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Research article

# Effects of *Carica papaya* seed powder as a plant-based aromatase inhibitor on growth performance, feed utilisation, reproductive health, biochemical indices, and intestinal histomorphology in hybrid red tilapia (*Oreochromis spp.)*

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## Abstract

Plant-derived aromatase inhibitors, such as *Carica papaya*, are increasingly employed in aquaculture for sex reversal, immunostimulant and growth enhancement. However, their effects on fish physiology, particularly nutrient utilisation, body composition, and serum biochemistry, remain poorly documented, especially in hybrid red tilapia. This study evaluated the effects of dietary inclusions of *Carica papaya* seed powder (CPSP) at dosage levels 0, 30, 60, and 90 g/kg feed on fish physiological parameters. A completely randomised design with three replicates per treatment was used, comprising a 60-day treatment phase and a 90-day recovery phase. During the treatment, higher CPSP (60–90 g/kg) significantly increased weight gain and protein efficiency ratio (PER) ($p < 0.05$) but impaired feed conversion ratio (FCR) ($p < 0.05$), with no significant differences in specific growth rate (SGR) ($p > 0.05$). At the recovery phase, fish previously fed 60–90 g/kg CPSP exhibited significantly improved SGR, FCR, and PER ($p < 0.05$). Lipid, ash, and moisture content were significantly elevated in fish from the 90 g/kg group ($p< 0.05$), while carbohydrate markedly declined ($p > 0.05$). Reproductive metrics showed that 90 g/kg CPSP significantly increased 11-ketotestosterone ($p< 0.05$) with suppression in oestradiol and GSI ($p < 0.05$). Serum biochemistry indicated lower aspartate aminotransferase (AST) and alanine aminotransferase (ALT) but higher alkaline phosphatase (ALP), albumin, and globulin in fish from 90 g/kg CPSP groups ($p < 0.05$). Cholesterol, glucose, and total protein levels markedly improved ($p < 0.05$). For improved fish health, 60 g/kg CPSP is recommended. Future research should prioritise scalable methods to commercialise papaya seeds as a cost-effective feed additive and sex-reversal agent in aquaculture.

**Keywords**: Aquaculture, Aromatase inhibitor, Biochemistry, Carica papaya, Masculinization, Fish physiology, Histology.

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# INTRODUCTION

In recent decades, global aquaculture has emerged as a critical subsector of fisheries, providing an affordable source of protein for rural communities and mitigating pressure on overexploited wild fish stocks (FAO, 2024).The rapid expansion of aquaculture has driven the adoption of innovative practices, including the use of plant- and animal-based feed additives (Gule and Geremew, 2022; Vijayaram et al., 2024) , natural feed supplements derived from crustacean by products such as Chitin and its derivative, chitosan, extracted from crustacean shells,(Ashraf et al., 2024) and use of plant extracts for masculinization and immunostimulant agents (Wokeh et al., 2021) have been widely adopted for sustainable aquaculture production. Among farmed finfish, tilapia ranks as the second most farmed species globally, with production systems putting into use plant-based substrates as fish feed, growth promoters, immunostimulants, and sex-reversal agents (Gule and Geremew, 2022; Zuluaga-Hernández et al., 2023). By 2030, global tilapia production is projected to exceed 10.8 million metric tonnes, potentially surpassing carp farming (Fao, 2024; Majumdar et al., 2024 ). Key tilapia species commonly farmed for aquaculture purposes includes but not limited to Nile tilapia (*Oreochromis niloticus*) (Niyibizi et al., 2022) , the genetically improved farmed tilapia (GIFT) strain of *O. niloticus* (Ponzoni et al., 2011), Mozambique tilapia (*O. mossambicus*) (Inokuchi et al., 2022), Zanzibar tilapia (*O. urolepis hornorum*) (Watanabe et al., 2002; Chuhila et al., 2024; Harikrishnan et al., 2024), three-spotted tilapia (*O. andersonii)* (Haihambo and Gabriel, 2022), blue tilapia (*O. aureus*) (Marshall, 1996), and hybrid red tilapia (*Oreochromis spp.),* which has been widely embraced among fish farmers in most Asian countries due to its appealing colour which resembles marine fishes such as red snapper and high market preference (Watanabe et al., 1989; Pongthana et al., 2010; Nwachi et al., 2020). Hybrid red tilapia species are preferably farmed for their adaptability to a wide range of salinity of up to 65ppt , rapid growth rates associated with hybrid vigour, and ability to thrive in diverse environmental conditions (Correia et al., 2019; Syanya et al., 2025). However, prolific breeding in hybrid red tilapia remains a significant challenge, often addressed through sex-reversal and masculinisation techniques. This process has traditionally been achieved in red tilapia through the use of traditional steroid hormones such as methyl testosterone (Basavaraja and Raghavendra, 2017; Zaki et al., 2021; Yostawonkul et al., 2023). This steroid hormone approach has of late been considered unfit for the masculinization of fish meant for human consumption and its use has since been burned in aquaculture in the United States of America among other EU countries and non-steroidal aromatase inhibitors such as tamoxifen citrate as agent of masculinization is slowly being adopted in red tilapia (Singh et al., 2012; Syanya et al., 2025). With respect to these challenges, the use of plant-based aromatase inhibitors as masculinisation agents has been widely embraced in aquaculture. Plant-based aromatase inhibitors are capable of inhibiting the aromatase enzyme activity in the ovary of the fish and supressing the release of Oestrogen hormone hence enhancing masculinisation as was documented in Nile tilapia ( *O. niloticus)* (Farrag et al., 2013) and Mozambique tilapia (*O. mossambicus* ) (Omeje et al., 2019).

Additionally, these phytochemical from plants have been shown to improve immune system and enhanced growth performance in fish (Gomes et al., 1995; Dawood and Koshio, 2020; Radwan et al., 2023; Syanya et al., 2023).In line with global efforts to transition from synthetic compounds and chemicals used in aquaculture to organic and plant-based alternatives, plant-based aromatase inhibitors such as *Carica papaya* seed powder are increasingly being used in aquaculture not only as masculinization agent but also as immune and growth promoter and in aquaculture production. These phytochemical compounds, are derived from plant sources such as *Carica papaya* seeds and leaf extracts (Somdare et al., 2023), *Moringa oleifera* (Makkar et al., 2007), Aloe vera (*Aloe barbadensis miller*) (Syed et al., 2022), and Neem leaf extracts (*Azadirachta indica*)

(Enyidi and Nduh-Nduh, 2016; Abu-Elala et al., 2023).These plant based phytochemicals offer a sustainable alternative to traditionally used synthetic steroid hormones like 17α-methyltestosterone for masculinization among other chemicals such as antibiotics to , enhance fish health and growth in aquaculture. Phytochemicals from different plants have further been documented to influence growth performance, immune function, biochemical composition, and organ histological parameters. For instance , *Moringa oleifera* has been shown to enhance immunity in African catfish (*Clarias gariepinus*)) (Adi et al, 2020), while *Tribulus terrestris* acts as both a growth promoter and a masculinisation agent in Nile tilapia (*O. niloticus)* and hybrid red tilapia (Ghosal and Chakraborty, 2020; Ghosal et al., 2021; Zaki et al., 2021). Similarly, *Carica papaya* seed meal has been found to improve feed efficiency, growth, and immunity during sex reversal and masculinisation in *O. niloticus* and *O. mossambicus* (Hamid et al., 2022; Ngozi, 2023). Despite these advancements, the effects of *Carica papaya* seeds powder as source of aromatase inhibitors on fish physiology, serum biochemistry, body biochemical composition and histomorphology of the gut remain undocumented, particularly in hybrid red tilapia. While higher inclusion levels of *Carica papaya* seeds powder have shown to improve male ratios in Nile tilapia, prolonged exposure can adversely affect haematological and serum biochemical parameters, as was documented in *O. niloticus* and *C. gariepinus* (Ayotunde et al., 2010; Okomoda, 2017; Ekinadose et al., 2021). Nevertheless, the cost-effectiveness, convenience in usage, environmental user-friendliness, and widespread availability of Carica *papaya* seeds make it a viable option for sustainable tilapia masculinisation with respect to traditionally used methyl testosterone in aquaculture (Ekinadose et al., 2021; Jamal et al., 2024). It offers additional benefits to fish such as enhanced growth, stress and disease resistance, and boost fish immune function (Farrag et al., 2013; Okomoda, 2017; Suman et al., 2024; Syanya, Litabas, et al., 2023).However, further research is needed to assess its long-term effects, particularly on hybrid red tilapia, where data remain scanty. The histopathological effects of *Carica papaya* seeds powder on fish, particularly, have not been well documented, as the available studies on Nile tilapia (*O. niloticus)* showed significant histological changes in the gills, liver, and muscles of fish-fed diets containing both steroidal and natural plant-based aromatase inhibitors (Ekinadose et al. , 2021). However no similar work has been done on hybrid red tilapia especially on the effect of *Carica papaya* seed powder on gut and intestinal morphology, *Carica papaya* seeds are believed to contain cytochrome P450 aromatase enzyme inhibitors, which can alter hormonal balance by increasing androgen levels and reducing oestrogen levels, thereby inducing male traits in genetically female fish. Studies on Nile tilapia have demonstrated that diets supplemented with *Carica papaya* seed powder resulted to elevated testosterone levels and reduced oestrogen levels (Magblenou et al., 2019; Chen et al., 2020; Radwan et al., 2023), Plant based phytochemicals has also been reported to be used as herbal medicinal agents in avian to enhance immunity (Kamil et al., 2023). Similarly dietary cinnamon supplementation has been documented to improve growth, haematology, serum biochemistry, intestinal microbiota, enzymes, and Histomorphological changes of the intestine of *Heteroclarias spp.* (Jimoh et al., 2023). Above all, despite the widespread usage of *Carica papaya* seeds in other fish species, their effects as a masculinisation agent, growth promoter, immune enhancer on hybrid red tilapia remain poorly documented especially the effects of different supplementation levels of *Carica papaya* seed powder on serum biochemistry, body biochemical nutrient composition, haematological parameters, serum enzyme activities, and the histomorphology of the fish intestines and feed utilisation. This study therefore aims to investigate this research gap on the potential utilization of use *Carica papaya* seed powder as agent of masculinization and associated effects on sex hormone profile, growth, feed utilisation, serum biochemistry, biochemical composition, organ indices and gut histomorphometry in hybrid red tilapia. The study further aims to comprehensively evaluate the

physiological, biochemical and growth effects of dietary *Carica papaya* seed powder inclusion in the fish diet and give guidelines for best sustainable aquaculture practices to enhance fish health and productivity of hybrid red tilapia (*Oreochromis spp*.).

# MATERIAL AND METHODS

## Source of experimental fish

Hybrid red tilapia fry (*Oreochromis spp*.), with an average initial weight of 0.017 g and aged 1-week post-hatching, were collected from a natural pond at the Mekera Fish Hatchery and Fish Farm in the Thrissur district of Kerala, South India. A total of 360 fry were collected, graded for uniformity, and transported in oxygenated polythene bags to the Hatchery Complex at the School of Industrial Fisheries, Cochin University of Science and Technology Kerala India. Upon arrival, the fry were acclimatised in a 700-litre fibreglass tank equipped with continuous aeration and a daily 50% water exchange for five days. During this acclimatisation period, the fry were fed a basal diet containing 45% crude protein at a rate of 8% of their body weight per day.

## Experimental design

The experiment was conducted in two distinct phases: Phase 1 (Treatment Phase**)** and Phase 2 (Recovery Phase**)**. The treatment phase involved indoor culture in 100-litre rearing tanks, each filled with 80 litres of freshwater under constant aeration. The experiment was conducted at the hatchery complex of the School of Industrial Fisheries, Cochin University of Science and Technology. Over the 60-day treatment period, the fry were fed a basal diet supplemented with varying inclusion levels of *Carica papaya* seed powder. The experimental design followed a completely randomised design (CRD) with three replications. At the start of Phase 1, 23 fish were randomly assigned to each replicate tank based on the *Carica papaya* seed powder*.* supplementation dosage levels in the fish feed. The treatment groups and corresponding *Carica papaya* seed powder inclusions were as follows: PCB0 (Control): 0 g *Carica papaya* seed powder/kg feed, PPLB1 (30g *Carica papaya* seed powder/kg feed). PPMB2 (60g *Carica papaya* seed powder/kg feed) and PPHB3 (90 g *Carica papaya* seed powder/kg feed).

The *Carica papaya* seed powder dosage limits used in this experiment were determined based on previous studies conducted on *Oreochromis mossambicus* (Omeje, 2016; Omeje et al., 2019), albeit with some modifications to suit the hybrid red tilapia used in this experiment. At the end of Phase 1, the final weight and total length of the fish in each replicate were recorded. These measurements served as the initial growth parameters for the subsequent recovery phase.

Phase 2 (Recovery Phase) commenced immediately after Phase 1 and lasted for 90 days. At phase2, fingerlings from the treatment phase were transferred to hapas (net enclosures) installed in an outdoor tank. The outdoor tank had a capacity of 14,432 litres (4.1 m × 3.2 m × 1.5 m), and twelve hapas, each measuring 0.8 m (length) × 0.7 m (width) × 1.5 m (depth), were used. Fish from each replicate tank in Phase 1 were transferred into correspondingly labelled hapas, maintaining the same replication arrangement. Throughout the recovery phase, the fingerlings were fed exclusively on a commercial diet containing 35% crude protein at a rate of 6% of their body weight twice daily until satiation This phase aimed to evaluate growth performance, feed conversion efficiency, and the long-term physiological effects of *Carica papaya* seed powder on fish health. Water qualities of the culture tanks was rigorously monitored and maintained throughout both phases. During Phase 1, water temperature and pH were measured daily, and aeration was provided to ensure dissolved oxygen levels remained above 5 mg/L. Additionally, 15% of the water in each culture tank was replaced daily with fresh, dechlorinated water from water storage tanks. Faeces and uneaten feed were syphoned out daily before

feeding. In Phase 2, water quality parameters, including dissolved oxygen and ammonia, were measured three times per week to ensure optimal rearing conditions. To control algal blooms in the outdoor tank, 50% of the water was replenished every two weeks. The experiment was conducted between July 2024 and January 2025, with all treatments and measurements performed in triplicate to ensure statistical robustness.

## Preparation of basal and experimental diets

Based on the guidelines of Omeje (2016) , basal feeds were prepared using ingredients sourced from Broadway's local market in Ernakulam, Kerala. The basal diet was formulated using the following ingredients (per 100 g diet): fish meal, soybean meal, wheat flour binder, millet flour rice flour , vegetable oil, vitamin premix and mineral premix. (Table 1a).

The ingredients were ground into a fine powder using a grinder from the Fish Processing Laboratory, School of Industrial Fisheries, Cochin University of Science and Technology, passed through a 200 µm sieve mesh, and stored in ziplock bags for experimental feed formulation. Approximately 600 g of *Carica papaya* seeds were extracted from 120 ripe pawpaw fruits obtained from AFC Fruits and Juice in Kalamassery Market, Ernakulam District, Kerala, India. The seeds were rinsed in clean water, sun-dried, and stored in a dry container. They were then finely ground using the same grinder, passed through a 200 µm sieve mesh, and stored in ziplock bags in a cool, dry place and labelled as pawpaw seed powder. The basal diet, with a high protein content of 45%, served as the control diet. To prepare the experimental diets, the control diet was mixed with *Carica papaya* seed powder at different dosage levels (0, 30, 60, and 90 g per kg of feed) to create the experimental diets: PCB0 (control, 0 g/kg), PPLB1 (30 g/kg), PPMB2 (60 g/kg), and PPHB3 (90 g/kg). The formulated feeds were pelletised, oven-dried, and crumbled into mash form for easy palatability by the fry. Feeds were prepared every two weeks to account for their short shelf life. Proximate composition analysis of the pawpaw seed powder and basal diet was conducted following AOAC. (2005) procedures. The *Carica papaya* seed powder proximate composition consisting of percentage moisture ash, crude fibre, crude fat, crude protein, and carbohydrates as shown in (Table 1b). However, Proximate composition analysis of basal diet were as indicated in (Table 1c). The dosages *of Carica papaya* seed powder. used for this experiment were based on previous studies by (Khalil et al., 2014; Omeje, 2016; Omeje et al., 2020) in Mozambique tilapia( *O. mossambicus)*,and Nile tilapia (*O.niloticus*) albeit with modifications tailored for hybrid red tilapia. Fry were fed the experimental diet for 60 days, followed by a 90-day recovery period where the fingerlings were then exclusively fed on commercial diet.

**Table 1** Basal feed ingredients and proximate composition analysis (1A). Proximate composition analysis of *Carica papaya* seed powder (1B). And, proximate composition analysis of formulated Basal diet (1C)

**1A**

| Feed Ingredient Composition | Plant-based aromatase inhibitor (*Carica papaya* seed powder) inclusion level | | | |
|---|---|---|---|---|
| **Treatment level** | **PCB0** | **PPLB1** | **PPMB2** | **PPHB 3** |
| Fish meal | 55.69 | 55.69 | 55.69 | 55.69 |
| Soya bean meal | 38.56 | 38.56 | 38.56 | 38.56 |
| Wheat flour (Binder) | 1.85 | 1.85 | 1.85 | 1.85 |
| Millet flour | 1.70 | 1.70 | 1.70 | 1.70 |
| Rice flour | 2.20 | 2.20 | 2.20 | 2.20 |
| Total (g) | 100 | 100 | 100 | 100 |
| Vegetable oil | 1 | 1 | 1 | 1 |
| Vitamin premix | 1 | 1 | 1 | 1 |
| Mineral premix | 1 | 1 | 1 | 1 |
| *Carica papaya* seed powder(g) inclusion | 0 | 30 | 60 | 90 |

**1B**

| Biochemical composition of Basal feed (%) | Mean ± SE |
|---|---|
| Moisture Content | 7.99 ± 0.047 |
| Ash | 6.21 ± 0.036 |
| Crude fibre | 5.84 ± 0.825 |
| Crude Lipid | 24.88 ± 0.022 |
| Carbohydrates | 26.39± 0.174 |
| Crude Protein | 28.69 ± 0.019 |

**1C**

| Biochemical composition of Basal feed (%) | Mean ± SE |
|---|---|
| Moisture Content | 9.27 ± 0.092 |
| Ash | 9.54 ± 0.071 |
| Crude protein | 44.96 ± 0.748 |
| Crude Lipid | 11.98 ± 0.031 |
| Carbohydrates | 24.25± 0.286 |

## Growth performance parameters analysis

Growth parameters were assessed during both the treatment phase (Phase 1) and the recovery period (Phase 2). Every three weeks, seven fish per replicate (n = 21 per treatment) were randomly sampled to evaluate the following growth metrics: mean weight gain (MWG), specific growth rate (SGR), average daily growth (ADG), Fulton's condition factor (K), feed conversion ratio (FCR), feed conversion efficiency (FCE), protein efficiency ratio (PER), and percentage weight gain. The sample size of 21 fish per treatment (7 fish per replicate, with 3 replicates per treatment) was chosen to ensure that there is statistical robustness and reliability of the results as well as reduce handling stress on fish, which may otherwise affect fish growth during the culture period, especially when the whole population was taken as a sample in each treatment group. These parameters were determined using established methodologies as described in previous studies by Ng and Hanim, (2007), Omeje (2016) Hosseinpour Zelaty et al. (2024), Syanya et al. (2025), Syanya Fredrick et al. (2025) as indicated below.

Average Weight gain (AWG) = Final weight of fish – Initial weight at stocking. /number of days cultured.

Percentage weight gain = (Final weight or Length) – (Initial weight or Length)/ Initial weight or length x 100.

Specific growth rate (SGR) % = Log (Final fish weight)- Log (Initial fish Weight)/ number of culture days x100.

Feed conversion ratio (FCR) = Feed consumed / fish Weight gain

Where feed consumed is the quantity of feeds given (g) during the culture period.

Weight gain = Final weight of fish (g) – Initial fish weight.

Feed conversion efficiency (FCE) = Weight gain/ total Feeds consumed (g)

Protein efficiency ratio (PER) = Weight gain/ Crude protein fed.

Feed consumed / Day = (Initial weight of feed container with feeds /treatment) – (final weight (of feed container after feeding at the end of the day).

Fulton's condition factor (K) = 100 × (Weight of fish)/ (Length of fish )$^3$ (100.W/L$^3$)

## Proximate body composition analysis

The proximate composition of fish samples from each treatment group was determined in triplicate following the standard methods of the Association of Official Analytical Chemists (AOAC, 2005). A total of 24 fish per treatment (n = 24), with 8 fish per replicate treatment, were selected for analysis. The fish were filleted, and the fillets were homogenised through maceration using a mortar and pestle. This sample size was determined based on the quantity of moisture-free samples required for accurate analysis. All proximate composition analyses were conducted at the processing laboratory of the School of Industrial Fisheries, Cochin University of Science and Technology. Moisture content was determined by oven-drying samples at 105°C until a constant weight was achieved. Crude protein content (calculated as N × 6.25) was analysed using the Kjeldahl method with a semi-automatic Kjeldahl system, following sulphuric acid digestion of the samples and sample titration. Crude lipid content was measured via Soxhlet extraction, and crude ash content was determined by incinerating samples at 550°C in a muffle furnace. Carbohydrate content was calculated by difference, subtracting the sum of protein, ash, moisture, and lipid percentages from 100%. All analyses were conducted following established procedures adopted from AOAC (2005), Islam et al. (2020), Hanan et al. (2022), Syanya et al. (2025).

## Fish organ indices parameters analysis

For statistical robustness and reliability, four fish were randomly sampled from each replicate hapa (n = 12 per treatment). These fish were weighed and dissected to extract organs such as Liver, gonads, Visceral organs in order to determine somatic indices, including the hepatosomatic index (HSI), viscerosomatic index (VSI), stomach index, and gonadosomatic index (GSI). Data collected included total body weight, liver weight, stomach weight, gonad weight, and visceral weight. The somatic indices were calculated using established formulas as outlined in previous studies by Sadiq Bukhari et al. (2012), Azarm and Lee (2014), Basavaraja and Raghavendra (2017), Syanya et al. (2025) as described below.

Stomach index (S I) = Weight of the stomach / Fish weight gain ×100

Gonadosomatic index (GSI) = Weight of the gonads / Total fish weight × 100

Hepatosomatic index (HSI) = Weight of the Liver/ Total fish weight × 100

Visceral somatic index (VSI) = Total visceral weight/ Total fish Weight × 100

## Masculinity features analysis

Masculinity features were assessed phenotypically through manual sexing. At the end of the recovery phase (Phase 2), when the fish were presumed to have reached sexual maturity, they were harvested, counted, and sexed based on phenotypic characteristics and morphological traits. Data were expressed as percentages. The phenotypic traits used for sex identification exclusively found in

hybrid red tilapia included body colouration (mottled red in males and uniformly dull in females), body shape (elongated in males and less elongated in females), genital papilla morphology (elongated and pointed in males while short and blunt in females), dorsal and anal fin morphology (longer in males and shorter in females), and the number of openings on the genital papilla (two in males and three in females). The number of males and females in each replicate per treatment was recorded, quantified, and verified, and their final weights were measured. The phenotypic sex identification method followed the procedure described by (Yostawonkul et al., 2023; Syanya et al., 2025).

## Reproductive hormone level analysis

To enhance the statistical robustness and reliability of the hormone level data in the study, blood samples for sex hormone analysis were collected from two fish per replicate (n = 6 samples per treatment) via the caudal vein using heparinised 5 mL disposable plastic syringes and 24-gauge hypodermic needles. The samples were promptly transported to the laboratory for processing. This sampling strategy ensured adequate biological replication and minimised variability, thereby improving the reliability of the hormone level measurements. Blood samples from each treatment group were centrifuged at 3500 × g for 15 minutes at 4°C using an Eppendorf centrifuge (Model 5810R), and the resulting plasma was stored at -20°C until further analysis. Reproductive hormones, specifically 17β-oestradiol (E2) and 11-ketotestosterone (11-KT), were quantified using species-specific enzyme-linked immunosorbent assay (ELISA) kits. The 11-KT levels were measured using a multispecies competitive ELISA kit (Cat. No.: ELK9704, Size: 48T; Cayman Chemical, Enzo Life Sciences, USA) with a colorimetric microplate reader. The 17β-oestradiol concentrations were determined using a fish-specific E2 ELISA kit (Catalogue No.: CSB-E19564Fh, Elk Biotechnology Co., Ltd., USA). The cross-reactivity of the assays was <10% for 11-KT with similar androgens and <1% for E2 with other steroid hormones. The sensitivity ranges were 0.02–0.05 ng/mL for 11-KT and 10–15 pg/mL for E2. Recovery rates were 85–110% for 11-KT and 80–105% for E2. Intra-assay coefficients of variation (CVs) were <8% for 11-KT and <10% for E2, while inter-assay CVs were <12% for 11-KT and <15% for E2. Standard curves were established within the ranges of 0.1–10 ng/mL for 11-KT and 20–2,000 pg/mL for E2, which was used for validation. All assays were performed in triplicate following the manufacturer's instructions and protocols adapted from Omeje et al. (2019), Fan et al. (2023), Syanya et al. 2025).

## Serum enzyme biochemical indices analysis

At the end of Phase 2 (90-day recovery period), blood samples were collected from six fish per treatment group (n = 6, with two fish sampled per replicate) to ensure data reliability during analysis. Fish were anaesthetised using oregano essential oil at a concentration of 0.25 mL/L of water, which allowed for rapid induction and prolonged recovery during the blood collection process. Blood was drawn from the caudal vein using a 5 mL syringe fitted with a 24-gauge needle, following the procedure adapted from (Fazio et al., 2012; Syanya et al., 2025). Blood samples were allocated into different tubes for specific analyses: EDTA tubes for haematological analysis, sodium fluoride tubes for glucose testing, and serum tubes for serum enzyme function tests. Haematological parameters were analysed within one hour of collection using an automated haematological analyser (HeCo Vet C, SEAC, Florence, Italy), following protocols established by Fazio et al. (2012), Fazio, (2019). The parameters assessed included red blood cell count (RBC), haematocrit (Hct), haemoglobin concentration (Hgb), white blood cell count (WBC), platelet count, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), and mean corpuscular haemoglobin concentration (MCHC). A specialised lysing reagent (SEAC code 71010460), containing potassium cyanide, quaternary ammonium salts, and surfactants, was used to prepare samples. The analyser employed customised software specific for fish blood to

account for nucleated RBCs, ensuring accuracy by subtracting their nuclei from WBC counts. Human blood with stabilised platelets was used as a control for instrument calibration. For serum analysis, blood samples in non-EDTA tubes were centrifuged at 3500 × g for 15 minutes to separate the supernatant. Biochemical parameters, including total protein, albumin, globulin, alanine aminotransferase (ALT), aspartate aminotransferase (AST), and cholesterol, were measured using an automated UV spectrophotometer following protocols established for Nile tilapia (Fazio et al., 2024). Total protein was quantified using the biuret method with an automated UV spectrophotometer (SEAC Slim, Florence, Italy). Alkaline phosphatase (ALP) activity was determined by incubating plasma with para-nitrophenyl phosphate in ammonium bicarbonate buffer (pH 7.9, 32°C) and measuring absorbance at 450 nm, as described by Adham et al. (2002), Abo-Taleb et al. (2024), Syanya et al. (2025).

## Intestine histology and morphometry analysis

At the end of Phase 2, four fish from each treatment group (n = 4) were dissected. A section of the anterior intestine, located just below the duodenum, was extracted and fixed in 10% neutral buffered formalin for 24 hours. The samples were labelled according to their respective treatment groups and sent to the DDRC Laboratory, Pallimuk, Ernakulam, Cochin City for histological slide preparation. Tissue processing involved dehydration through a graded series of alcohol baths, clearing in xylene, and infiltration with molten paraffin wax. The tissues were then embedded in fresh paraffin wax blocks. Sections were cut at a thickness of 5 µm using a microtome and stained with haematoxylin and eosin (H&E) for histological examination. The prepared slides were mounted with a DPX mounting medium and examined under a compound light microscope based on the procedures of (Dinh and Nguyen, 2022). Twelve slides were prepared per treatment group (3 slides per fish sample) for microscopic analysis (n = 12 slides). Each slide was examined under a compound microscope with a NIKON digital camera (Model: DS-Fi1) for histological evaluation. Image capture was performed at the Biotechnology Laboratory, Department of Biotechnology, Cochin University of Science and Technology, India. The captured images were further analysed using ImageJ software. Histometric parameters of the intestine, including villus height, villus width, and intestinal muscle thickness, were measured from randomly selected grid squares on transverse sections of each of the 12 slides. Images were captured at 40× magnification, with six random images per slide saved in TIFF format. For each sample, three zones were processed, and 10 random grid fields were measured and analysed using ImageJ software, following the methodology of Badran et al. (2024). Villus height, width, and intestinal muscle thickness were averaged from nine measurements per slide, using a conversion scale of 10 pixels = 1 µm. Goblet cells were quantified using periodic acid–shiff (PAS) staining. Nine equal-sized villi per slide were examined, and goblet cells were counted based on the procedure described by Chen et al. (2016). Results were expressed as the number of goblet cells per square millimetre, following the methods outlined by Palladino et al. (2023), Loureiro Paschoalini et al. (2024).

## Statistical analysis

All statistical analyses were conducted using SPSS version 24 software. Data normality was assessed using the Kolmogorov-Smirnov test, while homogeneity of variance was evaluated using Levene's test. A one-way analysis of variance (ANOVA) was performed to compare means across groups, followed by Tukey's HSD (Honestly Significant Difference) pairwise tests to identify significant differences. A threshold of $P < 0.05$ was used to determine statistical significance. Results are presented as *mean ± standard error (SE).* The standard error of the mean (SEM) was preferred for this study because of its ability to assess the precision of a sample mean as an estimate of the population mean. The sex ratio of fish from each replicate per treatment was calculated and compared to the

expected 1:1 (male: female) ratio in percentages. Intestinal morphometric parameters, measured using ImageJ software, were analysed using one-way ANOVA and Tukey's pairwise tests to determine significant differences among mean values.

# RESULTS

## Water quality Parameters during phase 1 and 2

During the 60-day treatment (Phase1), water quality parameters were maintained as indicated in Table 2. The temperature across all treatments averaged 28.3°C. pH ranged from 7.251 ± 0.034 to 7.511 ± 0.058. Dissolved oxygen (DO) was highest at 6.5 mg/L, while the lowest value was 6.2 mg/L. Ammonia levels across all treatments were kept below 0.02 mg/L (Table 2). The water quality parameters of tank water with Hapas during Phase 2 (recovery phase) were within optimal ranges for aquaculture. The temperature during the culture period averaged 28.99°C and pH 7.46, suitable for hybrid red tilapia. Dissolved oxygen was (7.02 mg/L). Ammonia (0.153 mg/L) and nitrite (0.013 mg/L) remained low, indicating good water quality. Total hardness (39.27 mg/L) and alkalinity (45.20 mg/L) were within acceptable limits, supporting buffering capacity (Table 3). These conditions ensured a stable environment for fish recovery and growth.

**Table 2** Water quality parameters (mean ± S.E.) during Phase1 (treatment period).

| Parameter | Water quality parameters (Mean± S.E) | | | |
|---|---|---|---|---|
| | PPB0 | PPLB1 | PPMB2 | PPHB3 |
| Temperature ($^0$c) | 28.335 ± 0.052 | 28.349 ± 0.051 | 28.343 ± 0.050 | 28.351 ± 0.056 |
| PH | 7.456 ± 0.069 | 7.300 ± 0.055 | 7.251 ± 0.034 | 7.511 ± 0.058 |
| Dissolve oxygen (mg/L) | 6.331 ± 0.214 | 6.466 ± 0.164 | 6.276 ± 0.064 | 6.500 ± 0.262 |
| Ammonia (mg/L) | 0.013 ± 0.001 | 0.012 ± 0.004 | 0.012 ± 0.003 | 0.014 ± 0.007 |

**NB:**.(PCB0 (control, 0 g/kg), PPLB1 (30 g/kg), PPMB2 (60 g/kg), and PPHB3 (90 CPSPg/kg feed)

**Table 3** Water quality parameters (Mean ±SE) during Phase 2 (Recovery phase).

| Phase 2 water quality of the tank water with Hapas | |
|---|---|
| Water quality parameters | Mean ± SE |
| Temperature (0c) | 28.987 ± 0.065 |
| PH | 7.455 ± 0.027 |
| Dissolved oxygen (mg/L | 7.023 ± 0.062 |
| Ammonia NH3 (mg/L | 0.153 ± 0.016 |
| Total water hardness (mg/L) | 39.266 ± 2.132 |
| Total Alkalinity (mg/L) | 45.200 ± 1.806 |
| Nitrite NO2- (mg/L) | 0.013 ± 0.002 |

**Note:** Water temperature and pH were measured daily, while DO, $NH_3$, total water hardness, alkalinity, and $NO_2^-$ were measured weekly during the culture period.

## Effects of dietary supplementation of *Carica papaya* seed powder as an agent of masculinisation on growth performance and feed utilisation in hybrid red tilapia.

The findings indicate that inclusion of CPSP in the fish diet at varying dosage levels influenced growth and feed utilisation during the treatment period (Phase 1). Higher dietary inclusion levels (60–90 g/kg) significantly improved final weight,

weight gain (WG), and average weight gain (AWG) ($p < 0.05$) (Table 4). Total feed consumption increased with higher CPSP levels in the fish diet. However, feed conversion ratio (FCR) worsened during the treatment period (Phase1), and feed conversion efficiency (FCE) declined significantly at the highest CPSP inclusion level (90 g/kg feed) ($p < 0.05$). Protein efficiency ratio (PER) significantly improved in fish from moderate and higher CPSP dosage treatment levels (60–90 g/kg) ($p < 0.05$) (Table 4). No significant differences in specific growth rate (SGR) were observed in fish across all treatment groups ($p > 0.05$) (Table4).

**Table 4** Effects of different supplementation levels of *Carica papaya* seed powder on growth parameters and feed utilisation at phase1 (Treatment phase).

| Growth Parameters | Treatment groups (*C. papaya* dosage inclusion level) | | | |
|---|---|---|---|---|
| | PPB0 | PPLB1 | PPMB2 | PPHB3 |
| Initial Weight (g) | 0.079 ± 0.01 | 0.07± 0.01 | 0.07 ± 0.00 | 0.08 ± 0.01 |
| Final weight (g) | 15.02 ± 0.29 $^{a}$ | 14.56 ± 0.26 $^{a}$ | 15.29 ± 0.24 $^{c}$ | 16.13 ± 0.20 $^{b}$ |
| Weight gain (g) | 14.94 ± 0.29 $^{a}$ | 14.49 ± 0.26 $^{a}$ | 15.22 ± 0.23 $^{b}$ | 16.05 ± 0.21 $^{c}$ |
| Average weight gain / day (g) | 0.24 ± 0.01 $^{a}$ | 0.24 ± 0.00 $^{a}$ | 0.25 ± 0.00 $^{c}$ | 0.26 ± 0.00 $^{d}$ |
| Specific growth rate (%/day) | 3.88 ± 0.08 | 3.86 ± 0.05 | 3.91 ± 0.04 | 3.92 ± 0.11 |
| Total feed consumed (g) | 24.43 ± 0.72 $^{a}$ | 27.41 ± 0.84 $^{b}$ | 31.14 ± 0.99 $^{c}$ | 38.43 ± 1.01 $^{d}$ |
| Feed conversion Ratio | 1.63± 0.03 $^{d}$ | 1.89 ± 0.04 $^{e}$ | 2.04 ± 0.05 $^{e}$ | 2.39 ± 0.05 $^{f}$ |
| Feed Conversion Efficiency | 0.61 ± 0.01 $^{a}$ | 0.53 ± 0.01 $^{b}$ | 0.49 ± 0.01 $^{b}$ | 0.42 ± 0.01 $^{d}$ |
| Protein efficiency ratio | 0.33 ± 0.01 $^{a}$ | 0.32 ± 0.01 $^{a}$ | 0.33 ± 0.01 $^{b}$ | 0.35 ± 0.00 $^{b}$ |

**Note:** Growth parameters are presented as mean ± standard error (S.E.) based on three determinations. Where one-way ANOVA indicated significant differences ($p < 0.05$), Tukey's pairwise test was performed. Means with different superscript letters within each row indicate significant differences at ($p< 0.05$). (PCB0 (control, 0 g/kg), PPLB1 (30 g/kg), PPMB2 (60 g/kg), and PPHB3 (90 CPSPg/kg feed)

During the recovery period (Phase 2), significant improvements in growth parameters and feed utilisation were observed in fish previously fed higher levels of CPSP supplementation. Fish from higher CPSP treatment groups (60–90 g/kg) exhibited significantly increased final weight, weight gain (WG), and average weight gain (AWG) ($p < 0.05$) (Table 5). Specific growth rate (SGR) also improved significantly in fish previously fed higher CPSP levels (90 g/kg feed), with an SGR of 0.662 compared to 0.597 in the control group ($p < 0.05$).

Feed conversion ratio (FCR) and feed conversion efficiency (FCE) were significantly enhanced in fish from higher CPSP supplementation groups, as evidenced by lower FCR and higher FCE values at the highest dose (90 g/kg feed) ($p < 0.05$) (Table 5). Additionally, the protein efficiency ratio (PER) significantly improved with increasing CPSP inclusion levels. Fish from the 90 g CPSP/kg feed treatment group showed the highest PER (1.58), indicating that CPSP enhanced protein retention and supported sustainable fish growth at recovery phase (Table 4).

## Effects of *Carica papaya* seed powder supplementation on proximate biochemical composition.

*Carica papaya* seed powder, significantly influenced the nutrient biochemical composition of hybrid red tilapia. The findings indicate that moisture content was significantly higher in fish from the higher CPSP supplementation treatment group (90 g/kg feed; 81.83%) compared to fish from the control group ($p < 0.05$) (Table 6). Similarly, fish from the 60 g CPSP/kg feed treatment group exhibited elevated ash content (4.000%) compared to the control group (2.896%) ($p < 0.05$). In

contrast, protein levels remained consistent across all treatment groups, with no significant differences observed ($p > 0.05$). Notably, lipid content was significantly higher in fish from the higher CPSP inclusion group (90 g/kg feed; 2.79%) compared to the control (1.29%) ($p < 0.05$) (Table 6). Conversely, carbohydrate content was significantly lower in fish from the higher CPSP treatment group (90 g/kg feed; 1.66%) compared to the control group (5.48%) ($p < 0.05$) (Table 6).

**Table 5** Effects of different supplementation levels of *Carica papaya* seed powder on growth parameters and feed utilisation at phase 2 (Recovery phase).

| Growth parameters | Treatment groups (*C. papaya* dosage inclusion level) | | | |
|---|---|---|---|---|
| | PPB0 | PPLB1 | PPMB2 | PPHB3 |
| Initial Weight (g) | 15.02 ± 0.29 $^{c}$ | 14.56 ± 0.26 $^{c}$ | 15.29 ± 0.24 $^{d}$ | 16.13 ± 0.20 $^{e}$ |
| Final weight (g) | 51.66 ± 0.67 $^{a}$ | 55.42 ± 0.61 $^{b}$ | 60.46 ± 0.31 $^{c}$ | 63.59 ± 0.57 $^{d}$ |
| Weight gain (g) | 36.64 ± 0.52 $^{c}$ | 40.85 ± 0.77 $^{d}$ | 45.17 ± 0.22 $^{e}$ | 47.45 ± 0.57 $^{f}$ |
| Average weight gain (g / day) | 0.40 ± 0.01 $^{a}$ | 0.45 ± 0.01 $^{b}$ | 0.50 ± 0.02 $^{c}$ | 0.52 ± 0.01 $^{d}$ |
| Percentage weight gain (%) | 245.45 ± 5.32 $^{a}$ | 283.30 ± 8.91 $^{c}$ | 296.84 ± 5.06 $^{c}$ | 295.09 ± 5.47$^{c}$ |
| Specific growth rate (%/day) | 0.59 ± 0.01 $^{a}$ | 0.64 ± 0.01 $^{b}$ | 0.66 ± 0.01 $^{b}$ | 0.66 ± 0.01 $^{b}$ |
| Total feed consumed (g) | 100.87 ± 2.30 $^{a}$ | 103.13 ± 2.25 $^{a}$ | 74.84 ± 1.58 $^{b}$ | 68.50 ± 1.75 $^{b}$ |
| Feed conversion Ratio | 1.96 ± 0.04$^{c}$ | 1.86 ± 0.03 $^{c}$ | 1.64 ± 0.03 $^{d}$ | 1.40 ± 0.04 $^{e}$ |
| Feed Conversion Efficiency | 0.51 ± 0.01$^{a}$ | 0.53 ± 0.01 $^{a}$ | 0.61 ± 0.01$^{b}$ | 0.72 ± 0.02 $^{c}$ |
| Protein efficiency ratio | 1.22 ± 0.01 $^{a}$ | 1.36 ± 0.02 $^{b}$ | 1.50 ± 0.01 $^{c}$ | 1.58 ± 0.01 $^{f}$ |

**Note:** Growth parameters are presented as **mean ± standard error (S.E.)** based on three determinations. Where one-way ANOVA indicated significant differences ($p < 0.05$), Tukey's pairwise test was performed. Means with different superscript letters within each row indicate significant differences at $p < 0.05$. (**NB**:.(PCB0 (control, 0 g/kg), PPLB1 (30 g/kg), PPMB2 (60 g/kg), and PPHB3 (90 CPSPg/kg feed)

**Table 6** Proximate biochemical composition of red tilapia across different *Carica papaya* seed powder treatment levels.

| Proximate biochemical composition | Treatment groups (*Carica papaya* dosage inclusion level) | | | |
|---|---|---|---|---|
| | PPB0 | PPLB1 | PPMB2 | PPHB3 |
| Moisture Content (%) | 80.35 ± 0.32 $^{a}$ | 81.24 ± 0.08 $^{b}$ | 81.43 ± 0.08 $^{b}$ | 81.83 ± 0.01 $^{b}$ |
| Ash Content % | 2.89 ± 0.11 $^{a}$ | 3.04 ± 0.04 $^{a}$ | 4.00 ± 0.04 $^{b}$ | 3.20 ± 0.17 $^{c}$ |
| Protein content (%) | 9.97 ± 0.42 | 10.98 ± 0.42 | 10.05 ± 0.31 | 10.50 ± 0.39 |
| Lipid content (%) | 1.29 ± 0.06 $^{a}$ | 1.78 ± 0.02 $^{b}$ | 1.97 ± 0.06 $^{b}$ | 2.79 ± 0.10 $^{c}$ |
| Carbohydrates content % | 5.48 ± 0.50 $^{a}$ | 2.94 ± 0.37 $^{c}$ | 2.54 ± 0.29 $^{c}$ | 1.66± 0.32 $^{c}$ |

**Note:** Biochemical composition parameters are presented as **mean ± standard error (S.E.)** based on three determinations. Where one-way ANOVA results were significant ($p < 0.05$), Tukey's pairwise test was performed. Means with different superscript letters within each row indicate significant differences at $p < 0.05$. (NB:.(PCB0 (control, 0 g/kg), PPLB1 (30 g/kg), PPMB2 (60 g/kg), and PPHB3 (90 CPSPg/kg feed)

## Effects of *Carica papaya* seed powder supplementation on haematological parameters as indicators of fish health in hybrid red tilapia.

Analysis of haematological parameters in fish across different CPSP supplementation levels revealed that haemoglobin (Hb) and haematocrit (Hct) levels

were significantly elevated in fish from higher CPSP (90g/Kg feed) treatment levels (8.583 g/dL and 26.10%, respectively), compared to the control group (8.078 g/dL and 25.20 %) ($p < 0.05$) (Table 7). Similarly, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), and mean corpuscular haemoglobin concentration (MCHC) were significantly elevated in fish from the higher CPSP treatment group (90 g/kg feed) ($p < 0.05$). In contrast, platelet and white blood cell (WBC) levels remained relatively stable, showing no significant differences across all treatment levels ($p > 0.05$) (Table 7). Additionally, lymphocytes and monocytes were significantly elevated in fish from the higher CPSP treatment group (90 g/kg feed) compared to the control ($p < 0.05$) (Table 7).

**Table 7** Haematological parameters across different *Carica papaya* seed powder treatment levels in hybrid red tilapia.

| Haematological parameters | Treatment groups (*Carica papaya* dosage inclusion level) | | | |
|---|---|---|---|---|
| | PPB0 | PPLB1 | PPMB2 | PPHB3 |
| Haemoglobin (g/dL) | 8.07 ± 0.08 $^{a}$ | 8.30 ± 0.07 $^{b}$ | 8.47 ± 0.03 $^{c}$ | 8.58 ± 0.03 $^{c}$ |
| Haematocrit (%) | 25.20 ± 0.06 $^{a}$ | 25.66 ± 0.16 $^{a}$ | 25.57 ± 0.19 $^{a}$ | 26.10 ± 0.15 $^{b}$ |
| Mean Corpuscular Volume (fL). | 151.11 ± 0.36 $^{b}$ | 153.19 ± 0.28$^{c}$ | 153.48 ± 0.23 $^{c}$ | 153.42 ± 0.37 $^{c}$ |
| Mean Corpuscular Haemoglobin (fL). | 47.77 ± 0.32 $^{a}$ | 48.90 ± 0.37 $^{a}$ | 51.61 ± 0.26 $^{b}$ | 52.36 ± 0.33 $^{b}$ |
| Mean Corpuscular Haemoglobin Conc (%) | 31.80 ± 0.12$^{a}$ | 32.91 ± 0.42 $^{b}$ | 32.95 ± 0.23 $^{b}$ | 32.96± 0.10 $^{b}$ |
| Platelets /microlitre (µL) | 319.96 ± 0.33 | 320.12 ± 0.43 | 320.27 ± 0.31 | 319.89 ± 0.27 |
| White Blood cells (x $10^{9}$ cells/µL) | 84.98 ± 0.33 | 85.19 ± 0.16 | 85.16 ± 0.16 | 85.64 ± 0.14 |
| Lymphocytes (x$10^{3}$ cells/µL) | 89.43 ± 0.17 $^{a}$ | 90.69 ± 0.14 $^{a}$ | 92.40 ± 0.13 $^{b}$ | 91.70 ± 0.64 $^{b}$ |
| Monocytes (%) | 2.38 ± 0.07 $^{b}$ | 2.57 ± 0.00 $^{c}$ | 2.60 ± 0.08 $^{c}$ | 2.67 ± 0.05 $^{c}$ |
| Eosinophils (%) | 1.26 ± 0.01 $^{a}$ | 1.45 ± 0.07 $^{b}$ | 1.58 ± 0.06$^{b}$ | 1.77± 0.04 $^{c}$ |

**Note:** Haematological parameters are presented as mean ± standard error (S.E.) based on three determinations. Where one-way ANOVA results were significant ($p < 0.05$), Tukey's pairwise test was performed. Means with different superscript letters within each row indicate significant differences at $p < 0.05$. ( **NB:** PCB0 (control, 0 g/kg), PPLB1 (30 g/kg), PPMB2 (60 g/kg), and PPHB3 (90 CPSPg/kg feed)

## Effects of *Carica papaya* seed powder supplementation on serum enzyme biochemical indices as indicators of fish health in hybrid red tilapia

Different dosage levels of CPSP**,** used as an aromatase inhibitor and masculinisation agent, significantly influenced serum liver enzyme activities in red tilapia. The findings indicate that aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels significantly decreased with increasing CPSP supplementation, suggesting enhanced liver activity and improved liver health in fish ($p < 0.05$). Alkaline phosphatase (ALP) levels were significantly elevated in fish fed higher CPSP dosage level (90 g/kg feed; 31.81 U/L) compared to the fish control group (27.47 U/L; $p < 0.05$) (Table 8). Additionally, albumin (ALB) and globulin (GLO) levels significantly improved in fish from the higher CPSP treatment group (90 g/kg feed; 1.57 g/dL and 5.6 g/dL, respectively) compared to fish from the control group (1.46 g/dL and 4.94 g/dL, respectively), indicating enhanced protein metabolism and immune response ($p < 0.05$). Serum cholesterol and glucose levels were also significantly elevated in fish from the higher CPSP treatment group ($p < 0.05$) (Table 8). Furthermore, total protein levels increased in fish from the higher CPSP supplementation group (90 g/kg feed; 6.805 g/dL) compared to fish from the control group (6.331 g/dL), reflecting improved protein synthesis and utilisation ($p < 0.05$) (Table 8).

## Effects of *Carica papaya* seed powder supplementation on fish organ indices as indicators of fish health in hybrid red tilapia.

Dietary supplementation of hybrid red tilapia's diet with CPSP as a masculinisation agent significantly influenced fish organ indices in relation to body weight. The findings indicate that the stomach index (SI), hepatosomatic index (HSI), and visceral somatic index (VSI) significantly increased in fish from higher CPSP supplementation level, demonstrating a dose-dependent effect (90 g CPSP/kg feed; $p < 0.05$) (Table 9). In contrast, the gonadosomatic index (GSI) was significantly reduced in fish from the higher CPSP treatment group (90 g CPSP/kg feed; 0.777) compared to the control group (2.781; $p < 0.05$) (Table 9).

**Table 8** Blood Serum profile of red tilapia across different *Carica papaya* treatment levels.

| Serum Biochemistry parameters | Treatment groups (*Carica. papaya* dosage inclusion level) | | | |
|---|---|---|---|---|
| | PPB0 | PPLB1 | PPMB2 | PPHB3 |
| Aspartate Amino transferase (µ/L) | 60.50 ± 0.49 [a] | 58.63 ± 0.53 [c] | 58.43 ± 0.28 [c] | 56.43 ± 1.32 [d] |
| Alanine Aminotransferase (µ/L) | 36.36 ± 0.23 [a] | 35.71 ± 0.42 [b] | 35.00 ± 0.50 [b] | 30.43 ± 0.35 [c] |
| Alkaline phosphate (µ/L) | 27.47 ± 0.22 [a] | 29.09 ± 0.47 [b] | 31.02 ± 0.27 [c] | 31.81 ± 0.34 [c] |
| Albumin (g/dL) | 1.46 ± 0.01 [d] | 1.48 ± 0.01 [d] | 1.55 ± 0.00 [e] | 1.57 ± 0.01 [e] |
| Globulin (g/dL) | 4.94 ± 0.06 [a] | 5.60± 0.02 [b] | 5.65 ± 0.01 [b] | 5.69 ± 0.03 [b] |
| Serum Cholesterol (mg/dL) | 132.43 ± 0.42 [a] | 133.45 ± 0.53 [a] | 140.15 ± 0.26 [c] | 141.51 ± 0.33 [c] |
| Glucose (mg/dL) | 92.50 ± 0.99 [c] | 93.32 ± 1.23 [c] | 106.79 ± 0.67 [d] | 109.16 ± 0.68 [d] |
| Total protein (g/dL) | 6.33 ± 0.04 [a] | 6.48 ± 0.04 [a] | 6.69 ± 0.01 [b] | 6.80 ± 0.01 [b] |

**Note:** Serum biochemical parameters are presented as **mean ± standard error (S.E.)** based on three determinations. Where one-way ANOVA results were significant ($p < 0.05$), Tukey's pairwise test was performed. Means with different superscript letters within each row indicate significant differences at $p < 0.05$. (**NB**:.(PCB0 (control, 0 g/kg), PPLB1 (30 g/kg), PPMB2 (60 g/kg), and PPHB3 (90 CPSPg/kg)

**Table 9** Effects of *Carica papaya* seed powder supplementation levels in the diet of hybrid red tilapia on organ indices.

| Fish organ indices | Treatment groups (*Carica. Papaya* seed powder dosage inclusion level) | | | |
|---|---|---|---|---|
| | PPB0 | PPLB1 | PPMB2 | PPHB3 |
| Stomach index | 2.15 ± 0.02 [a] | 2.54 ± 0.06 [b] | 3.83 ± 0.17 [c] | 5.25 ± 0.06 [d] |
| Hepatosomatic index | 1.95 ± 0.057[a] | 2.01 ± 0.06 [b] | 2.09 ± 0.04 [b] | 2.25 ± 0.05[c] |
| Gonadsomatic index | 2.78 ± 0.17 [a] | 1.88 ± 0.12 [b] | 1.16 ± 0.06 [c] | 0.77 ± 0.09 [c] |
| Visceral Somatic index | 9.60 ± 0.15 [a] | 9.45 ± 0.25 [b] | 9.78 ± 0.09 [c] | 10.48 ± 0.27 [c] |

**Note:** Fish organ indices are presented as **mean ± standard error (S.E.)** based on three determinations. Where one-way ANOVA results were significant ($p < 0.05$), Tukey's pairwise test was performed. Means with different superscript letters within each row indicate significant differences at $p < 0.05$.**NB**:.(PCB0 (control, 0 g/kg), PPLB1 (30 g/kg), PPMB2 (60 g/kg), and PPHB3 (90 CPSPg/kg)

## Effects of *Carica papaya* seed powder supplementation on fish masculinity and reproductive hormone profile in hybrid red tilapia

Different supplementation levels of CPSP in fish feed as an agent of masculinisation significantly influenced sex hormone profiles and fish condition

factor (K). Oestradiol which is a primary female sex hormone responsible for reproductive functions and secondary sexual characteristics in fish was significantly lower in fish from the higher CPSP treatment group (90 g CPSP/kg feed) at 473.66 ng/ml compared to fish from the control group which showed a significantly higher oestradiol level (2757.66 ng/ml) ($p < 0.05$) (Table 10). However, 11-ketotestosterone hormone, a major androgen (male sex hormone) in fish, regulating male reproductive development and behaviour, showed a dose-dependent increase with higher CPSP inclusion. Fish from 90 g CPSP/kg feed showed significantly higher 11-ketotestosterone hormone levels (4056.33 ng/ml) compared to fish from the control group at 1585.00 ng/ml (11-ketotestosterone hormone level) ($p < 0.05$) (Table 10). Fish Fulton condition factor (K), an indicator of fish health and nutritional status, was significantly better in fish from the higher CPSP treatment group (90 g/kg feed) with a K value of 1.093 compared to fish from the control group with a K value of 0.804 ($p < 0.05$) (Table 10). A higher K value indicates better fish condition.

**Table 10** effects of *Carica papaya* seed powder on fish condition factor and sex hormone profile

| Parameters | Treatment groups (*C. papaya* dosage inclusion level) | | | |
|---|---|---|---|---|
| | PPB0 | PPLB1 | PPMB2 | PPHB3 |
| **Estradiol hormone profile (ng/ml)** | 2757.66 ± 101.03 [a] | 1543.33 ± 94.55 [b] | 692.33 ± 39.29 [c] | 473.66 ± 39.29 [c] |
| **11 Ketotestosterone hormone profile (ng/ml)** | 1585.00 ± 24.58 [a] | 1882.33 ± 28.93 [b] | 3393.00 ± 90.06 [c] | 4056.33 ± 65.38 [d] |
| **Fulton condition factor** | 0.80 ± 0.01 [a] | 0.82 ± 0.02 [a] | 0.96 ± 0.04 [b] | 1.09 ± 0.04 [b] |

**Note:** Hormone and condition factor parameters are presented as **mean ± standard error (S.E.)** based on three determinations. Where one-way ANOVA was significant ($p < 0.05$), Tukey's pairwise test was performed. Means with different superscript letters within each row indicate significant differences at $p < 0.05$. **NB**:.(PCB0 (control, 0 g/kg), PPLB1 (30 g/kg), PPMB2 (60 g/kg), and PPHB3 (90 CPSPg/kg)

The percentage proportion of males increased significantly in fish from higher CPSP treatment group (90 g/kg feed) at 96.7% males compared to control with male proportions of 50% ($p < 0.05$), indicating a strong masculinising effect (Figure 1a). However, the percentage of females significantly decreased with higher CPSP *inclusion levels* (90 g/kg feed), with only 3.7% females compared to the control with a 50% female proportion ($p < 0.05$). (Figure 1b). This is likely due to reduced oestrogen production due to inhibitory nature of CPSP on the aromatase enzyme activities which altered sex differentiation pathways in fish due to bioactive compounds found in CPSP.

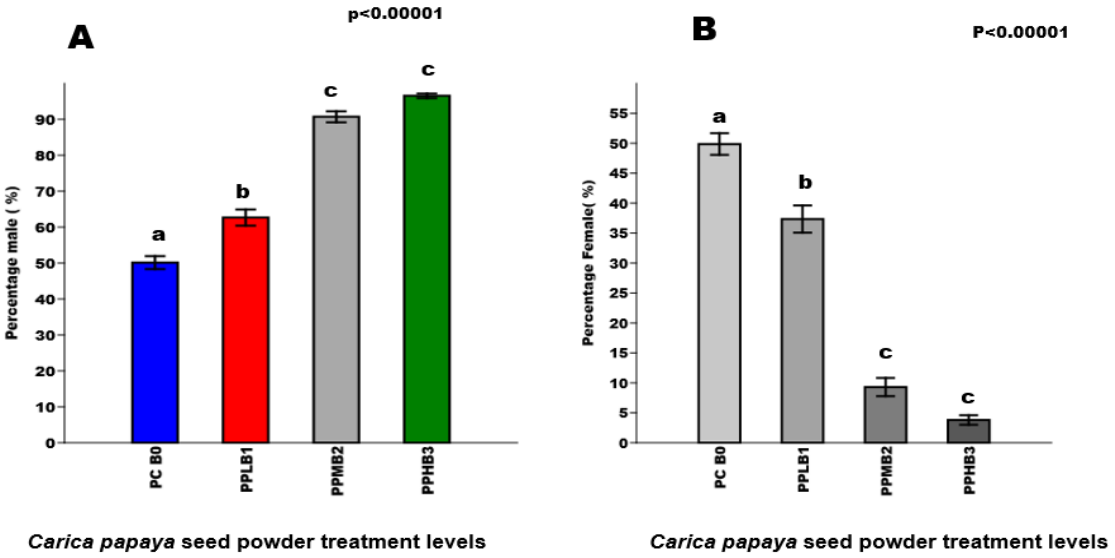


**Figure 1** The percentage proportion of masculinised males (**A**) and the percentage proportion of females **(B)** across different CPSP treatment levels (PCB0 - Control, PPLB1 - 30g/kg, PPM - 60g/kg, and PPH - 90g/kg CPSP) (Mean % ± S.E). Where ANOVA was significant, a Tukey's pairwise test was conducted, and bar graph plots with different letters indicate significant differences in mean percentage proportions at $p < 0.05$.

## Effects of *Carica papaya* seed powder supplementation on anterior gut Intestinal histomorphometry

The intestinal histomorphometry analysis revealed significant changes in intestinal villus morphology in response to varying levels of CPSP supplementation in hybrid red tilapia diet as agent of masculinization. Fish from the high-dose treatment group (90 g/kg feed) exhibited significantly longer intestinal villi, with an average villus length of 247 µm**,** compared to the control group, which had an average villus length of 195 µm ($p < 0.05$; Figure 2t). In contrast, the width of the intestinal villi showed a significant decrease with increasing levels of CPSP supplementation. Fish from the high-dose treatment group (90 g/kg feed) had an average villus width of 96 µm, while the control group exhibited a wider average villus width of 126 µm ($p < 0.05$; Figure 2s).

The length of the villus intestinalis crypts was significantly elevated in fish from the high-dose of CPSP treatment group (90 g/kg feed), with an average crypt length of 51 µm, compared to the control group, which had an average crypt length of 33 µm ($p < 0.05$; Figure 3p). Similarly, the number of goblet cells in the intestinal tissue was significantly higher in fish from the high-dose CPSP treatment group (90 g/kg feed), with an average of 7.5 goblet cells, compared to the control group, which had an average of 5 goblet cells ($p < 0.05$; Figure 3v).

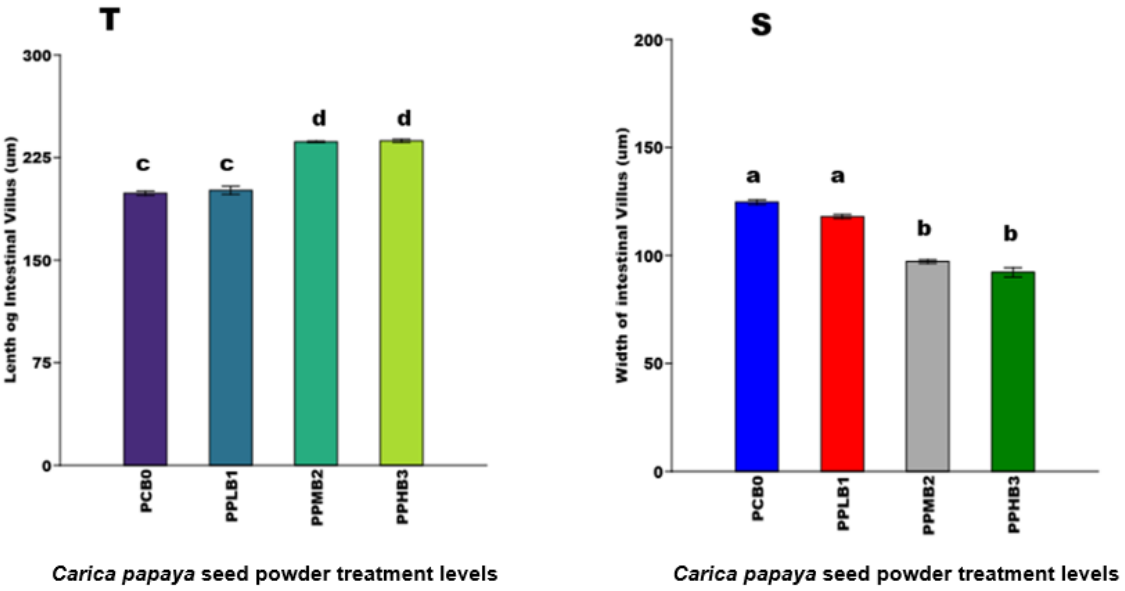


**Figure 2** Intestinal histometric analysis of anterior part of hybrid red tilapia intestine structure showing Intestinal villus length **(T)** and Villus width proportions **(S)** across different CPSP treatment levels (PCB0 - Control, PPLB1 - 30g/kg, PPM - 60g/kg, and PPH - 90g/kg CPSP. ) (**Mean ± S.E**). Where ANOVA was significant, a Tukey's pairwise test was conducted, and bar graph plots with different letters indicate significant differences in mean proportions at a p< 0.05.

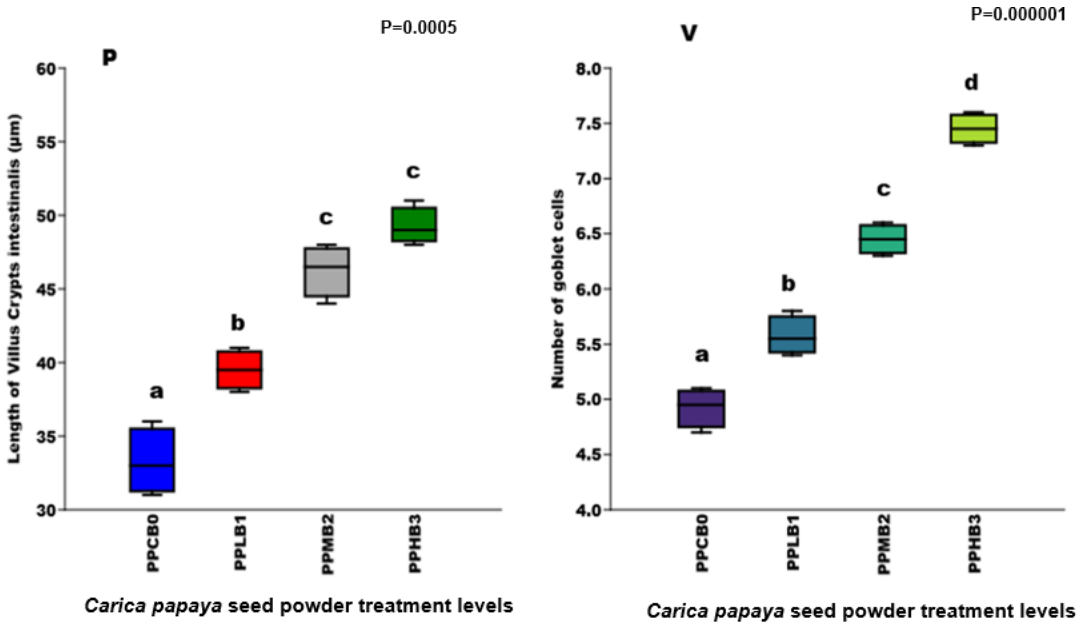


**Figure 3** Intestinal histometric analysis of hybrid red tilapia intestines structure showing Length of Villus Crypts intestinalis **(P)** and Number of goblet cells**(V)** across different CPSP treatment levels (PCB0 - Control, PPLB1 - 30g/kg, PPM - 60g/kg, and PPH - 90g CPSP/kg feed. (***Mean ± S.E*****).** Where ANOVA was significant, a Tukey's pairwise test was conducted, and bar graph plots with different letters indicate significant differences in mean proportions at a p-< 0.05.

Microscopic analysis of the anterior intestine revealed significant structural variations across different levels of CPSP treatment level, used as a masculinisation agent in hybrid red tilapia. The slide images visually highlighted key intestinal features, including the interstitial wall diameter (IW), villus height (VH), villus width (VW), intestinal crypts (CrP), lumen (LU), goblet cells (GC), mucosa (M), submucosa (SM), intestinal villi (Vi), and microvilli (Mi). These components were consistently observed across all treatment groups (Figure 4). The intestinal villi, which project outward (evaginate), and the crypts, which fold inward (invaginate), are critical structural elements. The villi arise from mucosal folds and are covered by a simple columnar epithelium with microvilli. These features were more pronounced in treatment groups receiving higher CPSP dosage levels (60 g/kg and 90 g/kg feed) (Figures 4c and 4d, respectively). No significant differences in intestinal histology specifically in villus height (VH), villus width (VW), and intestinal crypts (CrP) were observed between the control group and the low-dose of CPSP treatment group (30 g/kg feed) (Figure 4a and 4b). However, the mucosa and submucosa layers were significantly thicker in fish from the higher CPSP *treatment groups* (60 g/kg and 90 g/kg feed) compared to fish from the control group (Figure 4c and 4d).

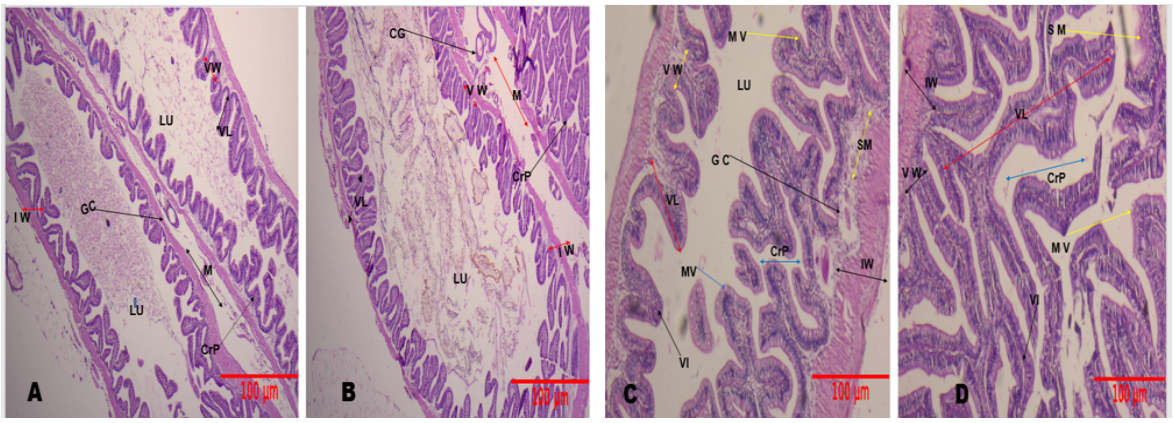


**Figure 4** Histomorphology structure of the small intestine of hybrid red tilapia treated with different dosage levels of plant-based Aromatase inhibitor CPSP, where **A-** (Control), **B** (30g/kg), **C (**60g/kg), and **D** (90g/kg) of CPSP supplementation in the fish diet,. Magnification was **(× 100),** staining was done using Haematoxylin-Eosin (HE). **IW** – Interstitial wall diameter, **VH** – Villus height, **VW** – Villus length, **CrP** – Intestinal crypt, **LU** – Lumen, **GC** – Goblet cells, **M** – Mucous, **SM** – Submucosa, **Vi** – Intestinal villi, **MV –** Microvilli.

# DISCUSSION

Plant-based aromatase inhibitors are increasingly utilised in aquaculture for various purposes, including masculinisation, immunostimulant, growth promotion, and as herbal remedies for fish diseases. Among these, *Carica papaya* is one of the most widely used plant-based aromatase inhibitors, particularly for fish masculinisation. Despite its extensive application in tilapia species, such as Nile tilapia (*O. niloticus*) (Iipinge, 2019; Wokeh et al., 2021; Jamal et al., 2024) and Mozambique tilapia (*O. mossambicus*) (Omeje, 2016; Omeje et al., 2020), its effects on fish physiology , body and serum biochemistry remain poorly documented, especially in hybrid red tilapia and other tilapia species. In aquaculture, *Carica papaya* seed powder (CPSP) is recognised as one of the most effective plant-based sex-reversal agents (Lohiya et al., 1994; Farrag et al., 2013; Jamal et al., 2024). This is attributed to the presence of cytochrome P450 aromatase inhibitors in the seeds and leaves of the *Carica papaya* plant. These inhibitors suppress the activity of the aromatase enzyme in the fish ovary, which is responsible for converting androgens into oestrogens, a process that promotes feminisation. Similar to non-steroidal

aromatase inhibitors such as tamoxifen citrate and letrozole (Séralini and Moslemi, 2001; Gao et al., 2010;Doering et al., 2021), *Carica papaya* seeds have been shown to skew sex ratios toward males in fish populations. This occurs through the suppression of oestrogen production in the ovary and the enhancement of 11-ketotestosterone levels—a key hormone responsible for masculinisation in fish and other animals (Afonso et al., 2001; Uchida et al., 2004; Doering et al., 2021).

Farrag et al. (2013) and Omeje (2016) reported a significant improvement in masculinisation at higher inclusion levels of *Carica papaya* seeds in the diet of *O. niloticus* and *O. mossambicus.* A similar finding is documented in our findings where *a* significant shift in sex ratios among hybrid red tilapia populations when *Carica papaya* seed powder is included in their diet at higher levels. Specifically, fish from the 90 g/kg feed treatment group exhibited a male proportion of 96.7%**,** a stark contrast to the control group, which maintained an equal sex ratio of 50% males and 50% females. This pronounced masculinising effect is likely driven by the bioactive compounds present in *Carica papaya* seeds which influence sex hormone production and differentiation pathways (Ayodipupo Babalola et al., 2024; Patel et al., 2024). The primary mechanism behind this shift is the inhibition of aromatase**,** an enzyme responsible for converting androgens (11 Keto testosterone) into oestrogens (oestradiol). Compounds such as carpaine and benzyl isothiocyanate in *Carica papaya* seeds act as natural aromatase inhibitors, reducing oestrogen production in fish ovary (Azizah et al., 2019; Khalil et al., 2014). Lower oestrogen levels were reported to suppress female sex differentiation, as oestradiol is essential for the development of female reproductive traits. This findings are in tandem with studies on synthetic aromatase inhibitors, such as letrozole and fadrozole, which have been shown to skew sex ratios toward males in various fish species (Lal Anand et al., 2024; Lal et al., 2023). Conversely, the significant increase in 11-ketotestosterone levels (4056.33 ng/ml) at higher CPSP inclusion levels (90 g/kg feed) suggested enhanced masculinisation of fish at higher dosage level. 11-ketotestosterone as an androgen hormone plays a critical role in male gonadal development and the expression of female secondary sexual characteristics (Omeje et al., 2019). The gonadosomatic index (GSI)**,** a measure of reproductive tissue development, showed a significant reduction in fish from higher CPSP treatment levels (0.777 at 90 g/kg feed) compared to fish from the control group. This decline reflects the suppression of gonadal growth due to the altered hormonal balance caused by CPSP aromatase inhibition properties in the ovary of the fish, particularly through inhibition of oestrogen synthesis and the promotion of androgen dominance. These findings are in tandem with Farrag et al. (2013) in Nile tilapia(*O. niloticus*) where a similar variability in sex hormone was documented when higher *Carica papaya* seed powder was used. Above all fish condition factor (K)**,** an indicator of overall health and nutritional status, increased significantly in fish from higher CPSP treatment group. This suggests a potential trade-off between growth performance and reproductive performance during the treatment period which enhanced fish health.

The improved condition factor may have been attributed to diversion of energy resources from reproductive tissue development to somatic growth, a phenomenon often observed in fish exposed to sex-reversal agents or hormonal manipulations which concurs with study by (Omeje, 2016; Omeje et al., 2018). *Carica papaya* seed powder (CPSP) supplementation in fish diets has been equally reported to significantly influence growth performance and feed utilisation during masculinisation in various tilapia species, including Nile tilapia (*O. niloticus*) (Farrag et al., 2013; Hamid et al., 2022; Radwan et al., 2023) and Mozambique tilapia (*O. mossambicus*) (Omeje, 2016). Similarly, our findings demonstrate that higher CPSP inclusion levels (60 g/kg and 90 g/kg feed) during Phase 1 (treatment phase) significantly enhanced growth parameters such as final weight, weight gain (WG), and average weight gain (AWG) in hybrid red tilapia. This improvement in growth can be attributed to the bioactive compounds in *Carica papaya* seeds which may stimulate appetite and enhance nutrient assimilation in fish. Feed consumption

increased in fish from higher CPSP treatment group (90 g/kg feed), reflecting heightened metabolic demands for growth and masculinization. However, the feed conversion ratio (FCR) worsened, and feed conversion efficiency (FCE) declined among fish from higher CPSP treatment group (90 g/kg feed) during the treatment phase (Phase 1). This suggests that excess feed energy may have been diverted toward maintenance and sex reversal rather than growth. Notably, the protein efficiency ratio (PER) improved significantly at moderate CPSP doses (60 g/kg feed), indicating optimal protein utilisation for tissue synthesis at this level. The lack of significant differences in specific growth rate (SGR) across treatments suggests that CPSP's growth-promoting effects were more pronounced at specific stages rather than uniformly throughout the treatment phase. This aligns with findings by Hamid et al. (2022) and Radwan et al. (2023) who reported similar growth patterns in tilapia fed higher concentrations of *Carica papaya* leaf extract.

During Phase 2 (recovery phase), fish previously subjected to higher CPSP levels (60–90 g/kg feed) exhibited improved growth performance and feed utilisation, indicating a compensatory growth effect as more energy that were previously used for body metabolic activity and sex reversal has been reverted towards growth. Final weight, WG, and AWG were significantly higher, while SGR improved notably in fish from 60 g CPSP /kg feed. Lower FCR and higher FCE at 90 g CPSP /kg feed during recovery suggest efficient nutrient conversion and utilization by the fish, likely due to the lingering metabolic effects of CPSP. This finding is consistent with studies by Omeje et al. (2018) and Farrag et al. (2013) in *O. niloticus*. The significant increase in PER in fish from 90 g CPSP /kg feed treatment group (1.58) further supports the role of CPSP in enhancing protein retention and sustaining fish growth. Additionally, CPSP, as a plant-based aromatase inhibitor, was found to influence the proximate body biochemical composition of hybrid red tilapia. The use of CPSP as a masculinising agent has also been found to influence the nutritional and biochemical composition of hybrid red tilapia. The findings indicate that fish from higher dietary supplementation of CPSP (90 g/kg feed) significantly showed increased moisture content (81.83%) compared to fish from the control group. This suggests that CPSP in the fish diet enhances water retention in fish tissues, potentially improving physiological function and osmoregulatory efficiency (Suman et al., 2024). Fish from the 60 g CPSP /kg feed treatment group exhibited significantly higher ash content (4.000%), indicating enhanced mineral deposition, likely due to improved nutrient bioavailability at this treatment dosage level. However, protein levels showed no significant differences across different CPSP treatment levels compared to the control, suggesting that CPSP supplementation does not impair protein synthesis or deposition in fish. Similar findings were reported in O*. niloticus* -fed higher *Carica papaya* seed meal as a masculinising agent (Farrag et al., 2013).

However, fish from higher CPSP inclusion group (90 g/kg feed) showed significantly higher lipid content (2.793%) compared to control and indication of improved energy storage capacity as deposited fat tissues. This may be linked to reduced energy allocation to reproductive activities due to the aromatase-inhibiting effects of CPSP. Conversely, carbohydrate content significantly decreased in fish from the 90 g/kg CPSP treatment group (1.660%) compared to the control (5.483%). This reduction suggests increased utilisation of carbohydrates for energy metabolism, likely to meet the growth and physiological demands induced by CPSP at higher dosage levels as an aromatase inhibitor. These results align with studies by (Radwan et al., 2023; Jamal et al., 2024) in Nile tilapia (*O.niloticus*). Therefore, CPSP supplementation in fish diet appears to influence nutrient partitioning, enhancing moisture, mineral, and lipid content while promoting carbohydrate utilisation for energy in hybrid red tilapia.

Haematology is a critical component in assessing fish health, immunity, and metabolic activity (Benli and Yildiz, 2004; Kumar et al., 2011). This study demonstrates that *Carica papaya* seed powder (CPSP) significantly influences haematological parameters in hybrid red tilapia. Higher CPSP inclusion (90 g/kg

feed) elevated haemoglobin (Hb; 8.583 g/dL) and haematocrit (Hct; 26.10%), suggesting improved oxygen transport capacity, basically due to CPSP's antioxidant properties and in enhancing erythropoiesis. This aligns with findings by Hoseinifar et al. (2011) in juvenile beluga (*Huso huso*) fed oligofructose. Increased mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), and mean corpuscular haemoglobin concentration (MCHC) in fish fed higher CPSP levels indicate enhanced red blood cell efficiency, and oxygen packaging and transportation potentially improving fish physiological performance and health. Similar results were reported by Okomoda (2017) in Nile tilapia (*O. niloticus*) and Omeje et al. (2019) in *O. mossambicus*, who both observed elevated MCV, MCH, and MCHC with higher CPSP inclusion, which was associated with enhanced immunity and improved fish health. Stable platelet and white blood cell (WBC) levels in fish across all CPSP treatment groups and compared to control suggest CPSP does not disrupt immune homeostasis functions in the fish body. *Carica papaya* seeds contain bioactive compounds with immunostimulatory properties, as noted by Radwan et al. (2023) in Nile tilapia, which act as immune boosters. Elevated lymphocytes and monocytes, which are key body immunity defence cells in fish at higher CPSP levels, reflect enhanced immune responses, likely due to the immunomodulatory effects of CPSP's bioactive compounds, consistent with findings by Mansour et al. (2022) and Radwan et al. (2023) in Nile tilapia and African catfish (*Clarias gariepinus*). Similarly, increased eosinophil concentration in fish fed higher CPSP inclusion levels indicates strengthened defence against pathogens, though this may also suggest mild inflammation, as supported by Owolabi and Abdulkareem (2021) in *C. gariepinus* fed *Carica papaya* and *Mangifera* diets. Haematology results of this study give a clear indication that CPSP supplementation especially at higher dosage levels enhances haematological parameters, hence supporting fish health and immunity. This makes *Carica papaya* seeds a key important compound in aquaculture, not only as an agent of masculinisation but also associated with a myriad of different functions in the fish body system.

Serum liver enzymes, such as aspartate aminotransferase (AST) and alanine aminotransferase (ALT) are considered key indicators of fish health, reflecting metabolic and hepatic function (Hastuti et al., 2020; Asghar et al., 2024; Eissa, Awlya, et al., 2024). The use of CPSP as a masculinising agent in fish significantly influences serum biochemical indices in hybrid red tilapia. Reduced AST and ALT levels in fish fed higher CPSP supplementation indicate improved liver health and liver enzyme activities , as lower transaminase activity suggests reduced hepatic stress or damage, enhancing metabolic efficiency (Sayed and Moneeb, 2015; El-Sayed Ali et al., 2018; Yehia et al., 2024). Elevated alkaline phosphatase (ALP) levels (31.818 µ/L) in fish fed 90 g/kg CPSP reflect enhanced bone metabolism and physiological activity, consistent with findings by Hoseinifar et al. (2011) in juvenile beluga (Huso huso). Increased albumin (ALB) and globulin (GLO) levels in fish from higher CPSP inclusion highlight improved protein metabolism and enhanced fish immune response, as these proteins are vital for nutrient transport, liver protein synthesis and immunity Kumar et al. (2011), Abd El-Naby et al. (2024) Eissa et al. (2024). Higher serum cholesterol and glucose levels in fish fed elevated CPSP levels indicate enhanced energy reserves and metabolic activity, likely due to bioactive compounds in CPSP. Similar results were reported in *O. mossambicus* fed higher CPSP doses of about 60g/kg (Omeje, 2016). Increased total protein levels (6.805 g/dL at 90 g CPSP/kg feed) further demonstrate enhanced protein synthesis and utilisation, supporting growth and health. These findings reveal that CPSP has potential to improve liver function, metabolic activity, and immune response in hybrid red tilapia, making it a valuable dietary supplement for enhancing fish health and productivity in aquaculture systems.

The intestinal histomorphometry analysis revealed significant structural changes in response to varying dosage levels of *Carica papaya* seed powder (CPSP) supplementation in the fish det. Fish fed a higher CPSP dosage level (90

g/kg feed) exhibited longer intestinal villi, indicating enhanced nutrient absorption capacity within the intestine of the fish Longer villi increase the surface area for nutrient uptake and hence better assimilation. This finding is supported by studies linking dietary bioactive compounds in *Carica papaya* leaves to improved intestinal morphology in tilapia (Somdare et al., 2023). Conversely, villus width decreased significantly in fish from higher CPSP dosage levels. This is an indication of villus elongation process, which further optimise nutrient transport efficiency within the fish gut. Crypt length was significantly greater in fish from higher CPSP treatment group compared to control, suggesting enhanced cell regeneration and food turnover, a critical factor for maintaining intestinal integrity and function not only in fish but also in other animals. This aligns with findings by Heluy et al. (2020) who reported similar effects in Nile tilapia fed plant-based oregano essential oil as immunostimulants and growth promoter. Additionally, goblet cell numbers increased significantly in fish from higher CPSP inclusion levels, an indication of improved mucus production and gut barrier function, which are essential for pathogen defence and nutrient absorption within the intestine of fish as was witnessed in hybrid red tilapia fed Daphnia as dietary supplementation (Abo-Taleb et al., 2024). Microscopic analysis revealed thicker mucosa and submucosa layers in fish from higher CPSP treatment groups, further supporting enhanced gut health and structural integrity of the fish intestine. These changes were more pronounced in fish from higher CPSP dosage levels (60 g/kg and 90 g/kg feed), likely due to bioactive compounds in CPSP, such as flavonoids and alkaloids, which promote intestinal health and mucin secretion function (Radwan et al., 2023).

Generally, based on this study for sustainable aquaculture purposes, CPSP supplementation at higher doses have been observed to improve intestinal histomorphometry, enhancing nutrient absorption, gut integrity, and fish immunity and immunostimulant in hybrid red tilapia, as evidenced by increased villus length, crypt depth, and goblet cell numbers.

## CONCLUSIONS

This study demonstrates that *Carica papaya* seed powder (CPSP) is an effective plant-based aromatase inhibitor for hybrid red tilapia (*Oreochromis spp*.) masculinisation agent. CPSP significantly influences growth, hormonal profiles, feed utilisation, and physiological fish health. Higher CPSP inclusion (90 g/kg feed) achieved 96.7% masculinisation while significantly reducing oestradiol hormone levels and elevating 11-ketotestosterone hormone, which is a main androgen in fish. This, as a result, supported more male differentiation, hence increasing the male percentage. However, the expected 100% all-male sex reversal was not achieved due to the fact that the treatment started late due to the fact that fry were to undergo conditioning before treatment began. Growth performance improved during treatment and recovery phases, with 60 g/kg CPSP optimising specific growth rate (SGR) and protein efficiency ratio (PER). However, the feed conversion ratio (FCR) worsened at 90 g/kg during the treatment phase but later improved significantly during the recovery phase. Serum liver enzyme biochemical analyses revealed enhanced fish health markers, including increased haemoglobin, haematocrit, albumin, and globulin, and improved liver serum enzyme activity (AST, ALT), indicating better fish body metabolic and immune function. The gut histomorphological structural improvements, such as longer intestinal villi and deeper crypts, confirmed enhanced nutrient absorption by the fish, hence improved food assimilation in the fish body.

This study had the following limitations that should be addressed in future research. First, the potential hepatotoxicity of *Carica papaya* seed powder (CPSP) was not evaluated as it was beyond the scope of this study. This raises concerns about the long-term effects of CPSP on fish liver health, necessitating further investigation into safe dosage thresholds and mechanisms of toxicity. Similarly, the

study focused exclusively on hybrid red tilapia, limiting the generalisability of findings to other species, such as cyprinids, which may exhibit different responses to CPSP supplementation. Furthermore, the economic feasibility of large-scale CPSP production and its incorporation into commercial fish feeds was not assessed. This is critical for practical application in aquaculture, as cost-effectiveness and scalability are essential for widespread adoption. The expected 100% sex reversal were not obtained since the experiment started late and some of the fry might have started to sexually differentiate. Addressing these limitations in future studies will enhance the understanding and applicability of CPSP as a sustainable alternative in aquaculture.

## RECOMMENDATION

For sustainable aquaculture practices, a *Carica papaya* seed powder (CPSP) dosage of 60 g/kg feed is recommended based on the findings of this study. This level effectively optimises masculinisation, growth performance, feed efficiency, and overall fish health. CPSP serves as a natural, plant-based alternative to synthetic steroids like methyltestosterone, offering a safer and more environmentally friendly option for monosex fish seed production. Future research should focus on developing scalable methods to harness locally available *Carica papaya* seeds, particularly in tropical regions where *Carica papaya* plants are abundant. This includes optimising processing techniques to commercialise CPSP as a feed additive, immunostimulant, and sex-reversal agent for aquaculture purposes. By integrating CPSP into aquaculture systems, the industry can reduce reliance on synthetic hormones and therapeutic medications in aquaculture and hence promote sustainability and enhance the economic viability of fish farming.

## AUTHORS' CONTRIBUTIONS

**FJS, ARNH, and MLJ**: Conceptualisation, data collection, methodology, data curation, writing—original draft, and final manuscript preparation.
**HM**: Research supervision, formal data validation, final editing, review, and English editing/proofreading.
**PM**: Reviewing, proofreading, final editing, and English copy editing.

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## CONFLICT OF INTEREST

All authors collectively and unanimously declare that they have no competing conflict of interests in this work and that no funding was obtained to fund this research work.

## ETHICAL APPROVAL STATEMENT

The study protocol for handling fish was ethically approved by the Institutional Animal Ethics Committee, Department of Biotechnology, Cochin University of Science and Technology, Kerala, India (Ethical Approval Number:

**363/GO/Re/S/01/CCSEA/90).** The research adhered to the *Animal Research: Reporting of In Vivo Experiments* (ARRIVE) guidelines.

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