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Effect of different protein levels on reproductive performance of snakehead murrel *Channa striatus* (Bloch 1793)

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Abstract

In this study the effect of different protein levels on reproductive performance of *Channa striatus* was conducted. Snakehead juveniles (65.5±0.2 g) were randomly distributed into nine homogenous groups of 75 fish each. Three isocaloric experimental diets differing in protein levels were prepared. The experiment lasted for 8 months and gonadosomatic index, absolute fecundity, egg diameter, number of mature oocytes, hatching rate, larval length, survival rate and amino acid and proximate composition of tissue, liver and ovary were monitored. Growth, gonadosomatic index (GSI) and absolute fecundity increased with increase in protein level. Protein and lipid content of ovary was highest in fish fed 450 g kg^{-1} protein. The percentage of mature oocyte, egg diameter, hatching rate and larval length were the highest in the group fed 450 g kg^{-1} protein. There was no significant difference between the amino acid profiles of muscle tissue in all treatments. Amino acid profile in the liver showed that isoleucine, leusine, phenylalanine and tyrosine were significantly higher in fish fed the 450 g kg^{-1} protein diet.

Keywords: *Channa striatus*, Protein nutrition, Reproductive performance, GSI, Fecundity

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Introduction

Snakehead, *Channa striatus*, is an obligatory air-breathing carnivorous fish with an important role in the economy of the aquaculture industry (Chen, 1990). The aquaculture of this species is growing worldwide because its flesh is claimed to help the body in rejuvenating, and is consumed also as a medicinal food for healing wounds in the Far East (Laila *et al.*, 2011). Although protein (Mohanty and Samantaray, 1996), lipid (Paiko *et al*., 2010) and carbohydrate (Arokiaraja *et al.*, 1999) in nutrition of juvenile *C. striatus* have already been documented, nevertheless no data is available in relation to broodstock nutrition of *C. striatus*.

The broodstock nutrition plays a vital role in gonadal development and fish reproduction (Lupatsch et al., 2010). An improvement in broodstock nutrition, has been shown to greatly improve not only egg and sperm quality but also seed production (Izquierdo *et al*., 2001). Recently more attention has been paid to the level of different nutrients such as protein and amino acids in broodstock diet.

Protein and amino acids have been identified as key factors for successful reproduction (Izquierdo *et al*., 2001; Li *et al*., 2009). Since the majority of fish eggs are cleidoic, early development of fish depends on maternal provision of amino acids during oogenesis (Finn and Fyhn, 2010). Dietary proteins appear to influence egg quality (Washburn *et al*., 1990, Harel *et al*., 1995), therefore inadequate protein in broodstock diet triggers poor reproductive performance (Gunasekera *et al*., 1997, Bhujel, 2000). Our goal is to determine the effect of various protein levels on reproductive performance of *C. striatus*.

Materials and methods

Fish husbandry

Snakehead juveniles with an initial body weight of 65.5±0.2 g (bought from a commercial hatchery in Rawang, Malaysia) were reared in the Aquaculture Research Complex of Universiti Sains Malaysia from November 2010 to Jun 2012 (8 months) in rectangular cement tanks and under natural photoperiod. Fish were randomly distributed into 9 homogenous groups of 75 fish each, after acclimation to laboratory conditions for 2 weeks, feeding on commercial grow out diets (Goldcoin Specialties Sdn-Bhd Johor Malaysia). Each group was reared in a 1500 L capacity cement tank connected to a flow through water system half-filled with a flow rate set at 2.5 L min^{-1}. Triplicate groups of fish were fed one of three experimental diets differing in their protein levels. Each diet was fed to fish by hand to visual satiation twice daily for 7 days per week and sampling was done bi-monthly.

Experimental diets

Tables 1 and 2 show the proximate composition and amino acid profile of the diets respectively. Three isocaloric formulated experimental diets, differing in protein levels (350, 400 and 450 g kg^{-1}) were prepared. It is broadly accepted that both fingerlings and adult fish require high protein levels, therefore, in the absence of nutritional information for the production of broodstock haruan, three protein levels were chosen according to data published on protein requirement of *C. striatus*

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fingerlings (Webster and Lim, 2003; Paiko *et al*., 2010; Zehra and Khan, 2011). Fish meal and casein were used as the sources of protein. Fish meal is an excellent source of essential amino acid, whereas the cost of fish meal limits its application level in diets for the culture of aquatic animals. Casein is a protein of milk and contains adequate amounts of essential amino acids.

Table 1: Ingredient used and proximate composition of the diets (g kg^{-1} DM).

	Protein levels (g kg^{-1})		
	350	**400**	**450**
Fish meal [1]	437.5	500.0	562.5
Casein	90.9	103.9	116.9
Corn starch	330.0	253.0	174.8
Soybean oil	50.0	48.2	41.8
Fish oil	4.70	0.00	0.00
Cellulose	6.90	14.9	24.0
Vitamin [2]	30.0	30.0	30.0
Mineral [3]	30.0	30.0	30.0
CMC [4]	20.0	20.0	20.0
Proximate composition			
Crude protein	357.0	412.1	460.7
Crude lipid	95.2	98.7	98.4
Ash	101.6	108	116.0
Fiber	41.0	31.0	22.0
NFE[5]	405.2	350.2	302.9
GE[6] (MJ.kg^{-1})	19.15	19.52	19.92

1-Danish fish meal containing (g kg^{-1} DM) , protein : 640, total fat:103

2-Vitamin contains (kg^{-1} dry weight), Vitamin A: 50.000 MIU, Vitamin D3: 10 MIU, Vitamin E: 130 g,Vitamin K3: 10 g, Vitamin B1: 10 g Vitamin B2: 25 g, Vitamin B6: 16 g, Vitamin B12: 100 mg, Niacin: 200 g, Pantothenic acid: 56g, Folic acid: 8 g, Biotin: 500 mg, Antioxidant: 0.2 g, Anti-cake: 20 g

3-Mineral premix, contains (kg^{-1} dry weight): calcium phosphate 397 g ; calcium lactate 327 g; ferrous sulphate 25g; magnesium sulphate 137g; potassium chloride 50g; sodium chloride 60g; potassium iodide 150 mg; copper suphate 780 mg; manganese oxide 800mg; cobalt carbonate 100mg; zinc oxide 1.5g; sodium selenite 20mg

4-Carboxyl Methyl Cellulose (sodium salt), binder

5-Nitrogen free extract calculated as 1000-(protein + lipid + ash + fiber) g kg^{-1}

6-Gross energy calculated based on 17.2, 39.5 and 23.6 KJ g^{-1} for carbohydrate, lipid and protein.

Table 2 : Amino acid composition (% of total protein) of experimental diets.

	Diet (g kg^{-1} diet) protein level		
	350	**400**	**450**
Indispensable			
Arg	2.23^{c}	2.63^{a,b}	3.16^{a}
His	0.83^{c}	0.92^{b}	1.16^{a}
Ile	1.60^{c}	1.86^{b}	2.05^{a}
Leu	2.80^{b}	3.19^{a}	3.45^{a}
Lys	2.40^{a}	2.74^{a}	2.56^{a}
Phe	1.56^{c}	1.77^{b}	2.25^{a}
Thr	1.97^{b}	1.98^{b}	2.37^{a}
Val	1.79^{c}	2.07^{b}	2.24^{a}
Dispensable			
Asp	2.80^{b}	3.25^{a}	3.01^{a}
Ala	1.83^{b}	2.10^{a}	2.14^{a}
Glu	5.36^{b}	6.08^{a}	5.82^{a}
Gly	1.80^{c}	1.94^{b}	2.24^{a}
Pro	1.93^{c}	2.19^{b}	2.36^{a}
Ser	1.65^{c}	1.82^{b}	2.12^{a}
Tyr	1.32^{c}	1.55^{b}	1.93^{a}

†Mean ± SD value with different alphabet in each row are significantly different (p<0.05)

†Key amino acids for reproductive performance are in bold

† Values are mean of triplicate determination (n=3)

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Gonadosomatic index and fecundity

The fish in each tank were bulk weighed to calculate the mean weight and those fish within the average fish size in each tank were randomly selected for subsequent analysis. Fish from each tank were anaesthetized using clove oil (1 ppm), individually weighed, and dissected to obtain and weight of the whole ovary. An ovary was fixed in 4% formalin for 48 hours for subsequent histological investigation. The second ovary was used to determine the absolute fecundity using the gravimetric method which relies on accurately weighed subsamples of the ovary and manual counts of all their oocytes using a microscope equipped with a camera. Three subsamples of the selected ovary were randomly obtained along the anterior-posterior axis of either left or right ovary lobe, weighed and placed in Gilson liquid (Appendix A) as explained by Humason (1961) and shaken periodically to loosen the oocytes. Three 1.0 g sub-samples of the ovary were taken to count the number of oocytes. The mean from three sub-samples were used to calculate absolute fecundity (number of oocyte female^{-1}) by counting the number of oocytes per subsample of ovary to the total ovary weight. Total numbers of oocytes (N) in both ovaries were calculated using the following formula described by Bagenal (1978).

$$N = \frac{Wt}{Ws} \times Ns$$

Where: W_t=total weight of two ovaries, W_s=subsample weight, and N_s =number of counted oocytes in the subsample. The ratio of ovary weight to body weight, GSI, was determined by weighing the whole fish and its ovary. GSI was calculated by using the formulas described by Brooks *et al*. (1997).

$$GSI = \frac{total\ ovary\ weight}{whole\ body\ weight} \times 100$$

Determination of mature oocyte

Ovary specimens (5 mm thick) from the middle of freshly dissected ovaries were fixed in 4% formalin for 24 hours. The tissues were passed through a graded alcohol series following standard procedure for tissues (Vandyk and Pieterse, 2008), then the ovary slices were cleaned in toluene and embedded routinely in paraffin wax. The pieces were then cut at 8 µm, using a rotary microtome (A. O. Spencer, model Reicheit-Jurg 820, Leica, Germany) but in order to aid adhesion of the cut sections, glass slides were coated with a thin glass film. The slides were allowed to dry overnight at 40°C, using a slide warmer. Later, the segments were deparaffinized, hydrated and stained with Hematoxyline and Eosin. The details of this standard procedure is presented in Appendix B. Histological slides were then examined and the number of mature oocytes was counted after oocyte stages were recognized under a compound light microscope and photographed using the attached digital camera (model Olympus Xcam-Alpha, Germany). The different oocyte development stages were identified as described by Gentek *et al*. (2009).

Proximate composition of tissues and diets

Feed ingredients, experimental diets, fish muscle, liver and ovary were analyzed for dry matter (DM) proximate composition of crude protein, crude lipid, fiber and ash content following standard (AOAC, 1997). For muscle, liver and ovary, three replicates per group were taken (one

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sample per replicate), freeze-dried (freeze drier model Labconco Freezone 2.5, Labconco Corporation, Kansas City, USA), then mixed. Later, three sections of mixed samples were used for proximate analysis. Crude protein was determined according to KJeldahl procedure (Crude protein=nitrogen×6.26). Samples were extracted with chloroform methanol (2:1, v/v) to determine crude lipid, crude ash was measured by ashing in a muffle furnace for 5 h at 550 °C and crude fiber by loss on ignition of dried residue after successive digestion with 5% H_2SO_4. Nitrogen free extract (NFE) was calculated by subtracting the sum of crude protein, crude fat, ash and crude fiber from the total dry matter content. The gross energy content of diets was determined based on 17.2, 39.5 and 23 KJ g^{-1} for carbohydrate, lipid and protein, respectively. All determinations were carried out in triplicate (*n = 3*) and results are presented as mean ± SD.

Amino acid profile of tissues and diets

The amino acid profile of the experimental diets and fish tissues were determined by hydrolysing 0.1 g (dry weight) of the sample with 6N HCl at 110 C° for 24 hr and then derivatized with AccQ reagent (6-aminoquinolyl-N-hydroxysuccinimdyl carbamite) before undertaking chromatographic separation using an AccQ Tag™ reversed phase (3.9×150 mm) analytical column (Waters). Amino acid analysis was performed on a HPLC (High Performance Liquid Chromatography) system comprising a Waters 1525 Binary HPLC Pump, 717 Plus auto-sampler (Waters®) and Waters 2475 Multi λ Fluorescence detector (wavelength excitation 250nm, emission 395nm). Chromatographic peaks were integrated, identified and quantified with Breeze™ software, version 3.20 by comparing to known standards (Amino acid standard H, Pierce, Rockford, Illinois, USA). In this study, sulphur amino acids, methionine and cysteine were not determined and all analyses were carried out in triplicate.

Spawning, egg and larval collection

Female fish with swollen abdomen and reddish swollen vent were selected for induced spawning based on the details described by Hussain and Latifa (2008). Sexual readiness in the males was determined by visual observation of the slightly pointed genital papilla. The fish in each tank were bulk weighed to calculate the mean weight and those ripe fish within the average fish size in each tank were randomly selected for spawning. According to the method described by Haniffah *et al*. (1996), three females and six males from each replicate were randomly collected and distributed at a ratio of one female to 2 males into circular fiberglass tanks filled with de-chlorinated tap water to the depth of 30.0 cm for induced spawning. Each breeding set was anesthetized and injected intramuscularly with ovaprim at 0.5 mL kg^{-1} body weight for both male and female. The aquatic macrophyte, *Eichhorinia crassipes* was placed in the breeding tanks to provide a suitable hiding place and the breeding tanks were covered with black meshed plastic sheets to reduce stress. Spawning occurred 24-36 hours after hormonal injection. Three egg batches were collected randomly from each replicate to

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calculate egg diameter, hatching and survival rate. One batch was fixed in 4% formalin, and transferred to the laboratory to calculate individual egg diameter using a light microscope equipped with a camera. Two egg batches were count and distributed into three containers filled with the same water used in the breeding tanks to calculate hatching and survival rates. The rest of the eggs were left in the tanks until hatching. Three days after hatching, three batches of larvae from each treatment were randomly collected, fixed in 4% formalin and total larvae length was measured in the laboratory using a light microscope equipped with a camera. The hatching and survival rates were calculated as follows.

$$Hatching\ rate = \frac{number\ of\ egg\ hatched}{total\ number\ of\ eggs\ in\ the\ batch} \times 100$$

$$Survival\ rate = \frac{number\ of\ live\ larvae\ after\ a\ week}{number\ of\ total\ larvae} \times 100$$

Statistical analysis

All data were analysed statistically by one-way analysis of variance (ANOVA), using the general linear model. Where differences between means were significant, subsequent comparison was made using Duncan's multiple range test. Value of $p<0.05$ were considered significant at 0.05 probability level. All analyses were performed using SPSS software, version 17.

Results

Growth of fish increased with increase in protein levels. While fish weight was found significantly higher in fish fed the diet of 450 g kg^{-1} during all sampling, significant highest weight gain was recorded in fish fed protein at 450 g kg^{-1} diet (289.7±5.5 g), followed by fish fed protein at 400 g kg^{-1}diet (250.3±4.0 g) and at 350 g kg^{-1} diet (230.3±3.5 g) at the end of the experiment (Fig. 1).

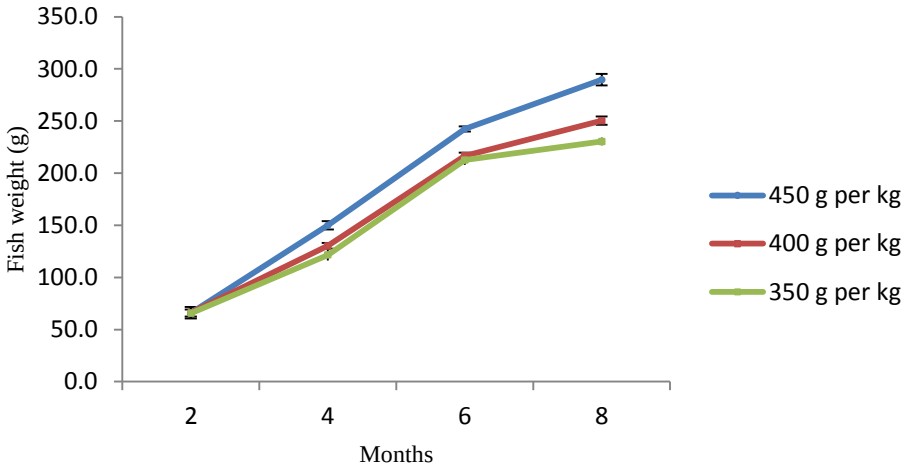

Figure 1: Growth performance of *Channa striatus* fed diets containing varying protein levels over 8 months feeding trial.

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Following weight gain trends, GSI values also increased with the increase in protein levels and was found significantly higher in fish fed protein at 450 g kg^{-1} during all the samplings. Moreover at the end of the trial, the significant highest GSI was detected in fish fed 450 g kg^{-1} diet crude protein (11.1±0.1) and followed by those fed 400 g kg^{-1} crude protein (9.6±0.9) and 350 g kg^{-1} diet (8.3±0.3) crude protein (Fig. 2).

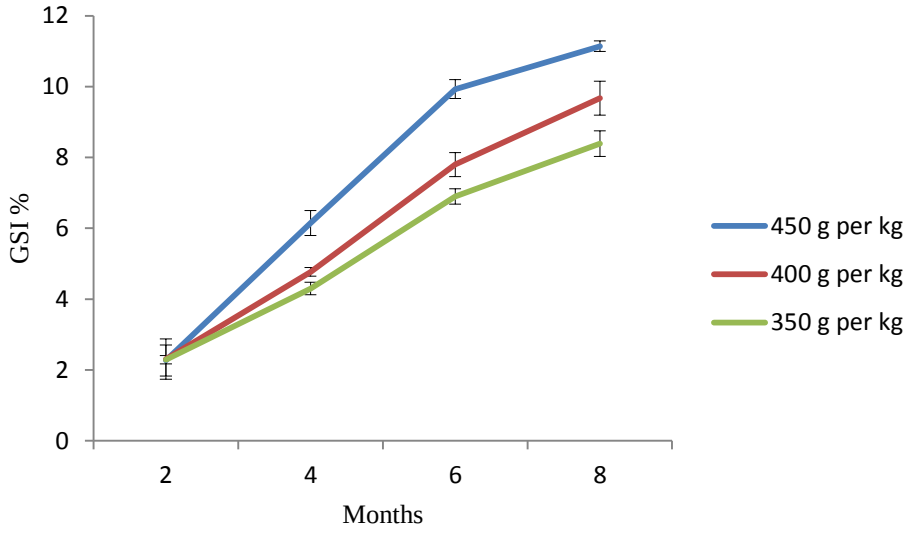

Figure 2: GSI changes in *Channa striatus* fed diets containing varying protein levels over the 8 months feeding trial.

Absolute fecundity was also significantly influenced by dietary protein levels. At the end of the study, the significant highest fecundity (48599±1775) was observed in fish fed 450 g kg^{-1} crude protein and followed by groups fed 400 g kg^{-1} diet (36734±1883) and 350 g kg^{-1} diet (28786± 883) crude protein (Fig. 3).

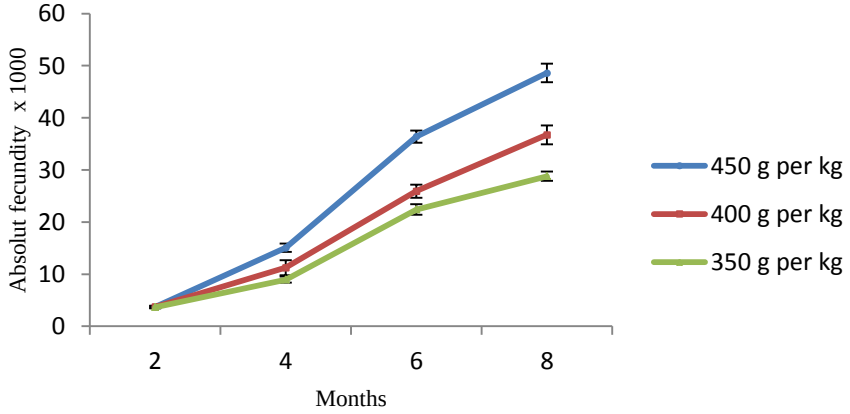

Figure 3 : Absolute fecundity of *Channa striatus* fed diets containing varying protein levels over 8 months feeding trial.

The crude protein content of muscle and liver were not significantly different between groups, while protein content in ovary was found to be significantly higher in fish fed 450 g kg^{-1} protein diet compared to other treatments.

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The lipid content of muscle and ovary was found to be significantly higher in fish fed diet of 400 g kg^{-1} protein compared to other groups, while lipid content in liver was detected higher in fish fed 400 and 450 g kg^{-1} and lower in fish fed diet of 350 g kg^{-1} protein content.

The ash content of muscle and ovary were not significantly different between groups while ash content in liver was found to be significantly higher in fish fed diet of 450 g kg^{-1} crude protein compared to other groups (Table 3).

Table 3: Tissue proximate composition (g kg^{-1} DM) of female *Channa striatus* fed diets containing different protein levels over 8-month feeding trial.

	Protein levels (g kg^{-1})		
	350	**400**	**450**
Muscle			
Crude protein	80.8 ± 0.5^{a}	79.9 ± 0.6^{a}	80.0 ± 0.7^{a}
Crude lipid	5.5 ± 0.1^{c}	8.3 ± 0.51^{a}	7.5 ± 0.7^{b}
Ash	4.2 ± 0.1^{a}	4.2 ± 0.02^{a}	4.3 ± 0.1^{a}
Liver			
Crude protein	29.8 ± 0.9^{a}	30.2 ± 0.8^{a}	29.1 ± 0.2^{a}
Crude lipid	56.7 ± 0.3^{b}	59.0 ± 0.7^{a}	59.6 ± 0.2^{a}
Ash	$2.29 \pm 0.02^{b, c}$	2.26 ± 0.03^{c}	2.36 ± 0.05^{a}
Ovary			
Crude protein	30.4 ± 0.2^{c}	$31.1 \pm 0.1^{a,b}$	31.6 ± 0.8^{a}
Crude lipid	64.65 ± 0.5^{b}	66.0 ± 0.4^{a}	65.2 ± 0.1^{b}
Ash	2.1 ± 0.4^{a}	1.9 ± 0.6^{a}	2.1 ± 0.1^{a}

† Mean ± SD value with different alphabet in each row are significantly ($p<0.05$).
† Values are mean of triplicate determination ($n=3$).
† Values are based on dry weight.

Histomorphological structure of *C. striatus* ovaries showed that the percentage of mature oocyte was significantly highest in fish fed 450 g kg^{-1} crude protein diet, followed by groups fed 400 and 350 g kg^{-1} crude protein in the June sampling. High egg diameter was recorded in fish fed 450 g kg^{-1} crude protein while no difference was found between fish fed 400 and 350 g kg^{-1} in both April and June. No differences were detected in total larval length between fish fed 350 and 400 g kg^{-1} crude protein while higher larval length was observed in fish fed 450 g kg^{-1} crude protein in both April and June. Hatching rate was found significantly highest in fish fed 450 g kg^{-1} crude protein followed by fish fed 400 and 350 g kg^{-1} crude protein (Table 4).

There was no significant difference between amino acid profiles of muscle in all treatments. Key amino acids that are important in reproduction, including arginine, leusin, asparagine, glutamine, proline and tyrosine were detected to be higher but showed no significant difference in the muscle of fish fed diet of 450 g kg^{-1} protein content (Table 5).

Amino acid profile in the liver showed that isoleucine, leusine, phenylalanine and tyrosine were significantly higher in fish fed 450 g kg^{-1} protein diet. No significant difference was detected between other AAs among all groups (Table 6, Fig. 4).

Amino acid profile in the ovary showed arginine was the highest in fish fed 400 g kg^{-1} crude protein followed by fish fed 350 and 450 g kg^{-1} protein diet. Lysine was

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higher in fish fed 350 and 400 g kg^{-1} crude protein and lower in fish fed the 450 g kg^{-1} protein diet. There were no differences in the other amino acid content (Leu, Ala, Glu, Pro, and Tyr) of the ovary among all treatments (Table 7).

Table 4 : Reproductive performance of female *Channa striatus* fed diets containing different protein levels over 8-months feeding trial.

	Protein levels (g kg^{-1})		
	350	**400**	**450**
Fish weight (g)			
April	213.0±2.6[b]	221.3±3.5[b]	251.0±3.0[a]
June	226.8±2.7[c]	246.0±2.6[b]	284.6±4.5[a]
%Mature oocytes			
June	83.9 ±2.9[c]	89.8 ±1.51[b]	94.3 ±1.8[a]
Egg diameter (mm)			
April	1.35 ± 0.07[b]	1.34±0.02[b]	1.43 ± 0.04[a]
June	1.40 ± 0.04[b]	1.41±0.01[b]	1.45 ± 0.04[a]
Hatching rate (%)			
April	53.05±0.61[c]	57.7 ± 0.5[b]	60.8 ±0.6[a]
June	55.7 ±0.5[c]	58.8 ±0.3[b]	62.6 ± 0.8[a]
Larval length (mm)			
April	4.37±0.01[b]	4.39 ± 0.05[b]	4.53±0.01[a]
June	4.45±0.03[b]	4.48 ± 0.04[b]	4.59±0.04[a]
Larvae survival (%)			
April	86.6±0.7[a]	87.1±0.5[a]	87.5±0.3[a]
June	87.0±0.2[a]	87.2 ± 0.3[a]	87.1 ±0.1[a]

†Mean ± SD value with different alphabets in each row are significantly different (p<0.05)
† Values are mean of triplicate determination (n=3)

Table 5: Amino acid composition (% of total protein) in muscle of female *Channa striatus* fed diets containing different protein over 8-months feeding trial.

	Protein levels (g kg^{-1})		
	350	**400**	**450**
Indispensable			
Arg	5.22±0.42[a]	4.88±0.60[a]	5.37±0.20[a]
His	1.87±0.14[a]	1.76±0.22[a]	2.02±0.08[a]
Ile	3.04 ±0.14[a]	2.95± 0.31[a]	3.23± 0.02[a]
Leu	5.22 ±0.21[a]	5.10 ±0.51[a]	5.57± 0.04[a]
Lys	4.78±0.33[a]	4.58±0.39[a]	4.99±0.20[a]
Phe	3.28± 0.21[a]	3.15± 0.36[a]	3.56± 0.14[a]
Thr	3.39±0.28[a]	3.21±0.41[a]	3.66±0.15[a]
Val	2.98±0.14[a]	2.91±0.32[a]	3.15±0.01[a]
Dispensable			
Asp	4.87±0.29[a]	4.68±0.42[a]	5.09±0.29[a]
Ala	3.26±0.26[a]	3.08±0.27[a]	3.33±0.08[a]
Glu	8.18±0.48[a]	7.84±0.72[a]	8.45±0.25[a]
Gly	3.47±0.36[a]	3.30±0.36[a]	3.62±0.07[a]
Pro	2.48±0.20[a]	2.33±0.18[a]	2.54±0.05[a]
Ser	2.91±0.19[a]	2.78±0.34[a]	3.10±0.08[a]
Tyr	2.66±0.20[a]	2.55±0.32[a]	2.80±0.15[a]

†Mean ± SD value with different alphabets in each row are significantly (p<0.05) different
†Key amino acids for reproductive performance are in bold
† Values are mean of triplicate determination (n=3)

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Table 6: Amino acid composition (% of total protein) in liver of female *Channa striatus* fed diets containing different protein levels over 8-months feeding trial.

	Protein levels (g kg^{-1})		
	350	**400**	**450**
Indispensable			
Arg	2.46 ± 0.10^{a}	2.38 ± 0.10^{a}	2.51 ± 0.13^{a}
His	0.83 ± 0.05^{a}	0.84 ± 0.02^{a}	0.63 ± 0.47^{a}
Ile	1.32 ± 0.02^{b}	1.31 ± 0.04^{b}	1.44 ± 0.06^{a}
Leu	2.44 ± 0.05^{b}	2.37 ± 0.08^{b}	2.61 ± 0.10^{a}
Lys	2.35 ± 0.08^{a}	2.31 ± 0.11^{a}	2.51 ± 0.12^{a}
Phe	1.47 ± 0.03^{b}	1.44 ± 0.04^{b}	1.57 ± 0.04^{a}
Thr	1.55 ± 0.05^{a}	1.52 ± 0.05^{a}	1.64 ± 0.07^{a}
Val	1.58 ± 0.04^{a}	1.56 ± 0.06^{a}	1.68 ± 0.08^{a}
Dispensable			
Asp	2.57± 0.18^{a}	2.57 ±0.17^{a}	2.77± 0.18^{a}
Ala	1.73 ± 0.05^{a}	1.67 ± 0.09^{a}	1.78 ± 0.09^{a}
Glu	3.97 ±0.17^{a}	3.77 ±0.22^{a}	4.10 ±0.28^{a}
Gly	1.67 ± 0.08^{a}	1.67 ± 0.10^{a}	1.77 ± 0.08^{a}
Pro	1.53 ± 0.02^{a}	1.54 ± 0.10^{a}	1.61 ± 0.07^{a}
Ser	1.68± 0.06^{a}	1.63 ±0.06^{a}	1.73 ±0.07^{a}
Tyr	1.15 ± 0.02$^{b, a}$	1.11 ± 0.03^{c}	1.21 ± 0.06^{a}

†Mean ± SD value with different alphabets in each row are significantly (p<0.05) different
†Key amino acids for reproductive performance are in bold
† Values are mean of triplicate determination (n=*3*)

Table 7: Amino acid composition (% of total protein) in ovary of female *Channa striatus* fed diets containing different protein levels over 8-months feeding trial.

	Protein levels (g kg^{-1})		
	350	**400**	**450**
Indispensable			
Arg	2.24 ± 0.18$^{b, c}$	2.37 ± 0.07^{a}	2.10 ± 0.05^{c}
His	0.93 ± 0.05^{a}	0.91 ± 0.03^{a}	0.88 ± 0.03^{a}
Ile	2.30 ± 0.08^{a}	2.34 ± 0.05^{a}	2.36 ± 0.08^{a}
Leu	2.50 ± 0.11^{a}	2.51 ± 0.05^{a}	2.56 ± 0.12^{a}
Lys	2.95 ± 0.11^{a}	2.88 ± 0.04$^{a, b}$	2.77 ± 0.02^{c}
Phe	1.47 ± 0.08^{a}	1.48 ± 0.02^{a}	1.50 ± 0.07^{a}
Thr	1.65 ± 0.08^{a}	1.61 ± 0.05^{a}	1.69 ± 0.08^{a}
Val	1.88 ± 0.07^{a}	1.86 ± 0.04^{a}	1.89 ± 0.03^{a}
Dispensable			
Ala	2.83 ± 0.06^{a}	2.80 ± 0.08^{a}	2.74 ± 0.05^{a}
Asp	2.57 ± 0.04^{a}	2.43 ± 0.08^{a}	2.47 ± 0.13^{a}
Glu	4.08 ± 0.08^{a}	3.95 ± 0.12^{a}	3.90 ± 0.06^{a}
Gly	1.48 ± 0.09^{a}	1.40 ± 0.05^{a}	1.38 ± 0.00^{a}
Pro	1.68 ± 0.10^{a}	1.72 ± 0.06^{a}	1.69 ± 0.02^{a}
Ser	2.51 ± 0.10^{a}	2.43 ± 0.08^{a}	2.46 ± 0.02^{a}
Tyr	1.66 ± 0.05^{a}	1.60 ± 0.05^{a}	1.69 ± 0.08^{a}

†Mean ± SD value with different alphabets in each row are significantly (p<0.05) different
†Key amino acids for reproductive performance are in bold
† Values are mean of triplicate determination (n=*3*).

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The comparison distribution of amino acids that play a role in reproduction i.e. Arg, Leu, Asp, Glu, Pro and Tyr in the liver and ovary is shown in Fig. 4. The data shows that with the exception of Leu, dietary protein resulted in similar deposition levels of these amino acids in the liver and ovary and these AAs levels were not affected by dietary protein level intake.

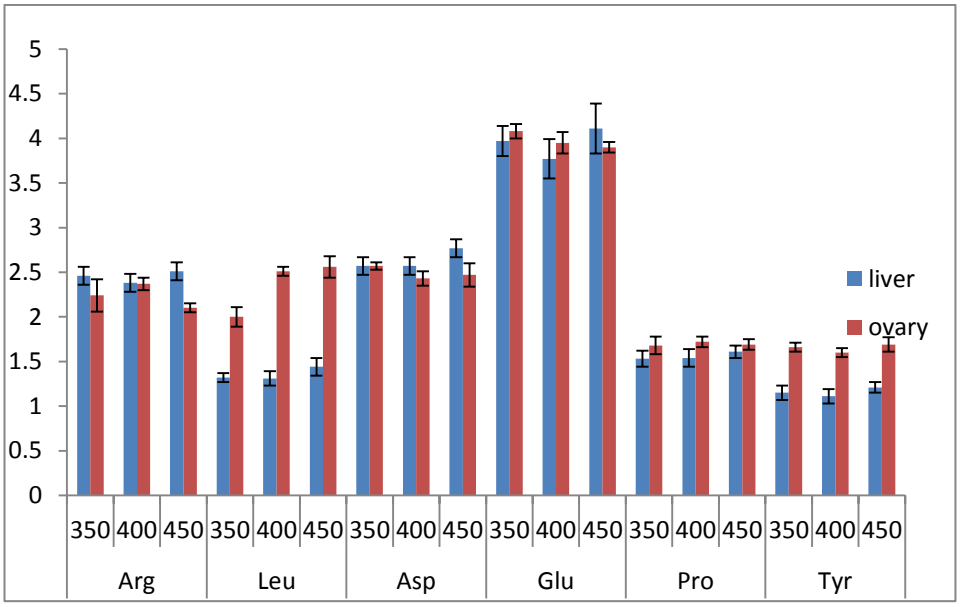

Figure 4: Composition of the amino acids with the important role in fish reproduction in liver and ovary of *Channa striatus* fed diets containing different protein levels over 8-months feeding trial.

Discussion

The current study was designed to investigate the effects of dietary protein levels on the reproductive performance of *C. striatus*. The protein requirement of *C. striatus* fingerlings for optimal growth has already been reported to be between 430 to 460 g kg^{-1} (Webster and Lim, 2003; Paiko *et al*., 2010). Since generally it has been suggested that the protein requirements for optimum reproductive development of broodstock in most fish is similar to that of fingerlings (Desilva and Anderson, 1995), the three protein levels (350, 400 and 450 g kg^{-1}) selected for this study were based on this assumption. Higher protein levels were not chosen because any excess protein energy, would have been diverted and used for de-amination and excretion of the excess protein which would not be stored and used by the fish (Ufodike and Ekokotu, 1986). Furthermore since the provision of protein levels higher than 460 g kg^{-1} would be uneconomical, the highest protein levels to be tested were set at 450 g kg^{-1}.

The present study demonstrated that feeding higher protein levels resulted in significantly higher growth, GSI and fecundity and is consistent with studies reported previously. Chong *et al*. (2004), observed higher fecundity when swordtail brooders were fed higher protein content and concluded that this was due to the higher weight gain of the female broodstock. A significant contribution of dietary protein level toward fish reproductive performance is also related to the effect on body size, where maturation of gonads and eggs occur

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earlier in larger broodstock (El-Sayed *et al*., 2003). Dahlgren (1980) also reported that female guppy *Poecilia reticulata* showed higher weight gain, GSI and fecundity when fed 470 g kg^{-1} proteins than those which were fed 250 and 350 g kg^{-1} protein diet. Studies on Nile tilapia (Siddiqui *et al*., 1998; El-Sayed *et al*., 2003; El-Sayed and Kawanna, 2008) and sea bass (Cerdá *et al*., 1994) indicated also that the fecundity increased with increasing protein levels in broodstock diets. However, numerous studies have also shown that once the dietary protein requirement of female broodstock is provided, further increases in dietary protein intake may increase fish weight but it does not necessarily enhance fish reproductive performance (Dahlgren, 1980, Gunasekera *et al*., 1996b, Alhafedh *et al*., 1999). For example, in a study by Desilva and Radampolak (1990) on the effect of varying protein levels on growth and fecundity in *Oreochromis niloticus*, the optimal protein level for growth of both male and female was 300 g kg^{-1}. However, the greatest percentage of spawned females occurred among those fed 250 or 300 g kg^{-1} protein.

Induced spawning was used to breed the *C. striatus* in the present study. Hormonal induced spawning is widely used in aquaculture to induce ovulation in target species (Bobe and Labbé, 2010). During the final oogenesis stages (also referred to as final oocyte maturation), the follicle-enclosed oocyte progressively acquires the ability (i) to resume meiosis, and (ii) to subsequently develop into normal embryo after fertilization (Bobe *et al*., 2008). In vertebrates, these two particularly overlapping processes are referred to as oocyte maturational competence acquisition (Bobe and Labbé, 2010). It is possible to artificially induce meiosis in a meiotically competent oocyte. Indeed, the ovarian stages at the time of spawning induction and physiological status of the female are critical for the quality of the ovulated eggs. In the current study, the number of mature oocytes and hatching rate were higher in fish fed 450 g kg^{-1} crude protein compared with the other treatments. It is likely that at this dietary protein intake, fish maturity and number of mature oocytes improved and subsequently, after induced spawning led to an increase in the percentage hatchability of eggs in the fish. Izquierdo *et al*. (2001) mentioned that optimum levels of nutrients in broodstock diets were necessary to improve egg morphology and hatching rates as well as for the normal embryo development. Similar results were reported in Nile tilapia (Gunasekera *et al*., 1996a; El-Sayed and Kawanna, 2008), common carp (Manissery *et al*., 2001), sea bream *Pagrus major* (Watanabe *et al*., 1984) and African catfish (*Clarias gariepinus*) (Sotolu, 2010) where egg hatchability increased with increasing protein levels in broodstock diets.

The size and appearance of the eggs can be used to evaluate or estimate the overall developmental potential of the eggs after fertilization (Bobe and Labbé, 2010). In aquaculture, generally there has been the perception that, bigger eggs are better (Brooks *et al*., 1997). Egg size may vary both within a species and between populations of the same species (Beacham, 1982; Beacham and Murray, 1987). Ecological differences in egg size of fish in different populations include temporal

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and spatial changes in food particle size and food availability (Sargent *et al*., 1987). Egg size in fish was also affected and modulated by the nutritional status of females during ovarian improvement (Tyler *et al*., 1994). Also it has been suggested that, larger hatchlings may have higher growth rate (Moodie *et al*., 1989) because of their larger yolk reserves. Although there are exceptions, it has generally been found that large fish eggs contain more yolk and lead to larger fry which have a potential for higher growth (Rana and Macintosh, 1988).

Egg diameter and total length of three-day post hatch larvae in the present study was significantly influenced by dietary protein levels whereby values increased with increasing dietary protein levels from 350 to 450 g kg^{-1} protein diet. A positive correlation between egg size and paternal body weight has also been reported in several species (Sehgal and Toor, 1991; Bromage *et al*., 1992), therefore, higher protein content causes bigger fish and subsequently bigger egg size. Besides weight gain, higher dietary protein intake would lead to larger eggs due to higher protein deposition and nutrient accumulation in the fish egg (Tyler and Sumpter, 1996; Gunasekera *et al*., 1997; Gunasekera *et al*., 1996a). Similar results have been reported on common carp (Manissery *et al*., 2001) and Nile tilapia (El-Sayed *et al*., 2003). Zakeri *et al*. (2009) showed that the total length of 3-day post hatch larvae of sea bream (*Acanthopagrus latus*) fed 500 g kg^{-1} protein diet was greater than fish fed lower protein levels. El-Sayed and Kawanna (2008) also reported that eggs from Nile tilapia broodstock fed low (300 g kg^{-1}) protein diet, resulted in lower larval length, than those fed higher (400 g kg^{-1}) protein diet.

There is no difference between protein content and amino acid profile in the liver among all groups. It can be attributed to the sufficient protein intake deposited in the liver in all treatments which is precursor of vitellogenin transferred to the ovaries. However, protein content of ovary was higher in fish fed 450 g kg^{-1} protein diet. Oocyte maturation involves transportation and accumulation of vitellogenin from the liver into the oocyte (Tyler and Sumpter, 1996), hence better nutrient deposition in the ovaries and eggs can be expected from an adequate nutrient intake. Therefore there is a relation between liver and ovary composition with protein intake. Gunasekera *et al*. (1996a) reported higher protein content in the eggs and ovary of *O. niloticus* fed 350 g kg^{-1} protein than those fed 100 and 200 g kg^{-1} protein diet and in a follow up study, noticed a lower protein content in the eggs of *O. niloticus* with a 100 g kg^{-1} in comparison with those receiving 200 and 300 g kg^{-1} protein diet.

The nutritional quality of diet e.g. protein content, appears not to affect the muscle amino acid composition (Gunasekera *et al*., 1996b). In the present study no significant differences were found in the amino acid profile of muscle of *C. striatus* fed diets of varying protein contents. Meyer and Fracalossi (2005) reported amino acid composition in the fish muscle did not depend on fish weight or dietary history. In this study, the major amino acids in *C. striatus* muscle were aspartic acid, glutamic acid, lysine, leucine and arginine. Similar results were reported

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by Zuraini *et al*. (2006) and Dahlan-daud *et al*. (2010) with regards to tissue amino acid profile of *C. striatus* in Malaysia. The amino acid composition of oocyte in a numbers of fish species, such as rainbow trout, Atlantic salmon, Chinook salmon, Coho salmon (Ketola, 1982), striped mullet (Tamaru *et al*., 1992) and channel catfish (Wilson and Poe, 1985) is well documented. These findings are comparable to those of the present study in that, total amino acid composition in oocyte is dominated by the essential amino acids, leucine and lysine while histidine was found in the lowest quantities regardless of dietary protein. At all dietary protein levels tested, glutaminc acid, serine, alanine and asparatic acid represented a major proportion of the total non-essential amino acid while glycine was found in small amounts.

The results from this study indicate that the total amino acids in the ovaries were not affected by dietary protein intake. Coldebella *et al*. (2011) reported the protein level is the diets did not affect the amino acids in the ovaries of *Rhamdia quelen* females when fed experimental diets containing 280, 340 and 400 g kg^{-1} crude protein. This result shows that the amino acid requirements for ovarian development were met by all protein levels.

In conclusion, this study showed that the 450 g kg^{-1} protein diets resulted in the best growth and reproductive performance such as fecundity, hatching rate, and egg diameter and larvae length. This observation was in spite of the similar levels of the key amino acids necessary for reproductive development in fish ovary, when the fish were fed the three different protein levels. Thus it is possible that other parameters such as the significantly higher fish weight, egg diameter and number of mature oocytes in the fish fed the 450 gkg^{-1} protein diet played an important role in influencing the good reproductive performance of fish fed this diet. It is therefore suggested that for best and economical dietary protein input for long term feeding of *C. striatus* fingerlings to sexual maturity is between 400 to 450 g kg^{-1} proteins. Future investigation should be carried out to understand the effect of free amino acids on fish reproduction. Diets of 450 g kg^{-1} protein could provide optimum levels of these amino acids crucial to stimulating the secretion of hormones from endocrine cells (Newsholme *et al.,* 2005) and increasing reproduction efficiency. This result has significant practical implications in *C. striatus* feed development as it is expected to pave the way for more in-depth studies on broodstock nutritional requirements of this species.

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