REPUBLIC OF TÜRKİYE
ERCIYES UNIVERSITY
GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES
DEPARTMENT OF AGRICULTURAL SCIENCES AND TECHNOLOGIES

EFFECTS OF ADDING AZOLLA AND BLACK SOLDIER FLY LARVAE TO COMMON CARP FEEDS ON GROWTH PERFORMANCE, MEAT QUALITY, AND BLOOD COMPONENTS

Prepared By
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Supervisor
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Ph.D. Thesis

February 2026
KAYSERİ

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COMPLIANCE WITH SCIENTIFIC ETHICS

I hereby declare that all the information presented in this study has been obtained in accordance with academic and ethical principles. I also affirm that, as these principles require, all materials and results that do not originally belong to this study have been fully and accurately cited and referenced.

Yazen Wisam HASAN

Signature

"Effects of adding Azolla and black soldier fly larvae to common carp feeds on growth performance, meat quality, and blood components" titled Ph.D. Thesis has been prepared in accordance with the Erciyes University Graduate School Thesis Proposal and Thesis Writing Guidelines.

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Yazen Wisam HASAN

Erciyes University, Graduate School of Natural and Applied Sciences
PhD Thesis, February 2026
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ABSTRACT

Two experiments evaluated the effects of replacing soybean meal (SBM) with Azolla pinnata (AP) and black soldier fly larvae (BSFL) on common carp performance, flesh quality, behaviors, blood components, fatty and amino acid content, and histology. For 60 days, 192 common carp (*Cyprinus carpio*) weighing 37 to 39 grams were randomly placed in 24 laboratory tanks with oxygen and temperature control, 8 fish per tank. The fish were fed morning and evening after one week of acclimatization. Experiment-I had three groups: 1: Control diet (C, soybean diet, no Azolla), 2: 6% Azolla replaced with SBM, and 3: 12%. The Experiment-II groups were: 1: Control diet (C, soybean diet, BSFL addition), 2: 25% SBM-replaced BSFL, and 3: 50% SBM-replaced BSFL. The diet of fish was 3.5% to 4% of their live weight. No significant changes in final weight, feed intake, weight gain, or feed conversion ratio were observed in fish fed Azolla, although the 6% Azolla group had higher carcass weight and survival rates. The 6% and 12% Azolla groups showed better nutritional content compared to controls, but muscle pH remained unchanged. The 12% Azolla group exhibited increased protein content and higher fatty acid and amino acid levels, while showing some tissue damage in organs during histopathological analysis. In the second experiment, a 25% Black Soldier Fly Larvae (BSFL) diet improved growth metrics and behavior; however, the control group had higher carbohydrate content. Both BSFL groups saw better nutritional values and minimal meat color change. Histological comparisons showed some damage in the 25% BSFL group while the control group remained undamaged. Therefore, Azolla and BSFL can effectively replace soybean meal in fish feed, yet caution is advised regarding substitution levels to avoid physiological issues in fish organs.

Keywords: Fish, common carp, Azolla, black soldier fly larvae, behaviors, performance, growth, histology

SAZAN BALIĞI YEMLERINE AZOLLA VE KARA ASKER SINEĞI LARVASI EKLENMESININ BÜYÜME PERFORMANSI, ET KALITESI VE KAN BILEŞENLERI ÜZERINDEKI ETKILERI

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ÖZET

İki deneyde, soya fasulyesi küspesinin (SBM) Azolla pinnata (AP) ve kara asker sineği larvaları (BSFL) ile değiştirilmesinin, sazan balığının performansı, et kalitesi, davranışları, kan bileşenleri, yağ ve amino asit içeriği ve histolojisi üzerindeki etkileri değerlendirildi. 60 gün boyunca, 37 ila 39 gram ağırlığındaki 192 sazan balığı (Cyprinus carpio), oksijen ve sıcaklık kontrolü bulunan 24 laboratuvar tankına rastgele yerleştirildi, tank başına 8 balık. Balıklar, bir haftalık adaptasyondan sonra sabah ve akşam yemlendi. Deney-I'de üç grup vardı: 1: Kontrol diyeti (C, soya fasulyesi diyeti, Azolla yok), 2: %6 Azolla'nın SBM ile değiştirilmesi ve 3: %12. Deney-II grupları ise şunlardı: 1: Kontrol diyeti (C, soya fasulyesi diyeti, BSFL ilavesi), 2: %25 SBM yerine BSFL ve 3: %50 SBM yerine BSFL. Balıkların diyeti, canlı ağırlıklarının %3,5 ila %4'ü oranındaydı. Azolla ile beslenen balıklarda son ağırlık, yem alımı, ağırlık artışı veya yem dönüşüm oranında önemli bir değişiklik gözlenmedi, ancak %6 Azolla grubunda karkas ağırlığı ve hayatta kalma oranları daha yüksekti. %6 ve %12 Azolla grupları kontrollere göre daha iyi besin içeriği gösterdi, ancak kas pH'sı değişmeden kaldı. %12 Azolla grubu, artan protein içeriği ve daha yüksek yağ asidi ve amino asit seviyeleri sergilerken, histopatolojik analizde organlarda bazı doku hasarları gösterdi. İkinci deneyde, %25 Kara Asker Sinek Larvası (BSFL) diyeti büyüme ölçütlerini ve davranışı iyileştirdi; ancak kontrol grubunda daha yüksek karbonhidrat içeriği vardı. Her iki BSFL grubu da daha iyi besin değerleri ve minimal et rengi değişikliği gösterdi. Histolojik karşılaştırmalar, %25 BSFL grubunda bazı hasarlar gösterirken, kontrol grubu hasarsız kaldı. Bu nedenle, Azolla ve BSFL, balık yeminde soya fasulyesi ununun yerine kullanılabilir, ancak balık organlarında fizyolojik sorunları önlemek için ikame miktarlarının dikkatlice değerlendirilmesi gerekir.

Anahtar Kelimeler: Balık, sazan, Azolla, kara asker sineği larvaları, davranışları, performansı, büyümesi, histolojisi

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INTRODUCTION

Fish farming and its production projects are among the most important industrial sectors worldwide, contributing significantly to maintaining food security (Verdegem et al., 2023). Fish provide a high percentage of high-quality proteins, healthy fats, and many minerals and vitamins essential for human health (De Boer et al., 2020; S. P. Mishra, 2020). In recent years, there has been a significant increase in demand for food, particularly protein. The fisheries sector has become one of the most important and rapidly developing sectors, responding to the growing need for sustainable production and a balance between demand and environmental resources (Rosamond Naylor et al., 2000). The aquatic industry is of great importance due to its numerous species and its great potential to provide nutritious and balanced food, as well as to create job opportunities and boost incomes in both rural and urban areas (Teh and Sumaila, 2013). Through its contribution to aquatic ecosystems and the sustainable management of natural resources, the fisheries industry not only plays an economic role but also helps maintain environmental balance (Gómez and Maynou, 2021;Kotb et al., 2025). It contributes significantly to the ecological functions of aquatic environments, influencing food web dynamics through its diverse feeding behaviors (Malinowski et al., 2023; Rosle et al., 2024). Among the fish species, the common carp is a cornerstone of freshwater fish production (Rahman, 2015) due to its relatively larger body weight compared to other species, and it can reach high weights of up to 10 kilograms, making it a commercially valuable fish in local and international markets (Koehn et al., 2000). Carp fish has a diverse diet that includes both plant and animal food sources, which enables the integration of alternative food sources into its feeding programs and contributes to the development of more sustainable and efficient agricultural systems (Henriksson et al., 2021).

Azolla is a water fern that is high in protein and can fix nitrogen. This makes it a good choice for fish feed as a dietary supplement (Yohana et al., 2023). In a study involving

pangasius fish, the incorporation of cultured Azolla into aquaculture water at a rate of 10 g per kg of feed led to enhanced weight gain, specific growth rate (SGR), protein utilization efficiency, and feed conversion ratio (FCR) relative to conventional feed, without negatively impacting survival rate or body composition. This indicates its potential as a high-value partial substitute for fishmeal protein in standard aquaculture fish feeds without detriment to growth or overall health (Rahmah et al., 2022). Another study on *Labeo catla* fish found that adding up to 30% Azolla pinnata meal to their food made them grow faster, gain weight and length, and convert feed more efficiently than the control group. This shows that Azolla can boost production and be a good and long-lasting source of protein for aquaculture feed (S. Kumar et al., 2025).

The European Union has established regulations regarding the use of insects as a reliable protein source in animal feed. (Conway and Jaiswal, 2024). European Regulation 2017/893, which went into effect in 2017, lifted the ban on using processed animal protein (PAP) from insects in aquaculture feed (Commission, 2017). This meant that aquaculture feeds could use PAP from species like the larvae of the black soldier fly (*Hermetia illucens*), the yellow mealworm (*Tenebrio molitor*), and others, as long as certain hygiene and production standards were met (Commission, 2021). Studies have shown that adding black soldier fly (*Hermetia illucens*) larval meal to gilthead seabream (mussels) feeds can partially replace fishmeal without changing the fish flesh's chemical and sensory properties. If the protein ratios and nutrition are carefully considered, insect protein can be added to fish diet for the best results. (Copelotti et al., 2025). The research and the standards it looked at are important because they aim to find ways to make animal feeds that are more environmentally friendly and less reliant on traditional marine protein sources that are expensive and harmful to the environment. At the same time, they want to make sure that other sources, like Azolla and insects, help with healthy growth, good feed conversion, physiological indicators, meat quality, and the health of the food chain (Serra et al., 2024).

In this thesis study investigated that the effects of replacing soybean meal (SBM) with *Azolla pintane* (AP) and black soldier fly larvae (BSFL) on common carp performance, flesh quality, behavior, blood components, fatty and amino acid content, gene expression, and histology.

CHAPTER 1

LITERATURE REVIEW

1.1. Problem Status

Feed is one of the most influential factors in aquaculture efficiency, and the use of traditional feed formulations based on well-known raw materials such as fishmeal and soybeans, along with some grain crops, has been established (Serra et al., 2024). While this type of feed has helped support fish growth for extended periods, relying on it is no longer as convenient as it once was. In recent years, the rising costs of these ingredients have become increasingly apparent, especially given the limited fish stocks used in fishmeal production and the growing demand for soybeans in sectors outside of aquaculture (Oliva-Teles et al., 2015). Furthermore, the environmental aspects associated with these materials are receiving increasing attention, both in terms of pressure on natural fisheries and the environmental changes resulting from agricultural expansion (Recepoğlu et al., 2025). The concerns extend beyond economic and environmental factors. The use of these ingredients places the aquaculture sector in direct competition with human food needs, raising questions about the long-term sustainability of this production method (F. Bohnes et al., 2021). This has prompted researchers to reconsider the feed ingredients used and to search for less reliant options on traditional resources (Vatsanidou et al., 2025). In this context, various alternative feed sources, including specific microorganisms and byproducts from agriculture and food processing, have been suggested (Areti et al., 2024). Several studies have shown that these alternatives can be used as suitable fish feed when managed properly, which reduces the need for imported or limited resources (Macusi et al., 2023). Moreover, using these alternatives improves resource efficiency and reduces waste, while also allowing for local feed production. Therefore, alternative feeds can be seen as a practical way to support the growth of

aquaculture in a more economically and environmentally sustainable way (Fantatto et al., 2024).

1.2. Purpose of the Study

This investigation aims to identify more sustainable, economically feasible, and environmentally sound feed alternatives for aquaculture, while also evaluating their performance relative to existing practices. To mitigate dependence on conventional feed components such as fishmeal and soybean-derived products, which are both costly and ecologically detrimental, the research explores alternative protein and energy sources. These include insects, algae, agricultural by-products, and proteins derived from microbial sources. The central objective of this study is to determine a more environmentally responsible and economically advantageous feeding strategy through a comparative analysis of these diverse options. Improved fish health and growth, along with reduced feed costs and enhanced global food security, would be the result. This approach aims to balance economic progress with responsible management of natural resources, ultimately fostering the long-term development of the aquaculture industry.

1.3. Importance of Study

This study is of great importance because it addresses one of the most significant challenges facing the aquaculture sector: the high and unsustainable costs of current feed. Feed costs represent the largest portion of production expenses in this sector, and the continued reliance on ingredients such as fishmeal and soybean derivatives places an increasing economic burden on producers, in addition to creating environmental challenges related to the depletion of marine resources and excessive agricultural expansion (Naylor et al., 2021). Additionally, relying on conventional feed sources poses significant environmental concerns, including excessive consumption of marine resources and the ecological impacts of large-scale farming methods (Tuomisto et al., 2017). Accordingly, this research highlights the importance of investigating alternative feed resources that offer practical solutions by integrating economic feasibility with environmental responsibility. Protein sources originating from insects, algae, agricultural by-products, and microbial biomass represent viable alternatives (Jafarzadeh et al., 2024; Kaur et al., 2025; S. Wu et al., 2025). The implementation and integration of these feed alternatives hold the potential to diminish production expenses, improve growth

performance and fish health, and foster circular economy principles through waste valorization and pollution mitigation (Siankwilimba et al., 2025). Moreover, the utilization of locally sourced feed resources can decrease reliance on imported ingredients and bolster food security (Getahun et al., 2025). Consequently, the significance of this research transcends mere technical feed enhancement, encompassing environmental protection, the economic stability of fish farms, and the enduring sustainability of the aquaculture sector. This research endeavors to support current requirements while ensuring the future of aquaculture by advocating for a balanced approach that harmonizes economic advancement with the conservation of natural resources.

1.4. Azolla Fern (*Azolla pinnata*)

Azolla *(Azolla pinnata)* , a member of the *Azollaceae* family, is a floating aquatic plant (Qiu and Yu, 2003). Azolla has unique characteristics that make it a valuable subject for ecological, agricultural, and scientific studies of the natural world (Sebastian et al., 2021). Because it grows quickly and can cover large areas of water, Azolla has many important uses in agriculture and environmental management (Chanapanchai et al., 2025). Azolla is typically found in stagnant or slow-moving freshwater environments, such as ponds and lakes, and is known for its impressive ability to adapt to different environmental conditions (Yuan et al., 2025). Azolla, a type of aquatic fern, includes several species, with Azolla pinnata and Azolla filiculoides being the most common. Although these species have different ecological and physical characteristics, they all share a remarkable ability to grow quickly, which is due to their ability to thrive in water (Nitta et al., 2022). Another species, *Azolla caroliniana*, is used specifically in agriculture and environmental purification (Youquan Yang et al., 2025). A defining feature of Azolla is its symbiosis with the cyanobacteria, specifically Anabaena azollae, residing within its root system. These bacteria are capable of atmospheric nitrogen fixation, a process wherein they transform atmospheric nitrogen into forms accessible to the plant for its development (Timofeeva et al., 2023). Consequently, this characteristic allows Azolla to flourish in environments deficient in nitrogen, as it depends on these bacteria to satisfy its nutritional requirements. In accordance with this symbiotic relationship, Azolla plays a role in enhancing soil fertility and facilitating the proliferation of other plant species (da Silva et al., 2022). Azolla is widespread in tropical and temperate environments around the world, including areas such as India, Southeast Asia, Australia, and Africa, and may extend to

some temperate regions of Europe (Yang et al., 2025). Azolla's native environment requires water rich in nutrients and warm temperatures for optimal growth, which is why it thrives in tropical and subtropical areas (Hagag et al., 2025). From an agricultural standpoint, Azolla is a significant plant, often used as green manure, particularly in rice farming. Azolla provides a natural source of nitrogen, which helps improve soil fertility (Das and Kim, 2025). Additionally, Azolla is utilized to ameliorate soil quality in agricultural areas where nutrient levels are deficient. For agriculturalists, Azolla represents a beneficial component of sustainable agricultural practices, owing to its capacity to enhance fertility in a natural and ecologically sound manner (Adnyana et al., 2025). Azolla proves useful in water purification, particularly when it comes to removing chemical contaminants such as lead and zinc (Gamal et al., 2026). Azolla's inherent ability to absorb these pollutants makes it a crucial tool for purifying tainted water. Several environmental projects utilize this property to enhance water quality in polluted ecosystems. Conversely, notwithstanding its numerous advantages, Azolla is regarded as an invasive species within certain ecological contexts. If not carefully managed, Azolla can completely cover the water's surface, blocking sunlight from reaching other aquatic life and reducing oxygen levels, which can harm aquatic ecosystems (Nikkhah et al., 2024). Additionally, if Azolla grows too much, it can reduce the variety of plants and animals in water because it covers the surface, making it hard for other plants to grow and for aquatic creatures to find food (Chandrababu and Parvathy, 2022). Azolla has also been used in some research projects to address environmental pollution due to its ability to absorb toxins and heavy metals. It also plays an important role in maintaining sustainable agricultural environments by improving soil fertility in natural and environmentally friendly ways (Kollah et al., 2016; Kour et al., 2024).



Figure 1.1. Shows Azolla Fern (Azolla pinnata).

1.4.1. Azolla in Animal and Fish Nutrition

Azolla fern has unique properties that make it of great importance in many environmental and agricultural applications, including animal nutrition (Chanapanchai et al., 2025). Azolla has a high ability to grow rapidly, making it an ideal choice for agricultural and aquatic animal husbandry. Azolla is a rich source of proteins and nutrients that support the growth and overall health of animals, especially poultry, cattle, other ruminants, and fish (Agarwal et al., 2024). Azolla's ability to flourish quickly positions it as a strong contender for use in agriculture and aquaculture. This fern is a rich source of proteins and other essential nutrients, benefiting the health and development of animals, especially poultry, cattle, and fish (Youquan Yang et al., 2025). Boasting a protein content that varies from 25% to 35% of its dry weight, Azolla is an excellent protein source for animal feed (Hagag et al., 2025). Beyond protein, Azolla is rich in essential amino acids, vitamins (notably B and C), and minerals like iron and calcium (M. Kumar et al., 2018). Furthermore, Azolla is rich in dietary fiber, which aids digestion and supports the digestive systems of animals (Yuan et al., 2025). Azolla is a healthy option, given its low fat content, making it useful in agriculture (Chanapanchai et al., 2025). Azolla's high nutritional value makes it a popular choice in poultry feed. Adding fresh or dried Azolla to feed formulations helps poultry grow and increases egg production (Danayit, 2024). Research conducted in Ethiopia demonstrated that the inclusion of Azolla in poultry feed correlated with increased egg production and enhanced growth rates in poultry (Yimam, 2023). Furthermore, Azolla is regarded as a viable substitute for conventional protein

sources, such as soybean, thereby mitigating agricultural expenses and fostering sustainability (Hagag et al., 2025). Azolla protein-rich composition and essential nutrients support growth and general health. Beyond that, Azolla's sustainable qualities present a financially sound choice for farmers looking to improve feed quality while keeping costs down (Normuhammedova and Rajamurodov, 2025). In a study of El Naggar and Elmesiry, (2022), it was found that including Azolla in cattle feed improves feeding efficiency, average daily weight gain, and milk production by 15-20%, which increases milk protein levels and overall animal weight. Therefore, Azolla can help boost milk production and promote overall growth. Furthermore, Azolla can reduce feed costs, as it is less expensive than other alternatives, such as soybeans. Besides its use in poultry and livestock feed, Azolla is becoming more common in fish nutrition, especially in sustainable aquaculture (Acharya et al., 2023). Azolla contains proteins and amino acids that support healthy fish growth, including essential amino acids that improve fish health (Y. K. Abbas and Mohanned, 2023; Gadzama and Mutimaamba, 2025). A study showed a significant improvement in growth rates and weight gain for Nile tilapia fed fresh Azolla (Azolla pinnata) as a supplementary feed, compared to the control group that received only commercial feed. Feed utilization efficiency also improved (M. Refaey et al., 2023). A study by Kumar et al. (2025) examined the impact of adding Azolla powder to the diets of *Labeo catla* fish on their growth, health, and feeding efficiency. The results indicated that adding 30% Azolla meal to the diet can greatly improve these fish's growth, health, and feeding efficiency, making it a cost-effective and long-lasting alternative to traditional aquaculture feeds. From an ecological perspective, Azolla presents a sustainable option, potentially reducing reliance on expensive industrial feeds and conventional agricultural inputs such as soybeans (Rashad, 2021; Youquan Yang et al., 2025). Economically, Azolla presents a cost-effective solution for animal nutrition. Its capacity to thrive in aquatic environments allows farmers to utilize it as a substitute for conventional feeds that depend on imported agricultural inputs (Paryanto et al., 2023). This efficient application of Azolla can contribute to reduced feed expenses and foster economic sustainability for agricultural producers. Notwithstanding its advantages in animal nutrition, Azolla necessitates meticulous management to mitigate potential adverse consequences (Chekol et al., 2024). Excessive ingestion of Azolla can precipitate digestive issues in animals, a consequence of ammonia buildup within the feed (Yohana et al., 2023). The provision of an appropriate Azolla quantity in animal feed is essential;

both excessive and insufficient amounts can prove harmful (Normuhammedova and Rajamurodov, 2025). Azolla presents considerable advantages as an aquatic plant, demonstrating its utility in animal nutrition across various species, including poultry, cattle, and fish. However, integrating Azolla into animal diets requires careful management to fully realize its nutritional value without compromising animal health (Md Nasir et al., 2022).

1.4.2. Active Compounds in Azolla

Azolla is a significant protein source, with bioavailable proteins constituting 25–35% of its dried biomass. These proteins are rich in essential amino acids, including lysine, methionine, and phenylalanine, all of which are crucial for animal growth (Boukrouh, 2025). Azolla contains linoleic acid (omega-6) and alpha-linolenic acid (omega-3), two essential fatty acids that support neurological and cardiovascular well-being (S Said et al., 2023). Azolla is also a source of essential vitamins. It boasts vitamin C, an antioxidant known to bolster the immune system, alongside B vitamins like B_1, B_2, and B_{12}, all of which are crucial for the nervous and digestive systems to operate correctly (Al-Shwilly, 2022). Furthermore, Azolla is rich in vitamin A, a nutrient essential for healthy eyesight and normal growth (Agarwal et al., 2024). Minerally, Azolla is packed with important minerals, including calcium, iron, potassium, and magnesium. These elements play a key role in strengthening bones and teeth, regulating bodily fluids, and promoting healthy blood (Nayel et al., 2024). Consequently, these minerals are indispensable for the health of animals that ingest Azolla. Azolla is distinguished by its phenolic compounds, particularly flavonoids and tannins; these compounds exhibit antioxidant properties, thus aiding in the safeguarding of cells from damage caused by free radicals (Elrasoul et al., 2020). Furthermore, these phenolic compounds contribute to the reduction of inflammation and the combatting of bacterial infections, rendering Azolla advantageous for the well-being of aquatic animals (Tran et al., 2020). Azolla contains additional components, including carotenoids such as beta-carotene, which is converted to vitamin A in the body and thus helps strengthen the immune system (Yuan et al., 2025). Azolla is a nutrient-dense natural source, distinguished by its essential enzymes, unlike other plant-based meals. Azolla has the digestive enzymes present, including amylase, lipase, and protease, facilitate the breakdown of organic materials in the animal's intestines and enhance nutritional absorption (Korsa et al., 2024; M. Refaey et al., 2023). Azolla's

compounds play a crucial role in absorbing heavy metals and toxins such as lead, cadmium, and mercury from water, contributing to the mitigation of water pollution and the preservation of aquatic ecosystems (Peyda et al., 2023). Additionally, initiatives focused on the restoration of contaminated land and industrial areas use Azolla to address environmental pollution (Zeid et al., 2024). The symbiotic relationship between the Azolla plant and cyanobacteria, specifically Anabaena azollae, exemplifies a successful natural partnership (Nawaz et al., 2024). These bacteria facilitate the uptake of atmospheric nitrogen, transforming it into an organic form accessible to the plant, thereby allowing Azolla to flourish in nitrogen-poor conditions. Consequently, this process eliminates the need for costly chemical fertilizers while simultaneously enhancing soil fertility in a natural and sustainable manner (Timofeeva et al., 2023). The importance of this interaction resides in its establishment of a balanced ecosystem predicated on mutual advantage. Also, this mutually beneficial relationship positions Azolla as an advantageous species for organic farming practices and the restoration of compromised agricultural terrains (Herath et al., 2023; Korsa et al., 2024). Moreover, the bioactive constituents present in Azolla augment their capacity to assimilate atmospheric nitrogen, thereby mitigating the requirement for chemical inputs, which diminishes pollution stemming from synthetic fertilizers and safeguards both water and soil integrity (Youquan Yang et al., 2025). Consequently, Azolla incorporation into agricultural systems can facilitate environmental sustainability and bolster initiatives aimed at promoting environmentally conscious agricultural practices (S. Das and Kim, 2025).

1.4.3. Applications of Azolla And its Effects on Fish and other Animals

Based on previous studies indicating that Azolla can be a sustainable alternative to traditional feed due to its content of proteins, essential amino acids, vitamins, and minerals that help animals grow and maintain their health, several studies on fish and other animals were reviewed to demonstrate all the characteristics of the Azolla plant. In a study conducted by Toutou et al. (2025) in Egypt examining the impact of substituting a portion of animal protein with Azolla meal in the diet of grey mullet (*Liza ramada*),the results indicated that the incorporation of 30% Azolla enhanced growth rates and strengthened the fish's immunological response. Moreover, the study revealed a notable improvement in liver health and an increase in the activity of antioxidant enzymes. The study also observed a reduction in mortality rates, demonstrating Azolla's capacity to

improve disease resistance in fish without adversely affecting water quality or the safety of the aquatic environment (Yohana et al., 2023). The effects of substituting fishmeal with Azolla in the diets of red tilapia (Oreochromis sp.) across a range of salinity levels. The outcomes revealed that the application of Azolla at a concentration of 20–40% improved feed conversion efficiency and accelerated growth rates, while also contributing to improved water quality and a decrease in ammonia concentration (Sallam et al., 2024). In a study conducted by Abu-Zahra et al. (2025) sought to assess the efficacy of Azolla in reducing the deleterious effects of the herbicide pendimethalin in Nile tilapia (*Oreochromis niloticus*). The research results indicated that incorporating 30% Azolla into the diet contributed to mitigating oxidative stress and diminishing tissue injury in the liver and gills resulting from herbicide exposure. Azolla additionally enhanced the immune response and re-established equilibrium in essential blood parameters (Mishra et al., 2016). The study advised the inclusion of Azolla as a feed additive due to its protective properties against toxic agents in aquatic environments. In a study by Magouz et al. (2020) present investigation centered on the application of Azolla as a dietary supplement for Nile tilapia *(Oreochromis niloticus).* The findings indicated that fish receiving fresh Azolla demonstrated enhanced digestive enzyme activity (amylase, lipase) and improved nutrient absorption efficiency, attributable to modifications in intestinal morphology. Furthermore, growth rates were elevated, and meat quality was enhanced, characterized by reduced fat content and increased protein levels; thus, Azolla presents an advantageous option for small-scale aquaculture operations seeking cost-effective, nutritionally beneficial alternatives (M. Refaey et al., 2023). Additional study sought to assess the impact of *Azolla caroliniana* on the growth and immune function of Nile tilapia. In a study conducted by Toutou et al. (2025) that dietary incorporation of Azolla resulted in a substantial enhancement in growth rates and ultimate weight, alongside a greater than 60% elevation in both cutaneous and serological immunity against bacterial pathogens. Furthermore, Azolla contributed to improved disease resistance and a reduction in mortality rates within intensive aquaculture settings (Lumsangkul et al., 2022). In a study looked at Nile tilapia to compare how Azolla (Azolla pinnata) and the green alga *Nannochloropsis oculata* affected their growth and immune responses. When Azolla was added to the feed at a 25% concentration, the fish showed increased total weight and daily growth rates. At the same time, their immune systems improved, and they became more resistant to bacterial infections. Furthermore, researchers noted improved water quality

within the ponds, which suggests Azolla's contribution to ecological balance (Mansour et al., 2020). In a study conducted by Korsa et al. (2024) mentioned Azolla production and animal usage in detail. It found that the plant contains 25–30% crude protein, minerals, and important amino acids, including lysine and methionine. In sustainable farming systems and resource-limited settings, adding 10–20% Azolla to cow and poultry diets boosts output and immunological performance. Azolla also absorbs nitrogen and phosphorus, reducing agricultural pollution (Kour et al., 2024). A study conducted examined Azolla as a cow and sheep feed. Azolla high content of vital amino acids, vitamins, and minerals boosts digestibility and animal weight gain Chekol et al. (2024). In a study conducted by Roy et al. (2018) found that feeding dairy cows up to 20% Azolla increased milk output and quality due to its carotenoid and chlorophyll content, which boosts milk nutrition, recommended adjusting the amount to avoid feeding moisture at high inclusion rates. Hagag et al. (2025) investigated Azolla as a sustainable poultry and small ruminant feed protein. Broiler chicks and dairy goats fed 10–20% dry Azolla powder was tested. Compared to the control group, chickens gained 12% and feed conversion efficiency improved, while goats produced 9% more milk. Azolla's antioxidants lowered cholesterol and improved meat quality. Reducing animal protein imports using Azolla is cost-effective and environmentally friendly. Ogbon et al. (2025) investigated how adding Azolla powder to rabbit diets affected growth. Rabbits receiving diets containing 20% Azolla exhibited the highest daily growth rates, improved feed conversion ratio (FCR), and no adverse effect on feed intake. Azolla enhances biological efficiency and reduces dietary costs by promoting digestive health and enzyme activity. Azolla improved the number of eggs laid each day, strengthened the eggshells, and increased the levels of carotenoids and chlorophyll in the eggs, making the yolks more colorful and nutritious (Mahamuda and Islam, 2021). While Azolla offers numerous advantages, its over-application can elicit adverse consequences for fish and other fauna. Within aquaculture settings, Azolla's rapid proliferation has the potential to entirely obscure the water's surface, thereby impeding the penetration of sunlight to other aquatic life forms (Devi et al., 2024). Consequently, Azolla can precipitate a decline in dissolved oxygen levels, which in turn can detrimentally affect the well-being of fish and other aquatic organisms. Therefore, the growth of Azolla requires careful watching in fish farming areas to ensure the water environment stays healthy for fish growth (da Silva et al., 2022). In poultry and livestock, consuming large amounts of Azolla can cause

digestive problems. This is mainly due to its high fiber content. In some cases, eating too much Azolla can lead to gastrointestinal issues, such as poisoning or intestinal disorders (Al-Shwilly, 2022; Yeshe and Gourkhede, 2022).

1.5. The Black Soldier Fly *(Hermetia Illucens)*

Hermetia illucens is a *Stratiomyidae* fly. A unique-looking aquatic insect, it adapts well to different situations. With its lustrous black color, this insect stands out from others (Ewusie et al., 2019). The families *Stratiomyidae* and *Hermetiinae* include black soldier flies and other helpful flies (Cichlidae et al., 2022). Black soldier flies are considered beneficial insects in many environmental and agricultural fields, especially in processing organic waste and converting it into usable products such as animal protein and organic feed (Siddiqui et al., 2022). Black soldier fly is a medium-sized fly, typically measuring between 1.2 and 2 cm in length. It is characterized by its black body and transparent wings, which allow it to fly long distances. The eyes of the black soldier fly are proportionally large, aiding in environmental navigation. Female insects are usually larger than males. They lay their eggs in places with a lot of decaying organic matter, like waste and compost. After that, the larvae hatch and start eating the decaying organic material, which helps to reduce the amount of waste (Amrul et al., 2022; Boafo et al., 2022; M. Cai et al., 2022).This particular species belongs to the order Diptera and has garnered considerable interest due to its capacity to process organic waste, converting it into valuable materials for both animal husbandry and industrial applications (Müller et al., 2017). Black soldier fly larvae thrive in warm, humid environments, particularly where decaying organic materials like food waste and agricultural byproducts are readily available (Bruno et al., 2025). These flies are present in various environments such as landfills, poultry farms, and agricultural areas. This diversity of environments plays an important role in breaking down organic waste and converting it into useful resources (Ogello et al., 2025). A key advantage of black soldier flies is their ability to quickly and effectively process organic waste. The larvae ingest substantial amounts of organic matter, subsequently converting it into nutrient-rich components, including proteins and fats, thereby establishing themselves as a suitable source of animal feed (Ding et al., 2023). Furthermore, the larvae possess utility in the creation of organic fertilizers and soil amendments (Lopes et al., 2022). Consequently, larvae are viewed as a sustainable alternative, potentially substituting conventional feed sources like soybeans and corn,

thereby mitigating both environmental and economic burdens (Heuel et al., 2021). Black soldier flies inhabit diverse global locales, favoring environments rich in decomposing organic material and characterized (Salam et al., 2022). One of the most important features of the black soldier fly in many countries is its role in reducing and utilizing organic waste, thus promoting environmental sustainability. In recent years, there have been projects to breed this insect in many commercial farms specializing in waste treatment and conversion into organic feed (Tariq et al., 2025).



Figure 1.2. Shows the black soldier fly from larval to adult stage.

1.5.1. Black Soldier Fly Larvae in Animal and Fish Nutrition

Black soldier flies (*Hermetia illucens*) are a fly species that is becoming more popular in animal and aquaculture feed, mainly because they have high nutritional value and can turn organic waste into protein- and fat-rich nutrients (Y.-S. Wang and Shelomi, 2017). The larvae of these flies are a beneficial feed option because they are rich in proteins, essential amino acids, vitamins, and minerals, which help improve animal health and increase productivity. This alternative feed is more environmentally friendly and cost-effective (Dîrvariu et al., 2025). In study of Praseatsook et al. (2025) it was show that the proteins in these larvae can be broken down into short peptides, which have both antioxidant and anticancer benefits these peptides reduce the presence of free radicals, protecting cells from oxidative damage, suggesting their potential use in therapies. Black soldier fly larvae are known to produce phenolic compounds when they ingest agricultural waste containing these compounds (Camperio et al., 2025). These compounds contribute to the larvae's enhanced resistance to oxidative stress while also imparting anti-

inflammatory and antibacterial characteristics (Rodríguez-González et al., 2025). Furthermore, black soldier fly larvae have been found to contain flavonoids, sterols, and tocopherols, which can substantially augment natural immunity and promote the health of animals that consume them (Muslykhah et al., 2024). The enzymes present in black soldier fly larvae are among the most important active components, capable of breaking down complex organic materials like cellulose, chitin, and lignin (Xiang et al., 2023). Müller et al. (2017) found that enzymes such as cellulase, lipase, and protease help the larvae break down food waste, resulting in nutrient-rich biomass, this ability makes black soldier fly larvae a useful tool for managing organic waste and converting it into valuable products like animal feed or fertilizer (Tariq et al., 2025). In addition, black soldier fly larvae are rich in important fatty acids, including omega-3 and omega-6 (Mohamad Zulkifli et al., 2022). The lipids found within black soldier fly larvae represent a significant reservoir of fatty acids, with lauric acid accounting for approximately 40–50% of the total fatty acid composition, this acid has antibacterial, antiviral, and antifungal properties, which is why larval extracts are used to improve the health of the intestines in both fish and poultry (Lee et al., 2025). Black soldier fly larvae are equipped with antimicrobial peptides (AMPs), such as defensin and cecropin, which are naturally occurring compounds that specifically target bacterial cell walls, consequently hindering their replication (Nakagawa et al., 2023). In a study conducted Lu et al. (2022), it was shown that these substances enhance the quality of the microbial environment during the waste conversion process and contribute to the reduction of pathogenic bacteria. *Hermetia illucens*, or black soldier fly larvae, exhibit a wide range of bioactive compounds and minerals, which renders them highly promising in the fields of agriculture, nutrition, and waste management (Macwan et al., 2023). Contemporary studies indicate that the specific organic substrate for their cultivation significantly influences the mineral and micronutrient composition of these larvae; it becomes easier to precisely manipulate their nutritional characteristics. (Tambeayuk et al., 2024). Black soldier fly larvae are abundant in calcium, phosphorus, magnesium, iron, zinc, copper, and manganese, thereby providing a comprehensive source of essential minerals suitable for animal feed applications (Shumo et al., 2019). Furthermore, Mohamad Zulkifli et al. (2022) reported that the mineral composition of the larvae is contingent upon the employed heating and drying techniques, with desiccated larvae displaying a greater mineral concentration compared to their fresh equivalents. As Chia et al. (2020) referred in study demonstrated,

larvae raised on used barley and corn residues exhibit differences in mineral accumulation, particularly calcium, zinc, and manganese. At the same time, depending on the feed, larvae can accumulate some heavy elements such as cadmium and lead when fed on contaminated organic waste (Addeo et al., 2024).

1.5.2. Active Compounds in Black Soldier Fly Larvae

Recent scientific inquiry has increasingly focused on the potential of black soldier fly larvae (*Hermetia illucens*) as a sustainable protein source for animal and aquaculture feed, owing to their considerable nutritional benefits and capacity to transform organic waste into protein- and energy-dense substances. Research findings suggest that the proteins derived from black soldier fly larvae are abundant in bioactive peptides, which exhibit antioxidant, antimicrobial, and anti-inflammatory characteristics (Camperio et al., 2025). Furthermore, black soldier fly larvae are distinguished by their elevated animal protein content, typically constituting 40% to 45% of their dry weight. These larvae provide a complete set of essential amino acids, including lysine, methionine, phenylalanine, and threonine. These amino acids are crucial for building muscle and tissue, which in turn improves animal productivity (Jorge Iñaki et al., 2022). Recent studies of their proteins have found over 70 active peptides and 50 bioactive proteins in these larvae. This includes defensive peptides like defensins and cecropins, these peptides are very effective against various bacteria (Tarahi et al., 2025). Black soldier fly larvae are high in fat (25–35% of dry weight), characterized by a high concentration of medium-chain fatty acids (MCFAs), particularly lauric acid, which constitutes approximately 40% of the total fat. Lauric acid possesses potent antibacterial, antiviral, and antifungal properties and is currently used as a dietary supplement and ingredient in animal feed to improve gut health and immunity (Estrada et al., 2024). Recent research has shown that modifying the larvae's diet by adding fish byproducts or vegetable oils improves their fatty acid profile, thus increasing their nutritional value as a natural alternative to fish oil in fish feed (Sudha et al., 2022). Oleic acid, linoleic acid, and palmitic acid, among other lipids, are also present, and these have been associated with benefits such as improved cardiovascular health, decreased inflammation, and better nutrient absorption in animals (Almeida et al., 2022). Furthermore, phenolic compounds and flavonoids are important constituents of these caterpillars, particularly when they are fed plant residues abundant in polyphenols. These caterpillars absorb and accumulate these compounds, thereby becoming a significant

source of natural antioxidants, including quercetin, catechin, and gallic acid. These compounds are known to enhance antioxidant resistance, safeguard cells from damage, and bolster the immune system, which suggests their potential utility in functional foods and pharmaceuticals (Rodríguez-González et al., 2025). The caterpillar's outer shell contains chitin, a biopolymer that is industrially converted into chitosan. Chitosan possesses antimicrobial and antioxidant properties and is used in medicine to accelerate wound healing and in animal feed to improve digestion and intestinal health and reduce pathogens. Chitosan is also a key ingredient in the biofilm and skincare industries due to its moisturizing and tissue-regenerating properties (Alhasyani et al., 2025). Furthermore, larvae contain a range of active vitamins and minerals, such as vitamins B, D, E, and K, and minerals like iron, zinc, magnesium, phosphorus, and calcium. These factors contribute to improved metabolic processes and the activation of enzymes that protect cells and help them resist oxidative stress. Additionally, research shows that adding these substances to animal feed can improve the quality of meat, fish, and poultry (Macwan et al., 2023). These substances are also crucial for improving intestinal immunity, decreasing inflammation, and raising animal productivity in aquaculture, poultry, and livestock projects. Their use also decreases the need for antibiotics and artificial additives, making them a more environmentally friendly and safer choice. Furthermore, the fatty and protein extracts from these larvae are used in cosmetics as natural ingredients with antioxidant, moisturizing, and UV-protective properties (Almeida et al., 2020). From an environmental approach, the rearing of black soldier fly larvae represents a noteworthy instance of circular economy principles in action. These larvae can be cultivated using food scraps and agricultural byproducts, thereby contributing to the transformation of organic waste into valuable food sources (Lopes et al., 2022). Research conducted by Luciana et al. (2019) demonstrated that these larvae possess the capacity to diminish organic waste by up to 70% within a fortnight, while simultaneously producing considerable quantities of animal protein and compost, which in turn contributes to soil fertility enhancement. Furthermore, Lopes et al. (2020) when used fish farm waste as larval food, it enhanced recycling efficiency, thus utilizing waste and the resulting waste is almost non-existent, which promotes environmental sustainability.

1.5.3. Applications of Black Soldier Fly Larvae and their Effects on Fish and other Animals

Regarding fish nutrition, recent scientific literature indicates that black soldier fly larvae *(Hermetia illucens)* represent a significant shift in alternative feeds within aquaculture systems, given their high nutritional value and positive effects on growth, health, and product quality. A study by Devic et al. (2018) on Nile tilapia showed that including black soldier fly larvae powder in diets during the advanced incubation stage did not lead to any decrease in growth rates or feed conversion efficiency, nor did it affect the fish's body composition. This finding indicates that the insect protein is easily digested and works well with the fish's digestive system. It also suggests that chitin might help keep the intestines healthy. Similarly, Zhou et al. (2018), in a study conducted on Jian carp, demonstrated that the gradual replacement of fishmeal with black soldier fly larvae powder led to a significant improvement in final weight and daily growth rate, along with an improved fatty acid composition in muscle tissue, particularly an increase in unsaturated fatty acids, reflecting an improvement in the nutritional value of the fish meat produced. The present findings corroborate Limbu et al. (2022) observations concerning the dietary effects of black soldier fly larvae meal on Nile tilapia fry. Specifically, a marked enhancement in growth and survival rates was documented, alongside improved protein and energy utilization efficiency, and a substantial decrease in feed expenses. Consequently, this supports the economic feasibility of incorporating this protein source within commercial aquaculture practices. In contrast, study by Rawski et al. (2020) investigated the possible replacement of fishmeal and fish oil with whole black soldier fly larvae meal in the diets of Siberian sturgeon. The results showed no changes in the physical properties of the feed or how well the fish accepted it, along with a good energy supply efficiency. This suggests that insect fats could be a useful energy source in fish feed. Furthermore, research conducted by (Fisher et al., 2020) corroborated these results when applied to Atlantic salmon. The research indicated that the inclusion of black soldier fly larvae powder, up to a concentration of 20%, did not negatively impact growth rates or feed consumption; instead, it improved the digestibility of both protein and energy. This observation corroborates the potential of this protein source, even for marine fish species with heightened nutritional requirements. These results align with the conclusions of Oteri et al. (2021), which corroborated that black soldier fly larvae constitute a strategic

approach to fostering environmental sustainability in aquaculture. This is achieved by diminishing dependence on marine resources and augmenting fish production efficiency and meat quality. In the poultry sector, there has been considerable interest from researchers, and studies have indicated that the larvae of the black soldier fly have properties that could be beneficial to poultry (Cattaneo et al., 2025). (de Souza Vilela et al., 2021) demonstrated that incorporating black soldier fly larvae into broiler chicken diets resulted in a significant improvement in live weight and FCR, as well as enhanced immune response and reduced inflammation markers. This is attributed to the larvae's content of medium-chain fatty acids and immune-supporting bioactive compounds. In addition, study by (Attia et al., 2023) showed that partially replacing soybean meal and fishmeal with black soldier fly larvae meal improved the growth of grill chickens and reduced feed costs. This change did not negatively affect the quality of the meat or the characteristics of the carcass. Recent studies on ruminants indicating that black soldier fly larvae are a promising protein source. These larvae can be added to ruminant diets without negatively affecting rumen function or animal health (Su et al., 2025). In study of Astuti and Wiryawan (2022) provided a comprehensive analysis of incorporating black soldier fly larvae into bovine feed, revealing that this protein source, when used judiciously, improved rumen digestibility. This approach contributes to reducing nitrogen loss rates and improving the efficiency of its utilization within the animal's body, which positively impacts the productivity of sheep and goats, in addition to reducing the harmful environmental effects associated with the accumulation and emission of nitrogen waste (Tariq et al., 2025). These findings support the results of Lu et al. (2024), who studied the effect of introducing black soldier fly larvae into goat diets. Their results showed improved rumen protein degradation and increased intestinal digestibility, without any negative effects on rumen microbial activity or the overall health of the animals. This conclusion supports the idea of using this protein source as a partial replacement for soybean meal in modern ruminant diets.

1.6. Fish Production and the Importance of Fish in Animal Products

The escalating population growth has heightened the demand for superior animal protein, resulting in swift qualitative advancements in aquaculture, now recognized as one of the most rapidly expanding sectors in global food production (D’Abramo, 2021). As a result, fish production has gained significant importance in recent years, becoming a key

component of global food security. This growth has helped alleviate pressure on wild fish stocks (Wasik et al., 2025). Fish are valuable in human nutrition for their role in providing essential nutrients that support overall bodily function. Rather than focusing solely on protein, regularly including fish in the diet contributes to improved metabolic efficiency, tissue maintenance, and energy balance (Noreen et al., 2025). Fish contribute to growth, tissue regeneration, and the maintenance of normal physiological processes. Therefore, they are of particular nutritional importance in communities with limited access to diverse protein sources (Ong et al., 2021). This positive effect is attributed to the presence of bioactive compounds in fish, which affect numerous bodily systems. Therefore, the nutritional value of fish extends beyond being a source of protein; it is an essential element that contributes to functional balance and the maintenance of overall health in the long-term (Kundam et al., 2018). Fish is a nutritionally complete food, providing vitamin D and essential minerals such as calcium, phosphorus, and iodine, which are necessary for bone health, boosting immunity, and regulating metabolism (Chungkham and Hei, 2019; Noreen et al., 2025). Fish is distinguished by its balanced range of macro- and micronutrients, which are difficult to replicate in other animal foods, and it represents a healthier and more beneficial option compared to red meat for supporting overall health (Drewnowski et al., 2024). Consequently, fish has been recognized as a vital part of nutritious dietary practices. In addition to its nutritional advantages, fishing and aquaculture substantially enhance economic development by creating job opportunities and promoting income expansion (Sinha and Banerjee, 2025). In underdeveloped nations, where the fish trade is vital for sustaining livelihoods and enhancing national economies, the significance of the global fish market is underscored (Elahi et al., 2024). The incorporation of processing by-products is another significant benefit of fish production. Fish waste can be transformed into fishmeal, fish oil, or organic fertilizers, thereby aiding waste reduction and enhancing resource efficiency (Coppola et al., 2021). Fish production principle is consistent with sustainable production principles and foster more environmentally conscious food systems, these traits enable the production of significant amounts of animal protein while reducing land and water use compared to conventional livestock methods (F. A. Bohnes et al., 2022).

1.7. Fish Production in the Middle East

The fish production sector in the Middle East is considered a developed and vital sector, contributing to food security and meeting demand in both local and international markets. A noticeable trend of increased interest in the fisheries sector has been observed due to rising demand resulting from rapid population growth. However, the limited natural fish resources have also influenced the development of the aquaculture sector. Aquaculture ecosystems across the Middle East exhibit diversity influenced by environmental conditions, such as clay lakes, floating cages, and closed systems. This diversity has allowed aqua culturists to attain elevated output, optimize scarce water resources, and leverage contemporary feeding methods.

1.7.1 Current State of Fish Production in Key Countries

Egypt is a major player in the Middle Eastern fish industry, consistently generating a significant portion of the region's annual catch. Carp and tilapia are the primary species cultivated. The country has seen steady advancements in intensive aquaculture methods, feed quality, and overall production levels. Projections indicate that Egypt's fish production could surpass 2.2 million tons by 2025 (Rossignoli et al., 2023). Saudi Arabia's aquaculture industry has seen impressive expansion, driven by intensive saltwater projects designed to satisfy both local and global markets. In 2023, production surged, climbing 55.56% to exceed 140,000 tons. This growth is anticipated to persist, aligning with the Kingdom's goal of achieving fish self-sufficiency by 2030. This will bolster food security and generate numerous employment prospects within the aquaculture sector and its associated fields (Al-Mahish and Alsafra, 2025). Iraq possesses considerable promise for fish farming, especially in its marshes and rivers, which offer a natural habitat ideal for cultivating carp and tilapia. The country's output was roughly 25,737 tons, underscoring the fisheries sector's significance as a food source and provider of local protein. This sector is important for the economy, supporting local communities and creating jobs in breeding, harvesting, and sales (Harlioglu et al., 2024). The United Arab Emirates, despite its scarcity of freshwater, has pioneered saltwater aquaculture initiatives. These initiatives focus on species such as grouper and shrimp, with the objective of fulfilling domestic demand and reducing reliance on imports. This demonstrates the ability to enhance the production of high-quality fish in a resource-

limited environment (A. Ahmed, 2025). Iran: Iran is a major player in Middle Eastern aquaculture. In 2023, the country produced roughly 1.06 million tons of fish, with plans to ramp up production to 1.5 million tons in the near future. Benefiting from a varied ecosystem, Iran is leveraging both freshwater and saltwater resources. The country is actively improving aquaculture techniques to optimize productivity within its fisheries, and it has established support programs aimed at cultivating disease-resistant fish and enhancing overall output (J. Cai et al., 2024). Turkey's progress during the past two decades has been nothing short of remarkable. Fish output escalated from 61,163 tons in 2002 to over 547,500 tons by 2023, representing an extraordinary rise over 800%. Turkey's strategically significant location has rendered it a crucial contributor to the diversity of fish it produces. The nation is a leading producer of sea bass and shrimp in the Mediterranean, and its adoption of intensive farming methods and sophisticated feeding systems has enhanced fish quality and facilitated sustainable development (Saygı et al., 2025).

Table 1.1. Fish production in some countries over the last three years

Country	2023 (tons)	2024 (tons)	2025 (tons) (Projected)
Egypt	2,100,000	2,150,000	2,200,000
Saudi Arabia	140,000	150,000	160,000
Iraq	25,000	26,000	27,000
United Arab Emirates	2,800	3,000	3,200
Iran	1,100,000	1,150,000	1,200,000
Türkiye	1,007,921	933,194	1,020,000

1.8. Carp fish, their Environment, Nutrition, and Economic and Nutritional Importance

The common carp (*Cyprinus carpio*), a globally prevalent freshwater fish, is of considerable importance in aquaculture at both global and regional levels. This species is classified within the *Cyprinidae* family, which includes a multitude of species that inhabit freshwater environments (Rahman, 2015). The carp, celebrated for its exceptional adaptability, swift growth, and significant nutritional benefits, is native to Central Asia, where it flourished in prominent rivers such as the Volga and Danube. During the Middle

Ages, it was introduced to Europe by humans, and it subsequently became integrated into European culinary practices. Furthermore, it spread to the Middle East, Africa, and America (Shalgimbayeva et al., 2021). Carp's environmental adaptability and straightforward cultivation methods have contributed to its status as a leading species in aquaculture, currently ranking among the top three fish species produced globally. This species demonstrates significant adaptability to a range of environmental settings, thriving in rivers, lakes, ponds, and earthen ponds, with a preference for calm or slow-moving waters (Mohammed et al., 2024). Depending on the climate, carp can be grown in areas with temperatures as high as 35 degrees Celsius, while it prefers a range of 20 to 28 degrees Celsius for growth. Şerban et al. (2024) found that carp can survive in habitats with low dissolved oxygen levels, tolerant to 3 mg/L. Their growth is best at values above 5 mg/L. Soliman et al. (2025) says carp thrive in neutral to slightly alkaline pH 6.5–8.5 environments. The reason they work well in semi-intensive aquaculture systems that combine natural and supplement feeding methods is because they can thrive in waters that are already rich in nutrients from things like phytoplankton and zooplankton (Berzi-Nagy et al., 2021). Carp are omnivorous, which means they eat a wide variety of foods, a feature that helps them get the nutrients they need. In their natural habitats, carp eat decaying aquatic plants, worms, insect larvae, small crustaceans, and algae. However, in modern fish farming, they are often fed artificial diets, mostly made from plant materials (Jalii et al., 2025). Research by Wang et al. (2023) shows that carp need between 28% and 35% crude protein, depending on their growth stage and age. They also need 5% to 8% fat to provide energy and important fatty acids. Carp exhibits a distinctive carbohydrate metabolism; consequently, feed formulations containing up to 40% carbohydrates can reduce the reliance on expensive protein sources (Xiao et al., 2025). Furthermore, carp require a spectrum of essential amino acids, including lysine and methionine, alongside minerals such as calcium and phosphorus, and vitamins B12 and D, all of which are crucial for overall health, immune function, and optimal feed conversion, thereby supporting healthy development (Y. Lu, 2024). Conversely, unlike carnivorous species that primarily depend on fishmeal, carp can attain substantial growth rates through the utilization of cost-effective feeds, a result of their specific dietary needs (Fantatto et al., 2024). Carp plays a significant role in the global fisheries sector, contributing to its economic significance beyond its rapid growth and cost-effectiveness (Hossain et al., 2022). Due to constituting about 20% of aquaculture production, carp are

deemed a crucial component of the global food supply by the Food and Agriculture Organization (FAO) (Boyd et al., 2022). Carp is the most commonly farmed fish in aquaculture, mainly due to its highly desirable in the market and can thrive in many cultivating environments. This is especially true in countries like China, India, and numerous Eastern European nations (Engin and Koşan, 2025). In the Middle East, particularly in Iraq, carp holds a significant status, constituting the largest share of local fish production and being a favored option among consumers (Harlioglu et al., 2024). The elevated demand bolsters the feasibility of carp fish cultivation, particularly considering the increasing costs of red meat and poultry, as carp serves as a cost-effective alternative that delivers high-quality animal protein at a reasonable price (Hua et al., 2019). Carp aquaculture serves as a valuable tool for both rural and urban economic advancement, as it generates opportunities directly and indirectly throughout the entire value chain, from cultivation and breeding to marketing and food processing (Ababouch et al., 2023). Consuming carp offers numerous health advantages, as it is a source of complete The high-quality (Noreen et al., 2025). The high-quality proteins found in carp flesh include all the essential amino acids needed for the development and maintenance of tissues (Ljubojević Pelić et al., 2017; Schaeffer et al., 2012).The unsaturated fatty acids it contains, particularly omega-3, are beneficial for the heart, lungs, and nervous system. They also lower the risk of chronic diseases (Herc et al., 2024). Carp also contains vital minerals such as phosphorus and calcium, essential for bone and dental development, as well as iron, which plays a crucial role in hemoglobin formation and enhances the blood's capacity to transport oxygen (Lall and Kaushik, 2021). It is a vital source of essential vitamins, including B12 and D, that are indispensable for maintaining a healthy nervous system and immune function. Due to these factors, carp is a nutritious choice for individuals of all ages and an effective method for preventing deficiency-related disorders (Islam et al., 2023). Carp holds significance beyond its commercial value and nutritional worth; it also possesses social and cultural ramifications. Masgouf, a traditional Iraqi dish featuring carp, is a symbol of hospitality and cultural heritage (Ameen et al., 2024). The common carp (*Cyprinus carpio*) is a model for successful aquaculture. It combines easy breeding, adaptability to different environments, and the availability of inexpensive feed. It also has high nutritional and health value, along with economic and social importance (Mihály-Karnai et al., 2025). The continuous development of carp aquaculture systems, coupled with the incorporation of sustainable feed alternatives, will enhance food

security, reduce dependence on imports, and promote sustainable economic growth, particularly in Middle Eastern countries.



Figure 1.3. Shows the common carp (*Cyprinus carpio*)

1.9. The Importance of Carp in Scientific Research

Researchers have established that the common carp (*Cyprinus carpio*) serves as an excellent model organism, particularly in studies concerning fish nutrition and the utilization of non-conventional feedstuffs (Nedoluzhko et al., 2020). Due to its unique biological, economic, and ecological attributes, this fish presents significant advantages for scientific inquiry, distinguishing it from other fish species (Coroian et al., 2015). Initially, the carp's omnivorous diet, which encompasses a broad spectrum of both plant and animal matter, provides considerable adaptability in the utilization of alternative feed components. This includes both plant-based sources, such as oilseed meal, grain by-products, and algae, and unconventional animal sources, including insect larvae and worms (Sobczak et al., 2021). Consequently, the carp's inherent nutritional versatility makes it a suitable species for assessing the effectiveness of alternative feeds, as well as for evaluating their acceptance and bio-efficiency in fostering growth (Wong et al., 2025). Carp, moreover, are characterized by swift growth and the ability to attain advantageous FCRs, even when provided with less costly diets (Mohammed et al., 2024). Considering their moderate protein needs, which fall between 28% and 35%, as opposed to the higher protein requirements of carnivorous fish (40% to 50%), carp present an ideal model for exploring methods to reduce reliance on expensive fishmeal and replace it with locally available substitutes (Host et al., 2025). This, in turn, establishes a scientific basis for

creating economical and sustainable feeds, suitable for widespread application in aquaculture. Carp displays significant adaptability to a variety of environmental conditions. They are capable of withstanding a broad spectrum of temperatures and can endure in environments characterized by relatively low levels of dissolved oxygen. This attribute supports research activities in various contexts, thereby eliminating the requirement for highly controlled environments (Şerban et al., 2023). Consequently, researchers can evaluate the impacts of novel feeds in conditions that closely approximate those of actual fish farms, thereby augmenting the realism and practical applicability of the findings (Wang et al., 2025). Furthermore, carp holds considerable economic value, being one of the most widely produced fish globally and accounting for a substantial portion of aquaculture in numerous Middle Eastern nations, including Iraq, Iran, and Turkey (Engin and Koşan, 2025). In this case, the consequences are enormous, since research on alternate carp meals will directly influence the fishing industry, cutting costs and improving sustainability. Therefore, using carp as a model organism is important for academic research and has significant practical and economic value (Mihály-Karnai et al., 2025). Moreover, from a practical perspective, carp are easy to grow, catch, and manage in research ponds. Their suitable size, the ease of breeding them in captivity, and the availability of many young fish allow for long-term studies and a detailed look at their nutritional and physiological responses (Patil et al., 2019). In addition, the existing research provides a strong basis for current studies, making it easier to compare and analyze the results (Muhyaldeen et al., 2019).

1.10. Traditional Feeds, Their Problems and Non-Traditional Feeds

The evolution of the fishing industry has been profoundly affected by the traditional fish feed, which comprises of several essential ingredients. As feed accounts for 60-70% of the overall expenses associated with a fish production operation, these materials have encountered several environmental obstacles (Serra et al., 2024). Fish feed consists of two primary components: animal protein and plant protein. The principal protein sources in fish feed are followed by an assortment of cereals and feed additives (Ebeneezar et al., 2021). The reliance on conventional feed in production projects, while considered a healthy and effective resource, has resulted in several environmental and economic difficulties that jeopardize the fish production business (Turlybek et al., 2025). Fishmeal is considered one of the best types of animal protein used in the manufacture of various

fish feeds because it contains all the essential amino acids that fish need, in addition to being easily digestible (Macusi et al., 2023). There has been a negative impact on natural fisheries in fishmeal production, which has had an economic impact on the price of fishmeal, especially with the increase in aquaculture worldwide (Huntington and Hasan, 2009; Zhang et al., 2019). Reliance on natural fisheries for fishmeal production has serious environmental consequences. In addition to increasing production costs, there has been a decline in natural stocks of marine and freshwater fish, and the depletion of overall stocks in natural fisheries, leading to an imbalance in aquatic ecosystems (Atalah and Sanchez-Jerez, 2020). Soybeans are a critical source of plant-based protein in the fish feed and other animal feed sectors. Production is constrained in several countries, including the United States, Brazil, and Argentina, resulting in elevated costs and heightened demand for the crop (Z. Kari et al., 2023). The detrimental environmental effects of soybean farming include the overconsumption of water, pesticides, and chemical fertilizers, alongside the degradation of forests and natural green areas, rendering it environmentally unsustainable in the long run (Dreoni et al., 2022). Due to the impacts and problems associated with traditional feed production, the focus in recent years has been on developing new feed alternatives that are more environmentally sustainable, more productive, and less costly. Research has focused on finding local, renewable, inexpensive, and environmentally sustainable protein sources that can be used as feed alternatives without threatening human food supplies or causing significant environmental damage (Gil et al., 2024). As a result, efforts have been initiated to formulate diverse feed compositions that provide fish with vital nutrients necessary for growth and production, thereby decreasing dependence on expensive and environmentally unsustainable traditional feed sources (Mahmud et al., 2025). Recently, scientific research and practical implementations have explored the use of unconventional materials in feed production, including insects, algae, agricultural refuse, aquatic vegetation, and other alternative sources (I. Ahmed et al., 2023a; Fawole et al., 2020; Shahin et al., 2023). Alternative feed sources, such as black soldier fly larvae produced from agricultural and organic detritus, have been investigated as replacements for fishmeal and soybean meal. This approach to feed production supports recycling initiatives and environmental sustainability (Wu et al., 2025). Azolla, a type of aquatic plant, offers a beneficial alternative for inclusion in plant-based diets, given its high protein, fiber, and vitamin content. Also, it can function as a substitute for plant-based

protein in fish nutrition, according to (Yohana et al., 2023). These alternative feeds can improve the environment by reducing pressure on fisheries and unregulated and haphazard farming practices. They also contribute to lowering production costs by utilizing available resources (FAO, 2018). Furthermore, these non-traditional feeds or feeding systems contain bioactive compounds that can improve fish health and enhance the flavor of their meat (Tufail et al., 2025). Carp are versatile feeders, and these options offer an excellent opportunity to provide balanced diets that promote carp growth and productivity without relying solely on costly traditional sources (Suprayudi et al., 2022). Using non-traditional feed alternatives can contribute to food security and find a middle ground between productivity, environmental efficiency, and economics by shifting from traditional feed sources to more sustainable alternatives. This helps ensure the long-term sustainability of aquaculture and yields economic benefits (Gamage et al., 2024).

As long as global warming continues and the world's population increases, it is inevitable that increasing food production will become more difficult. Therefore, it is necessary to use feed sources other than existing ones in animal nutrition and to continue searching for new options. Therefore, in this study, the effects of replacing soybean meal with *Azolla pintane* and black soldier fly larvae on common carp performance, carcass yield, meat quality, fatty and amino acid content, attraction to feed, blood components, and organs histology were investigated.

CHAPTER 2

MATERIALS AND METHODS

Two experiments were conducted to examine the effect of replacing soybean meal with different sources, including dried Azolla fern and dried black soldier fly larvae, in different proportions, and comparing them with a control unit that did not include any additives. The experiment was conducted on common carp for 60 days.

2.1. Place and Time to Carry out the Experiment

The experiment was conducted in a specialized fish laboratory consisting of 24 tanks in a closed system. It was equipped with an automatic circulation system, an oxygen system, and a mechanical and biological filtration system. The laboratory experiment took place between October 1, 2024, and December 1, 2024.

2.2. Source of Fish

A total of 250 two-month-old common carp were obtained from a licensed local fish hatchery in Salah al-Din Governorate, Dujail District, at an average weight of 37-38 g per fish. The fish were transported in a large tank truck filled with water and equipped with an oxygen generator. They were then immediately transferred to the experimental unit of the researcher's fish laboratory in the Al-Amiriya region, Baghdad Governorate, Iraq. The fish experimental unit consists of 24 tanks, each with a volume of 100 liters, equipped with two water filtration and cleaning systems, a water-cooling system, a pump, and an oxygenator. The water temperature during the experiment was 25-28 °C.

2.3. Feed Sources and Formulation

The feed materials were obtained from local markets. Azolla fern was obtained from a local farm in Salah al-Din Governorate, Balad District, and black soldier fly larvae were

obtained from a local farm in Mosul Governorate. The other materials were obtained locally except for soybean meal and fish meal, and the additives were imported from international sources, as shown in Table 2.1.

Table 2.1. The feed formulation and the amount of replacement

Ingredients (%)	**Control %**	**Azolla 6%**	**Azolla 12%**	**BSFL 25%**	**BSFL 50%**
Fish meal	24	24	24	24	24
Soybean meal	30	28.2	26.4	22.5	15
Black Soldier Fly Larvae	0.0	0.0	0.0	7.5	15
Azolla	0.0	1.8	3.6	0.0	0.0
Yellow corn	20	20	20	20	20
Wheat	10	10	10	10	10
Barley	6	6	6	6	6
Millet	6.5	6.5	6.5	6.5	6.5
Oil	2.0	2.0	2.0	2.0	2.0
Vitamins and minerals	1.0	1.0	1.0	1.0	1.0
Salt	0.5	0.5	0.5	0.5	0.5
Total	100	100	100	100	100

Table 2.2. The chemical analysis of the feeds

Chemical Analysis (%)	**Control**	**Azolla 6%**	**Azolla 12%**	**BSFL 25%**	**BSFL 50%**
Dry matter%	92.62±0.18	92.11±0.11	92.51±0.13	92.56±0.14	93.81±0.07
Protein%	29.40±0.31	28.08±0.10	27.98±0.30	28.03±0.07	26.75±0.12
Lipid%	4.10±0.18	3.25±0.11	4.20±0.06	6.50±0.19	9.50±0.16
Ash%	9.50±0.24	10.50±0.11	10.50±0.11	9.00±0.29	10.50±0.07
Carbohydrates %	3.11±0.06	2.71±0.15	2.31±0.08	4.70±0.15	3.72±0.08

2.4. Experimental Design

The fish were raised in laboratory tanks in the researcher's lab, which contained 24 tanks. 12 tanks were used for each experiment, and participants were divided into three treatment groups, each containing eight carp, with four replicates. A total of 96 carp were used in each experiment, for a total of 192 fish across both experiments. The fish were weighed and distributed randomly according to their weight. Five types of feed were prepared as shown in Table 2.1: the control group and the Azolla pinnata group, which

consisted of two groups of Azolla 6% and 12% replaced by soybean meal and two groups of black soldier fly larvae 25% and 50% also replaced by soybean meal. Each group had four replicates. The feed was stored in the freezer at (+4°C).

2.5. Administer the Fish in the Laboratory

Upon arrival from the fish farm, 192 fish were immediately placed in the researcher's laboratory. A central cooling system conditioned and dispersed the water among the tanks, maintaining a constant temperature of 26°C in each tank. The water was cycled continuously, twenty-four hours a day. Following the random placement of the fish in the tanks, they were deprived of food for one week to facilitate their acclimatization to the new laboratory setting. An oxygen pump system was employed in each tank at a concentration of 12.2 mg/L per tank. The tanks underwent cleaning daily, while all pumps were serviced weekly. A saline solution was introduced into the primary tank, which disperses the water. Water was delivered to mechanical filters and subsequently returned to the tanks. The daily nutritional requirements of the fish were assessed according to their needs. To determine the daily feed requirement for the fish, use the equation: daily feed required = average weight of fish in each pond * temperature-dependent constant / 100.

2.6. Fish Feeding

During the first week of feeding, the fish were not given any feed additives so that they could become used to the characteristics of the meal. Following that, the fish were given a meal that included Azolla group and a black soldier fly larvae group, as addition to a diet that just served as a control. Feeding was administered at a rate of between 3.5-4% of the live fish body weight (BW). Weekly, fish were weighed, and their live weights were carefully monitored to make adjustments to the amount of feed they consumed, ensuring they grew and developed healthily. Intake of feed was raised in proportion to the weight of the fish. Eight fish were dwelling in each tank. For the purpose of determining the amount of feed that was provided to each tank, a sensitive balance was utilized.

2.7. Determination of Behavioral Responses of Fish

The general behavior of the fish during the experiment, as well as the various activities, responses to food and movement, were monitored. The feeding responses and locomotor behavior of common carp were studied through multiple tests. Behavioral responses before and after feeding, as well as responses to feed and feed palatability were observed by calculating the amount of remaining feed.

2.8. Study Attributes

2.8.1. Determination of Body Weight (BW)

After the initial weighing of the fish at the beginning of the experiment, the process was repeated weekly until the experiment's conclusion. An electronic balance was used to determine the weight of each group and its replicates. To ensure consistency, the fish weight was divided by their total weight, and a weighted average was calculated using the equation below:

Average live fish weight (g) = Total fish weights (g) per replicate / Total number of fish in the replicate.

2.8.2. Determination of Body Weight Gain (BWG)

The progression of weight gain was established using the following equation:

Weight gain [g] = live body weight at the end of the experiment [g] – live body weight at the beginning of the experiment [g]

2.8.3. Determination of Feed Consumption (FC)

The amount of feed consumed per week was calculated at the end of each week, the feed was weighed weekly for the purpose of knowing the feed consumed per week according to the following formula:

Quantity of food consumed during the period = amount of food at the beginning of the week [g] - amount of food remaining at the end of the week [g]

2.8.4. Determination of Feed Conversion Ratio (FCR)

FCR= Amount of feed consumed in a period [g] / The body weight gain in the same period [g]

2.8.5. Determination of Length and Width of Fish

The length and width of the fish were measured at the beginning and end of the experiment to observe changes in length and width between groups. A tape measure was used for calculations. The length of the fish was measured from the beginning of the mouth to the back fin, and the width was measured from the dorsal fin to the abdominal area.

2.9. Determination of Carcass Traits

At the conclusion of the experiment, after 60 days, 8 fish were randomly selected from each group, weighed separately using an electronic scale, and then dissected. The carcasses were weighed, cleaned, and the internal viscera were extracted.

2.9.1. Determination of Hot and Cold Carcass Yield

The carcass was weighed after cleaning and removing the viscera [hot weight] and then placed in the refrigerator for 24 hours, and then weighed [cold weight]. Hot and cold carcass ratios were calculated as follows: carcass weight/body weight ×100.

2.9.2. Determination of pH of meat

The pH of the carcass was assessed using distilled water, measured in the lateral muscles of the fish's body. A lateral incision was executed in the lateral musculature from the cephalic region to the caudal end.

2.9.3. Determination of Carcass Color

The meat color was assessed in fresh cross-sections of the fish at the time of slaughter and quantified for three regions: the breast, belly, and tail, using a chromameter (CR-400, Minolta Co., Ltd., Japan) with a 50 mm color measuring area in accordance with the CIE L*a*b* color scale. L* denotes luminosity, with a scale from 0 for absolute black to 100 for pristine white. a* and b* are color coordinates (+A* = red, -A* = green, +B* = yellow,

-B* = blue). The instrument was calibrated using Minolta calibration meters (L* = 93.8, a* = 0.3136, b* = 3192).

2.9.4. Determination of Cooking Loss (%)

Samples were equilibrated at varying weights after being systematically cut and placed in sealed nylon bags. The samples was then placed in a water bath at 80 °C for one hour, and the resultant cooking loss was quantified using the equation provided by (Honikel, 1998).

Cooking loss = weight after cooking/weight before cooking * 100

Percentage of cooking loss% = 100 - loss of cooking.

2.9.5. Determination of Thawing Loss (%)

Samples of fish meat were measured at different weights and frozen for 24 hours, and then quoted for the measuring (Honikel, 1998).

Thawing loses = weight after freezing / weight before freezing * 100

Melting loss ratio% = 100-melting loss.

2.10. Determination of Chemical Composition

The meats chemical composition (dry matter, crude protein, fat and ash, %) were determined according to process of AOAC (1990).

2.10.1. Determination of Dry Matter of Meat (%)

Fish meat samples, approximately 5 grams each, were weighed and placed in aluminum pots, with the weight of the pots included. These pots, containing the fish meat samples, were then placed in a laboratory oven (UFP 500 Universal Laboratory Drying Oven, Memmert GmbH/Germany) for 72 hours at a temperature of 70˚C. After the drying process, the weight of the meat was quantified post-drying in accordance with the following equation:

The weight of the dried meat is the total weight of the meat and cup minus the weight of the cup.

Weight of meat after drying = total weight of meat and cup - cup weight

After obtaining the weight of dry meat, the dry matter ratio was measured according to the following equation:

Dry matter ratio% = weight of meat after drying/weight of meat before drying * 100



Figure 2.1. Memmert UFP 500 Universal Laboratory Drying

2.10.2. Determination of Humidity of Meat (%)

Humidity was estimated utilizing the following equation

Humidity ratio% = 100 - Dry matter ratio

2.10.3. Determination of Crude Protein of Meat (%)

The Kjeldahl method was used to estimate the protein content in the samples, based on the method mentioned by (van Dijk, D 2000) and others, by taking a known weight of the sample, approximately 5 g, and placing it in a beaker. 25 ml of concentrated sulfuric acid was added to the sample, and an appropriate amount of a mixture of potassium sulfate and copper sulfate was added. The digestion process was carried out by heating the contents. After the digestion was completed, the mixture was converted into a clear, pale blue liquid. The liquid was transferred quantitatively to the distillation flask of the

Kjeldahl apparatus, which contains a concentrated solution (40%) of sodium hydroxide and is connected to a condensing distillation flask ending in a test tube immersed in a receiving flask containing a known volume of boric acid (20%), with drops of methyl red indicator and bromocresol blue dye added to it. Then the estimation flask is heated until the collected distilled liquid in the flask reaches approximately 25 ml, then it is filtered. The liquid is collected with hydrochloric acid (0.1) and a buffer solution (blank) is prepared from the above chemicals except for the sample. The protein percentage is calculated according to the following equation:

Protein % = Volume of HCl consumed x Molarity x 0.014 x 6.25 / Sample weight x 100

2.10.4. Determination of Crude Ash of Meat (%)

Following the drying and grinding of the samples as previously described, the weights of the samples (0.450-1000 mg) were recorded and placed in small ceramic vessels of varying weights. The weights of these vessels were then measured before being placed in the incinerator to determine ash content at a temperature of 550 degrees Celsius for 5 hours. The ceramic vessels were removed and allowed to cool before being placed in glassware designed to eliminate moisture from the environment. The glassware consists of airtight containers, after which the jars were weighed, and the ash content was calculated using the following formula:

Crude Ash% = ash weight / sample weight before burning * 100



Figure 2.2. Oven used for crude ash

2.10.5. Determination of Crude Fat of Meat (%)

The SER 148 Series Solvent Extractors were used to extract fat from meat by preparing samples weighing 3-3.5 g, which were placed in cotton cups, coated with cotton, and affixed to the device. A 50 ml petroleum ether solution was then introduced into the glass flasks of the apparatus. Following a duration of 90 minutes, the jugs were divided and weighed to ascertain the fat percentage.



Figure 2.3. Solvent extractors for crude fat

2.10.6. Determination of Carbohydrate Ratio of Meat (%)

Carbohydrates were extracted from meat by measuring muscle glycogen. A small amount of a sample of fresh meat was minced and treated with a hot potassium hydroxide solution to extract the glycogen, then precipitated using ethanol, collected by centrifugation and re-dissolved in water. Its content was then quantified either by the enzymatic method after conversion to glucose or by the iodine conjugation method, according to the standard methods used in the analysis of glycogen in meat (Dalrymple and Hamm, 1973; Hefferan and Goodnight, 1969)

2.11. Blood Parameter Analysis

2.11.1. Determination of Blood Film Analysis

One sample from each replicate (4 samples) was selected for treatment. Blood was drawn from the caudal vein of the fish, located at the tail fin, using a 5 cm3 plastic medical syringe. 2 ml of fish blood was collected and placed in a 5 ml tube containing Ethylene Diamine Tetraacetic Acid (EDTA) and transported directly to the laboratory. Whole blood was used for blood cell characterization tests, including hematocrit, hemoglobin, red blood cells, white blood cells, lymphocytes, monocytes, neutrophils, eosinophils, and basophils.

2.11.2. Determination of Biochemical Blood Parameters

One sample from each replicate (4 samples) was selected for treatment. 2 ml of fish blood was collected from the caudal vein, located behind the anal fin, using a 5 cm3 plastic medical syringe and placed in a barrier-free tube. Coagulation gel tube (EDTA) was used. Serum was obtained after centrifugation at 4000 rpm for 3 minutes. The serum was placed in sterile tubes for biochemical analysis. Tests were performed using a German-made Reflotron Plus device. Serum analyses included glucose, cholesterol, triglyceride, total protein, albumin, globulin, high-density lipoprotein (HDL), very-low-density lipoprotein (VLDL), low-density lipoprotein (LDL), urea, and creatinine.

2.11.3. Determination of Liver Enzymes

The levels of ALP, AST, and ALT were quantified using various kits produced by the German company Human. The measurements were conducted according to the instructions provided with the kits, and the sample was analyzed using a spectrophotometer at a designated wavelength based on the test type.

2.12. Determination of Fatty Acid Analysis of Meat

1- Fat Extraction from the Sample: Fats were estimated according to the AOAC 1995 method using a Soxholet fat extraction device.

2- Fat Esterification: The sample was prepared according to the AOAC 1995 method, which relies on fat esterification by reacting it with methanolic potassium hydroxide, which was prepared by dissolving 11.2 g of potassium hydroxide in 100 ml of methanol. 1 g of fat was then taken, and 8 ml of methanolic potassium hydroxide and 5 ml of hexane were added. The sample was shaken quickly for 30 seconds, then allowed to separate into two layers. The upper layer (the hexane layer), which contains the esterified fat, was taken and injected into the device.

3- Chromatographic analysis of the sample: Fatty acid compounds were analyzed using a gas chromatography device (GC-2010) Shimadzu model, made in Japan, where a flame ionization detector (FID) was used and a capillary separation column type (SE-30) with lengths of (30m *0.25 mm) was used according to the following conditions:

Table 2.3. Indicates the conditions for sample chromatographic analysis.

Item	Temperature
Injection zone temperature	280 C
Detector temperature	310 C
Separation column temperature	120 – 290 (10 C /min)
Gas flow rate	100 Kpa

2.13. Determination of Amino Acid Analysis of Meat

1- Extraction Process: Amino acids were extracted according to the method presented by Dahl-Lassen et al. (2018). A 3-gram sample was weighed and placed in a 25-ml volumetric flask. 25 ml of 1M hydrochloric acid was added at 55°C for 3 hours. The

sample was then dried using a rotary evaporator and 5 ml of sodium citrate, pH 2.2, was added. The sample was filtered using a 0.45um plastic filter and placed in the apparatus for injection.

2- Derivatization process: 1 ml of the extracted sample was taken, and 200 microliters of Ortho phthalein aldehyde (OPA) (5%) was added to it. The sample was shaken for 2 minutes, after which 100 microliters of the final mixture were taken and injected into the Amino Acid Analyzer.

The analysis was conducted in the laboratories of the Scientific Research Authority / Environment and Water Research Center using an amino acid analyzer (made in Korea). The method provided by the scientist (Scriver CR, 2001) where the carrier phase was used consisting of (methanol: acetonitrile: 5% formic acid) in proportions (20: 60: 20) at a flow rate of (1 ml/minute), a separation column (C18 – NH2 (250 mm * 4.6 mm) was used to separate the amino acids, while a fluorescence detector was used to detect the amino acids at wavelengths (Ex = 445 nm, Em = 465 nm). The (clarity 2015) program was used to analyze the amino acids.

2.14. Determination of Histopathological Analysis of Organs

12 samples were prepared for histological analysis in accordance with Munro (1971). Samples from the experimental groups were euthanized at the conclusion of the experiment on day 61. Two samples were collected for each treatment and immersed in a 10% formalin solution until histological slices were prepared. The steps included the following:

1. After slaughtering the 2 fish for each group, the liver, pancreas, kidneys, and musculature were excised and immersed in a 10% formalin fixation solution.

2. Washing: After a 48-hour fixation, the samples were washed with distilled water for thirty minutes.

3. Drying: The samples were then subjected to increasing concentrations of ethyl alcohol, first with 50-70%, progressing to 85-96%, and concluding with 100% ethyl alcohol. The drying process took 5 hours.

4. Purification: All samples were treated with a dilution solution (xylene) to remove the alcohol.

5. Infiltration: To make sure the paraffin wax penetrated the gaps between the tissue cells, the samples were heated to 60°C in an oven.

6. Embedding: To embed samples, molten wax was poured into a metal mold that held the sample in the direction it needed to be cut. After cooling, the mold was placed in the fridge at 4°C until the next stage.

7. Sectioning: A microtome was used. The sample molds were attached to the mold holder and aligned with a sharp knife. These 5-micrometer slices were cut sequentially. Spraying the slices into a 40°C German bath ensured consistent sectioning. Glass slides containing Sayer's solution were then used to secure the parts. After drying at normal temperature, they were stained.

8. Staining: The tissue slices were stained using hematoxylin and eosin staining. For this purpose, a xylol solution is used to remove the wax from the glass slides that contain the tissue sections. The next step is to wash with plain water for two minutes at each of the following decreasing ethyl alcohol concentrations: absolute ethyl alcohol 100, 90, 80, and 70. The sections are then stained with hematoxylin for 3 minutes, rinsed with plain water for 5 minutes, stained with eosin for 30 seconds, and then washed with distilled water. A sequence of increasing ethyl alcohol concentrations is administered, beginning with 70, 80, 96, and 100, followed by xylene for two intervals of three minutes each. A drop of D.P.X is applied to the tissue sections, which are then covered with a cover glass to inhibit desiccation.

9. Examination and Imaging: The slides were scrutinized by a brightfield compound microscope (Laxco™ LMC-3000 Series); pictures were captured by a digital microscope camera (CMOS type, C-mount), model MU1003, AmScope, USA, compatible with the Laxco™ LMC-3000 Series Brightfield Compound Microscope; and they were analyzed.

2.15. Statistical analyses

All statistical were conducted in SPSS v22. Data were checked for normality (Shapiro–Wilk) and variance homogeneity (Levene’s test). One-way ANOVA tests was used to test treatment effects, and Tukey’s post hoc test identified pairwise differences at $p<0.05$.

CHAPTER 3

RESULTS

3.1. Body Weight, Weight Gain, Feed Intake, and Feed Conversion Ratio of fish

Table 3.1. shows the effect of replacing soybean meal with two levels of Azolla (6% and 12%) body weight (g), feed intake (g), BWG (g), and FCR. The results indicated that there were no significant differences between the experimental groups. The 6% Azolla group outperformed the other experimental groups in final weight measurement, with the weights of the fish in the group containing Azolla in the diet being numerically better. Regarding body weight gain, no significant differences were recorded between the experimental groups. There were no significant differences in feed intake (g), and 12% Azolla group had the numerically lowest feed consumption. There were no significant differences in FCR between the groups (P>0.05).

Table 3.1. Effect of Azolla levels on body weight, weight gain, feed intake, feed conversion ratio (FCR) of fish

	Control	**Azolla 6%**	**Azolla 12%**	**Total**	**P**
BW-initial, g	294.50±4.77	308.25±2.84	298.00±4.26	300.25±2.75	0.093
BW-last, g	740.25±21.91	769.00±13.42	737.50±27.50	748.92±12.13	0.547
BWG, g	445.75±19.18	460.75±13.68	439.50±23.97	448.67±10.48	0.736
Feed intake, g	787.46±2.61	783.43±3.73	763.44±10.75	778.11±4.73	0.069
FCR, g feed/g BWG	1.78±0.07	1.70±0.04	1.75±0.08	1.74±0.04	0.759

BW-initial: body weight initial, BW-last: body weight last, BWG: body weight gain, FCR: Feed conversion ratio, g feed/g, P: probability, *, $^{a, b}$: the significance of the means has different letters in the same row were significant P<0.05.

Table 3.2. shows the effect of replacing soybean meal with two levels of black soldier fly larvae, 25% and 50%, on body weight (g), feed intake (g), BWG (g), and FCR. Significant differences were found between the experimental groups, with the BSFL 25% group

(P<0.05) having the highest final weight, followed by the BSFL 50% group, and the control group having the lowest. Significant differences were also observed in body weight gain, BSFL 25% group's BWG was higher than the BSFL 50% and control groups, and BSFL 50% groups BWG was higher than the control group (P<0.01). There were also statistically significant differences in feed intake, with the BSFL 50% group' feed intake was higher than BSFL 25% and control groups, and control group' feed intake was lower than others (P<0.01). As for the FCR, there were statistically significant differences (P<0.05), with the BSFL 25% group leading the way, followed by the BSFL 50% group, and the control group having the lowest among the experimental groups.

Table 3.2. Effect of Black soldier fly larvae on body weight, weight gain, feed intake, and feed conversion ratio (FCR) of fish

Item	**Control**	**BSFL 25%**	**BSFL 50%**	**Total**	**P**
BW-initial, g	292.75±5.98	290.00±2.97	298.50±7.50	293.75±3.21	0.59
BW-last, g	735.25±19.36^{c}	837.50±19.84^{a}	782.50±7.77^{b}	785.08±15.30	0.006**
BWG, g	442.50±15.69^{c}	547.50±19.24^{a}	484.00±9.44^{b}	491.33±15.28	0.003*
Feed intake, g	782.33±2.81^{c}	802.25±1.93^{b}	815.25±3.35^{a}	801.65±3.70	0.00**
FCR	1.75±0.07^{c}	1.47±0.05^{a}	1.69±0.04^{b}	1.65±0.05	0.005*

BW-initial: body weight initial, BW-last: body weight last, BWG: body weight gain, FCR: Feed conversion ratio, g feed/g, P: probability BSFL: Black soldier fly larvae, *, ^{a,b}: the significance of the means has different letters in the same row were significant P<0.05.

3.2. Behavioral Responses of Fish

The fish behaviors are determined in Table 3.3, which shows the effect of Azolla 6% and 12%, respectively, on the behavior of different fish. Fish activity was monitored daily during the experiment. There were notable differences and variations in activity observed before feeding, where the highest activity was in the group fed on Azolla, at 12%, followed by 6% Azolla, and then the control group. There were significant differences in daily activity levels between groups. The Azolla 12% group showed better movement and activity, with some fish searching for food. The activity levels of the 6% group were similar to the first group, where the fish consumed the food quickly and calmed down. In contrast, the control group maintained average activity levels before and after feeding. There were no significant differences in external responses before feeding, although there were responses to external interactions between groups, with the Azolla 6% group being

more responsive to external stimuli. There were no significant differences in feeding attraction. There were significant differences in response to feeding in the Azolla 6% group, with a high response compared to the other groups, as daily attraction to feeding effects varied between groups. The Azolla 6% group showed the highest level of interest in feeding. The Azolla 12% group responded slightly less to the first group's desire to feed, while the control group was lower than both groups. The Azolla 6% group showed the best results, achieving a survival rate of 100% by the end of the experiment. The Azolla 12% group had a slightly lower survival rate, at 96%, with one death recorded. The control group had the lowest survival rate, at 93%, with two deaths recorded.

Table 3.3. Effect of Azolla levels on fish feeding behavioral responses

Behaviors	**Control**	**Azolla 6%**	**Azolla 12%**	**Total**	**P**
Activity before feeding %	71.96±1.65^{c}	78.54±0.80^{b}	80.61±1.24 ^{a}	77.04±1.30	0.003**
Activity after feeding %	58.38±2.35	62.35±0.88	64.00±1.83	61.58±1.18	0.129
Interestingly before feeding %	90.44±0.40	91.62±0.52	91.25±0.30	91.10±0.26	0.178
Attraction to feeding %	72.42±1.16	75.94±0.87	75.84±1.29	74.73±0.76	0.089
Respond to the provided feed %	87.91±0.79^{c}	91.77±0.65^{a}	89.84±0.44^{b}	89.84±0.58	0.007**
Survival rate%	93.75±3.61	100	96.88±3.13	96.88±1.63	0.323

P: probability, *, $^{a, b}$: the significance of the means has different letters in the same row were significant P<0.05.

Fish behavior was monitored in Table 3.4, which indicates the effect of Black soldier fly larvae 25% and 50%, respectively, on the behavior of different fish. Fish activity was monitored daily during the experiment. There were significant differences (P<0.05) in activity before feeding, with the highest activity being in the BSFL 25% and BSFL 50% groups, followed by the control group, respectively. No significant differences were recorded in activity after feeding between the experimental groups. Significant differences (P<0.05) were observed in interest before feeding, with BSFL 25% being the most responsive, followed by the BSFL 50% group, and finally the control group. A highly significant effect was recorded in attraction to feeding (P<0.05), with fish groups fed a diet containing black soldier fly larvae performing better than the control group, especially the BSFL 25% group. Significant differences (P<0.05) were observed in response to feed provided for most groups fed a diet containing black soldier fly larvae. This indicates high palatability of the experimental groups BSFL 25%, followed by BSFL 50%, and finally the control group. The BSFL 25% and BSFL 50% groups showed the

best results, achieving a survival rate of 100% at the end of the experiment. The control group had the lowest survival rate of 93%, with two deaths recorded.

Table 3.4. Effect of Black soldier fly larvae levels in feed on fish feeding behavioral responses

	Control	**BSFL 25%**	**BSFL 50%**	**Total**	**P**
Activity before feeding %	69.71±1.10	81.62±1.53[a]	81.25±0.87[b]	77.53±1.78	0.003**
Activity after feeding%	59.63±4.00	62.87±0.72	61.30±0.23	61.27±1.29	0.638
Interesting before feeding%	88.44±0.59	92.45±0.26[a]	91.46±0.36[b]	90.78±0.56	0.002**
Attraction to feeding%	71.67±2.13	82.45±0.84[a]	82.19±0.25[b]	78.77±1.67	0.00**
Respond to the provided feed %	85.41±1.62	93.65±0.89[a]	91.98±0.30[b]	90.35±1.21	0.001**
Survival rate%	92.00±4.83	100	100	97.33±1.85	0.18

BSFL: Black soldier fly larvae, P: probability, *, [a, b]: the significance of the means has different letters in the same row were significant P<0.05.

3.3. Length and Width of Fish

Fish length and width measurements were taken at the beginning and end of the experiment using a tape measure. Table 3.5 shows the effect of replacing the soybean meal with Azolla levels on body length and width. No significant changes were recorded between length and width at the end of the experiment, but slightly higher length and width were observed compared to the control group.

Table 3.5. Effect of Azolla levels on the length and width of fish

Item	**Control**	**Azolla 6%**	**Azolla 12%**	**Total**	**P**
Length-initial, cm	14.36±0.19	14.74±0.15	14.70±0.18	14.60±0.10	0.27
Width-initial, cm	3.90±0.05	4.04±0.05	4.04±0.06	3.99±0.03	0.11
Length-last, cm	20.86±0.29	21.40±0.25	21.79±0.45	21.35±0.20	0.18
Width-last, cm	7.34±0.07	7.48±0.07	7.68±0.14	7.50±0.06	0.07

P: probability, *, a, b: the significance of the means has different letters in the same row were significant P<0.05.

Table 3.6 shows the effect of replacing the soybean meal with black soldier fly larvae levels. Significant differences were found between the experimental groups (P<0.05) when measuring fish length and width in the experimental groups whose diets contained

black soldier fly larvae. The best results were achieved by the 25% BSFL group, followed by the 50% BSFL group, and lastly, the control group.

Table 3.6. Effect of black soldier fly larvae levels on the body length and width of fish

Item	**Control**	**BSFL 25%**	**BSFL 50%**	**Total**	**P**
Length-initial, cm	14.24±0.25	14.58±0.16	14.55±0.23	14.45±0.12	0.483
Width-initial, cm	3.96±0.09	3.98±0.05	4.00±0.07	3.98±0.04	0.931
Length-last, cm	20.74±0.31^{c}	22.85±0.47^{a}	22.10±0.60^{b}	21.90±0.32	0.016*
Width-last, cm	7.21±0.13^{c}	7.95±0.17^{a}	7.71±0.15^{b}	7.63±0.11	0.008**

BSFL: Black soldier fly larvae, P: probability, *, a, b: the significance of the means has different letters in the same row were significant P<0.05.

3.4. Carcass Traits of Fish

Table 3.7 shows the effect of Azolla levels on *Common carp* carcass characteristics. Significant differences were found between individual fish weights, with the 6% Azolla group having the highest carcass weight among the individual experimental groups. As for hot carcass weight, there were no significant differences between the experimental groups, as it was observed that the fish groups fed Azolla were better than their counterparts in the control group. As for cold carcass weight, no statistically significant differences were recorded between the experimental groups, as the percentage of water or fluid loss was close between the experimental groups.

Table 3.7. Effect of Azolla levels to the common carp fish diet on fish carcass traits

Carcass traits	**Control**	**Azolla 6%**	**Azolla 12%**	**Total**	**P**
Slaughter weight, g	109.03±0.35^{c}	113.50±0.61^{a}	110.42±1.00^{b}	110.98±0.55	0.001**
Hot carcass, g	52.17±0.31	53.11±0.67	53.21±0.72	52.83±0.34	0.412
Cold carcass, g	49.85±0.29	50.47±0.68	50.73±0.74	50.35±0.34	0.576
Hot carcass yield, %	47.85±0.27	46.78±0.40	48.19±0.61	47.61±0.28	0.089
Cold carcass yield, %	45.72±0.26	44.46±0.43	45.96±0.62	45.38±0.29	0.068
Loss of carcass ratio, %	2.13±0.09	2.32±0.05	2.24±0.07	2.23±0.04	0.186

P: probability, *, $^{a, b}$: the significance of the means has different letters in the same row were significant P<0.05.

Table 3.8 shows the effect of different levels of black soldier fly larvae on common carp carcass characteristics. No significant differences were recorded between the experimental groups in hot carcass and cold carcass weight, but the groups with black soldier fly larvae diets experienced less cooling loss.

Table 3.8. Effect of Black soldier fly larvae levels to the common carp fish diet on fish carcass traits

Carcass traits	Control	BSFL 25%	BSFL 50%	Total	P
Slaughter weight, g	110.23±0.35	111.64±1.34	110.08±1.53	110.65±0.67	0.598
Hot carcass, g	53.37±0.31	52.98±0.42	52.61±0.37	52.98±0.21	0.356
Cold carcass, g	51.05±0.29	51.28±0.37	50.59±0.50	50.97±0.23	0.467
Hot carcass yield, %	48.42±0.27	47.49±0.45	47.86±0.81	47.92±0.32	0.506
Cold carcass yield, %	46.31±0.25	45.96±0.39	46.04±0.91	46.10±0.33	0.905
Loss of carcass ratio, %	2.10±0.09	1.53±0.16	1.83±0.33	1.82±0.13	0.201

BSFL: Black soldier fly larvae, P: probability, *, a, b: the significance of the means has different letters in the same row were significant $P<0.05$.

3.5. Chemical and Physical Properties of Meat

The chemical and physical characteristics of the meats of all experimental groups were measured and showed in Table 3.9. Statistically significant differences ($P<0.05$) were found in dry matter and protein content, with the Azolla 6% experimental group having the best results, followed by the Azolla 12% group, and finally the control group. Fat content was higher in Azolla 12%experimental group, with significant differences ($P<0.05$) compared to Azolla 6% experimental groups, with the lowest being the control group. No statistically significant differences were recorded between the experimental groups in ash content. Carbohydrate content was higher in the control group, with significant differences ($P<0.05$), while the 6% Azolla group was lower, and the 12% Azolla group had the lowest level among the experimental groups. No significant differences were observed in the measurement of meat pH between the experimental groups. As for the cooking loss and melting loss test, there were statistically significant differences between the experimental groups ($P<0.05$), where the percentage of cooking loss and melting loss was higher for the control group, Azolla 6%, and Azolla 12%, respectively. Meat color measurements were taken of three locations and there were no statistically significant differences were observed between the experimental groups in terms of yellowness (b*), redness (a*), and lightness (L*).

Table 3.9. Effect of Azolla levels to the common carp fish diet on fish meat traits

Meat tests	Control	Azolla 6%	Azolla 12%	Total	P
Dry matter %	32.94±0.58^{c}	34.91±0.30^{a}	33.08±0.28^{b}	33.64±0.29	0.004**
Protein %	14.27±0.53^{c}	17.88±0.56^{a}	16.15±0.93^{b}	16.10±0.49	0.006**
Lipid %	3.53±0.20^{c}	3.93±0.10^{b}	4.10±0.14^{a}	3.85±0.10	0.043*
Ash %	1.72±0.20	1.89±0.11	1.77±0.19	1.79±0.10	0.772
Carbohydrate %	12.41±0.38^{a}	11.18±0.26^{b}	11.10±0.37^{c}	11.56±0.23	0.022*
Physical tests					
PH	6.64±0.10	6.89±0.11	6.97±0.16	6.83±0.08	0.171
Cooking loss%	4.07±0.31^{a}	3.45±0.19^{b}	3.05±0.20^{c}	3.53±0.16	0.022*
Thawing loss%	3.66±0.15^{a}	3.23±0.14^{b}	3.08±0.11^{c}	3.32±0.09	0.018*
Meat Color					
L* Chest	45.02±0.63	46.01±0.57	46.00±0.39	45.68±0.31	0.349
L* Belly	44.55±0.55	45.02±0.53	45.20±0.79	44.92±0.35	0.759
L* Tail	42.03±0.36	43.02±0.51	43.59±0.59	42.88±0.31	0.107
a* Chest	1.36±0.10	1.07±0.09	1.14±0.12	1.19±0.06	0.144
a* Belly	1.47±0.19	1.31±0.21	1.30±0.24	1.36±0.12	0.822
a* Tail	-0.95±0.31	-1.51±0.35	-1.35±0.29	-1.27±0.18	0.454
b* Chest	0.95±0.04	0.94±0.04	0.93±0.07	0.94±0.03	0.986
b* Belly	1.89±0.07	2.01±0.09	2.11±0.11	2.00±0.05	0.254
b* Tail	0.91±0.64	0.47±0.71	0.01±0.73	0.46±0.39	0.659

P: probability, L*: lightness, a*: Redness, b*: Yellowness, *, a, b: the significance of the means has different letters in the same row were significant P<0.05.

Table 3.10. shows the effect of black soldier fly larvae levels in common carp diets on meat traits. Chemical analyses of the meat revealed statistically significant differences in dry matter, protein, fat, ash content, and carbohydrate content (P<0.05). The dry matter content was highest in the BSFL 25% experimental group, followed by the BSFL 50% experimental group. The lowest dry matter content was recorded among the experimental groups, in favor of the control group. Protein content was superior in the experimental groups fed black soldier fly larvae, with significant differences observed, with the BSFL 25% group having the highest value, the BSFL 50% group having the slightly lower value, and the control group having the lowest value. Significant differences were found in the lipid percentage, with the highest percentages recorded in the BSFL 50% group, followed by the BSFL 25% group. The control group had the lowest percentages. In the ash percentage test, the experimental groups (BSFL 25%, BSFL 50%, and control) had higher percentages, respectively. In the carbohydrate percentage test, the control group

had higher percentages than its counterparts (BSFL 25% and BSFL 50%). In the physical characteristics of meat quality measured by the pH scale, no statistically significant differences were found between the experimental groups. In the cooking loss and thawing loss tests, significant differences were found between the experimental groups, with the highest percentages favoring the control group, BSFL 25%, and BSFL 50%, respectively. As for the meat color tests, which were taken from three regions: the chest, abdomen, and tail, and the colors L*: lightness, a*: redness, b*: yellowness, it was indicated that no significant differences appeared between the experimental groups that were fed on black soldier fly larvae and the control group.

Table 3.10. Effect of Black soldier fly larvae levels to the common carp fish diet on fish meat traits

Meat tests	Control	BSFL 25%	BSFL 50%	Total	P
Dry matter %	33.44±0.49^{c}	35.81±0.52^{a}	34.90±0.14^{b}	34.72±0.31	0.003**
Protein %	14.57±0.53^{c}	20.68±0.21^{a}	19.88±0.20^{b}	18.37±0.60	0.000**
Lipid %	3.78±0.23^{c}	4.26±0.15^{b}	4.40±0.19^{a}	4.14±0.09	0.012*
Ash %	1.60±0.16^{c}	2.16±0.04^{a}	1.95±0.03^{b}	1.90±0.07	0.001*
Carbohydrate %	12.78±0.31^{a}	9.88±0.25^{b}	8.69±0.39^{c}	10.45±0.40	0.000**
Physical tests					
PH	6.89±0.19	6.74±0.04	6.85±0.03	6.82±0.06	0.616
Cooking loss %	4.45±0.34^{a}	3.60±0.04^{b}	3.15±0.05^{c}	3.73±0.16	0.001**
Thawing loss %	4.03±0.29^{a}	3.39±0.06^{b}	3.25±0.06^{c}	3.56±0.12	0.010*
Meat Color					
L* Chest	44.89±0.65	44.81±0.57	44.80±0.39	44.83±0.30	0.991
L* Belly	45.18±0.63	43.15±0.53	43.33±0.79	43.89±0.41	0.076
L* Tail	42.28±0.55	41.48±0.51	42.05±0.59	41.93±0.31	0.583
a* Chest	1.48±0.18	1.09±0.14	1.01±0.12	1.19±0.09	0.072
a* Belly	1.60±0.26	1.11±0.14	0.98±0.24	1.23±0.13	0.14
a* Tail	-0.82±0.40	-0.91±0.35	-0.76±0.30	0.83±0.20	0.957
b* Chest	1.07±0.13	0.81±0.07	0.77±0.09	0.88±0.06	0.096
b* Belly	2.01±0.13	1.64±0.09	1.74±0.11	1.80±0.07	0.079
b* Tail	1.04±0.67	0.72±0.71	0.26±0.73	0.67±0.39	0.738

BSFL: Black soldier fly larvae, P: probability, L*: lightness, a*: Redness, b*: Yellowness, *, a, b: the significance of the means has different letters in the same row were significant $P<0.05$.

3.6. Hematological Parameters of the Fish

Table 3.11 shows effect of Azolla levels to the common carp fish diet on the hematological parameters of the fish. Blood tests revealed no statistically significant differences among the experimental groups, including hematocrit, hemoglobin, white blood cells, lymphocytes, monocytes, neutrophils, eosinophils, and basophils. There was a significant difference in red blood cells in the experimental groups, as there was an

increase in the number of blood cells in the Azolla 12% group, followed by the Azolla 6% group, followed by the control group. As for blood serum tests, there were no significant differences in the biochemical tests of glucose, cholesterol, triglycerides, albumin, globulin, albumin/globulin, high-density lipoprotein, very-low-density lipoprotein, low-density lipoprotein, urea, and creatine. However, there was a significant difference in total blood protein in the Azolla 12% group compared to its counterparts in the experimental groups. As for liver function tests, which include aspartate aminotransferase, alanine aminotransferase, and alkaline phosphatase, it was noted that there were no statistically significant differences, except for a decrease in some samples compared to the control group.

Table 3.11. Effect of Azolla levels to the common carp fish diet on the hematological parameters of the fish

Blood tests	**Control**	**Azolla 6%**	**Azolla 12%**	**Total**	**P**
HCT %	36.00±1.96	35.75±3.97	34.00±2.27	35.25±1.52	0.868
HGB g/l	120.00±8.83	98.00±8.89	109.25±8.72	109.08±5.34	0.262
RBC ($\times 10^6/\mu L$)	2.99 ± 0.24^c	3.80 ± 0.36^b	5.22 ± 0.65^a	4.00±0.36	0.019*
WBC ($\times 10^3/\mu L$)	58.81±4.96	63.48±3.37	61.27±2.67	61.18±2.06	0.695
Lymphocytes ($\times 10^3/\mu L$)	42.07±3.76	46.50±1.89	44.38±1.90	44.31±1.49	0.526
Monocytes ($\times 10^3/\mu L$)	3.59±0.71	3.38±0.16	3.45±0.13	3.47±0.23	0.943
Neutrophils ($\times 10^3/\mu L$)	9.83±1.18	9.94±1.30	9.94±0.86	9.90±0.59	0.997
Eosinophils ($\times 10^3/\mu L$)	2.78±0.18	3.10±0.28	2.95±0.27	2.94±0.14	0.67
Basophils ($\times 10^3/\mu L$)	0.55±0.07	0.56±0.06	0.57±0.03	0.56±0.03	0.968
Blood serum tests					
Glucose (mg/dl)	76.02±6.24	70.16±3.29	66.87±2.71	71.01±2.55	0.366
Cholesterol (mg/dl)	123.19±7.89	122.54±7.81	115.22±3.48	120.31±3.67	0.66
Triglyceride (mg/dl)	74.64±3.53	69.99±6.10	77.36±5.58	73.99±2.86	0.614
Total protein (g/dl)	3.78 ± 0.07^b	3.73 ± 0.11^c	4.19 ± 0.14^a	3.90±0.08	0.029*
Albumin (g/dl)	1.42±0.02	1.46±0.13	1.55±0.09	1.48±0.05	0.604
Globulin (g/dl)	2.36±0.09	2.27±0.12	2.64±0.19	2.42±0.09	0.194
Albumin/Globulin(A/G)	0.61±0.04	0.65±0.08	0.60±0.08	0.62±0.04	0.848
HDL (mg/dl)	50.13±2.96	53.00±3.81	49.42±1.57	50.85±1.60	0.67
VLDL (mg/dl)	14.93±0.71	14.00±1.22	15.47±1.12	14.80±0.57	0.613
LDL (mg/dl)	52.02±4.11	55.55±5.44	50.32±4.97	52.63±2.63	0.748
Urea (mg/dl)	16.12±2.63	12.34±0.90	16.31±2.78	14.92±1.31	0.413
Creatin (mg/dl)	0.18±0.02	0.14±0.03	0.15±0.01	0.16±0.01	0.343
AST/GOT (U/L)	42.92±12.02	31.77±13.99	27.00±8.36	33.89±6.43	0.629
ALT/GPT (U/L)	25.00±2.23	21.61±2.95	33.07±5.66	26.56±2.50	0.158
ALP(U/L)	50.39±5.83	43.16±2.74	41.32±4.24	44.96±2.61	0.356

HCT: Hematocrit, HGB: Hemoglobin, RBC: Red Blood Cells, WBC: White Blood Cells, HDL: High-Density Lipoprotein, VLDL: Very Low-Density Lipoprotein, LDL: Low-Density Lipoprotein, AST: Aspartate Aminotransferase, ALT: Alanine Aminotransferase, ALP: Alkaline Phosphatase, P: probability, *, $^{a, b}$: the significance of the means has different letters in the same row were significant $P<0.05$.

Table 3.12 shows the effect of black soldier fly larvae in the diet of common carp on hematological parameters. Blood tests showed no statistically significant differences between experimental groups, including hematocrit, hemoglobin, red blood cell and white blood cell counts, lymphocytes, monocytes, neutrophils, eosinophils, and basophils. Serum tests showed no statistically significant differences between experimental groups in biochemical tests of glucose, cholesterol, total protein, triglycerides, albumin, globulin, albumin/globulin, high-density lipoprotein (HDL), very-low-density lipoprotein (VLDL), low-density lipoprotein (LDL), urea, and creatine. Liver function tests, including aspartate aminotransferase, alanine aminotransferase, and alkaline phosphatase, showed no statistically significant differences.

Table 3.12. Effect of Black soldier fly larvae levels to the common carp fish diet on the hematological parameters of the fish

Blood tests	Control	BSFL 25%	BSFL 50%	Total	P
HCT %	35.50±2.40	34.50±4.37	30.50±4.13	33.50±2.06	0.62
HGB g/l	117.50±9.18	112.25±13.00	99.50±15.65	109.75±7.10	0.613
RBC (×10⁶/µL)	3.24±0.20	3.29±0.45	3.02±0.73	3.18±0.27	0.924
WBC (×10³/µL)	58.31±5.24	62.20±3.68	64.80±2.91	61.77±2.27	0.546
Lymphocytes (×10³/µL)	42.57±3.50	44.59±2.57	47.19±2.46	44.78±1.61	0.547
Monocytes (×10³/µL)	3.84±0.58	3.25±0.29	3.83±0.18	3.64±0.22	0.502
Neutrophils (×10³/µL)	10.08±1.31	10.80±1.43	10.16±0.61	10.34±0.62	0.895
Eosinophils (×10³/µL)	2.80±0.19	2.99±0.37	3.13±0.19	2.97±0.14	0.684
Basophils (×10³/µL)	0.52±0.08	0.59±0.06	0.50±0.02	0.54±0.03	0.571
Blood serum tests					
Glucose (mg/dl)	75.27±6.69	71.99±7.24	82.76±4.86	76.67±3.58	0.497
Cholesterol (mg/dl)	122.44±8.30	119.90±7.09	127.99±2.67	123.44±3.54	0.677
Triglyceride (mg/dl)	72.89±4.28	67.82±5.71	78.59±2.61	73.10±2.65	0.272
Protein (g/dl)	3.70±0.15	3.69±0.27	3.59±0.20	3.66±0.11	0.92
Albumin (g/dl)	1.39±0.04	1.50±0.08	1.57±0.07	1.49±0.04	0.191
Globulin (g/dl)	2.34±0.09	2.19±0.25	2.02±0.15	2.18±0.10	0.462
Albumin /Globulin (A/G)	0.59±0.05	0.71±0.07	0.79±0.05	0.69±0.04	0.092
HDL (mg/dl)	49.63±2.78	58.76±1.68	55.59±2.73	54.66±1.71	0.072
VLDL (mg/dl)	14.43±0.86	13.56±1.14	15.72±0.52	14.57±0.53	0.27
LDL (mg/dl)	50.77±3.75	47.58±6.19	56.68±3.56	51.68±2.69	0.411
Urea (mg/dl)	15.62±2.73	13.20±2.66	12.75±2.88	13.86±1.49	0.738
Creatin (mg/dl)	0.18±0.02	0.17±0.01	0.11±0.03	0.15±0.01	0.123
AST/GOT (U/L)	32.92±9.68	16.91±2.77	17.12±2.70	22.32±3.87	0.153
ALT/GPT (U/L)	24.00±2.29	28.15±2.87	33.32±5.40	28.49±2.28	0.266
ALP (U/L)	48.89±5.21	49.93±2.42	50.35±2.97	49.72±1.96	0.96

BSFL: Black soldier fly larvae, HCT: Hematocrit, HGB: Hemoglobin, RBC: Red Blood Cells, WBC: White Blood Cells, HDL: High-Density Lipoprotein, VLDL: Very Low-Density Lipoprotein, LDL: Low-Density Lipoprotein, AST: Aspartate Aminotransferase, ALT: Alanine Aminotransferase, ALP: Alkaline Phosphatase, P: probability, *, $^{a, b}$: the significance of the means has different letters in the same row were significant P<0.05.

3.7. Fatty Acid Composition of Fish Meat

Table 3.13 shows the effect of Azolla levels on the common carp fish diet on the fatty acid composition of fish. The results showed that there were significant differences and statistically significant differences between the experimental groups (P<0.05), which include palmitic acid, stearic acid, linoleic acid, alpha-linolenic acid, eicosapentaenoic acid, docosahexaenoic acid, and oleic acid, showing significant superiority in the Azolla 12% group, followed by the 6% group and the control group, respectively.

Table 3.13. Effect of Azolla levels on the common carp fish diet on the fatty acid composition of fish meat

Fatty acids	Control	Azolla 6%	Azolla 12%	Total	P
Palmitic acid (C16:0)	13.72±0.69^{c}	14.62±0.61^{b}	16.10±0.49^{a}	14.81±0.39	0.034*
Stearic acid (C18:0)	2.46±0.29^{c}	3.01±0.42^{b}	4.03±0.27^{a}	3.16±0.23	0.011*
Linoleic acid (C18:2n-6)	11.33±0.51^{c}	13.33±0.62^{b}	14.94±1.20^{a}	13.20±0.55	0.02*
Alpha-linolenic acid (C18:3n-3)	2.22±0.34^{c}	3.12±0.33^{b}	4.18±0.40^{a}	3.17±0.26	0.004**
Eicosapentaenoic ac id (C20:5n-3)	19.11±0.77^{c}	22.33±0.72^{b}	23.48±0.80^{a}	21.64±0.57	0.002**
Docosahexaenoic acid (C22:6n-3)	16.07±0.85^{c}	17.05±0.81^{b}	18.93±0.63^{a}	17.35±0.49	0.046*
Oleic acid (C18:1n-9)	11.06±0.50^{c}	12.56±0.47^{b}	13.73±0.78^{a}	12.45±0.40	0.017*

P: probability, *, a, b: the significance of the means has different letters in the same row were significant P<0.05.

Table 3.14 shows the results of the effect of black soldier fly larvae levels in the diet of common carp on the fatty acid composition of the fish. The results showed significant differences (P<0.05) between the experimental groups, which included palmitic acid, stearic acid, linoleic acid, alpha-linolenic acid, eicosapentaenoic acid, docosahexaenoic acid, and oleic acid, with a significant advantage in the black soldier fly larvae group (50%), followed by the black soldier fly larvae group (25%), and the control group, respectively.

Table 3.14. Effect of Black soldier fly larvae levels on the common carp fish diet on the fatty acid composition of fish meat

Fatty acids	Control	BSFL 25%	BSFL 50%	Total	P
Palmitic acid (C16:0)	13.97±0.84^{c}	15.04±0.28^{b}	16.50±0.38^{a}	15.17±0.37	0.015*
Stearic acid (C18:0)	2.58±0.28^{c}	3.26±0.36^{b}	4.31±0.41^{a}	3.38±0.25	0.008**
Linoleic acid (C18:2n-6)	11.46±0.54^{c}	14.05±0.79^{b}	17.55±0.59^{a}	14.35±0.63	0.000**
Alpha-linolenic acid (C18:3n-3)	2.35±0.31^{c}	3.59±0.41^{b}	4.68±0.42^{a}	3.54±0.29	0.001**
Eicosapentaenoic acid (C20:5n-3)	18.86±0.86^{c}	23.10±0.98^{b}	24.34±0.88^{a}	22.10±0.70	0.001**
Docosahexaenoic acid (C22:6n-3)	16.19±0.78^{c}	18.42±0.95^{b}	19.26±0.20^{a}	17.96±0.48	0.018**
Oleic acid (C18:1n-9)	10.81±0.33^{c}	13.62±0.55^{b}	17.82±0.62^{a}	14.08±0.66	0.000**

BSFL: Black soldier fly larvae, P: probability, *, a, b: the significance of the means has different letters in the same row were significant P<0.05.

3.8. Amino Acids Composition of Fish Meat

Table 3.15, shows Effect of Azolla levels on the common carp fish diet on the amino acid composition of fish meat. There were statistically significant differences in the following amino acid levels: Aspartic acid, Glycine, Lysine, Serine, Threonine, Isoleucine, Alanine, Valine, Tyrosine, Arginine, Cysteine, Methionine, Proline, Histidine, Lucien, Glutamic acid, and Phenylalanine. The experimental groups containing Azolla showed better results than the control group, with the experimental groups (Azolla 12% and Azolla 6%) showing the best results and the control group showing the lowest.

Table 3.15. Effect of Azolla levels on the common carp fish diet on the amino acids composition of fish meat

Amino acids %	**Control**	**Azolla 6%**	**Azolla 12%**	**Total**	**P**
Aspartic acid	7.99±0.16^{c}	8.35±0.38^{b}	13.06±1.24^{a}	9.80±0.90	0.005**
Glycine acid	4.00±0.12^{c}	4.87±0.64^{b}	9.06±0.53^{a}	5.98±0.82	0.001**
Lysine acid	5.89±0.14^{c}	6.12±0.66^{b}	11.46±1.00^{a}	7.82±0.97	0.002**
Serine acid	3.56±0.09^{c}	4.01±0.65^{b}	9.07±1.09^{a}	5.55±0.96	0.003**
Threonine acid	3.89±0.05^{c}	4.10±0.64^{b}	10.53±0.91^{a}	6.17±1.14	0.001**
Isoleucine acid	3.13±0.14^{c}	3.88±1.22^{b}	8.77±0.54^{a}	5.26±0.96	0.004**
Alanine acid	4.17±0.13^{c}	4.74±0.93^{b}	10.25±0.64^{a}	6.39±1.02	0.001**
Valine acid	5.77±0.09^{c}	6.30±0.73^{b}	11.45±0.69^{a}	7.84±0.95	0.001**
Tyrosine acid	2.36±0.41^{c}	2.98±0.27^{b}	5.77±0.55^{a}	3.70±0.57	0.003**
Arginine acid	3.63±0.12^{c}	4.09±0.10^{b}	7.15±0.68^{a}	4.96±0.59	0.002**
Cysteine acid	1.56±0.09^{c}	2.01±0.61^{b}	5.46±0.40^{a}	3.01±0.65	0.001**
Methionine acid	3.00±0.11^{c}	3.88±0.09^{b}	7.11±0.84^{a}	4.66±0.67	0.002**
Proline acid	2.85±0.08^{c}	3.12±0.11^{b}	5.92±0.95^{a}	3.96±0.56	0.014**
Histidine acid	1.98±0.12^{c}	2.74±0.16^{b}	5.11±0.94^{a}	3.28±0.55	0.017**
Leucine acid	10.58±0.45^{c}	11.55±0.13^{b}	15.57±1.49^{a}	12.57±0.89	0.018**
Glutamic acid	8.09±0.16^{c}	9.12±0.10^{b}	13.88±1.29^{a}	10.36±0.97	0.003**
Phenylalanine acid	4.15±0.13^{c}	5.69±0.13^{b}	6.79±0.73^{a}	5.54±0.44	0.015**

P: probability, *, a, b: the significance of the means has different letters in the same row were significant $P<0.05$.

Table 3.16 shows effect of Black soldier fly larvae levels on the common carp fish diet on the amino acid composition of fish meat. There were statistically significant differences were observed in the levels of Aspartic acid, Glycine, Lysine, Serine, Threonine, Isoleucine, Alanine, Valine, Tyrosine, Arginine, Cysteine, Methionine, Proline, Histidine, Lucien, Glutamic acid, and Phenylalanine. The experimental groups containing Black soldier fly larvae performed better than the control group. Specifically, the experimental groups containing 25% and 50% BSFL showed the best results, while the control group showed the lowest.

Table 3.16. Effect of Black soldier fly larvae levels on the common carp fish diet on the amino acids composition of fish meat

Amino acids %	Control	BSFL 25%	BSFL 50%	Total	P
Aspartic acid	7.98±0.16^{c}	8.12±0.50^{b}	9.81±0.57^{a}	8.64±0.37	0.048*
Glycine acid	3.97±0.14^{c}	4.20±0.11^{b}	6.11±0.42^{a}	4.76±0.36	0.002**
Lysine acid	5.86±0.17^{c}	6.00±0.47^{b}	8.45±0.42^{a}	6.77±0.46	0.005**
Serine acid	3.53±0.10^{c}	3.98±0.67^{b}	6.88±0.27^{a}	4.80±0.57	0.003**
Threonine acid	3.86±0.06^{c}	4.00±0.63^{b}	7.49±0.26^{a}	5.12±0.63	0.001**
Isoleucine acid	3.11±0.11^{c}	3.69±0.71^{b}	5.99±0.14^{a}	4.26±0.49	0.007**
Alanine acid	4.14±0.10^{c}	4.80±0.60^{b}	6.91±0.17^{a}	5.28±0.46	0.004**
Valine acid	5.74±0.10^{c}	6.01±0.48^{b}	8.70±0.37^{a}	6.82±0.51	0.002**
Tyrosine acid	2.26±0.48^{c}	2.77±0.48^{b}	4.11±0.22^{a}	3.05±0.34	0.046*
Arginine acid	3.61±0.11^{c}	4.08±0.61^{b}	5.98±0.08^{a}	4.56±0.40	0.008**
Cysteine acid	1.53±0.08^{c}	2.06±0.27^{b}	4.16±0.61^{a}	2.58±0.45	0.007**
Methionine acid	2.98±0.10^{c}	3.69±0.25^{b}	5.08±0.19^{a}	3.92±0.32	0.001**
Proline acid	2.84±0.09^{c}	3.08±0.60^{b}	5.64±0.21^{a}	3.85±0.48	0.003**
Histidine acid	1.93±0.09^{c}	2.65±0.53^{b}	4.98±0.12^{a}	3.19±0.49	0.001**
Leucine acid	10.56±0.44^{c}	11.98±0.53^{b}	13.65±0.24^{a}	12.06±0.49	0.006**
Glutamic acid	8.06±0.19^{c}	9.88±0.73^{b}	11.91±0.48^{a}	9.95±0.61	0.006**
Phenylalanine acid	4.12±0.10^{c}	4.98±0.57^{b}	6.90±0.10^{a}	5.33±0.45	0.003**

BSFL: Black soldier fly larvae, P: probability, *, a, b: the significance of the means has different letters in the same row were significant P<0.05.

3.9. Histopathological Analysis

Figures (1 to 6) illustrated the control group's histopathological figures of the liver, kidneys, and skeletal muscle, which showed normal cytoarchitecture. The Azolla 6% group referred to histopathological figures of this group's liver, pancreas, kidney, and muscle flesh, similar to those of the control group (Figs. 7 to 12). The Azolla 12 % group referred to histopathological figures of the liver, which showed marked non-plastic hyperplasia and hypertrophy of acinar cells of the pancreas with marked massive venous dilation. At the same time, the hepatocytes revealed a normal appearance (Figs. 13 & 14). Other figures showed mild portal fibrosis with portal dilation and marked portal aggregation of mononuclear leukocytes (Fig. 15). The kidney histopathological figures of the renal tissue showed generalized moderate cellular swelling with desquamations of the lining cells of renal tubules and normal appearance of glomerular cells and hemopoietic tissue (Figs. 16 & 17). The skeletal muscle histopathological figures show marked muscular dystrophy associated with moderate degeneration and necrosis of myofibers, consequently, atrophy and tissue depletion (Fig. 18).

Figures (1 to 6) illustrated the control group's histopathological figures of the liver, kidneys, and skeletal muscle, which showed normal cytoarchitecture. The histopathological for the experimental group, black soldier fly larvae (BSFL 25%), figures of liver showed marked hyperplasia of the acinar cells of pancreas forming glandular cords like extension within hepatic tissue; there was marked massive venous dilation, while the hepatocytes revealed normal appearance (Fig. 19). The acinar cells of pancreas revealed marked like aplastic cells with marked variable shape and sizes and intense basophilic nuclei (Fig.20). The histopathological figures of the kidney showed moderate cellular swelling with desquamations of the lining cells of renal tubules, and normal appearance of glomerular cells hemopoietic tissue (Fig.21 & 22). The histopathological figures of the skeletal muscle show marked disarrangement of the myofibers with moderate atrophy and tissue depletion (Fig. 23). The histopathological figures findings for the experimental group, black soldier fly larvae (BSFL 50%), liver showed hepatitis with mild hyperplasia of acinar cells of pancreas and perivascular infiltration of lymphocytes with pancreatic lobules (Fig.24 & 25). The hepatic tissue revealed mild steatosis with infiltration the polymorphous leukocytes and mononuclear leukocytes (Fig.26). The histopathological figures of the kidney showed little cellular swelling with desquamations of the lining cells of renal tubules and tubular dilation. The glomeruli and hemopoietic tissue revealed normal cytoarchitectures (Fig.27 & 28). The histopathological figures of the skeletal muscle show normal appearance and arrangement of the myofibers (Fig.29).

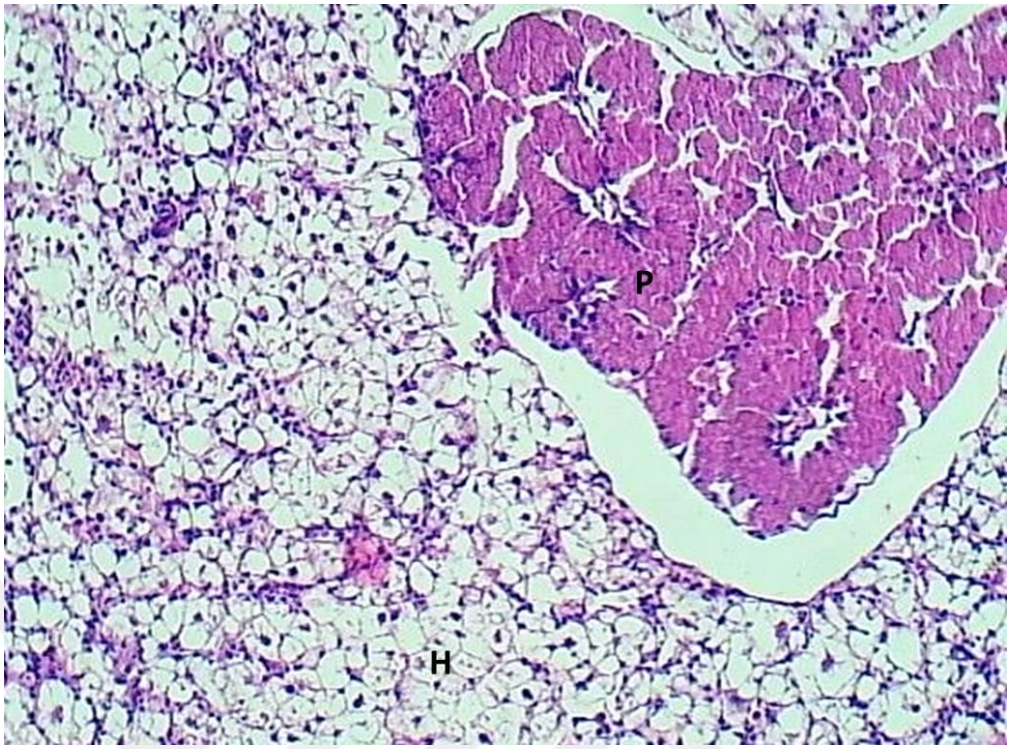


Figure 3.1. Section of liver and pancreas (Control) shows normal appearance of hepatocytes (H) & pancreatic acini (P).H&E.100x

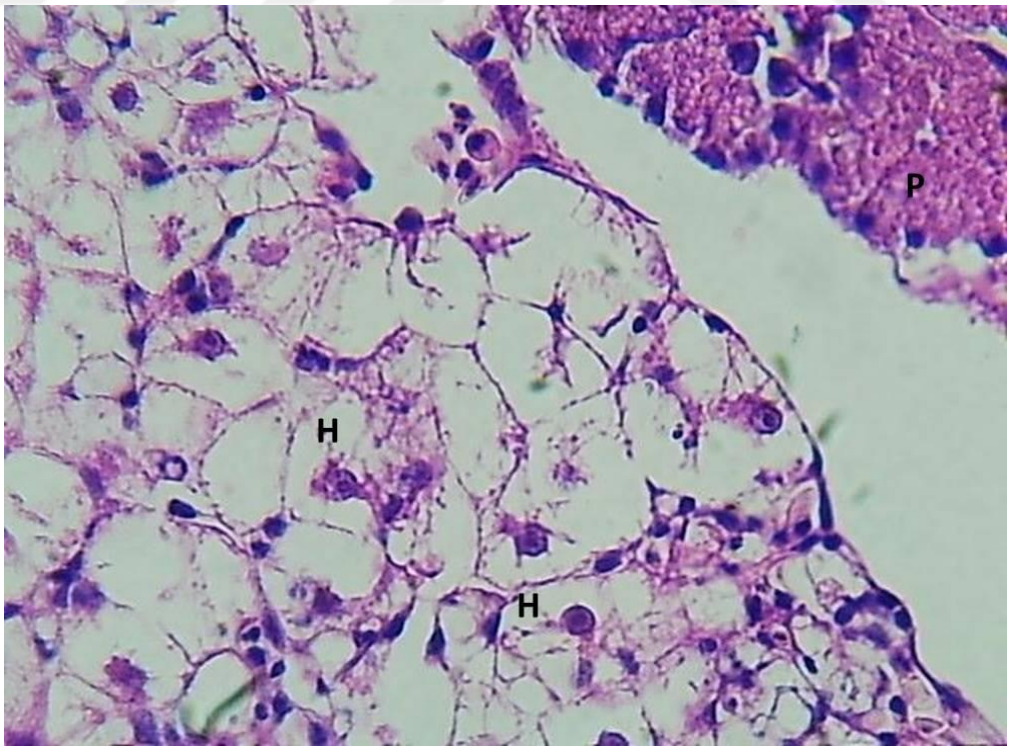


Figure 3.2. Section of liver and pancreas (Control) shows normal appearance of hepatocytes (H) & pancreatic acini (P).H&E.400x

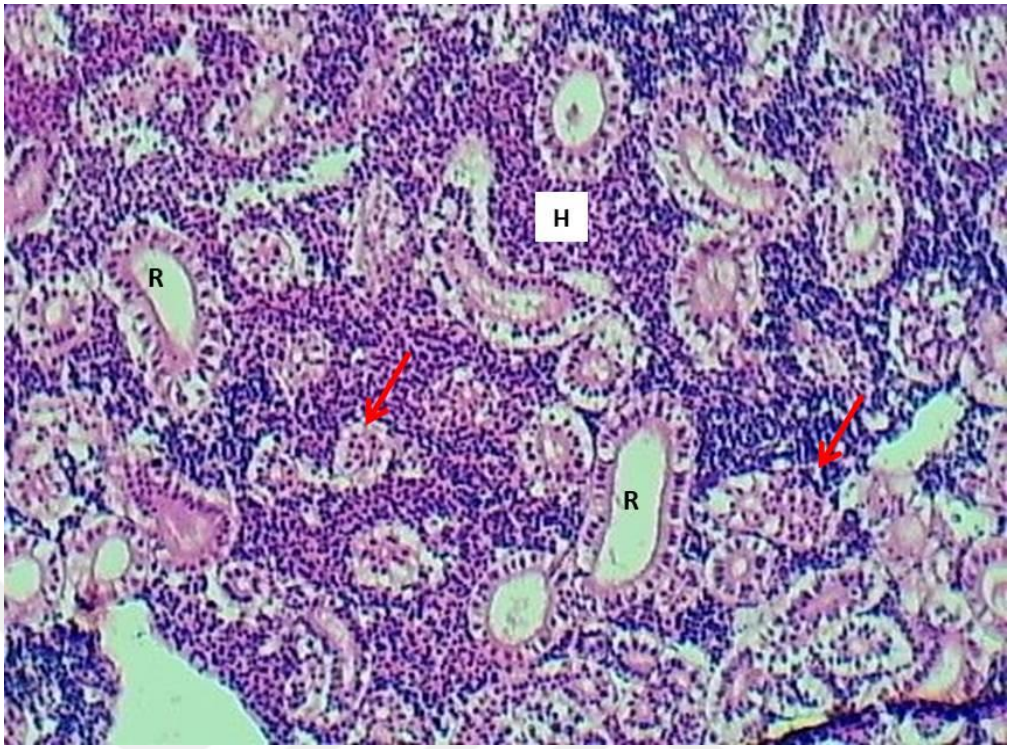


Figure 3.3. Section of kidney (Control) shows normal appearance of renal tubules (R), glomeruli (red arrows) & hemopoietic tissue (H).H&E.100x

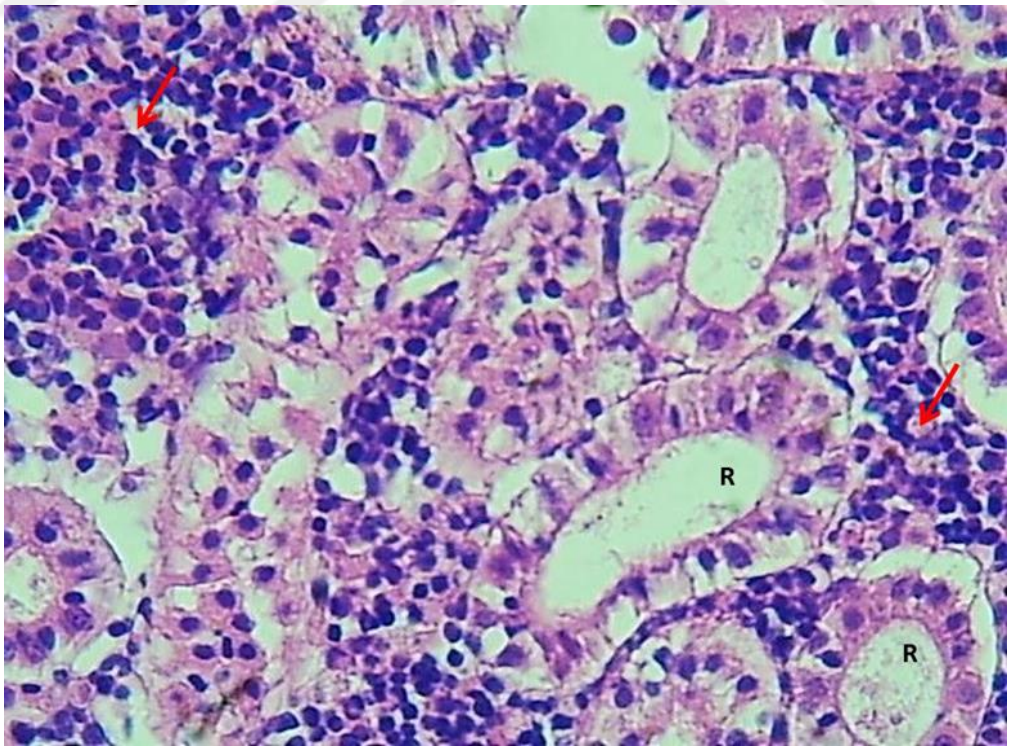


Figure 3.4. Section of kidney (Control) shows normal appearance of renal tubules (R), glomeruli (red arrows) & hemopoietic tissue (H).H&E.400x



Figure 3.5. Section of skeletal muscle (Control) shows normal appearance of myofibers (m) & endomysium loose connective tissue (arrow).H&E.100x

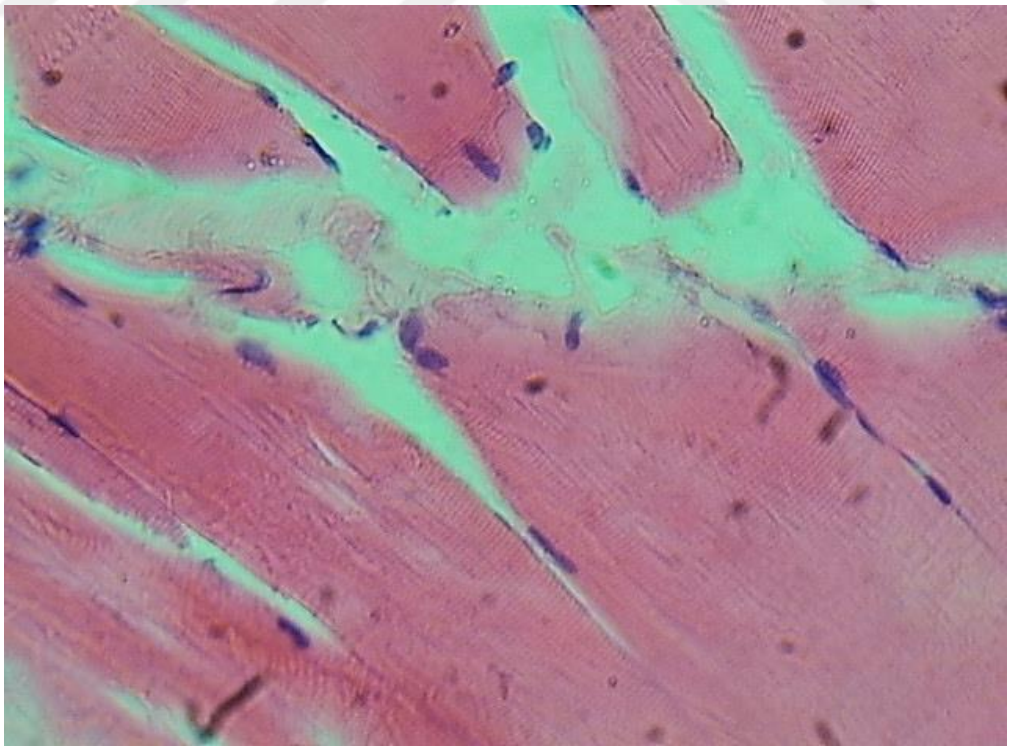


Figure 3.6. Section of skeletal muscle (Control) shows normal appearance of myofibers, & endomysium loose connective tissue. H&E.400x

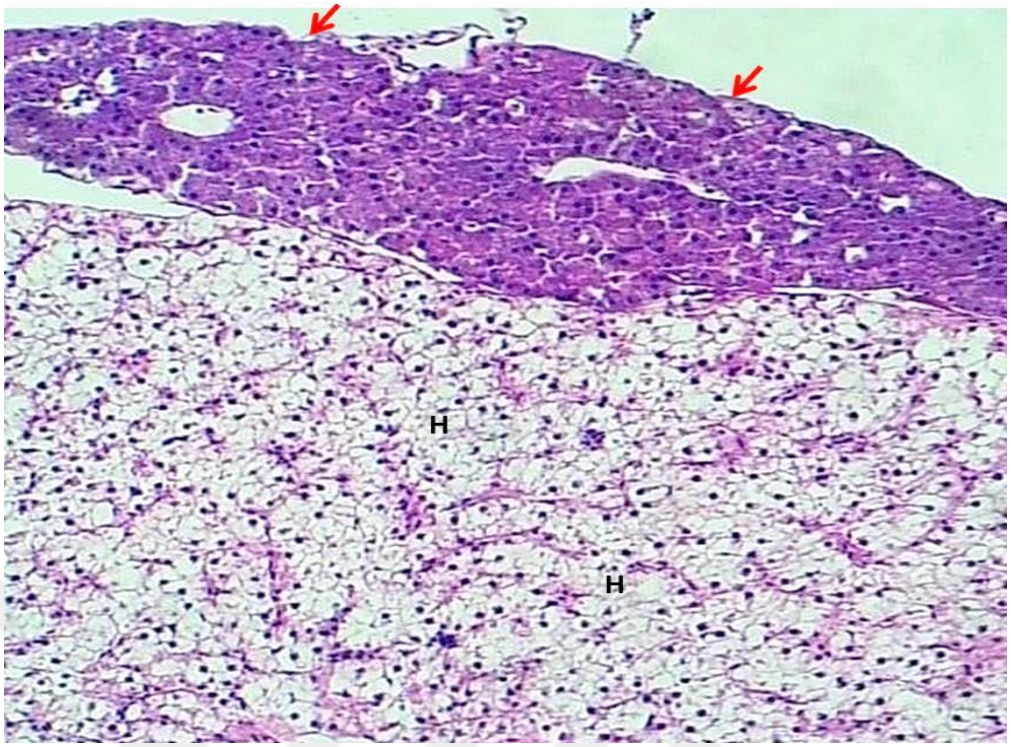


Figure 3.7. Section of liver and pancreas (Azolla 6%) shows normal appearance of hepatocytes (H) & pancreatic acini (arrows).H&E.100x

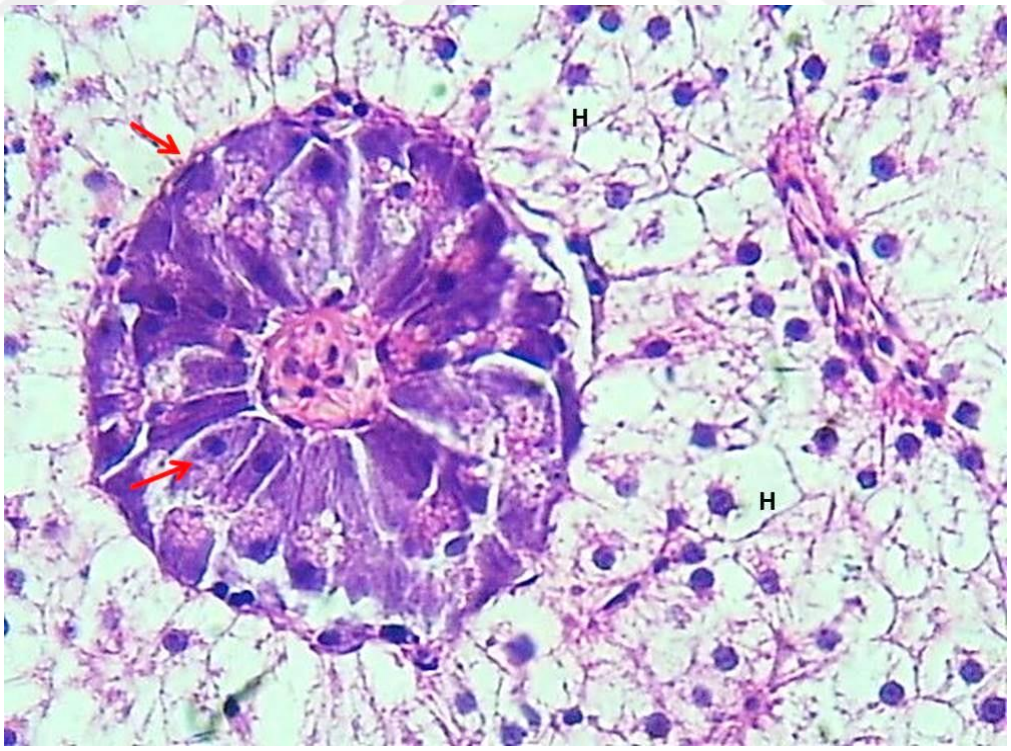


Figure 3.8. Section of liver and pancreas (Azolla 6%) shows normal appearance of hepatocytes (H) & pancreatic acinar cells (arrows).H&E.400x

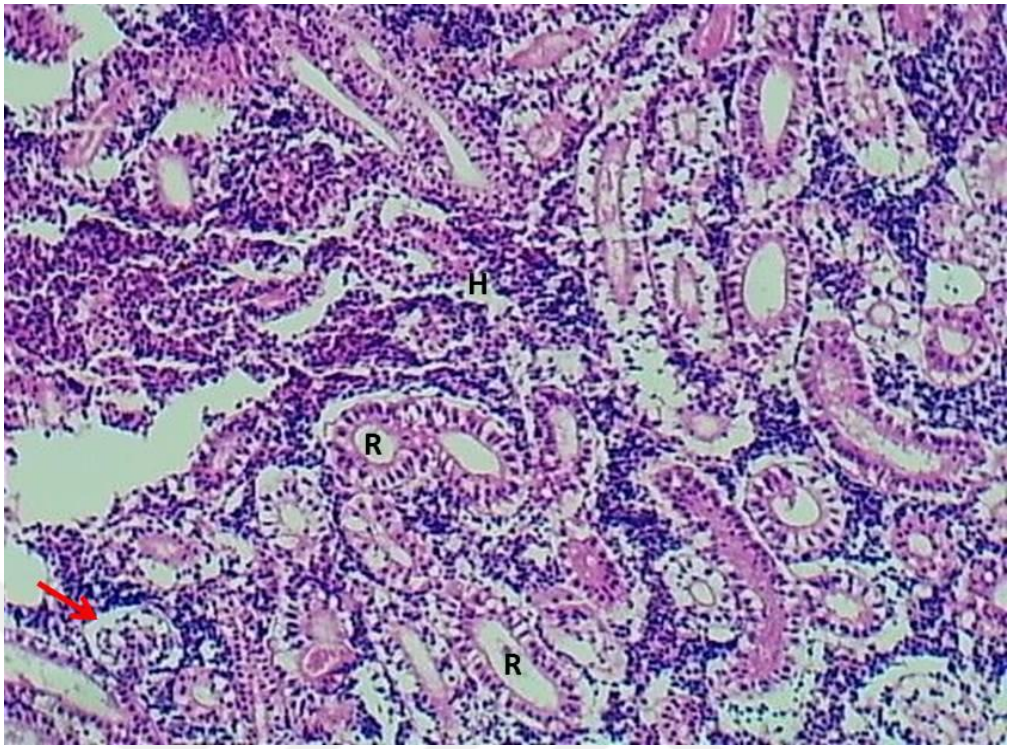


Figure 3.9. Section of kidney (Azolla 6%) shows normal appearance of renal tubules (R), glomeruli (red arrows) & hemopoietic tissue (H).H&E.100x

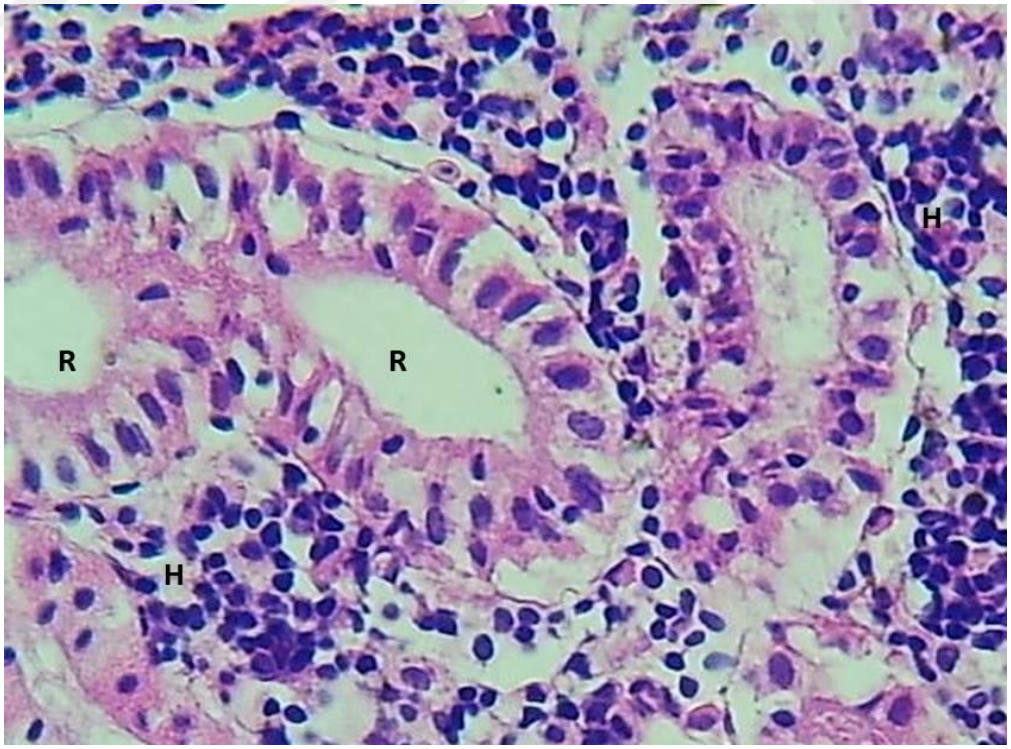


Figure 3.10. Section of kidney (Azolla 6%) shows normal appearance of lining cells of renal tubules (R) & hemopoietic tissue (H).H&E.400x

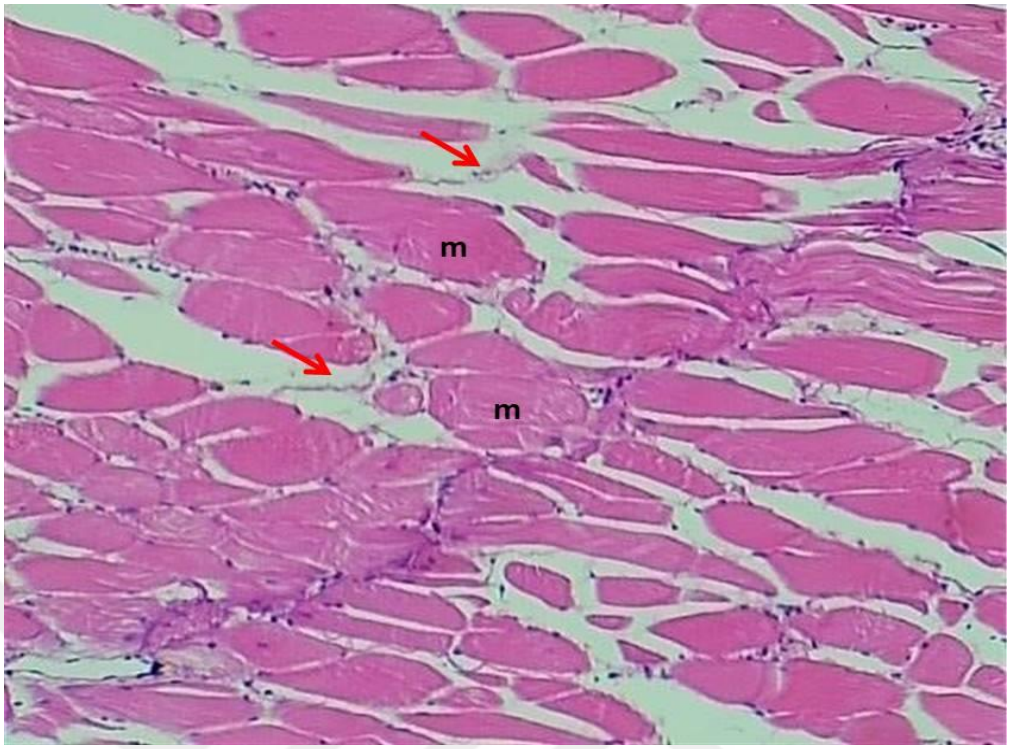


Figure 3.11. Section of skeletal muscle (Azolla 6%) shows normal appearance of myofibers (m) & endomysium loose connective tissue (arrow).H&E.100x

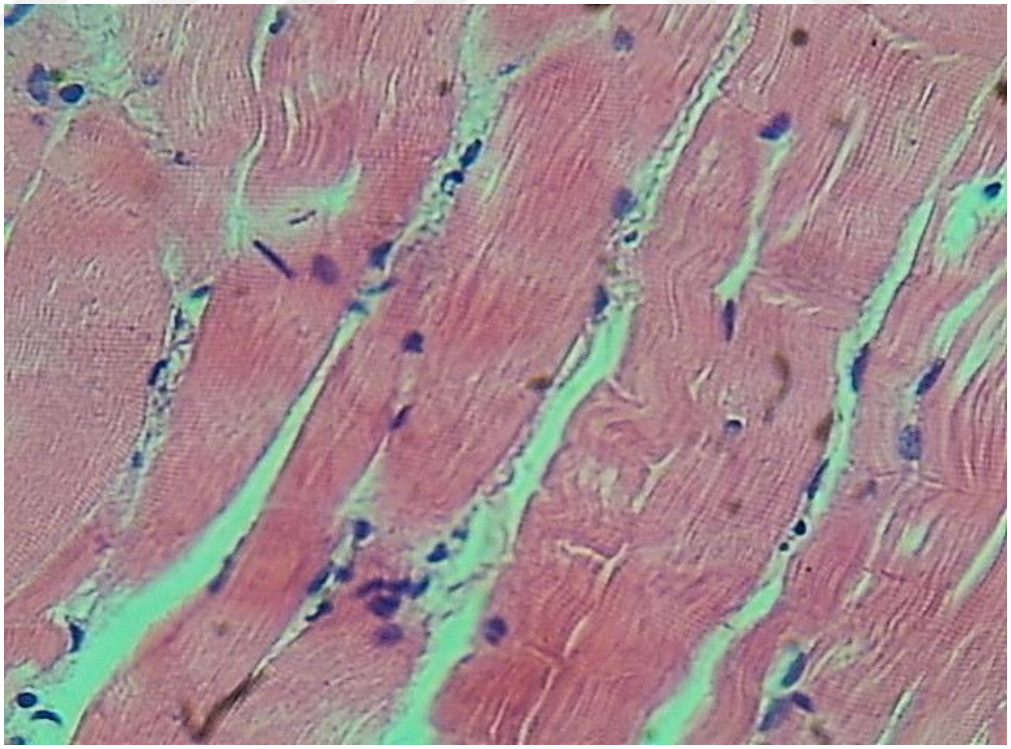


Figure 3.12. Section of skeletal muscle (Azolla 6%) shows normal appearance of myofibers & endomysium loose connective tissue. H&E.400x

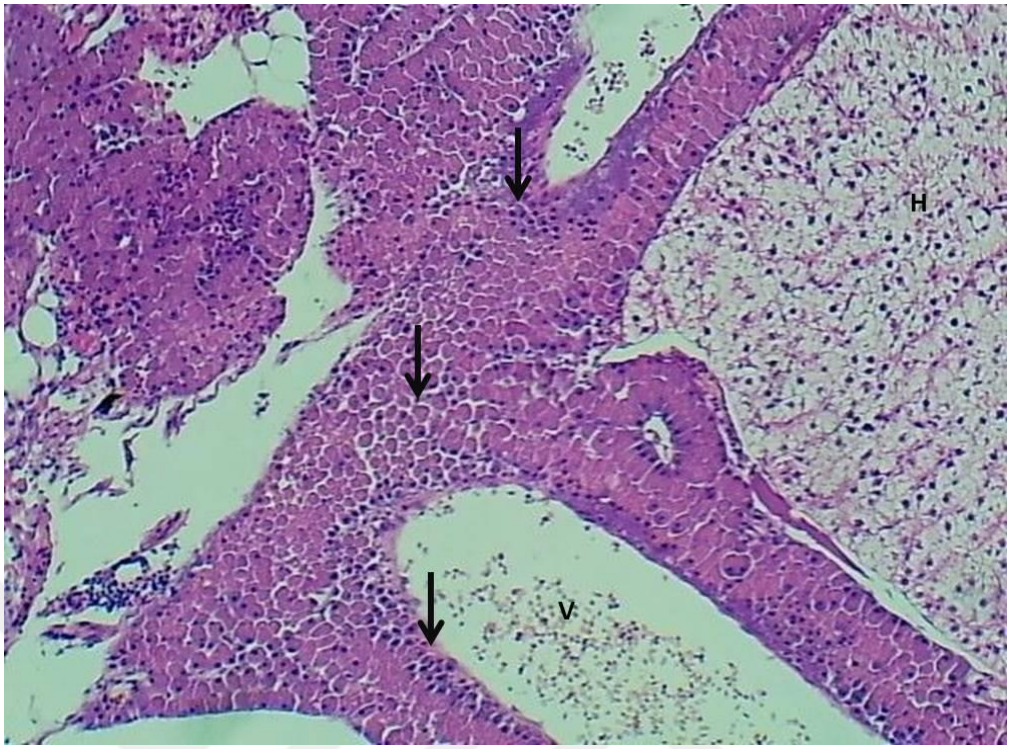


Figure 3.13. Section of liver and pancreas (Azolla 12%) shows marked non plastic hyperplasia of acinar cells of pancreas (arrows) with marked massive venous dilation (V) & normal hepatocytes (H).H&E.100x

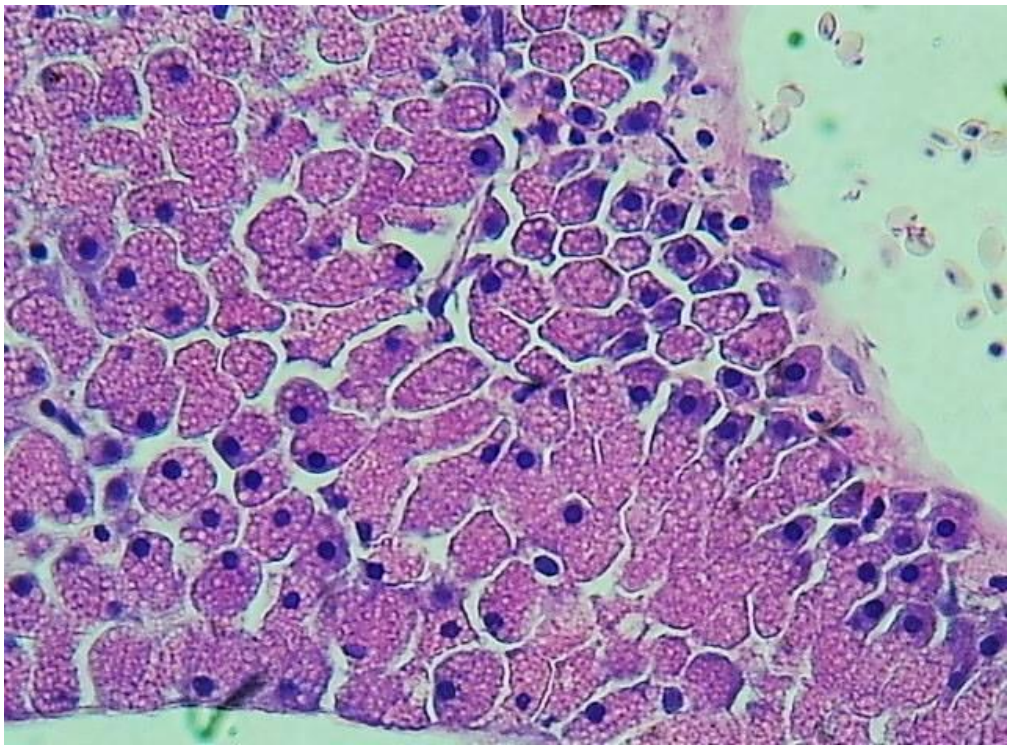


Figure 3.14. Section of liver and pancreas (Azolla 12%) shows marked non plastic hyperplasia of acinar cells of pancreas with marked hyperactive cells .H&E.400x

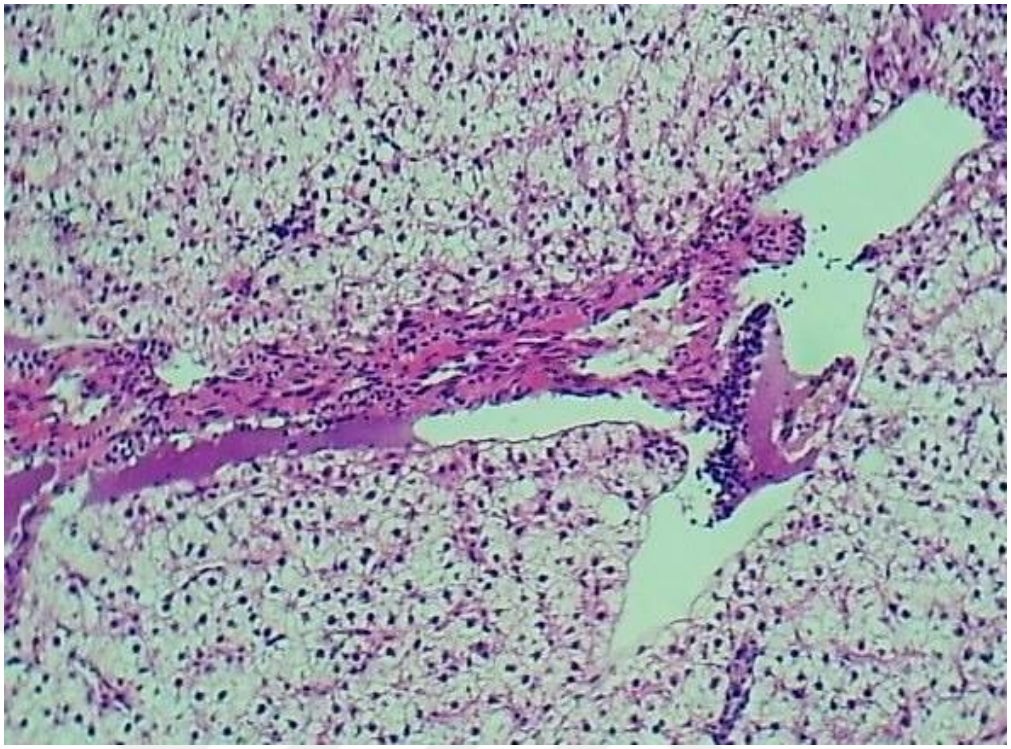


Figure 3.15. Section of liver and pancreas (Azolla 12%) shows mild hepatic fibrosis with portal aggregation of mononuclear leukocytes.H&E.100x

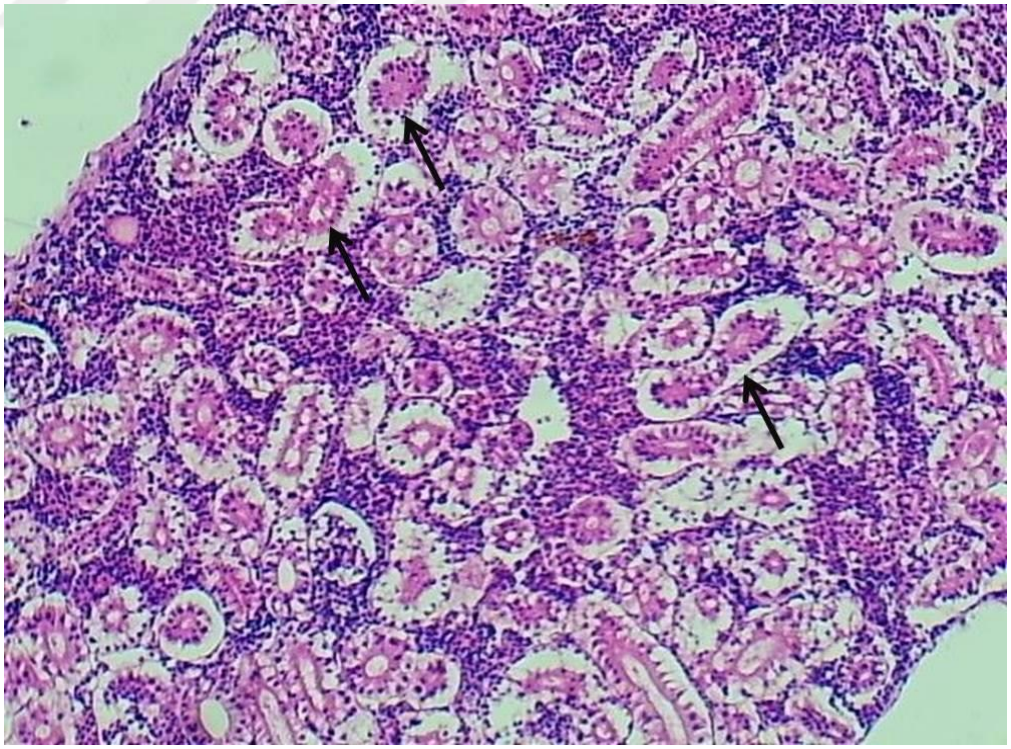


Figure 3.16. Section of kidney (Azolla 12%) shows marked cellular swelling of renal tubule with lining cells desquamation (arrows) with normal glomeruli & hemopoietic tissue (H).H&E.100x

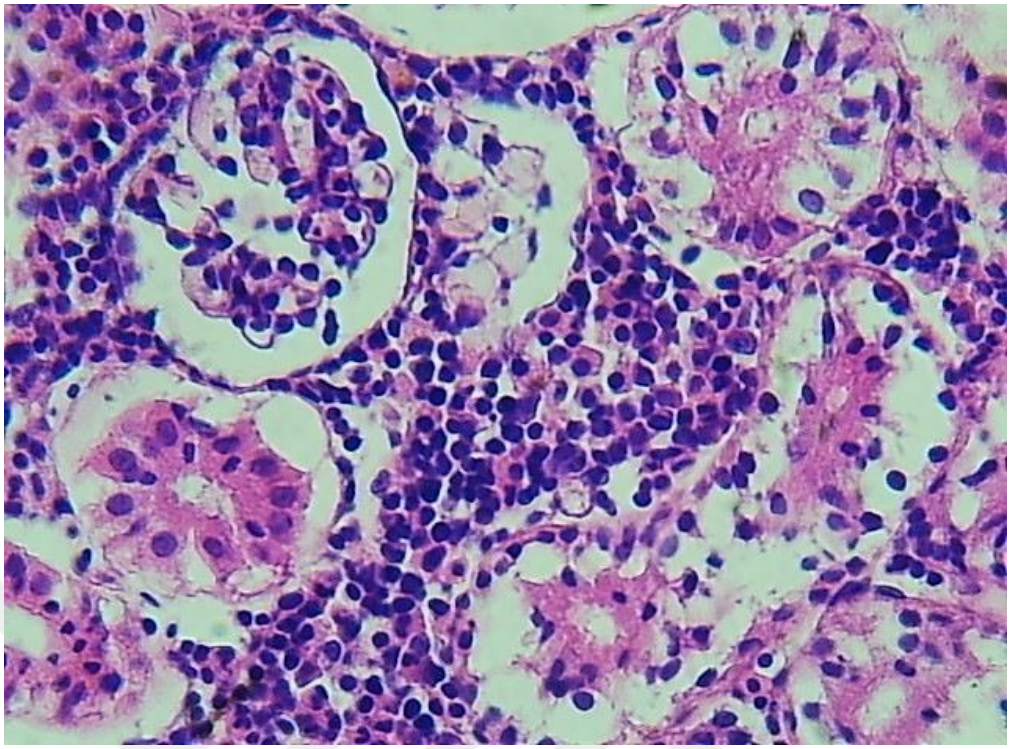


Figure 3.17. Section of kidney (Azolla 12%) shows cellular swelling of renal tubule with lining cells desquamation and normal glomeruli & hemopoietic tissue. H&E.400x

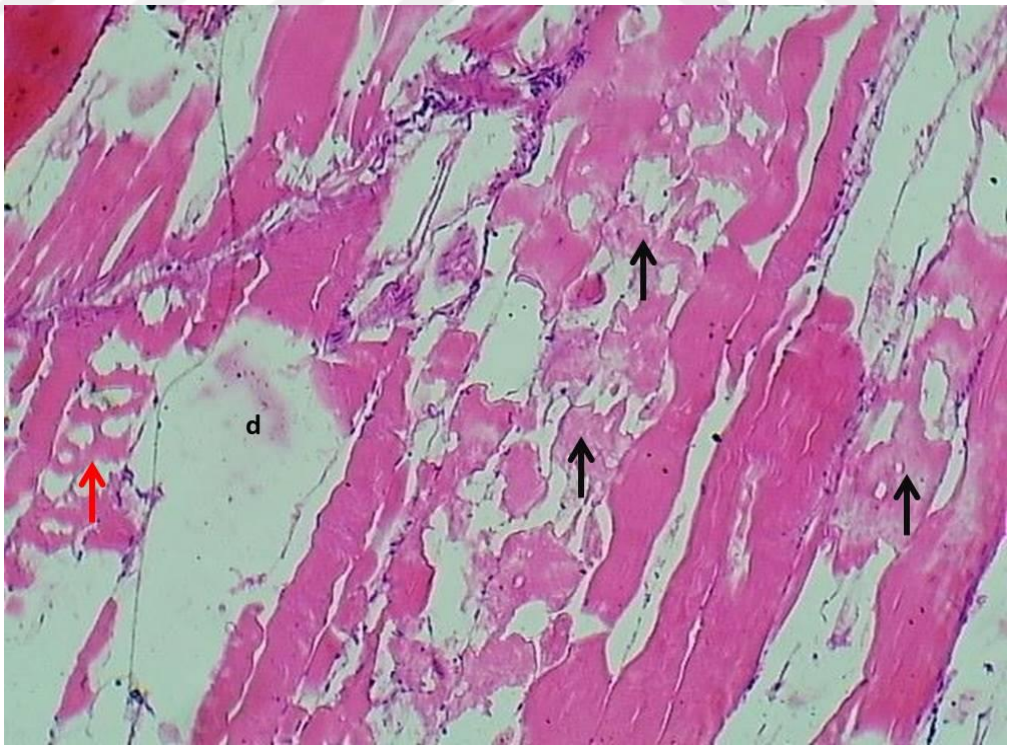


Figure 3.18. Section of skeletal muscle (Azolla 12%) shows marked muscular dystrophy associated with degeneration and necrosis of myofibers (black arrows), atrophy (Red arrow) and tissue depletion (d).H&E.100x



Figure 3.19. Section of liver and pancreas (BSFL 25%) shows marked non plastic hyperplasia of acinar cells of pancreas forming glandular cords like extensions (arrows) with marked massive venous dilation (V) & normal hepatocytes (H).H&E.40x

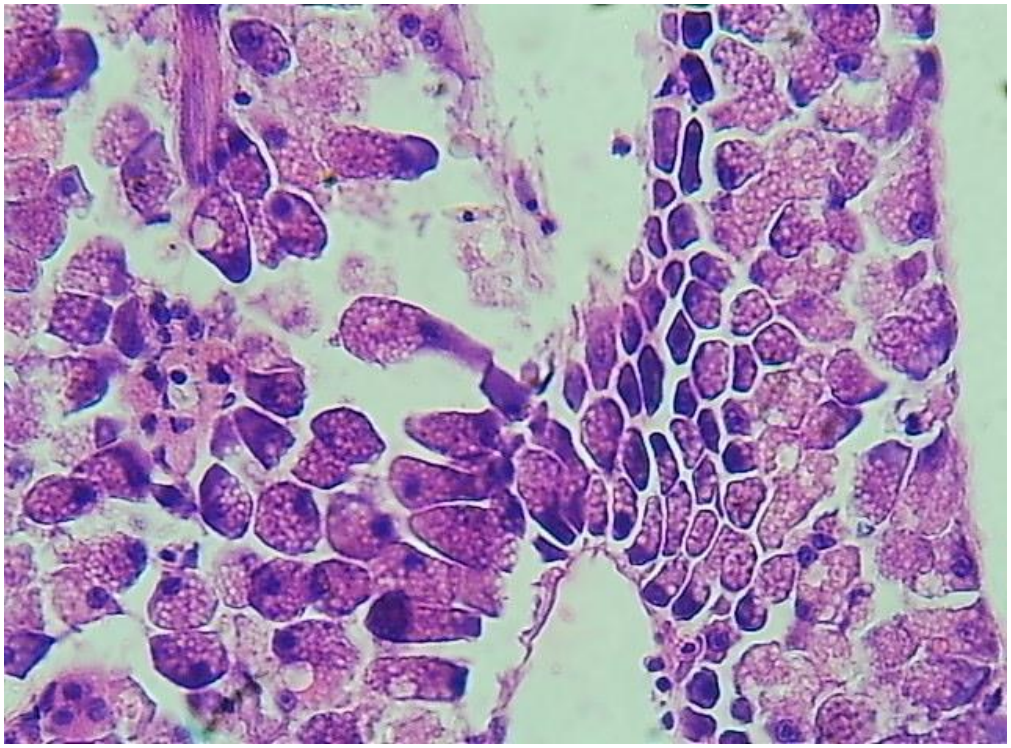


Figure 3.20. Section of liver and pancreas (BSFL 25%) shows marked plastic hyperplasia of acinar cells with marked variable shape and sizes.H&E.400x

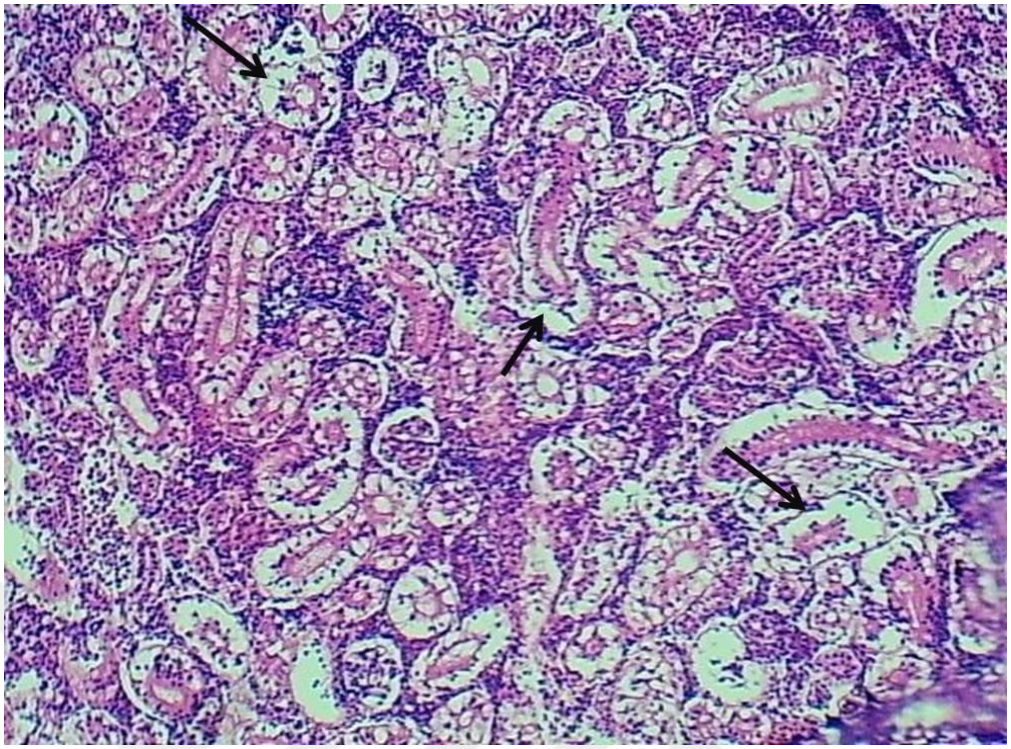


Figure 3.21. Section of kidney (BSFL 25%) shows mild cellular swelling of renal tubule with lining cells desquamation (arrows) with normal glomeruli & hemopoietic tissue.H&E.100x

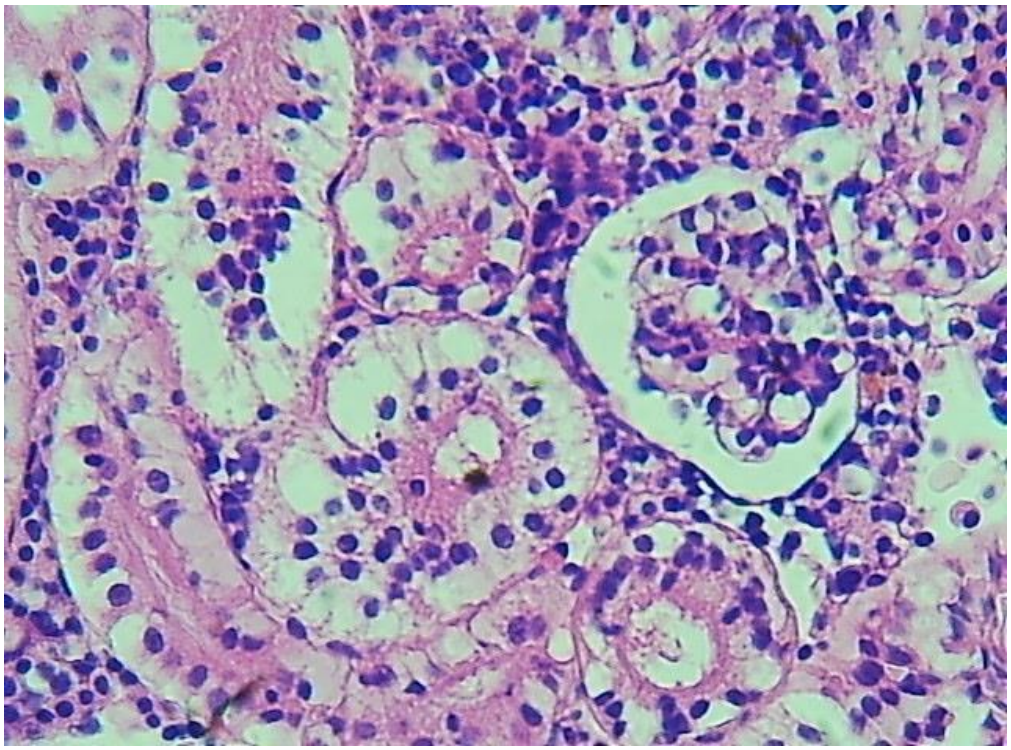


Figure 3.22. Section of kidney (BSFL 25%) shows mild cellular swelling of renal tubule with lining cells desquamation with normal glomeruli & hemopoietic tissue.H&E.400x



Figure 3.23. Section of skeletal muscle (BSFL 25%) shows disarrangement muscle myofibers with little atrophy of myofibers (Arrows) and tissue depletion.H&E.100x

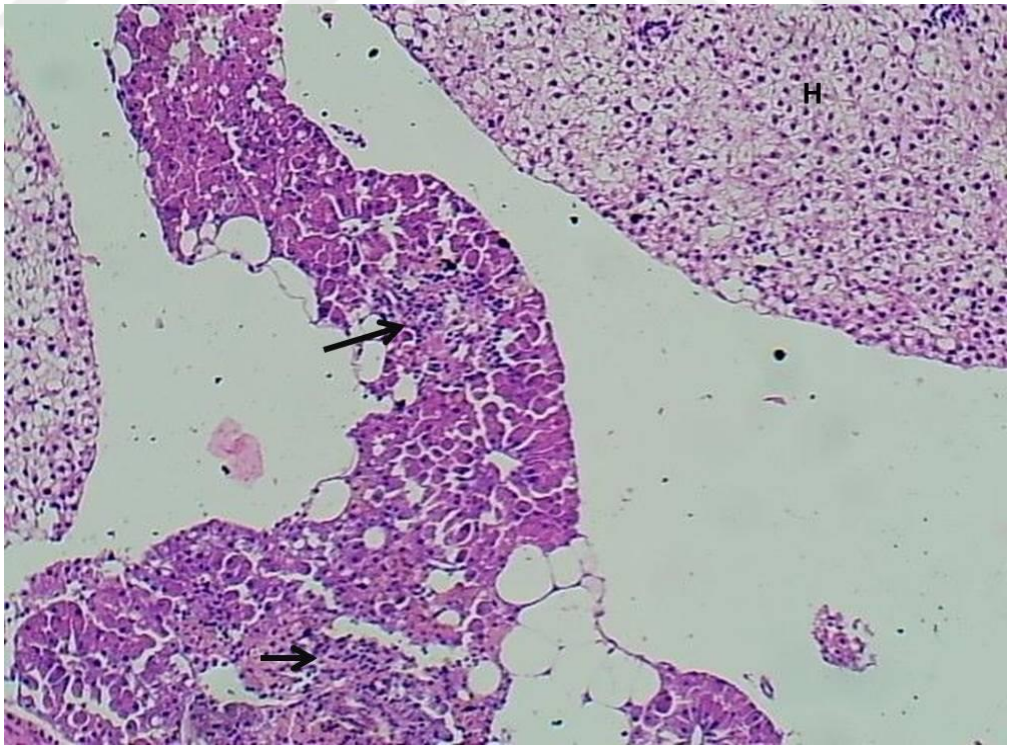


Figure 3.24. Section of liver and pancreas (BSFL 50%) shows marked hepatitis and mild hyperplasia of acinar cells of pancreas & infiltration of leukocytes (arrows).H&E.100x

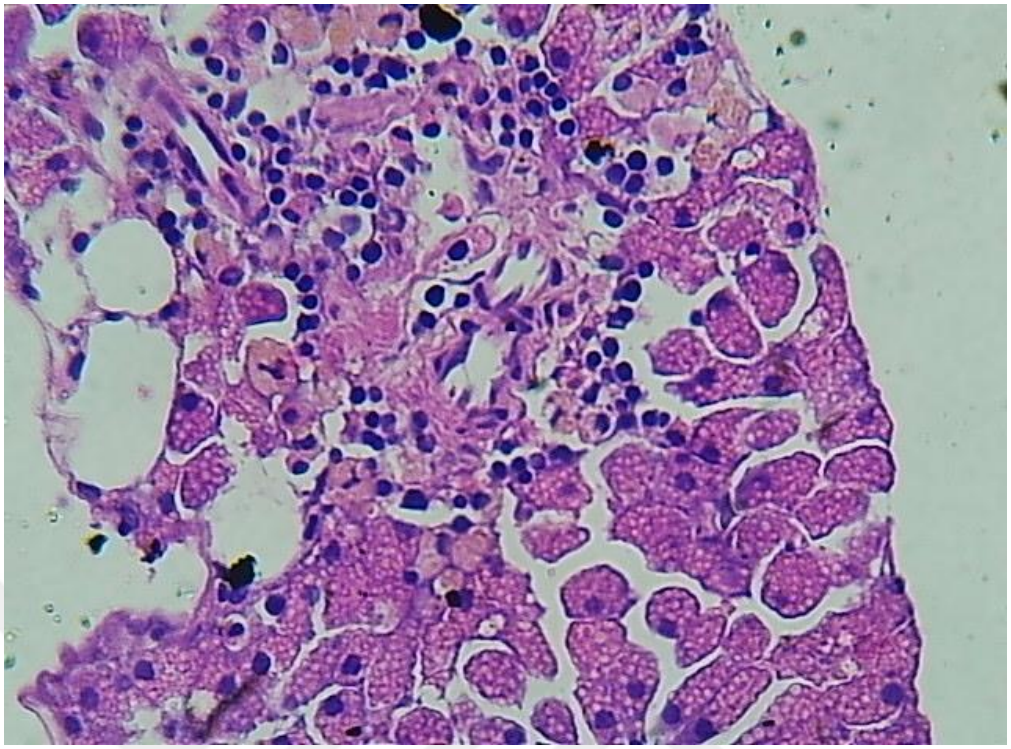


Figure 3.25. Section of liver and pancreas (BSFL 50%) shows perivascular infiltration of lymphocytes within pancreas.H&E.400x

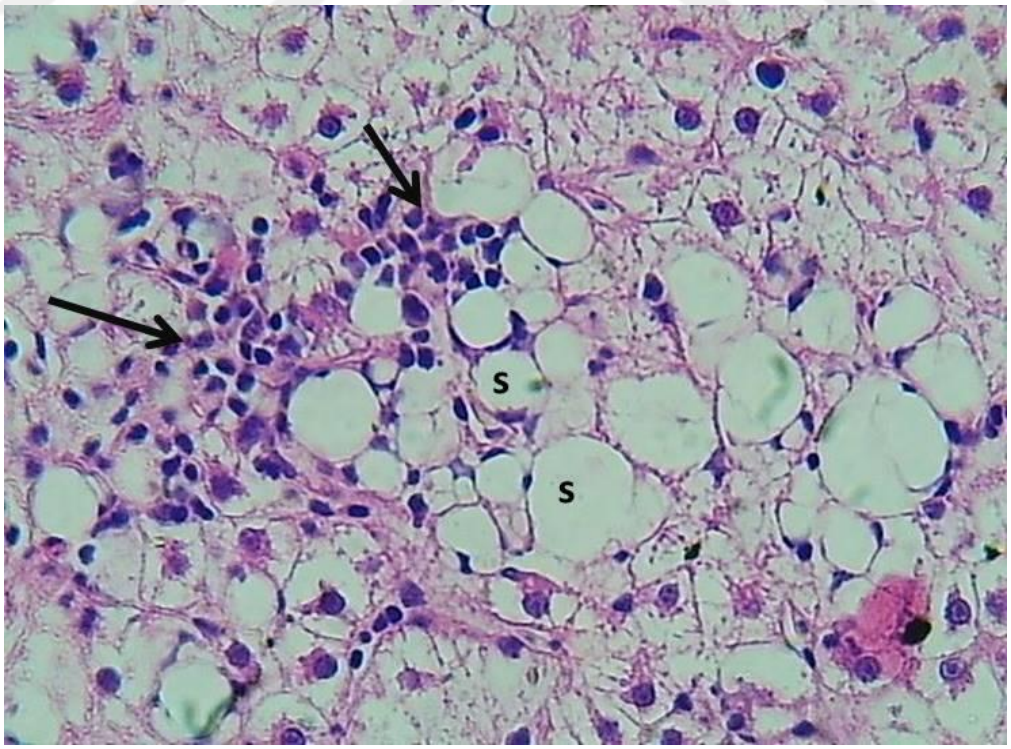


Figure 3.26. Section of liver and pancreas (BSFL 50%) shows marked hepatitis and mild hyperplasia of acinar cells of pancreas & infiltration of leukocytes (arrows).H&E.400x

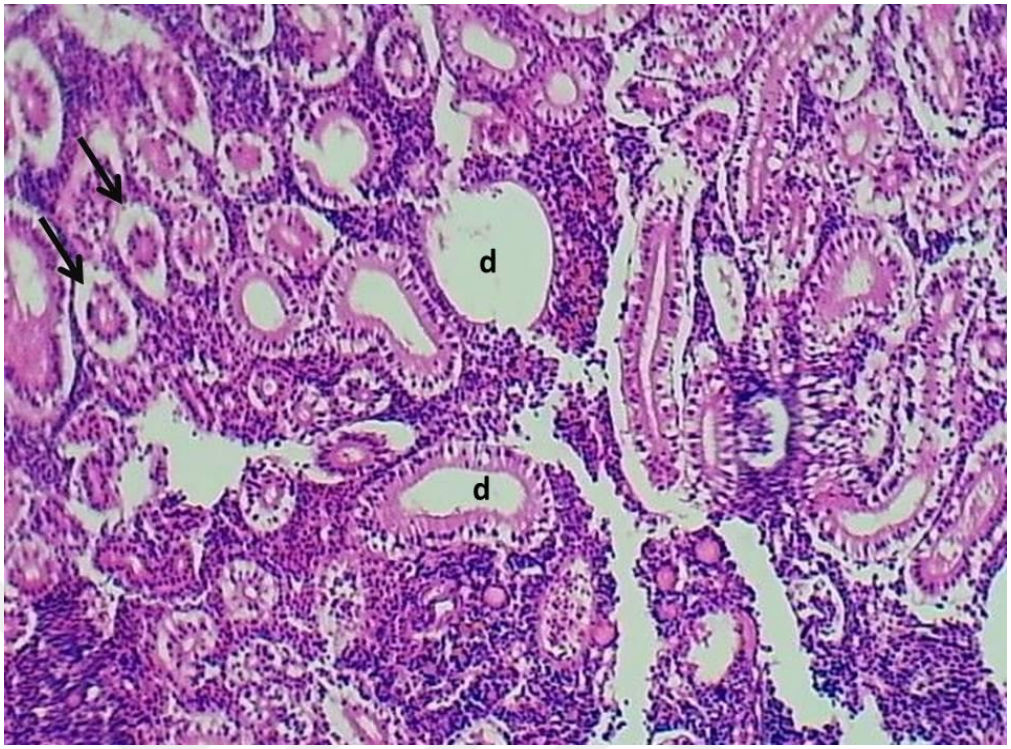


Figure 3.27. Section of kidney (BSFL 50%) shows little cellular swelling of renal tubule with lining cells desquamation (Arrows) & tubular dilation (d), normal glomeruli & hemopoietic tissue.H&E.100x

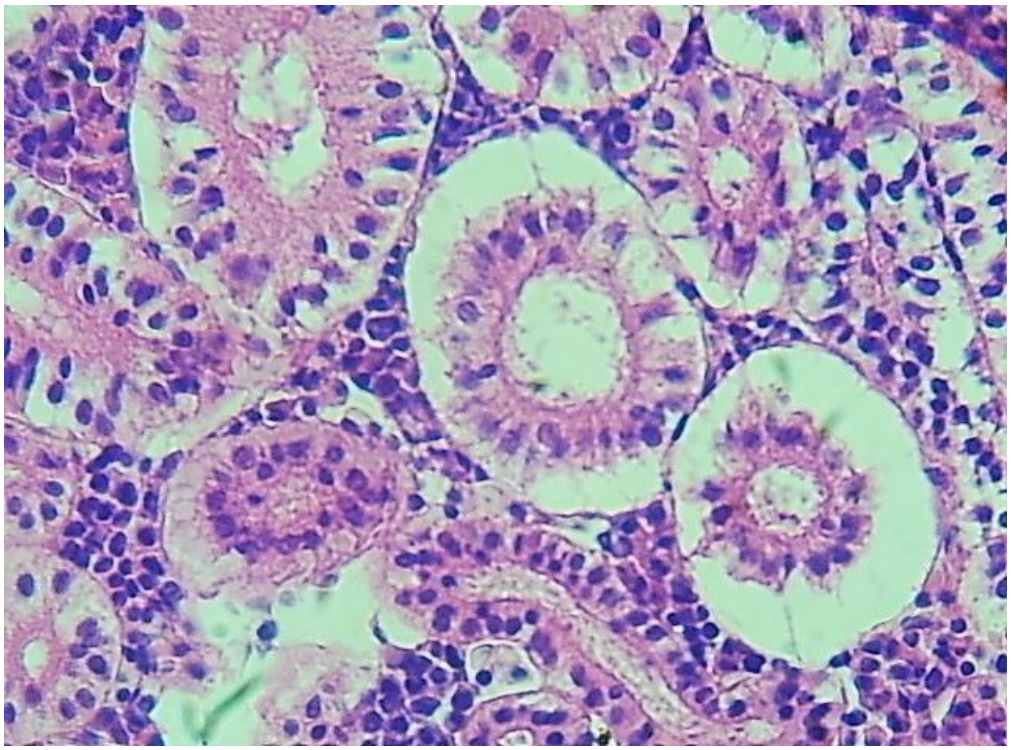


Figure 3.28. Section of kidney (BSFL 50%) shows mild cellular swelling of renal tubule lining cells desquamation with little figure of intra luminal occlusion by eosinophilic precipitate .H&E.400x

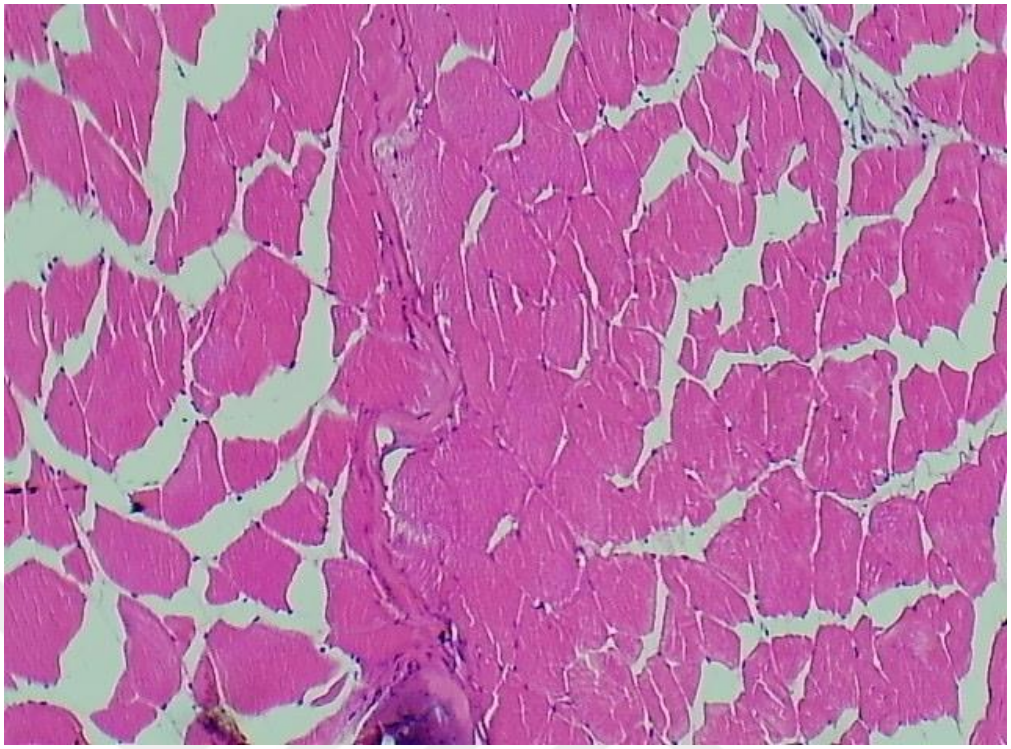


Figure 3.29. Section of skeletal muscle (BSFL 50%) shows normal appearance and arrangement of muscle myofibers.H&E.100x

CHAPTER 4

DISCUSSION

4.1. Body Weight, Weight Gain, Feed Intake, Feed Conversion Ratio of Fish in Azolla feeding

There were no statistically significant differences in body weight, feed consumption, and feed consumption ratio in this experiment. Ahmed et al. (2023a) reported that in a study conducted on carp, where it is recommended to include 10% of Azolla meal in practical diets for C. carpio var, as mentioned that increasing Azolla ratios may affect growth, body weight, FCR, and protein efficiency ratio of fish. This was confirmed by Can (2015) in a study, which stated no significant differences in the Azolla groups (20,30%). However, there were similar results to those of the current study, which indicated that up to 10% of fish meal protein could be replaced with an Azolla meal without any adverse effect on the growth performance of carp fish. Naji and alzurfi (2023) mentioned in his study that there was a decrease in the growth and weight of fish when using high levels of Azolla. However, low levels showed remarkable growth in groups of Azolla with low concentrations. This was confirmed by Gangadhara et al. (2015) in their study on the effect of including Azolla in the diet on the growth and survival of Laboe fimbriates fish, where he found that there are no statistically significant differences in weight, weight gain, and FCR for fish fed on Azolla percentages, except that it can be included in the diet to reduce costs. Al-dubakel et al. (2021) In his study on the partial replacement of fishmeal with Azolla meal on grass carp fish, he indicated that Azolla does not affect growth parameters. Replacing 15% of the fish meal with Azolla meal is recommended in a different study conducted by Kamil and Taha (2022) that concluded that the mixture (Azolla and lentils) at a rate of 5% can be used as an alternative to soybeans as the best criterion for the growth of common carp. Scientific evidence suggests that food

supplements and additives can enhance fish immunity and help promote fish health (Ogunkalu, 2019).

4.2. Body Weight, Weight Gain, Feed Intake, and Feed Conversion Ratio of Fish in Black Soldier Fly Larvae Feeding

The current study investigated the effect of replacing soybean meal with black soldier fly larvae meal at a ratio of 25% and 50% on fish. It had positive effects on the final weight of common carp, feed intake, weight gain, and FCR (Hu et al., 2023). Black soldier fly larvae contain a high percentage of nutrients needed by fish (Lu et al., 2022). The chemical composition of black soldier fly larvae varies depending on the substrate on which the larvae are reared (Zandi-Sohani and Tomberlin, 2024). Insect meal is a rich source of protein and fat, consumes decomposing organic waste, and has characteristics that have increased interest in its use in animal feed (Mannaa et al., 2024). The results of the current study show that using black soldier fly larvae instead of soybean meal had positive effects on weight, feed intake, FCR, and final weight. This is what Musoni et al. (2023) reported in their study, indicating that the best results in weight gain and specific growth rate of Nile tilapia among the different diets were achieved when replacing soybean meal with black soldier fly larvae. 50% of the soybean meal was supplemented with black soldier fly larvae. The current study is similar to Shafaq et al. (2023) study on rohu (*Labeo rohita*) and catla (*Catla catla*) juveniles, where growth and FCR were significantly higher on the feed supplemented with black soldier fly larvae compared to the control group, with catla fish exhibiting slightly higher growth indicators. Singha et al. (2025) indicated that adding 5% whole black soldier fly larvae to the feed was superior to all other dietary treatments in terms of growth for trout. Wang et al. (2025) reported that replacing 50% of the fish meal with black soldier fly larvae had advantages for juvenile southern catfish (*Silurus meridionalis*) in terms of specific growth rate, weight gain, and protein efficiency ratio ($p < 0.001$). In a contrasting study on European sea bass, Abdel-Tawwab et al. (2020) reported no statistically significant differences in fish growth, feed utilization efficiency, or survival rate ($P > 0.05$). However, it can be used to reduce production costs. The current results are similar to a study on red hybrid tilapia, where FCR and growth performance were superior in the experimental group fed black soldier fly larvae at a ratio of 30% and 20%, respectively, compared to the other groups (Aisyah et al., 2022). The improved growth of fish fed black soldier fly larvae in their

diet can be attributed to the naturally occurring protein and fat content of these larvae (Aristi et al., 2020). Black soldier fly larvae are comparable to fishmeal or soybean meal, making them an excellent feed source for fast-growing fish (Y. Chen et al., 2025). Furthermore, black soldier fly larvae contain polyphenols, beneficial fatty acids, and other compounds that support antioxidant activity, immunity, and gut lining health (Alhasyani et al., 2025).

4.3. Behavioral Responses of Fish in Azolla Feeding

In this study, there were no differences in daily behaviors except for significant differences in activity before feeding, as the experimental groups' fish were constantly searching for food and in dynamic movement. The results indicated that the response to feed, as found in the groups fed on Azolla, was higher (91.77±0.65a, 89.84±0.44b), respectively. This is what Al-Dubakel et al. (2020) indicated in a study on crass carp, which had no significant effect on daily behaviors, responses, and activities. However, the experimental units fed on Azolla had substantial differences in their response to feeding, which may be related to the nature of the taste and the structure of the leaves. This is consistent with the current study, as the results indicated a high response of fish to the feed provided to the experimental groups containing Azolla in their diet (Kamil and Taha, 2022a). Das et al. (2018) referred to experimental fish containing Azolla in their diet as having higher pre- and post-feeding activity and behavioral responses than the control group. Still, there were no statistically significant differences between the groups. There were slight differences in the survival rate; however, there were no statistical differences. The survival rate in the groups fed Azolla in their diet was higher than that of the control group, as no deaths were recorded in the Azolla 6% diet group. This was confirmed by Nurhidayati et al. (2022) in the study on the effect of fermented Azolla on the growth and survival of carp fish, as he stated that the use of Azolla and its inclusion in the carp diet at a rate of 50% improved the survival rate more than at high rates. This is what Ahirwal et al. (2024) pointed out in their study on grass carp fish, where he mentioned that the survival rate was higher and statistically significant ($p < 0.05$) in the group containing Azolla in its diet. Ahmed et al. (2023a) mentioned that the survival rate was significantly higher in the group fed with Azolla at 50% or less. This indicates that replacing part of the fish meal with Azolla did not negatively affect the survival of carp fish (Can, 2015). While Ismail et al. (2022) discussed the improving effect of the level of

inclusion of Azolla fermented by *Bacillus subtilis* in Nile perch feed and focused on several aspects related to fish health, where the results were positive in terms of enhancing fish health and improving the survival rate at the 5 and 15% Azolla content in the diet (Yohana et al., 2023), where he found an increase in the effectiveness of immunity, antioxidants, resistance to pathogens and digestive enzymes. Moreover, Azolla pinnata has been proven effective in improving the growth and health of grouper fish by improving the survival rate, as it can be a good choice in fish feeding and enhances performance in rearing conditions (A. Ibrahim et al., 2021).

4.4. Behavioral Responses of Fish in Black Soldier Fly Larvae Feeding

The present investigation revealed a beneficial impact of varying levels of black soldier fly larvae on fish behavior, specifically indicating enhanced survival rates and feed palatability when black soldier fly larvae were incorporated. Hamza et al. (2025) found that black soldier fly larvae powder serves as a valuable protein and fat source for juvenile Nile tilapia, with the potential to fully substitute fishmeal in their diet, concurrently promoting improved growth performance. Kariuki et al. (2024) stated that black soldier fly larvae powder can replace fishmeal (FM) in Nile tilapia diets without negatively impacting fish growth, while simultaneously increasing profitability. This finding was supported by a study conducted on juvenile Nile tilapia using diets in which fishmeal protein was partially or completely replaced with black soldier fly larvae powder, and behavioral tests were performed to determine feed selection (Younas et al., 2025). The fish showed significant differences in their preferences between diets with varying levels of black soldier fly larvae powder, indicating that feeding behavior changes depending on the percentage of this powder added (Oliveira et al., 2024). The current results are consistent with those of a study on European sea bass, which concluded that partial replacement of fishmeal protein in the diet with up to 50% black soldier fly protein (BSFLM) is effective without adverse effects on fish health (Abdel-Latif et al., 2021). In a contrasting study on striped catfish, there was a weakening of the various responses when the fishmeal replacement percentage with BSFLM was increased to 100%, leading to a significant decrease in growth and feeding efficiency in the striped catfish (Sudha et al., 2022). Chivite et al. (2025) observed that Atlantic salmon fry consuming diets with reduced insect powder demonstrated more natural feeding patterns, whereas those receiving higher insect powder concentrations displayed feeding aversion. The enhanced

behavioral profile of fish fed black soldier fly larvae meal can be ascribed to its elevated protein content and the presence of essential amino acids, which are crucial for growth and energy production. Consequently, this results in improved feeding behavior and increased feed intake (S. Lu et al., 2022). Black soldier fly larvae contain high levels of fat, which provides fish with energy and flavor (Rawski et al., 2020). The fat content makes the feed more palatable and energy-dense, contributing to increased voluntary feed consumption and positive feeding responses (Su et al., 2025). Antimicrobial peptides (AMPs), lauric acid, and chitin are among the components of black soldier fly larvae meal (BSFL). These components promote gut health and immune function, helping fish manage stress and maintain their natural behavior patterns (Radhakrishnan et al., 2023). Chitin, a polysaccharide found in black soldier fly larvae, has the potential to produce bioactive effects at low concentrations. This could improve gut health and reduce discomfort or inflammation (Alhasyani et al., 2025). As a result, better gut health might lead to increased survival and activity, which then affects overall health and consumption habits (Dîrvariu et al., 2025).

4.5. Length and Width of Fish in Azolla Feeding

This study investigated the effect of including Azolla in fish diets at 6% and 12% levels on morphometric characteristics, specifically body length and width. Statistical results showed no significant differences between the different treatments in morphometric measurements, either at the beginning or end of the experiment. The absence of significant differences in initial length and width values reflects the homogeneity of the experimental fish, thus enhancing the reliability of the results and confirming that any subsequent growth responses are primarily attributable to the applied dietary treatments, rather than to initial differences between groups. This finding aligns with the results of Refaey et al. (2023) study on Nile tilapia, which examined the effects of Azolla as a dietary supplement. At the conclusion of the feeding trial, all experimental groups demonstrated anticipated and typical increases in both body length and width, thereby suggesting that the prevailing environmental parameters—specifically water quality, fish density, and temperature—coupled with the overall dietary composition, were conducive to normal fish development. While a marginal trend towards greater final length and width measurements was noted in the groups receiving Azolla-enriched diets relative to the control group, Sallam et al. (2024) observed that these enhancements did not achieve

statistical significance. Comparable findings have been documented in recent investigations involving diverse fish species, which indicate that moderate Azolla supplementation facilitates normal growth without inducing substantial morphometric alterations (I. Ahmed et al., 2023b; Carnegie et al., 2023). (Sallam et al., 2024) further observed that substituting fishmeal with Azolla in the diets of red tilapia did not produce notable alterations in fish length, even when supplementation levels were comparatively elevated, assuming the diet was well-balanced and fulfilled the fish's protein and energy needs. A recent investigation involving carp fry Bharti et al. (2024) revealed that modifications to plant-based diet components did not yield significant disparities in final length or width, notwithstanding fluctuations in body composition and fat content. This research corroborated that weight serves as the most sensitive indicator of dietary alterations, whereas morphometric characteristics remained within the typical growth parameters. The absence of significant morphometric differences between treatments is attributed to the nutritional and physiological properties of Azolla (Abdelatty et al., 2021). Despite its good protein and some mineral content, Azolla has relatively high levels of crude fiber, in addition to anti-nutritional compounds such as tannins and phytic acid (Yohana et al., 2023). Some recent studies have indicated that Azolla components may reduce the efficiency of nutrient digestion and absorption, thus limiting the energy available for linear body growth, especially when its inclusion in the diet is increased (Naji and alzurfi, 2023). In this context, recent studies suggest that body length and width, which are related to shape, are less affected by changes in diet than indicators of weight gain. This is especially true when the initial diet is balanced and meets the nutritional needs of the fish species being studied (Abu-Zahra et al., 2025; Ismail et al., 2022).

4.6. Length and Width of Fish in Black Soldier Fly Larvae Feeding

The present investigation assessed the effects of replacing soybean meal with black soldier fly larvae at 25% and 50% inclusion levels on the growth metrics of common carp (Cyprinus carpio), specifically their length and width. The findings revealed no statistically significant variations in initial length and width across the experimental groups, thereby confirming that the observed growth modifications were primarily linked to the dietary treatment, rather than any pre-existing size differences. This observation suggests a considerable degree of uniformity in fish sizes at the outset of the experiment. This consistency is crucial in fish nutrition research because it ensures that any observed

growth changes are due to the dietary changes, rather than differences in size that existed before the experiment. Recent studies on common carp and its various subspecies (such as mirror carp and koi) have underscored the importance of standardizing initial morphometric measurements when evaluating alternative protein sources (Linh et al., 2024; Xu et al., 2020). Studies of the growth of common carp and other carp species have yielded similar results to those of Tacon and Metian (2015), who stated that uniformity of initial size is an important factor for accurately assessing the effects of different feeds. At the end of the experiment, common carp fed diets containing black soldier fly larvae (BSFL) showed a significant increase in final body length and width compared to the control group ($P < 0.05$). Fish fed a diet containing 25% BSFL exhibited marked improvements in morphometric parameters, whereas the inclusion of 50% BSFL produced a less substantial effect. This observation suggests that a moderate BSFL inclusion is particularly beneficial for promoting elongation. The enhanced growth of carp and other fish species may be attributed to the high protein content, the balanced essential amino acid profile, and the distinctive lipid composition found in black soldier fly larvae. These characteristics collectively contribute to improved bone and muscle development in these aquatic organisms (Belghit, Liland, Gjesdal, et al., 2018; Henry et al., 2015; Makkar et al., 2014; Seyedalmoosavi et al., 2021). These results are consistent with the study of Jahan et al. (2021), which investigated the incorporation of BSFL in diets for common carp fry, demonstrating that moderate replacement levels facilitate optimal growth while preserving the integrity of body structure. In a contrasting study on koi (*Cyprinus carpio var. koi*), the inclusion of BSFL in the diet improved overall growth and feed efficiency without negatively impacting body dimensions or shape (Linh et al., 2024). Vargas et al. (2018) research investigated the impact of feeding zebrafish diets formulated from black soldier fly (*Hermetia illucens*) larvae. The study's findings indicated that these diets did not induce morphological deformities or any observable growth-related abnormalities in the zebrafish subjects. The reduced clarity concerning the beneficial impact observed at the 50%BSFL replacement level can be attributed to a range of physiological and nutritional influences. A key factor is the increased chitin concentration within black soldier fly larvae; this structural component is not fully assimilable by bony fish (Muslimin et al., 2023). Consequently, the inclusion of this substance in the diet might potentially diminish the digestibility of both protein and energy (Abenaim and Conti, 2025). Belghit et al. (2018b) attributed this to the fact that

increased chitin content in insect-based diets may limit the energy available for skeletal growth, even if survival rates and overall fish health are not affected. Xu et al. (2020) confirmed the current findings, indicating that high levels of BSFL components in mirror carp diets may affect feed efficiency, even if they do not cause a sharp decrease in growth or survival rates.

4.7. Carcass Traits of Fish Fed with Azolla

There was a clear response in the experimental groups receiving Azolla supplementation with respect to carcass weight. These findings are in agreement with Yohana et al. (2023), who reported that dietary inclusion of Azolla can enhance muscle deposition in fish, thereby contributing to improved carcass weight and meat quality. The increase in carcass weight may be attributed to the nutritional value of Azolla, particularly its protein content and bioactive compounds that support muscle growth. In this context, Abdel-Tawwab (2008) reported an increase in carcass moisture content in fish fed Azolla-based diets, while recommending that the optimal inclusion level of dried Azolla meal should not exceed 25% in tilapia diets to avoid potential negative effects on nutrient utilization. Similarly, previous studies have demonstrated that incorporating Azolla at levels ranging from 10% to 50% in diets of fish belonging to the *Cyprinidae* family positively influences growth performance, carcass characteristics, and protein conversion efficiency (Magouz et al., 2020; Mosha, 2018). Regarding hot and cold carcass traits, as well as the difference between them, no significant differences were observed between the Azolla-fed groups and the control group. This finding is consistent with earlier reports indicating that Azolla supplementation does not markedly affect hot or cold carcass yields, despite its positive influence on overall growth performance (Baldawy and Mohammad, 2022; Magouz et al., 2020). These results suggest that the observed improvements in carcass weight are primarily associated with increased muscle growth rather than changes in post-slaughter carcass losses. Moreover, several studies have highlighted the broader physiological benefits of Azolla supplementation, including enhanced growth performance, improved feed utilization, strengthened immune responses, and better intestinal morphology in fish, which collectively contribute to improved carcass traits and overall productivity (Refaey et al., 2023; Yohana et al., 2023).

4.8. Carcass Traits of Fish fed with Black Soldier Fly Larvae

The findings of this investigation revealed that substituting a segment of the standard feed with 25% and 50% black soldier fly larvae did not yield statistically significant variations ($P > 0.05$) across the examined carcass attributes. These included carcass weight, hot and cold weights, hot and cold carcass ratios, and the percentage of carcass loss. Consequently, these outcomes suggest that the incorporation of BSFL at these concentrations does not adversely impact meat settling efficiency or the overall quality of the carcass in common carp. The lack of significant differences in carcass weight suggests that insect protein sources, particularly BSFL, are capable of supporting normal muscle growth in fish in a manner comparable to conventional protein sources. These results are consistent with those of (Belghit, Liland, Gjesdal, et al., 2018), who demonstrated that replacing fishmeal with BSFL in Atlantic salmon diets did not significantly affect lean meat production, despite some minor changes in body composition. These findings also align with those of (Gebremichael et al., 2021), who confirmed that adding up to 50% black soldier fly larvae to carp diets maintains carcass productivity and quality without adverse physiological effects. Furthermore, the lack of impact on hot and cold carcass weight and its ratios reflects the stability of physiological processes related to tissue water retention after slaughter and chilling. This indicates that the inclusion of BSFL did not affect muscle tissue integrity or its physical characteristics. Wang et al. (2025) reported similar results when using ingredients derived from black soldier fly larvae in mirror carp diets. No significant differences were observed in carcass quality indicators, although some growth indicators were improved (Xu et al., 2020). The absence of statistically significant disparities among the treatments concerning carcass loss suggests that the incorporation of BSFL did not adversely impact tissue integrity or the efficacy of the slaughtering and chilling procedures (Majeed et al., 2025). This finding holds economic significance, given that the rate of loss is directly correlated with production yield (Saidani et al., 2025). Gasco et al. (2018) corroborated that the application of insect-based feed in fish diets frequently yields minimal effects on carcass attributes, even when alterations are evident in certain metabolic or weight-related metrics. The lack of significant differences in carcass characteristics can be ascribed to the relative insensitivity of these traits to dietary modifications, in contrast to productive performance indicators like final weight or specific growth rate. As long as the diet is balanced in terms of protein and energy, the distribution of muscle and fat tissue remains

within the normal range. Studies indicate that the quality of fish flesh generally remains stable when different protein sources, like insect proteins, are used, especially when added in moderate amounts (Copelotti et al., 2025; Henry et al., 2015; Li et al., 2017). The lack of impact on dressing percentages can also be explained by the absence of significant changes in visceral or head weight compared to total body weight, as confirmed by Jahan et al.'s findings when using black soldier fly larvae meal as a fishmeal replacement in carp fingerling diets (Jahan et al., 2021). In contrast, in a different study Herawati et al. (2023), a decrease in carcass percentage occurred when using black soldier fly larvae oil, and they attributed this to an increase in visceral fat deposition, which led to a decrease in carcass efficiency. This difference is attributed to the difference in the form of the material used (oil versus whole larvae or powder), the fat level in the diet, and the duration of the experiment (Srikha et al., 2024). On the other hand, although BSFL contains chitin, a component that may reduce protein and energy digestibility at high levels, the results of this study indicate that the inclusion rates used (25% and 50%) did not reach a level that negatively affected meat settling or carcass characteristics (Mikołajczak et al., 2022). This is consistent with the findings of Kroeckel et al. (2012), who observed that the potential negative effects of chitin first appear in growth indicators and feed efficiency before impacting carcass quality or survival.

4.9. Chemical and Physical Properties of Meat Quality in Azolla Feeding

The results indicated significant differences in the chemical composition of fish meat concerning dry matter, protein, fat, and carbohydrates (34.91±0.30, 17.88±0.56, 4.10±0.14, 12.41±0.38) except for ash, where no significant differences were found, as the highest percentages were in the groups fed a diet containing Azolla. These results are consistent with the results of (I. Ahmed et al., 2023a; Al-Aaraji and Taha, 2020), who indicated in their study the use of multiple concentrations of Azolla for common carp fish and stated that the best percentage for including Azolla in the diet is 10%, as he found that there were significant differences between the experimental groups for protein, fat and moisture content of the meat compared to high concentrations of systems containing Azolla (Elrasoul et al., 2020). (Abdelatty et al., 2020) indicated that increasing the concentration of Azolla in the diet negatively affects the composition of the meat. This is in agreement with the study of Ahirwal et al. (2024) that there were significant differences in protein and fat content in carp fish meat. The study suggested that growth performance

and protein content in fish carcasses can be achieved using a diet containing 30% Azolla meal. The results conclude that using Azolla as a feed additive in carp fish diets may improve fish's demographic and health performance and thus positively affect the meat content. This is attributed to Azolla being a good source of amino acids, minerals, and vitamins (Chekol et al., 2024; Kamil and Taha, 2022b; M. Kumar et al., 2018). There were no significant differences in the ash level between the experimental groups for fish meat, as stated by Carnegie et al. (2023) in their study of Azolla filiculoides as a supplementary source of nutrition in the intensification of environmental agriculture in the culture of small fish, with a focus on the biological effects on gourami fish, as the study noted a positive impact on the composition of the meat in terms of increasing the protein percentage and improving the fat content, which makes the meat more functional and highly beneficial (Goswami et al., 2022). As mentioned earlier, there were significant differences in the percentage of carbohydrates, as the control group was superior to the Azolla groups, which indicates that the increase in fish meat in terms of excess carbohydrates may lead to a change in the nutritional balance of fish meat, which reduces the content of proteins and healthy fats (Kamalam J and Panserat, 2016). Studies have stated that one of the causes of inflexibility in physiological and digestive processes, hormonal differences, and the resulting metabolism of glucose clearance from the blood is due to the poor application of carbohydrate-rich diets in carnivorous fish (Karasov and Douglas, 2013; Nakrani et al., 2025). There was no significant effect on the pH scale of fish meat in the experimental food groups, as shown in Table 4. The results were consistent with the study of the qualitative characteristics of the most consumed fresh fish species in Saudi Arabia, as the results showed significant differences between all studied fish species in pH at $P>0.05$. However, there were no significant differences in the quality of the fish (Boukrouh, 2025; Elshehawy et al., 2016; Lougovois, 2005). This is consistent with previous studies, as the pH value of fresh fish is less than 7, but after cooling, the pH begins to decrease, which means that the fish is fresh and of unacceptable quality. On the other hand, if the pH value is higher or tends towards 7, it begins to decompose and spoil (K. A. Abbas et al., 2008; Kurek et al., 2024; Sari et al., 2020). Table 4 shows significant differences in the percentages of cooking and melting losses, as the control group recorded the highest percentages ($P<0.05$) in cooking and melting losses (4.07 ± 0.31) (3.66 ± 0.15) respectively, compared to the groups that contained diets supplemented with Azolla at a rate of 6% and 12% (3.45 ± 0.19) (3.05 ± 0.20) and

(3.23±0.14b) (3.08±0.11) respectively. This indicates that the cooking and melting losses in the groups that contained Azolla in their diet were better than the control group (Vahedi et al., 2021), i.e., they contain a low percentage of water in the tissues of fish meat. These results are consistent with the results of (M. M. Refaey et al., 2023), who stated that adding Azolla to Nile tilapia feed showed positive results in improving meat quality from all physical tests, and this was confirmed by (Abdelatty et al., 2020) where the inclusion of Azolla reduced the loss rate during cooking breast meat linearly ($P < 0.01$). Still, he indicated that Azolla can be included in broiler feed by up to 5% without adversely affecting meat quality or performance (Kinkpe et al., 2024). As for the meat color (L*, a*, b*), no significant changes or differences were shown, as three areas of the fish were taken (breast, belly, and tail). The results are consistent with Bhat et al, (2012), who mentioned the highest percentage of dry matter in the carcass, crude protein, crude fat, ash, and energy content, and the lowest moisture and carbohydrate content in the feed groups containing high protein ratios. (Fiogbe et al., 2004) mentioned that the diet containing 45% Azolla showed growth and could be a supplementary diet for tilapia. Gümüş et al. (2022) indicated in their study that there was no difference between the average values of L*, a*, and b* ($P>0.05$). There was also no difference between males' and females' average L*, a*, and b* values in scale and mirror carp fillets ($P>0.05$). In a different study, fish performance continued to deteriorate with the increase in Azolla diet levels. Body protein and fat content were negatively correlated with Azolla diet levels (El-sayed, 2008) . In another study on African and European catfish, no significant difference was found between the mean color values according to L*, a*, and b* distributions for both fish species ($P > 0.05$) (E. Gümüş et al., 2021).

4.10. Chemical and Physical Properties of Meat Quality in Black Soldier Fly Larvae Feeding

The present investigation's findings indicated that incorporating black soldier fly larvae (*Hermetia illucens*) into the diets of common carp, at inclusion rates of 25% and 50%, engendered notable enhancements in the meat's chemical profile. Specifically, there were increases in dry matter, crude protein, fat, and ash content, alongside a significant reduction in carbohydrate content. This observed enhancement suggests a more effective nutrient deposition within the muscle tissues, a consequence of the efficient physiological utilization of the BSFL diet's constituents, especially the readily digestible protein. These

results are consistent with those of Secci et al. (2018) when using *H. illucens* in trout diets, where they indicated that insect larvae protein has a good amino acid balance and high feed conversion efficiency, contributing to increased protein deposition within muscles. The observed elevation in protein content within the fish flesh of those fed BSFL can be attributed to the fact that the protein derived from black soldier fly larvae possesses ideal ratios of essential amino acids, which are crucial for the development of muscle tissue; consequently, this promotes muscle protein synthesis more effectively than traditional diets (Saputra and Lee, 2023). Hua et al. (2019) elucidated that the integration of insect protein into fish feed formulations enhances protein utilization efficiency and augments deposition within muscle tissue, a result of the improved digestibility and bioavailability of amino acids. Furthermore, Makokha et al. (2023) suggested that the amino acid profile of *H. illucens* protein, specifically its lysine content, is instrumental in facilitating muscle growth and enhancing the chemical composition of fish flesh. Furthermore, Li et al. (2017) study on the common carp (*Cyprinus carpio*) revealed that incorporating black soldier fly larvae powder into their diet resulted in improvements in both body composition and meat quality, without adversely affecting their growth or general well-being. A study by Iaconisi et al. (2018) on trout showed that diets containing *H. illucens* improved the chemical composition of fillets without negatively affecting muscle tissue quality. Renna et al. (2017) demonstrated that using defatted black soldier fly larvae powder in trout diets improved fillet quality and muscle structure without causing muscle tissue disturbances, emphasizing that protein quality plays a crucial role in this improvement. The elevated fat content seen in fish flesh is largely attributable to the unique lipid composition of black soldier fly larvae; these larvae contain fatty acids that can be assimilated into muscle tissue, thus improving the meat's energy density (Z. Wang et al., 2025). Chaklader et al. (2021) study revealed that incorporating *H. illucens* into barramundi feed led to a marginal rise in fillet fat levels, alongside enhancements in both physical and sensory attributes, while not detrimentally affecting meat quality. Moreover, Hender et al. (2021) noted that a modest elevation in meat fat content is a technologically beneficial trait, as it contributes to enhanced tenderness, juiciness, and flavour profiles. Moutinho et al. (2020) research indicated that the use of *H. illucens* powder in European sea bass (*Dicentrarchus labrax*) diets resulted in moderate alterations in fillet fat content, remaining within technologically acceptable parameters, and without adversely affecting meat quality or sensory properties. Bruni et al. (2020)

study of trout revealed that the inclusion of full-fat black soldier fly larvae prompted changes in fat metabolism, yet fillet quality remained unaffected. The significant increase in ash content implies an improvement in the meat's mineral profile, likely due to an augmented absorption efficiency of particular minerals, a result of insect-based feeding regimens. Borgogno et al. (2016) observed that diets incorporating *H. illucens* could potentially augment mineral bioavailability, thereby promoting greater mineral deposition within fish muscle tissue (Lock et al., 2015). Conversely, the marked decrease in glucose concentrations could be explained by a metabolic shift that prioritizes the use of proteins and lipids as primary energy substrates. This metabolic adaptation is often observed in fish fed high-protein diets (Skiba-cassy et al., 2020). The physical results showed a significant decrease in both cooking and thawing losses in the BSFL treatments. This suggests that the muscle tissue retained more water. This is an important technical aspect of meat quality because it directly affects meat juiciness and the amount of meat obtained after cooking (H. N. Lee et al., 2025). These findings support Lemke et al. (2023) research, which found that adding *H. illucens* improves muscle protein stability and reduces losses during cooking, likely because of changes in the muscle fiber structure. Additionally, Chaklader et al. (2022) found that lower cooking losses are linked to less lipid peroxidation and better protein-water interactions in muscle tissue. These results are further supported by (M. R. Chaklader et al., 2023) research, which revealed that incorporating *H. illucens* into fish diets enhances muscle fiber structure and decreases losses during heat treatment, owing to improved protein-water interaction within muscle tissue. Conversely, no significant differences were observed in pH or meat color measurements (L*, a*, b*) across the breast, abdomen, and tail regions. The consistent pH values suggest that the incorporation of black soldier fly larvae did not influence post-mortem glycolysis rates or the chemical processes that maintain acid stability within the muscle tissue. These results are consistent with the observations of Popova et al. (2020), who reported that diets incorporating *H. illucens* do not significantly affect pH or meat coloration when provided at moderate, nutritionally balanced levels (Cullere et al., 2019). Furthermore, Ma et al. (2025) more recent study supported the idea that myoglobin concentrations and post-mortem metabolic processes primarily dictate meat color, rather than the source of dietary protein; this explains the lack of observed impacts on color characteristics in the present study. The elevated protein content observed in the fish flesh of those fed diets containing black soldier fly larvae is likely attributable to the superior

digestibility and bioavailability of essential amino acids present in *Hermetia illucens* protein (Bamba et al., 2025). Consequently, this enhances muscle protein synthesis more effectively compared to conventional diets. Deren and Fedorenko (2023) indicated that incorporating insect protein into fish diets improves protein utilization efficiency and increases its deposition in muscle tissue without negatively impacting meat structure or quality. As Lu et al. (2022) demonstrated, the amino acid balance of black soldier fly larva protein, particularly its lysine content, plays a crucial role in supporting muscle growth and enhancing the chemical composition of fish meat. These findings are consistent with Hender et al. (2021), who showed that insect-based diets improve the nutritional value of meat. This is due to the efficient digestion and use of protein in muscle tissue, which aligns with the significant increase in protein content seen in this study.

4.11. Hematological Parameters of the Fish in Azolla Feeding

The effect of Azolla on fish blood parameters in blood tests is shown in Table 6, which shows no significant differences between the experimental groups with statistical significance. Red blood cell count increased in the groups whose diet contained Azolla 12% and 6% compared to the control group ($P<0.05$) where (5.22±0.65), (3.80±0.36), and (2.99±0.24) respectively. These results are consistent with the results of the study by Magouz et al., (2020), which stated that feeding tilapia with Azolla did not have any differences in the measured blood parameters and indicated the presence of non-significant differences in the measured parameters except for the number of red blood cells, which increased significantly ($P<0.05$) in fish fed Azolla by 20% compared to the control group. In their study on carp, Ahmed et al. (2023a) reported that all hematological parameters showed a linear decreasing trend with an increasing percentage of groups containing Azolla in the diet. Naji and alzurfi (2023) reported that feeding carp with diets containing Azolla did not affect the hematological functions and blood profile but were close to those of the control group. Nekoubin (2012) supported the present results through his study on grass carp, where in terms of hematological parameters, he reported no difference in the hematological parameters, and no standard differences were observed in the concentration of hemoglobin, red blood cells, and white blood cells among the treatments. Studies have reported that using Azolla as a plant protein source for restrictive feeds can improve growth, some physical parameters, hematological parameters, and antioxidant properties without adversely affecting the health status of fish, birds, and

ruminants and can contribute significantly to increasing the profit margin (Y. K. Abbas and Mohanned, 2023; M. Das et al., 2018; Kamel and Hamed, 2021). There were no significant differences in the blood plasma of fish in this study except for total protein, as shown in Table 6, where the protein percentages in the groups whose diet contained Azolla were 12% and 6%, and the control group were (4.19±0.14), (3.73±0.11), (3.78±0.07) respectively. M. M. Refaey et al. (2023) indicated similar results in their study on tilapia fish fed a diet containing Azolla, where no significant differences ($P > 0.05$) were detected in the activity of alanine transaminase, aspartate transaminase, and serum creatinine between treatments. Fasakin et al. (2001) indicated in v. In different results, Mosha (2020) stated that 10% of Azolla had a significant response on growth performance, serum biochemical parameters, and immunity compared to the control group and other treatments. The current study results show decreased blood parameters, especially alanine aminotransferase (ALT) and aspartate aminotransferase (AST). However, there were no significant differences between groups. It is consistent with the results of Ahmed et al. (2023a) a decrease in serum biochemical parameters was observed with increasing inclusion of the Azolla meal, except for glucose, cholesterol, alanine aminotransferase (ALT), and aspartate aminotransferase (AST). Azolla contains compounds that may help reduce hepatotoxicity and improve the biological functions of the liver (Elrasoul et al., 2020). Azolla may act as an antioxidant (Dai et al., 2012), reducing the levels of free radicals that lead to tissue damage and thus may lead to lower levels of ALT and AST if there is an improvement in liver health (Dohaei et al., 2020).

4.12. Hematological Parameters of the Fish in Black Soldier Fly Larvae Feeding

The results of the current study showed that introducing black soldier fly larvae (*Hermetia illucens*) into common carp diets at levels of 25% and 50% did not lead to significant differences in key hematological parameters, including hematopoietic cell volume (HCT), hemoglobin concentration (HGB), and red blood cell count (RBC). This indicates that this alternative protein source does not negatively affect hematopoiesis or oxygen transport efficiency to tissues, which are among the most important indicators of the fish's overall health. These results are consistent with those of Abdel-Tawwab et al. (2020) when using black soldier fly larvae powder in fish diets. No significant deviations were observed in hematological or biochemical measures compared to the control group, suggesting that this protein source effectively maintains homeostasis in fish without

causing physiological distress. The present study's conclusion is consistent with Li et al. (2017) examination of incorporating defatted black soldier fly larvae powder into carp diets. Their findings indicated that the hematological parameters remained within the expected physiological range, suggesting no adverse effects or nutritional stress associated with this protein source. The results confirmation the research of Xu et al. (2020), which showed that the addition of black soldier fly larvae paste to mirror carp diets did not induce any atypical changes in hematological values. This suggests that the food source is compatible with the fish's circulatory system. In the same vein, these results are consistent with the study by Jahan et al. (2021), who confirmed that replacing fishmeal with BSFL in carp fingerling diets did not affect normal white blood cell counts, indicating the immunological safety of this source. The results of the current study also showed stability in white blood cell and differential counts, indicating that the introduction of BSFL into the diet did not stimulate abnormal immune responses. In the study by Tippayadara et al. (2021), there was agreement with the results of the current study, which confirms that there are no statistically significant differences in white blood cell count and differential cell count when using fishmeal with black soldier fly larvae powder in Nile tilapia diets, and concluded that this particular component does not weaken the immune system or provoke an excessive inflammatory response. Similarly, Fawole et al. (2020) study revealed that the partial substitution of fish feed with *H. illucens* in the diets of African catfish did not adversely impact hematological and immunological parameters; instead, it preserved values within the established physiological norms. These results are similar to those of Gebremichael et al. (2021), who indicated that BSFL diets do not lead to immune imbalance or an increase in immune stress indicators in carp, but rather maintain immune system stability. In terms of the serum biochemical indicators, the current study showed no significant differences in glucose levels. The data indicate that the fish did not exhibit metabolic distress stemming from the alteration in protein source. These findings align with the observations of (Hansen, 2021), whose research indicated that incorporating black soldier fly larvae into the diets of ornamental fish did not compromise glucose homeostasis or the stress response. No significant differences were recorded in total protein, albumin, globulin, or A/G ratio levels, indicating a well-balanced nutritional and immune status in the fish. These results are consistent with those of Priyadarshana et al., (2022), who demonstrated that diets containing BSFL do not lead to disturbances in blood proteins or liver function

in farmed fish. The results showed that there were no major changes in cholesterol, triglyceride, and lipoprotein levels (HDL, LDL, VLDL), meaning that adding black soldier fly larvae did not affect how fats are processed or moved in the blood. The findings presented here corroborate the work of Abd El-Gawad et al. (2025), who reported that diets incorporating BSFL help maintain balanced blood lipid levels and enhance fish well-being, without compromising hepatic function. Regarding renal function, the current study's findings demonstrated stable urea and creatinine concentrations. This suggests an absence of either excessive protein intake or impairments in the elimination of metabolic byproducts. These observations are consistent with Abdel-Latif et al. (2021) research on European sea bass, which indicated that the inclusion of BSFL in their diet does not negatively impact kidney function or related biochemical indicators. Regarding liver enzymes, the values for AST, ALT, and ALP were all within the normal range. Despite a near-significant AST value, this does not suggest any actual liver damage. These findings align with the research of Yildirim Aksoy et al. (2023), which demonstrated that black soldier fly products, when administered at moderate levels, do not induce liver stress and may even promote the overall health of fish. The lack of significant differences in hematological and biochemical values in the current study is attributed to the fact that black soldier fly larvae (*Hermetia illucens*) represent a balanced and highly digestible protein source, meeting the nutritional requirements of common carp without causing physiological disturbances (Shati et al., 2022). The stability of indices such as hematopoietic cell count (HCT), hematopoietic ganglion cell (HGB), and red blood cell count (RBC) indicates the integrity of hematopoiesis and oxygen transport efficiency, as these values typically only change under stress or malnutrition (Abdel-Tawwab et al., 2020). Furthermore, the unchanged white blood cell count and differential count indicate that the introduction of black soldier fly larvae did not induce undesirable immune or inflammatory responses, consistent with the findings of (Fawole et al., 2020; Tippayadara et al., 2021). Blood glucose, protein, and lipid concentrations showed stability, indicating efficient metabolic homeostasis. This suggests that basic metabolic processes are not affected by changes in protein intake, as reported by Moutinho et al. (2020). Moreover, the maintenance of liver enzymes and renal function markers within normal ranges signifies the lack of metabolic strain or toxic repercussions, as corroborated by (Fischer et al., 2022; Srikha et al., 2024).

4.13. Fatty Acid Composition of Fish Meat in Azolla Feeding

In this study, Table 7 shows the fatty acid content in the fat extracted from fish meat in the experimental groups, where all fatty acids, including palmitic acid, stearic acid, linoleic acid, alpha-linolenic acid, eicosatetraenoic acid, docosahexaenoic acid, and oleic acid had high percentages in the experimental groups fed on Azolla compared to the control group. There were significant differences. The results are consistent with Abou et al. (2013) in their study on feeding tilapia on diets containing Azolla, where they stated that the diet affects the fatty acid composition in fish and noted that there is a significant effect in fish fed in ponds compared to those fed in tanks. Fish fed on high-Azolla (AM) diets were rich in eicosatetraenoic acid (EPA) and docosahexaenoic acid (DHA), which contributed to increasing the percentage of healthy fatty acids such as n-3 compared to n-6 in ponds. However, this ratio decreased when fish were fed in tanks. The results also indicated an increasing trend in the ratio between n-3 and n-6 in ponds. Abou et al. (2011) demonstrated in his study on tilapia that increasing the level of Azolla in fish feed reduces linolenic acid and enhances arachidonic acid, Eicosatetraenoic acid, and docosahexaenoic acid (D. Ibrahim et al., 2022). The n-3/n-6 ratio is also improved in fish fed on AM, which enhances the quality of fatty acids in fish without affecting growth. In a different study, Oktavianawati et al. (2016) reported that the groups fed on Probiotics were better than the group fed on Azolla in their research on Batin fish and that oleic acid and palmitic acid are the main components of unsaturated and saturated fatty acids, respectively, in all fish samples. Fatty acids in fish meat are affected by seasonal variations and fish species (Guler et al., 2011). The reason for the increase in fatty acids is that Azolla is a rich source of long-chain polyunsaturated fatty acids such as DHA and EPA, which are fatty acids with significant health benefits for humans (K. Chandrababu et al., 2024). When Azolla is incorporated into feed, fish tend to store these fatty acids in their bodies, which enhances the level of omega-3 in their meat (Carnegie et al., 2023). Studies have shown that feeding on diets containing Azolla enhances the digestion and absorption of fish because it contains other components of nutrients, such as proteins and fibers, that can increase the absorption rate of fatty acids (Chanapanchai et al., 2025). Feeding Azolla improves the ratio of saturated and unsaturated fatty acids. Oleic acid (C18:1n-9) and linoleic acid (C18:2n-6) are increasing and cause increased lipid formation in fish meat (Yohana et al., 2023).

4.14. Fatty Acid Composition of Fish Meat in Black Soldier Fly Larvae Feeding

The results of this investigation show that replacing 25% and 50% of the common carp's feed with black soldier fly larvae significantly altered the fatty acid composition of the muscle tissue. This observation highlights the direct effect of dietary fat sources on lipid metabolism in omnivorous fish species. Devic et al. (2018) conducted research on common carp, revealing that the incorporation of *Hermetia illucens* larvae triggered notable metabolic alterations in muscle fat (Abdel-Latif et al., 2021). This effect stemmed from the partial congruence between the dietary fat composition and the muscle tissue, which elucidates the disparities observed between the control and replacement treatments in the present study. The pronounced elevation in saturated fatty acids, especially palmitic and stearic acids, at the higher replacement level, can be ascribed to the elevated concentration of these acids within the fat of black soldier fly larvae, a characteristic previously documented by (Borgogno et al., 2016). When fish were fed diets containing insects, the results showed they absorbed and stored saturated fatty acids in their muscle tissue, with little oxidation. Herawati et al. (2023) also found that increasing the amount of substitution improved this effect. This is because the fish used these acids for energy, which explains the differences seen with different substitution levels. It can be observed that the large increase in oleic acid, which is a monounsaturated fatty acid, is due to the high concentration of oleic acid in the fats of black soldier fly larvae. Furthermore, freshwater fish prefer monounsaturated fatty acids as their main source of energy. (Belghit, Liland, Gjesdal, et al., 2018) observed that fish consumed black soldier fly larvae showed improved fatty acid stability. This stability reduced their susceptibility to oxidation, thus protecting the fatty acids in their muscle tissue. Limbu et al. (2022) suggested that this accumulation doesn't negatively affect fish performance or health; instead, it represents a normal physiological response to a change in dietary fat source. When the diet included more black soldier fly larvae, the amount of linoleic acid, a type of omega-6 fatty acid, increased. This increase in the larvae's fat content, compared to diets without the larvae, is a key factor. Moutinho et al. (2020) found that linoleic acid, when consumed in large amounts, is directly stored in muscle tissue. In contrast, (X. Ma et al., 2020) showed that freshwater fish have a limited ability to break down linoleic acid, leading to its accumulation and significant differences in the results of the dietary treatments. A significant increase in eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) was observed despite the relative deficiency of these acids in black soldier

fly larvae. This is attributed to the high conversion capacity of common carp to convert short-chain omega-3 fatty acids into long-chain fatty acids. The study by Maulu et al. (2022) found this biotransformation is one of the most important metabolic adaptation mechanisms in freshwater fish fed insect-based diets, while Li et al. (2017) confirmed that the activity of desaturating and elongating enzymes allows for the preservation of the nutritional value of meat fat even in the event of a reduction in the direct dietary supply of EPA and DHA. Studies on meat quality have also supported this interpretation. Secci et al. (2018) showed that changes in fat composition, resulting from using different fat sources, don't always lead to worse meat quality or sensory characteristics. In contrast, Cullere et al. (2019) found that the balance of saturated and unsaturated fatty acids is the most important factor in determining fat quality, not just the source of the fat. Overall, the significant differences seen between the study groups reflect a complex interaction. This interaction involves the fat composition of the black soldier fly larvae diet, how efficiently fatty acids are selectively absorbed and precipitated (Ewald et al., 2020), and the metabolic flexibility of common carp (Malekpouri et al., 2016). Therefore, the degree of substitution is clearly related to the extent of lipid changes, which supports the dose-response effect. Specifically, increasing the level of substitution intensifies the metabolic effect, resulting in more pronounced and statistically significant differences. These observations align with the experimental research undertaken by Renna et al. (2017), which investigated the effects of diets incorporating black soldier fly larvae on freshwater fish. Their outcomes indicated that an elevated substitution level induced significant modifications in muscle lipid composition, attributable to the direct incorporation of insect lipid constituents. The present findings align with the conclusions of (Limbu et al., 2022) in their experimental investigation of tilapia, revealed that elevated levels of BSFL substitution within the diet foster progressive alterations in muscle fatty acid composition, with statistically significant disparities observed across the dietary treatments (H. N. Lee et al., 2025). This result suggests the fish's capacity to adapt its metabolic processes when utilizing an alternative lipid source. Consequently, this supports the proposition that black soldier fly larvae represent a viable and sustainable feed component for aquaculture applications (Jin et al., 2025). The differences seen in the experimental treatments are due to changes in the types of fats in the diet, which result from varying amounts of black soldier fly larvae used. When more larvae are added, certain fatty acids, particularly saturated and monounsaturated ones, become more available (Nekrasov et al., 2022). This

can exceed the common carp's ability to process them. As a result, these fatty acids build up in the muscle tissue, leading to significant differences, as shown by the studies of (Borgogno et al., 2016; Devic et al., 2018). The high metabolic flexibility of freshwater fish also contributes to enhancing these differences, as they have an efficient ability to convert short-chain fatty acids into long-chain omega-3 fatty acids (Glencross et al., 2025), which explains the significant increase in EPA and DHA despite the relative poverty of BSFL fats in these acids, as indicated by (M. R. Chaklader et al., 2023; R. Chaklader et al., 2022). The increasing differences observed as the substitution rate rises from 25% to 50% indicates a dose-response relationship; higher levels of BSFL enhance the metabolic effect, leading to statistically significant differences (Herawati et al., 2023; Limbu et al., 2022). Therefore, the 50% substitution treatment is statistically better at producing significant differences and improving certain nutritionally important fatty acids. In contrast, the 25% treatment is the most practical and balanced option, achieving significant nutritional improvement while preventing a large increase in saturated fatty acids, as recommended by (Gasco et al., 2018; Iaconisi et al., 2018; Renna et al., 2017; Saputra and Lee, 2023).

4.15. Amino acid Composition of Fish Meat in Azolla Feeding

The results showed that the experimental fish groups fed Azolla had higher protein and amino acid contents than the control group, as Azolla is a fern rich in protein and amino acids (Mohamed et al., 2018). Azolla is rich in essential nutrients such as lysine, methionine, cysteine, threonine, tryptophan, arginine, isoleucine, leucine, phenylalanine, tyrosine, glycine, serine, and valine, all of which exceed the levels found in soybean meal except histidine (Kabir et al., 2020; S. Mosha, 2020). High levels of essential amino acids and crude protein in aquatic weeds, such as Azolla, may significantly contribute to improved growth and feed efficiency in various fish species (Rashad, 2021). The presented results are consistent with research conducted by Kamil and Taha (2022), which showed that the use of 2.5% Azolla in common carp fish significantly increased protein deposition and production rates ($P \leq 0.05$) compared to the control and other treatment groups. In a different study on batin fish conducted by Oktavianawati et al. (2016), reported that amino acid content decreased in fish groups fed Azolla and probiotics compared to the control group. To date, no comprehensive study has been conducted examining the effect of dietary Azolla on fish meat quality, particularly with regard to

amino acid composition. Mosha et al. (2020) recently conducted a study on the total carotenoid concentration in muscle tissue of two fish species. The results indicated that increased Azolla supplementation was associated with higher total carotenoid content in fish muscle (Vasudhevan et al., 2015).

4.16. Amino Acids Composition of Fish Meat in Black Soldier Fly Larvae Feeding

This study examined the impact of including Black Soldier Fly Larvae (BSFL) into the diets of common carp (*Cyprinus carpio*), substituting conventional protein sources at 25% and 50% ratios. The primary objective was to ascertain the impact of various replacement levels on the overall amino acid composition in the fish's tissues. The results showed a significant improvement in all studied amino acids with increasing BSFL inclusion levels. The 50% BSFL treatment recorded the highest values, followed by the 25% BSFL treatment, and then the control treatment. This indicates a response directly dependent on the level of protein replacement. The present results align with study of Secci et al. (2018), who investigated Nile tilapia, and Belghit et al. (2018b) , who researched Atlantic salmon; both studies demonstrated that the inclusion of BSFL enhanced protein quality and the accumulation of amino acids in muscle tissues. The significant increase in aspartic acid and glutamic acid observed with higher BSFL inclusion stems from the essential roles these acids play in deamination, nitrogen transport, and the regulation of protein metabolism within muscle cells, alongside their involvement in energy production pathways. This improvement suggests a more efficient utilization of dietary protein. These results are consistent with those of Renna et al. (2017) in their studies on common carp, as well as (Kariuki et al., 2024) when using BSFL in Nile tilapia (*Oreochromis niloticus*) diets, where a clear improvement in protein metabolism efficiency and an increase in the concentration of acidic amino acids in fish tissues were observed. The results also showed a progressive increase in glycine, serine, and proline with increasing BSFL inclusion levels. These amino acids are closely linked to collagen and connective tissue formation and the regulation of osmotic balance in muscles. The observed elevation is ascribed to the enhanced digestibility of BSFL protein and a corresponding increase in intestinal absorption efficiency, which facilitates a greater accumulation of these amino acids within muscle tissue (Yu et al., 2022). These findings support the results of (Basto et al., 2020), whose study on European sea bass (*Dicentrarchus labrax*) showed that adding insect protein improved the storage of non-essential amino acids, likely due to

better digestibility and use. A key finding of this research is the significant increase in branched-chain amino acids (BCAAs), such as valine, isoleucine, and leucine, which are important for muscle growth and metabolic processes related to growth. The black soldier fly larva protein's higher amino acid profile, when compared to plant-based proteins, explains the observed improvements in performance. These findings align with the observations of Radhakrishnan et al. (2023) regarding Atlantic salmon (*Salmo salar*), and also with those of Chaklader et al. (2023), whose investigations into Nile tilapia revealed analogous increases in branched-chain amino acids and improved meat quality. The notable increase in lysine, threonine, and methionine within the 50% BSFL treatment is attributed to the superior amino acid profile of black soldier fly larva protein compared to plant sources, given that these amino acids often act as growth-limiting factors in fish diets. Makkar et al. (2014), in their extensive review of insect protein, established that BSFL possesses a superior lysine and methionine content relative to conventional plant proteins. Cichlidae et al. (2022) demonstrated in their studies on tilapia, and Xu et al. (2020) in their research on common carp, that the incorporation of BSFL enhanced the biological value of dietary protein and augmented the accumulation of essential amino acids in muscle tissue, aligning directly with the findings of the present study. An appreciable elevation in arginine, histidine, and cysteine was noted with escalating BSFL inclusion levels, attributable to the functional roles of these amino acids in augmenting immune response, modulating oxidative stress, and facilitating physiological homeostasis in fish. H. Wang et al. (2025) demonstrated in their studies on Southern Catfish (*Silurus meridionalis*) that insect proteins contribute to improved immune markers in fish. Similarly, in recent studies on freshwater fish, indicated that the presence of chitin and its derivatives in BSFL stimulates the immune system and improves overall fish health, positively impacting metabolic efficiency and amino acid deposition in tissues (Fawole et al., 2020; Nakagawa et al., 2023). The significant differences between the 25% BSFL and 50% BSFL treatments can be attributed to the fact that the lower inclusion level may not be sufficient to fully elicit the nutritional and functional effects of the black soldier fly larval protein, whereas the higher level provides a greater quantity of high-quality protein and associated bioactive compounds, leading to a more pronounced improvement in digestibility, absorption, and amino acid deposition in muscle tissue. Renna et al. (2017), in their studies on common carp, indicated that positive responses to insect protein are more pronounced when certain inclusion levels are exceeded. Onsongo et al. (2018),

in their research on Broiler, demonstrated that higher levels of insect protein resulted in improved digestibility and protein utilization compared to lower levels (Hasnan et al., 2023). Furthermore, Serra et al. (2024), found an ideal level of insect protein inclusion. This level needed to be exceeded to achieve the best nutritional results without negatively affecting the fish's physical health. (Saleh et al., 2024) investigation within Nile tilapia and European Sea Bass determined that insect proteins enhance immune markers within the fish. These findings support the current study's results, where including BSFL at 50% was more effective at improving the amino acid profile than including it at 25%.

4.17. Histopathological Analysis of the Organs of Fish in Azolla Feeding

Histopathological results indicated no changes in the control group's liver, pancreas, kidneys, and muscles, which displayed standard cellular structure. The 6% Azolla group was similar to the control group, showing no abnormal histopathological changes. However, some changes in the 12% Azolla group showed pathological histopathological features in the liver, marked non-plastic hyperplasia, and hypertrophy of the pancreatic acinar cells, with significant venous dilation. This is consistent with a study by Tahseen et al. (2022), who stated in their study on the use of Azolla in carp nutrition that Azolla plants are an effective medium for the bioaccumulation of lead and zinc (Sood et al., 2012). Consequently, this accumulation poses a risk of transfer to the fish, which could lead to histopathological changes and tissue damage. Carnegie et al. (2023) stated that using less than 30% Azolla in the feed can maintain the growth rate of juvenile giant gourami. In his study on tilapia, M. M. Refaey et al. (2023) reported that fish fed Azolla at levels of 10% and 40% had higher mucosal and submucosal thicknesses, while the intestinal lumen area increased significantly ($P < 0.05$) with increasing Azolla levels compared to the control group. Ismail et al. (2022) demonstrated this in his study on Nile tilapia fed different levels of Azolla, which showed a significant increase in intestinal villi, including villus height, width, area, and goblet cell count. Gonçalves et al. (2024) suggested that increasing the surface area of the villi can enhance nutrient absorption. In study on carp, Altaee et al. (2024) reported a difference in the degree of change in diseased liver and kidney tissues depending on the amount of Azolla included in the feed. Yousif et al. (2021) pointed out that liver cell deterioration may result from several factors, including oxygen deficiency, the effects of heavy metals on the circulatory system, changes in vascular permeability, and others.

4.18. Histopathological Analysis of the Organs of Fish in Black Soldier Fly Larvae Feeding

Histopathological analysis of the present investigation revealed that the fish within the control cohort exhibited a preserved cellular architecture across the liver, kidneys, and skeletal muscles. This observation suggests a stable physiological condition and the lack of any nutritional or metabolic strain affecting the examined tissues. This normal histological appearance is consistent with the findings of Renna et al. (2017), who considered the preservation of vital tissue integrity a key indicator of the efficiency of conventional fish diets. In the group of fish that were fed black soldier fly larvae, which made up 25% of their diet, we saw that the pancreatic acinar cells and some parts of the liver tissue were noticeably bigger. The liver cells, on the other hand, looked normal. These changes are likely an early adaptive response of the pancreas to the introduction of a new protein source (Z. Chen et al., 2025). This source had a different structure than usual proteins, which required increased secretion of digestive enzymes. Studies have indicated that the inclusion of black soldier fly larvae at moderate levels may lead to adaptive changes in digestive tissues without causing direct liver damage (Basili and Fiera, 2024; Fiorilla et al., 2024). The enlarged blood vessels in the livers of these fish likely result from increased blood flow, which is a consequence of their faster metabolism after the dietary change (McArley et al., 2021). Researchers have previously observed this change in tissue structure in fish fed different protein diets (Yuting Yang et al., 2021). Fish fed with various protein sources have shown a similar pattern in their livers (Williams et al., 2024). The presence of undifferentiated acinar cells with darkly stained nuclei probably indicates a temporary cellular stress or increased cell division. This situation is a common physiological response in secretory tissues when diets change suddenly (Assan et al., 2021). The kidneys of the BSFL 25% cohort exhibited moderate cellular swelling, accompanied by the shedding of cells from the renal tubules, although the glomeruli and hematopoietic tissues maintained their typical architecture. These alterations are likely a consequence of elevated nitrogenous metabolites stemming from insect protein metabolism, thereby imposing an increased workload on the renal tubules, yet not progressing to a state of significant pathological harm (Bhatnagar et al., 2007). Rawski et al. (2020) suggested that these tubular modifications are frequently reversible, representing a transient adaptive mechanism, this is what confirmed by (Cullere et al., 2019). Significant atrophy and tissue loss, accompanied by damage to muscle fibers, were

observed in the skeletal muscles of the 25% black soldier fly larvae group. These findings may result from a transient imbalance in amino acids or a reallocation of energy towards metabolic alterations, rather than muscle protein synthesis (H. Wang et al., 2025). Chaklader et al. (2023) suggested that suboptimal levels of insect protein inclusion may temporarily affect muscle fiber integrity during the initial adaptation phases (jonas levi and Martinez, 2017). In contrast, the 50% BSFL cohort exhibited a unique histological profile, marked by mild hepatitis, perivascular lymphocytic infiltration, and slight fatty degeneration. These modifications may be attributed to an increased concentration of lipids or bioactive compounds associated with BSFL protein, consequently activating the hepatic immune response. (Ahirwal et al., 2024) asserted that these mild inflammatory responses are typically adaptive and do not signify irreversible tissue damage (Abdel-Latif et al., 2021). Despite the liver changes, the kidneys in the 50% BSFL group showed normal glomerular structure, with only minor changes in the renal tubules. This suggests a strong ability to adapt to a moderate increase in metabolic demands. The skeletal muscle in this group maintained its usual fiber structure and arrangement. This finding suggests that the improved balance of amino acids and energy sources was due to the increased inclusion of BSFL. As a result, this led to the preservation of tissue integrity and the continued synthesis of muscle proteins (Borgogno et al., 2016; M. R. Chaklader et al., 2023; Secci et al., 2018). The observed differences in tissue structure in this study are attributed to the varying amounts of black soldier fly larvae in the diet and the resulting changes in the fish's diet and metabolism (Yamamoto et al., 2022). Betta et al. (2023) research indicated that incorporating moderate amounts of BSFL protein could potentially hinder adaptive responses within certain tissues. Conversely, an appropriate nutritional equilibrium appears to enhance metabolic stability and tissue integrity when BSFL protein inclusion is elevated. Donadelli et al. (2024) also suggested that the subtle changes in the liver and kidneys associated with insect protein consumption represent adaptive physiological mechanisms, rather than unequivocal signs of disease. This is supported by the findings of Radhakrishnan et al., (2023) It was found that improved amino acid balance at higher inclusion levels positively impacts skeletal muscle health (Zhao et al., 2022). These studies thus confirm that the histological effects of BSFL depend primarily on the inclusion level and the fish's dietary adaptation, rather than on a direct toxic effect of the protein source.

CHAPTER 5

CONCLUSION AND RECOMMENDATION

5.1. Conclusion

1. The introduction of 6% and 12% Azolla into the common carp diet, substituting soybean meal, did not yield statistically significant alterations in growth performance metrics. Conversely, total weight gain and daily growth rates were maintained within acceptable ranges, particularly when the replacement ratios were meticulously regulated. Furthermore, substituting 25% and 50% of soybean meal with black soldier fly larvae in common carp feed formulations led to enhanced weight gain, growth rates, and feed efficiency.

2. Fish fed a diet containing Azolla or black soldier fly larvae did not exhibit any negative behavioral changes. They ate normally, moved regularly, and responded consistently to feeding. This indicates that the fish tolerated these components and that the replacement ratios used did not contain any appetite-suppressing substances. The use of Azolla significantly affected dressing percentage and lean meat percentage, while it had no effect on black soldier fly larvae. The findings indicate that the feed sources used did not negatively impact the partitioning of growth between muscle and internal organs, a factor of significance from both economic and production perspectives.

3. The fish flesh's pH and coloration exhibited only minor alterations. Conversely, incorporating black soldier fly larvae or Azolla into the fish diets resulted in enhancements across multiple parameters, including crude protein, fat, ash, dry matter, and carbohydrates. The groups fed Azolla and black soldier fly larvae demonstrated superior moisture retention. Furthermore, a decrease in moisture loss during both the cooking and post-discarding phases was observed.

4. Blood analyses revealed that the individual administration of Azolla or black soldier fly larvae did not significantly detriment the core hematological indices of common carp. These indices encompassed red and white blood cell counts, hemoglobin concentration, and packed cell volume. Consequently, these findings suggest the maintenance of the fish's robust health and physiological state, alongside an absence of hematological stress, impaired oxygen transport efficacy, or an atypical immunological reaction.

5. The inclusion of Azolla and black soldier fly larvae in fish diets improved the lipid composition of the fish flesh. This was demonstrated by an increase in certain saturated and medium-chain fatty acids, alongside the maintenance of a balanced proportion of unsaturated fatty acids. The incorporation of Azolla and black soldier fly larvae enhanced the fat diversity in the fish without compromising the nutritional quality of the final product.

6. Diets that included Azolla and black soldier fly larvae showed a beneficial profile of essential amino acids, including lysine, methionine, threonine, and valine. This specific composition promoted protein deposition within muscle tissue, consequently supporting normal growth and maintaining meat quality.

7. Histological analyses of the liver, muscles, kidneys, and pancreas indicated only minor pathological alterations. Although the groups receiving Azolla and black soldier fly larvae at the prescribed levels exhibited no adverse effects, elevated ratios did negatively influence the histological well-being of the fish. Regularity in cell structure and tissue integrity was observed at the moderate levels, supporting the physiological findings and confirming the safety of these feed components at the recommended ratios.

5.2. Recommendation

1. This investigation supports the use of Azolla and black soldier fly larvae, considered separately, as supplementary feed components in the diets of common carp. Both options demonstrate the capacity to support typical growth and feeding patterns without significantly compromising fish productivity or general health.

2. The research recommends determining optimal substitution ratios for each ingredient independently, contingent upon the fish's developmental stage and nutritional needs. This approach aims to ensure a balanced feed composition and mitigate potential negative consequences associated with excessive application.

3. The research suggests that appropriate pretreatments, including drying or grinding, should be employed for Azolla before its incorporation into the diet, with the aim of improving digestibility and maximizing utilization efficiency.

4. The study recommends an assessment of the fat content within feed containing black soldier fly larvae and an examination of its effects on the chemical composition of the meat, particularly with regard to fatty acids, to ensure the food safety of the fish product.

5. The study advocates for the observation of Azolla or larvae's influence on carcass attributes and meat quality, considering physical and chemical properties, as well as the composition of essential amino acids, given their importance in maintaining the nutritional and market value of the fish.

6. The research findings underscore the necessity of routine evaluations of physiological and hematological indicators to ensure the well-being of fish and to identify any anomalies stemming from the continuous application of these feed components.

7. The study recommends histological examinations of key organs, including the liver, intestines, and muscles, to investigate the enduring cellular consequences associated with the use of Azolla or black soldier fly larvae.

8. The study advocates for the implementation of comprehensive, long-term investigations to evaluate the cumulative effects of these feed sources across diverse aquaculture systems.

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