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The effects of dietary protein and carbohydrates ratios (P: C) on growth and development of ovarian tissues in Asian walking catfish, *Clarias magur* (Hamilton, 1822)

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Abstract
A 90-day experiment of different protein (CP) and carbohydrate CHO) levels in the diet of female *Clarias magur* broodstock with regard to growth, nutrient utilization and ovarian tissue development was carried out. Six different CP and CHO ratio based moist feed such as D1 (40:36), D2 (35:44), D3 (30:51), D4 (25:59), D5 (20:66) and D6 (15:74), were fed ad *libitum* to fish with respective treatments. Diets contains Low-protein–high-carbohydrate diet, i.e., below 30% CP and above 51% CHO experienced decreased weight gain percentage (WG%), specific growth rate (SGR), gonado-somatic index (GSI), hepato-somatic index (HSI) and increased feed conversion ratio (FCR) and protein efficiency ratio (PER). No significant differences (p>0.05) were found in WG%; SGR, GSI and HSI in D1, D2 and D3 fed groups. These data indicate that 35% CP and 44% CHO and above increased ovarian tissues, fecundity and for satisfactory growth required 30% CP and 51% CHO.

Keywords: *Clarias magur*, protein-carbohydrate ratio, gonadal tissue development, female broodstock

Introduction
Catfish farming is one of the largest and fast-developing aquaculture segments in the world. Asian catfish, *Clarias magur* is an air-breathing and omnivorous catfish with efficient food conversion [1] and excellent nutritional profile [2]. These traits make suitable for intensive commercial culture. However, their aquaculture production is declined due to a lower success in hatchery seed production. Often the seed is collected from wild sources such as wetlands, ponds and swamps [3]. Considering the high demand for this fish, it is important to formulate strategies, to overcome the problems associated with its seed production [4]. One of the important strategies is to focus on broodstock nutrition to optimize egg quality, hatching and fry production. Knowing the exact requirement of main nutritional ingredients like protein and carbohydrates are a major step in the formulation of a balanced diet.
Nutrition plays a crucial role in the breeding performance directly by affecting the fecundity, fertilization rate, egg quality of fish [5-10]. Proper feeding of the brooders before the breeding season can guarantee healthy gonad development and successful spawning. Several investigations have reported the variations in nutritional requirements of broodstock [11-12]. It is also cleared that many of the deficiencies and problems encountered during the early rearing phases of newly hatched finfish larvae are directly related to the feeding regimes and imbalanced dietary nutrients [12]. The concept of Ovarian recrudescence was investigated with different protein levels in the diets of fish results has no effect on the main physiological and reproductive parameters to lowered dietary protein [13]. The warm water herbivorous and omnivorous fish can utilize carbohydrate efficiently than the predatory /carnivorous fish with less ability. The best combination of both Protein - Carbohydrate ratios yet to be standardised in the diet of *C. magur* which is a highly accepted catfish in India having excellent flavour, high-quality protein and single central bone with high aquaculture potential [14]. In addition, energy contents [15-16], amino acids [17-21] and protein to carbohydrate ratio [22-23] have a pivotal role in the cascade of this process and influence the egg quality as well as gonadal development. With these pretexts, the present experiment was conducted to investigate the role of varying dietary protein and carbohydrate levels in the diet on growth and tissue/gonadal

Development of female *Clarias magur*.

Materials and Methods

Procurement of experimental animals and acclimatization

The brood fish (ranging from 89.5 to 95 g) of Asian magur, *Clarias magur* were used for the experimental purpose. The fishes were procured from local water bodies and the market of Jorhat, Assam, India. They were carefully transferred to 5000 L capacity rectangular cemented tanks (washed with disinfectants) kept in the wet laboratory of Fisheries Research Center (FRC), Assam Agricultural University (AAU), Jorhat, Assam in wet lab of the center and were acclimatized for ten days before conducting an experiment. The stock was acclimatized after a mild dip treatment with 2 ppm Potassium permanganate solution and fishes were fed with moist rice bran and mustard oil cake containing ~ 20% crude protein, as followed in the general farm practice.

Experimental design, set-up, feed preparation and fish maintenance

The experiment was conducted in 18 concrete rectangular tanks (5.9 X 2.6 X 1.6 m^3, 2450 L capacity) previously treated and cleaned with potassium permanganate solution (4 ppm). One hundred and twenty-six (126) female brooders were randomly distributed into six distinct experimental groups [D1 (40% CP), D2 (35% CP), D3 (30% CP), D4 (25% CP), D5 (20% CP) and D6 (15% CP)] in triplicates. The feed formulation and proximate composition analysis is mentioned in Table-1. Seven fishes (average body weight 92.15±2.76-105.29±1.9 g) were stocked in each of 18 tanks. The fishes were further fed the basal diet for one week before the commencement of the actual feeding trial. The experimental groups were fed with moist feed with 5% of body weight twice a day at 8:00 am and 06:00 pm for 90 days. The experimental tanks were cleaned every week with a 50% water replacement. Water quality was monitored throughout the experimental period *viz*., temperature (22.00 - 29.90 °C), pH (7.53 - 7.77), dissolved oxygen (6.60 - 7.06 mg L^{-1}), total hardness (179.67 - 194.67 mg L^{-1}), total alkalinity (153.33 - 158.67 mg L^{-1}) and ammonia-N (0.01 - 0.03 mg L^{-1}).

Table 1: Formulation and proximate composition of the experimental diets

	Diets[1]					
	D1	**D2**	**D3**	**D4**	**D5**	**D6**
Protein : Digestible Carbohydrate Ratio	1.05:1	1:1.26	1:1.70	1:2.32	1:3.25	1:4.87
Fish meal replacement (%)	0	20	40	60	80	100
Ingredients (g kg^{-1})						
FM[2] (Major protein source)	500	400	300	200	100	0
Starch (Purified)	0	100	200	300	400	500
WF[3] (Basal Carbohydrate Source)	168	158	148	138	128	118
PHM[4] (Basal Animal Protein Source)	100	100	100	100	100	100
SBM[5] (Basal Plant Protein Source)	130	140	150	160	170	180
Cod liver oil	20	20	20	20	20	20
Sunflower oil	60	60	60	60	60	60
CMC[6]	5	5	5	5	5	5
Vit-Min Mix[7]	15	15	15	15	15	15
Vitamin – C	2	2	2	2	2	2
Total	1000	1000	1000	1000	1000	1000
Proximate composition (g kg^{-1}) on dry matter basis (Mean±SE)						
Moisture	511.2	515.5	521.0	536.1	521.9	532.8
*Crude protein	407.1	355.2	305.1	251.4	199.7	147.6
*Crude fat	82.5	82.2	81.9	81.8	82.4	81.8
*Total ash	132.9	111.2	89.6	67.3	46.3	24.7
*Crude fibre	16.1	15.6	14.3	13.3	12.5	11.7
*Nitrogen free extract	361.4	435.8	509.1	585.9	659.1	734.2
Gross energy (Kcal/100g, calculated)[10]	470.2	466.6	463.0	459.4	455.8	452.2

[1]D1, 500 g kg^{-1} FM and 0 g kg^{-1} Starch; D2, 400g kg^{-1} FM and 100 g kg^{-1} Starch; D3, 300 g kg^{-1} FM and 200 g kg^{-1} Starch; D4, 200 g kg^{-1} FM and 300 g kg^{-1} Starch; D5, 100 g kg^{-1} FM and 400 g kg^{-1} Starch; D6, 0 g kg^{-1} FM and 500 g kg^{-1} Starch; [2]FM, Fish meal; [3]WF, Wheat flour; [4]PHM, Prawn Head Meal; [5]SBM, Soybean meal; [6]CMC, Carboxymethyl Cellulose; [7]Composition of Vitamin-Mineral mixture (Minamil) (quantity kg^{-1} diet)Vitamin A, 20,00,000 IU; Vitamin D3, 4,00,000 IU; Vitamin E, 320 mg; Vitamin B2, 1.2 g; Vitamin B6, 0.4 g; Vitamin B12, 4 mg; Calcium Pantothenate, 1.2 g; Nicotinamide, 8g; Choline Chloride, 60 g; Vitamin K_3, 0.40 g; Calcium, 320 g; Phosphorus, 20 g; Manganese, 12 g; Iodine, 0.40 g; Iron, 3.2 g; copper, 1 g; Cobalt, 0.2 g; Selenium,10 mg; Zinc, 8 g; Vitamin C, 300 mg; [8]Gross energy (Kcal/100g) = [4.5 x CP + 9.1 x EE + 4.1 x TC] (Halver, 1976)

Sampling and Analysis

The fishes were starved overnight before sampling; two fish were collected from each replicate and anesthetized by using clove oil (60 µl / L water). The final body weight of the experimental fish was measured at the end of the experiment. The whole ovary and liver were separated from the body after dissection to measure its size, weight and morphological observation. The data on the body and gonad weights were used to compute the growth, nutrient utilization parameters, gonado-somatic index (GSI) hepato-somatic index (HSI) and survival using the following standard formulae.

$$\text{Weight gain (\%)} = \frac{\text{Final wet weight (g)} - \text{Initial wet weight(g)}}{\text{Initial wet weight (g)}} \text{x}100$$

$$\text{Specific growth rate (SGR)(\%)} = \frac{(\text{Log}_e\text{final weight} - \text{Log}_e\text{initial weight})}{\text{Duration of the experiment (days)}} \text{x}100$$

$$\text{Feed conversion ratio (FCR)} = \frac{\text{Feed consumption (g on dry matter basis)}}{\text{Body weight gain (g on a wet weight basis)}}$$

$$\text{Protein efficiency ratio (PER)} = \frac{\text{Body weight gain (g on wet weight basis)}}{\text{Protein consumption (g on dry matter basis)}}$$

$$\text{Feed efficiency ratio (FER)} = \frac{\text{Body weight gain (g on wet weight basis)}}{\text{Feed consumption (g on dry matter basis)}}$$

$$\text{Survival (\%)} = \frac{\text{Total number of fish harvested}}{\text{Total number of fish stocked}} \text{x } 100$$

$$\text{GSI} = \frac{\text{Weight of gonad (g)}}{\text{Weight of fish (g)}} \text{X } 100$$

$$\text{HSI} = \frac{\text{Weight of liver (g)}}{\text{Weight of fish (g)}} \text{X } 100$$

For fecundity study, one gram from the three cross-sectional samples was taken from the anterior, middle and posterior position of the two lobes of each ovary and was kept in 10% neutral buffered formalin (NBF) solution. The eggs were counted by taking a photograph of spread oocytes in Petri-plate and then the mean number of ova was calculated. The total number of eggs (Absolute fecundity) in the entire ovary was calculated by multiplying the mean of the subsamples with a total weight of the ovary. Individual egg weight was calculated with the following formula.

Fecundity = Number of eggs in 1g of ovary × total ovary weight (g)

Statistical analysis
The data obtained in this study were analysed with the computer using the SPSS package version 22.0. The analysis carried out includes and one-way analyses of variance (ANOVA) with Duncan's multiple range tests were carried out to determine the significant differences between the means at a 5% level of significance.

Results and Discussion
Growth performance, nutrient utilization and survival
Growth performance, nutrient utilization and survival of fishes are shown in Table 2. Growth performance and nutrient utilization of fish was expressed in terms of weight gain percent, SGR, FCR and PER. The body weight gain percent and SGR of D1, D2 and D3 fed groups were not significantly (P>0.05) different from the fishes of D4, D5 and D6 groups. Fishes of D1 group shown the highest weight gain and SGR followed by D2, D3, D4, D5 and D6 groups. Dietary protein is always considered to be a primary nutrient in fish diets [25], as fish being evolved in an environment rich in protein. Thus sufficient dietary protein supplement is needed for rapid growth [26]. The D3 fed group containing 30% level of dietary protein was found to be optimum for the growth and further lowering the protein level growth decreases as described by several authors in species *Labeo rohita* and *Lutjanus argentimaculatus* [27-28]. The fish fed with high carbohydrate diet (59%) had shown significantly lower weight gain, SGR and higher feed conversion ratio than those of fish fed the ≤ 51% carbohydrate in the diet (p<0.05) may be due to their inability to utilize high digestible carbohydrate which results in poor growth and feed utilization [29]. Previous studies also suggested that high dietary carbohydrate levels reduce growth rate and feed utilization [30].
The PER value was highest in D3 fed group and significantly (p<0.05) lowest in D6 and D5 group. This result also supports the trend of growth which shows 30% protein and 51% carbohydrate fed group exhibited optimum growth performance and nutrient utilization. Few studies corroborate with findings of the present study. The higher weight increment was observed in fish fed dietary protein >300 g kg^1 diet [31]. Gunasekera & Lam [32] also reported that the broodstock of *O. niloticus* fed low (100 g kg^{-1}) protein diet results in lower weight gain than those fed high (200 and 350 g kg)1) protein diets.
The FCR values were in the range of 3.92±0.12 to 9.52.±0.84 which were significantly (P<0.05) different among all the treatments and lowest was in D1, D2 and D3 whereas the highest FCR in other fed groups due to high carbohydrates and least protein in the diet. The trials of Haque & Mazid [33] with low protein levels 24% and less in *Clarias batrachus* showed similar results with our study.

Table 2: Growth performance, nutrient utilization and survival rate of female brood stock of *Clarias magur* with different experimental diets

	Diets[1]					
	D1	**D2**	**D3**	**D4**	**D5**	**D6**
Parameters						
Weight gain (%)	76.30 ± 1.62[a]	74.07 ± 1.33 [a]	70.74± 1.61 [a]	52.59 ± 2.25[b]	32.96±2.59[c]	13.70±2.25[d]
SGR (%)[2]	0.63±0.02[a]	0.61 ±0.02 [a]	0.59±0.01 [a]	0.47±0.02[b]	0.32±0.02[c]	0.14±0.02[d]
FCR[3]	3.92±0.09[d]	4.04±0.09[d]	4.02±0.12[d]	5.37±0.26[c]	6.545±0.69[b]	7.92±0.84[a]
PER[4]	0.64 ±0.01[bc]	0.70±0.01 [ab]	0.72±0.02 [a]	0.77±0.02 [a]	0.64±0.03 [c]	0.48±0.02[d]
Survival (%)*	100±0.00	100±0.00	100±0.00	100±0.00	100±0.00	100±0.00

Data expressed as Mean ± SE, n = 3;
Mean values in the same row with different superscripts differ significantly (P<0.05).

[1]D1, 500 g kg^{-1} FM and without Starch; D2, 400g kg^{-1} FM and 100 g kg^{-1} Starch; D3, 300 g kg^{-1} FM and 200 g kg^{-1} Starch; D4, 200 g kg^{-1} FM and 300 g kg^{-1} Starch; D5, 100 g kg^{-1} FM and 400 g kg^{-1} Starch; D6, 0 g kg^{-1} FM and 500 g kg^{-1} Starch; [2]SGR, Specific growth rate; [3]FCR, Feed conversion ratio; [4]PER, Protein efficiency ratio.

Ganado-somatic index, hepato-somatic index (HSI) and fecundity
Mean GSI, HSI, ovary length and fecundity of *C. magur* females were affected by various levels of protein-carbohydrate ratios in the diet (Table 3). The fish fed with high protein diets, i.e., D1 and D2 had shown significant increment in GSI, ovary length and fecundity compared to the lower protein i.e., D3, D4, D5 and D6 groups. Among the experimental groups, fish fed with 35% protein in diet shown the highest GSI and higher numbers of oocytes per body weight of fish (Absolute fecundity) compared to all other groups. Pathmasothy [34] reported higher GSI in *Leptobarbus hoevenii* which was fed higher (320 and 400 g kg) 1) protein diets, similarly in *L. rohita*, lowest GSI was observed at 200 g kg^{-1} dietary protein [31]. The HSI values of the D6 group were significantly (P<0.05) higher than all other fed groups

whereas D2, D3 and D5 group showed higher values. The present results indicated that increasing dietary protein (above 35%) led to increasingly better gonad development in the female *C. magur* resulting in an increment of gonad weight.

The relative fecundity of D1 and D2 group found to be significantly (P<0.05) lowest 387.96 ± 0.51 and 387.16 ± 0.32 than all the other fed groups results are comparable with Khan *et al*. [31] Diets containing lower (250 and 300 g kg^{-1}) protein produced higher relative fecundity in *L. rohita*. Similar results also observed by De Silva & Radampola [35] who noted that *O. niloticus* fed low (20 g kg^{-1}) protein diet produced higher relative fecundity than those fed high (250 and 300 g kg^{-1}) protein diets.

The highest total fecundity was recorded from D2 and D1 fed groups measuring 8873.71 ± 5.92 and lowest in D6 (6106.01 ± 248) fed due to the small size of the ovary. Santiago *et al*. [36] noted a higher number of eggs kg^{-1} body weight in bighead carp, *Aristichthys nobilis*, fed 400 g kg^{-1} than those receiving 200 g kg^{-1} protein diet. This indicates that higher protein results in higher total fecundity irrespective of the size of the eggs.

Table 3: Relationship between the fish ovary (length, weight) and fecundity of *C. magur* female brooders fed different experimental diets.

	Diets[1]					
	D1	**D2**	**D3**	**D4**	**D5**	**D6**
Parameters						
GSI (%)[2]	14.35 ± 0.17^{ab}	14.76 ± 0.18^{a}	13.29 ± 0.03^{c}	13.21 ± 0.43^{c}	13.65±0.13^{b}	12.97±0.34^{d}
HSI (%)[3]	1.45±0.02^{c}	1.61 ±0.02^{b}	1.58±0.01^{b}	1.39±0.01^{c}	1.61±0.04^{b}	1.90±0.05^{a}
Length of Ovary (cm)	6.74±0.01^{a}	6.79±0.02^{a}	6.01±0.12^{b}	6.10±0.12^{b}	5.73±0.03^{c}	5.32±0.01^{d}
Relative Fecundity (No. of eggs /g)	387.96 ± 0.51^{e}	387.16 ± 0.32^{e}	404.22 ± 0.42^{d}	408.92 ± 0.15^{c}	433.15 ± 0.18^{b}	459.79 ± 0.08^{a}
Absolute Fecundity (Total numbers of eggs/female)	8792.5 ±23.95^{ab}	8873.71±5.92^{a}	8382.17±10.52^{b}	7412.36 ±130.71^{c}	7080.56 ±203^{d}	6106.01±248^{e}

Data expressed as Mean ± SE, n = 3

Mean values in the same row with different superscripts differ significantly (P<0.05).

[1]D1, 500 g kg^{-1} FM and without Starch; D2, 400g kg^{-1} FM and 100 g kg^{-1} Starch; D3, 300 g kg^{-1} FM and 200 g kg^{-1} Starch; D4, 200 g kg^{-1} FM and 300 g kg^{-1} Starch; D5, 100 g kg^{-1} FM and 400 g kg^{-1} Starch; D6, 0 g kg^{-1} FM and 500 g kg^{-1} Starch; [2]GSI, Gonado-somatic Idex; [3]HSI, Hepato-somatic Index.

Morphological attributes of the ovary

The ovaries are bi-lobed in *C. magur*, the weight; length and colour of the ovaries were varied with the diets fed (Fig.1). Each ovary is covered with peritoneal covering shows significant (P<0.05) changes in length and weight highest observed in D1 and D2 fed groups and followed by D3, D4, D5 & D6 groups with lowest in D6 group. The peritoneal covering of D1, D2 and D3 were found to be transparent comparably with other groups that are not transparent and highly vascularised, especially the D6 group showed dark red colour may be due to late-developing phase.

Conclusion

The study clearly shows that the level of protein in the diets has a profound influence on growth and the high reproductive performance of *C. magur*. According to the results, a diet supplemented with 30% of the protein-based diet and 51% carbohydrates rich diets recommended for better growth but 35% protein and 44% carbohydrates are optimum for superior response in early gonadal development and maturation and their performances of *C. magur*. The data generated during this study will be useful in developing protein and carbohydrate balanced diets for magur (*C. magur*) during broodstock rearing. However, the protein level of 35% is recommended, which is optimum for the growth of ovarian tissues as the estimation of total growth is obsolete due to the presence of gravid gonads in the body of broodfish.

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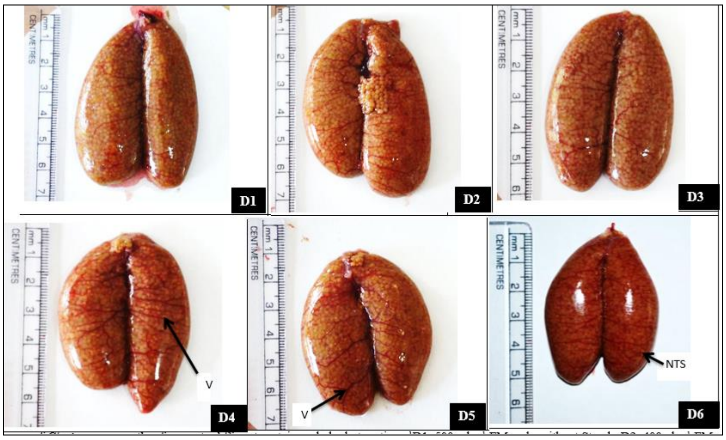

Fig 1: Fully developed ovaries of *Clarias magur* with reference to different protein-carbohydrate ratios : [1]D1, 500 g kg^{-1} FM and without Starch; D2, 400g kg^{-1} FM and 100 g kg^{-1} Starch; D3, 300 g kg^{-1} FM and 200 g kg^{-1} Starch; D4, 200 g kg^{-1} FM and 300 g kg^{-1} Starch; D5, 100 g kg^{-1} FM and 400 g kg^{-1} Starch; D6, 0 g kg^{-1} FM and 500 g kg^{-1} Starch; V, Vascularised and NTS, No transparent ovarian sac.

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