

Influence of Pomegranate (*Punica granatum*) Peel Extract as a Dietary Additive on Growth, Blood Indices, and Innate Immune Response of *Mystus bleekeri*

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Abstract

The present investigation was conducted to assess the impact of dietary supplementation of pomegranate (*Punica granatum*) peel meal on growth performance, hematological characteristics, biochemical indices, and immune responses in *Mystus bleekeri*. Experimental fish were fed diets containing different inclusion levels of pomegranate peel meal (0% as control, 0.5%, 0.75%, 1%, and 2%) over a 60-day feeding trial.

The findings indicated that fish receiving 0.75% supplementation showed a significant improvement in weight gain and specific growth rate (SGR) compared to other treatment groups ($P < 0.05$). Conversely, the lowest growth performance was observed in fish fed the highest inclusion level (2%). Feed conversion ratio (FCR) was also most efficient in the 0.75% group, reflecting enhanced feed utilization.

Hematological parameters such as red blood cell count, hemoglobin concentration, and hematocrit values were notably elevated in fish fed 0.75% pomegranate peel meal, indicating better physiological condition. An increasing trend in white blood cell count was observed with higher supplementation levels, suggesting stimulation of immune function. Biochemical parameters showed variation among treatments, with optimal responses generally recorded at moderate inclusion levels.

At the molecular level, expression of the pro-inflammatory cytokine IL-1 β was significantly upregulated in fish fed 0.75% diet, while the anti-inflammatory cytokine IL-10 was downregulated, indicating activation of innate immune mechanisms. In summary, supplementation of pomegranate peel meal at 0.75% in the diet is optimal for enhancing growth, improving hematological status, and modulating immune responses in *Mystus bleekeri*.

Key words: *Mystus bleekeri*, pomegranate peel meal, growth performance, hematology, cytokines, immunity

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Introduction

Aquaculture is currently one of the most rapidly developing sectors in global food production, contributing significantly to the supply of affordable and nutritious protein, thereby supporting food security worldwide (Food and Agriculture Organization, 2018). Nevertheless, the long-term sustainability of aquaculture is challenged by intensive farming practices, which increase the risk of disease outbreaks and their rapid transmission among cultured species (Santos and Ramos, 2018; Zaki et al., 2020). Although antibiotics and chemical therapeutics have traditionally been employed to manage such diseases, their excessive and unregulated use has led to serious concerns, including the development of antibiotic-resistant pathogens, accumulation of harmful residues in aquatic organisms, and negative impacts on environmental and human health (Dawood et al., 2019; Kothari et al., 2019). As a result, there is a growing need to identify safer and environmentally sustainable alternatives.

In this context, natural feed additives derived from plants have attracted considerable attention due to their richness in biologically active compounds such as phenolics, flavonoids, and antioxidants. These compounds are known to promote growth, enhance physiological functions, and strengthen the immune system of fish (Van Hai, 2015; Abdel-Latif et al., 2020).

Such additives can be incorporated into aquaculture diets either as powdered materials or as extracts at appropriate inclusion levels to achieve optimal benefits (Dawood et al., 2018).

Fruit processing industries generate large quantities of by-products, which, if not properly utilized, can contribute to environmental pollution (RedCorn et al.,

2018). Pomegranate (*Punica granatum*) is widely processed for juice and pharmaceutical purposes, resulting in substantial amounts of peel waste (Iqbal et al., 2008). This peel is a rich source of polyphenolic compounds with strong antioxidant and immunomodulatory properties (Al-Zoreky, 2009). Previous research has demonstrated that pomegranate-derived products can enhance growth performance, feed efficiency, immune responses, and oxidative stability in several fish species, including Nile tilapia (*Oreochromis niloticus*) (El-Sayed et al., 2014; Badrey et al., 2019; Toutou et al., 2019). Moreover, pomegranate extracts have been reported to improve disease resistance and overall health condition in cultured fish (Harikrishnan et al., 2010; Acar et al., 2018).

Hematological parameters, serum biochemical indices, and immune-related markers such as cytokines are widely used as reliable indicators to assess the

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physiological status and health condition of fish under different dietary regimes (Yılmaz and Ergün, 2018).

Mystus bleekeri, a freshwater catfish species of economic importance, holds considerable promise for aquaculture development. However, limited information is available regarding the application of plant-based feed additives in this species. In particular, studies evaluating the effects of pomegranate peel meal on growth, hematological parameters, and immune responses in *Mystus bleekeri* are lacking.

Therefore, the present study was undertaken to investigate the effects of dietary inclusion of pomegranate peel meal at different levels on growth performance, hematological and biochemical parameters, and immune responses, with special emphasis on cytokine expression (IL-1 β and IL-10), in *Mystus bleekeri*.

MATERIALS AND METHODS

Experimental Site

The present study was conducted for a period of 60 days in the Zoology Laboratory of Annada College, Hazaribag, Jharkhand. All experimental trials, including fish rearing and feeding, were carried out under controlled laboratory conditions.

Experimental Fish and Design

Healthy fingerlings of *Mystus bleekeri* were collected from Tilaiya Dam, Jharkhand, with an average initial body weight of 24.0 ± 0.50 g. Prior to the experiment, the fish were subjected to a prophylactic dip treatment in 1% saline solution for 5 minutes to eliminate external pathogens, followed by acclimatization for 10 days in circular fiber-reinforced plastic (FRP) tanks (1000 L capacity).

Continuous aeration was maintained throughout the experimental period using an air compressor. During acclimatization, fish were fed a basal diet containing approximately 30% crude protein at a feeding rate of 3% of their body weight per day, divided into two equal meals.

A total of 180 fingerlings were randomly distributed into five experimental groups corresponding to different

dietary treatments (0% as control, 0.5%, 0.75%, 1%, and 2% pomegranate peel meal), with three replicates per treatment, following a completely randomized design (CRD). Fish were fed to apparent satiation twice daily throughout the 60-day feeding trial.

To maintain optimal water quality, uneaten feed and fecal matter were removed by siphoning approximately 50% of the tank water on alternate days, taking care to minimize stress to the fish. Water quality parameters were regularly monitored and maintained within suitable ranges: dissolved oxygen (6.0–6.8 mg L⁻¹), pH (7.2–7.6), and temperature (24–27°C), which are appropriate for the culture of *Mystus bleekeri*.

Preparation of Pomegranate Peel Meal (PPM)

Fresh fruits of pomegranate (*Punica granatum*) were procured from the local market and thoroughly washed with distilled water to remove any surface impurities. The peels were carefully separated from the edible portions.

The collected peels were dried in a hot air oven at a controlled temperature until the moisture content was reduced to approximately 8% (dry basis). The dried peels were then finely ground into powder using a mechanical grinder.

For extraction, the powdered peel was mixed with 70% ethanol in a glass container and placed in a thermostatically controlled shaking water bath to facilitate the extraction of bioactive compounds. The mixture was subsequently filtered through Whatman No. 1 filter paper to remove solid residues.

The filtrate was concentrated by evaporating the solvent using a rotary vacuum evaporator (EYELA system). The concentrated extract was further dried using a lyophilizer to obtain a stable powdered extract. The final product was stored in airtight containers at 4°C under dark conditions until further use.

Experimental diets with graded inclusion levels of 0%, 0.5%, 0.75%, 1%, and 2% were prepared based on the composition presented in the Table 1.

Table 1. Composition of Experimental Diets (%)

Ingredients (%)	Control (0%)	0.5% PPM	0.75% PPM	1% PPM	2% PPM
Fish meal	25	25	25	25	25
Soybean meal	20	20	20	20	20
Mustard oil cake	15	15	15	15	15
Rice bran	20	19.5	19.25	19	18
Wheat flour	15	15	15	15	15
Pomegranate peel meal	0	0.5	0.75	1	2

Vitamin–mineral premix	2	2	2	2	2
Oil	3	3	3	3	3
Total	100	100	100	100	100

Growth Performance Parameters

The growth performance and feed utilization of *Mystus bleekeri* were evaluated using the following standard formulas:

- **Weight Gain (WG, g)**
= Final body weight – Initial body weight
- **Specific Growth Rate (SGR, % day⁻¹)**
= [(ln Final weight – ln Initial weight) / Number of days] × 100
- **Feed Conversion Ratio (FCR)**
= Feed intake (g) / Weight gain (g)
- **Survival Rate (%)**
= (Number of fish survived / Total number of fish stocked) × 100

Hematological Analysis

At the end of the feeding trial, blood samples were collected from fish using sterile syringes. Hematological parameters such as red blood cell (RBC) count, white blood cell (WBC) count, hemoglobin (Hb), and hematocrit (HCT) were determined following standard laboratory procedures.

Cytokine Expression Analysis

The expression levels of immune-related cytokines, including IL-1 β (pro-inflammatory) and IL-10 (anti-inflammatory), were analyzed using molecular techniques such as quantitative PCR (qPCR). RNA was

extracted from fish tissues, converted into cDNA, and amplified using gene-specific primers to evaluate relative gene expression levels.

Statistical Analysis

All experimental data were expressed as mean \pm standard error (SE). Statistical analysis was performed using one-way analysis of variance (ANOVA) to determine significant differences among treatment groups. When significant differences were observed, Duncan’s multiple range test was applied for comparison of means. A significance level of $P < 0.05$ was considered statistically significant. Statistical analyses were carried out using standard software such as SPSS

Results

Growth Performance

Dietary supplementation of pomegranate (*Punica granatum*) peel meal significantly influenced the growth performance of *Mystus bleekeri*. Fish fed with 0.75% supplementation exhibited a significant increase ($P < 0.05$) in weight gain and specific growth rate (SGR) compared to other treatment groups. In contrast, the lowest growth performance was recorded in fish receiving the highest inclusion level (2%). Feed conversion ratio (FCR) was also significantly improved in the 0.75% group, indicating better feed efficiency (Table 2).

Sl. No.	Diet (Pomegranate Peel Meal %)	SGR (%/day)	Significance (P<)	FCR	Significance (P<)
1	Control (0%)	1.25 \pm 0.05	—	2.10 \pm 0.08	—
2	0.5%	1.45 \pm 0.06	0.05	1.90 \pm 0.07	0.05
3	0.75%	1.80 \pm 0.04	0.01	1.60 \pm 0.05	0.01
4	1%	1.40 \pm 0.05	0.05	1.95 \pm 0.06	0.05
5	2%	1.10 \pm 0.03	0.001	2.30 \pm 0.09	0.001

Table 2: Effect of dietary pomegranate (*Punica granatum*) peel meal inclusion levels on specific growth rate (SGR) and feed conversion ratio (FCR) of *Mystus bleekeri*.

Hematological Parameters

Hematological analysis revealed significant variations among the treatment groups. Fish fed with 0.75% pomegranate peel meal showed significantly higher ($P < 0.05$) red blood cell (RBC) count, hemoglobin (Hb), and

hematocrit (HCT) values compared to other groups, indicating improved physiological condition. Additionally, white blood cell (WBC) count increased progressively with increasing levels of supplementation, suggesting enhanced immune activity (Table 3).

Table 3 : Effect of Dietary Pomegranate Peel Meal on Hematological Parameters

Sl. No.	Diet (Pomegranate Peel Meal %)	Body Weight (g)	Total Blood Volume	Significance (P<)	Relative % Blood Volume	Significance (P<)	Hemato crit (%)	Significance (P<)
1	Control (0%)	25.0 ± 0.91	0.378 ± 0.051	—	1.643 ± 0.221	—	45.0 ± 1.22	—
2	0.5%	23.0 ± 0.59	0.278 ± 0.028	0.05	1.108 ± 0.113	0.05	44.0 ± 0.96	0.50 (NS)
3	0.75%	24.0 ± 1.47	0.417 ± 0.042	0.05	1.737 ± 0.125	0.05	48.0 ± 0.75	0.01
4	1%	32.0 ± 0.40	0.253 ± 0.026	0.01	1.100 ± 0.117	0.01	28.0 ± 0.71	0.001
5	2%	30.0 ± 0.80*	0.222 ± 0.021	0.001	0.965 ± 0.091	0.001	33.0 ± 0.84	0.001

Biochemical Parameters

Biochemical parameters showed noticeable differences among the dietary treatments. Fish receiving moderate levels of pomegranate peel meal, particularly 0.75%, demonstrated improved biochemical profiles compared to the control and higher inclusion groups. However, no consistent trend was observed across all treatments.

Immune Response (Cytokine Expression)

Dietary inclusion of pomegranate (*Punica granatum*) peel meal significantly influenced immune-related gene expression in *Mystus bleekeri*. A marked upregulation (P < 0.05) of the pro-inflammatory cytokine **IL-1β** was observed in fish fed with 0.75% supplementation, indicating activation of innate immune responses. The expression level increased progressively from control to 0.75% inclusion, followed by a slight decline at higher

inclusion levels (1% and 2%), though still remaining above control (Fig 1).

In contrast, the expression of the anti-inflammatory cytokine **IL-10** showed a significant reduction (P < 0.05) in fish fed with 0.75% pomegranate peel meal. The lowest expression was recorded in the 0.75% group, suggesting suppression of anti-inflammatory signaling and thereby promoting a stronger immune response. However, at higher inclusion levels, IL-10 expression showed a slight recovery but remained lower than the control (Fig 2).

These findings indicate that **0.75% dietary inclusion level is optimal for enhancing immune responsiveness**, characterized by increased pro-inflammatory and decreased anti-inflammatory cytokine expression.

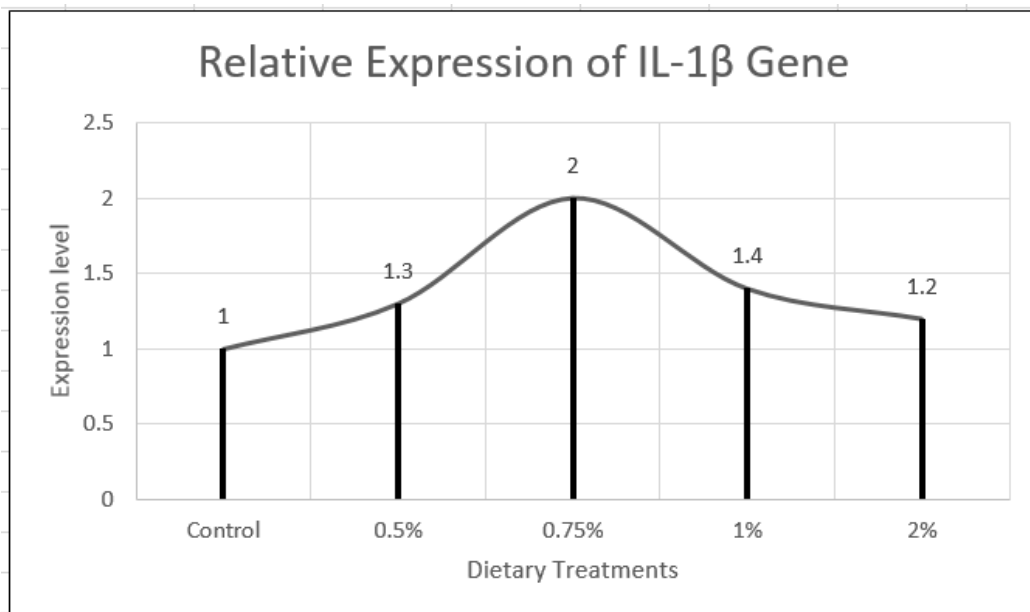


Figure 1: Relative expression of IL-1 β gene in *Mystus bleekeri* under different dietary treatments. Fish fed with pomegranate peel meal showed variation in IL-1 β expression, with the highest upregulation observed at 0.75% inclusion level compared to control and other treatment groups. Values are expressed as mean \pm standard error (SE).



Figure 2: Relative expression of IL-10 gene in *Mystus bleekeri* under different dietary treatments. Fish fed with pomegranate peel meal showed a decrease in IL-10 expression compared to the control group, with the lowest expression observed at 0.75% inclusion level. Values are presented as mean \pm standard error (SE).

Discussion

The application of plant-based feed additives has emerged as a sustainable and eco-friendly strategy to enhance fish health and immunity, reducing reliance on antibiotics and chemotherapeutics that may cause environmental hazards and antimicrobial resistance

(Dawood et al., 2019; Van Hai, 2015). Herbal products rich in bioactive compounds are known to improve growth performance, hematological status, and immune responses in fish (Song et al., 2014; Hoseinifar et al., 2015).

In the present study, dietary inclusion of pomegranate (*Punica granatum*) peel meal showed significant improvement in growth performance at 0.75% inclusion level, as indicated by higher weight gain and SGR. Similar findings have been reported in fish and other animals, where moderate inclusion of plant-based additives enhanced growth, while higher levels negatively affected performance, possibly due to high fiber content and bioactive compounds influencing nutrient utilization (Badrey et al., 2019).

Hematological analysis further supported these findings, revealing significant variations among the treatment groups. Fish fed with 0.75% pomegranate peel meal exhibited significantly higher ($P < 0.05$) total blood volume, relative blood volume, and hematocrit values compared to other groups, indicating improved physiological condition and better oxygen-carrying capacity. The improvement in hematological parameters

at moderate inclusion levels suggests enhanced metabolic efficiency and overall health status. In contrast, higher inclusion levels (1% and 2%) resulted in a significant decline ($P < 0.01$ – 0.001) in these parameters, which may be attributed to excess bioactive compounds or reduced nutrient digestibility at elevated concentrations. Similar dose-dependent effects of plant-based additives on hematological indices have been reported in previous studies (Hoseinifar et al., 2015). The immunological findings demonstrated a significant upregulation of IL-1 β expression and downregulation of

IL-10 in fish fed 0.75% pomegranate peel meal. Cytokines play a crucial role in regulating immune responses, where IL-1 β acts as a key pro-inflammatory mediator initiating defense mechanisms, while IL-10 functions as an anti-inflammatory cytokine that suppresses excessive immune reactions (Gioacchini et al., 2008; Dinarello, 1997). The elevated IL-1 β expression suggests enhanced activation of innate immunity, including macrophage activation and leukocyte recruitment, which are essential for pathogen defense (Buonocore et al., 2005; Lu et al., 2008).

Conversely, the reduction in IL-10 expression indicates suppression of anti-inflammatory signaling, thereby allowing a stronger immune response. Similar trends have been reported in fish fed immunostimulants such as β -glucans and herbal extracts, where increased pro-inflammatory cytokines coupled with decreased IL-10 enhanced disease resistance (Swain et al., 2011; Watanuki et al., 2006).

Overall, the present findings suggest that pomegranate peel meal at an optimal inclusion level (0.75%) enhances growth, hematological status, and immune responsiveness by modulating physiological and molecular mechanisms, thereby improving the health status of *Mystus bleekeri*.

Conclusion

In conclusion, dietary supplementation of pomegranate peel meal at 0.75% significantly improved growth performance, hematological parameters, and immune responses in *Mystus bleekeri*. The enhancement of total blood volume, relative blood volume, and hematocrit values indicates improved physiological condition, while the upregulation of IL-1 β along with downregulation of IL-10 reflects strengthened innate immunity. These findings highlight the potential of pomegranate peel meal as a natural immunostimulant in aquaculture. Further studies focusing on molecular pathways and disease challenge trials are recommended to validate its application as a functional feed additive.

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