Effect Of Cheap Plant Protein Diet On Growth & Digestive Enzyme Activity Of Gold Fish (Carassius Auratus Auratus)

Manju Devi, Preety Madan, Sudesh Rani

Abstract: Objective: Studies were conducted to investigate the effect of diets, supplemented with soybean as cheap protein source, on growth & digestive enzyme activities (protease, amylase, cellulase & lipase) of gold fish fry. For this nine experimental diets were formulated with 40% protein level. Animal protein (fish meal) & plant protein (soybean meal) with basal ingredients like wheat flour, sesame oil cake, & rice bran were incorporated in the diets. Diet with fish meal were considered as reference diet & diets 1-8 with different level of raw (1-4) & processed (5-8) soybean meal (65, 130, 195, 260 gkg
ds) were taken as experimental diet. Experiment was set up in duplicate groups (weight 0.55g fry, 15 fish per aquarium). Results: Diet-8 contained 100% processed soybean meal (260gkg
ds) was significantly increased growth parameters, specific growth rate (SGR), Protein efficiency ratio (PER) & Visceromastic index (VSI) in gold fish fry. Values for digestive enzymes, amyrase, cellulase & Lipase were recorded higher in diet 8 as these parameters were remained minimum in diets supplemented with raw soybean ad reference diet. Maximum values for Liver glycogen & minimum values muscle glycogen were recorded observed in fry fed on 8th diet. Conclusions: present study indicated that 100% replacement of fish meal with processed full soybean meal is possible in gold fish only after removing anti nutritional constituents & evaluation of amino acid contents.

Index Terms: Growth parameters, Digestive enzyme activity, Fish meal, Soybean

1 INTRODUCTION
Fish meal is found to be main ingredient in the diet of fish from last many years; this is because of its palatability & good protein quality. But in present era its availability is a limiting factor due to microbial infestation & high price in market. Increasing demand, unstable supply of fish meal have resulted to investigate the alternative protein sources, especially plant protein to replace fish meal in diet of fresh water & marine fish species (Pongmanreet & Watanable, 1993). Hence it becomes essential to evaluate the alternative dietary protein sources. This is the reason there is need of such protein source which is easily available, cheap & remains free from any kind of alterations. Sufficient data is available on different plant protein sources, out of these, soybean is found to be most potential & main source because of its high nutritive & commercial value (Boonyaratpalin, et al. 1998). Hence, soybean meal with high protein content & balanced amino acids, is now widely used to replace fish meal in several fish species, gilthead sea bream, Sparus aurata (Gomez- Requeni, et al, 2004), red sea bream (Pagrus major); (Biswas, et al, 2007) & Korean rockfish, Sebastes schlegeli (Lim et al, 2004). Understanding effects of increasing dietary soybean inclusion & decreasing fish meal on growing fish is a central issue in developing more suitable & environment friendly feeding strategies. Some authors (Viola, et al, 1983 & Lim, 1992) studies that the high dietary level of plant proteins or complete substitution of animal proteins has resulted in poor growth & feed efficiency in fish. This is due to presence of anti nutritional factors (ANFs) & improper balance of essential amino acids. Raw Soybean contains ANFs that inhibit some digestive processes of fish. Proper heat treatment at particular temperature & pressure is found to inactivate these ANFs (121˚ for 15 minutes, 15 lbs, pH 5-6). After removing ANFs is becomes more nutritional & palatable for fishes (Jana et al, 2012; Sudesh, 2014). Therefore present experiment was planned to study nutritional value of soybean meal on growth & digestive enzymes activities of the gold fish.

2 MATERIAL & METHODS
2.1 Experimental diet preparation
Diet preparations were done by following the procedure of Jana et al, 2012. Soybean is main protein source used in experimental diets. Eight diets were prepared (1-4 raw soybean based, 5-8 processed soybean based) at four inclusion levels of processed as well as raw soybean viz- 65, 130, 195, 260 g kg
ds. In all diets 40% protein level was maintained. Fish meal (as chief protein source) was used to prepare reference diet. Wheat flour was added as a binder. 1% chromic oxide acts as external digestibility marker. All diets were supplemented with a mineral premix (MPA) to fulfill the mineral requirements of fishes. By using a mechanical pelletizer, 0.5mm thicker pellets were obtained which were then dried in oven (60-62˚ C) before using in feeding trials.

2.2 Experimental design
The experiment was conducted in duplicates in 18 glass aquaria (60 ×30 × 30 cm). The experiment was carried in fisheries Laboratory of Department of Zoology, Maharshi Dayanand University, Rohtak. Before starting the experiment fishes were acclimatized for a period of 10 days under laboratory conditions & were fed using commercial diet. After acclimation period gold fish fry (mean body weight 0.55 g) were randomly distributed in aquaria (holding capacity 35 L of tap water with continuous aeration) in groups of 15 fry in each aquarium. The fry were h & fed @ 5% of their total body weight (BW0.75) twice a day (9:00 am & 5:00 pm) over a period of 60 days. Weight of fishes was recorded every 15 days by electronic balance.
2.3 Parameters
At the end of experiment, the fish from all the treatments were individually weighed to the nearest gram & measured to the nearest millimeter & processed for subsequent analyses. From each treatment, ten fishes were randomly sampled. Fishes were dissected on ice tray, Viscera & liver were removed & weighed for the calculation of viscerosomatic index (VSI) & hepato-somatic index (HSI). Growth parameters like Live weight gain (g), Growth % gain, specific growth rate (SGR), Protein efficiency ratio (PER), feed conversion ratio (FCR), gross protein retention (GPR), & gross energy retention (GER) were calculated using standard method (Steffens, 1989). Whole Intestines were processed for assessment of digestive enzyme activity; protease (Walter 1984), amylase (Tietz, 1970), lipase (Worthington, 1991); Zamani, et al, 2009) & cellulose (Sadasivam & Manickam, 1996). Estimation of glycogen from Liver & Muscle was done (Dubois et al, 1956). Proximate analysis was done following the Standard methods of the Association of Official Analytical Chemists (1995).

2.4 Statistical analysis
One way ANOVAs was applied using OPSTAT of CCSHAU, Hisar.

3 RESULTS & DISCUSSION

3.1 Growth
Gold fish in all dietary treatments grew differently. Acceptance of experimental diet was judged by growth performance Table 1, proximate composition Table- 3 & digestive enzyme activity Table-2 of fishes. In present study high growth was recorded in diet-8, here fish meal was totally replaced with 100% processed soybean meal (main plant protein). Hence high live weight gain (1.06±0.01), growth % gain (192.72±1.1) & specific growth rate (1.79±0.001) were recorded in fish fed on diet 8th. Value for PER were also found to be in increased order from 1 to 8th diets. Similar pattern for HSI & VSI were recorded which indicated increased growth in fish fed on processed soybean based diets. Reverse trend for FCR & muscle glycogen were observed in fishes fed soybean diets i.e. low value was recorded in diet-8 (2.98±0.01) where more growth was observed whereas high in diet 4th (5.00±0.02) where less growth was observed (Table-1). High crude fiber & Low fat % was recorded in fish fed on diet-8 (Table 3).

3.2 Digestive enzyme activities
Results on digestive enzyme activities revealed that Increased Specific protease enzyme activities were recorded in diets were processed soybean was added, whereas value for these remained low in diets 1-4 (where raw soybean was incorporated in diets). There was no significant difference (p<0.05) in the specific protease activity of reference as well as processed soybean diets (Table 2). High value for specific amylase, cellulase & lipase activities were recorded in fish fed processed soybean diets than raw soybean diets (Table 2).

Lack of organic alternate protein sources is major obstacle in the growth of the organic aquaculture sector (Craig & McLean, 2005). Moreover, issues concerning the palatability & availability of amino acid in organic food must be addressed (Li, et al, 2007). The present study shows that growth & digestive enzyme activities in gold fish fed on soybean based diets under laboratory conditions were similar to that attained by animals maintained fishmeal-based feeds. How much quantity of plant protein should be used in fish diet is depends on its species, availability, cost & acceptability by fish. Presence of nutrient & anti nutritional factors (Lim & Dominy, 1990) is also very important for its incorporation in diet. Generally, it has been examined that a partial replacement of fish meal by plant protein source is well supported by fish. Hence more than 30% is already replaced in most commercial fish feeds (Burel, et al. 2000). It was found that anti-nutritional factors such as phytic acid & trypsin inhibitor can negatively affect the feed taste, feed intake & nutrient absorption, resulting in a decrease in fish growth when used in high percentages replacing dietary fish meal (Barrows, et al, 2008); (Burr, et al, 2012); (Manju, et al, 2017). The results of present study also support this finding as growth & digestive enzyme activity were decrease in fish fed on diets 1-4 where raw soybean (have anti-nutritional factors) was incorporated. Other researchers also reported a decrease in growth performance used different levels of plant protein sources in feeds of some fish species (Drew, et al, 2007); (Jallil, et al, 2012); (Palmegiano, et al, 2006). Barrows, et al, (2008) in his findings soybean meal in place of fish meal should be limited to less than 25% (or 10% to 15% dietary inclusion rates) for achieving the highest growth in rainbow trout. In addition, the decreased growth performance with increasing amount of plant protein resources in fish diets is possibly due to the higher dietary fiber & carbohydrate contents than animal protein resources (Gatlin, et al, 2007); (Hardy, 2010); (Krogdahl, et al, 2010). In recent study (Brett Glencross, 2016) a minor decline in feed intake was seen at the 40% inclusion level of the soybean meal & the feed conversion was unaffected suggesting that it was this decline in feed intake that was the primary problem. These results are in contradiction to present study. Present investigation confirmed & clearly indicated that soybean is a rich source of protein & can be consider as alternative protein source in fish feed.

4 CONCLUSIONS
The replacement of fish meal by plant protein (Soybean meal) sources increased growth & digestive enzymes activity in goldfish. This indicated that the total removal of dietary fishmeal by plant protein can be effectively & economically to the farmers.

5 ACKNOWLEDGEMENTS
This study was founded by the university research scholarship, Maharshi Dayanand University, Rohtak, & Haryana, India.
Table 1. Effect of soybean meal (Plant Protein) on growth performance of fry of Carassius auratus auratus.

Table 2. Effect of soybean meal (Plant Protein) on digestive enzymes activity of gold fish.

Table 3. Effect of soybean meal (Plant Protein) on proximate composition of gold fish under laboratory conditions.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Reference</th>
<th>Diet-1</th>
<th>Diet-2</th>
<th>Diet-3</th>
<th>Diet-4</th>
<th>Diet-5</th>
<th>Diet-6</th>
<th>Diet-7</th>
<th>Diet-8</th>
<th>C.V.</th>
<th>C.D.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial wt.</td>
<td>0.55±10</td>
<td>0.55±10</td>
<td>0.55±10</td>
<td>0.55±10</td>
<td>0.55±10</td>
<td>0.55±10</td>
<td>0.55±10</td>
<td>0.55±10</td>
<td>0.55±10</td>
<td>-</td>
<td>-</td>
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<tr>
<td>Final wt.</td>
<td>1.09</td>
<td>1.00</td>
<td>0.98</td>
<td>0.92</td>
<td>0.64</td>
<td>1.10</td>
<td>1.24</td>
<td>1.42</td>
<td>1.61</td>
<td>1.42</td>
<td>0.01</td>
</tr>
<tr>
<td>Live wt. gain</td>
<td>0.54±0.01</td>
<td>0.45±0.01</td>
<td>0.43±0.01</td>
<td>0.37±0.01</td>
<td>0.09±0.01</td>
<td>0.59±0.01</td>
<td>0.69±0.01</td>
<td>0.87±0.01</td>
<td>1.06±0.01</td>
<td>1.61</td>
<td>0.02</td>
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<tr>
<td>Growth % gain</td>
<td>98.18±0.4</td>
<td>81.81±0.5</td>
<td>78.18±0.4</td>
<td>67.27±1.1</td>
<td>16.36±0.8</td>
<td>107.27±1.1</td>
<td>100.00±1.5</td>
<td>158.18±0.7</td>
<td>192.72±1.1</td>
<td>0.47</td>
<td>1.21</td>
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<td>Specific growth rate (SGR)</td>
<td>1.14±0.01</td>
<td>0.99±0.01</td>
<td>0.96±0.01</td>
<td>0.85±0.01</td>
<td>0.25±0.02</td>
<td>1.11±0.16</td>
<td>1.35±0.01</td>
<td>1.58±0.01</td>
<td>1.79±0.01</td>
<td>1.59</td>
<td>0.54</td>
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<tr>
<td>Feed conversion ratio (FCR)</td>
<td>4.80±0.01</td>
<td>4.70±0.01</td>
<td>4.84±0.01</td>
<td>5.00±0.02</td>
<td>2.62±0.01</td>
<td>4.58±0.05</td>
<td>4.56±0.02</td>
<td>3.64±0.01</td>
<td>2.98±0.01</td>
<td>0.30</td>
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<tr>
<td>Protein efficiency ratio (PER)</td>
<td>0.06±0.01</td>
<td>0.03±0.01</td>
<td>0.05±0.01</td>
<td>0.06±0.01</td>
<td>0.02±0.01</td>
<td>0.07±0.01</td>
<td>0.08±0.01</td>
<td>0.10±0.01</td>
<td>0.12±0.05</td>
<td>3.89</td>
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<tr>
<td>Liver glycogen</td>
<td>3.29±0.01</td>
<td>3.84±0.01</td>
<td>3.80±0.01</td>
<td>3.44±0.01</td>
<td>3.20±0.01</td>
<td>5.93±0.05</td>
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<td>7.08±0.01</td>
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<td>Muscle glycogen</td>
<td>0.33±0.01</td>
<td>0.37±0.01</td>
<td>0.38±0.01</td>
<td>0.50±0.02</td>
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<td>0.22±0.01</td>
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<td>0.18±0.01</td>
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<tr>
<td>VSI</td>
<td>0.12±0.01</td>
<td>0.26±0.01</td>
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<td>0.23±0.01</td>
<td>0.42±0.01</td>
<td>0.50±0.01</td>
<td>0.45±0.01</td>
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<td>1.36</td>
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<td>HSI</td>
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<td>1.03±0.01</td>
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<td>1.07±0.01</td>
<td>1.04±0.01</td>
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<td>2.11±0.01</td>
<td>2.75±0.01</td>
<td>2.28±0.01</td>
<td>0.28</td>
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All the values are mean ± S.E of mean.

<table>
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<tr>
<th>Enzyme activity</th>
<th>Reference</th>
<th>Diet-1</th>
<th>Diet-2</th>
<th>Diet-3</th>
<th>Diet-4</th>
<th>Diet-5</th>
<th>Diet-6</th>
<th>Diet-7</th>
<th>Diet-8</th>
<th>C.V.</th>
<th>C.D.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total protease activity</td>
<td>5.69±0.01</td>
<td>4.63±0.01</td>
<td>4.47±0.01</td>
<td>4.21±0.01</td>
<td>4.18±0.01</td>
<td>4.78±0.01</td>
<td>5.82±0.01</td>
<td>6.86±0.01</td>
<td>7.41±0.01</td>
<td>3.21</td>
<td>0.06</td>
</tr>
<tr>
<td>Specific protease activity</td>
<td>1.41±0.01</td>
<td>1.34±0.05</td>
<td>1.28±0.01</td>
<td>1.26±0.01</td>
<td>1.17±0.01</td>
<td>1.42±0.01</td>
<td>1.40±0.01</td>
<td>1.50±0.01</td>
<td>1.58±0.01</td>
<td>2.05</td>
<td>0.05</td>
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<td>Total amylase activity</td>
<td>1.57±0.01</td>
<td>1.48±0.01</td>
<td>1.42±0.01</td>
<td>1.43±0.05</td>
<td>1.39±0.01</td>
<td>1.79±0.01</td>
<td>1.87±0.01</td>
<td>1.90±0.01</td>
<td>1.90±0.01</td>
<td>1.57</td>
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<tr>
<td>Specific amylase Activity</td>
<td>0.30±0.01</td>
<td>0.19±0.01</td>
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<td>0.16±0.01</td>
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<td>0.43±0.01</td>
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<td>Total cellulase activity</td>
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<td>2.75±0.01</td>
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<td>3.40±0.01</td>
<td>3.67±0.01</td>
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<td>5.94±0.01</td>
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<tr>
<td>Specific cellulase activity</td>
<td>0.17±0.01</td>
<td>0.16±0.01</td>
<td>0.22±0.05</td>
<td>0.25±0.01</td>
<td>0.15±0.01</td>
<td>0.41±0.01</td>
<td>0.42±0.01</td>
<td>0.44±0.01</td>
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<tr>
<td>Total lipase activity</td>
<td>2.80±0.05</td>
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<td>1.38±0.01</td>
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<td>1.40±0.01</td>
<td>1.38±0.01</td>
<td>1.41±0.01</td>
<td>1.47±0.01</td>
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<tr>
<td>Specific lipase activity</td>
<td>0.23±0.01</td>
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<td>0.06±0.05</td>
<td>0.05±0.01</td>
<td>0.07±0.01</td>
<td>0.08±0.01</td>
<td>0.07±0.01</td>
<td>0.08±0.01</td>
<td>0.10±0.01</td>
<td>1.35</td>
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</tbody>
</table>

All the values are mean ± S.E of mean. aMg of tyrosine mg−1 of protein/h. bMg of maltose mg−1 of protein/h. cMg of glycogen mg−1 of protein/h. dMg/mg of protein/h.
### Table 3

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Reference</th>
<th>Diet-1</th>
<th>Diet-2</th>
<th>Diet-3</th>
<th>Diet-4</th>
<th>Diet-5</th>
<th>Diet-6</th>
<th>Diet-7</th>
<th>Diet-8</th>
<th>C.V.</th>
<th>C.D.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dry matter</td>
<td>93.8±0.01</td>
<td>96.2±0.01</td>
<td>89.0±0.05</td>
<td>91.0±0.02</td>
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<td>91.4±0.01</td>
<td>89.8±0.01</td>
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<tr>
<td>Crude fat</td>
<td>13.33±0.05</td>
<td>10.00±0.01</td>
<td>0.04±0.02</td>
<td>0.42±0.01</td>
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<td>0.06±0.01</td>
<td>0.08±0.01</td>
<td>0.07±0.01</td>
<td>0.03±0.01</td>
<td>1.26</td>
<td>0.15</td>
</tr>
<tr>
<td>Crude fibre</td>
<td>3.50±0.01</td>
<td>3.00±0.01</td>
<td>4.00±0.03</td>
<td>3.00±0.02</td>
<td>2.00±0.01</td>
<td>2.50±0.01</td>
<td>3.00±0.01</td>
<td>4.00±0.05</td>
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<td>1.32</td>
<td>0.08</td>
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<tr>
<td>Ash</td>
<td>3.24±0.01</td>
<td>3.42±0.01</td>
<td>3.54±0.02</td>
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<td>3.89±0.01</td>
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<td>3.21±0.02</td>
<td>3.10±0.04</td>
<td>2.58±0.01</td>
<td>2.01</td>
<td>0.02</td>
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</table>

All the values are mean ± S.E of mean

### REFERENCES


