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EFFECT OF VARYING TIME OF HEAT PROCESSED SOYBEAN DIETS ON GROWTH OF *CIRRHINUS MRIGALA* FRY

Poonam, *Sudesh Rani, Manju Devi and Reeti Panchal

Department of Zoology, M.D. University Rohtak (124001), Haryana, India

**Author for Correspondence*

ABSTRACT

The fry (0.55g) of *Cirrhinus mrigala* were fed on six (1-6) isonitrogenous (40% protein) diets supplemented with a mineral premix with amino acid (MPA) for 60 days, in duplicates. Fry were randomly distributed @ 15 fish per aquarium for each dietary treatment. Six diets were formed out of which one diet was used as control contained fish meal as main protein source and remaining 5 diets contained soybean as protein source. Soybean was autoclaved for 0, 5, 10, 20, and 40 min at 121°C at 15 lbs to remove anti-nutritional factors prior to inclusion into diets. Growth performance was evaluated by feeding the fish twice @ 5% BW d⁻¹ for the whole duration i.e. 60 days. The results revealed that fish fed on diet containing 40 min processed soybean based diet and fishmeal containing diet showed maximum increase in growth (% gain in body weight), food conversion ratio (FCR), and specific growth rate (SGR), nutrient retention (GER, GPR) and apparent protein digestibility (APD) values and it get decreasing with decrease in processing time of soybean. Fish fed on raw soybean based diet showed minimum growth performance with low values of VSI and HIS but having maximum liver and muscle glycogen values. This study indicated that autoclaving the soybean for 40 min improved its nutritional value in practical feeds for *Cirrhinus mrigala* fry.

Keywords: Fish Meal, Anti-Nutritional Factors, Nutritional Value, Autoclaved

INTRODUCTION

Traditionally, farmers use diets for fish which have fishmeal as primary source of protein. But with increasing demand, less availability and having adulteration makes its use in aqua feeds less beneficiary. Soybean can be a better option to replace fishmeal as it has a good amino acid profile, further it is easily available in the local market at cheapest source of protein. Other important characteristics of soybean are its good palatability. But partial and total replacement of soybean for fishmeal has observed some deleterious effects on growth parameters, feed utilization efficiency and digestibility of certain fish as observed by many workers (Macgoogan and Gatlin, 1997; Lim *et al.*, 2004; Wang *et al.*, 2006; Hernandez *et al.*, 2007). The reason for this is the presence of some ANFs (anti-nutritional factors) in raw soybean (Lim and Lee, 2009) which makes the nutrition unavailable to fish and reduce their digestibility (Barrow *et al.*, 2008; Peres *et al.*, 2003; Francis *et al.*, 2001) feed conversion efficiency and growth (Antolovic *et al.*, 2012; Adewumi *et al.*, 2006; Abel *et al.*, 1980). Hence, before its inclusion into aquafeed formulation, these heat labile ANFs should be removed by proper processing of soybean by conventional methods like toasting, soaking, boiling, hydrothermal treatment and fermentation (Tiamiyu *et al.*, 2015b). Careful and controlled heating is required to optimize the nutritional components of soybean and this can be done by providing proper heat temperature to this legume. Further which duration of time is best to remove ANFs is not clear. Hence, to achieve this target, present experiment has been conducted to examine the growth of *Cirrhinus mrigala* fry fed on diets with varying time autoclaved soybean.

MATERIALS AND METHODS

Feed ingredients like sesame oilcake, soybean, wheat flour, fish meal, rice bran, chromic oxide, mineral premix, cod liver oil were purchased from local market of Rohtak. Before inclusion in diets, full fat soybean was autoclaved for different time i.e. 0 min, 5 min, 10 min, 20 min, and 40 min at 121°C at 15 lbs to inactivate ANFs. 6 diets named D1, D2, D3, D4, and D5 with 40% protein contents, were prepared by following the procedure of Rani (2014) (Table 1). All the feed ingredients were finally ground with the

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help of a grinder. A fishmeal containing diet was considered as control diet. Sesame oilcake and rice bran were used as base materials; chromic oxide (Cr_2O_3) as external digestibility marker; wheat flour as a binder and supplemented with a mineral premix with amino acid (Lysine + Methionine) (MPA). Finally a mechanical pelletizer (figure 1) was used to form noodle like strands which were broken to 0.5 mm thick pellets. These pellets were dried at room temperature and then were stored in freeze until being fed.

Table 1: Ingredient Contents (g kg⁻¹) and Proximate Analysis (% Dry Weight) of Experimental Diets

Diet Constituents	Control Diet	D1	D2	D3	D4	D5
Sesame oilcake	650	650	650	650	650	650
Rice bran	26.23	35	35	35	35	35
Wheat flour	26.23	35	35	35	35	35
Fish meal	260	-	-	-	-	-
Raw soybean heated for 0 min.	-	260	-	-	-	-
Soybean heated for 5 min.	-	-	260	-	-	-
Soybean heated for 10 min	-	-	-	260	-	-
Soybean heated for 20min	-	-	-	-	260	-
Soybean heated for 40min	-	-	-	-	-	260
Cod liver oil	17.54	-	-	-	-	-
Mineral + amino acid premix**	10	10	10	10	10	10
Chromic oxide	10	10	10	10	10	10
Proximate analysis						
Moisture	0.735 ±0.040	0.850 ±0.010	0.950 ±0.01	0.970 ±0.010	1.135 ±0.004	1.185 ±0.040
Dry Matter	99.265 ±0.040	99.15 ±0.007	99.055 ±0.010	99.030 ±0.010	98.865 ±0.004	98.815 ±0.040
Ash Content	11.95 ±0.04	9.75 ±0.53	10.15 ±0.11	9.93 ±0.06	10.25 ±0.53	9.05 ±0.11
Crude fat	4.20 ±0.12	5.30 ±0.52	5.57 ±0.09	5.89 ±0.01	5.52 ±0.19	6.35 ±0.18
Crude Protein	40.55 ±0.32	39.55 ±0.39	40.11 ±0.04	39.60 ±0.42	40.55 ±0.25	39.70 ±0.14
Crude fiber	2.95 ±0.04	2.80 ±0.21	1.10 ±0.07	1.75 ±0.11	2.55 ±0.25	3.20 ±0.07
Gross Energy (Kj g ⁻¹)	20.990 ±0.290	20.750 ±0.010	20.805 ±0.015	20.835 ±0.036	20.835 ±0.016	20.915 ±0.024

All values are mean ± SE of mean

*(Source: Rani, 2014)

**Mineral premix and amino acids: Each kg contains: Copper 312 mg, Cobalt 45 mg, Magnesium 2.114g, Iron 979 mg, Zinc 2.130 g, Iodine 156 mg, DL-Methionine 1.920 g, L-Lysine Mono Hydrochloride 4.4 g, Calcium 30%, Phosphorus 8.25%

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Figure 1: Mechanical Pelletizer

Experimental Design

The experiment was conducted in the fisheries lab of Department of Zoology, Maharshi Dayanand University Rohtak (India) from July 2016 for further 60 days. Total 180 fries of *Cirrhinus mrigala* fish were purchased from Sultan Aqua & Research Foundation, Nilokheri, Karnal, Haryana, India. Prior to the commencement of the experiment; fish were acclimatized in glass aquaria (60×30×30 cms) under laboratory conditions for 10 days. The temperature of water and lighting schedule of laboratory were maintained at 25±1°C and LD 12:12 respectively. The aquaria water was renewed daily with tap water adjusted to the laboratory temperature. Fry (mean body weight, 0.55g) were randomly distributed @ 15 fish per aquarium with duplicate for each dietary treatment. All fish were hand fed daily twice at 8:00 am and in afternoon at 4:00 pm with feeding rate 5% of live body weight per day until satiation for the whole duration and feed ration was adjusted every 15 days after bulk weighing. Each group of fish was exposed to their respective diet for 4 hour during each ration. The leftover feed were siphoned out, stored and dried separately in oven at (60-62 °C) for calculating the FCR. The fecal matter was taken out by siphoning, every morning then dried in an oven at 60 °C and subsequently analyzed for calculating digestibility. After termination of experiment, all the fish were weighed individually to the nearest gram and proceeded for subsequent analyses.

Analytical Techniques

The proximate analysis of experimental diets, fecal matter samples, fish carcass (initial and final) was analyzed following the procedure of AOAC (1995). Live weight gain (g), growth percent gain, specific growth rate (%d⁻¹) (SGR), gross protein retention (GPR), protein efficacy ratio (PER), gross energy retention (GER) were determined using standard methods of Sevier *et al.*, (2000). Cr₂O₃ levels in diets as well as in the fecal samples were estimated spectrophotometrically following the methods of Furukawa and Tsukahara (1966). Apparent protein digestibility (APD) of the diets was calculated according to Cho *et al.*, (1982). Energy content of the diets of fish was calculated using the average caloric conversion factors of 0.3954, 0.1715 and 0.2364 KJg⁻¹ for lipid, carbohydrate and protein respectively (Henken *et al.*, 1986).

Statistical Analysis

ANOVA followed by Turkey HSD test was applied to estimate the significance of the differences among treatments.

RESULTS AND DISCUSSION

No mortality was observed during experiment period. Each feed was equally accepted by fish. Proximate analysis (% dry weight) of experimental diets is shown in Table 1. Gross energy for diets as well as for

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fish was significantly ($p < 0.05$) higher in treatments where the fish were fed diets containing processed soybean and fish meal as primary protein source.

Table 2: Effect of Replacement of Fish Meal with Soybean Autoclaved for Varying Time Period (0 Min, 5 Min, 10 Min, 20 Min and 40 Min) on Growth Performance, Nutrient Retention, Feed Conversion Efficiency and Digestibility in Experimental Fish *Cirrhinus Mrigala* Fry under Laboratory Conditions (Temp $25 \pm 1^\circ\text{C}$ and LD 12:12)

Parameters	Control (fishmeal)	D1 (0min)	D2 (5min)	D3 (10min)	D4 (20min)	D5 (40min)
Initial weight (g)	0.55 \pm 1	0.55 \pm 1	0.55 \pm 1	0.55 \pm 1	0.55 \pm 1	0.55 \pm 1
Final weight (g)	1.46	1.09	1.14	1.28	1.34	1.45
	± 0.04	± 0.04	± 0.02	± 0.03	± 0.02	± 0.02
Live weight gain (g)	0.92	0.54	0.59	0.74	0.79	0.91
	± 0.05	± 0.05	± 0.02	± 0.04	± 0.02	± 0.02
Growth % gain in body weight	166.37	97.27 ± 8.18	107.28	133.64	143.64	164.55
	± 8.19		± 3.64	± 6.37	± 3.64	± 2.73
Specific growth rate% (SGR)	1.63	1.13	1.22	1.42	1.50	1.63
	± 0.05	± 0.07	± 0.04	± 0.05	± 0.03	± 0.03
Feed conversion ratio (FCR)	5.05	6.04	5.89	5.59	5.11	5.06
	± 0.24	± 0.03	± 0.43	± 0.47	± 0.03	± 0.14
Protein efficiency ratio (PER)	0.280	0.240	0.260	0.280	0.250	0.260
	± 0.030	± 0.020	± 0.010	± 0.007	± 0.02	± 0.001
GER	28.53	16.67	19.69	23.47	25.73	26.82
	± 0.24	± 0.32	± 0.19	± 0.47	± 0.06	± 0.33
GPR	27.00	16.75	18.63	21.25	24.25	27.13
	± 0.75	± 0.50	± 0.38	± 0.25	± 0.25	± 0.63
APD	94.83	88.61	91.19	92.11	93.63	94.25
	± 0.00	± 0.24	± 0.26	± 0.04	± 0.25	± 0.08

All the values are mean \pm S.E. of mean

Growth performance in terms of live weight gain, growth% gain in body weight, and specific growth rate increased significantly ($p < 0.05$) with increasing time period from D1 to D5. Highest growth performance was observed in the fish fed on D5 and control diet (Table 2), FCR was greatly improved with indicating significant differences ($p > 0.05$) among various treatments. PER values were observed significant ($p > 0.05$) among different treatments. Nutrient retention (GER, GPR) and APD values were increased in group fed on D5 in comparison to the group fed on D1, but the difference was not found to be significant. No significant difference was observed between values of VSI and HSI (Table 4). Liver and muscle glycogen values decreased from D1 to D5, without any significant difference. The fish fed diet D5 had the highest carcass lipid and protein content, while the group of fish fed on D1 had the lowest, with no significant difference.

Carcass (%) moisture and (%) fiber content were decreased from D1 to D5 with no significant difference. (%) ash content decreased significant value from D1 to D5 (Table 3).

Fish fed on D1 and D2 have shown significantly less growth rate than fish fed with D5 and control diet. Fish fed with D5 diet had an FCR of 5.06 compared with 6.04 and 5.89 for the fish fed with diets D1 and D2 respectively. Fish fed the control and D5 diets had shown improvement in carcass protein content but decreased HSI and VSI.

Higher gross energy values were observed in fish fed fishmeal and D5 and these values decreased with lowering processing duration of raw soybean in diets revealing higher feed intake by fish fed diets D4, D5 and control. Increased digestibility and nutrient retention were observed in fish fed on raw soybean.

Research Article

Table 3: Effect of Replacement of Fish Meal with Soybean Autoclaved for Varying Time Period (0 Min, 5 Min, 10 Min, 20 Min and 40 Min) on Proximate Carcass Composition (% Dry Weight Basis) and Gross Energy in Fish *Cirrhinus Mrigala* Fry under Laboratory Conditions (Temp 25±1°C and LD 12:12)

Parameters	Initial	Control (Fishmeal)	D1 (0min)	D2 (5min)	D3 (10min)	D4 (20min)	D5 (40min)
Moisture	7.84 ±0.05	6.89 ±0.08	8.83 ±0.06	8.37 ±0.11	8.08 ±0.05	7.71 ±0.07	6.81 ±0.03
Ash Content	2.50 ±0.10	1.95 ±0.05	2.60 ±0.10	2.50 ±0.10	2.15 ±0.05	2.20 ±0.20	1.80 ±0.10
Crude fat	8.25 ±0.25	11.00 ±0.50	7.00 ±0.50	7.75 ±0.25	8.50 ±0.00	9.95 ±0.25	10.25 ±0.25
Crude protein	13.40 ±0.10	16.45 ±0.25	14.10 ±0.10	14.47 ±0.04	14.76 ±0.04	15.18 ±0.13	15.95 ±0.05
Crude fiber	3.792 ±0.002	3.815 ±0.095	4.875 ±0.025	4.425 ±0.075	4.235 ±0.035	4.050 ±0.050	3.600 ±0.100
Gross energy (Kj g ⁻¹)	5.39 ±0.01	6.43 ±0.08	6.04 ±0.04	6.13 ±0.02	6.26 ±0.03	6.35 ±0.02	6.48 ±0.03

All the values are mean ± S.E. of mean

Table 4: Effect of Replacement of Fish Meal with Soybean Autoclaved for Varying Time Period (0 Min, 5 Min, 10 Min, 20 Min and 40 Min) on Muscle/Liver Glycogen, Hepato-Somatic Index (HSI) and Viscero-Somatic Index (VSI) in Fish *Cirrhinus Mrigala* Fry under Laboratory Conditions (Temp 25±1°C and LD 12:12)

Parameters	Initial	Control (Fishmeal)	D1 (0min)	D2 (5min)	D3 (10min)	D4 (20min)	D5 (40min)
Liver	4.27	3.80	9.94	8.31	5.12	4.64	3.66
Glycogen	±0.24	±0.17	±0.39	±0.29	±0.33	±0.21	±0.11
Muscle	1.311	1.211	1.281	1.271	1.271	1.261	1.211
Glycogen	±0.002	±0.000	±0.010	±0.003	±0.000	±0.003	±0.004
HIS	1.13 ±0.11	1.67 ±0.07	1.14 ±0.03	1.18 ±0.05	1.42 ±0.05	1.57 ±0.02	1.68 ±0.02
VSI	7.24 ±0.54	9.35 ±0.15	6.15 ±0.05	6.49 ±0.25	7.175 ±0.42	9.03 ±0.06	9.36 ±0.10

All the values are mean ± S.E. of mean

Raw soybean was observed containing endogenous ANFs i.e. lectin, phyto-haemagglutinin, anti vitamins and protease inhibitors (Liener, 1980) causing hindrance in its protein digestion. These protease inhibitors are mainly trypsin and chymotrypsin inhibitors. According to Lovell (1989), heat treatment of soybean not only improves palatability, digestibility and metabolized energy but also inactivate trypsin inhibitors. In previous studies, poor growth performance in tilapia, mrigal and *Clarias gariepinus* fed on diets containing soybean has been attributed to ANFs present in raw soybean (Rani 2014; Azaza *et al.*, 2009; Fafioye *et al.*, 2005; Peres *et al.*, 2003), which supports our findings as we also observed poor growth performance in fish fed raw soybean based diets i.e. Di. Also other parameters like low level of carcass protein, fat, ash and energy, a higher percentage of moisture and fiber were observed in these fish which fed on D1 and the values were significantly different from the other diets. Feeding *Cirrhinus mrigala* fries on processed soybean under laboratory conditions have revealed that growth parameters were significantly ($p < 0.05$) high for fish fed on diet D5 as compared to D1 with lowest FCR values and these results are in agreement with previous work done (Azaza *et al.*, 2009; Fafioye *et al.*, 2005; Peres *et al.*,

Research Article

2003). From these results, it is concluded heat treatment reduces ANFs in full-fat soybean seeds. Among different experimental diets, high growth was observed in fries fed on D5. This difference may attribute to inactivation of ANFs for increased in time of autoclaving the soybean causing more availability of protein content of soybean to fish fry. Besides this heat processing for long duration has a great impact on the improvement of FCR value which has a negative correlation with growth. Best growth results with control and D5 diet may be attributed to low protein and fat content with their low conversion ration. Growth depression in fries fed on D1, D2, and D3 is due to the presence of high level of inactivated trypsin inhibitors. *Cirrhinus mrigala* fry has shown good growth results could be attributed to high protein content in the feed (approximately 40%) which is more than its recommended dietary requirement i.e. 35% (Das and Ray, 1991). Both growth performance and FCR values are improved with decreasing trypsin inhibitors activity of experimental diets to tolerable levels with processing for longer time period i.e. for 40 min. Similar results were also shown by Okomoda *et al.*, (2016) for 40 min hydrothermally processed *Canavalia ensiformis* for *Clarias gariepinus* fingerlings diet at inclusion level 27%. Different fish have shown different result towards duration of heat treatment like, 30 min autoclaved soybean meal is sufficient to improve the nutrient value of improving growth performance and feed utilization in practical diets of Nile tilapia fingerlings (Azaza *et al.*, 2009), heat-treated soybean at 30 and 60 min were potent enough to its inclusion in diets of *Clarius gariepinus* fingerlings (Fafioye *et al.*, 2005), soybean autoclaved using dry cycle at 130 °C and 22 psi for 40 min was observed to decreased trypsin inhibitors, improved nutritional value of raw soybean meal for diets of cat fish (Peres *et al.*, 2003).

Conclusion

From this experiment, it seems that the growth depression is due to insufficiently treated soybean in experimental diet. This indicates the ANFs of D1, D2, and D3 diets caused growth reduction in respective diets, where inadequate heating of soybean causes incomplete destruction of trypsin inhibitors. But with an increase in duration of heat-treatment caused significant improvement in growth rate, feed utilization efficiency results of experimental fish fry, it concluded that 40 min heat treatment of full fat soybean seed is adequate to improve the growth performance and nutrient retention for *Cirrhinus mrigala* fish fry.

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Research Article

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