as to form a dough-like consistency. The dough was steam-cooked in a pressure cooker, cooled under fan and fortified with vitamin and mineral mixture. Feed pellets (1 mm dia) were then prepared using a small hand pelletizer. The pellets were oven dried at 60 °C till moisture content was reduced to about 7-8%. Dry pellets were crumbled to appropriate size and stored at ambient temperature in airtight jars until used for feeding eight groups of experimental animals in triplicate.

Experimental animal and set up : About one thousand hatchery bred *Anabas testudineus* fry were procured from ICAR-Central Institute of Freshwater Aquaculture, Bhubaneswar, India and were acclimatized in 4 number of 500 l capacity FRP tanks under aeration and feeding with commercial diet containing 30% protein and 8% lipid. After acclimatization, uniform sized (1.23 ± 0.01 g) healthy fishes were randomly distributed in 24 glass aquaria (12"×12"×15" and 30 l capacity) @ 10 fish in each tank and thereby, for each dietary treatment, three replicated glass aquaria tanks were maintained. The experimental animals were then fed with the respective experimental diets at 4% of their body weight, twice daily at 09:00 and 16:00 hr for 60 days. The experimental fishes were weighed individually at beginning and end of the experiment to determine the initial and final weight. The batch weighing of fishes was done at an interval of 7 days and the feeding rate was adjusted accordingly. All tanks were aerated continuously from 08.00 to 15.00 hr throughout the experimental period as accessory respiratory organ might not have been developed at early stage. The water quality parameters like water temperature, pH and dissolved oxygen were recorded once in a week, following the standard methods of APHA (2018), and were found in the ideal range of 28°±2° C, 5.9 to 7.2 and 6.6 to 8.1 mg l^{-1}, respectively.

Table 1 : Ingredients and proximate composition of the test diets (% dry matter basis).

Ingredients /	Composition of test diets							
Chemical composition	D1 (35/9)[1]	D2 (35/13)	D3 (40/9)	D4 (40/13)	D5 (45/9)	D6 (45/13)	D7 (50/9)	D8 (50/13)
Ingredients composition								
Fish meal	24	24	27	27	32	32	37	37
Groundnut oil cake (defatted)	24	24	27	27	32	32	37	37
Rice bran	25	25	22	22	17	17	11	11
á-Cellulose	23.5	19.5	20.5	16.5	15.5	11.5	11.5	7.5
Binder (wheat flour)	1	1	1	1	1	1	1	1
Vegetable oil	-	3	-	3	-	3	-	3
Cod liver oil	0.5	1.5	0.5	1.5	0.5	1.5	0.5	1.5
Vitamin and mineral mixture[2]	2	2	2	2	2	2	2	2
Chemical composition								
Crude Protein	35.2	35.1	40.2	40.2	45.3	45.2	50.2	50.1
Crude lipid	9	13	9	13	9	13	9	13
Digestible energy[3]	400	440	400	440	400	440	400	440
Protein to Energy (P/E) ratio (mg protein/ kcal)	88.0	79.7	100.5	91.3	113.2	102.7	125.5	113.8

[1]35/9 : numerator refers to dietary protein and denominator refers to dietary lipid content; [2]Vitamins/100 mg: Vitamin-A, 70,000 IU; Vitamin D, 70,000IU; Vitamin B_2, 0.016; Vitamin E, 6.0 units; Vitamin K, 0.008; Choline Pantothenate, 0.02; Nicotinamide, 0.08; Vitamin B_{12}, 0.048. Minerals/100 mg: Calcium chloride, 1.2; Calcium, 6.0; Manganese, 0.22; Iodine, 0.008; Iron, 0.06; Zinc, 0.12; Copper, 0.016; Cobalt, 0.004; [3]Digestible energy was calculated considering: 1 g protein, lipid and carbohydrate equal to 4.0, 9.0 and 4.0 kcal, respectively (Pike and Brown, 1967).

At the beginning of experiment, 50 fry were sacrificed for analyses of whole body chemical composition in triplicate as per the methods described below. At the end of the experiment, six fish from each tank were killed and used for whole body chemical composition analysis in duplicate.

Analytical methods : Chemical composition of the experimental diets and fish body were analyzed following the methods of Association of Official Analytical Chemists (AOAC, 2019). Briefly, moisture content was determined by oven drying the samples at 100 ± 2°C untill constant weight was obtained. Crude protein was estimated based on the method of Kjeldahl nitrogen estimation (Crude protein = N x 6.25). Similarly, samples were extracted with petroleum ether repeatedly for 8 hr in a Soxhlet extraction apparatus for the estimation of crude fat. Ash content was determined in a muffle furnace for 6 hr at 550 ºC. The energy value of diets was calculated based on standard physiological fuel values of 4.0, 4.0 and 9.0 kcal for g of protein, carbohydrate and lipid, respectively (Pike and Brown, 1967).

The growth performance, feed efficiency and nutrient retention parameters like average daily growth percent (ADG%), specific growth rate (SGR% day^{-1}), food conversion ratio (FCR), protein efficiency ratio (PER), protein productive value (PPV) and lipid productive value (LPV) *etc.,* were calculated by the formulae given by Mohanta *et al*. (2013) and De Silva and Anderson (1994).

Statistical analyses : The difference among the treatments (mean ± standard error) was tested by One-way analysis of variance (ANOVA) and comparison between the treatments were analysed by Duncan's Multiple Range Test (P< 0.05) (Duncan, 1955; Snedecor and Cochran, 1968). The statistical package used for the study was IBM SPSS Statistics for windows, Version 20.0. Armonk, NY, USA.

Results and Discussion

In the present study, two levels of lipid (9% and 13%) were used at each of the four protein levels (35%, 40%, 45% and 50%) to determine the optimum protein and lipid requirement of *Anabas testudineus* at its fry stage based on the growth performance, feed efficiency and nutrient gain efficiency and the findings have been given in Table 2. It is evident from the table that the growth performance among the treatment groups differed significantly (P<0.05). Significantly (P< 0.05) the highest weight gain was observed in the diets with 45 % protein and 9 % lipid level (diet D5), followed by 45 % protein and 13 % lipid level (diet D6). Accordingly, specific growth rate (SGR% day^{-1}) was significantly (P< 0.05) higher with diets having 45 % protein at both the lipid levels of 9% (diet D5) and 13% (diet D6). Similarly, the feed efficiency parameters among the treatment groups differed significantly (P<0.05). The food conversion ratio (FCR) was significantly (P< 0.05) lowest with 45% CP level (D5 and D6) and significantly (P< 0.05) highest with 50% CP level (D7 and D8), irrespective of the lipid levels (9 or 13%). Accordingly, the feed efficiency ratios followed similar trend (Table 2). In the similar line, the nutrient retention parameters among the treatment groups found to vary significantly (P<0.05). The highest PER values were recorded with diets having 45% protein (D5 and D6) and the lowest with diets having 50% protein (D7 and D8) at both the lipid levels ($P < 0.05$). Significantly higher ($P < 0.05$) values for protein retention, protein productive value and lipid productive value were recorded in D5 with 45 % protein and 9 % lipid level, followed by D4 with 40% protein and 13% lipid level (8.07), which did not differ significantly ($P > 0.05$) (Table 2).

Analysis of all the above results e.g., weight gain, percentage weight gain, SGR, FCR, FER, PER PR, PPV and LPV of climbing perch, *Anabas testudineus* fry (Table 2) indicate that the growth performance, feed efficiency and nutrient gain efficiency of the test animal increased steadily with the increase in dietary protein level up to 45%, when compared independently at each of the dietary lipid levels used in this study. However, with the increase in dietary protein level beyond 45%, all these parameters were found to decrease significantly ($p < 0.05$) both at 9% and 13% dietary lipid levels. In agreement to the findings of this study, many species of fishes such as, *Channa striatus* (Samantaray and Mohanty, 1997), silver barb (Mohanta *et al.,* 2008), *Megalobrama amblycephala* (Li *et al.*, 2010) and, Thai strain of *Anabas testudineus* (Hossain *et al.*, 2012) and hybrid grouper (Rahimnejad *et al.*, 2015), have recorded increased weight gain, SGR, FER and PER and decreased FCR with increase in dietary protein up to a certain level, beyond which these parameters have shown reverse trend, when fed with iso-caloric diets. Each fish has an optimum level of dietary protein requirement at its different life stages (Bartolo *et al*., 2018). If protein level in the diet is less than optimum requirement, body will withdraw protein from the tissues for biosynthesis of essential biomolecules like enzymes, hormones etc., so as to carry on the vital life functions, thereby resulting in reduced growth (Li *et al*., 2010). On the other hand, if the diet contains excess protein and since protein cannot be stored in the body, the surplus protein is metabolized to produce energy, thus reducing the overall protein conversion efficiency in fish (Bortolo *et al*. 2018). Besides, at higher dietary protein, beyond optimum requirement level, more energy is required to eliminate toxic nitrogenous wastes produced during protein metabolism (Wu and Gatin, 2014). Thus, a part of energy available from dietary protein is utilized for elimination of nitrogenous waste rather than being utilized for

Table 2 : Growth performance, feed efficiency and nutrient retention efficiency of *Anabas* fry

Parameters	Treatment Group/ Diet Number(Protein/Lipid)							
	D1 (35/9)	**D2 (35/13)**	**D3 (40/9)**	**D4 (40/13)**	**D5 (45/9)**	**D6 (45/13)**	**D7 (50/9)**	**D8 (50/13)**
Initial weight (g)	1.23 ± 0.01	1.25 ± 0.03	1.21 ± 0.01	1.22 ± 0.02	1.25 ± 0.01	1.23 ± 0.02	1.22 ± 0.02	1.23 ± 0.01
Final weight (g)	$3.80^{c*} \pm 0.17$	$3.67^{c} \pm 0.05$	$4.86^{b} \pm 0.00$	$4.24^{b} \pm 0.01$	$6.60^{a} \pm 0.24$	$6.06^{a} \pm 0.08$	$3.05^{d} \pm 0.04$	$3.06^{d} \pm 0.02$
Weight gain (g)	$2.56^{c} \pm 0.18$	$2.42^{c} \pm 0.02$	$3.64^{b} \pm 0.01$	$3.02^{b} \pm 0.01$	$5.35^{a} \pm 0.25$	$4.83^{a} \pm 0.07$	$1.83^{d} \pm 0.05$	$1.83^{d} \pm 0.01$
Weight gain (%day^{-1})	$208.94^{c} \pm 17.51$	$193.6^{c} \pm 3.18$	$301.65^{b} \pm 4.58$	$247.5^{bc} \pm 6.41$	$428.0^{a} \pm 22.5$	$392.68^{a} \pm 5.27$	$150.0^{d} \pm 17.15$	$148.78^{d} \pm 6.23$
ADG%$^{\#}$	$42.77^{c} \pm 2.08$	$40.33^{c} \pm 1.33$	$60.66^{b} \pm 1.16$	$50.32^{b} \pm 1.28$	$89.22^{a} \pm 2.18$	$80.55^{a} \pm 1.18$	$30.49^{d} \pm 2.83$	$30.60^{d} \pm 2.25$
SGR (%)	$1.86^{c} \pm 0.09$	$1.78^{c} \pm 0.02$	$2.30^{b} \pm 0.02$	$2.06^{b} \pm 0.03$	$2.77^{a} \pm 0.07$	$2.65^{a} \pm 0.01$	$1.52^{d} \pm 0.04$	$1.52^{d} \pm 2.71$
FCR	$1.86^{b} \pm 0.04$	$1.82^{b} \pm 0.01$	$1.56^{bc} \pm 0.01$	$1.60^{bc} \pm 0.02$	$1.45^{c} \pm 0.03$	$1.46^{c} \pm 0.01$	$2.02^{a} \pm 0.01$	$2.00^{a} \pm 0.01$
FER	$0.53^{bc} \pm 0.02$	$0.55^{bc} \pm 0.01$	$0.64^{ab} \pm 0.01$	$0.63^{ab} \pm 0.01$	$0.69^{a} \pm 0.03$	$0.68^{a} \pm 0.01$	$0.49^{c} \pm 0.01$	$0.50^{c} \pm 0.02$
PER	$2.08^{b} \pm 0.06$	$2.15^{b} \pm 0.01$	$2.33^{a} \pm 0.04$	$2.24^{a} \pm 0.04$	$2.34^{a} \pm 0.07$	$2.28^{a} \pm 0.01$	$1.30^{c} \pm 0.01$	$1.32^{c} \pm 0.01$
PR	$4.74^{e} \pm 0.09$	$4.72^{c} \pm 0.96$	$4.93^{c} \pm 0.72$	$8.07^{a} \pm 0.29$	$8.31^{a} \pm 0.31$	$6.46^{b} \pm 0.19$	$1.12^{d} \pm 0.01$	$1.23^{d} \pm 0.01$
PPV	$0.21^{d} \pm 0.01$	$0.13^{d} \pm 0.03$	$1.03^{b} \pm 0.01$	$1.19^{b} \pm 0.06$	$1.93^{a} \pm 0.08$	$1.05^{b} \pm 0.04$	$0.37^{c} \pm 0.01$	$0.41^{c} \pm 0.02$
LPV	$0.73^{e} \pm 0.09$	$1.11^{c} \pm 0.02$	$1.35^{b} \pm 0.04$	$1.89^{ab} \pm 0.05$	$2.48^{a} \pm 0.02$	$1.45^{b} \pm 0.13$	$0.80^{d} \pm 0.03$	$1.03^{d} \pm 0.05$
Survival (%)	80	75	85	80	90	90	80	85

*Mean values with same super script within the row are not significantly different ($P < 0.05$).

Table 3 : Whole body chemical composition of *Anabas testudineus* fry (on wet weight basis)

Parameters (%)	Before	After feeding trial							
	feeding trial	**D1 (35/9)**	**D2 (35/13)**	**D3 (40/9)**	**D4 (40/13)**	**D5 (45/9)**	**D6 (45/13)**	**D7 (50/9)**	**D8 (50/13)**
Moisture	$74.8^{a\#} \pm 2.37$	$76.1^{a} \pm 2.42$	$76.8^{a} \pm 2.40$	$74.8^{a} \pm 2.63$	$73.2^{a} \pm 2.43$	$73.2^{a} \pm 2.48$	$74.9^{a} \pm 2.64$	$75.0^{a} \pm 2.72$	$74.9^{a} \pm 2.64$
Crude Protein (CP)	$13.2^{c} \pm 1.37$	13.58 ± 1.23	$13.85^{c} \pm 1.18$	$15.31^{ab} \pm 1.40$	$16.69^{a} \pm 1.43$	$16.91^{a} \pm 1.49$	$16.03^{a} \pm 1.41$	$14.08^{bc} \pm 1.27$	$14.15^{bc} \pm 1.3$
Crude Fat (EE)	$3.22^{b} \pm 1.28$	$3.30^{b} \pm 1.06$	$3.46^{b} \pm 1.27$	$3.65^{ab} \pm 1.06$	$3.68^{ab} \pm 1.04$	$3.84^{a} \pm 1.34$	$3.96^{a} \pm 1.39$	$3.56^{ab} \pm 1.07$	$3.78^{ab} \pm 1.06$
Total Ash	$5.07^{ab} \pm 1.02$	$4.59^{b} \pm 1.23$	$4.19^{b} \pm 1.29$	$5.04^{ab} \pm 1.31$	$5.24^{a} \pm 1.37$	$5.62^{a} \pm 1.69$	$5.04^{ab} \pm 1.31$	$5.25^{a} \pm 1.38$	$5.28^{a} \pm 1.40$
NFE*	3.71 ± 1.03	2.17 ± 0.53	1.71 ± 0.32	1.02 ± 0.01	0.51 ± 0.02	1.57 ± 0.31	0.07 ± 0.01	2.11 ± 0.46	1.83 ± 0.23

*:Nitrogen Free Extract=100- (Moisture + Crude Protein + Crude Lipid + Ash); # :Mean values with same superscript within the row are not significantly different ($P < 0.05$).

growth (Cho *et al*., 2005).These may be the reasons behind poor growth and nutrient utilization of climbing perch fry fed with either lower or higher dietary protein than 45% at both the levels of dietary lipid used in this study.

On the basis of the results obtained in this study, the optimum level of dietary protein for *A. testudineus fry* was found close to 45%, as it produced significantly (p<0.05) better growth performance, feed efficiency and nutrient gain efficiency (Table 2). The findings of the present study reveal that the optimum protein requirement of climbing perch, *A. testudineus* in fry was 45% is higher than the protein requirement of other carnivorous fishes like 37-40% for Indian catfish, *Clarias batrachus* (Khan and Jafri, 1990), 40% for gulsha, *M. cavasius* (Hossain *et al.*, 2005), 42 % for Seabass (*Lates calcarifer*) (Ambasankar, 2013) and 34% for *Anabas testudineus* fingerling (Bhaskar *et al.*, 2017). However, this was similar to Hossain *et al*. (2012), who found insignificant difference in growth performance in Thai strain of *Anabas testudineus* with 40% and 45% protein diet. However, this level was lower than the dietary protein requirement levels for other carnivorous fishes such as 48-53% brown trout fry (Salmo trutta) (Arzel *et al*., 1995), 48% in Channa argus (Sagada *et al.*, 2017), 50 % hybrid grouper (Rahimnejad *et al*., 2015), 54-60% giant snake head fry (*Channa marulius*) (Raizada *et al*., 2012) and 55% snakehead fry (*Channa striatus*) (Samantaray and Mohanty, 1997). Such differences state that each fish species has a specific dietary protein requirement that is otherwise called as optimum requirement level (Bortolo *et al.*, 2018), and maintenance of such optimum dietary protein level is highly essential while formulating cost-effective feed for the target fish.

While comparing the growth performance of *Anabas testudineus* fry between the two lipid levels, at any protein level between 35% to 45%, irrespective of the dietary protein level, it was observed that 9% lipid level showed better growth performance and feed efficiency than 13% lipid level, although the difference was not statistically significant (P>0.05). However, at 50 % dietary protein, the growth performance was almost same at both the lipid (Table 2). The present findings on the growth performance, feed and protein efficiency decreased at higher lipid level is in agreement with earlier findings of Samantaray and Mohanty (1997) in *Channa striatus;* Erfanullah and Jafri (1998) in walking cat fish and Ali *et al.* (2012) in *A. testudineus* and Rahimnejad *et al.* (2015) in hybrid grouper. Better growth performance of *Anabas testudineus* fry at 9.0% dietary lipid level irrespective of the dietary protein level indicates two things. First, this level would be almost close to the optimum dietary lipid requirement for the fish. The optimum dietary lipid requirements for different fish species have been reported as 9.0% for for catfish (Anwar and Jafri, 1992), 8.0% for walking cat fish (Erfanullah and Jafri, 1998), and 8.0% for blue gourami (Mohanta *et al*., 2013), 14% in hybrid grouper (Rahimnejad *et al.*, 2015) and 12 to 15% in Northern snakehead fish (Sagada *et al.*, 2017). Hence, further study by taking multiple close ranges of lipid level on either side of 9.0% is required to ascertain the optimum dietary lipid requirement for *Anabas testudineus* fry. The second inference that can be drawn from the present findings is that lipid does not have protein sparing effect in *Anabas testudineus* fry as this fish would have limited ability to utilise lipid as a source of energy and relies more on protein as a primary energy source (Rahimnejad *et al*., 2015). Otherwise, the higher lipid level of 13% at sub-optimal level of protein (40%) as in D4 would have resulted an almost equal or better growth than that of D5 containing higher protein (45%) and lower lipid (9%) levels (Table 2) (Schuchardt *et al.*, 2008; Mohanta *et al.*, 2013). The present finding is in contrast to the findings of Alam *et al.* (2009) in black sea bass; Liu *et al.*(2011) in Asian catfish and Sagada *et al.* (2017) in *Channa argus*, who have found better growth and protein retention at higher lipid level. At the same time, the findings of this study is in agreement with Schuchardt *et al.* (2008) in red porgy; Mohanta *et al*. (2013) in blue gourami and Arenas *et al*. (2021) in juveniles *O. chrysurus*, who did not find protein sparing effect of lipid in their respective test animals.

The whole body chemical composition of *Anabas testudineus* was significantly (p<0.05) affected due the change in dietary protein and energy level in the diet, with an exception to moisture content (Table 3). Significantly (P>0.05) higher whole body protein content was observed in the treatment group with 45 or 40% dietary protein, irrespective of dietary lipid level, which lies within the average range suggested by Varghese and Mathew (2020). The present study is in agreement with the findings of Hossain *et al*. (2012), who reported a significantly increasing trend in carcass protein content of Thai strain of *Anabas testudineus* with increase in dietary protein level from 25-40%, which decreased at 50% dietary protein level. Similar reports on increase in carcass protein content up to certain dietary protein level for other species have been reported by Jauncey (1982) and Khan *et al*. (1993). On the other hand, significantly (P>0.05) higher whole body lipid content was observed in the treatment group D6 (45 P /13 L) and D5 (45 P /9 L) as compared to the initial values and also other treatment groups.

From the present study we found that the koi, *Anabas testudineus* fry fed with 45% protein and 9% lipid had significantly better growth performance (i.e., percentage weight gain, SGR and PER), feed efficiency (FCR and FER) and nutrient gain (PPV and LPV). Hence, it can be concluded that the optimum dietary protein and lipid requirement of koi fry is 45% and 9%, respectively. The present findings would be helpful in formulating nutritionally balanced and cost-effective practical nursery diets for koi, which is essentially required for quality seed production of this species.

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Add-on Information

Author's contribution : B.D. Pawar: Carried out the research work; **K. Samantaray:** Guided the student including allotment of topic, experimental design, analysis of result, correction of manuscript etc.; **B. Sahu:** Advisory Committee member, refined the manuscript, revised as per the reviewer's comments and the corresponding author; **K.N. Mohanta:** Experimental design, representation of experimental results, critical analysis of the findings, editing the manuscript, complying the referee comments and expert consultation; **B.K. Khuntia:** Guided the student in preparing the manuscript and representation of data, **M.K. Tripathy:** Helped in rearing of the fishes and monitoring water quality parameters; **S.K. Patra:** Undertook the statistical analysis and bibliographic collection.

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