

Research Article



Effect of Soybean and Sunflower Meal on Growth and Blood Chemistry of *Labeo Rohita* under Control Conditions in Pakistan

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Abstract | In Pakistan, the aquaculture industry has the potential to provide employment, increase exports and enhance the National GDP. However, it is also important to investigate the effects of alternative feed ingredients and formulation on cultured fish. Current study was carried out to evaluate the effects of plant based feed (Soybean and Sunflower meals) on different parameters (growth and blood chemistry) of *Labeo rohita*. In present study, 300 fish were given three different types of plant based feeds. Treatment 1 was natural feed (T₁), Treatment 2 sunflower meal (T₂) and Treatment 3 soybean meal (T₃). Statistically significant differences ($p < 0.05$) were observed in weight gained by fish in T₂ and non-significance for other treatments. The total increase in length (3.81 ± 0.28 cm) was recorded for T₂. Significant increase in the length ($p < 0.05$) was recorded in T₂ and were non-significant ($p > 0.05$) amongst all other treatment groups. The total feed conversion ratio, 1.06 ± 0.4 was observed for T₂ and 1.17 ± 0.2 was observed in T₃. The sunflower based feed showed a significant difference favoring higher numbers of monocytes in T₂. While non-significant differences ($p > 0.05$) were shown by the platelets count among all the given treatments. It was observed that physico-chemical parameters were constant throughout the study for all treatments.

Received | May 26, 2023; **Accepted** | October 28, 2023; **Published** | December, 2023

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Citation | Aman, S., Ashraf, M., Aman, I and Ullah, R. 2023. Effect of Soybean and Sunflower Meal on Growth and Blood Chemistry of *Labeo Rohita* under Control Conditions in Pakistan. *Journal of Innovative Biology and Environmental Sciences*, 4(2): 14-20.

Keywords | Sunflower meal, Soybean meal, *Labeo rohita*, Plant based feeds, Aquaculture

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Introduction

Aquaculture refers to the cultivation of aquatic organism such as, fish, mollusks, crustaceans and algae which are cultivating in freshwater, brackish water

and salt water under the different cultivating systems (Subasinghe *et al.*, 2009). Aquaculture has become the fastest growing sector in food production, contributing 43% to the total global food consumption by humans in the year 2007

(Bostock *et al.*, 2010). In Pakistan, aquaculture has showed a great potential to provide means for employment and enhancement of the National GDP (Shahzad, 2022).

Plant based feed demand is rapidly increasing worldwide and most of the studies are focusing on the early development stages of the fish, since juvenile fish stage shows more growth performance (Jones *et al.*, 2005). Plant based feed is the preferred feed due to its cost effectiveness and statistical relevance in significant growth differences achieved over shorter time spans (Bai *et al.*, 2022). However, it is also important to investigate the effects of alternative feed ingredients and formulation on fish in the grow-out phase. Plant based products containing soybean and sunflower meals have growth potential, rapid availability and cost effectiveness.

Soybean meal is a suitable plant protein source and viable substitute for fish meal (FM) in aqua-feed due to its nutritional composition (Sørensen *et al.*, 2009). It's important to note that the effectiveness of soybean and sunflower meals in promoting fish growth depend upon various factors, including the fish species, age, and the specific formulation of the fish feed. Similarly, the processing and quality of feed ingredients can also influence their performance in fish feed (Krishnan and Jez, 2018). Plant protein sources are not only easily available and cost effective but are also readily available worldwide (Saleh *et al.*, 2021). Incorporating soybean and sunflower meals into fish feed contributes to the sustainability of aquaculture by reducing the reliance on fishmeal, which is often derived from wild-caught fish stocks. This helps mitigate the environmental impact of aquaculture (Pillay, 2008).

2. Materials and Methods

2.1. Sampling site

The present study was conducted at the Department of Fisheries and Aquaculture, University of Veterinary and Animal Sciences, Ravi Campus, Pattoki. Fish (*L. rohita*) were kept in six different ponds, each covering an area of 0.03 ha.

2.2. Experimental design

Experiments were carried out for the duration of six months (180 days) in a completely randomized design (CRD). Feed with different diets made from plant based ingredients viz., soybean and sunflower were given to the fish. Feed was formulated in Food Science Institute, University of Agriculture Faisalabad (PARB Project No.195). Ponds were well fertilized and fish were fed plant based floating fish feed at the rate of 2% of body weight, twice a day. Three hundred fish were stocked in ponds. Sampling was done after every fortnight and feed was readjusted with the passage of time according to the increase in weight.

The control or Treatment 1 fish group (T₁) was provided natural pond diet, Treatment 2 fish group (T₂) was fed sunflower meal diet whereas, the Treatment 3 fish group (T₃) received soybean meal based feed. The type of feed ingredients and their percentage used in different feed formulation are provided in Table 1 and Table 2.

Table 1: Treatment 2 ingredients and CP (Sunflower meal based diet)

Ingredients	Inclusion Level (%)	Contribution of CP%
Fish meal	24	12
Sunflower meal	46	11.96
Rice polish	10	1.4
Fish oil	4	3
Wheat flour	9	1.2
Vit. Premix	2	1
Ascorbic acid	1	2
Minerals	2	1
Citric acid	2	3
Total	100	35%

Table 2: Treatment 3 ingredients and CP (Soybean meal based diet)

Ingredients	Inclusion Level (%)	Contribution of CP%
Fish meal	12	6
Soybean Meal	56	16.2
Rice polish	12	1.6
Wheat flour	9	1.2
Vit. Premix	2	1
Fish oil	4	3
Ascorbic acid	1	2
Minerals	2	1
Citric acid	2	3
Total	100	35%

2.3. Physicochemical parameters

All physicochemical parameters of the pond water were recorded using dissolved oxygen (DO) meter and multimeter on daily basis (Cond 330i WTW 82362, Weilheim Germany). Nitrates and phosphates were monitored on weekly basis by using commercial Nitrate Chemical Test Kit (HI3874 Hanna, USA).

2.4. Fish growth parameters

The significant parameters for fish growth i.e. weight and length were documented before stocking and after the conclusion of the experimental studies. Growth rates were recorded using formulae:

For calculation of net weight gain:

Net weight gain = Average of final weight (g) – Average of initial weight (g)

Feed Conversion Ratio (FCR) = Feed intake (g) ÷ Wet weight gain (g)

Percent weight gain = (Final weight (g) - Initial weight (g) ÷ Initial weight (g) × 100

2.5. Hematological parameters

Neubaur's chamber hemocytometer was used for the blood cell count (erythrocytes and leucocytes) by following the method described by [Blaxhall and Daisley \(1973\)](#). Leucocytes were identified by differential staining according to protocols described by [Kocobatmaz and Ekingen \(1984\)](#).

2.6. Proximate analysis

Proximate analysis of fish was performed according to recommended methods

proposed by the Association of Official Analytical Collaboration ([Horwitz, 1975](#)).

2.7. Statistical analysis

Data set was analysed using Duncan's multiple post hoc tests. ANOVA technique was applied using statistical software SAS (9.1).

3. Results

3.1. Physicochemical parameters

The Physicochemical parameters of the pond water were monitored throughout the entire duration of the experiment at random intervals. It was noted that all parameters under observation remained at average levels throughout the treatment period and there were no significance difference observed ([Figure 1](#)).

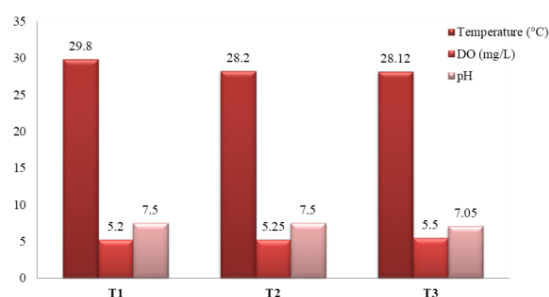


Figure 1: Physicochemical parameters of different treatment groups.

3.2. Fish growth parameters

Both growth and net weight gain was monitored and calculated. The weight gained by the fish in treated and untreated groups exhibited statistically significant differences ([Table 3](#)). The treated group T₂ ($p < 0.05$) had a significant weight gain whereas it remained non-significant ($p > 0.05$) for all other treatments. The highest total increase in length was recorded for T₂ (3.81 ± 0.28 cm). Similarly, the total feed conversion ratio for T₂ (1.06 ± 0.4) was greater than T₃ (1.17 ± 0.2).

3.3. Hematological parameters

The highest red blood cells (2.4 ± 0.4) were found in fish fed on the control treatment (T₂). RBCs were 0.5 ± 0.4 and 1.8 ± 0.5 , in

Table 3: Growth performance of *Labeo rohita* fed on plant based diet

Parameters	Treatments		
	T ₁ (Control group)	T ₂ (Sunflower Feed)	T ₃ (Soybean Feed)
Initial weight (g)	219.50±8.54	306.2±3.38	274.0±4.23
Final weight (g)	226.0±8.7	547±6.7	560.0±8.10
Net Gain in weight (g)	6.50±1.00	241.0±3.8	102.00±4.19
Initial length (cm)	28.97±0.19	27.44±0.40	29.32±0.61
Final length (cm)	30±0.0	31.25±0.12	32.75±0.05
Increase in length(cm)	2.77±0.02	3.81±0.28	3.43±0.11
% Weight gain	2.96± 0.5	78.0±0.3	37.86±2.5
FCR	0000	1.06±0.4	1.17±0.2

T₁ and T₂ respectively. The sunflower based feed showed a significant difference between T₃ and T₁. The highest and similar number of lymphocytes were found in T₂ followed by all treatments. The extreme numbers of monocytes in T₂ were observed followed by T₁ and T₃. However, there was no significant difference ($p>0.05$) observed in others. While non-significant difference ($p>0.05$) was shown by the platelets count among all the given treatments. The hemoglobin uppermost level was 11.25±0.8 in T₂, 10.5±0.8 in T₃ and 9.0±0.8 in T₁. T₂ showed significant differences ($p<0.05$) between them. Total proteins of serum showed no significant difference among overall treatments. Total cell count showed a significant difference between all treatments. Sunflower showed no significant difference in cells except red blood cells (Table 4).

Table 4: Hematological parameters of *Labeo rohita* fed on plant based diet

Parameters	T ₁	T ₂	T ₃
RBCs (10 ⁶)	1.5±0.4 ^a	2.4±0.4 ^{ab}	1.8±0.5 ^b
WBCs (10 ⁶)	0.04±0.08 ^a	0.04±0.06 ^a	0.04±0.03 ^a
Platelets (10 ⁶)	0.04±0.06 ^a	0.04±0.07 ^a	0.02±0.05 ^a
Total proteins (g/dl)	5.15±1.77 ^a	7.62±0.18 ^a	7.45±1.29 ^a
Hb (g/dl)	9.0±0.7 ^a	11.25±0.7 ^a	10.5±0.7 ^{ab}
Lymphocytes (%)	86±2.9 ^a	96±1.5 ^a	94±2.12 ^a
Monocytes (%)	1.25±0.2 ^a	1.75±0.2 ^a	1.55±0.2 ^a

*Figures with superscripts letters a and b significantly differ from each other

2.6. Proximate analysis

The post-trial body composition of *Labeo rohita* was determined via the proximate analysis. The highest CP (69.42±0.07) was found in T₂ among all treatments. The highest ash contents were found in T₃

(16.28±0.29) followed by other two treatments. While in the control treatment, maximum amount of fat (15.85±0.04) was observed. There were significant differences ($p<0.05$) from T₂ and T₃ showing values of 12.27±0.47 and 12.31±0.2 respectively (Figure 2).

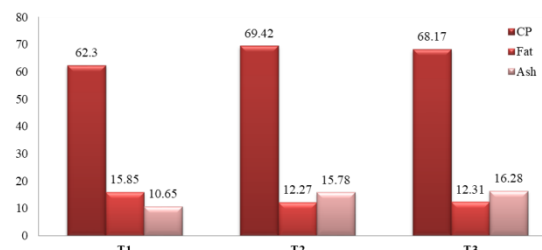


Figure 2: Post-trial body composition of *Labeo rohita* fed on plant based diet

4. Discussion

Our findings are aligned with the research conducted by Shin et al. (2010) in which they observed the reduced feed conversion ratio and improved growth performance when fed with olive flounder polyphenols at 150 mg/kg. Similarly, improvements in growth were observed in *Pangasianodon gigas* when 5% of Fish meals were replaced with spirulina (Tongsiri et al., 2010). Xu et al. (2019) reported that the enhancement in growth of the fish, meat quality and ant anti-oxidative activity of *Ctenopharyngodon idella* when they have given the polyphenols enriched diets. Reduction in feed conversation ratio and improvement in weight gain was observed when fed on grape seed proanthocyanidins in *Nile tilapia* (Babaheydari et al. 2014). *Cyprinus carpio* also showed the growth improvement when the fish were fed on extract of marshmallow (Fallahpour et al., 2014). The growth and immunity response in *Megalobrama amblycephala* was improved when high fat supplemented diet with polyphenols was fed (Jia et al., 2019). Similarly, Munglue (2016) concluded that the *Oreochromis niloticus* also showed the improvement in growth performance and gained maximum weight when fed with 1% *Nelumbo nucifera* peduncle extract.

Studies conducted by [El-Mesasllamy et al., 2016](#) showed that *Oreochromis niloticus* were fed with 0.5, 1 and 1.5% supplementation of *roselle calyx*, another rich source of polyphenols respectively and showed the better growth rate. In current study, growth-related variables specifically weight gain and length exhibited a strong positive correlation with dissolved DO and temperature. [Hussain et al. \(2011\)](#) documented that the decline in the fish growth as temperature fell below the normal range. The physico-chemical parameters such as pH, temperature and dissolved oxygen have a substantial impact on fish growth ([Ali et al., 2000](#); [Ahmad et al., 2008](#); [Noor et al., 2010](#)).

5. Conclusion

Results of current study showed that plant based feeds (Soybean and Sunflower Meal) showed better growth performance including gain in weight, length and FCR and also showed the best growth performance and significant positive improvement in body composition of *Labeo rohita* when fed on soybean and sunflower based plant based feeds. Hence, SBM-based diet and SFM-based diet are cost effective which will help in production of healthy fish, enhance immunity and overcoming the fish meal high price challenge in aquaculture industry.

6. Acknowledgments

The authors acknowledge all the laboratory staff of University of Veterinary and Animal Sciences, Lahore.

7. Author's Contribution

Sadaf Aman and Muhammad Ashraf designed and carried out the study. Iqra Aman and Rahman Ullah analysed and drafted the manuscript.

8. Conflict of Interest

The authors declare no conflict of interest.

9. Novelty Statement

This study is the preliminary study which will enhance and attract farmers towards fish farming as it provides good and cheaper source of diet to aquaculture.

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