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**EFFECTS OF PARTIAL AND TOTAL REPLACEMENT OF FISHMEAL  
WITH INSECT MEALS IN COMMON CARP AND AFRICAN CATFISH  
(*Clarias gariepinus*) FEEDS**

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#### DEDICATION

*I dedicated this thesis work to my father Gebremichael Gebretsadik, my mother Turinge Yebo, my husband Mebratu Mengesha, my kids Metibeb Mebratu, Binabesh Mebratu, Mariyona Mebratu and all my family members and best friends for their unreserved love, encouragement, and their partnership in the success of my life*

#### STATEMENT OF AUTHOR

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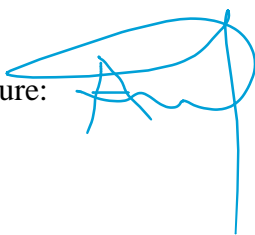
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## LIST OF ABBREVIATIONS

AA	Amino acid
ABM	Animal by-product meal
ADC	Apparent digestibility coefficient
ADCDM	Apparent digestibility coefficient of dry matter,
ADCP <sub>r</sub>	Apparent digestibility coefficient of protein
ADF	Acid detergent fiber
AI	Atherogenicity indices
ALP	Alkaline phosphatase
AMY	Amylase
ANOVA	Analysis of Variance
AOAC	Association of Official Analytical Chemists
APM	Animal product meal
BPM	Bacterial protein meal
BSF	Black soldier fly
CF	Crude fat
CFIA	Canadian Food Inspection Agency
CHOL	total cholesterol
CL	Cooking loss
CMC	Carboxymethyl cellulose
CP	Crude protein
CRD	Completely randomized design
DE	Digestible energy

DHA	docosahexaenoic acid
DL	Drip loss
DM	Dry matter
DPA	docosapentaenoic acid
EAA	Essential amino acid
EE	Ether extract
EFA	Essential fatty acid
EFSA	European food safety authority
EPA	eicosapentaenoic acid
EU	European Union
EUMOFA	European Market Observatory for Fisheries and Aquaculture Products
FA	Fatty acid
FAFR	Food Act and Feeds Regulation
FAO	Food and Agriculture Organization
FBW	Final body weight
FCR	Feed conversion ratio
FDA	Federal Food and Drug Administration
FFDCA	Federal Food, Drug, and Cosmetic Act
FM	Fishmeal
FO	Fish oil
GLOB	Globulin
GLU	Glucose
GRAS	Generally Recognized as Safe
HI	Hermetia illucens
HIS	Hepato-somatic index
IBW	Initial body weight
IM	Insect meal
IPIFF	International platform of insects for food and feed
K	Condition factor



Kg	Kilogram
LcPUFA	Long-chain poly unsaturated fatty acid
MBM	Meat and bone meal
MUFA	Mono unsaturated fatty acid
MW	Mealworm
NRC	National research council
PAPs	Processed Animal Proteins
PBP	Poultry by-product,
PER	Protein efficiency ratio
PHOS	Phospholipid
PPC	Pea protein concentrate
PPV	protein productive value
PUFA	Poly unsaturated fatty acid
RAS	Recirculating aquaculture system
RGR	Relative growth rate
RIL	Relative intestine length
RPC	Rice protein concentrate
SD	Standard deviation
SFA	Saturated fatty acids
SGR	Specific growth rate
SR	Survival rate
SWP	Silkworm pupae
TI	Thrombogenicity indices
TL	Thawing loss
TL	Total length
TP	Total protein
USD	United state dollar
VSI	Viscero-somatic index
WG	weight gain

## 1. INTRODUCTION

An increase in fish consumption has led to a rapid increase in global fish demand. However, due to the natural resource maximum limits and climate change, capture fisheries will not be able to satisfy the increasing demand. The expansion of aquaculture may fill this gap and relieve pressure on capture fisheries, which have been gradually decreasing. However, the feed, particularly its aquaculture protein source, is another challenging aspect. Fishmeal contributes to the global aquaculture feed supply with the highest ratio, accounting for 60 - 80% due to its excellent nutritional profile (protein, amino- and fatty acid), absence of antinutritional factors, suitability for fish health and promotion of immunity, palatability, ease of nutrient digestibility. Wild fish production is decreasing worldwide, from time to time, and is expected to reach ecological limits in a short period. The use of by-products from fisheries and aquaculture in aquafeeds has increased but will be inadequate for predicted aquafeed demands by 2050 (Rana et al., 2009). This will upshot the growing aqua sector to face a supply shortage of fishmeal, and its replacement in aquafeeds is probable. Various actions have been taken to reduce the proportions of fishmeal in aquafeeds over the past two decades (Tacon and, Metian, 2015), for instance, increasing the inclusion of plant-derived ingredients (Fry et al., 2016), animal origin products and by-products (Tang et al., 2018; Jing et al., 2013), single-cell proteins (Gai et al., 2016). However, the inclusion of plant and animal-origin ingredients in aquafeeds, concerning the environment, places greater pressure on water and land resources' utilization and competition with the human food sector and terrestrial livestock sector (the plant origin) (Fry et al., 2016; Kok et al., 2020). Nowadays, insect meal draws increasing interest as an alternative to fishmeal in aquaculture, especially in fish diets because of its favorable nutrition profile, used as the natural diet of many fish species in the natural environment (Barroso et al., 2014; Henry et al., 2015), the feasibility of commercial-scale production, eco-friendliness, and consumer acceptance. It was reported that approximately 2.5 billion people, mainly in Africa, Asia, and Latin America, eat insects as part of their common diets, similar to eating meat or fish (FAO, 2020). Among the different insect species, yellow mealworm (*Tenebrio molitor*) and black soldier fly (*Hermetia illucens*) seem to be very promising and approved to be used as food and animal feed by European Union (Bovera et al., 2015). Currently, mealworm (MW) and black soldier fly (BSF) have been reported as suitable for partial or total replacement of fishmeal in aqua feeds for a variety of marine and freshwater fish species,

considering fish growth performance, nutrient utilization, digestibility, and fillet quality (Belghit et al., 2019; Caimi et al., 2021). Freshwater fish species account for over 50% of the world's aquaculture production, with tilapia (*Oreochromis niloticus*), common carp (*Cyprinus carpio*), and African catfish (*Clarias gariepinus*) representing the primary culture species. These species are cultured in numerous countries as they have adaptable feeding habits, respond well to a wide variety of culture technologies and are well accepted by consumers (Davis et al., 2009). According to FAO (2013), common carp is the third most widely cultivated and commercially important freshwater fish species in the world. It is considered as a significant species for commercial aquaculture in Asia and some European countries as it has a very high adaptive capacity environment as well as food. This species was also introduced to Ethiopia in Lake Koka by a Catholic priest Aba Samuel from Italy in the late 1960s (Dadebo et al., 2015). The African catfish is one of the major fish species cultured in Africa and is native to the African continent, is also introduced for aquaculture in different parts of the world. Considering the relatively lower production costs when this species is used for aquaculture than with many other fish species, as well as broader environmental requirements for breeding and the excellent quality of the meat as a protein source, African catfish production is a viable aquaculture endeavor (Khan and Pannikar, 2009). Further growth of intensive production should be expected, particularly considering that African catfish is a warmwater species, which can be successfully cultured on a mass scale on different continents (Africa, Asia, Europe) (Khan and Pannikar, 2009). The species is also one of the most important individual commercial freshwater fish in many parts of Africa. This species can be cultivated under tropical circumstances or in areas with access to geothermal waters, in heated recirculating water systems. It is considered to be a very hardy fish in aquaculture terms, which can be densely stocked in waters with low oxygen saturation, making it ideal for culture in areas with a limited water supply. Many insects have been used successfully in African catfish feed. Such as mealworm (*Tenebrio Molitor*) (Ng et al., 2001), the common house fly maggot (*Musca domestica*) (Aniebo, et al., 2009) cricket (*Gryllus bimaculatus*) (Tran et al., 2015), Taufek et al., 2016), caterpillars of *Cirina butyrospermi* (Anvo et al., 2016) and grasshoppers (*Caelifera*) (Olaleye, 2015). Likewise, some insects are tested on carp. For instance, BSF (Xu et al., 2020), silkworm (*Bombyx mori*) (Nandeeshha et al., 2000; Ji et al., 2015).

However, information is scarce regarding the combined effect of BSF and MW meals in the practical diets of African catfish investigated simultaneously on the same experimental condition

at the same time. Furthermore, regarding feeding insect meal, flesh quality, fillet yield, and slaughter traits of neither African catfish nor common carp were documented.

## **2. OBJECTIVES**

The main objective of the Ph.D. work was to investigate the effects of partial and total replacement of fishmeal with insect meals in common carp and African catfish feed.

Specific objectives

- ✚ To evaluate the production parameters (SGR, PER, FCR, WG), biometric indices (HSI, VSI, K), and survival rate of juvenile common carp fed on total and partial replacement levels of fishmeal with black soldier fly meal.
- ✚ To examine the production parameters, biometric indices, and whole-body proximate composition of juvenile common carp fed on total or partial replacement levels of fishmeal with mealworm meals.
- ✚ To examine production parameters, diet apparent digestibility, and blood biochemistry of African catfish fed on a simultaneously replaced fishmeal with total or partial replacement level of black soldier fly, mealworm and, 1:1 combination of black soldier fly and mealworm meals.
- ✚ To analyze fillet yield and flesh quality of common carp and African catfish fed on partial replacement of fishmeal with black soldier fly, mealworm and, 1:1 combination of black soldier fly and mealworm meals

### **3. LITERATURE REVIEW**

An overview of the insect as an alternative protein feed and a background of its benefit, highlighting the environmental, nutritional, and legal regulations, as well as sustainability aspects, are presented. The proximate composition of black soldier fly and mealworm larvae is highlighted, literature on the amino and fatty acid profile of black soldier fly and mealworm larvae, and the inclusion of black soldier fly and mealworm in different fish species is presented. The nutrient requirement of fish with special emphasis on African catfish and common carp are highlighted, and the nutritional values of fish are also discussed. Furthermore, the meat quality of fish fed insect meals is highlighted.

Aquaculture (the farming of fish, crustaceans, and other aquatic animals and plants) is one of the fastest-growing industries. However, a major barrier to the sustainable growth of the industry is the feed cost, particularly fishmeal and fish oil (van Huis, 2013). Approximately 10% of fish production is recycled into meal industries to make fishmeal, and ocean fish stocks are being depleted by overfishing to provide the feed. Increasing restrictions on unregulated fishing and catch quotas are another issue in the supply shortage of fishmeal in aquaculture industries (van Huis, 2013). On the other hand, aquaculture mainly uses wild-caught fish for fishmeal production and therefore diverts a food source that could be consumed by humans (Costa et al., 2020). Consequently, the continued availability of fishmeal in the future is not sustainable due to stiff competition from human consumption (FAO, 2012). Nowadays, commercialized fish farming relies on the use of feeds based on fishmeal and fish oil as optimal ingredients (Zarantoniello et al., 2019). According to the reports of Gaines et al. (2005), the compositional quality of the nutrients found in FM distinguishes it from other dietary supplements, particularly the essential amino acids profile and long-chain polyunsaturated omega-3 fatty acids. Furthermore, high-quality FM and fish oil (FO) provide all essential amino acids in a balanced amount, phospholipids and fatty acids (DHA; docosahexaenoic acid and EPA; eicosapentaenoic acid) for optimal development, growth, and reproduction (Maldjian et al., 2005). However, the increased demand for FM, overfishing pressure, finite nature, and climate change has resulted in a supply shortage with affiliated price (Dong et al., 1993). Thus, in aquaculture to remain sustainable, different efforts have been exerted to find alternative protein sources to replace FM in aqua feeds particularly in fish diets with varying success (Beyhan, 2018).

### **3.1 Global production of fishmeal**

Fishmeal is a product obtained by cooking, pressing, drying, and grinding fresh raw fish or shellfish from typically using small, fatty species like *Engraulis encrasicolus* (*anchovy*), sprat (*Sprattus sprattus*), *Clupea pallasii* (*herring*) and euphausia (*krill*). It is an excellent protein source mainly used as feed for aquaculture species and livestock (EUMOFA, 2021). The pressure for aquaculture to improve the efficiency of fishmeal used also reflects the increasing competition for fishmeal in the global animal feed markets between aquaculture and livestock producers (Rana et al., 2009). The share of world fisheries intended for fishmeal production has declined over the past 20 years. Accordingly, from 2001 to 2010, the average yearly fishmeal production was above 5.5 million tons, while from 2011 to 2020 it decreased and around 5 million tons. The variations from one year to another are affected to a great extent by the supply of forage fish and particularly by the Peruvian anchoveta fisheries, the latter being the world's largest in terms of volume, varying between 3 and 7 million tons a year. The huge variations in the landings of this species are associated with the periodic climate changes, which bring warm water into the upwelling areas. If this situation continues to happen in years, catches might decline by several million tons in one season (EUMOFA, 2021). Likewise, industrial fisheries in the EU are conducted by both EU-registered vessels and non-EU vessels landing in EU ports. The number of fishmeal plants has plummeted in the last 20 years. Thus, these declining trends in world fishmeal production capacities are an urgent alarming situation to seek an alternative to substitute fishmeal in aquaculture feed particularly farmed fish to sustain the sector as well as to meet the increasing demand for fish meat.

### **3.2 Alternative proteins in fish feed**

#### **3.2.1 Plant origin protein sources**

Many plant feedstuffs including full-fat soybean (*Glycine max*), soybeans meal (extruded product or protein concentrate), corn gluten meal, extruded peas, extruded lupin, and rapeseed meals have been tested in the diets of freshwater and marine fish as alternative protein sources with varying successes (Kikuchi, 2001). However, studies prove that the low palatability, high levels of fiber and non-starch polysaccharides, inadequate fatty acid and amino acid profiles, and the impairment of the integrity of fish intestinal enterocytes are the major limiting factors of plant source

alternatives in a fish diet (Gai et al., 2012; Gasco et al., 2018b). Among the mentioned plant-origin proteins, soybean meal is the key alternative to FM in fish farms. However, it has been complained about land-use competition and significant environmental deterioration (Kikuchi, 1999). In addition, soybean meal contains anti-nutritional factors that may cause inflammations in the digestive tract of fish (Gasco et al., 2018b). Numerous studies on many fish species have been done so far with regard to plant origin inclusion. For instance, Oliva-Teles et al. (2015) reported a significant ( $p < 0.05$ ) reduction of crude protein digestibility compared to fishmeal in rainbow trout (*Oncorhynchus mykiss*). Plant proteins still present disadvantages such as anti-nutritional factors (NRC, 2011) that can affect the availability of AA (Cai and Burtle, 1996), high levels of fiber and non-starch polysaccharides, inadequate fatty acid (FA) and amino acid profile and low palatability (Gatlin et al., 2007). On the other hand, a significant reduction of voluntary feed intake of juvenile turbot (*Psetta maxima*) decreased with the increase of plant protein incorporation in diet was reported by Fournier et al. (2004).

### **3.2.2 Animal origin protein sources**

Animal-origin proteins, animal products meals (APM), and animal by-product meals (ABM) are the other alternative protein sources to replace fishmeal in a practical diet of fish. (ABM), unsuitable for human consumption, are good alternatives to use as ingredients in fish diets. They are in general much less expensive than fishmeal (Steffens, 1994). However, individual ABMs such as blood meal, hydrolyzed feather meal or meat, and bone meal often have deficiencies and or excesses in essential amino acids. On the other hand, the presence of zoonotic disease, poor palatability, and inconsistency of the nutritional content might be dependent on parts of trimmings and the type of by-products. Meat and bone meal (MBM) is one of the potential alternative protein sources to replace FM due to its high protein content ranging between 45-65% and low cost compared to FM. It has been strictly banned in the feed of ruminant animals due to the presence of bovine spongiform virus (mad cow disease) (Taylor and Woodgate, 2003). However, in the diets of many aquatic animals, MBM has been successfully used for the replacement of FM (Tacon and Jackson, 1985). MBM is reported to have poor palatability due to the high content of ash. However, MBM has a more favorable amino acid profile, high protein, lipid, and calcium. Poor palatability of meat and bone meal (MBM) due to the high content of ash was confirmed by Robaina et al. (1997). Low palatability can also affect feed intake and result in a reduction of growth performance as well as feed conversion ratio or retention. The increasing inclusion level of meat and bone meal was

reported as it showed negative effects on different fish species, for instance, juvenile *Pseudobagrus ussuriensis* (Tang et al., 2018), tilapia (*O. niloticus*) (Jing et al., 2013), hybrid striped bass (*Morone chrysops x Morone saxatilis*) (Brignon, 2002), swamp eel (*Monopterus albus*) (Cao, 2007) and bluegill (*Lepomis macrochirus*) (Karthik et al., 2014).

### **3.2.3 Single-cell proteins**

Numerous microbes (bacteria, microalgae, and yeast) have been used to produce a wide range of single-cell proteins (Ravindra, 2000). They have been studied as a feed ingredient in diets for rainbow trout (Aas et al., 2006; Øverland et al., 2006), Atlantic halibut (*Hippoglossus hippoglossus*) (Aas et al., 2007), and Atlantic salmon (*Salmo salar*) (Storebakken et al., 2004). The report of Gai et al. (2016) demonstrated that the lowest SGR, FCR, PER, crude protein, and nitrogen digestibility recorded on Rainbow trout fed bacterial protein meal (BPM) and a mixture of bacteria and pea protein concentrates (PPC) containing diet compared to fishmeal. The reason for the declining digestibility of nitrogen was reported most possibly due to the presence of a negative effect on the bacterial membrane and cell wall components (Gai et al., 2016).

The major challenges of single-cell proteins as fish feed reported are: -

- Low palatability resulting in low intake (yeast) (Solomon et al., 2017),
- Low digestibility of bacteria (Tibbetts, 2018),
- Need of yeast protein to improve its protein and EAA content (Ritala et al., 2017),
- Economical scale- up and cell disruption to release nutrients of microalgae as protein sources (Tibbetts, 2018).

### **3.2.4 Insects and insect meals**

Insects represent an innovative feed and food source, rich in high-quality protein, fat, minerals and vitamins (Rumpold and Schlüter, 2013). They may represent promising candidates as they have short generation interval or lifecycle, less environmental burden, needs small initial capital investment, leave a small ecological footprint and have a limited need for arable land and water (Mancini et al., 2019). They are natural food sources for some salt or freshwater fish species (Howe et al., 2014). However, the present price of insect meals seems still not competitive if compared with other protein sources (Koeleman, 2014); an increase in demand inevitably will lead to an



increase of production scale and thereby a reduction of insect meal prices in the future (Mancuso et al., 2016).

### 3.3 Legal regulations on the use of insect protein

#### 3.3.1 Global regulation on insect meals

According to the reports of Sogari et al (2019) the regulatory system on the use of insects as feed or food differs widely between countries around the globe and the differences are not always associated with the “traditional” use of insects as food. The following table adopted from the previous review of other studies shows the differences in the use of insects as food or feed in some selected countries (see Table 1).

Table 1. Feed legislation on the use of insects as feed (Sogari et al., 2019)

Country	Authority	Regulation	Insects as Feed
European Union (EU)	EFSA	EU Decisions/regulations	PAPs authorized in aquaculture Authorized fat from insects in feed Positive list of rearing insects on selected substrates and only approved for seven species of insect
USA	FDA	FFDCA	Additive approval list or GRAS needed for insects. HI larvae included as ingredient for animal food
Canada	CFIA	FAFR	Feed raw material needs authorization, HI product authorized for poultry.
North Korea	Ministry of Agriculture, Food and Rural Affairs	Not exist	Applicable
South Korea	Ministry of Agriculture, Food and Rural Affairs	Not exist	It is not compulsory
China	Ministry of Health	Ministry of Health, Safety and Welfare	Applicable

\*Where EFSA: European Food Safety Authority; PAPs: Processed Animal Proteins; FDA: Federal Food and Drug Administration; FFDCA: Federal Food, Drug, and Cosmetic Act; GRAS:

Generally Recognized as Safe; HI: *Hermetia illucens*; CFIA: Canadian Food Inspection Agency; FAFR: Food Act and Feeds Regulation.

### **3.3.2 EU regulation on insect meals**

In December 2016, EU Member States voted a proposal presented by the European Commission to authorize the use of insect proteins as fish feed in aquaculture. The EU Regulation 2017/893 entries into force as of 1st July 2017). EU member States approved in favor of authorizing the use of insect and processed animal proteins (PAPs), in aquaculture since (13 December 2016).

## **3.4 Benefits of insect protein**

### **3.4.1 Environmental benefit**

Conventional protein sources for food or feed need larger agricultural land and are the source of greenhouse gas and climate change (van Huis and Oonincx, 2017). In contrast, insects are an overlooked source of protein and a way to adapt to climate change (Awayesheh and Picard, 2022). About 27% of all agricultural produce is wasted a yearly and 22% if only the edible part is taken into account. It globally equals 1.6 and 1.3 billion tons, respectively (FAO 2013). Insects, like black soldier fly are feeding on organic materials from other processes, thus preventing additional waste from going into landfills, and providing added value (circular agriculture) (Ramos-Elorduy et al., 2002; Oonincx et al., 2015), diverting materials from landfills, which produce 20% of the global anthropogenic methane emission and are the second highest producers of greenhouse gas (Makkar, 2018). Using insects as inputs for another function closes the cycle and also positively impacts climate change (Awayesheh and Picard, 2022). This circular value chain will continue to help decrease emissions, as disposed organic materials no longer end up in landfills, producing methane and other harmful gases in the process. Due to the respiration and metabolism of insects and their feces, CO<sub>2</sub>, CH<sub>4</sub>, N<sub>2</sub>O, and NH<sub>3</sub> can be emitted. However, direct emission levels seem to be lower than for conventional livestock (Oonincx and de Boer, 2012). Interestingly, mealworms do not emit ammonia excretion (Oonincx et al., 2010), and have a water footprint per ton that is 3.5 times less than that of beef (Miglietta et al., 2015). In general, the major environmental advantages of insect farming compared to livestock production are as follows: (1) requires less land and water; (2) lower greenhouse gas emissions; (3) high feed conversion efficiencies; (4) insects can transform low-value organic by-products into high-quality food or feed.

### **3.4.2 Sustainable feed ingredient**

The use of insects for feeding farmed animals represents a promising alternative because of the nutritional properties of insects and the possible environmental benefits, given the sustainability (van Huis and Oonincx, 2017; Sogari et al., 2019). Large-scale production of insect meals for aquaculture feed has a positive impact on the sustainability and profitability of the fish farming sector (Alfiko et al., 2021). Globally, many companies are interested in /invested in the production of insect feed. Among these, few companies are into animal feed production. Most of the other companies concentrate only on insect feed production and primarily produce insect meals for animal and aqua feeding purposes. There have been over half a billion USD invested in dozens of insect farming and insect feed production companies. Insects are projected to provide 3 to 5 million tons of protein for animal feed by 2030 for Europe alone (Brian, 2019). According to recent data, insect production at the EU level of about 6000 tons per year, corresponding to an average of 2000–3000 tons of insect-derived processed animal proteins (PAPs) (IPIFF, 2019; Mancuso et al., 2019). They are expected to be increasingly used in Europe and around the globe as a replacement for conventional animal-derived proteins for aquaculture (Lock et al. 2018) and terrestrial livestock (Bovera et al., 2018; Khan, 2018). Forecasted insect protein production to be used in both the human food and the animal feed sectors for 2025 surpasses 1.2 million tons (Mt) reaching about 10% of the EU share of the total protein supply (IPIFF, 2019; Mancuso et al., 2019). Thus, the increasing production capacity will be a promising opportunity to sustain the aquaculture sector and meet the increasing feed demand for farmed fish.

### **3.4.3 Nutritional profile**

The average crude protein content of insects varies between 50 and 82% (dry matter base) (Rumpold and Schluter, 2013) and that might be dependent on the method of processing (Fasakin et al., 2003). Defatted insect meals can be richer in crude protein than soybean meals or fishmeal (Tran et al., 2015). The nutrition profile of insect meals can be easily manipulatable by the substrate where they grow (Spranghers et al., 2017; Ewald et al., 2020), or by the appropriate harvesting time of the developmental stage (Mohan et al., 2020). The lipid content of non-defatted insects can be high, up to 36% at MW larvae which is also strongly influenced by the stage of development and by the diet. In addition, insects are rich in trace elements such as copper, iron,

magnesium, manganese, phosphorus, selenium, and zinc. They are also rich in vitamins like riboflavin, pantothenic acid, biotin, and folic acid (Rumpold and Schlüter, 2013), as it is illustrated in Figure 1.

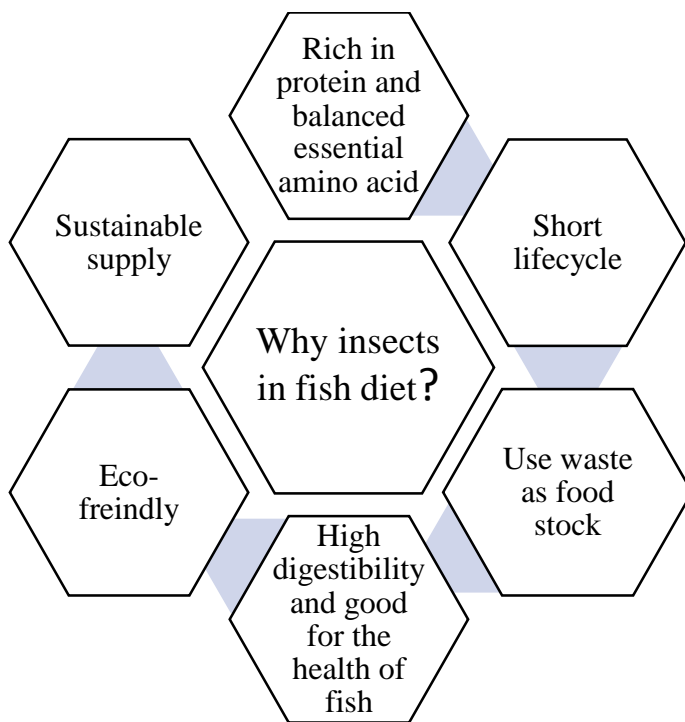


Figure 1. Reasons for insects in fish diet (IPIFF, 2019; Rumpold and Schluter, 2013; Tran et al., 2015)

### **3.5 Challenges in the utilization of insect meals in fish diet**

Besides they are considered to be environmentally friendly, sustainable, and good nutritional profile, there are issues to be taken into account while deciding to formulate the feed for aquaculture, particularly for farmed fish. The following points are some of the challenges to be considered.

#### **3.5.1 Disparity in nutritional composition**

The inconsistency of nutritional profile is considered one of the major issues of insect meal inclusion in aqua feeds (St-Hilaire et al., 2007; Smets et al., 2020). The nutritional composition of insects mainly depends on the developmental stage, processing method, and substrate on which the insects feed. One has to take into account these factors while formulating a fish diet.

### 3.5.2 Chitin

Insects' chitin is considered as fiber the presence of structure similarities to cellulose. Chitin is synthesized by a large number of living organisms, such as arthropods and insects (exoskeletons); crustaceans (shells); scale of scaly fish (common carp, herring), algae, plants, and fungi (cell walls) (Santos et al., 2020). The exoskeleton of arthropods (cuticle) is built primarily of chitin fibers, and chitin is a polysaccharide of glucosamine and N-acetylglucosamine, which both contain nitrogen atoms. Due to the strong linkage of proteins in chitin fibers and changes in chitin with life stage, estimations of the amounts of chitin and non-digestible protein in insect cuticles are not constant. According to the reports of Jonas-Levi and Martinez (2017), hard cuticles have high protein ranging between 70% and 85% (dry weight) and low chitin contents of 15–30%, whereas soft cuticles contain approximately 50% each of chitin and proteins. According to Finke (2007), the digestibility of proteins/AAs in insects can vary and depends on how much of the AAs are bound to chitin or scleroprotein, which is mainly present in adult insect cuticles (Becker and Yu, 2013). However, these proteins or amino acids can be available for fish nutrition due to the presence of chitinolytic activity from enzymes such as chitinase in the stomach and chitobiase in the intestine (Lindsay, 1983). On the other hand, chitin could act as a nutrient source as well as an anti-nutrient (Eggink et al., 2022), then the high amount (> 10%) of chitin is reported to have a negative effect on diet digestibility and growth performance of fed organisms (Kroeckel et al., 2012), whereas a low quantity of chitin can enhance immunological effects and microbiota modulation. (Gasco et al., 2020; Gasco et al., 2018a; Huyben et al., 2019). Another issue to be taken into account during the quantification of crude protein. The Kjeldahl method is still widely used to quantify the crude protein content of insects, which ranges from 8 to 70% of dry mass. This procedure evaluates the total concentration of nitrogen (N), which is converted to protein by multiplying it by the nitrogen-to-protein conversion factor for meat (6.25). Given that the insect cuticle contains large amounts of fibrous chitin, a polysaccharide-rich in N, and proteins tightly embedded in its matrix, and is not digested by humans or domesticated animals, using the Kjeldahl method overestimates the digestible protein content of insects. Thus, there are ways to cope with this. Janssen et al. (2017) proposed the following method to quantify the crude protein content of insects in any developmental stage after they studied and found results by a specific nitrogen-to-protein conversion factor (Kp) of  $4.76 \pm 0.09$  calculated for larvae from *Tenebrio molitor*, *Alphitobius diaperinus*, and *Hermetia illucens*, using amino acid analysis, then after protein

extraction and purification, a Kp factor of  $5.60 \pm 0.39$  was found for the larvae of three insect species studied.

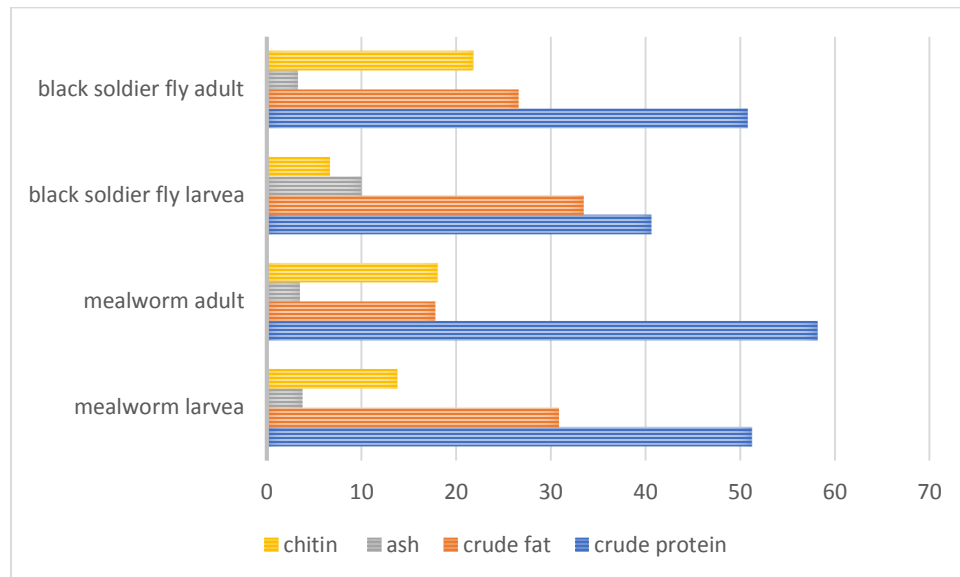


Figure 2. Proximate composition and chitin content of MW and BSF by life stage data analyzed from (Liu et al., 2020; Liu et al., 2017; Igor et al., 2019; Mohan et al., 2020; Gasco et al., 2020; Gasco et al., 2018b; Huyben et al., 2019)

### 3.6 Potential insects in fish feed in European union

There are seven insect species (Black Soldier Fly, Common Housefly (*Musca domestica*), Yellow Mealworm, Lesser Mealworm (*Alphitobius diaperinus*), House cricket (*Acheta domesticus*), Banded cricket (*Grylloides sigillatus*) and Field Cricket (*Gryllus assimilis*)) (Bovera et al., 2015). In addition, according to Byrne (2021), the EU authority authorized the use of processed Silkworm (*Bombyx mori*) protein in aquafeeds in the EU from end of 2021 onwards.

#### 3.6.1 Black soldier fly

According to Newton et al. (2005), the black soldier fly is a harmless insect native to the warm tropical and temperate zones of the American continents. Climate change and human activities enabled its spread to other continents such as Asia, Europe and Australia (Olivier, 2009). Accordingly, the BSF is now becoming distributed to almost 80% of the world between latitudes 46°N and 42°S (Martinez-Sanchez et al., 2011). On the Africa continent, BSF has only been

reported to naturally occur in South Africa, Guinea and Ghana respectively, though used for waste and feed production.

The BSF is a tremendously resilient species and potentially used as an alternative protein source for animal feeds, clearance of organic wastes, by-products, and side streams (Taiwo and Otoo, 2013), can be reared on food and organic wastes by converting them into a protein-rich and fat-rich biomass suitable significantly important for animal feeding, biodiesel and chitin production (Sheppard et al., 2002).

BSF is a very promising species for partial or total replacement of fishmeal in aqua feeds for a variety of marine and freshwater fish species, considering fish growth performance and nutrient utilization, positive physiological effect on fish, acceptable flesh quality and approved to be used as food and animal feed by the European Union (Zarantoniello et al., 2019; Belghit et al., 2019). BSF larvae can efficiently utilize or recycle a proportion of the fish oils from fish processing leftovers and it is possible to maintain a year-round breeding adult colony in a greenhouse with access to full natural light throughout the year (Barry, 2004).

### ***3.6.1.1 Life cycle of black soldier fly***

The BSF undergoes a complete metamorphosis, having four live stages (egg/embryo, larva, pupa and adult) (Li et al., 2011), see Figure 3. The transformation from one life cycle to another depends on many factors but mostly depends on the substrate, temperature and humidity. Accordingly, the lifecycle from egg to adult is estimated to last about 40-43 days under optimum rearing conditions but under unsuitable rearing conditions, the period can stretch up to six months with the longest part of the lifecycle spent at the larval and pupal stages.

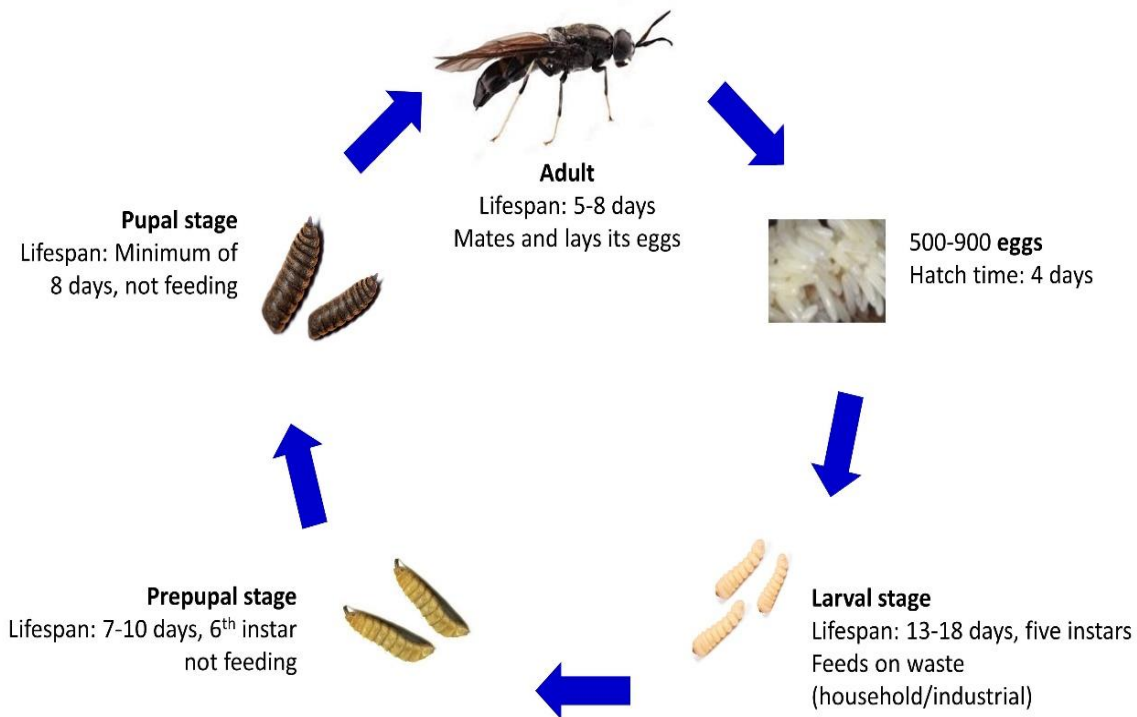


Figure 3. Life cycle of black soldier fly ( <https://encyclopedia.pub/entry/7597> )

### 3.6.1.2 Proximate composition of black soldier fly

Black soldier fly contains the approximately similar proximate composition to soya and is mainly dependent on the life cycle and substrate, as it is shown in Table 2.

Table 2. Proximate composition of BSF fed on different substrate

Feed type	Crude protein (g kg <sup>-1</sup> )	Crude fat (g kg <sup>-1</sup> )	Ash (g kg <sup>-1</sup> )	Sources
Chicken feed	412	n/a	100	
Digestate (g kg <sup>-1</sup> )	422	n/a	197	Spranghers et al., 2017
Vegetable waste (g kg <sup>-1</sup> )	399	n/a	96	
Restaurant waste (g kg <sup>-1</sup> )	431	n/a	27	
	Crude protein (%)	Crude fat (%)	Ash (%)	
Chicken	41.1	n/a	9.3	Shumo et al., 2019
Manure (%)				
Kitchen waste (%)	33.0	n/a	9.6	



Spent Grain (%)	41.3	n/a	11.6	
Bread (%)	39.2	57.8	3.9	
Fish (%)	52.6	46.7	5.7	
Food waste (%)	36.6	40.7	16.3	
Fresh muscle (%)	44.6	33.1	18.7	Ewald, et al., 2020
Ensiled muscle (%)	27.3	11.2	33.0	
Rotten muscle (%)	42.3	29.7	22.6	

n/a: no information

### 3.6.1.3 Amino and fatty acid profile of black soldier fly

The amino acid profile of the black soldier fly is better than soybean meal and can be a better replacement for fishmeal in the fish feed formulation (Barroso et al., 2014). Black soldier fly is well known for its high lauric acid content. The fatty and amino acid profiles are variable depending on the life cycle, substrate type and environmental condition (Haasbroek., 2016), (Tables 3 and 4).

Table 3. Amino acid profile of black soldier fly

Essential Amino acid (gkg <sup>-1</sup> )	Haasbroek, 2016	Al-Qazzazet al., 2016	Schiavone et al.,2017
Lysine	100	28.63	21
Valine	101	21.93	27
Phenylalanine	65	16.29	16.6
Tryptophan	6	0.49	n/a
Methionine	26	26.46	6.46
Threonine	16	22.76	17.2
Isoleucine	58	12.22	18.5
Leucine	104	37.33	28.6
Arginine	67	93.31	21.5
Histidine	58	14.76	12.3
Cystine		n/a	n/a

n/a: no information

Table 4. Fatty acid profile of black soldier fly larvae

Fatty acid	Ewald et al., 2020	Barroso et al.,2017	Liland et al., 2017
	%		
Capric (C10:0)	n/a	0.9	n/a
Lauric (C12:0)	7.5	22.6	23.9
Myristic (C14:0)	2.3	5.3	6.7
Palmitic(C16:0)	19.2	17.9	16.6
Palmitoleic (C16:1n-7)	0.8	1.9	2.5
Stearic(C18:0)	6.9	4.6	4.1
Oleic(C18:1n-9)	26.9	22.9	17.9
Linoleic(C18:2n-6)	31.4	15	18.6
$\alpha$ -linolenic(C18:3n-3)	3.6	0.9	1.6
$\gamma$ -linolenic(C18:3n-6)	0	n/a	n/a
EPA(C20:5n-3)	0.0	1.6	1.0
DPA(C22:5n-6)	0.0	0.0	n/a
Dpan-3(C22:5n-3)	n/a	0.5	1.1
DHA(C22:6n-3)	0.0	0.0	n/a

n/a: no information

#### 3.6.1.4 Black soldier fly meal in fish feeds

Black soldier fly meal successfully replaced the total and partial portion of dietary protein of FM in different inclusion levels; 12.5 % in Atlantic salmon (Weththasinghe et al., 2021); 19.5% in European seabass (*Dicentrarchus labrax*) (Magalhães et al., 2017); 15% in rainbow trout (*Oncorhynchus mykiss*) (Caimi et al., 2021), partial replacement level found to be with the best result in African catfish (Huda et al., 2020). More than 80% digestibility for total dry matter and macronutrients (i.e., proteins, lipids) for all BSFL inclusion levels, are reported in salmon (Weththasinghe et al., 2021). However, inclusion levels beyond 330 gkg<sup>-1</sup> would significantly decrease the palatability of the diet, protein digestibility, feed intake and growth performance in turbot (Kroeckel et al., 2012), see the details below (Table5).

Table 5. black soldier fly meal inclusion in fish diet

<b>Fish species</b>	<b>IBW(g)</b>	<b>duration (days)</b>	<b>Inclusion level</b>	<b>Measured parameters</b>	<b>results</b>	<b>Sources</b>
Rainbow trout ( <i>Oncorhynchus mykiss</i> )	100.1	131	0, 10, 20, 30, 40 and 50% (0, 30, 60, 90, 120 and 150 gkg <sup>-1</sup> )	growth performance, physical characteristics, proximate and fatty acid (FA) compositions of the fillets, gut and liver histology, and diet digestibility	No differences in terms of growth performance, fillet physical parameters, DM, CP, Histopathology of liver and gut, ADC of DM, CP, EE and gross energy Significant difference in terms of SFA as by inclusion	Caimi et al., 2021

Fish species	IBW(g)	duration (days)	Inclusion level	Measured parameters	results	Sources
Jian carp ( <i>Cyprinus carpio</i> var. <i>Jian</i> )	10.1	56	0, 25, 75 and 100% (0, 35, 70, 105 and 140gkg <sup>-1</sup> )	Growth performance and body composition	No significant difference on growthproximate, serum, and amino acidcomposition, biochemical parameters Significant reduction in PUFA	Zhou et al., 2018
Nile tilapia ( <i>O. niloticus</i> )	5.7	32	0, 30, 50 and 80 gkg <sup>-1</sup> )	Growth performance, feed utilization efficiency and body composition	No significance difference on Growth Performance, feed utilization efficiency (FCR and PER), feed intake, whole body composition Significance difference recorded on fatty acid proportion (omega3 negatively affected by inclusion level	Devic et al., 2018

Fish species	IBW(g)	duration (days)	Inclusion level	Measured parameters	results	Sources
Turbot ( <i>Psetta maxima</i> )	54.9	56	0%, 17%, 33%, 49%, 64%, and 76% (165, 332, 486, 640 and 756g kg <sup>-1</sup> )	growth potential, feed intake and nutrient digestibility efficiencies	FBW was negatively affected by BSF inclusion compared to control. Worsen result observed BSF>33% Feed intake was significantly lower at BSF>33% ADC's for BSF was low and were 45.2% for organic matter, 63.1% for crude protein, 78.0% for crude lipid,	Kroeckel et al., 2012
Atlantic salmon ( <i>S. salar</i> )	34	42	0,6.25,12.5and 25% (0, 80.7,161.3 and 322.7g kg <sup>-1</sup> )	nutrient digestibility, nutrient utilization and growth performancs	25% replacement significantly decreased growth, ADC and feed utilization.	Weththasin ghe et al., 2021

### 3.6.2 Mealworm

Mealworm (*Tenebrio molitor*) is a beetle species of the *Tenebrionidae* family, which is distributed worldwide, edible, an exceptional source of novel alternative dietary proteins for animal feed and is already being produced on an industrial scale (FAO, 2013; EFSA, 2015). Mealworms are rich sources of essential amino acids (methionine), lipids, and fatty acids, which vary based on the

developmental stage of the worms (Shafique et al., 2021). In addition, it grows well on organic waste (Khusro et al., 2012). It is usually produced on mixed grain diets with the help of moisture-sourced vegetables, although it can also consume meat or feathers depending on the country's regulation on substrate used for, amongst other alternatives, due to its omnivorous nature of efficiently utilizing all kinds of plant and animal materials (Ramos-Elorduy et al., 2002). Mealworm contains, on a dry basis, high amount of crude protein (47–60%) and lipid (31–43%), a relatively low ash content (> 5%) and fresh larvae contain about 60% water, as well as a good source of vitamins and minerals (Makkar et al., 2014). Mealworm has been used as a potential alternative dietary protein source for replacing soybean meal or fishmeal and is produced at industrial level (Ramos-Elorduy et al., 2002; Bovera et al., 2016). Similarly, it has already been tested to replace fishmeal for farming rainbow trout (*O. mykiss*), European sea bass (*Dicentrarchus labrax* L.), common catfish (*Ameiurus melas*), Tilapia (*O. niloticus*), yellow catfish (*Pylodictis olivaris*) (Su et al., 2017; Antonopoulou et al., 2019). According to Finke and Oonincx (2014), the nutritional quality of insect proteins has generally been described as being good. However, their significance for use as alternative feed sources depends on their digestibility and amino acid profile.

MW is economically among the most important species used for the large-scale conversion of plant biomass into protein. The energy used to produce 1 kg of fresh mealworms is similar to that used in the production of beef and pork, but the land area required was much less compared to beef, chicken and pork. The production of ammonia and greenhouse gases was significantly lower for mealworms compared with livestock (Grau et al., 2017).

#### **3.6.2.1. Life cycle of mealworm**

The life cycle of mealworm takes not more than 3 months to be beetle, even faster and shorter when there is good feeding and conducive environments (Khusro et al., 2012). The detail is presented on Figure 4.

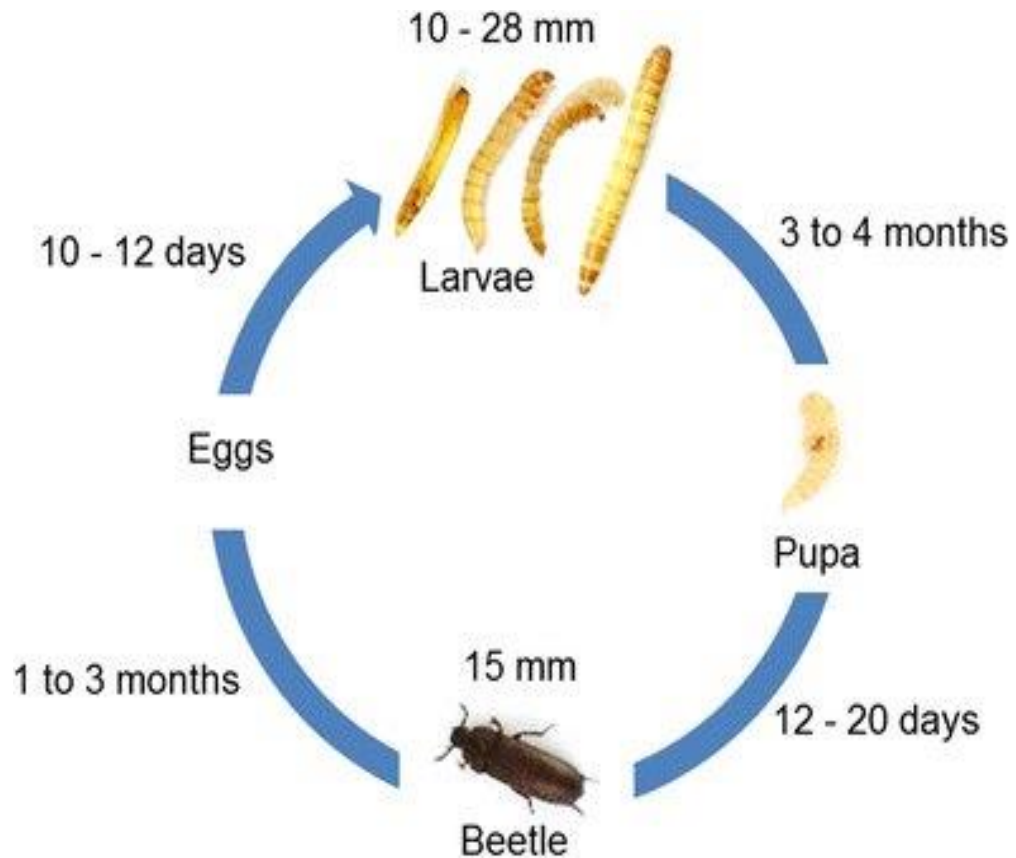


Figure 4. lifecycle of mealworm (*Tenebrio molitor*) (<https://www.breedinginsects.com/yellow-mealworm-biology/>)

### 3.6.2.2 Nutritional composition of mealworm

As many other species, the nutritional composition of mealworm varies depending on numerous factors. Some of the factors include substrate, processing technology, developmental stage (Sánchez -Muros et al., 2016; 286; Toviho and Bársony, 2022). The nutritional composition of mealworm is presented in (Table 6)

Table 6. Proximate composition of mealworm grown on the different substrates

Feed type	CP (%)	Crude fat (%)	Ash (%)	Sources
Cereal bran	58.42	n/a	n/a	(Sánchez - Muros et al., 2016)
Mixture of cereal grains	55.83	25.19	4.84	Igor et al., 2019
Organic wheat flour	37.78	40.10	3.48	Ruschioni et al., 2020
Middling	50.14	34.04	3.90	
Middling +olive pomace75:25	47.58	32.14	3.86	
middling/olive pomace 50:50	39.39	35.32	3.92	
middling/olive pomace 25:75	38.05	36.06	4.57	Song et al., 2018
Watermelon rinds	43.38	32.84	4.40	
Banana peels	38.53	40.13	2.48	
Broiler's egg shell	42.49	33.57	3.44	

n/a: no information

### 3.6.2.3 Amino acid and fatty acid content of mealworm larvae

The essential amino- and fatty acid profile of insects varies according to substrates used, environmental conditions, species and developmental stages (Anvo et al., 2017). Igor et al. (2020) reported that the fatty and amino acid profile of mealworm affected by substrates, protein, and fat content. The high content of fatty acids in feed affects their antioxidant activity, which is highly required in human diets (Wojciak and Dolatowski, 2012). According to the findings of Ravzanaadii et al. (2012), mealworm contains high amounts of unsaturated fatty acids, mainly linoleic and oleic acid, and palmitic acid as saturated fatty acid. The essential amino and fatty acid profile of mealworm are presented in Tables 7 and 8.



Table 7. Essential amino acid profile of mealworm

Essential amino acid (gkg <sup>-1</sup> )	Adámková et al., 2020	Igor et al., 2020	Zhao et al., 2016
Lysine	25.5	26.7	59
Valine	32.2	65	65
Phenylalanine	17.8	30.5	109
Tryptophan	25.0	38.5	n/a
Methionine	4.8	17.6	21
Threonine	20.1	14.7	36.5
Isoleucine	24.8	41.2	50.7
Leucine	38.9	29.6	83
Arginine	27.4	36	55
Histidine	16.5	n/a	24
Cystine	4.1	n/a	n/a

n/a: no information

Table 8. Fatty acid profile of mealworm larvae

Fatty acid %	Ravzanaadii et al., 2012	Costa et al.,2020	Igor et al., 2020	Anvo et al., 2017
Capric (C10:0)	n/a	n/a	n/a	0.00
Lauric (C12:0)	n/a	n/a	n/a	0.00
Myristic (C14:0)	3.05	4	n/a	4.45
Palmitic(C16:0)	16.72	15.3	16.20	21.33
stearic(C18:0)	2.49	2.7	2.2	7.92
Palmitoleic (C16:1)	n/a	2.8	n/a	1.79
Oleic(C18:1n-9)	43.17	37.8	40.83	35.83
Linoleic(C18:2n-6)	30.23	33.2	29.8	22.83
$\alpha$ -linolenic(C18:3n-3)	1.36	1.5	n/a	0.11
$\gamma$ -linolenic(C18:3n-6)	0.05	n/a	n/a	0.00
EPA(C20:5n-3)	0	n/a	0.02	0.00
DPA(C22:5n-6)	0	n/a	n/a	0.00
DHA(C22:6n-3)	0	n/a	0.07	0.00

n/a: no information

### 3.6.2.4 Mealworm larvae in fish feed

Feeding trials in several aquaculture species proved that fresh and dried mealworms are acceptable as an alternative protein source for aquaculture (Alfiko et al., 2021) The potential of MW for partial or total replacement of the fishmeal (FM) in the aquafeeds has been previously assessed in various fish species, such as European perch (*Perca fluviatilis*) (Tran et al., 2022); European sea bass (*D labrax*) (Gasco et al., 2016; Rema et al., 2019). Piccolo. (2017) reported lower apparent digestibility coefficients (ADC) of crude protein (CP) and ether extract (EE) for gilthead sea bream (*Sparus aurata*) fed a diet containing 500 gkg<sup>-1</sup> of mealworm meal than those fed low mealworm meal level (250 gkg<sup>-1</sup>) and fishmeal (FM)-based diets. See the following table (Table 9) for more details.

Table 9. inclusion of mealworm meal in fish diet

Fish species	IBW (g)	duration (day)	Inclusion level	Measured parameters	Results	Sources
Blackspot sea bream ( <i>Pagellus bogaraveo</i> )	110.67-246.36	131	0, 25, 50% (0, 210, 400 gkg <sup>-1</sup> )	growth performances marketable, physical and chemical traits	<p>No significant difference on:</p> <ul style="list-style-type: none"> <li>• Daily intake ratio FCR, SGR</li> <li>• slaughter traits and carcass yield</li> <li>• water holding capacity and texture characteristics (hardness, cohesiveness, resilience, gumminess and adhesiveness),</li> </ul> <p>Significance difference on: -</p> <ul style="list-style-type: none"> <li>• Σn-6 higher at MW50</li> <li>• Σn-3FA lower at MW50</li> </ul> <p>Lower pH at MW50</p>	Iaconisi et al., 2017

Fish species	IBW (g)	duration (day)	Inclusion level	Measured parameters	Results	Sources
African catfish ( <i>C. gariepinus</i> )	5.16	49	0, 20, 40, 60, 80 and 100% (60.3, 86.8, 173.5, 2, 347 and 433.8gkg <sup>-1</sup> )	Growth performance and feed utilization efficiency	Up to 40% replacement no significant difference on growth and feed utilization MW100% significant reduction	Ng et al., 2001
Red hybrid tilapia ( <i>O. niloticus</i> )	4.67	90	0, 25, 50, 75 and 100% (0, 81.2, 163, 244 and 325gkg <sup>-1</sup> )	Growth and feed utilization efficiency	<ul style="list-style-type: none"> <li>No significant difference up to 75%</li> <li>Significant reduction of growth at 100%</li> </ul> significant reduction beyond 25% on growth	Zainab et al., 2022
European Perch ( <i>Perca fluviatilis</i> )	20.81	105	0, 25, 50, and 75% (0, 68, 135 and 203gkg <sup>-1</sup> )	production performance, serum biochemistry, nutrient digestibility, fillet traits, intestinal microbiota	The aspartate aminotransferase activities in perch's serum increased with increasing dietary MW solid nitrogen waste significantly increased with elevated MW level fillet composition was not affected by MW level the diversity of fish gut microbiota was not modified by MW level	Tran et al., 2022

IBW: initial body weight, SGR specific growth rate, ADC apparent digestibility coefficient, FCR feed conversion ratio

### 3.7 Nutrient requirements of the fish

Understanding the optimum nutrient requirements, optimizing commercial feed formulations and managing feed inputs are all the central points to the success of the fish farming industry (Davis

et al., 2009). Successful production of good quality fish can be achieved by feeding the fish with nutritionally balanced feeds (Prabu et al., 2017). Nutrition and feeding are the significant criteria that should be focused on for economical and sustainable aquaculture. Sustainable production of aquatic organisms can be obtained by formulating and producing low-cost, low-polluting, and nutrient-rich high-quality artificial feeds (Prabu et al., 2017). Like terrestrial animals, around 40 essential nutrients are required by aquatic organisms which include protein, carbohydrates, fatty acids, vitamins, minerals, growth factors and other energy sources essential for maintaining growth, reproduction and other normal physiological functions (Prabu et al., 2017).

### **3.7.1 Protein**

The optimal dietary protein level required for maximal growth in farmed fishes is reported to be 50–300% higher than that of terrestrial farm animals (Cowey, 1975). In the main, these quantitative differences have been attributed to the carnivorous/omnivorous feeding habits of fishes and their apparent preferential use of protein over carbohydrates as a dietary energy source.

According to NRC (1993) the protein requirements mean, the minimum amount needed to meet the requirements for amino acids and to achieve maximum growth. Protein is considered as the major concern during the formulation of fish feed to provide the amino acid needs of fish (Wilson, 2003). It is the most expensive fish feed and the most significant factor that contributes to the growth performance of cultured species by serving three purposes (providing energy, supplying amino acids, meeting requirements for functional proteins- enzymes and hormones and structural proteins) in the nutrition of fish (Deng et al., 2011). Protein requirements vary among the fish species and they range from 25% to 57% (NRC, 2011; Ogino and Saito, 1970; Coutinho et al., 2016). The major factors for the variability of protein requirements include dietary protein-to-energy balance, amino acid compositions and digestibility of proteins, amount of non-protein energy sources in test diets, fish species and size, and water temperature (Wilson and Halver, 1986; Wilson, 2003).

### **3.7.2 Amino acid**

An absolute requirement for 10 amino acids (arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine) has been demonstrated in all fish species examined so far (NRC, 1993). Some amino acid deficiencies lead to well-defined

pathologies; deficiency is generally manifest as a reduction in weight gain. In certain fish species, however, a deficiency of tryptophan or methionine does lead to pathologies and the relationship between amino acid intake and the incidence of pathology may provide useful information with respect to requirements (Cowey, 1994). The essential amino acid requirement of African catfish and common carp is presented in Table 10.

Table 10. essential amino acid requirement of African catfish and common carp (Ogino, 1980; Wilson and Moreau, 1996; Robinson and Li, 2007)

	African catfish	Common carp
Crude protein (%)	35-50	28-43
Crude fat	7.4–12	4.6-15
EFA (%)	0.5-0.75	0.5-1
Amino acids (%)		
Lysine	1.23	2.2
Valine	0.71	1.4
Phenylalanine	1.20	2.5
Tryptophan	0.12	0.3
Methionine	0.46	1.2
Threonine	0.53	1.5
Isoleucine	0.62	0.9
Leucine	0.8	1.4
Arginine	1.03	1.6
Histidine	0.37	0.8

### 3.7.3 Lipids

Lipids are one of the utmost valuable nutrients in the formulation of fish feed. They are not the only source of energy, but also provide phospholipids, essential fatty acids, and sterols. Lipids are necessary to maintain the function of physiological processes and to stabilize the biological structure and function of the membrane system of the organism and are needed for basic functions (growth, reproduction and maintenance) (Sargent et al., 1989). They also assist in the absorption and transport of fat-soluble vitamins, hormone precursors, vitamin D, and poly-unsaturated fatty

acids, such as arachidonic acid (NRC, 1993). Due to their comparative physiological advantages (poikilothermic, excretion of ammonia without converting to urine or uric acid and buoyancy), fish have a lower energy requirement resulting in digestible energy: protein ratio (8 to 10 kcal of DE/g of CP for fish vs. 15 to 20 kcal of DE/g of CP for livestock) (cattle) (Lovell, 1991).

#### **3.7.4 Essential fatty acids**

Like other vertebrates, fish cannot synthesize either omega 6 (18:2(n-6)) or omega3(18:3(n-3)) de novo. Thus, one or both of these fatty acids must be supplied in the diet, depending on the EFA requirements. Polyunsaturated fatty acid (PUFA) allows optimal physiological performance in the growth process including visual development, optimal pigmentation and immunity, and maintenance of cell membrane fluidity that are expressed in better growth and survival of fish larvae (Das, 2006). The EFA requirement of the fish is thus related, to some extent, to their ability to modify these fatty acids metabolically. However, fish require n-3 fatty acids and land animals require n-6 (Lovell, 1991). A small amount of lipid should be included to supply EFA. African catfish apparently require 0.5 percent to 0.75 percent omega-3 fatty acids in the diet (Robinson et al., 2001)

#### **3.7.5 Carbohydrates**

Carbohydrates are the major constituent of plants, comprising 50% to 80% of the dry weight of various plants (Robinson et al., 2001). They form the structural framework of plants and are the primary form of energy stored in seeds, roots, and tubers. Plants synthesize carbohydrates from solar energy, carbon dioxide, and water through the process of photosynthesis. Animal tissues contain small amounts of stored carbohydrates. Glucose in the blood of animals is relatively constant, at about 0.05% to 0.1%. Circulating glucose is utilized for energy and is replenished from stores of glycogen in the liver. Generally, glycogen stores in the liver are small, representing only about 3% to 7% of liver weight in most animals. Excess ingested carbohydrate is converted to and stored primarily as lipids (Robinson et al., 2001). The carbohydrate requirement of fish depends on their feeding habits (carnivorous, omnivorous and herbivorous). Carbohydrate digestion and capacity is variable among fishes, where carnivorous fish are less able to utilize it than omnivorous and herbivorous species (Krogdahl et al., 2005). Carbohydrate inclusion in carnivorous fish diets is limited to 20 % (NRC, 2011). If carbohydrates are not provided in the diet, other nutrients such

as protein and lipids are catabolized for energy and to provide metabolic intermediates for the synthesis of other biologically important compounds (Wilson, 1994). The recommended carbohydrate level for common carp is 30 to 40%, while for channel catfish is 25 to 30% (Hardy, 1991; Wilson, 1991)

### **3.7.6 Minerals**

Unlike most terrestrial animals, fish can absorb some minerals (inorganic elements) not only from their diets but also from their external aquatic environment (NRC,1993). Calcium (Ca), magnesium (Mg), sodium (Na), potassium (K), iron (Fe), zinc (Zn), copper (Cu), and selenium (Se) are generally derived from the water to satisfy part of the nutritional requirements of fish (NRC, 1993). While, Phosphates and sulfates are more effectively obtained from feed sources (Hardy, 1989). The functions of calcium and phosphorus are direct involvement in the development and maintenance of the skeletal system and participate in several physiological processes. Furthermore, fish scales are also an important site of calcium metabolism and deposition (NRC,1993). The calcium requirement of fish is met in large part by absorption through gills and skin in fresh water and by drinking seawater. On the other hand, the calcium requirement is dependent the water chemistry, the concentration of Ca in the rearing environment and the availability of dietary Ca and species differences (Hossain and Yoshimatsu, 2014). A low concentration of calcium (0.34 percent or less) is required in the diet of carp and eel for optimum growth, while for catfish and tilapia reared in calcium-free water require 0.45 percent and 0.7 percent calcium in the diet, respectively, for optimum growth (Robinson, 1991). Phosphorus is an important constituent of nucleic acids and cell membranes, and is directly involved in all energy-producing cellular reactions. Feed is the main source of phosphate for fish because the concentration of phosphate is low in natural waters. The dietary supply of phosphate is more critical than that of calcium because fish must effectively absorb, store, mobilize, and conserve phosphate in both freshwater and seawater environments. In most fish, the main phosphorus deficiency signs include poor growth, feed efficiency, and bone mineralization (Robinson et al., 2001).

### **3.7.7 Vitamins**

Vitamins are organic compounds, distinct from amino acids, carbohydrates, and lipids, that are required in trace amounts from an exogenous source (usually the diet) for normal growth,

reproduction, and health (NRC,1993). They are classified into two groups: water-soluble and fat-soluble. The water-soluble vitamins are required in relatively small amounts, have primarily coenzyme functions, and are known as the vitamin B complex (Halver, 1989). The water-soluble vitamins, choline, inositol, and vitamin C, are required in larger quantities and have functions other than coenzymes. Vitamins A, D, E, and K are the fat-soluble vitamins that function independently of enzymes or, in some cases, such as vitamin K, may have coenzyme roles. In mammals, the absence of vitamins leads to characteristic deficiency diseases, but in fish, such diseases are less specifically identified. Some vitamins may be synthesized from other essential nutrients to spare a portion of the dietary requirement. For example, channel catfish appear to synthesize choline if adequate methyl donors such as methionine are present in the diet (Halver, 1989).

### 3.8 Production systems of African catfish and common carp

Based on the production input level and output level, the aquaculture system has been divided into three types: extensive, semi-intensive, and intensive types (Baluyut and Balnyme,1995). Intensive culture has an advantage of high productivity and less space than extensive, but it requires high operating costs and a higher survival rate of fish.

African catfish can be cultured in different aquaculture production systems including a recirculating aquaculture system (RAS), a (semi) flow through system or ponds (Khan and Pannikar, 2009). It is mainly cultivated under mixed-sex semi-intensive systems in earthen and liner ponds or intensive with high stocking rate. Ecologically, African catfish are inhabiting freshwater lakes, rivers, swamps and lagoons as well as man- made habitats. They are well known for their polyculture features with the Nile tilapia in many African countries. Common carp can be produced in extensive, natural food and supplementary feed-based monocultural production systems, in stagnant water ponds. Artificial feed-based intensive monocultural production can be carried out in cages, irrigation reservoirs, and running water ponds and tanks, or in RAS.

Common carp are stocked with Chinese carps, and/or Indian major carps, tilapia, mullet, etc., in polycultural systems. This constitutes a natural food and supplementary feed-based production method, in which fish that have different feeding habits and occupy different trophic niches are stocked into the same ponds. The quantity of fish should be in accordance with the productivity of natural food organisms. Carp culture can be integrated with animal husbandry and/or plant production. Integration can be direct (animals above fish ponds), indirect (wastes of animals are



used in the ponds as manure), parallel (rice-cum-fish), or sequential (fish production between crops).

### **3.9 Feeding habit and the digestive physiology of African catfish and common carp**

A holistic understanding of feeding behavior and digestive physiology is important for designing diets for fish to meet requirements for the best presentation of prey and micro- diets, and their optimal ingestion, digestion and absorption (Rønnestad et al., 2013). According to Evans and Claiborne (2006), the feeding activities of fishes are classified into three categories based on the nature of the food consumed by all fish species: (1) herbivores: fish that eat plant material; (2) carnivores: that consume animals and (3) omnivores: that consume both plant and animal materials). In its natural environment, the African catfish has been described as an omnivorous predatory fish which feeds on a wide variety of food items from minute zooplankton, crustaceans to fish half of its own length or 10% of its own body weight, terrestrial insects, aquatic insects, debris of higher plants, fruits (Bruton, 2010). In its artificial (controlled) environment, the African catfish (animal-oriented omnivore) is adaptive to a wide range of environments which consist of plant-origin animal-origin insects and yeast but with protein rather than carbohydrates. A fish's digestive system is adapted to their food habits. The prominent digestive organs of African catfish include the mouth, esophagus, liver, stomach and intestine. The mouth is usually large for engulfing prey whole, or in large chunks (Moawad et al., 2017).

Common carp were mainly benthic in habitat choice, feeding on benthic macroinvertebrates when only plankton and benthic macroinvertebrates were available in the system. In the absence of benthic macroinvertebrates, their feeding niche shifted from near the bottom of the tanks to the water column where they spent 85% of the total time and fed principally on zooplankton. Common carp readily switched to artificial feed when available, which led to better growth. Common carp preferred to graze individually (Rahman et al., 2010)

The common carp (*C. carpio*) is omnivorous in its feeding habits in Lake Koka, with considerable seasonal variation but insignificant onto genetic change in its diet (Dadebo et al., 2015), and as it is a stomachless species, absorption and digestion of nutrients take place in the intestine (Ziółkowska et al., 2021). The species is a bottom feeder. The common carp is a stomachless species.

### **3.10 Nutrient requirements of African catfish and common carp**

The feed supplementation depends on the type of farming system (extensive, semi-intensive intensive), type of facilities (tank, cage, natural pond), developmental stages (larvae, fry, fingerling, grower, finish...). Early catfish producers depended primarily on natural pond organisms to provide nutrients essential for fish growth (Robinson et al., 2001). Fish production can be often enhanced by the addition of fertilizers to pond water to stimulate the growth of natural food organisms. Formulated feeds, mixtures of feedstuffs processed into various forms, have been used to supplement natural productivity. Supplemental feeds for catfish are mostly steam-pelleted (sinking) feeds that provide protein and energy (Robinson et al., 2001). Due to the reduction of natural food, intensive culturing of catfish is becoming predominant throughout the globe in an intensive stocking density. Nutritionally complete feeds that provide all required nutrients required by catfish, as well as sufficient energy needed for their metabolism, can be developed and manufactured either by steam pelleting into sinking pellets or by extrusion to make pellets that would float on the water surface. However, dietary energy may result in an increased weight gain, but body fat is also likely to increase. Energy requirements can be changed with environmental temperature and each 10 °C increment doubles the growth of catfish until the optimal temperature (30°C) requirement (Robinson et al., 2001). Protein comprises about 70% of the dry weight of fish muscle (NRC, 2011; Robinson et al., 2001). Thus, a continuous supply of protein is important for maintenance and growth. Water temperature, feed allowance, fish size, amount of nonprotein energy in the diet, protein quality, natural food available, and management practices are the major determinant factors for deciding the actual level of protein requirement of catfish. However, studies recommending 35-50% crude protein and energy requirements (DE/P ratio of 8.5–9.5 kcal/gram) is adequate for use in commercial catfish feeds (Robinson and Li (2007). Likewise, the common carp requires for crude protein levels ranging from 30 to 38 percent and seems to satisfy the common carp to the optimal level.

The daily requirement of common carp for protein is about 1 gkg<sup>-1</sup> body weight for maintenance and 12 gkg<sup>-1</sup> body weight for maximum protein retention (FAO, 2022). As an omnivorous fish, the common carp can effectively utilize both lipids and carbohydrates as dietary energy sources. The enrichment of the digestible energy content from 13 to 15 MJ/kg diet by the addition of lipids at levels of 5–15 percent to diets did not result in a higher growth rate or improved net protein utilization (Takeuchi et al., 1979). On the other hand, Pantazis, (2005) reported that the crude

protein level of 46%, a crude lipid level of 10-17%, and a carbohydrate level of 26-32% is best performing for *C. garipepinus*. Increasing dietary lipid seem to increase its body deposition. From the essential fatty acids, common carp requires both n-6 and n-3 fatty acids. Supply of 1 percent of each of these fatty acids leads to the best growth and feed efficiency in juvenile common carp (Takeuchi and Watanabe, 1977). An adult carp reached the maximum daily feeding rate at 28°C, being 2.84% of body mass (Song-bo et al.,2012).

### **3.11 Flesh quality of fish fed insect meal as fishmeal replacement**

Since marine fish are rich in omega 3 highly unsaturated fatty acid (n-3HUFA), which is significantly important for human health, as an anti-inflammatory, significant modification or replacement of their fatty acid content could affect the perception of consumers and resulting market value of fish cultured at a commercial level for human consumption (Amberg and Hall, 2008). Furthermore, the modification of fillet lipid and fatty acid composition directly affects the total volatile compounds and affects the flesh aroma as well as flavor (Turchini et al.,2004). The replacement of fishmeal with insect meal can raise the amount of fat or alter the nature of lipids in fish as a result, change the taste of the fish fillets (Bondari and Sheppard, 1981). According to the reports of Caimi et al. (2021), the absence of significant ( $p > 0.05$  ANOVA) differences was recorded in terms of the fillet's physical characteristics, dry matter (DM), crude protein (CP) and ether extract (EE) contents. While total saturated and monounsaturated FA increased and polyunsaturated FA (particularly n-3 FA) decreased while increasing the Black soldier fly meal inclusion in the diet of rainbow trout (*O. mykiss*). On the other hand, less fondness has been shown for catfish and tilapia fed solely with whole BSF larvae due to the different aroma and texture from that of fish fed a commercial diet or fed partly with BSF larvae (25 or 50%) (Bondari and Sheppard, 1981). Similarly, Azri et al. (2022) reported the PUFA content of African catfish decreased as the fishmeal in the diet was partially replaced with black soldier fly meal. Apart from this, no difference was reported in organoleptic properties was found in African catfish fed maggot meal (Aniebo et al., 2011), in Atlantic salmon fed defatted BSF (Lock et al., 2016), in cyprinids fed SWP (silkworm pupae) oil (Nandeeshia et al., 1999, 2000) or non-defatted silkworm pupae meal (Nandeeshia et al., 2000) compared to control fish. It can be understood that the above results suggest the partial inclusion of insect meal (10–50%) in the diet of fish does not affect the FA profiles, aroma, or flavor enough to be detected by consumers. Defatting black soldier fly larvae

were reported to feed with higher protein values than those commonly found in soybean meals and then increase the muscle protein composition of the fish (Ng et al., 2001).

### **3.12 Nutritional composition of fish meat**

The nutritional value and physical properties of fish meat can vary considerably between species and between individuals of the same species. Also, the contents of protein and lipids, and the size of muscle fibers, are closely related to the origin (fishing or farming), age, body weight, type of feeding, migratory behavior and reproductive status (Solari, 2006). It is widely known that reproductive activity causes stored energy expenditure in the form of lipids or proteins, depending on environmental conditions (Gjedrem et al., 2012). Iaconisi et al. (2018) reported that replacing of mealworm meal in rainbow trout (*O. mykiss*) did not show significant differences in proximate composition of fillets (raw and cooked), whilst the fatty acid (FA) profile was strongly affected by the diet containing insect meal.

#### **3.12.1 Protein content of fish meat**

Fish meat is considered a protein of high biological value, not only because it has all the essential amino acids, but also because it presents digestibility rates superior to those of beef, eggs, and milk (Watanabe et al., 1996). The crude protein content in fish flesh varies depending on the species, the nutritional aspects, the production cycle and the body part. Research on muscle protein content in commercial catfish reported levels between 12% and 21%, depending on the origin (cultured or natural), reproductive cycle and type of feeding (Thammapat et al., 2010).

#### **3.12.2 Lipid content and fatty acid composition of fish meat**

According to lipid content, fish meat can be classified as lean (< 2% fat), low fat (2-4%), medium fat (4-8%) and blue or fat (> 8%). This classification involves not only individual characteristics of the nutritional quality of the meat, but also the visual aspect, yield during processing and taste (Wimalasena and Jayasuriya, 1996). Fat content varied between species; freshwater fish, 0.6-14 g/100g and marine fish, 0.5-9 g/100g. It is well known that marine sources have high PUFA content and any modification or replacement can result in significant changes in the PUFA profile (Zhou et al., 2018). Fabrikov (2021) reported that the main drawback of insect meal inclusion in the fish diet is reduction of valuable n-3 PUFAs in fillets, such as EPA and DHA. Thus, great

attention should be given while replacing conventional and optimal dietary protein sources (FM) in aquafeed, for avoiding to excessively penalize the intrinsic healthiness properties of the fish flesh for humans (Iaconisi et al., 2018). Eating fish and fish products has long been recognized as a health-promoting food. Increasing evidence points to the fatty acids in fish, and particularly the long chain (C20 and longer) omega-3 fatty acids it contains, and also playing vital roles in the diet of human beings and animals contributing to good health (Pike, 1999).

It has been reported that the consumption of fatty acids of PUFA n-3. Particularly, EPA and DHA, promote the reduction of the risk of many diseases in humans, such as brain development and retinal disorders, neurological dysfunctions, inflammatory processes, auto-immune disorders and cardiovascular diseases (Pike, 1999). According to health recommendations, the omega-6/omega-3 ratio should be lower than 4, thereby reducing the incidence of chronic food-related illnesses (Cordain et al., 2005). On the other hand, the recommended PUFA/SFA ratio is to be higher than 0.4 in animal products, so as to reduce the risk of cardiovascular, autoimmune and other chronic diseases (Simopoulos, 2002). Many scholars confirm that when FM is replaced with insect meal, the FA profile, especially omega -3 decreases dramatically. For example, Iaconisi et al. (2018) confirmed that the FAs C16:0, C18:1n-9 and C18:2n-6 increased whilst EPA and DHA progressively diminished in fillets when FM replacement with mealworm meal increased. In rainbow trout feeds regarding the FA quality indices (both for raw and cooked fillets), the MW50 group had a higher thrombogenicity index than the MW0 group; PUFA/SFA and n-3/n-6 ratio gradually decreased with the increase of FM replacement in the diets (Iaconisi et al., 2018). Similarly, Fabrikov (2021) confirmed that significant reduction of PUFA profiles of fillets and lipid indices (n-3/n-6 ratio, atherogenicity AI, and thrombogenicity TI indices) of fillets. when three fish species: sea bream (*Sparus aurata*), tench (*Tinca tinca*) and rainbow trout (*O. mykiss*) fed at replacement level of 30% (109gkg<sup>-1</sup>) BSF and (107gkg<sup>-1</sup>) MW meal for the experimental period of 45 days in sea bream, 46 days in rainbow trout and 100 days in tench.

Table 11. Effect of the dietary inclusion of MW (*T. molitor*) larvae meal on fatty acid profile (% of total fatty acids) of rainbow trout cooked fillet (Iaconisi et al., 2018)

	Diet			p	RSD <sup>1</sup>
	TM0	TM25	TM50		
Number	6	6	6		
Fatty acids					
C14:0	3.29 <sup>A</sup>	2.82 <sup>B</sup>	2.44 <sup>C</sup>	< 0.0001	0.237
C16:0	14.15 <sup>C</sup>	15.51 <sup>B</sup>	17.15 <sup>A</sup>	< 0.0001	0.727
C18:0	3.89	4.01	4.12	0.096	0.171
SFA	22.34 <sup>b</sup>	23.26 <sup>ab</sup>	24.51 <sup>a</sup>	0.007	1.016
C16:1n-7	4.21 <sup>A</sup>	3.36 <sup>B</sup>	2.76 <sup>C</sup>	< 0.0001	0.231
C18:1n-9	18.59 <sup>C</sup>	22.91 <sup>B</sup>	26.73 <sup>A</sup>	< 0.0001	0.749
C18:1n-7	2.58 <sup>A</sup>	1.88 <sup>B</sup>	1.17 <sup>C</sup>	< 0.0001	0.055
C20:1n-9	1.72 <sup>A</sup>	1.32 <sup>B</sup>	0.93 <sup>C</sup>	< 0.0001	0.089
MUFA	28.85 <sup>C</sup>	30.76 <sup>B</sup>	32.63 <sup>A</sup>	< 0.0001	1.010
C18:2n-6	7.26 <sup>C</sup>	15.36 <sup>B</sup>	20.90 <sup>A</sup>	< 0.0001	0.455
PUFAn-6	9.79 <sup>C</sup>	18.55 <sup>B</sup>	26.67 <sup>A</sup>	< 0.0001	0.549
C18:3n-3	1.81 <sup>A</sup>	1.49 <sup>B</sup>	1.06 <sup>C</sup>	< 0.0001	0.068
C20:5n-3	6.43 <sup>A</sup>	3.99 <sup>B</sup>	1.56 <sup>C</sup>	< 0.0001	0.190
C22:5n-3	2.50 <sup>A</sup>	1.52 <sup>B</sup>	0.70 <sup>C</sup>	< 0.0001	0.132
C22:6n-3	24.7A <sup>A</sup>	18.29 <sup>B</sup>	11.91 <sup>C</sup>	< 0.0001	1.328
PUFAn-3	37.52 <sup>A</sup>	26.54 <sup>B</sup>	15.79 <sup>C</sup>	< 0.0001	1.484

Table 12. Effects of replacing FM with black soldier fly larvae on fatty acid composition in muscle of Jian carp are means±SD (n = 3) (gkg<sup>-1</sup>) Values Values in the same line with different superscript letters are significantly different (p < .05) (Zhou et al., 2018)

	FM	R25	R50	R75	R100
12:0	0.0 ± 0.0 <sup>d</sup>	13.6 ± 1.6 <sup>c</sup>	30.4 ± 1.3 <sup>b</sup>	48.3 ± 6.3 <sup>a</sup>	56.8 ± 8.3 <sup>a</sup>
14:0	12.7 ± 1.3 <sup>c</sup>	15.0 ± 0.3 <sup>c</sup>	22.6 ± 2.3 <sup>b</sup>	30.7 ± 2.4 <sup>a</sup>	29.9 ± 3.0 <sup>a</sup>
16:0	197.5 ± 12.3 <sup>c</sup>	199.4 ± 3.0 <sup>c</sup>	209.8 ± 5.0 <sup>b</sup>	217.1 ± 16.1 <sup>a</sup>	215.3 ± 15.6 <sup>b</sup>
18:0	78.0 ± 9.0	82.2 ± 4.2	75.5 ± 2.4	89.7 ± 6.9	77.1 ± 9.1
SFA	288.1 ± 20.1 <sup>c</sup>	310.3 ± 2.1 <sup>bc</sup>	338.4 ± 5.4 <sup>b</sup>	385.8 ± 19.0 <sup>a</sup>	379.0 ± 8.0 <sup>a</sup>
16:1 n-7	13.6 ± 1.3	15.8 ± 0.5	19.2 ± 1.3	24.6 ± 1.8	20.7 ± 2.1
18:1n-9	247.5 ± 15.2	256.7 ± 17.4	254.5 ± 1.5	273.5 ± 6.9	269.6 ± 9.5
20:1n-9	10.5 ± 0.9 <sup>a</sup>	10.1 ± 1.2 <sup>ab</sup>	11.1 ± 0.7 <sup>a</sup>	8.0 ± 1.2 <sup>b</sup>	8.1 ± 0.4 <sup>b</sup>
MUFA	271.6 ± 15.2 <sup>b</sup>	282.6 ± 16.7 <sup>ab</sup>	284.7 ± 2.7 <sup>ab</sup>	306.2 ± 8.0 <sup>a</sup>	298.3 ± 11.4 <sup>ab</sup>
18:2n-6	251.8 ± 30.1 <sup>a</sup>	236.0 ± 3.8 <sup>ab</sup>	223.2 ± 4.0 <sup>ab</sup>	177.5 ± 13.2 <sup>c</sup>	210.8 ± 4.4 <sup>b</sup>
18:3n-6	6.4 ± 1.6 <sup>ab</sup>	4.6 ± 2.6 <sup>b</sup>	8.9 ± 1.1 <sup>a</sup>	8.9 ± 1.3 <sup>a</sup>	6.2 ± 0.9 <sup>ab</sup>
20:4n-6	25.2 ± 6.5	25.6 ± 2.8	28.9 ± 1.4	25.3 ± 1.8	34.3 ± 9.2
22:4n-6	3.8 ± 1.3	9.3 ± 5.5	7.9 ± 1.9	7.3 ± 3.9	10.4 ± 8.8
Sum n-6	287.2 ± 23.6 <sup>a</sup>	275.5 ± 9.4 <sup>a</sup>	268.9 ± 5.4 <sup>a</sup>	219.0 ± 9.5 <sup>b</sup>	261.7 ± 4.2 <sup>a</sup>

	FM	R25	R50	R75	R100
18:3n-3	37.3 ± 4.6 <sup>a</sup>	35.8 ± 0.6 <sup>a</sup>	26.6 ± 0.3 <sup>b</sup>	23.2 ± 1.5 <sup>b</sup>	24.7 ± 01.8 <sup>b</sup>
20:5n-3	20.7 ± 2.1 <sup>a</sup>	17.1 ± 5.7 <sup>a</sup>	13.9 ± 1.2 <sup>ab</sup>	13.2 ± 2.3 <sup>ab</sup>	8.9 ± 3.5 <sup>c</sup>
22:6n-3	95.1 ± 25.3 <sup>a</sup>	78.8 ± 8.9 <sup>ab</sup>	67.4 ± 2.2 <sup>ab</sup>	52.6 ± 4.0 <sup>b</sup>	27.4 ± 8.1 <sup>c</sup>
Sum n-3	153.1 ± 18.7 <sup>a</sup>	131.6 ± 15.1 <sup>ab</sup>	107.9 ± 3.1 <sup>bc</sup>	88.9 ± 7.5 <sup>c</sup>	61.0 ± 9.9 <sup>d</sup>
PUFA	440.3 ± 5.1 <sup>a</sup>	407.1 ± 15.0 <sup>b</sup>	376.8 ± 8.1 <sup>c</sup>	307.9 ± 16.1 <sup>d</sup>	322.7 ± 12.6 <sup>d</sup>
HUFA	144.7 ± 28.4 <sup>a</sup>	130.7 ± 14.4 <sup>ab</sup>	118.2 ± 6.2 <sup>abc</sup>	98.3 ± 1.6 <sup>b</sup>	81.0 ± 17.5 <sup>c</sup>

### 3.13. The Slaughter traits and fillet yield of fish

Fillet yields are central attributes for both the fish processing industry, consumers and fish farmers as they can be used to assess the economic value of different fish species (Solomon & Akogu 2005; Iaconisi et al., 2018). Fillet yield is the edible portion of the fish body and excludes the bones, fins, viscera organs and scales (Solomon and Akogu, 2005). The fillet yield can vary depending on many factors.

According to Tosin et al. (2021), the difference in fillet yield and body characteristics is attributed to the structural anatomy and other biological dynamics of the fishes. The edible fillet percentage of African cat fish is reported as 55% from 424g whole body weight. While, the fillet yield percentage reported for common carp (42-45%) by Varga et al. (2013) was slightly lower than African catfish but higher than other species (*S. nigrita* and *B. bajad*) reported as 34.96 and 39.09% respectively. According to Iaconisi et al. (2017), the fillet yield reported for blackspot sea bream (*Pagellus bogaraveo*) fed different replacement levels of MW with FM ranges from 60-64%. This can be varied from species to species and the weight of individual fish varies. See the (Table15)



Table 13. fillet yield of some fish species (Tosin et al., 2021)

Species	% Gut weight	% Head weight	% Frame weight (bone)	% Edible fillet weight	% non-edible fillet
<i>C. gariepinus</i>	5.71 ± 0.05	29.18 ± 0.21	7.72 ± 0.15	55.01 ± 1.05	44.95 ± 0.13
<i>M. rume</i>	4.13 ± 0.01	20.33 ± 0.10	7.93 ± 0.11	65.03 ± 1.02	34.99 ± 0.21
<i>S. nigrita</i>	10.80 ± 0.10	30.54 ± 0.25	5.44 ± 0.09	34.96 ± 0.23	65.05 ± 0.12
<i>B. bajad</i>	2.95 ± 0.02	22.36 ± 0.11	3.52 ± 0.05	39.09 ± 0.15	60.90 ± 0.40
<i>L. senegalensis</i>	6.65 ± 0.03	14.34 ± 0.95	7.17 ± 0.03	63.20 ± 0.41	36.65 ± 0.49

Table 14. Dress percentage (Tosin et al.,2021)

Species	Whole body weight (g)	Total length (cm)	Gut weight (g)	Head weight (g)	Frame weight (bone) (g)	Edible fillet weight (g)
<i>C. gariepinus</i>	424.00 ± 62.2	37.86 ± 1.95	24.22 ± 4.76	124.00 ± 16.2	32.7 ± 4.47	233.0 ± 28.9
<i>B. bajad</i>	235.00 ± 57.1	29.73 ± 0.91	6.92 ± 0.78	52.52 ± 4.70	8.26 ± 0.62	91.83 ± 8.95
<i>S. nigrita</i>	152.00 ± 14.1	23.28 ± 0.58	16.42 ± 3.58	46.45 ± 3.29	8.27 ± 0.76	53.17 ± 4.70
<i>M. rume</i>	83.26 ± 9.34	24.80 ± 0.81	3.44 ± 0.41	20.26 ± 1.88	6.60 ± 0.74	77.96 ± 25.0

Table 15. Effect of the dietary inclusion of mealworm (*Tenebrio molitor*) larvae meal (MW) on the morphometric and orthometric properties and somatic indices of blackspot sea bream (Iaconisi et al., 2017)

	Replacement level			p ≤ ....		RSD <sup>2</sup>
	MW0	MW25	MW50	D	BW	
<b>Number of fish</b>	8	8	8			
<b>Slaughter traits (g)</b>						
Body weight	292.53 <sup>a</sup>	251.34 <sup>b</sup>	244.36 <sup>b</sup>	0.0381		37.603
Eviscerated weight	241.36	242.66	240.20	0.5226	< 0.0001	4.246
Right fillet	63.25	62.43	61.63	0.8074	< 0.0001	4.331
Left fillet	60.68	64.44	63.27	0.2832	< 0.0001	4.149
Right skin	13.50	12.13	11.87	0.4753	0.0008	2.418
Left skin	13.75	12.22	12.34	0.3963	0.0002	2.104
Viscera	20.34	19.80	21.00	0.8499	0.0010	4.195
Visceral fat	7.97	6.59	6.30	0.6052	0.0023	3.010
Liver	3.37	3.90	4.63	0.1751	0.0121	1.155
Head	74.32	71.30	71.65	0.4836	< 0.0001	4.648
Frame	28.45	29.84	29.55	0.7793	0.0006	3.618
Fins	6.12	4.93	5.61	0.6257	0.5561	2.247
<b>Orthometric measurements</b>						
Fish total length, cm	24.89	24.21	24.73	0.3626	< 0.0001	0.935
Intestine length, cm	19.31	21.59	22.22	0.1173	0.1903	2.398
RIL	0.78	0.89	0.90	0.1391	0.5262	0.109
CF	1.71	1.86	1.72	0.3212	0.7979	0.205
<b>Percentage on live weight, %</b>						
Head	28.21	27.27	27.32	0.5934	0.3016	1.763
Frame	10.85	11.46	11.33	0.7124	0.3779	1.361
Fins	2.35	1.89	2.20	0.6037	0.2285	0.860
Total wastes	49.13	48.17	48.81	0.8307	0.2030	2.984
Dressed yield	91.86	92.37	91.41	0.5278	0.9422	1.679
With skin fillet yield	47.24	48.09	47.36	0.8427	0.3142	3.044
Without skin fillet yield	36.91	38.89	38.27	0.5245	0.4548	3.114
VSI	8.45	8.20	8.74	0.8552	0.9090	1.919
VFI	2.93	2.45	2.31	0.6174	0.1482	1.129
HSI	1.29	1.48	1.77	0.1402	0.5192	0.420

Table 16. Effect of the dietary inclusion of mealworm (*T. molitor*) larvae meal on morphometric and marketable traits of rainbow trout(n=6/group) (Iaconisi et al., 2018)

Parameters	Diet			p ≤ ...	RSD <sup>1</sup>
	MW0	MW25	MW50		
<b>Yields (g)</b>					
Body weight	351.00	408.67	395.33	0.124	47.700
Eviscerated weight	263.93	273.48	293.43	0.648	55.166
Right fillet	112.84	130.90	132.31	0.204	19.986
Left fillet	108.18	122.72	112.93	0.327	16.543
Right skin	18.42	18.42	21.42	0.526	5.180
Viscera	28.53	31.43	31.06	0.610	5.419
Liver	7.57	7.56	6.41	0.319	1.470
Frame	27.41	32.57	32.12	0.503	8.255
Fins	5.79	5.84	5.62	0.924	0.990
<b>Orthometric measurements</b>					
Intestine length, mm	136.67	136.67	135.83	0.998	23.787
Fish total length, mm	338.67	355.00	349.67	0.103	12.518
RIL	0.39 <sup>a</sup>	0.34 <sup>b</sup>	0.34 <sup>b</sup>	0.045	0.129
CF (g/cm <sup>3</sup> )	1.15	1.55	1.46	0.217	0.395
<b>Percentage on live weight, %</b>					
Dressed yield	75.21	66.69	74.32	0.313	10.222
fillet yield with skin	62.92	61.94	62.01	0.656	2.040
fillet yield without skin	53.53	54.91	56.09	0.684	5.030
VSI	8.07	7.69	7.83	0.716	0.810
HIS	2.15 <sup>a</sup>	1.86 <sup>ab</sup>	1.62 <sup>b</sup>	0.031	0.311
Frame	7.81	8.06	8.23	0.949	2.224
Fins	1.65	1.42	1.43	0.058	0.174
Total wastes	9.47	9.48	9.65	0.987	2.232

RIL: Relative Intestinal Length, CF: condition factor; HSI: hepatosomatic index; VSI: viscerosomatic index.

Table 17. Corporal indices and fillet yield (mean  $\pm$  SEM) of the tambaqui fed whole black soldier fly larvae to replace commercial feed (Ordoñez et al., 2022)

<b>Variables (%)</b>	<b>BSFL</b>	<b>BSFL:CF</b>	<b>CF</b>	<b><i>p</i>-Value</b>
Viscerosomatic index	7.01 $\pm$ 0.15 a	6.56 $\pm$ 0.16 ab	6.20 $\pm$ 0.20 b	0.025
Hepatosomatic index	1.40 $\pm$ 0.05	1.50 $\pm$ 0.07	1.61 $\pm$ 0.09	0.161
Visceral fat index	2.79 $\pm$ 0.33	2.36 $\pm$ 0.28	2.19 $\pm$ 0.12	0.282
Fillet	28.04 $\pm$ 0.58	28.49 $\pm$ 0.37	28.09 $\pm$ 0.25	0.719
Head	19.63 $\pm$ 0.85	19.78 $\pm$ 0.97	18.44 $\pm$ 0.41	0.539
Skin	5.47 $\pm$ 0.28	5.58 $\pm$ 0.22	5.51 $\pm$ 0.34	0.967

## **4. MATERIAL AND METHODS**

### **4.1 Common procedures for experiments**

#### **4.1.1 Origin of fish and keeping conditions**

All fish used for overall experiments were reared in Hungarian University of Agriculture and Life Science recirculating aquaculture system (RAS, Kaposvár). Also total of five experiments was carried out at the Hungarian University of Agriculture and Life Science in the recirculating aquaculture system (RAS, Kaposvár). The study design applied was a complete randomized design (CRD). Water parameters were checked regularly. Dissolved oxygen and temperature were measured daily by portable dissolved oxygen meter HI9147 (HANNA instrument Woonsocket RI USA, made in Europe (Romania)). Nitrogen forms:  $\text{NO}_3^-$ ,  $\text{NO}_2^-$ ,  $\text{NH}_4^+$  and pH are measured by (PF-12 plus photometer, MACHEREY NAGEL MN, city, country) every week. At the beginning and end of the experiments, individual weight and length of the fish were measured by Shimadzu scales, Japan accuracy  $\pm 1\text{g}$ ) and length ( $\pm 0.5\text{cm}$ ) respectively. In all experiments fish were kept in 250L volume capacity tanks connected to recirculating system containing a drum filter (Trome Belgium) moving bed bio filter (see picture 5) and sump. All tanks were aerated with a radial blower and air stone ( $3\pm 0.5\text{ L/min./tank}$ ). The total biomass was measured to adjust the daily feed portions every week in experiments 1 and 2, and every two weeks in experiment 3, and every four weeks in experiments 4 and 5 by a portable measuring scale (MMX, China- accuracy  $\pm 5\text{g}$ ). To minimize the stress caused by the manipulation and avoid feed wastage, the feeding was skipped one day after the trial setup, one day before and one day after the biomass measurement, and one day before closing the experiment. The tank was checked every day for mortalities and morbidities.

#### **4.1.2 Experimental set up**

Altogether five experiments were carried out. In the first experiment the different inclusion levels of black soldier fly meal (BSF) in the common carp diet was studied at the substitution level of fishmeal (FM) ( $120\text{gkg}^{-1}$ ) in the diet were 0, 50 and 100%. Production parameters and body condition indices were analyzed. In the second experiment the effects of mealworm meal (MW) on common carp were studied the substitution level of FM ( $100\text{ gkg}^{-1}$ ) were 0, 50 and 100%. Production parameters, body condition indices and eviscerated proximate composition were analyzed. In the third experiment BSF, MW, and 1:1 combination of both was studied on African catfish for production parameters, blood plasma biochemistry, apparent digestibility. Lastly, in

fourth and fifth experiments BSF, MW, and 1:1 combination of both was studied on African catfish and common carp for fillet quality and yield (Table18).

Table 18. overall experimental set up

experiment	Insect used	FM replacement level	The Fish	Duration (weeks)	Parameters
1	BSF	Total (120 gkg <sup>-1</sup> ) and partial (60 gkg <sup>-1</sup> )	Common carp	8	SGR, FCR, SR, Body indices
2	MW	Total (100 gkg <sup>-1</sup> ) and partial (50 gkg <sup>-1</sup> )	Common carp	6	SGR, FCR, SR, Body indices, Proximate composition
3	BSF, MW and combination	Total (200 gkg <sup>-1</sup> ) and partial (100)	African catfish	6	SGR, FCR, SR, Body indices, blood biochemistry, ADC
4	BSF, MW and 1:1 BSFMW	Partial (100 gkg <sup>-1</sup> )	Common carp	13	Fillet yield, coking, dripping and thawing loss, proximate composition
5	BSF, MW and 1:1 BSFMW	Partial (100 gkg <sup>-1</sup> )	African catfish	25	Fillet yield, slaughter trait, proximate composition Fatty acid profile

BSFMW: 1:1 combination of BSF and MW, BSF: black soldier fly, MW: mealworm



Figure 5. Experimental tanks at kaposvar campus aquaculture department, 250l volume capacity

#### 4.2 Experimental procedures and tank management (Experiment I)

The experiment was designed to investigate the effect of total and partial replacement of FM with BSF in carp feed. The experimental design is shown in Fig 6.

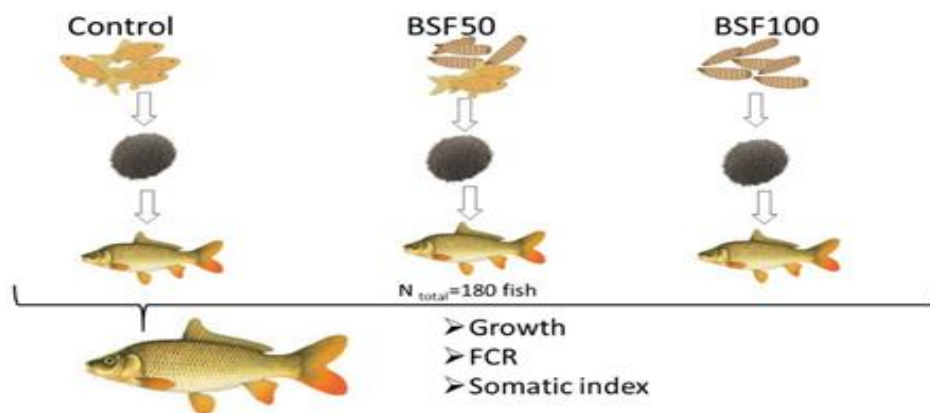


Figure 6. Scheme of the experimental setup and the measured parameters at the end (Experiment I)

### 4.2.1 Diet formulation

The following diet was formulated: BSF meal originated from a domestic producer (GRINSECT, kft, Hungary) in processed form having 23.7% moisture, 52.7% crude protein, 19.9% crude fat, and 7.8% crude fiber. The basal diet was set to 38.1% crude protein and 7.5% crude fat containing 120 gkg<sup>-1</sup> FM (Table 19) and was fed to BSF free (control). In the first treatment, 60 g of FM was replaced with BSF (BSF50) while in the second treatment, all of 120 g FM was replaced by BSF (BSF100). The experimental diets were set to be iso-nitrogenous and iso-energetic. The experimental diets were prepared by mixing dry ingredients with fish oil and gelatin and adding some slightly warm water. The homogenized and moisturized ingredients were then pelleted using a minced grinder and dried with cold ventilation for 48 hrs. The proximate composition of BSF and the experimental diets are shown in Table 19.

Table 19. Inclusion level of ingredients and proximate composition of experimental feeds

Ingredients	Inclusion levels (gkg <sup>-1</sup> )		
	Control	BSF50	BSF100
Soya	250	250	250
FM	120	60	0
BSF	0	60	120
PBP	200	200	210
FO	25	20	15
Premix	5	5	5
Gelatine	2	2	2
Corn	398	403	398
Proximate composition			
Moisture (%)	8.3	8.0	8.5
Crude protein (%)	38.1	38.7	38.8
Crude fat (%)	7.5	7.7	8.2
Crude fiber (%)	1.7	1.6	2.5
Ash (%)	7.3	6.2	5.4



\*FM: fishmeal, BSF: black soldier fly meal, PBP: poultry by-product, FO: fish oil, Premix: Cargill Ltd: vitamin A 1003400 IU; vitamin D3 80650 IU; vitamin E 5000 mg; vitamin K3 337 mg; Ca 12.2%; P 7.8%; Na 0.1%; Fe 670 mg; Zn 1070 mg; Mn 160 mg; Cu 200 mg; Se 20 mg.

#### **4.2.2 Fish stocking, feeding and tank management**

Experimental fish ( $n=180$   $w_0=35.2\pm 6.01$ g) were randomly distributed to three groups (Control, BSF50 and BSF100) in triplication (20 fish per tank) and acclimatized for one week to the experimental feed before the actual experimental work. The fish were offered a daily ratio of 2.5% of their body weight by hand distribution of feed, three times per day. Water parameters (temperature,  $\text{NH}_4^+$ ,  $\text{NO}_3^-$ ,  $\text{NO}_2^-$ , dissolved oxygen and pH) were checked regularly during the experiment, and the average values were T:  $24.0\pm 0.5^\circ\text{C}$ , Dissolved Oxygen:  $4.9\pm 0.5$  mg  $\text{L}^{-1}$  measured daily,  $\text{NH}_4^+$ :  $0.50\pm 0.02$  mg  $\text{L}^{-1}$ ,  $\text{NO}_3^-$ :  $20.5\pm 0.19$  mg  $\text{L}^{-1}$ ,  $\text{NO}_2^-$ :  $0.14\pm 0.02$  mg  $\text{L}^{-1}$ , and pH:  $7.1\pm 0.2$ . The length of the experiment was 8 weeks.

#### **4.2.3 Sample collection**

At the beginning of the experiment, ten fish were sacrificed for somatic measurements and the individual weight of all experimental fish were measured at the beginning and end of the experiment. Altogether, 18 fish (2 individuals per tank, 6 per treatment) were randomly taken and dissected for somatic indices. Pooled samples of the whole fish bodies per each treatment were sent to the Hungarian University of Agriculture and Life Sciences, Central Laboratory Department of Food and Feed Safety, for proximate composition analyses in duplicates. Samples were stored before proximate analysis in the fridge under  $-20^\circ\text{C}$ .

### **4.3 Experimental procedures and tank management (Experiment II)**

The experiment was designed to investigate the effect of total and partial replacement of FM with MW in common carp feed. The experimental design is shown in Figure 7.

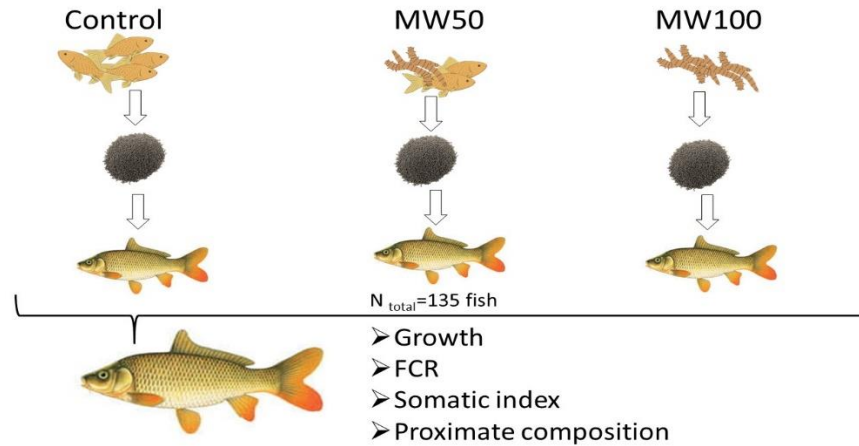


Figure 7. Schematic Diagram of the experimental setup and the measured parameters at the end (Experiment II)

#### 4.3.1 Diet formulation

Yellow mealworm meal originated from Berg and Schmidt Pte. Ltd Singapore; in dried and processed form and imported by Hecron-Agro Kft. Hungary. The nutritional value of mealworm meal was 91.96, 56.56, 6.20 and 7.01% dry matter, crude protein, crude fat and crude ash respectively in dry basis (Table 21). The basal diet was set to 35.2% crude protein and 6.7% crude fat and contained 10% fishmeal (Table 20). In the first experimental diet, 50 % of FM was replaced with MW (MW50), and in the second diet, FM was totally replaced by MW (MW100). The experimental diets were set as iso-nitrogenous and iso-energetic (Table 20). The experimental diets were prepared manually by mixing dried ingredients with oil, and slightly warm water and carboxymethyl cellulose (CMC) was used as binder. The homogenized and moisturized ingredients were then pelleted using a minced grinder and dried with cold ventilation for 48 hrs.

Table 20. Formulation (%) and proximate composition (% , wet weight) of the control and experimental diets used in the nutritional experiment in common carp juveniles

Ingredients (%)/Diets	Control	MW50	MW100
Fishmeal	10.0	5.0	0.0
Corn	45.0	45.5	46.0
Poultry byproduct conc.	20.5	21.0	21.0
Mealworm meal	0.0	5.0	10.0
Soy protein conc.	19.0	18.0	18.0
Sunflower oil	2.0	2.0	1.5
Fish oil	2.0	2.0	2.0
Vitamin/mineral premix	0.5	0.5	0.5
CMC	1.0	1.0	1.0
Proximate composition			
Dry Matter	10.1	10.2	10.3
Crude Protein	35.2	35.4	35.4
Crude Fat	6.7	6.9	7.1
Crude Ash	6.4	5.6	4.9
Gross energy (KJ g <sup>-1</sup> )	19.5	19.7	19.8

CMC: Carboxymethyl cellulose

Table 21. Composition of the tested mealworm meal (MW) (dry weight, %)

Ingredients	%
Dry Matter	91.96 ± 0.30
Crude Protein	56.56 ± 0.14
Crude Fat	6.20 ± 0.17
Crude Ash	7.01 ± 0.05
Crude Fibre	2.02 ± 0.33
Acid Detergent Fibre (ADF)	27.69 ± 0.12
Chitin	5.81 ± 2.08
ΣEAA	27.62
ΣAA	52.61

\*Protein was calculated by applying a nitrogen to protein conversion factor of  $K_p=4.76$  (Janssen et al, 2017);  $\Sigma EAA$  sum of essential amino acids,  $\Sigma AA$  sum of total amino acids

#### **4.3.2 Fish stocking, feeding and tank management**

Experimental fish (n:135  $w_0$ : 97.54±15.0 g) were randomly distributed to three groups (MW0, MW50 MW100) in triplication (15 fish per tank) and acclimatized for one week using experimental feed before the nutritional experiment. The fish were fed with 3 % of body weight manually three times per day. Water parameters (temperature,  $NH_4^+$ ,  $NO_3^-$ ,  $NO_2^-$ , dissolved oxygen and pH) were checked regularly during the experiment, and the average values were T: 24.0±0.5°C, Dissolved Oxygen: 5.0 ±0.5 mg L<sup>-1</sup>  $NH_4^+$ : 0.50±0.02 mg L<sup>-1</sup>,  $NO_3^-$ : 19.0±0.3 mg L<sup>-1</sup>,  $NO_2^-$ : 0.14±0.02 mg L<sup>-1</sup>, and pH: 7.2±0.2. The duration of the experiment was 6 weeks.

#### **4.3.3 Sample collection**

At the end of the experiment, 18 fish (2 individuals per tank, 6 fish per treatment) were randomly taken and dissected for somatic indices, and finally used body proximate composition analyses. The samples were kept in a fridge under -20 °C until sent to the Hungarian University of Agriculture and Life Sciences, Central Laboratory Department of Food and Feed Safety for proximate composition analyses in duplicates.

#### **4.4 Experimental procedures and tank management (Experiment III)**

The experiment was designed to investigate the effect of total and partial replacement of FM with BSF, MW and 1:1 combination of BSF and MW in African catfish feed. The experimental design is shown in Figure 8.

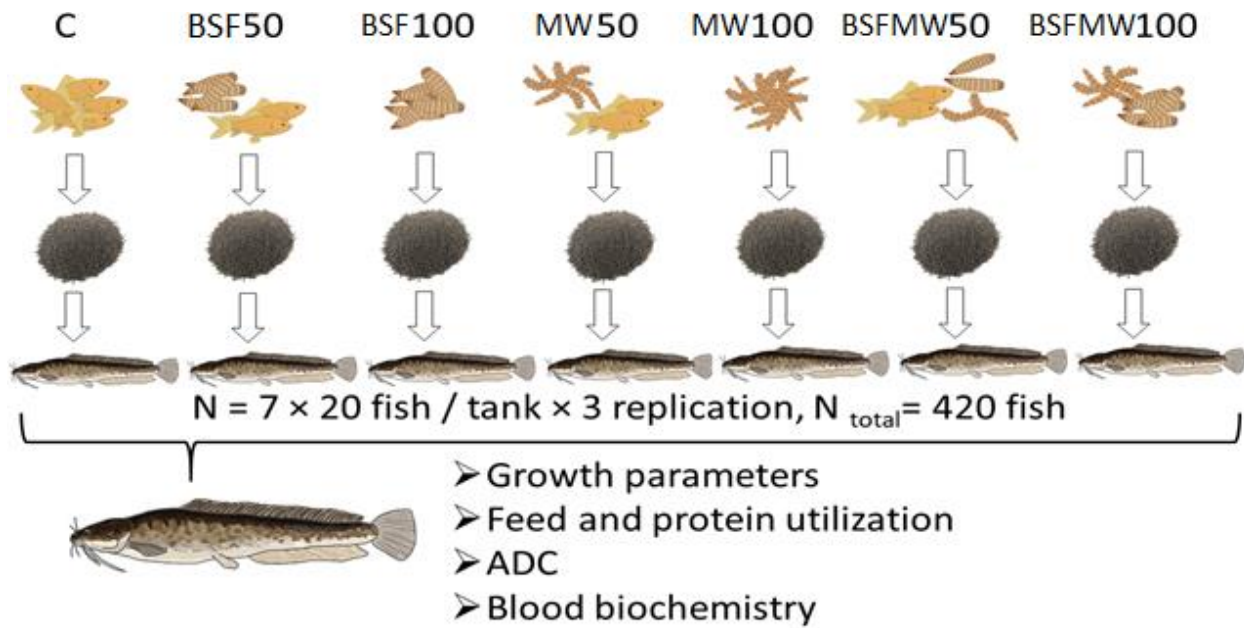


Figure 8. Schematic diagram of the experimental set up and the measured parameters at the end (Experiment III).

ADC: apparent digestibility coefficient of diet.

#### 4.4.1 Diet formulation

The experiment was designed to evaluate the effect of total and partial replacement of FM with BSF, MW, BSF, MW, and 1:1 combination (BSFMW). Accordingly, seven experimental diets, including the control diet, were prepared. The basal diet was set to  $44.97\% \pm 0.45$  crude protein and  $8.62 \pm 0.16\%$  crude fat, it contained 200 g/kg fishmeal (Table 22) and was MW and BSF free (control). In the first treatment, 100g of FM was replaced with MW (MW50), and in the second treatment, all of 200g FM was replaced with MW (MW100) in the third treatment, 100g of FM was replaced with BSF (BSF 50), and all of 200g FM was replaced by BSF (BSF 100) In the fourth treatment 100g of FM was replaced with 1:1 combination of MW and BSF (BSFMW50), and all of 200g FM was replaced by 1:1 combination of MW and BSF (BSFMW100).

Table 22. Formulation and chemical composition of the diets with different inclusion level of IM

<b>Ingredients (%)</b>	<b>Control</b>	<b>BSF50</b>	<b>BSF100</b>	<b>MW50</b>	<b>MW100</b>	<b>BSFMW50</b>	<b>BSFMW100</b>
Fishmeal-60	20	10	0	10	0	10	0
Mealworm (MW)	0	0	0	10	20	5	10
Black soldier fly (BSF)	0	10	20	0	0	5	10
Soy protein concentrat.	14.6	14.6	14.6	14.6	14.6	14.6	14.6
Wheat	33.4	33.1	32.9	32.6	31.7	32.9	32.3
Poultry meal	25	25	25	25	25	25	25
Titan oxid	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Vitamin, mineral premix	1.5	1.5	1.5	1.5	1.5	1.5	1.5
Rapeseed oil	4	4.3	4.5	4.8	5.7	4.5	5.1
Calcium phosphate	1	1	1	1	1	1	1
<b>Proximate Composition % wet weight basis</b>							
Dry matter	95.78 ± 0.21	97.22 ± 0.04	96.58 ± 0.02	96.37 ± 0.01	95.73 ± 0.01	97.10 ± 0.03	93.74 ± 0.10
Crude protein	44.35 ± 0.35	45.53 ± 1.09	44.48 ± 0.04	45.11 ± 0.46	46.46 ± 0.77	45.97 ± 0.20	42.90 ± 0.25
Crude fat	7.95 ± 0.05	8.45 ± 0.27	8.85 ± 0.03	8.29 ± 0.20	9.03 ± 0.12	8.50 ± 0.06	9.31 ± 0.45
Crude fibre	1.70 ± 0.28	3.27 ± 0.43	4.71 ± 0.09	2.97 ± 0.11	2.93 ± 0.18	2.95 ± 0.66	2.98 ± 0.74
Crude ash	10.63 ± 0.21	9.38 ± 0.02	8.00 ± 0.05	8.97 ± 0.01	7.36 ± 0.04	8.99 ± 0.03	7.22 ± 0.05
Gross energy (KJ g <sup>-1</sup> )	19.05 ± 0.04	19.94 ± 0.04	19.96 ± 0.05	19.72 ± 0.00	20.02 ± 0.06	19.87 ± 0.05	19.56 ± 0.21
Phosphorus	1.47	1.33	1.13	1.28	1.04	1.24	0.95
Calcium	2.10	1.79	1.46	1.79	1.42	1.62	1.29
Chitin	4.96 ± 0.07	5.98 ± 0.02	6.32 ± 0.01	6.42 ± 0.34	7.24 ± 0.03	5.91 ± 0.20	5.55 ± 0.18
ADF	7.69 ± 0.05	8.62 ± 0.21	10.14 ± 0.06	10.25 ± 0.05	13.43 ± 0.44	10.31 ± 0.15	10.62 ± 0.02

BSF black soldier fly, MW mealworm, BSFMW mixture of black soldier fly and mealworm

Table 23. Amino acid and fatty acid composition of the diets

<b>Essential Amino Acid</b>	<b>Control</b>	<b>BSF50</b>	<b>BSF100</b>	<b>MW50</b>	<b>MW100</b>	<b>BSFMW50</b>	<b>BSFMW100</b>
Arginine (ARG)	2.73±0.07	2.53±0.16	2.73±0.11	2.60±0.10	3.16±0.05	2.98±0.34	2.90±0.11
Cysteine (CYS)	0.39±0.01	0.38±0.00	0.41±0.00	0.42±0.05	0.50±0.02	0.49±0.03	0.46±0.01
Histidine (HIS)	1.10±0.01	1.00±0.06	1.09±0.06	0.88±0.05	0.93±0.01	1.06±0.06	0.97±0.04
Isoleucine (ILE)	2.02±0.06	1.81±0.16	2.11±0.04	1.88±0.02	2.27±0.07	2.43±0.27	2.25±0.12
Leucine (LEU)	3.56±0.11	3.17±0.27	3.64±0.06	3.50±0.01	4.39±0.21	4.39±0.50	4.08±0.22
Lysine (LYS)	3.06±0.11	2.59±0.34	2.96±0.01	2.83±0.23	3.51±0.36	4.01±0.44	3.38±0.14
Methionine (MET)	1.11±0.02	1.01±0.08	1.03±0.02	1.00±0.01	1.16±0.03	1.22±0.06	1.09±0.03
Phenylalanine (PHE)	2.06±0.04	2.00±0.07	2.19±0.09	1.96±0.14	2.27±0.05	2.21±0.16	2.21±0.10
<b>Essential Amino Acid</b>	<b>Control</b>	<b>BSF50</b>	<b>BSF100</b>	<b>MW50</b>	<b>MW100</b>	<b>BSFMW50</b>	<b>BSFMW100</b>
Threonine (THR)	2.00±0.05	1.80±0.14	2.00±0.05	1.85±0.05	2.23±0.01	2.27±0.23	2.14±0.11
Valine (VAL)	2.60±0.09	2.43±0.21	2.87±0.05	2.53±0.01	3.15±0.11	3.26±0.39	3.07±0.16
Tryptophan (TRP)	0.22±0.01	0.27±0.00	0.43±0.02	0.26±0.12	0.15±0.01	0.17±0.02	0.15±0.01
ΣEAA	18.24	16.56	18.59	17.17	20.59	21.23	19.62
<b>Non-Essential Amino Acid</b>	<b>Control</b>	<b>BSF50</b>	<b>BSF100</b>	<b>MW50</b>	<b>MW100</b>	<b>BSFMW50</b>	<b>BSFMW100</b>
Alanine (ALA)	2.90±0.10	2.51±0.30	2.86±0.00	2.69±0.01	3.21±0.31	3.64±0.46	3.14±0.10
Aspartic acid (ASP)	3.45±0.13	3.17±0.35	3.73±0.01	3.36±0.14	4.05±0.32	4.59±0.51	4.06±0.18
Glutamic acid (GLU)	6.02±0.17	5.37±0.45	6.16±0.05	6.14±0.21	7.38±0.42	7.82±0.86	7.06±0.36
Glycine (GLY)	2.83±0.07	2.57±0.23	2.74±0.07	2.53±0.15	2.88±0.01	3.11±0.37	2.82±0.17

<b>Essential Amino Acid</b>	<b>Control</b>	<b>BSF50</b>	<b>BSF100</b>	<b>MW50</b>	<b>MW100</b>	<b>BSFMW50</b>	<b>BSFMW100</b>
Proline (PRO)	3.03±0.09	2.73±0.23	3.16±0.05	3.03±0.05	3.62±0.18	3.87±0.47	3.56±0.16
Serine (SER)	2.68±0.10	2.50±0.17	2.84±0.04	2.76±0.09	3.46±0.02	3.33±0.44	3.21±0.16
Tyrosine (TYR)	1.30±0.04	1.41±0.11	1.76±0.08	1.25±0.07	1.52±0.01	1.54±0.12	1.62±0.07

<b>Fatty acids w%</b>	<b>Control</b>	<b>BSF50</b>	<b>BSF100</b>	<b>MW50</b>	<b>MW100</b>	<b>BSFMW50</b>	<b>BSFMW100</b>
12:0	n/a	3.92±0.00	7.80±0.01	0.39±0.00	0.17±0.00	2.05±0.03	4.03±0.02
14:0	2.11±0.01	2.49±0.00	3.00±0.00	2.02±0.00	1.85±0.01	2.19±0.02	2.45±0.01
16:0	18.03±0.15	16.92±0.00	16.34±0.02	16.93±0.04	16.26±0.01	16.96±0.09	16.17±0.06
16:1n-9	3.56±0.02	3.34±0.00	3.22±0.01	3.37±0.01	3.17±0.00	3.32±0.01	3.16±0.01
18:0	5.54±0.05	4.95±0.00	4.41±0.01	5.27±0.00	5.13±0.00	5.13±0.02	4.73±0.02
18:1n-9	35.05±0.28	34.63±0.01	33.58±0.12	36.67±0.05	38.00±0.07	36.02±0.00	35.24±0.11
18:2n-6	13.52±0.08	14.43±0.01	15.35±0.03	14.40±0.01	15.52±0.02	14.82±0.01	15.34±0.03
Fatty acids w%	Control	BSF50	BSF100	MW50	MW100	BSFMW50	BSFMW100
18:3n-3	2.11±0.01	2.21±0.00	2.23±0.01	2.31±0.00	2.44±0.00	2.27±0.01	2.35±0.01
20:4n-6	0.85±0.00	0.68±0.00	0.54±0.00	0.74±0.00	0.65±0.00	0.70±0.00	0.61±0.00
20:5n-3	2.34±0.00	1.87±0.00	1.52±0.00	2.16±0.00	1.96±0.02	1.92±0.04	1.81±0.03
22:6n-3	6.00±0.06	4.49±0.01	3.32±0.01	5.16±0.01	4.40±0.05	4.61±0.14	4.09±0.11
TOTAL SFA	27.19±0.22	29.61±0.01	32.87±0.04	26.01±0.05	24.90±0.02	27.65±0.16	28.60±0.13
TOTAL MUFA	41.36±0.26	40.42±0.01	38.78±0.13	42.79±0.06	43.88±0.07	41.88±0.01	40.88±0.10
TOTAL PUFA	26.96±0.02	25.60±0.00	24.48±0.06	26.92±0.01	27.12±0.08	26.30±0.18	26.17±0.13

PUFA: polyunsaturated fatty acid, SFA: saturated fatty acid, MUFA: mono unsaturated fatty acid



#### **4.4.2 Fish stocking, feeding and tank management**

Experimental fish (n:420, W0 200g±0.51) were randomly distributed to seven groups (control, MW50, MW100, BSF50, BSF100, BSFMW50 and BSFMW100) in triplication (20 fish per tank). The fish were acclimatized for one week to the experimental feed before the actual experimental work. The feed was fixed at 2% of their body weight and distributed by hand five times per day during six weeks of the experiment. To avoid feed wastage, feed was skipped one day before the biomass measurement and two days after the biomass measurements. Water parameters (temperature, NH<sub>4</sub><sup>+</sup>, NO<sub>3</sub><sup>-</sup>, NO<sub>2</sub><sup>-</sup>, dissolved oxygen, and pH) were checked regularly during the experiment, and the average values were T: 24.5±0.2°C, Dissolved Oxygen: 4.2±0.5 mg L<sup>-1</sup> measured daily, NH<sub>4</sub><sup>+</sup>: 0.50±0.02 mg L<sup>-1</sup>, NO<sub>3</sub><sup>-</sup>: 28.5±0.19 mg L<sup>-1</sup>, NO<sub>2</sub><sup>-</sup>: 0.16±0.02 mg L<sup>-1</sup>, and pH: 7.1±0.2 measured on a two-weekly basis.

#### **4.4.3 Sample collection**

##### **4.4.3.1 Production parameters and somatic indices**

At the end of the experiment, 126 fish (6 individuals per tank, 18 per treatment) were randomly taken and dissected for somatic indices.

##### **4.4.3.2 Fecal samples collection**

After six weeks of the feeding experiment, the fish was sacrificed in order to collect faeces from the intestine (Cho et al., 1982) (Figure 8). Total of 84 fish (4 fish per tank or 12 fish/ treatment group) were anesthetized with Norcaicum based anesthetics (Bureau et al., 1999). Fish were dissected and faeces were collected from the posterior part of the intestine according to (Cho et al., 1982). Faecal samples from the same experimental group tanks were pooled on Petri dish, frozen and lyophilized (freeze dried) then refrigerated (+ 4 °C) until analysed

##### **4.4.3.3 Blood serum collection**

Blood samples were collected from the caudal veins of a total of 63 fish (3 fish/tank, n = 9 fish/treatment with nonheparinized 1 ml syringes (Figure 9). The samples were kept on dry ice for 30 min to allow the formation of a clot. The serum was separated by centrifugation at 4 °C and 4000 RCF for 20 min (Hettich Zentrifugen, Tuttlingen, Germany). Serum samples were stored at -20 °C for further analysis.



Figure 9. Feaces sample collection for apparent deductibility coefficient evaluation (Experiment III)



Figure 10. Blood sample collection for blood biochemical analysis (Experiment III)

#### **4.4.4 Blood serum Biochemistry**

##### **4.4.4.1 Total protein assay**

Total protein (TP) concentration of the serum was determined by a colorimetric assay based on the biuret reaction, using a protein diagnostic reagent kit (Fluitest TP, Analyticon Biotechnologies AG, Germany).

##### **4.4.4.2 Total immunoglobulin assay**

To determine the total immunoglobulin (IG) level of serum samples, 50 µl plasma or mucus and 50 µl polyethylene glycol (PEG) was added to each well of a 96-well microtiter plate. After two hours of incubation at room temperature, plates were centrifuged at 1000 G for 15 min. Total protein contents of the supernatants were measured using FLUITEST Total Protein Kits (Analyticon Biotechnologies AG, Germany). These values were subtracted from the total protein levels of the samples, which had been measured previously. The result was equal to the total immunoglobulin concentration of plasma or mucus.

##### **4.4.4.5 Measuring other biochemical parameters**

Serum samples were stored at -20 °C was defrost at room temperature and steered to homogenized by vortex mixer at speed of 1800 round per minute, then 70 µL sample were pipetted by finnpipet ®F2. The sample was injected into the cartridge's specimen inlet (comprehensive plus 17v.). Then by using a Samsung PT10V blood analyzer and Comprehensive Plus test assays (Samsung, Republic of Korea, City), the levels of serum samples were measured for Alkaline phosphatase (ALP), amylase (AMY), total cholesterol (CHOL), glucose (GLU), globulin (GLOB) and phospholipid (PHOS).

#### **4.5 Experimental procedures and tank management (Experiment IV and V)**

All procedures and methods are similar for both experiments (IV and V) except sample size (n=92) fish for African catfish and n=36 common carp), experimental period which were 25 weeks in case of African catfish and 13 weeks in case of common carp. The fatty acid analysis not been carried out for common carp. Experiment IV and V were set after evaluation of inclusion level effect of BSF and MW on common carp and African catfish we decided to use the partial replacement to study the long-term effects.

A total of 92 ( $w_0=248.66\pm 40.27$ ) African catfish was randomly distributed to four tanks (23 fish/tank) fed 1.75% biomass weight. In order to avoid feed wastage and minimizing handling stress, withdrawal of feeding one day before and two days after measurement was applied and the fish kept for about 25 experimental period. The biomass measurement was undertaken every month since African catfish is sensitive to handling and manipulation stress as well as considering the pre slaughter effect on flesh quality.

A total of 36 ( $w_0=209\pm 40.21$ ) common carp (9 fish /tank) was also randomly distributed to four diet groups and fed 1.75% biomass. One day before and after measurement was feed withdraw time. Unlike African catfish common carp relatively tolerates handling stress and resumes to normal feeding regime within one day (own experimental experience). Temperature (min-max: 25.4-25.6°C),  $\text{NO}_3^-$  (min-max: 20-44.8 mg/l),  $\text{NO}_2^-$  (min-max: 0.14-0.24 mg/l), DO (min-max: 3.91-5.0 mg/l) and pH (min-max: 7.5-7.8).

#### 4.5.1 Diet formulation (Experiment IV and V)

The control diet contained 20% dietary fishmeal protein was replaced 50% (10%) dietary protein to evaluate the long-term effect on fillet quality and yield on African catfish and common carp. The feed was formulated in four isonitrogenous and iso-lipid varieties (control, BSF50, MW50 and BSFMW50) (Table 24).

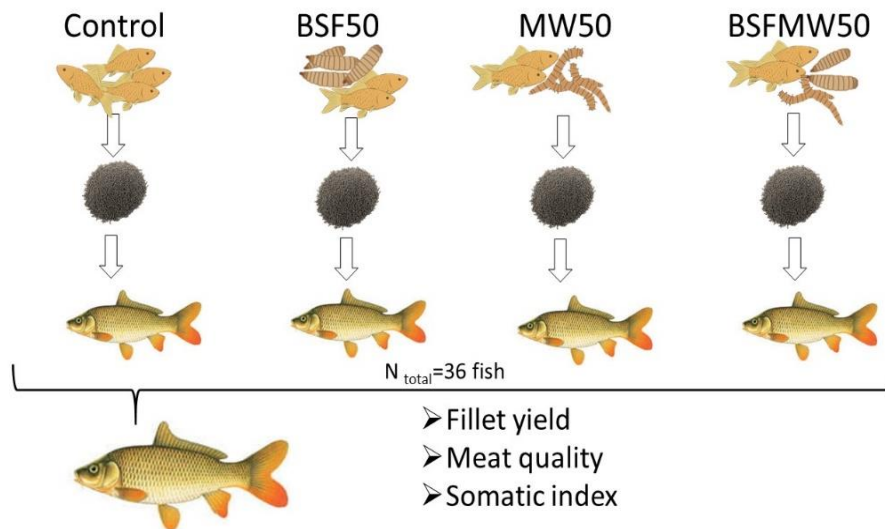


Figure 11. Schematic Diagram of the experimental setup and the measured parameters at the end (experiment IV)

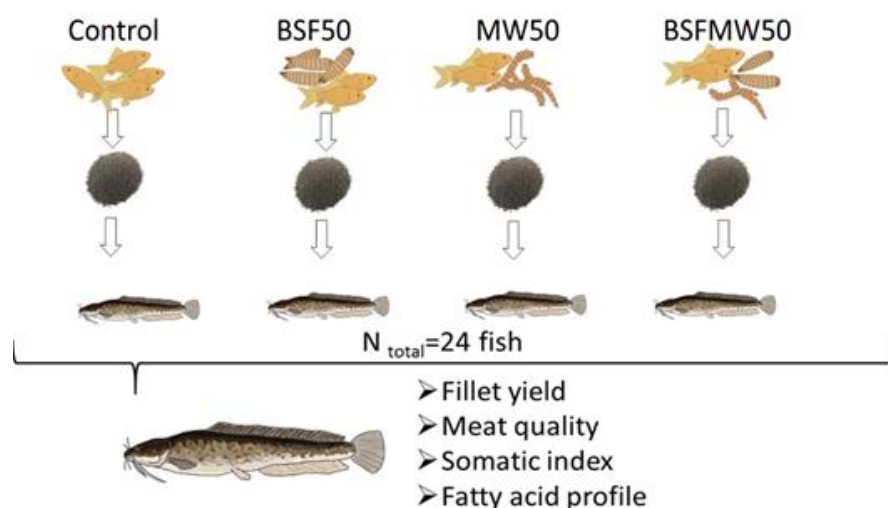


Figure 12. Schematic Diagram of the experimental setup and the measured parameters at the end (experiment V

Table 24. ingredient composition of control and experimental feeds for meat quality testing experiments on common carp and African catfish

Ingredients (gkg <sup>-1</sup> )	Control	BSF50	MW50	BSFMW50
Fishmeal (FM)	200	100	100	100
Mealworm meal (MW)	0	0	100	50
Black soldier fly (BSF)	0	100	0	50
Soy protein conc	146	146	146	146
Wheat	334	331	326	329
Poultry meal	25	25	25	25
Titan dioxide	1	1	1	1
Vitamin, mineral premix	15	15	15	15
Rapeseed oil	40	43	48	45
Calcium phosphate	10	10	10	10

#### 4.5.2 Sample collection

At the end of the experiment, six fish from each group were sacrificed by manual stunning method following administration of anesthesia (2-phenoxyethanol, Sigma Aldrich, Schnelldorf, Germany). After measuring body weights and standard lengths, fish were dissected, internal organs removed, head removed, skin removed, fillet removed from the bone by sharp knife as the detail procedures are shown in the (Figures13-16). Left and right fillet samples were stored at -20 °C until fatty acid and proximate quality analysis. Fillet flesh acidification (pH) was measured 45 min post-mortem (pH 45') and after 24 hrs (pH 24 h) with the use of a portable Testo 205 precision pH meter (Testo AG, Lenzkirch, Germany). Fillet yield was determined as the proportion of carcass weight to whole body mass. To determine the cooking loss, fillet samples (100 g) were closed into sealed bags and cooked at 75 °C for 20 min. The exudate weight, as expressed in the percentage of the initial sample weight was referred to as cooking loss. The thawing loss was determined by freezing fillet samples (50 g) at -20 °C and thawed at room temperature after 2 days. To determine drip loss, fillet samples (50 g) was sealed in bag and hanged over firig at 4°C over night and cautiously open and wiped then measured.



Figure 13. removal of African catfish head



Figure 14. removal of African catdish Skin





Figure 15. African catfish Fillet cutting for fillet yield measurement





Figure 16. African catfish fillet yield and trimming the remaining meat parts on the bone



Figure 17. pH measurement



Figure 18. Drip loss measurement



Figure 19. Cooking loss measurement

## **4.6 Chemical analysis (experiment I, II, III, IV and V)**

### **4.6.1 The chemical composition of test ingredients, feeds and faces**

The chemical composition of test ingredients, feeds and faces were analyzed by standard methods of the AOAC (2000). Crude protein (CP) was determined by Kjeldahl method (AOAC 928.08) using digestion block (KJELDATHERM, Gerhardt, Germany) via a distillation procedure (VAPODEST 450, Gerhardt, Germany). 0.5 g dry samples were digested with 10 mL cc H<sub>2</sub>SO<sub>4</sub> and 10 mL 30% H<sub>2</sub>O<sub>2</sub>, afterwards the generated ammonium sulphate was distilled off by using 2% H<sub>3</sub>BO<sub>3</sub>. The CP was calculated as  $N \times 6.25$  for diets and faces. The crude fat was determined from a 5 g dry sample according to the AOAC 945.16 Soxhlet method using an automatic system (SOXTHERM® Unit SOX416, Gerhardt, Germany) and diethyl ether (boiling point, 40–60°C) as a solvent. The crude ash content was estimated according to the AOAC 942.05 method. Two grams of the samples were weighed and placed in a furnace heated to 550°C and held for 4 h. The amount of the remained ash was recorded. Crude fiber content was determined from defatted samples (AOAC 928.08). The sample amount was 1.5-2 grams and the digestion procedure was carried out using 0.13 M H<sub>2</sub>SO<sub>4</sub> and 0.313 M NaOH in a GERHARDT Fibretherm FT12 apparatus (Königswinter, Germany). The acid-dissolved fiber (ADF) was determined with the same equipment by using ADF solution prepared from N-cetyl-trimethyl-ammonium bromide dissolved

in 0.5M H<sub>2</sub>SO<sub>4</sub> (100 g/5 L) and a few drops of anti-foaming agent. The chitin content was determined as the difference between ash free Acid Detergent Fiber (ADF) and protein linked to ADF (ADIP) (chitin %=ADF%-ADIP%) according to Finke (2007) and Marono et al. (2015). The gross energy was determined by a Parr Instruments 6400 calorimeter bomb (Moline, Illinois, USA) calibrated with benzoic acid. Apparent digestibility coefficients (ADCs) of dry matter and protein of the diets were determined by the following formula (Halver and Hardy, 2002; Cho et al., 1982 Bureau et al.,1999; Austreng, 1978).

#### **4.6.2 Amino Acid**

The amino acid content of samples was analyzed using the UPLC-DAD method (Waters Acquity UPLC H-Class, Milford, USA) after acid hydrolysis and pre-column derivatization with 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate (AQC) reagent. The analysis was performed with AccQ UPLC BEH C18 2.1x100 mm, 1.7 µm column (Waters) and AccQ Tag Ultra eluents A, B and water in gradient mode, the flow rate was 0.7 ml/min. The chromatograms were evaluated at 260 nm, using amino acid standards. Acid hydrolysis was carried out for amino acid analysis. Twenty-five milligrams of the samples were hydrolyzed by 6 NHCl containing 1% of phenol in a Milestone Ethos One Microwave digestion system. Hydrolysates were completed to 5 ml by 1 M borate buffer (pH 8.51).

#### **4.6.3. Titan, calcium and phosphorus**

Titan, calcium and phosphorus content was analyzed by the ICP method. The digestion of samples was made with mixtures of acids, including nitric acid (R.G. 65%) and hydrogen peroxide (R.G. 30%). The extraction was realized by using a microwave digestion technique under high pressure and a Milestone Ethos Plus (Soriso, Italy) microwave apparatus. The concentrations of elements (Titan, calcium and phosphorus) were measured by Thermo Scientific 6500 ICP-OES (Massachusetts, USA) equipment. The ADCs were measured using the indirect titan oxide method. For this purpose, the fish were fed the same experimental diets of the growth experiment, added with 0.1% titan oxide in all diet groups as an inert marker. The ADC of DM, CP, were calculated as reported by Cho et al., 1982 Bureau et al.,1999 and expressed as a percentage.

#### 4.6.4 Lipid analysis (Experiment V)

For fish fillet and diet samples, each sample was homogenized (IKA T25 Digital Ultra Turrax, Staufen, Germany) in a 20-fold volume of chloroform: methanol (2:1 vol: vol) and total lipid content was extracted (Folch et al., 1957). Solvents were ultrapure-grade (Sigma-Aldrich, St. Louis, MO, USA) and 0.01% w:v butylated hydroxytoluene was added to prevent FA oxidation. This latter fraction was evaporated to dryness under a nitrogen stream and was transmethylated with a base-catalyzed NaOCH<sub>3</sub> method (Christie, 1982).

Fatty acid methyl esters were extracted into 300 µL ultrapure n-hexane for gas chromatography (AOC 20i automatic injector; Shimadzu 2030, Kyoto, Japan) equipped with a Phenomenex Zebron ZB-WAXplus capillary GC column (30 m × 0.25 mm ID, 0.25 µm film, Phenomenex Inc., Torrance, CA, USA) and a flame ionization detector (FID) detector. Characteristic operating conditions were: injector temperature: 220 °C; detector temperature: 250 °C; helium flow: 28 cm/sec. The oven temperature was graded: from 60 (2 min holding) to 150 °C, from 150 to 180 °C: 2 °C/min and 10 min at 180 °C, from 180 to 220 °C: 2 °C/min and 16 min at 220 °C. The makeup gas was nitrogen. The calculation was performed with the LabSolutions 5.93 software, using the PostRun module (Shimadzu, Kyoto, Japan) with manual peak integration. Fatty acid results were expressed as the weight % of total FA-methyl esters. Calculation of the atherogenic index and thrombogenicity index was based on Ali (2021).

#### 4.7 Calculations

Growth parameters were compared by calculation of:

Relative Growth Rate (RGR %) which was calculated as:

$$\text{RGR} = 100 \times (W_f - W_i) / W_i$$

Specific growth rate (SGR %/d) which was calculated as:

$$\text{SGR} = 100 \times (\text{Ln}W_f - \text{Ln}W_i) / t$$

where:  $W_f$  = final average weight at the end of the experiment (g);

$W_i$  = initial average weight at the beginning of the experiment (g);

$t$  = experimental time in days.

Feed utilization parameters were compared by calculation of:

Feed conversion ratio (FCR g/g), which was calculated as:

$$\text{FCR} = \text{offered feed (g)} / (\text{final weight of fish (g)} - \text{initial weight of fish (g)})$$

Protein efficiency ratio (PER g/g) which was calculated as:

$$\text{PER} = \text{weight gain (g)} / \text{protein intake (g)}$$

The survival of fish (S %) was calculated as  $S = 100 \times (\text{number of fish at the beginning of the experiment} / \text{number fish at the end of the experiment}) (\%)$

The body condition indices were calculated by the following formulas:

The hepatosomatic index (HSI%):  $\text{HSI} = \text{LW} / \text{BW} \times 100$

Viscerosomatic index (VSI%):  $\text{VSI} = \text{VW} / \text{BW} \times 100$

Condition factor (k) =  $\text{BW} / \text{TL}^3 \times 100$

VW = visceral weight (g), LW = liver weight (g), BW = total body weight (g) and TL = total length (cm) respectively

Relative gut length (RGL%)  $\text{RGL} = \text{GL} / \text{BL} \times 100$

Apparent digestibility coefficient was calculated by the formula:

$$\text{ADC} \% = 100 - (\% \text{TiO}_2 \text{ in feed} \times \% \text{ nutrient in faeces} / \% \text{TiO}_2 \text{ in Faeces} \% \text{ nutrient in feed}) \times 100$$

GL = gut length (cm) and BL = total body length (cm)

Fillet physical parameters are calculated as follows

$$\text{DL} (\%) = [(\text{raw fillet weight (g)} - \text{raw fillet weight after 24h (g)}) / \text{raw fillet weight (g)}] \times 100$$

$$\text{CL} (\%) = [(\text{raw fillet weight (g)} - \text{cooked fillet weight (g)}) / \text{raw fillet weight (g)}] \times 100$$

$$\text{TL} (\%) = [(\text{raw fillet weight (g)} - \text{thawed fillet weight (g)}) / \text{raw fillet weight (g)}] \times 100$$

DL=drip loss

CL= cooking loss

TL =thawing loss

$$\text{Fillet yield} (\%) = \text{fillet(g)} / \text{BW(g)} \times 100$$

Profile index = standard length(cm)/height (cm)

#### **4.8 Ethical issues**

All procedures involving fish were conducted in line with the Hungarian legislation on experimental animals and approved by the National Scientific Ethical Committee on Animal Experimentation (identification number of the license: KA-3403). To minimize the fish's suffering, all necessary procedures (administration of anesthesia, skipping feeding before and after measurement) were applied.

#### **4.9 Statistical analysis**

In the beginning steps for statistical data analysis, tests of normality (Shapiro-Wilk normality test) and homogeneity of variances (Levene's tests) were performed, then the data were analyzed by one-way ANOVA using Rcmdr version 4.02 (developed by Ross Ihaka and Robert Gentleman at the University of Auckland, New Zealand) at a confidence interval (CI) of 95%. Significant differences level was considered for a p-value  $< 0.05$ . Means were compared using post hoc Tukey's multiple comparison test.

## 5. RESULTS AND DISCUSSIONS

### 5.1 Experiment I. Effect of different replacement level of FM with BSF in common carp diet.

#### 5.1.1 Growth performance and feed utilization

The final body weight (FBW), specific growth rate (SGR), feed conversion ratio (FCR), and protein efficiency ratio (PER) of common carp were significantly ( $p < 0.05$ ) improved as the fishmeal was totally replaced with black soldier fly meal. The highest value for FBW ( $64.58 \pm 15$ g) was recorded in total ( $120 \text{ gkg}^{-1}$ ) replacement. The lowest FBW ( $59.8 \pm 14.02$ g) was observed in the control group (Table 25). There was no significant difference between BSF50 and BSF100. Similarly, the difference in terms of FCR and PER was not significantly different between BSF50 and BSF100. A significant difference ( $p < 0.05$  ANOVA) in terms of growth performance was observed between the control and BSF100. In contrast to our finding, the absence of significant difference was reported after 15 weeks of experimental duration in Atlantic salmon (*S. salar*) with initial body weight of 250g, where dietary replacement of FM with BSF at a replacement level of  $200 \text{ gkg}^{-1}$  (100%) on evaluated parameters (FBW, FCR, HSI, VSI) (Lock et al., 2016). While Huda et al. (2020) reported that the replacement of FM with BSF at replacement level of 50% ( $175 \text{ gkg}^{-1}$ ) gave the best results in (SGR) in African catfish (*C. gariepinus*) with initial length ranging from 5-7cm stocked for the experimental duration of 35 days. On the other hand, Kroeckel et al. (2012) have reported that dietary replacement of FM with BSF larvae meal had significantly reduced growth performance and feed utilization of turbot (*Psetta maxima*). These different results can be explained, among others, by the differences in fish species, and differences of nutritional composition of BSF which is substantially dependent on many factors including the developmental stage of BSF, of which the insect meal was harvested (Liu et al., 2017; Smets et al., 2020), differences in the substrate on which the BSF has grown on (Ewald et al., 2020), different processing methods of the BSF meal (Fasakin et al., 2003). Furthermore, the age and initial weight differences of the experimental unit (fish), water chemistry, and health condition of the fish might have contributed significantly.



Table 25. growth performance traits\* (mean  $\pm$  SD) in the different treatment groups (experiment I)

Parameters	Control	BSF50	BSF100	<i>p</i> -value
IBW (g)	35.2 $\pm$ 5.93	35.23 $\pm$ 6.10	35.23 $\pm$ 6.02	0.999
FBW (g)	59.8 $\pm$ 14.02a	62.2 $\pm$ 14.4ab	64.58 $\pm$ 15.3b	0.01
WG (g)	24.6 $\pm$ 14.47a	26.9 $\pm$ 16.74ab	29.35 $\pm$ 16.29b	0.01
SGR (%)	0.94 $\pm$ 0.01a	1.01 $\pm$ 0.22ab	1.08 $\pm$ 0.24b	0.015
RGR (%)	73.56 $\pm$ 44.59	83.35 $\pm$ 58.50	88.48 $\pm$ 56.22	0.302
FCR	2.33 $\pm$ 0.07b	2.14 $\pm$ 0.07ab	1.99 $\pm$ 0.09a	0.006
PER	1.12 $\pm$ 0.03a	1.20 $\pm$ 0.04ab	1.29 $\pm$ 0.06b	0.04

\*Where: IBW = initial body weight, FBW = final body weight, SGR = specific growth rate, RGR = relative growth rate, PER= protein efficiency ratio, WG =weight gain, FCR= feed conversion ratio,

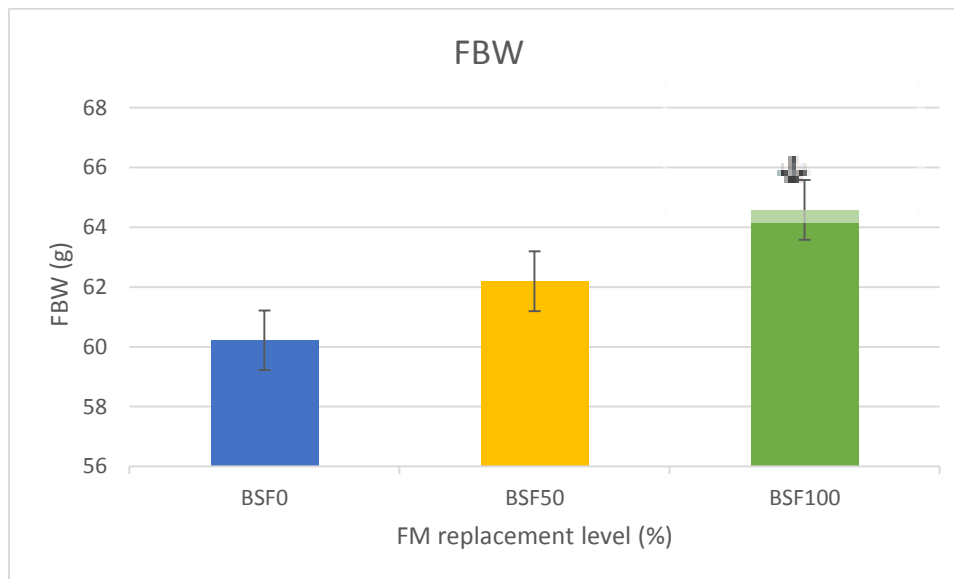


Figure 20. Effect of BSF in final body weight of common carp (mean $\pm$  SD, n=60 fish/ diet group, \* indicates significant difference  $p < 0.05$  ANOVA)

### 5.1.2 Survival rate and biometric indices

Survival rate (SR), which was not significantly affected by dietary FM replacement, and all fish survived in all diet groups. As well as the hepatosomatic index (HSI), relative gut length (RGL) was not significantly affected by either total or partial replacement level.

Biometric indices indicate the physiological condition of the specimens based on fat accumulation, gonadal development, general well-being, and adaptation to the environment (Nikolsky, 1963). According to Hoffmann et al. (2020), hepatosomatic index can be associated with the gonad developmental stage, since HSI increases at early ripening and decreases at late phase Arukwe and Goksøyr, 2003. On the other hand, HSI is an indicator effect of feeding on liver functionality which can be further associated with carbohydrate and fat metabolism (Iaconisi et al., 2018). In the present study, HSI ranged between 2.17 to 2.72% and was not significantly different ( $p > 0.05$  ANOVA) between diet groups. The condition factor (K) expresses the ratio between fish body weight and length, reflecting the interactions between biotic and abiotic variables in the physiological condition of the fish (Le Cren, 1951). A fish's condition factor (K) reflects physical and biological situations and fluctuations by interaction among feeding conditions, parasitic infections and physiological factors (Le Cren, 1951). Interestingly, in the current study, the condition factor (k) was not affected by the BSF replacement level. In the case of the viscerosomatic index (VSI) and the final length of carp were significantly different. The highest VSI (3.66%) was observed at control diet groups.

Table 26. survival rate and values of biometric indices \* (mean  $\pm$  SD) in the different treatment groups

Parameters	Control	BSF50	BSF100	P-value
SR (%)	100	100	100	-
HSI (%)	2.72 $\pm$ 1.00	2.50 $\pm$ 0.20	2.17 $\pm$ 0.47	0.355
VSI (%)	3.669 $\pm$ 0.60 <sup>a</sup>	3.01 $\pm$ 0.36 <sup>b</sup>	3.06 $\pm$ 0.39 <sup>b</sup>	0.048
k (g/cm <sup>3</sup> )	1.83 $\pm$ 0.12	1.75 $\pm$ 0.08	1.80 $\pm$ 0.16	0.568
TL (cm)	14.81 $\pm$ 1.03 <sup>a</sup>	15.37 $\pm$ 1.25 <sup>b</sup>	15.09 $\pm$ 1.16 <sup>ab</sup>	0.031
RGL (%)	1.87 $\pm$ 0.08	1.69 $\pm$ 0.16	1.79 $\pm$ 0.18	0.142

\*Where:SR=survival rate, HSI = hepato-somatic index, VSI = viscero-somatic index, K = condition factor, TL = total length, and RGL = relative gut length. The different letters in the same row indicate significant differences ( $p < 0.05$  ANOVA).

As mentioned above, due to the variations among fish species and size, insect species, rearing conditions of insects or fish, processing methods of insect meal, and duration of the experiment, the direct comparisons of results among different studies would be difficult and then the optimal FM substitution levels could vary accordingly (Jeong et al., 2020). In addition, standardization of insect protein production (insect growing substrate, harvesting time of insect developmental stage, processing method) is a central point to assure the correct formulation of diets for fish and also for other targeted animal species

## **5.2. Experiment II. Effect of different replacement level of FM with MW in common carp diet.**

### **5.2.1 Growth performance and feed utilization**

The result revealed that growth parameters (FBW (final body weight), SGR (specific growth rate) and WG (weight gain) were not significantly different ( $p > 0.05$ ) between treatments but the highest values were recorded at the replacement level of group MW50 (Table 27). The feed utilization parameters (PER) showed decreasing tendency at a total replacement. This finding is in agreement with the reports of Mamuad et al. (2021) who conducted an investigation on ornamental carp with initial weight and length ( $30 \pm 5$  g,  $130 \pm 5$  mm) by replacing FM with MW and found a reduction in feed utilization (feed intake and FCR) at 100% ( $180\text{gkg}^{-1}$ ) replacement level. Similarly, Nandeesh et al. (2000) confirmed after 90 days of feeding carp with different replacement levels of non-defatted silkworm pupae, partial replacement was found to be best in terms of fish growth (growth and FCR) and meat quality attributes Likewise, Ji et al. (2015), who conducted an investigation on Jian carp with an initial body weight of  $15.96 \pm 0.66$  g) for 57 experimental periods to see the effect of different replacement levels of FM with silkworm pupae (SW0, SW50, SW70 and SW80). At the end of the experiment, they confirmed that the partly (SW50) replacement of the fishmeal in common carp diets did not affect the performance (growth and feed conversion) of the fish. In the current study, the better growth performance observed in

MW50 replacement level than that of MW100 might be associated with the high dietary chitin content of MW which could impair the digestibility of other nutrients as the limited existence of chitinolytic action for most fish species (Ng et al., 2001; Lindsay et al., 1984; Kroeckel et al., 2012). According to Zhang et al. (2014), chitin mainly contributes to increased bulk, reduced feces retention time, and reduced enzyme accessibility to substrates. According to the reports of Iaconisi et al. (2017) MW was replaced up to 50% ( $400\text{gkg}^{-1}$ ) without any negative effect on the growth performance and FCR in 131 feeding days on blackspot sea bream (*Pagellus bogaraveo*) ( $W_0=171-174\text{g}$ ). Similarly, Piccolo et al. (2017) reported MW replacement up to 50% ( $500\text{gkg}^{-1}$ ) for 163 days of feeding with gilthead sea bream (*Sparus aurata*) ( $105.2 \pm 0.17\text{ g}$  average initial body weight) did not negatively affect growth performance but best result in terms of FBW, FCR, PER obtained at 25% ( $250\text{gkg}^{-1}$ ) replacement level. On the other hand, the total replacement of FM with defatted MW was not affected the FCR and increased growth performance after 4 weeks of feeding on red sea bream (*Pargus major*). (Ido et al., 2019). The FCR obtained from this study is better than the reports of Mamuad et al. (2021) who confirmed the FCR for common carp fed different inclusion level of *Ptecticus tenebrifer* and *Tenebrio molitor* (MW) ranging from 3.72-6.2. However, the overall FCR obtained from this study seems low when compared to commercially raised carp. This might be due to low feed uptake associated with handling stress which affects the appetite of the fish. Due to this reason the consumed feed might be overestimated to calculate FCR as the uneaten feed was not measured. The other reason might be less feeding frequency. According to the reports of Abdel-Aziz et al. (2021), the feeding rate below four resulted in reductions of FCR in common carp and the best FCR was obtained at a feeding frequency of four to six times with the recommended optimum feeding frequency of six times a day. The size of the fish used in this experiment was relatively larger (sensitive to handling stress) than the fish in the first experiment.

On the other hand, MW has successfully replaced the fishmeal on practical diets of Nile tilapia (*O. niloticus*) juveniles (Tubin et al., 2020) and the authors observed significantly higher growth ( $p < 0.05$ ) with the highest inclusion level. Su et al. (2017) confirmed that after five weeks of feeding, the total replacement of fishmeal with MW did not affect negatively the growth performance of yellow catfish (*Pelteobagrus fulvidraco*). The alteration of results might be related to differences in nutritional properties of the mealworm (Igor et al., 2019; Liu et al., 2020), chitin content that can affect the crude protein digestibility of MW (Marono et al., 2015), deficiency of micronutrients

and essential amino acids (Nogales-Merida et al., 2019) or fish species differences. Thus, it needs further investigation on chitin content and nutrient digestibility of MW for common carp.

Table 27. growth performance and feed utilization of common carp juveniles fed total or partial replaced FM with mealworm meal (Experiment II) (Mean  $\pm$  standard deviation)

Parameters	Control	MW50	MW100	p-value
IBW (g)	97.44 $\pm$ 14.13	97.62 $\pm$ 15.71	97.55 $\pm$ 15.62	0.99
FBW (g)	132.1 $\pm$ 34.39	134.7 $\pm$ 30.42	130.9 $\pm$ 26.62	0.83
WG(g)	568.33 $\pm$ 59.34	556.33 $\pm$ 94.51	501.60 $\pm$ 46.11	0.49
RGR (%)	35.56 $\pm$ 4.46	37.98 $\pm$ 6.05	34.23 $\pm$ 3.17	0.63
FBL (cm)	18.32 $\pm$ 1.36	18.45 $\pm$ 1.28	18.54 $\pm$ 1.32	0.73
IBL (cm)	17.12 $\pm$ 0.91	17.14 $\pm$ 0.92	17.36 $\pm$ 0.94	0.41
SGR (%/day)	0.72 $\pm$ 0.07	0.76 $\pm$ 0.10	0.70 $\pm$ 0.05	0.64
SR (%)	100	100	100	0.37
FCR(g/g)	3.35 $\pm$ 0.33	3.40 $\pm$ 0.48	3.68 $\pm$ 0.37	0.56
PER(g/g)	0.85 $\pm$ 0.08	0.84 $\pm$ 0.11	0.77 $\pm$ 0.07	0.52

IBW initial body weight, FBW final body weight, RGR relative growth rate, FBL final body length, IBL initial body length, SGR specific growth rate, SR survival rate, FCR feed conversion rate, PER protein efficiency ratio, WG weight gain

### 5.2.2 Biometric indices and survival rate

In this study, the biometric parameters such as hepatosomatic index (HSI) and condition factor (K) were not significantly affected by partial or total replacement of FM with MW (Table 28.). This result is in agreement with the findings of Hoffmann et al., (2020), who reported that HSI and K of sea trout larvae (*Salmo trutta*) were not significantly affected by partial or total replacement of FM with MW. Chemello et al. (2020) reported the absence of significant differences among treatments for the condition factor of rainbow trout fed up to 100% replacement of FM with MW. While Gasco *et al.* (2014) observed the lowest hepatosomatic indices were observed in rainbow trout fed the 25 and 50% yellow mealworm replacement with FM. The differences might be due to the fish age, gonad maturity stage and processing method of the mealworm meal (full-fat vs defatted) (Rizzo and Bazzoli, 2020; Chemello et al.,2020).

Table 28. Effect of replacing dietary protein of FM with mealworm meal on body condition of common carp juveniles (Experiment II) (Mean  $\pm$  standard deviation)

Parameters	Control	MW50	MW100	p-value
LW (g)	4.43 $\pm$ 1.56	6.03 $\pm$ 1.73	5.24 $\pm$ 1.28	0.23
GL (cm)	34.75 $\pm$ 4.12	37.01 $\pm$ 4.19	36.93 $\pm$ 3.40	0.53
BLW (g)	0.23 $\pm$ 0.11	0.24 $\pm$ 0.10	0.28 $\pm$ 0.10	0.76
KW (g)	0.62 $\pm$ 0.16	0.78 $\pm$ 0.19	0.88 $\pm$ 0.42	0.29
K (g/cm <sup>3</sup> )	2.17 $\pm$ 0.42	2.24 $\pm$ 0.13	2.02 $\pm$ 0.18	0.40
HSI (%)	0.03 $\pm$ 0.00	0.04 $\pm$ 0.00	0.04 $\pm$ 0.00	0.052
VSI (%)	0.03 $\pm$ 0.00	0.04 $\pm$ 0.00	0.04 $\pm$ 0.00	0.17
RGL (cm)	1.86 $\pm$ 0.18	1.92 $\pm$ 0.19	1.95 $\pm$ 0.20	0.72

LW: liver weight, GL: gut length, BLW: bile weight, KW: kidney weight, K: condition factor, HSI: hepatosomatic indices, VSI: viscera somatic indices, RGL: relative gut length

### 5.2.3 Whole body proximate quality

The proximate composition of the eviscerated carp was not significantly affected by the inclusion level of MW except for crude fat content which was significantly higher at the inclusion rate of 50% and 100% (Table 29.). Similar to our findings, Tubin et al. (2020) observed a significant ( $p < 0.05$ ) increase in the crude fat content of Nile tilapia as the level of MW replacement increased. Rema et al. (2019) observed no significant difference in the whole-body composition of rainbow trout fed different inclusion levels of MW. In accordance with the findings of Iaconisi et al. (2018), the absence of significant differences was observed in rainbow trout fillets' proximate composition (raw and cooked), whereas the fatty acid (FA) profile was strongly affected by the diet containing mealworm meal. Similarly, Ng et al. (2001) reported the absence of significant differences in the lipid composition of African catfish filets using different inclusion levels of MW.

Table 29. Effect of replacing dietary protein of FM with mealworm meal on meat proximate composition of common carp juveniles (Experiment II) (Mean  $\pm$  standard deviation)

Parameters	Control	MW50	MW100	p- value
Moisture (%)	70.50 $\pm$ 1.27	68.33 $\pm$ 0.55	69.40 $\pm$ 1.47	0.15
Crude protein (%)	13.80 $\pm$ 0.72	13.76 $\pm$ 0.05	13.80 $\pm$ 0.17	0.99
Crude fat (%)	12.56 $\pm$ 1.09a	15.20 $\pm$ 0.60b	14.03 $\pm$ 1.20ab	0.04

Ash (%)

1.93 ± 0.20

1.86 ± 0.25

1.76 ± 0.05

0.59

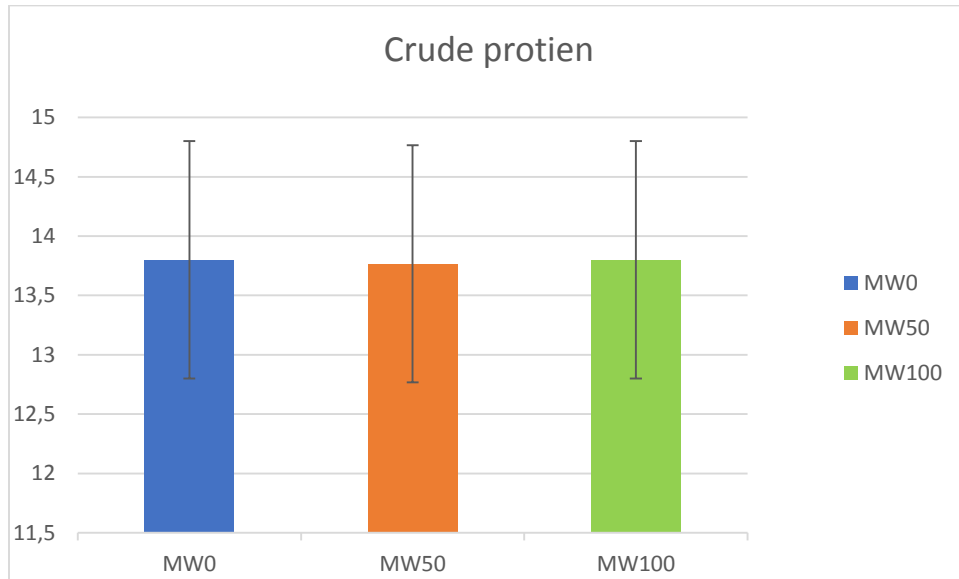


Figure 21. Effect of different inclusion level of mealworm meal in whole body crude protien composition of common carp (mean± SD, n=6 fish/ diet group)

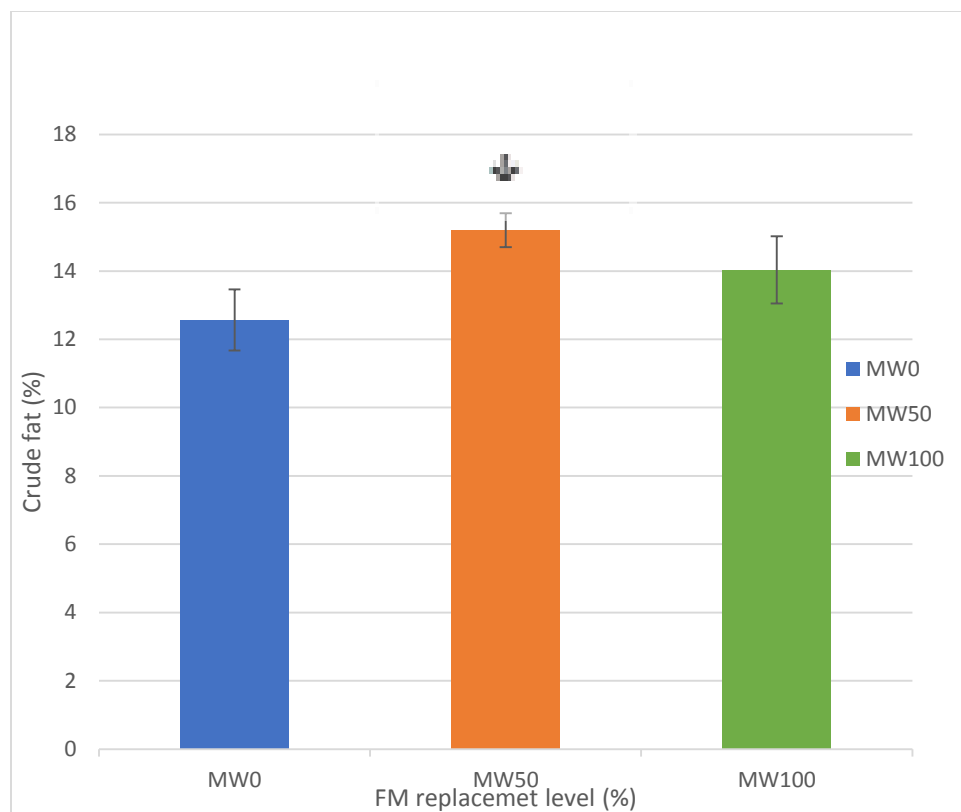


Figure 22. Effect of different inclusion level of mealworm meal in whole body crude fat composition of common carp (mean± SD, n=6 fish/ diet group, \* indicates significant difference  $p < 0.05$  ANOVA)

### 5.3 Experiment III. Effect of different replacement level of FM with BSF and MW in African catfish diet

#### 5.3.1 Growth performance, feed utilization and survival rate

The growth performance, feed utilization, and survival rate of African catfish (*C. gariepinus*) fed different experimental diets are presented in Table 30. The result revealed that there were no significant differences ( $p > 0.05$ ) in the FBW, SGR, and TL in fish fed the control (i.e., 100% fishmeal-based) diet and those fed diets containing MW50, MW100, BSF50, BSF100, BSFMW50 and BSFMW100 fishmeal replacement. This finding is in pact with the findings reported on European sea bass (*Dicentrarchus labrax*) fed with different inclusion levels of defatted mealworm meal (Basto et al., 2021) Siberian sturgeon (*Acipenser baerii*) fed with black soldier fly larvae meal (Józefiak., 2019) Nile tilapia (*O. niloticus*) fed with different inclusion levels of black soldier



fly (Devic et al., 2018). Similarly, Melenchón et al. (2021) reported the absence of a negative effect on the growth performance of rainbow trout (*Oncorhynchus mykiss*) fed where the FM was partially and totally (130 and 300gkg<sup>-1</sup>) replaced with black soldier fly and mealworm meal. Furthermore, a positive influence on the growth performance of mirror carp was observed when the fish were fed a diet containing a mixture of three insects (black soldier fly, mealworm, and silkworm pupae) oils (Xu et al., 2020). In our study, partial replacement of fishmeal (MW50, BSF50 and BSFMW50) exhibited approximately similar growth performance with the control diet. This finding is comparable with previous findings in the literature. Huda et al. (2020), and Ng et al. (2001) confirmed the replacement of FM with BSF and mealworm meal at partial inclusion level showed the best results in African catfish having 2.67, 6 and 5.1g initial body weight respectively. The decreasing tendency of growth performance of African catfish as the replacement level of fishmeal with BSF and mealworm meal increases could be associated with low palatability of black soldier fly and mealworm that can affect the intake. Low acceptability and intake due to low palatability have been confirmed when fishmeal is significantly or completely replaced in fish diets (St-Hilaire et al., 2007a). In contrast, black soldier fly meal successfully replaced 100% of fishmeal without a significant effect on growth. For instance, (140gkg<sup>-1</sup>) in Jian carp (Zhou et al., 2018). The variations of the results might be associated with differences in doses (gkg<sup>-1</sup>) of ingredients (insect meal) and other ingredient differences in the formulated diet, age and size of fish (Chemello et al., 2020), the feeding habits of fish (Henry.,2015), processing (either full-fat or mechanically defatted) and the high lipid content that reduces the availability of crude protein (Rema et al., 2019). Thus, it needs further investigation on which inclusion level and the other ingredients type can be standard. In the present study, 100% survival was observed in BSF50% groups, while some mortalities occurred in the other groups. The mortality was related rather to the handling stress than the feed. However, the positive impact of BSF50 to cope the handling stress needs further investigation. Interestingly, the survival was high (> = 90%) indicating the positive impact of this specific insect meal on the survival of African catfish.

The quality and utilization of protein in a diet are measured by the protein efficiency ratio (PER) and the protein productive value (PPV) (Albrektsen et al., 2006). Productive protein value (PPV) also known as the efficiency of protein utilization, can evaluate the protein in the diet by the ratio between the protein retained in fish tissues and the dietary protein fed (Gerking, 1971). In the present study, feed utilization did not significantly ( $p > 0.05$ ) differ between the different diet

groups. Nevertheless, the highest result was obtained with the control diet. The highest feed utilization in the control group might be associated with the lowest chitin content in that diet. It was confirmed that the growth performance, FCR and SGR of Nile tilapia decreased as the dietary chitin increased (Shiau, and Yu,1999). 10% chitin content decreased the growth performance and up to 5% increased the growth of Nile tilapia (Elserafy et al., 2021). The other reason for the reduction of feed utilization (FCR) might be associated with low feed intake due to the low palatability of insect meal. In fact, as fishmeal substitution with insect meal increases in the fish diet, the palatability decreases (Kroeckel et al., 2012). The other reason for low FCR might be the overestimated feed consumption due to the absence of measuring uneaten feed.

Table 30. Growth performance, survival and Feed Utilization Efficiency of African Catfish (n=60 fish/group (Experiment III) (Mean  $\pm$  standard deviation)

	Control	BSF50	BSF100	MW50	MW100	BSFMW50	BSFMW100	P-Value
FBW(g)	267.40 $\pm$ 59.33	257.63 $\pm$ 48.06	258.56 $\pm$ 47.00	260.88 $\pm$ 41.45	251.56 $\pm$ 49.55	263.83 $\pm$ 42.12	251.25 $\pm$ 39.74	0.44
SGR (%/day)	0.69 $\pm$ 0.02	0.62 $\pm$ 0.07	0.60 $\pm$ 0.07	0.61 $\pm$ 0.04	0.53 $\pm$ 0.04	0.64 $\pm$ 0.09	0.53 $\pm$ 0.02	0.09
PER(g/g)	0.98 $\pm$ 0.07	0.86 $\pm$ 0.11	0.83 $\pm$ 0.10	0.85 $\pm$ 0.05	0.73 $\pm$ 0.07	0.90 $\pm$ 0.16	0.77 $\pm$ 0.01	0.12
PPV (%)	16.38 $\pm$ 0.49	15.65 $\pm$ 2.64	12.90 $\pm$ 3.02	15.35 $\pm$ 1.32	11.85 $\pm$ 1.25	15.67 $\pm$ 3.46	14.69 $\pm$ 2.58	0.12
FCR(g/g)	2.30 $\pm$ 0.22	2.67 $\pm$ 0.39	2.56 $\pm$ 0.29	2.58 $\pm$ 0.19	2.97 $\pm$ 0.28	2.47 $\pm$ 0.35	2.96 $\pm$ 0.13	0.09
SR (%)	90.00 $\pm$ 0 a	100 $\pm$ 0 e	90.00 $\pm$ 0 a	91.25 $\pm$ 0 b	90.00 $\pm$ 0 a	92.50 $\pm$ 0c	93.75 $\pm$ 0 d	0.001

control (200g fishmeal (FM)), MW50 (100g of FM replaced with MW), MW100 (200g FM replaced with MW), BSF50 (100g of FM replaced with BSF), BSF 100 (200g FM replaced with B), BSFMW50 (100g of FM replaced with 1:1 combination of BSF and MW) and BSFMW100 (200g FM replaced by 1:1 combination of BSF and MW, SGR: specific growth rate, FBW: final body weight, SR: survival rate. PPV: protein productive value

### 5.3.2 Biometric Indices

The present study demonstrated that the biometric indices (Hepatosomatic indices, condition factor and total length) of the African catfish were not significantly affected ( $p > 0.05$ ) by the dietary replacement level of fishmeal by BSF and MW as well as the mixture of both (BSFMW) (Table 31).

Table 31. Biometric Indices of African catfish (n = 6 fish/group, mean and  $\pm$  SD) (Experiment III)

treatments	Control	BSF50	BSF100	MW50	MW100	BSFMW50	BSFMW100	P-value
K (g/cm)	0.82 $\pm$ 0.09	0.76 $\pm$ 0.06	0.78 $\pm$ 0.11	0.76 $\pm$ 0.06	0.82 $\pm$ 0.10	0.78 $\pm$ 0.05	0.77 $\pm$ 0.07	0.146
HSI (%)	1.56 $\pm$ 0.44	1.62 $\pm$ 0.40	1.70 $\pm$ 0.39	1.77 $\pm$ 0.36	1.73 $\pm$ 0.54	1.64 $\pm$ 0.42	1.72 $\pm$ 0.48	0.824
TL (cm)	32.08 $\pm$ 0.95	32.22 $\pm$ 2.23	32.90 $\pm$ 3.11	32.52 $\pm$ 1.24	31.50 $\pm$ 2.09	32.58 $\pm$ 1.52	32.61 $\pm$ 1.55	0.412

TL: total length, HSI: hepato- somatic index, K: condition factor

### 5.3.3 Diet Apparent Digestibility Coefficient

Due to the analytical error that happen the ADC result of the simultaneous replacement of fishmeal with black soldier fly and mealworm meals (BSFMW50 and BSFMW100) was excluded.

The apparent digestibility coefficient of dry matter and crude protein of diets containing partial and total replacements of fishmeal with black soldier fly and mealworm meals are presented in (Table 32). The results revealed higher apparent digestibility coefficients (> 91%) compared to the control diet in diets containing different levels of BSF and MW meals (Table 32).

In the present study, our result is comparable with the previous reports: Caimi et al. (2021) who reported the maximum dry matter digestibility of defatted black soldier fly was 87.7% for rainbow trout. On the other hand, our result is higher than the result of Taufek et al. (2016), who reported that the apparent digestibility of dry matter and crude protein of cricket meal for African catfish were 73.97% and 81.21% respectively. In the case of mealworm meal, our result is quite lower than the result of Chemello et al. (2020) who found > 94% and > 97% ADC for dry matter and crude protein in rainbow trout fed with a diet containing 100% replacement level of fishmeal with partially defatted mealworm meal. The chitin content of black soldier fly and mealworm meals and the developmental stage of the insects harvested (Liu et al., 2020; Liu et al., 2017; Igor et al., 2019; Mohan et al., 2020). High dietary chitin may impair the digestibility of other nutrients when it is greater or equal to 10% (Shiau, and Yu, 1999). However, the chitinase activity existing in some fish species, chitinolytic action seems to be inadequate for most of the fish (Lindsay et al., 1984) Therefore, chitin mainly contributes to increased bulk, reduced feces retention time, and reduced enzyme accessibility to substrates (Zhang et al., 2014).

Table 32. Apparent Digestibility Coefficient of Diets Containing Different Replacement Levels of B and M meals (%) (Experiment III)

Replacement level (%)	Control	BSF50	BSF100	MW50	MW100
ADCDM (%)	87.58	91.36	91.21	93.43	92.00
ADCPr (%)	91.60	93.49	93.06	93.66	93.14

ADCDM: apparent digestibility coefficient of dry matter, ADCPr: apparent digestibility coefficient of protein

### 5.3.4 Blood Biochemistry

Significant differences ( $p < 0.05$ ) were found in blood biochemistry parameters (Table 33). The serum total cholesterol value increased significantly ( $p < 0.05$ ) in fish fed with MW100. Feeding African catfish with BSF50 and MW100 significantly affected ( $p < 0.05$ ) the serum phospholipid concentration when compared to the control diet. However, the phospholipid levels between the insect meal (BSF, MW and BSFMW) based diets were not significantly different. In the case of glucose, globulin, immunoglobulin, alkaline phosphatase and amylase, there was no significant difference ( $p > 0.05$ ) between different replacement levels and control diet. Plasma glucose and total protein levels are associated with stress response (Sulaiman et al., 2017). In this study, the highest levels of plasma glucose (GLU) were observed in fish fed with the control (FM) diet, followed by BSF100 and the lowest value was observed in fish fed BSF50. Despite the numerical differences, the values were not statistically significant ( $p > 0.05$ ) between the treated and control diet groups. In the same sense, the previous report showed the absence of a significant difference ( $p > 0.05$ ) of GLU concentration in juvenile black porgy fed with mealworm meal as fishmeal substitution (Jeong et al., 2022).

High levels of energetic metabolites such as cholesterol can be indicators of liver pathology and a high-fat diet (Coz-Rakovac et al., 2005). In the present study, the cholesterol level of blood serum was significantly lower in the control and higher in fish fed with MW100, respectively. This result is in contrast with the finding of Tran et al. (2022) and Jeong et al. (2020) who confirmed that defatted mealworm meal did not significantly affect the serum cholesterol level of European perch and rainbow trout fed with feeds in which 20.3% and 28% dietary protein (fishmeal) was replaced with defatted and fully fattened mealworm meal, respectively. This might be associated with the physiological status of the fish and the fat content of mealworm meal that is related to the substrate

in which the insect grows. Indeed, the level of cholesterol was below 200 mg/dl indicating it was within the normal range (Mori et al., 1994). Similarly, Sándor et al. (2021) reported (88-90.83 mg/dl). Phospholipids help by preventing the accumulation of fats in the liver. It plays a major role in the transportation and removal of cholesterol from the cells. It forms the structural components of the cell membrane with the association of proteins. In the present study, the plasma phospholipid was significantly different ( $p < 0.05$ ) by insect meal inclusion level and the lowest value was observed at BSF50 followed by MW100, respectively. This result is in contrast with the findings of Mastoraki et al. (2020) who reported plasma phospholipids level of European sea bass was not affected by the fishmeal substitution with mealworm and black soldier fly meal

Table 33. Selected Blood Serum Biochemistry Parameters of African Catfish fed diets with different inclusion level of MW and BSF (Experiment III) (Mean  $\pm$  standard deviation)

	Control	BSF50	BSF100	MW50	MW100	BSFMW50	BSFMW100	<i>P</i> -value
ALP (U/L)	45.77 $\pm$ 7.2 9	42.88 $\pm$ 3.62	44.50 $\pm$ 10.50	46.50 $\pm$ 5.07	45.70 $\pm$ 8.0 8	46.28 $\pm$ 6.36	46.00 $\pm$ 5.65	0.94
CHOL (mg/dl)	115.22 $\pm$ 15 .65a	125.44 $\pm$ 9.08a b	125.50 $\pm$ 13.72 ac	133.75 $\pm$ 11.34 ac	<b>146.10</b> $\pm$ <b>18.98</b> c	137.85 $\pm$ 14.20 bc	136.88 $\pm$ 13.95b c	0.00 07*
GLOB (g/dl)	2.45 $\pm$ 0.21	2.60 $\pm$ 0.31	2.63 $\pm$ 0.45	2.53 $\pm$ 0.19	2.70 $\pm$ 0.35	2.70 $\pm$ 0.14	2.81 $\pm$ 0.26	0.24 2
GLU (mg/dl)	101.55 $\pm$ 31 .16	76.88 $\pm$ 13.60	94.62 $\pm$ 20.29	82.12 $\pm$ 22.49	81.20 $\pm$ 12. 18	82.71 $\pm$ 15.69	82.66 $\pm$ 18.42	0.14 1
TP (g/dl)	3.19 $\pm$ 0.56	3.06 $\pm$ 0.37	3.15 $\pm$ 0.58	3.30 $\pm$ 0.34	3.37 $\pm$ 0.0.6 5	3.20 $\pm$ 0.44	3.28 $\pm$ 0.83	0.73 5
PHOS (mg/dl)	7.11 $\pm$ 1.36 b	5.83 $\pm$ 0.41a	6.65 $\pm$ 0.76ab	5.91 $\pm$ 0.45ab	5.85 $\pm$ 0.70 a	6.31 $\pm$ 0.91ab	6.41 $\pm$ 0.70ab	0.01 2
IG (mg/mL)	0.19 $\pm$ 0.13	0.11 $\pm$ 0.09	0.17 $\pm$ 0.18	0.13 $\pm$ 0.10	0.22 $\pm$ 0.15	0.26 $\pm$ 0.11	0.24 $\pm$ 0.14	0.82 5
AMY (U/L)	21.44 $\pm$ 7.7 3	16.09 $\pm$ 3.26	24.13 $\pm$ 9.09	22.13 $\pm$ 10.37	16.00 $\pm$ 3.4 6	19.71 $\pm$ 3.68	18.13 $\pm$ 5.28	0.18 6

ALP: Alkaline phosphatase, CHOL: Total cholesterol, PHOS: Phospholipid, GLU: Glucose, GLOB: Globulin, TP: Total protein, AMY: Amylase

Mean and  $\pm$  SD (n=12 fish/group), Between groups differences were compared with one-way post hoc Tukey's multiple comparison test.

Different letters in the same rows indicates significance difference ( $p < 0.05$ )

### **5.3.5 Liver fatty acid profile**

The total lipid fat content presented high individual variability within the same treatment, and significant differences ( $p = 0.254$ ) between the diets were found. Saturated FA level was almost higher in diets containing black soldier fly. For instance, lauric acid was presented in the liver of fish fed with BSF50 and BSF100 diets, in the case of myristic acid significant difference was found between C and BSF100 groups. The stearic acid was significantly ( $p < 0.05$ ) higher in BSF50 compared to C and MW50, MW100 and BSFMW100 groups. Total SFA was the highest in BSF50 and BSF100, but significantly highest only compared to group M50. Significant differences ( $p < 0.001$ ) of Lc-PUFA between C and the rest of the treatments were detected. The n-6/n-3 ratio was lowest in the control group and significantly differing in BSF100, MW50, MW100, BSFMW50 and BSFMW100 treatments. The present study result is supported by the previous reports reported by Belghit et al. (2019) who confirmed that feeding black soldier fly meal to Atlantic salmon has increased lauric acid in the liver and other tissues. The authors demonstrated Lc-PUFA decreased when FM is replaced with black soldier fly meal in the diet, they reported that both total hepatic FA and the hepatic content of neutral lipids, including TAG, decreased significantly when insect (BSF) ingredients were included in the diets.

Table 34. Total lipid (% wet weight) and fatty acid composition (% of total fatty acids) of liver after feeding period using diets with different inclusion level of M and B (n=6) (Mean  $\pm$  standard deviation)

	C	BSF50	BSF100	MW50	MW100	BSFMW50	BSFMW100	<i>p-value</i>
Total lipid	6.44 $\pm$ 2.65	8.09 $\pm$ 3.75	7.31 $\pm$ 2.51	5.78 $\pm$ 2.05	9.12 $\pm$ 2.42	5.64 $\pm$ 3.25	7.87 $\pm$ 2.80	0.254
12:0	n/d	0.22 $\pm$ 0.04	0.41 $\pm$ 0.08	n/d	n/d	n/d	n/d	
14:0	1.27 $\pm$ 0.26 <sup>a</sup>	1.51 $\pm$ 0.24 <sup>ab</sup>	1.70 $\pm$ 0.18 <sup>b</sup>	1.58 $\pm$ 0.11 <sup>ab</sup>	1.42 $\pm$ 0.21 <sup>ab</sup>	1.56 $\pm$ 0.29 <sup>ab</sup>	1.53 $\pm$ 0.20 <sup>ab</sup>	0.026
16:0	28.34 $\pm$ 3.12	29.90 $\pm$ 0.90	30.15 $\pm$ 2.05	28.31 $\pm$ 2.35	29.93 $\pm$ 1.58	29.38 $\pm$ 2.96	30.82 $\pm$ 1.33	0.379
16:1n-7	3.44 $\pm$ 0.53	3.44 $\pm$ 0.31	3.05 $\pm$ 0.17	3.82 $\pm$ 0.55	3.49 $\pm$ 0.50	3.46 $\pm$ 0.53	3.43 $\pm$ 0.55	0.251
18:0	7.71 $\pm$ 1.57 <sup>ab</sup>	10.23 $\pm$ 1.95 <sup>b</sup>	9.67 $\pm$ 2.18 <sup>ab</sup>	7.44 $\pm$ 0.73 <sup>ab</sup>	7.36 $\pm$ 0.66 <sup>a</sup>	8.63 $\pm$ 1.15 <sup>ab</sup>	8.31 $\pm$ 2.15 <sup>ab</sup>	0.021
18:1n-9	34.93 $\pm$ 6.08	37.60 $\pm$ 2.46	35.62 $\pm$ 1.65	37.31 $\pm$ 0.56	37.68 $\pm$ 1.71	36.42 $\pm$ 4.48	38.36 $\pm$ 2.86	0.557
18:2n-6	4.52 $\pm$ 1.54	4.18 $\pm$ 1.67	4.89 $\pm$ 1.67	6.02 $\pm$ 0.98	5.46 $\pm$ 1.04	4.79 $\pm$ 1.48	4.60 $\pm$ 1.60	0.378
18:3n-6	0.38 $\pm$ 0.14	0.40 $\pm$ 0.23	0.41 $\pm$ 0.13	0.59 $\pm$ 0.10	0.58 $\pm$ 0.15	0.56 $\pm$ 0.19	0.47 $\pm$ 0.13	0.256
20:1n-9	1.79 $\pm$ 0.20	1.61 $\pm$ 0.13	1.77 $\pm$ 0.14	1.73 $\pm$ 0.23	1.79 $\pm$ 0.15	1.61 $\pm$ 0.28	1.77 $\pm$ 0.16	0.069
18:3n-3	0.43 $\pm$ 0.07	0.29 $\pm$ 0.17	0.32 $\pm$ 0.16	0.47 $\pm$ 0.11	0.40 $\pm$ 0.14	0.35 $\pm$ 0.10	0.31 $\pm$ 0.12	0.218
20:4n-6	1.22 $\pm$ 0.43	1.01 $\pm$ 0.50	1.35 $\pm$ 0.37	1.12 $\pm$ 0.26	1.13 $\pm$ 0.43	0.74 $\pm$ 0.32	0.84 $\pm$ 0.41	0.161
20:5n-3	0.54 $\pm$ 0.26	0.45 $\pm$ 0.17	0.25 $\pm$ 0.01	0.61 $\pm$ 0.24	0.29 $\pm$ 0.01	0.39 $\pm$ 0.21	0.21 $\pm$ 0.02	0.104
22:6n-3	3.02 $\pm$ 1.15 <sup>b</sup>	2.49 $\pm$ 0.94 <sup>ab</sup>	2.64 $\pm$ 0.74 <sup>ab</sup>	3.03 $\pm$ 0.95 <sup>b</sup>	2.14 $\pm$ 0.81 <sup>ab</sup>	1.75 $\pm$ 0.72 <sup>ab</sup>	1.60 $\pm$ 0.58 <sup>a</sup>	0.030
Total SFA	39.24 $\pm$ 2.19 <sup>ab</sup>	42.55 $\pm$ 1.94 <sup>b</sup>	42.59 $\pm$ 3.62 <sup>b</sup>	38.09 $\pm$ 1.98 <sup>a</sup>	39.72 $\pm$ 1.55 <sup>ab</sup>	40.77 $\pm$ 2.05 <sup>ab</sup>	41.66 $\pm$ 2.02 <sup>ab</sup>	0.009
Total MUFA	40.29 $\pm$ 6.43	42.81 $\pm$ 2.58	40.65 $\pm$ 1.63	43.09 $\pm$ 0.82	43.23 $\pm$ 2.00	41.61 $\pm$ 5.09	43.80 $\pm$ 3.08	0.532
Total PUFA	11.68 $\pm$ 4.45	10.08 $\pm$ 3.90	11.55 $\pm$ 3.22	13.65 $\pm$ 2.51	11.60 $\pm$ 1.78	9.91 $\pm$ 3.07	9.37 $\pm$ 3.51	0.347
n-6/n-3	2.15 $\pm$ 0.50 <sup>a</sup>	2.41 $\pm$ 0.42 <sup>ab</sup>	2.99 $\pm$ 0.95 <sup>bc</sup>	2.60 $\pm$ 0.77 <sup>b</sup>	3.25 $\pm$ 0.94 <sup>bc</sup>	3.33 $\pm$ 1.09 <sup>c</sup>	3.37 $\pm$ 0.60 <sup>c</sup>	0.001
Lc-PUFA	4.51 $\pm$ 1.80 <sup>b</sup>	3.79 $\pm$ 1.60 <sup>a</sup>	4.08 $\pm$ 1.21 <sup>a</sup>	4.65 $\pm$ 1.45 <sup>a</sup>	3.28 $\pm$ 1.34 <sup>a</sup>	2.63 $\pm$ 1.15 <sup>a</sup>	2.37 $\pm$ 1.07 <sup>a</sup>	0.001

Different superscript letters denote significant differences among fish fed the different dietary treatments ( $p < 0.05$ ). Total SFA include 6:0, 8:0, 10:0, 12:0, 14:0, 15:0, 16:0, 17:0, 18:0, 22:0; Total MUFA include 16:1n-9, 17:1n-7, 18:1n-9, 20:1n-9; Total PUFA include 18:2-n-6, 18:3n-6, 20:2n-6, 20:3n-6, 20:3n-3, 20:4n-6, 20:5n-3, 22:6n-3, SUM: Total SFA + Total MUFA + Total PUFA; n-6/n-3: Total n-6 PUFA/Total n-6 PUFA; Lc-PUFA: 20:4n-6+ 20:5n-3+ 22:6n-3. n/d: not determined

## 5.4. Experiment IV. Effect of partial replacement of FM with BSF and MW in common carp diet on fillet yield and flesh quality

### 5.4.1 Fillet yield, physical characteristics of flesh and biometric indices

The fillet yield obtained from this study was within the range of previous studies done on common carp strains of Hungary by Varga et al. (2013) who reported 42% to 45%. From this study, we observed the highest final body weight at 100 gkg<sup>-1</sup> replacement of fishmeal with black soldier fly meal. The pH value showed slightly lower records at 24hrs post-mortem. This result is in pact with the reports of Varga et al. (2010). This might be due to the muscle-to-meat conversion, by the breakdown of glycogen (Varga et al., 2010), or the fish might be exposed to early stress. According to the reports of Poli et al. (2005) early stress of the fish before death that attributes to post-mortem glycolysis favoring the accumulation of lactic acid in muscles and subsequently decreasing the pH value of muscles. On the other hand, the relatively mild pH fall is attributed to the low glycogen concentration of fish flesh, as compared to mammals (Varga et al., 2010). The sex of common carp was not significantly ( $p > 0.05$ ) affecting the pH at 45minutes or 24hrs.

Table 35. Effect of replacing of FM with black soldier fly and mixture of black soldier fly and mealworm meal on fillet yield and flesh quality of common carp (Experiment IV (Mean  $\pm$  standard deviation))

Parameters	<i>P</i> -value					
	Control	BSF	BSFMW	Feed	Sex	Feed $\times$ Sex
Liveweight (g)	436.2 $\pm$ 77.89	459.6 $\pm$ 199.3	405.6 $\pm$ 75.1	0.81	0.39	0.37
SL (cm)	22.58 $\pm$ 1.69	22.52 $\pm$ 3.79	21.40 $\pm$ 1.89	0.75	0.55	0.41
profile index	2.36 $\pm$ 0.14	2.39 $\pm$ 0.16	2.31 $\pm$ 0.12	0.74	0.63	0.98
Trunk(g)	275.39 $\pm$ 52.12	272.33 $\pm$ 119.32	244.2 $\pm$ 46.05	0.88	0.49	0.39
Fillet yield (g%)	43.09 $\pm$ 1.13	40.38 $\pm$ 2.71	40.62 $\pm$ 2.62	0.23	0.39	0.76
HSI	1.27 $\pm$ 0.48	1.90 $\pm$ 0.9	1.60 $\pm$ 0.41	0.33	0.72	0.56
GSI (%)	4.13 $\pm$ 1.18	2.02 $\pm$ 1.38	2.12 $\pm$ 1.23	0.03	0.66	0.23
VSI (%)	5.34 $\pm$ 0.64	5.93 $\pm$ 2.15	5.04 $\pm$ 0.27	0.76	0.14	0.89
K(g/cm <sup>3</sup> )	3.78 $\pm$ 0.43	3.89 $\pm$ 0.47	4.14 $\pm$ 0.57	0.59	0.96	0.70
pH 45min	7.12 $\pm$ 0.11	7.04 $\pm$ 0.16	7.05 $\pm$ 0.11	0.63	0.10	0.86



pH 24h	6.66±0.25	6.48±0.06	6.76±0.17	0.15	0.58	0.58
Cooking loss (%)	17.07±4.50	19.25±1.25	17.97±2.98	0.61	0.46	0.96
Dripping loss (%)	4.93±2.30	5.27±2.37	4.43±1.11	0.63	0.13	0.45
Thawing loss (%)	7.93±1.12	7.48±0.95	8.30±1.91	0.70	0.99	0.49

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K Condition factor, HSI hepatosomatic index, GSI gonado -somatic index, VSI visor somatic index, SL standard length

#### 5.4.2 Survival rate

From the present investigation, the highest mortality was observed at 100gkg<sup>-1</sup> replacement rate of mealworm meal with fishmeal. However, the reason of mortality was not known, we suspect cumulative effect from feed ingredients (MW) quality of the batch, low oxygen amount during that specific experimental period, and deterioration of the nutritional value of MW meal due to over- storage might be one reason. There are reports on the reduction of the survival rate of fish with increasing level of MW in different fish species. For instance, Tubin et al. (2020) reported the survival rate of Nile Tilapia (*O. niloticus*) juveniles significantly ( $p < 0.05$ ) decreased as MW inclusion increased and at 100% replacement mortality rate was 46.67%, according to the reports of Coutinho et al. (2021), the mortality rate increased as inclusion level of MW increased in the practical diet of meagre juveniles. Similarly, Gasco et al. (2016) observed a significant reduction of final body weight, weight gain, specific growth rate and feeding rate at an inclusion level of MW50%. In addition, Tran et al. (2022) confirmed the decreasing trend of survival rate as MW inclusion increases and a significant reduction of growth rate at 75% replacement level of FM with mealworm in the practical diet of European perch.

The reason for increasing mortality as the replacement levels of MW increases might be due to the more complex chitin-protein matrix existing in MW (Marono et al., 2015) and a lower trypsin susceptibility (Janssen et al., 2019). Such evidence is associated with the reduction of nutrient digestibility and consequently impairing growth and survival (Janssen et al., 2019). The other factor might be the processing method of mealworm. As evidence indicated the release of insects' proteases and phenoloxidases during the grinding of whole insects for insect meal production negatively affects protein digestibility, solubility and overall quality (Terwilliger, 2015; Janssen et al., 2019). Phenoloxidases are responsible for insect meal browning, resulting in the formation

of cross-linked structures between o-quinone and AA. There should be further investigation on optimum feeding dosages ( $\text{gkg}^{-1}$ ) and length of experimental period to feed mealworm meal for the health status of common carp.

Table 36. Effect of replacing of FM with black soldier fly and mixture of black soldier fly and mealworm meal on survival rate of common carp (Experiment IV) (%)

	Treatments			
	Control	BSF	BSFMW	MW
Survival rate	100	88.8	77.77	0

#### 5.4.3 Fillet proximate quality of common carp

Except for Ash, all proximate quality parameters were not altered by partial replacement. The ash content was significantly different between the control and partially replaced diet groups with the highest value at BSF50 (Table 37). Similarly, Li et al. (2022) confirmed significantly the highest ash content in the muscle of Tongue Sole (*Cynoglossus semilaevis*) fed partially (BSF50%) meal compared to control diet. In this study, most of the proximate quality of common carp fillets was not affected by the replacement of fishmeal with black soldier fly and a mixture of BSF and MW meal. This result is supported by the findings of Rema et al. (2019) who observed the absence of significant difference in the whole-body composition of rainbow trout (*Oncorhynchus mykiss*) fed different inclusion levels of MW.

This result is also supported by the findings of Iaconisi et al. (2018) and Tran et al. (2022) who observed the absence of differences in fillets' proximate composition of rainbow trout (*Oncorhynchus mykiss* W.) and European perch (*Perca fluviatilis*) which fed different inclusion of mealworm meal as Fm replacement respectively. Similarly, Zhou et al. (2018) and Caimi et al. (2021) reported the absence of significant differences interims of proximate composition - dry matter (DM), crude protein (CP) and ether extract (EE) contents by the replacement of BSF on Jian carp (*C. carpio* var. Jian) and rainbow trout (*Oncorhynchus mykiss*) respectively. Also, Devic et al. (2018) confirmed that the replacement of black soldier fly larvae meal did not alter the proximate quality of Nile Tilapia (*O. niloticus*). Similarly, 50% replacement of FM with

mealworm meal did not significantly affect the proximate composition of Nile Tilapia Nile (Sánchez-Muros et al., 2016).

Table 37. effect of partial replacement of FM on Proximate composition of common carp fillet (Experiment IV) (Mean  $\pm$  standard deviation)

Parameters	Control	BSF50	BSFMW50	P-value
Moisture	70.83 $\pm$ 1.55	74.30 $\pm$ 1.15	72.46 $\pm$ 2.64	0.16
Crude protein	17.66 $\pm$ 0.28	17.5 $\pm$ 0.20	17.3 $\pm$ 0.10	0.18
Crude fat	8.86 $\pm$ 0.66	7.03 $\pm$ 1.45	9.16 $\pm$ 2.73	0.36
Ash	1.00 $\pm$ 0.00a	1.1 $\pm$ 0.01b	1.05 $\pm$ 0.05ab	0.02

## 5.5 Experiment V. Effect of partial replacement of FM with BSF and MW in African catfish diet on fillet yield and flesh quality

### 5.5.1 Survival, slaughter traits and somatic indices

After 25 weeks of feeding, the survival ratio was 100% in all groups. The slaughter value parameters (eviscerated, a trunk with skin, trunk without skin, and skinned fillet) were not significantly different ( $p > 0.05$ ) between the treatments and control groups (Table 38). However, the hepatosomatic index was significantly increased ( $p < 0.05$ ) in the BSF50 diet group.

In the present study, the control diet (FM) (200 gkg<sup>-1</sup>) as replaced partially (100 gkg<sup>-1</sup>) with BSF50, MW50 and BSFMW50 meals did not affect the survival of African catfish and all fishes survived. Our result is in agreement with the previous studies that reported a 100% survival rate of the following species fed different inclusion levels of BSF and MW meals: Jian carp (Zhou et al., 2018), and African catfish (Ng et al., 2001). Similarly, Józefiak et al. (2019) reported that the survival rate of Siberian sturgeon was not affected by the inclusion level of BSF larvae meal.

The slaughter value parameters (eviscerated, trunk with skin, trunk without skin and skinned fillet) were not significantly different between treatments. Iaconisi et al. (2017) confirmed the absence of significant differences in slaughter traits (fillet yield) in blackspot seabream fed diets supplemented with full-fat mealworm larvae meal replaced up to 50% of FM compared with the control group. Similarly, Ordoñez et al. (2022) confirmed absence of a significant difference in fillet yield when tambaqui (*Colossoma macropomum*) fish with initial body weight 115.2  $\pm$  0.9 g

fed on BSF larvae as commercial feed replacement (only whole BSFL, 50% BSFL; 50% CF (BSFL:CF) and only CF (CF, as the control group)) after 120 days of feeding.

The condition factor (K) of a fish reflects physical and biological situations and fluctuations by interaction among feeding conditions, parasitic infections and physiological factors (Le Cren, 1951). In the present study, the condition factor of African catfish fed BSF was significantly lowest ( $p < 0.05$ ) than in other groups. This might be the situation of fish not exhibiting isometric growth (absence of length increases in equal proportion to body weights) (Balai et al., 2017). The hepatosomatic index (HSI) showed significantly lower values ( $p < 0.05$ ) in the control group. This might be associated with the gonad developmental stage (Hoffmann et al., 2020), since HSI increases at early ripening and decreases at the late phase (Arukwe and Goksøyr, 2003). This happens during gonadal maturation when vitellogenin (Vtg) and zona radiata proteins (Zrp) are synthesized by hepatocytes, which are subsequently transported through the bloodstream to the ovaries by receptor-mediated pinocytosis (Arukwe and Goksøyr, 2003). However, it needs further investigation on gonadal development stages and HSI relations and their effect on meat quality.

### **5.5.2. Fillet physical parameters**

Cooking and dripping loss were not significantly different ( $p > 0.05$  ANOVA) among the different diet groups and ranged between 17-23% and 2.78-3.9% (Table 38). The acidification value (pH) of fillet after 45 minutes of post-mortem did not exhibit a significant ( $p > 0.05$ ) difference in different diet groups. However, the pH 24 h post-mortem showed a significantly highest (6.61) in BSF diet group ( $p < 0.05$ ) (Figure 23). The cooking loss determined in the present study was approximately similar to earlier reports (Paleckaitis, et al., 2018), ranging between 17-21% and 4.06-4.39 in African catfish fed different extruded feeds, respectively. The absence of significant differences in cooking, thawing and dripping loss was reported in rainbow trout fed up to 100% (150 gkg<sup>-1</sup>) replacement rate of fishmeal with BSF meal (Caimi et al. (2021)). Furthermore, Iaconisi et al. (2018) found no marked difference in cooking loss of rainbow trout fed diets supplemented with up to 50% of full-fat MW larvae meal compared to the fish in the control diet.

The antemortem handling of fish and the stress suffered before and during slaughtering have a great impact on the quality of the final product, including low pH (Morzel et al., 2003; Wilkinson et al., 2008). Generally, flesh pH was reported to be significantly lower in the stressed fish than

in the rested group until 18 h post-harvest. Furthermore, the flesh pH of the stressed fish changes significantly over time (from 1 h up to 24 h), while the anesthetized fish exhibited a gradual decrease in pH, reaching ultimate values at 18 h post-mortem. In the present study, the fish fed BSF meal at 100 gkg<sup>-1</sup> inclusion level showed the highest value at pH 24 h post-mortem, and this might relate to the antemortem stress tolerance of the fish. The black soldier fly meal is rich in lauric acid, which has a role in stress resistance and immune system building, possibly indicative of the highest pH recorded (Xu et al., 2020).

Table 38. effect of partial replacement of FM on Fillet yield, somatic indices and fillet physical qualities of African catfish (Experiment V) (Mean ± standard deviation)

Parameters	Control	BSF	MW	BSFMW
IBW (g)	248.95±48.06	249.00±36.26	248.17±39.78	248.43±39.64
FBW (g)	690.52±0.58	740.02±9.79	693.51±9.09	822.18±0.39
Survival rate (%)	100	100	100	100
K (g/cm <sup>3</sup> )	1.16±0.14	0.99±0.11	1.18±0.14	1.00±0.08
HSI (%)	0.89±0.23b	1.33±0.39a	0.96±0.38ab	1.43±0.44a
pH 45'	7.19±0.20	7.15±0.34	7.09±0.09	7.14±0.15
VSI (%)	1.24±0.26	1.05±0.08	1.21±0.26	1.05±0.13
Cooking loss (%)	17.19±15.35	23.61±11.18	19.94±2.47	21.90±3.10
Dripping loss (%)	2.78±1.13	3.40±2.35	3.05±0.80	3.90±0.81
Trunk with skin (%)	60.11±5.27	64.00±4.28	58.48±8.31	62.96±3.49
Skinned trunk (%)	53.64±6.21	58.47±3.91	53.00±7.11	57.18±3.03
Thawing loss (%)	6.06±1.51	5.06±3.19	6.04±3.43	5.57±2.05
Skinned fillet (%)	39.24±6.14	44.2±5.52	39.58±7.28	42.12±2.80

BSF: black soldier fly, MW: mealworm, BSFMW: mixture of black soldier fly and mealworm, IBW: initial body weight, FBW: final body weight, K: condition factor, HSI: hepatosomatic index, VSI: viscerosomatic index, na: not applicable, pH 45': pH after 45 minutes of death. Different letters between rows shows significant difference ( $p < 0.05$ ).

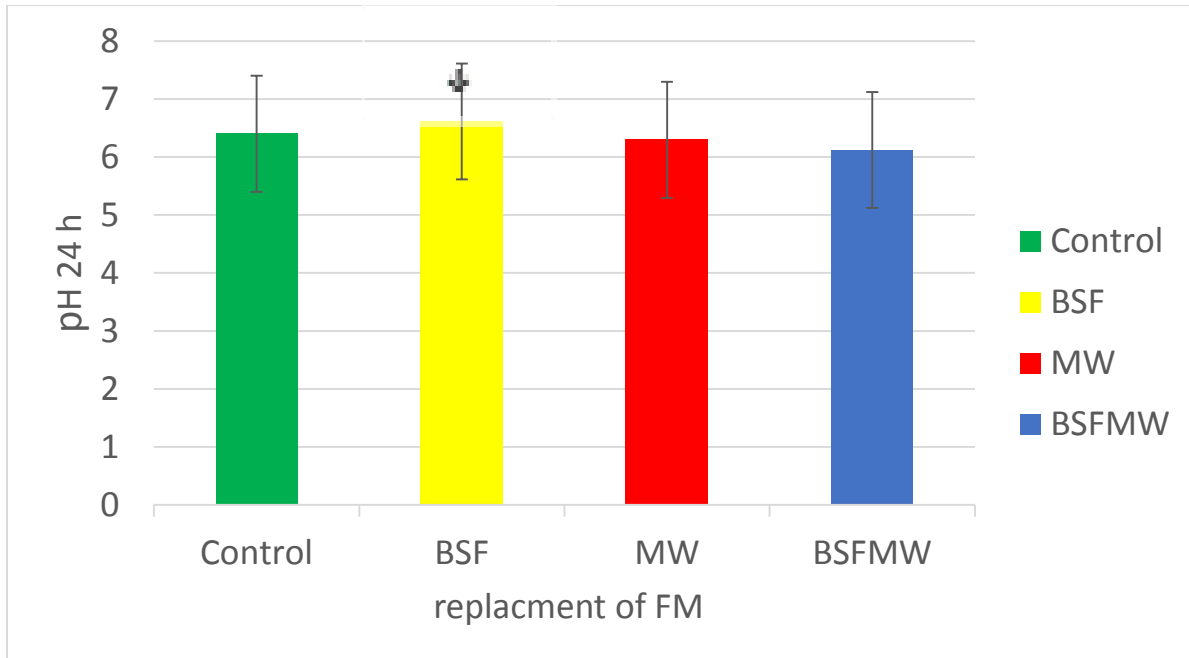


Figure 23 Fillet pH of African catfish after 24 h post-mortem of four groups (n = 6 fish/group) columns represent group means (n = 6) and error bars represent  $\pm$ SD. Between groups differences were compared with one-way post hock Tukey's multiple comparison test, presence of an asterisk (\*) represents significant difference ( $p < 0.05$  ANOVA).

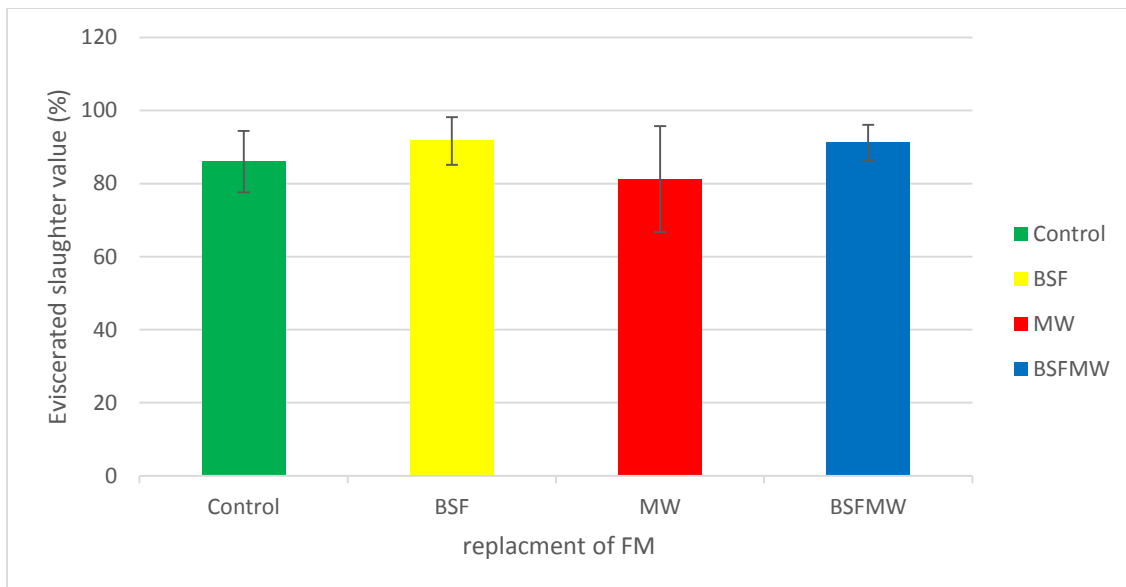


Figure 24. Eviscerated slaughter value (%) of African catfish fed partially replaced fishmeal four experimental groups (mean  $\pm$  SD of n = 6 fish per group). Lack of uppercase differences indicates the absence of significance ( $p > 0.05$ ) between groups.

### 5.5.3 Fillet proximate composition

This investigation revealed that the proximate composition of African catfish was not affected by the partial replacement of fishmeal with mealworm and black soldier fly meal (Table 39). In this study, the proximate composition of African catfish fed partially replaced fishmeal with BSF, MW and a 1:1 combination of BSFMW meals was not affected, as compared to the control group. There is supporting evidence previously reported on the lack of insect meal effect on the proximate quality of fish flesh: defatted MW meal on European perch (Tran et al. (2022), BSF on Nile Tilapia (Devic et al., 2018), and partially defatted BSF on rainbow trout (Caimi et al., 2021). Generally, the flesh of African catfish has a high total crude protein content (16.91–17.90%) and a comparatively low-fat content (3.95–7.57%) (Chwastowska Siwiecka et al., 2016; Paleckaitis et al. 2018. Interestingly, in the present study, the fillet protein content was above 18% in all groups. According to lipid content, fish meat can be classified as lean (<2% fat), low fat (2-4%), medium fat (4-8%), and blue or fat (> 8%) (Castro, 2002). In the present study, the fat content was relatively low and ranged between 3.45-3.90%.

Table 39. effect of partial replacement of FM on proximate composition of African catfish fillet (% fresh matter) (n =6 fish/group, mean ± standard deviation)) (Experiment V)

Parameters	Control	BSF	MW	BSFMW
Ash	1.26±0.09	1.27±0.08	1.29±0.09	1.20±0.06
Crude Fat	3.45±2.34	3.90±2.06	3.70±1.34	3.53±1.04
Moisture	76.83±3.06	75.62±2.70	76.31±1.66	75.88±1.17
Crude Protein	18.05±0.85	18.82±0.98	18.27±0.79	18.76±0.69

BSF: black soldier fly, MW: mealworm, BSFMW: combination of black soldier fly and mealworm

\*Lack of different letters in the same rows indicate absence of significant difference

### 5.5.4 Fillet fatty acid profile

The proportions of saturated and monounsaturated fatty acids increased as fishmeal was partially replaced with black soldier fly and mealworm meal, while polyunsaturated fatty acid (particularly omega-3 FA) proportion decreased (Table 40). The lauric acid (C12:0) proportion was significantly increased in the BSF diet group. While C17:0 was significantly highest in MW diet groups. Among polyunsaturated fatty acid groups, the C18:4n-3 significantly decreased in MW diet groups. Furthermore, atherogenic index was significantly highest in BSF diet groups. The

partial replacement of fishmeal with BSF, MW and BSFMW did not lead to significant alteration of total PUFA profile, specifically n-6 FA proportion was approximately similar in all diet groups. However, there was a decreasing tendency in (omega-3) poly-unsaturated FA levels, but not proven statistically. This finding is in pact with the findings of (Caimi et al. 2021), who have described the gradual decreasing trend in long-chain omega-3 PUFA (C20:5n-3 (EPA), C22:5n-3 (DPA) and C22:6n-3 (DHA)) as BSF inclusion increased from 3 to 15% in the diet of rainbow trout. Similarly, Gasco et al. (2016) and Sánchez-Muros et al. (2016) reported that whole body and muscle EPA, DPA, DHA and omega-3/omega-6 fatty acid ratio decreased as the inclusion level of MW meal increased in the practical diet of Juvenile European Sea bass and Nile tilapia.

The reason for the decreasing tendency of the total polyunsaturated fatty acid with fish-meal replacement as well as the increase of saturated and monounsaturated fatty acid might be due to the FA content and composition of insects (and derived meals) that can be substantially dependent on insect species, the substrate where the insects grow on, developmental stage and processing methods (Ewald et al., 2020). On the other hand, insects are generally rich in SFA and poor in PUFA (Stanley-Samuelson et al., 1988; Fabrikov., 2021; Renna et al., 2017), in particular, BSF larval fat consists mainly of C12:0, C14:0 and other SFA (Barragan-Fonseca et al., 2017; Fabrikov., 2021; Ewald et al., 2020). However, in the current study, the higher lauric acid content in BSF resulted in a decreasing tendency on polyunsaturated fatty acids and yet did not significantly affect ( $p > 0.05$ ) the fillet crude fat profile (mentioned earlier in proximate composition) compared to the fish fed on the control diet, so it would not be a quality issue (Belghit et al., 2019). Similarly, Jian carp and rainbow trout fed diets high in lauric acid did not have a reduction in the whole-body lipids (Li et al., 2016). On the other hand, as we mentioned in proximate quality parameters, the presence of high lauric acid impacted positively the post-mortem pH.

The other factor might be the effect of chitin on feed intake. Meanwhile, the indigestibility of chitin affects nutrient utilization by absorbing lipids and bile in the gastrointestinal tract, thus decreasing/limiting lipid digestion and absorption (Tharanathan and Kittur, 2003). The omega-6/omega-3 ratio of fish flesh in the present study ranged between 1.17-1.40. According to health recommendations, the omega-6/omega-3 ratio should be lower than 4, thereby reducing the incidence of chronic food-related illnesses (Cordain et al., 2005). In our study, the atherogenic index was significantly ( $p < 0.05$ ) higher at partial replacement of fishmeal with black soldier fly



meal. This might be due to the high lauric acid proportion of black slider fly meal. However, our finding is within the normal range (<1%) Similarly, (Łuczyńska et al., 2017) reported the absence of significant difference ( $p > 0.05$ ) in the thrombogenic index by partial replacement of fishmeal. In the present investigation, the ratio of PUFA to SFA was 0.67 to 0.78. The recommended PUFA/SFA ratio is to be higher than 0.4 in animal products, to reduce the risk of cardiovascular, autoimmune and other chronic diseases (Simopoulos, 2002).

Table 40. Fatty acid profile (w %) of the fish meat samples of different treatments (n=6 fish/group), mean and  $\pm$  standard deviation) (Experiment V)

<b>Fatty acid</b>	<b>Control</b>	<b>BSF50</b>	<b>MW50</b>	<b>BSFMW50</b>
C10:0	0.01 $\pm$ 0.00 a	0.01 $\pm$ 0.00 a	0.01 $\pm$ 0.00 b	0.01 $\pm$ 0.00 a
C12:0	0.52 $\pm$ 0.61 b	1.84 $\pm$ 0.89 a	0.25 $\pm$ 0.04 b	0.90 $\pm$ 0.47 ab
C14:0	2.15 $\pm$ 0.23	2.51 $\pm$ 0.27	2.21 $\pm$ 0.20	2.37 $\pm$ 0.26
C14:1n-5	0.03 $\pm$ 0.01	0.04 $\pm$ 0.01	0.04 $\pm$ 0.00	0.04 $\pm$ 0.00
C15:0	0.40 $\pm$ 0.05	0.37 $\pm$ 0.04	0.38 $\pm$ 0.06	0.37 $\pm$ 0.04
C16:0	25.2 $\pm$ 1.14	25.6 $\pm$ 1.37	24.2 $\pm$ 2.13	25.3 $\pm$ 1.84
C16:1n-7	3.42 $\pm$ 0.47	3.56 $\pm$ 0.65	3.36 $\pm$ 0.45	3.62 $\pm$ 0.66
C16:2	0.05 $\pm$ 0.01	0.05 $\pm$ 0.01	0.05 $\pm$ 0.01	0.05 $\pm$ 0.01
C16:3	0.03 $\pm$ 0.01	0.03 $\pm$ 0.00	0.03 $\pm$ 0.01	0.03 $\pm$ 0.01
C17:0	0.35 $\pm$ 0.04 a	0.32 $\pm$ 0.03 ab	0.29 $\pm$ 0.03 b	0.30 $\pm$ 0.03 ab
C18:0	6.19 $\pm$ 0.38	6.30 $\pm$ 0.33	7.83 $\pm$ 2.65	6.22 $\pm$ 0.67
C18:1n-7	2.64 $\pm$ 0.40	2.29 $\pm$ 0.32	2.10 $\pm$ 0.43	2.31 $\pm$ 0.34
C18:1n-9	29.9 $\pm$ 2.47	30.9 $\pm$ 1.91	31.9 $\pm$ 2.28	31.9 $\pm$ 3.09
C18:2n-6	11.3 $\pm$ 0.98	11.0 $\pm$ 1.31	12.0 $\pm$ 1.58	11.5 $\pm$ 2.30
C18:3n-3	1.26 $\pm$ 0.11	1.27 $\pm$ 0.13	1.48 $\pm$ 0.15	1.34 $\pm$ 0.30
C18:3n-6	0.47 $\pm$ 0.06	0.43 $\pm$ 0.07	0.45 $\pm$ 0.07	0.46 $\pm$ 0.13
C18:4n-3	0.25 $\pm$ 0.02 ab	0.19 $\pm$ 0.02 b	0.26 $\pm$ 0.05 a	0.23 $\pm$ 0.06 ab
C20:0	0.24 $\pm$ 0.04	0.23 $\pm$ 0.03	0.23 $\pm$ 0.04	0.23 $\pm$ 0.03
	<b>Control</b>	<b>BSF50</b>	<b>MW50</b>	<b>BSFMW50</b>
C20:1n-9	1.65 $\pm$ 0.22	1.64 $\pm$ 0.11	1.76 $\pm$ 0.28	1.63 $\pm$ 0.07
C20:2n-6	0.58 $\pm$ 0.13	0.48 $\pm$ 0.15	0.50 $\pm$ 0.15	0.48 $\pm$ 0.09

C20:3n-3	0.13±0.03	0.12±0.02	0.14±0.04	0.12±0.03
C20:3n-6	0.79±0.08	0.74±0.11	0.72±0.11	0.76±0.17
C20:4n-3	0.18±0.02	0.17±0.01	0.20±0.04	0.18±0.04
C20:4n-6	1.16±0.27	0.96±0.22	0.89±0.14	0.91±0.27
C20:5n-3	1.20±0.18	1.07±0.07	1.19±0.15	1.11±0.10
C21:0	0.02±0.00	0.02±0.01	0.02±0.01	0.02±0.01
C22:0	0.07±0.03	0.08±0.02	0.09±0.03	0.09±0.03
C22:1n-9	0.08±0.02	0.08±0.02	0.09±0.02	0.08±0.02
C22:1n-11	0.13±0.07 a	0.06±0.04 b	0.02±0.01 b	0.04±0.03 b
C22:5n-3	0.66±0.16	0.61±0.09	0.64±0.10	0.60±0.07
C22:6n-3	8.82±2.01	6.89±1.50	6.57±1.16	6.66±1.72
C24:0	0.05±0.01	0.05±0.01	0.05±0.01	0.04±0.01
C24:1n-9	0.08±0.02	0.07±0.03	0.07±0.02	0.06±0.01
Saturation	35.2±1.48	37.3±.54	35.6±3.62	35.8±1.79
Unsaturation	65.7±1.55	63.5±1.62	65.3±3.71	65.1±1.87
Mono-unsaturation	38.0±2.32	38.6±2.12	39.3±2.54	39.7±3.40
Poly-unsaturation	27.8±3.34	24.9±3.25	26.0±3.62	25.4±4.55
Omega-6	14.3±1.25	13.6±1.76	14.6±1.97	14.1±2.78
Omega-3	12.5±2.31	10.3±1.57	10.5±1.63	10.2±1.86
Omega-6: omega-3	1.17±0.22	1.33±0.12	1.40±0.10	1.38±0.19
Atherogenic index	0.45±0.05 b	0.57±0.06 a	0.43±0.05 b	0.49±0.03 ab
thrombogenicity index	0.52±0.06	0.59±0.07	0.58±0.12	0.58±0.09
PUFA/SFA	0.79±0.12	0.66±0.10	0.74±0.15	0.71±0.14

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BSF black soldier fly, MW mealworm, BSFMW combination of black soldier fly and mealworm, different uppercase letters in rows indicate significant difference ( $p < 0.05$ ).

## 6. CONCLUSIONS AND RECOMMENDATIONS

In Experiment I, total or partial replacement of fishmeal with black soldier fly meal showed an excellent result and the total replacement significantly improved ( $p < 0.05$  ANOVA) FBW, SGR, FCR, PER for 8 weeks of the experimental period on  $35.2 \pm 6.01$ g initial body weight common carp. The total or partial replacement level of fishmeal with black soldier fly meal did not significantly affect ( $p > 0.05$ ) the (HSI) of common carp. The condition factor between the control and experimental diet groups were not significantly different ( $p > 0.05$  ANOVA). Total stocked fish survived and there was no morbidity observed during the experimental period. The significant improvement in growth performance indicates that there is some room to elevate the inclusion level of BSF meal to some rate in the practical diet of common carp after evaluating and confirming the ADC, blood biochemistry, and product quality test at the minimum level (current portion).

In experiment II, it was confirmed that total or partial replacement of fishmeal with mealworm meal in the diet of common carp juveniles (initial body weight  $97.54 \text{g} \pm 15.0$ ) did not significantly affect ( $p > 0.05$  ANOVA) the growth performance, feed utilization, biometric indices and most proximate composition for up to six weeks of feeding regimes. All fish survived in all diet groups and there was no morbidity observed during the entire experimental period. Among the analyzed proximate compositions, crude fat exhibited significant differences between diet groups. The partial replacement has significantly higher crude fat content compared to the control diet groups. However, there were no statistically significant differences in terms of growth performance, feed utilization, and biometric indices, the best result was recorded in the partial replacement level. Thus, further investigation should be undertaken on nutrient digestibility, chitinolytic enzyme activity, and chitin level in mealworm-containing diet formulated for common carp juveniles. This result indicated that in longer-term experimentations, the replacement of fishmeal with 50% mealworm in the carp diet should be optimal.

In experiment III, in conclusion, the growth performance, feed utilization, and biometric indices of African catfish fed with partial or total replacement levels of fishmeal with black soldier fly, mealworm, and 1:1 combination meal were not significantly affected ( $p > 0.05$  ANOVA). The apparent digestibility coefficient of dry matter and crude protein of the diets was high ( $> 91\%$ ) in BSF and MW-containing diets than in the control group, indicating African catfish can digest more efficiently the diet containing BSF and MW than the control and this is confirmed in the other reports too. Based on this fact, BSF and MW can successfully replace FM ( $200 \text{gkg}^{-1}$ ) totally or

partially in the practical diet of African catfish without significant adverse effects on nutrient utilization and growth performance of African catfish. In the case of survival, some mortality was observed in all groups except BSF50. concerning to the partial replacement of BSF with FM, immunological assays are recommended to figure out if there is any positive effect of BSF on immune parameters (responses). The selected serum biochemistry results confirmed that there was no negative effect of diet on juveniles African catfish.

In experiment IV, black soldier fly and 1:1 combination feed at a rate of 100 gkg<sup>-1</sup> of dietary protein can successfully replace FM without any adverse effect on common carp's meat quality and slaughter traits. Significantly highest ash content was observed at BSF50 compared to the control group. A highest mortality was observed at an inclusion rate of 100 gkg<sup>-1</sup> of MW. Further investigations should be performed regarding the long-term inclusion level of mealworm feed in the practical diet formulation of the common carp.

In experiment V, the result of the study demonstrated that the partial replacement of fishmeal with BSF fly and MW and 1:1 combination meal up to 100gkg<sup>-1</sup> in the African catfish diets did not affect the fillet yield and fillet physical and chemical properties in a 25-weeks experimental setting. BSF50 group significantly improved the post-mortem 24 h pH content in the African catfish fillet. In conclusion, partial inclusion of black soldier fly, mealworm and 1:1 combination meal seems to be suitable for African catfish without compromising flesh quality and quantity. The PUFA profile was decreasing when FM was replaced with the above-mentioned insect meals at a partial level without exhibiting significant alteration. However, we highly recommend further investigation into the economic feasibility of long-term experiments and enrichment of the diet with PUFA source when needed to exceed the replacement level beyond the current portion.

As a general, we would like to draw a conclusion on African catfish that as the digestibility, growth, biometric index, fillet yield, and quality was not affected, it would be soundful to replace 200 gkg<sup>-1</sup> FM after enrichment of the diet with EFA particularly, the marine source.

## 7. NEW SCIENTIFIC RESULTS

1. Total (120 gkg<sup>-1</sup>) replacement of fishmeal with black soldier fly meal can improve the growth performance and feed utilization of juvenile common carp.
2. Total (100 gkg<sup>-1</sup>) or partial (50 gkg<sup>-1</sup>) replacement of fishmeal with mealworm meal did not significantly affect growth performance, survival, and proximate quality of juvenile common carp.
3. Total (200 gkg<sup>-1</sup>) replacement of fishmeal either with mealworm or black soldier fly meal is suitable for growth performance, feed utilization, apparent nutrient digestibility, and blood biochemistry concentration of African catfish.
4. Partial (50%) replacement of dietary fishmeal with black soldier fly meal for juvenile African catfish was found to be an excellent option.
5. Partial (50%) replacement of dietary fishmeal with black soldier fly meal exhibited significantly highest post-mortem pH compared to partial (50%) replacement of dietary fishmeal with mealworm meal, and 1:1 combination in African catfish fillet.
6. Partial (100 gkg<sup>-1</sup>) replacement of fishmeal with black soldier fly and 1:1 combination of black soldier fly and mealworm meals can be used in the practical diet of common carp without negative changes in fillet yield, physical, and proximate quality of flesh.
7. Black soldier fly, mealworm, and 1:1 combination of both can replace partial portion (100 gkg<sup>-1</sup>) of FM in practical diets of African catfish without negative changes in fillet yield, chemical and physical quality of flesh.

## **8. SUMMARY**

The number of undernourished people is increasing from time to time and grew by as many as 150 million from 2019 to 2022, due to a crisis driven largely by conflict, climate change, and the COVID-19 pandemic, however over 70% of the planet is covered with water and aquatic foods represent an essential component of the global quality food source to improve the nutrition, health, and well-being people in the globe. On the other hand, the global population is projected to increase to nine (9) billion by 2050, and the lifestyle will be very different, forcing an increased food/feed output from available agro-ecosystems putting more strain on the environment. Shortage of agricultural land, water, forest, fishery, and biodiversity resources as well as nutrients and non-renewable energy is expected and meeting meat demand from the conventional livestock sector will be unfortunate. It is estimated that about 50% more food will be needed to sustain the quality of human life. Increasing the consumption of meat from fish will be an excellent option to cope with the challenges. Increases in fish consumption have led to rapid increases in global fish demand. However, due to the natural resource maximum limits and climate change, capture fisheries will be not enough to meet the increasing demand. The expansion of aquaculture will fill this gap and relieve pressure on capture fisheries, which have been steadily declining. However, the feed particularly the protein source for aquaculture is another challenging aspect from a sustainability and high-cost point of view. Fishmeal has been used as the main protein source for its excellent nutritional value, high digestibility, absence of antinutritional factors, and acceptance by all fish species. Unfortunately, overfishing pressure, direct use of small fish for human consumption, climate change resulting in raw material supply shortage to fishmeal processing industries, and increasing demand for fishmeal resulted in supply shortage and elevated its price to unaffordable level as well as the sustainability of aquaculture is becoming under question. For aquaculture to remain sustainable, the feed sector needs to find sustainable alternative protein sources to substitute costly fishmeal. In the last decades, several alternative ingredients have been tested. For instance, protein sources of animal, plant, and single-cell origins, with varying success. Plant proteins still present disadvantages such as anti-nutritional factors, high fiber non-starch polysaccharides levels, inadequate fatty acid (FA) and amino acid profile, and low palatability. Moreover, some plant proteins compromise fish intestinal enterocyte integrity likewise, animal origin protein is inconsistent in nutrient contents and may have zoonotic disease problems. Besides, plant and animal-origin protein need large land, food, and water which can aggravate

climate change and increase environmental stress. Insects are arbitrated ecofriendly (leaves a small ecological footprint), renewable, and sustainable alternate protein source with an appealing quantity (high scalability future), quality, and acceptable nutritional composition for the production of aquafeeds. Yellow mealworm and black soldier fly seem to be very promising, widely used, and approved by the European Union to be used as food and animal feed. In the present study, the BSF and MW meals as a fishmeal replacement, have been investigated to evaluate total and partial replacement effects in the practical diets of two fish species (African catfish and common carp). A total of five experiments were set at Hungarian University of Agriculture and Life Science at the Department of Aquaculture by using recirculating aquaculture system (RAS). The tank used to stock the fish was 250L volume capacity connected to a recirculating system containing a drum filter (Trome Belgium) moving bed bio filter and sump. All tanks were aerated with a radial blower and air stone ( $3\pm 0.5$  L/min./tank).

In the first experiment, eight (8) weeks feeding experiment was carried out to evaluate the effects of fishmeal (FM) replacement with black soldier fly (BSF) meal on growth performance, and the biometric index of the common carp (*C. carpio*). Three experimental diets were made replacing BSF0 (control group), BSF50, and BSF100. Each diet was randomly assigned to a triplicate group a total of 180 fish (20 fish per tank) with an initial weight of  $35.2\pm 6.01$  g. Fish were fed three times daily to 2.5% of fish biomass. At the end of the experiment, the survival rate of the fish was 100%. Among calculated parameters, final body weight (FBW), specific growth rate (SGR), feed conversion ratio (FCR), and protein efficiency ratio (PER) were significantly improved ( $p < 0.05$  ANOVA) at BSF100 (total) replacement level. The biometric indices like, condition factor (k), and hepatosomatic index (HSI) were not affected by dietary replacement of FM with BSF inclusion level. To generalize the replacement level significantly ( $p < 0.05$ ) improved SGR, FCR, and PER of common carp.

In the second experiment, a six weeks experiment was set to evaluate the effects of total or partial replacement of fishmeal with defatted yellow mealworm (*Tenebrio Molitor*) meal on one-summer old common carp with the initial body weight of  $97.54 \pm 51$ g. Three experimental diets were prepared (control, MW50 and MW100). Fish were fed three times per day with 3.0 % of fish biomass. Neither morbidity nor mortality occurred during the experimental period. The growth performance of common carp was not significantly affected by the partial or total replacement levels of mealworm meal. However, the highest weight gain was observed at the inclusion level

of 50% mealworm meal (specific growth rate;  $0.76 \pm 0.10$ ). The fish body's crude fat content differed significantly between the control and experimental groups. To summarize, mealworm meal can be used as a potential alternative dietary protein source to replace fishmeal without adverse effects on the growth performance of one-summer-old common carp.

In experiment III, A six-week experiment was carried out to test the effects of total (100%) and partial (50%) replacement of fishmeal (FM) by black soldier fly meal (BSF), yellow mealworm meal (MW) and 1:1 combination of both black soldier fly and mealworm meal (BSFMW) in the diet of African catfish juveniles. A total of 420 fish with the initial body weight of  $200 \pm 0.51$ g were randomly distributed to seven diet groups (C, BSF50, BSF100, MW50, MW100, BSFMW50 and BSFMW100 respectively) in triplicates. At the end of the experiment, there were no significant differences ( $p > 0.05$ ) between groups in terms of final body weight (FBW), specific growth rate (SGR), feed conversion rate (FCR), protein efficiency ratio (PER), protein productive value (PPV) and total length (TL). The partial replacement (MW50, BSF50 and BSFMW50) was approximately similar to the control diet, while the highest growth and feed utilization were observed in the control group. The results revealed higher apparent digestibility coefficients ( $> 91\%$ ) compared to the control diet in diets containing different levels of BSF and MW meals. Among blood plasma biochemistry parameters, total cholesterol and phospholipid exhibited significant differences ( $p < 0.05$ ) between treatments and the control diet. To summarize, BSF and MW meal can substitute a partial and total portion of fishmeal in the practical diet of African catfish without negative impact on growth, feed utilization and digestibility, and blood biochemistry.

In experiment IV, a 13 weeks experiment was carried out to study the effect of the partial replacement of fishmeal (FM) with black soldier fly, mealworm and the combination of both on common carp flesh quality, a total of 36 fish with an initial weight of  $209.33 \pm 0.07$  g were randomly assigned to four groups of diet. The control diet contained  $200 \text{ gkg}^{-1}$  fishmeal while the experimental diets contained black soldier fly meal  $100 \text{ gkg}^{-1}$ , mealworm  $100 \text{ gkg}^{-1}$  and black soldier fly and mealworm 1:1 combination in  $100 \text{ gkg}^{-1}$ . The fish were fed at 1.75% of their biomass twice per day. After 9 weeks of the experimental period, the treatment with mealworm ( $100 \text{ g kg}^{-1}$ ) was excluded from the experiment due to the high fish mortality. At the end of the experiment (13th week) five fish per group were sacrificed by manual stunning method and measured for slaughter traits and flesh quality. The result revealed that there was no significant



difference ( $p > 0.05$ ) between various diet groups in terms of profile index, fillet yield, cooking loss, dripping loss, and acidification (pH) at 45 minutes and 24 hrs. Except for the ash, all proximate qualities of the fillet were not significantly different among different diet groups, While the ash content was significantly ( $p < 0.05$ ) higher in the black soldier fly meal 100 gkg<sup>-1</sup> replacement. This study proves that black soldier fly and a mixture of mealworm meal can replace 100 gkg<sup>-1</sup> of FM without any negative effect on flesh yield and quality parameters. Further investigation should be undertaken on the optimum inclusion level and length of experimental duration of mealworm on common carp.

In experiment V, A 25-week experiment was undertaken to explore the effect of partial replacement of dietary fishmeal (FM) with black soldier fly, mealworm meal, and a 1:1 mixture of both insect meals on fillet yield and quality in African catfish (*C. gariepinus*). A total of 92 fish with an average initial body weight of 248.66±0.08g were stocked into a recirculating aquaculture system (RAS). During the experimental period, there was no morbidity or mortality in any of the dietary groups. The result revealed that there was no alteration of fillet yield in different experimental groups. Among quality attributes, pH 24 h post-mortem exhibited a significant difference ( $p < 0.05$ ). The fatty acid profile was not negatively affected. The omega-6 to omega-3 fatty acid ratio ranged between 1.17 to 1.40 and was not significantly modified by partial replacement. The ratio of PUFA to SFA ranged between 0.66 to 0.79 and was not significantly different between diet groups. Similarly, the proximate composition of the fillet was not affected by the partial replacement. Furthermore, atherogenic and thrombogenicity indices were not negatively affected by FM replacement. This study demonstrates that black soldier fly and mealworm meals can partially replace fishmeal in the practical feeding of African catfish without any adverse effect on fillet quantity and quality.

## 9. ÖSSZEFOGLALÁS

Az éhező és alultáplált emberek száma folyamatosan növekszik világszerte. Számuk 2019 és 2022 között 150 millióval nőtt, melyhez hozzájárulnak a különböző válságok, háborús konfliktusok, az éghajlatváltozás és a COVID-19 világjárvány.

A vízi élőlények a világon számos helyen hozzájárulnak az emberek egészséges táplálkozásához és jólétük fenntartásához. Az előrejelzések szerint a világ népessége 2050-re kilenc milliárdra fog növekedni. A megnövekedett népesség ellátása az élelmiszer- és takarmánytermelés növekedését fogja eredményezni, ami fokozódó környezeti terhelést is jelent. Ez kihívást jelent a mezőgazdasági földhasználat, a biodiverzitás megőrzése, valamint tápanyagok és nem megújuló energia felhasználása szempontjából is. A hagyományos állattenyésztési ágazat hosszú távon nem lesz képes kielégíteni az állati eredetű élelmiszerek iránti igényeket. A becslések szerint körülbelül 50%-al több élelmiszerre lesz szükség a jelenlegi életminőség fenntartásához. A halhús fogyasztásának növelése kiváló lehetőséget nyújthat erre a problémára. A halfogyasztás növekedése a hal iránti globális igények növekedéséhez vezethet. A természeti erőforrások korlátai és az éghajlatváltozás miatt azonban a halászat nem elegendő a növekvő kereslet kielégítésére. Az akvakultúra terjeszkedése kiöltheti a hiányt, és enyhíti a halászatra nehezedő nyomást, amely volumene folyamatosan csökken. Az akvakultúra takarmány, és különösen a fehérjeigénye azonban további kihívást jelent a fenntarthatóság és a magas költségek szempontjából is. Az akvakultúra ágazat korábban a hallisztet használt fő fehérjeforrásként, kiváló tápértéke és emészthetősége miatt. A hallisztet jellemzően minden halfaj elfogadja, a táplálékfelvételt befolyásoló negatív hatásokkal nem rendelkezik. Sajnos a túlhalászás, az egyre kisebb testméretű halak élelmiszerként történő felhasználása, a halliszt-feldolgozóknak nyersanyagellátási hiányt okoz. Ezzel párhuzamosan a kereslet növekedése hiányt eredményez, és magas szintre emelte a halliszt árát, valamint negatívan befolyásolta a fenntarthatóságot. Az akvakultúra számára fontos a fenntartható alternatív fehérjeforrások használata a halliszt felhasználás csökkentése érdekében. Az elmúlt évtizedekben számos alternatív takarmány alapanyagot teszteltek, így például állati, növényi és egysejtű fehérjeforrásokat, változó sikerrel. A növényi fehérjéknek még mindig vannak hátrányai, például az emésztést gátló tényezők, valamint a magas rost és keményítő tartalom, a nem megfelelő zsírsav- (FA) és aminosavprofil, valamint nem megfelelő íz. Ezenkívül egyes növényi fehérjék szintén veszélyeztetik a halak bélhámsejtjeinek épségét. Az állati eredetű fehérjék tápanyagtartalma inkonzisztens, illetve fogyasztásuk zoonózisos megbetegedésekkel

járhat. Emellett a növényi és állati eredetű fehérjék megtermelése nagy földterületet, sok tápanyagot és vizet igényel, ami súlyosbíthatja a klímaváltozást.

A rovarok környezetbarát módon előállíthatók (kis ökológiai lábnyommal rendelkeznek), megújuló és fenntartható alternatív fehérjeforrásnak tekinthetők, illetve tömegtermelésük is megvalósítható. Mindemellett megfelelő minőséggel és elfogadható tápanyag-összetétellel rendelkeznek a haltápok előállításához. A lisztbogár (*Tenebrio molitor*) és a fekete katonalégy (*Hermetia illucens*) használata nagyon ígéretesnek tűnik a haltakarmányozásban, az Európai Unió is jóváhagyta élelmiszerként és takarmányként való felhasználásukat.

A doktori disszertációmban a haltakarmány halliszt tartalmának fekete katonalégy (BSF) és a lisztbogár (MW) liszttel történő teljes és részleges helyettesítésének hatását vizsgálok két halfaj (afrikai harcsa és ponty) esetén. A dolgozatban öt kísérlet kerül bemutatásra, melyek a MATE Akvakultúra és Környezetbiztonsági Intézet, Kaposvári Campus recirkulációs rendszerben (RAS) lettek végrehajtva.

Az első kísérletben a halliszt (FM) fekete katonalégy (BSF) liszttel történő helyettesítésének hatását a ponty (*Cyprinus carpio*) növekedésére takarmány hasznosítására és testindex paramétereire. Három kísérleti takarmányt készítettem: a BSF0 (kontroll) csak hallisztet tartalmazott, a BSF50 részlegesen (50%-ban) volt helyettesítve katonalégy liszttel a BSF100 takarmány pedig teljesen (100%-ban). Kísérleti takarmányonként három csoportot alakítottam ki háromszoros ismétlésben. Összesen 180 hal lett a kísérletekhez felhasználva (20 hal/kád;  $W_0$  35,2±6,01g). A halakat naponta háromszor ettettem, a napi takarmány adag a hal biomassa 2,5%-a volt. A kísérlet végén kiszámítottam a súlygyarapodást (WG), a specifikus növekedési rátát (SGR), a túlélési arányt, a takarmányértékeitést (FCR), ami esetén szignifikánsan ( $p < 0,05$  ANOVA) jobb értékeket kaptam a hallisztet teljesen fekete katonaléggel helyettesítő csoportnál. Míg a kondíciós faktort (CF) és a hepatoszomatikus indexet (HSI) értékeit a különböző bevonási szintek szignifikánsan nem befolyásolták.

A második, hat hetes kísérletben megvizsgáltam a halliszt zsírtalanított lisztbogár liszttel történő teljes vagy részleges helyettesítésének hatását egynyaras ponty (n:135;  $w_0$  97,54 ± 51g) takarmányában. Három kísérleti takarmány készült. A kontroll, 100gkg<sup>-1</sup> hallisztet tartalmazott, az MW50 a hallisztet részlegesen (50%-ban) lisztbogár liszttel helyettesített és MW100 a hallisztet teljesen (100%-ban) lisztbogár liszttel helyettesített tápok voltak. A halakat naponta háromszor ettettem a halbiomassa 3,0%-ával. Elhullás nem fordult elő a kísérleti időszakban. A ponty

növekedési teljesítményét nem befolyásolta szignifikánsan a lisztbogár liszt, sem részleges, sem teljes helyettesítési szintje. A legnagyobb súlygyarapodást azonban az 50%-os liszt kukac liszt esetében figyeltem meg (fajlagos növekedés (SGR);  $0,76 \pm 0,10$ ). A haltest nyerszsír tartalma szignifikánsan különbözött a kontroll és a kísérleti csoportok között. Összefoglalva, a liszt kukac liszt potenciálisan felhasználható a halliszt helyettesítésére anélkül, hogy káros hatással lenne az egynyaras ponty növekedési teljesítményére.

A harmadik, hathetes kísérletben a halliszt (FM) fekete katonalégyliszt (BSF) lisztbogár liszttel (MW) valamint a két alapanyag 1:1 arányú kombinációjával (BSFMW) történő teljes (100%) illetve részleges (50%) helyettesítésének hatásait vizsgáltam afrikai harcsa takarmányában. Összesen 420 ( $w_0: 200 \pm 0,51$ g) hal felhasználásával 7 csoportot alakítottam ki (C, BSF50, BSF100, MW50, MW100, BSFMW50 és BSFMW100), háromszoros ismétlésben. A kísérlet végén nem volt szignifikáns különbség ( $p > 0,05$ ) a csoportok között a végső testtömeg (FBW), a fajlagos növekedési ráta (SGR), a takarmányértékesítési ráta (FCR), a fehérje hasznosulási arány (PER) a fehérje produktivitási érték (PPV) és teljes hossz (TL) tekintetében. A halliszt részleges helyettesítése (MW50, BSF50 és BSFMW50) megközelítőleg hasonló volt a kontroll csoporthoz viszonyítva, míg a legnagyobb növekedést és legjobb takarmányértékesítést a kontroll csoportban tapasztaltam, bár az eredmények nem bizonyultak szignifikánsnak ( $p > 0,05$  ANOVA). Magasabb látszólagos emészthetőségi együttható ( $> 91\%$ ) volt kimutatható a kontroll takarmányhoz képest a különböző szintű BSF és MW takarmányokhoz tartozó kezelésekben, míg ez a tendencia nem figyelhető meg, a rovarlisztek 1:1 arányú keverékének alkalmazása esetén. A vérplazma biokémiai paraméterei közül az összkoleszterin és a foszfolipid szignifikáns különbséget mutatott ( $p < 0,05$  ANOVA) a kezelések és a kontroll csoport között.

A negyedik kísérletben 13 hetes időszakban vizsgáltam a halliszt részleges helyettesítését fekete katonalégyliszt, lisztbogár-liszttel és a kettő kombinációjával. A kísérletben elsődleges célja a húsminőségére gyakorolt hatás vizsgálata volt. Összesen 36 hallal végezett a kísérletet ( $w_0 = 209,33 \pm 0,07$ g). A kontroll takarmány  $200 \text{ g kg}^{-1}$  hallisztet, míg a kísérleti táp 50%-ban fekete katonalégylisztet, 50%-ban lisztbogár lisztet és 50%-ban fekete katonalégyliszt és liszt kukac 1:1 kombinációt tartalmazott. A halakat biomasszájuk 1,75%-ával etettem naponta kétszer. A 9 hetes kísérleti időszak után a liszt kukac ( $100 \text{ g kg}^{-1}$ ) kezelést a magas halpusztulás miatt kizártuk a kísérletből. A kísérlet végén (13. hét) csoportonként öt halat leöltünk és megmértük a vágási tulajdonságokat, valamint a húsminőségi paramétereket. Az eredmény azt mutatta, hogy nem volt

szignifikáns különbség ( $p > 0,05$  ANOVA) a különböző takarmányozási csoportok profilindexe, a filéhozama, a főzési veszteség, a csepegési veszteség és a savasodás (pH) között (45 perc és 24 óra elteltével). A hamu kivételével a filé összetétele nem különbözött szignifikánsan a különböző kísérleti csoportok között, míg a hamutartalom szignifikánsan ( $p < 0,05$ ) magasabb volt a  $100 \text{ g kg}^{-1}$  hallisztet helyettesítő csoportok esetén. Az eredményim alapján a fekete katonalégy és a fekete katonalégy liszt 1:1 keveréke képes  $100 \text{ g kg}^{-1}$  arányban bekeverve a hallisztet helyettesíteni a húshozamra és a minőségi paraméterekre gyakorolt negatív hatás nélkül. További vizsgálatokat kell végezni a liszt kukac-liszt optimális arányával kapcsolatban.

Az ötödik kísérletben egy 25 hetes vizsgálatot végeztem annak felderítésére, hogy a hallisztet (FM) részlegesen (50%-ban) helyettesítve fekete katonalégy- liszt kukac liszttel, továbbá a két rovarliszt 1:1 arányú keverékével milyen hatást gyakorol a filé hozamára és minőségére Afrikai harcsa esetén. Összesen 92, ( $w_0 = 248,66 \pm 0,08 \text{ g}$ ) halat telepítettek egy recirkulációs rendszerbe. A kísérleti időszak alatt egyik csoportban sem volt elhullás. A file kihozatalban nem találtam szignifikáns különbségeket a kezelések között. A minőségi jellemzők közül a pH (24 óra post-mortem) mutatott szignifikáns különbséget ( $p < 0,05$  ANOVA). Az omega-6 és omega-3 zsírsavak aránya 1,17 és 1,40 között mozgott, és a részleges pótlás nem változtatta meg jelentősen. A PUFA és az SFA aránya között mozgott, és nem volt szignifikáns különbség a csoportok között. Hasonlóképpen, a filé kémiai összetételét nem befolyásolta a részleges helyettesítés. Ezenkívül a halliszt pótlása nem befolyásolta negatívan az aterogén és trombogénitási indexeket. Ez a tanulmány azt bizonyítja, hogy a fekete katonalégy- és liszt kukac liszt részlegesen helyettesítheti a hallisztet az afrikai harcsa takarmányozásában, anélkül, hogy káros hatással lenne a filé mennyiségére és minőségére.

## 10. PRACTICAL APPLICATION OF THIS WORK

This thesis work can be practically applicable either in my home country (Ethiopia) or any other country which has access to the insect meal. Based on the result obtained from all five experiments, neither partial nor total replacement of fishmeal with mealworm or black soldier fly meals did not negatively influence the growth performance, physiology, nutrient utilization, and fillet yield as well as flesh quality of both African catfish and common carp. Besides, the production of insects is eco-friendly, sustainable, less land and water demanding sector, less labor and initial capital requiring, and nutritionally sound extra. Interestingly, African catfish is the second most exploitable fish species in Ethiopia next to Nile tilapia. The Agroecological condition is very favorable for the production of insects (mealworm and black soldier fly). Currently, Ethiopia is producing silkworms primarily for silk production. However, the utilization of insect meal in the fish diet is not common in my country (Ethiopia), I am a responsible person who had qualified and acquainted with present advancements in knowledge to contribute more in the area of fish nutrition. And I am certain that the knowledge I have gained from this study will help me to contribute to the ongoing research work and scientific approaches in fish nutrition and the research output I obtained from this thesis work as well as my career objective goes in line with the prime objectives of my institution and my country, which is conducting research that can alleviate poverty and secure food self-sufficiency and produce qualified graduate. Beyond this, this thesis work enabled me to see and admit the weakness and mistakes that I should take action on in the future before setting an experiment. The experiences and excellent skills I gained will enhance my professional competency, serve as a bridge to the future work of my organization and realize my dream of being a prominent researcher that enhances my capacity to contribute to the development Endeavors of my country.

## 11. ACKNOWLEDGEMENTS

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## 13. LIST OF PUBLICATION

### 13.1 Peer-reviewed papers published in foreign scientific journal related to the subject of dissertation

1. **Gebremichael A.**, Hancz C., Kucska B., 2021 Effect of total or partial replacing of fishmeal with black soldier fly (*Hermetia illucens*) meal on growth performance and body condition indices of common carp (*Cyprinus carpio*). *AACL Bioflux* 14(4):2280-2286. (Q3, IF=0.836)
2. **Gebremichael A.**, Varga D., Kiszlinger H., Kucska B., 2022 Fillet yield and flesh quality of common carp (*Cyprinus carpio*) fed with extruded feed containing black soldier fly (*Hermetia illucens*) and mealworm (*Tenebrio Molitor*). *AACL Bioflux* 15(5):2273-2281(Q3, IF=0.836)
3. **Gebremichael, A.**, Sándor Z.J, Kucska B. (2022). Does dietary inclusion of defatted yellow mealworm (*Tenebrio molitor*) affect growth and body composition of juvenile common carp (*Cyprinus carpio*)? *South African Journal of Animal Science* 52 (4),444-451 (Q3, IF=1.16)
4. **Gebremichael, A.;** Kucska, B.; Ardó, L.; Biró, J.; Berki, M.; Lengyel-Kónya, É.; Tömösközi-Farkas, R.; Egessa, R.; Müller, T.; Gyalog, G.; Sándor, Z.J. Physiological Response of Grower African Catfish to Dietary Black Soldier Fly and Mealworm Meal. *Animals* **2023**, 13, 968. <https://doi.org/10.3390/ani13060968> (Q1, IF=3.231)

### 13.2 International conference abstracts

1. Askale Gebremichael\*, Balázs Kucska<sup>1</sup> (2021): Effect of total or partial replacing of fishmeal with black soldier fly (*Hermetia illucens*) and mealworm (*Tenebrio molitor*) meal on growth performance and body condition indices of common carp (*Cyprinus carpio*) international symposium animal science day 29<sup>th</sup> international conference Gödöllő, Hungary September 13-17, 2021.
2. Askale Gebremichael\*, Zsuzsanna JakabneSandor and Balázs Kucska (2022): Effect of mealworm (*Tenebrio molitor*) and black soldier fly larvae (*Hermetia illucens*) meal on growth performance, survival and body ind of African catfish (*Clarias gariepinus*) 13<sup>th</sup>

annual conference of the Ethiopian Fisheries and Aquatic Sciences Association (EFASA)  
Addis Ababa Ethiopia May 13-14, 2022.

### 13.3 Conference abstracts published in Hungarian

1. Gebremichael Askale; Stettner, Gabriella; Varga, Dániel Kucska, Balázs Alternative feherjeforrás -liszt kukac (*Teneberio molitor*) alkalmazása harcsa (*Silurus glanis*) takarmányokban ISBN: 9789632699615 Journal Article/Conference paper in journal (Journal Article)/Scientific [32224291] [Approved]
2. Askale, Gerbreemichael; Yarsmin, Yunuis Zeebone; Omeralfaroug, Ali; Müller, Tamás; Kucska, Balázs A rovarliszt takarmányba történő részleges bevonásának hatása az Afrikai harcsa (*Clarias gariepinus*) ikrások reprodukciós paramétereire HALÁSZATFEJLESZTÉS 39 pp. 115-117. 3 p. (2022)
3. Müller, Tamás; Nguyen, Quyen; Getachew, Worku Alebachew; Bógó, Bence; Horváth, László; Csorbai, Balázs; Szabó, Tamás; Gebretsadik, Askale Gebremichael; Urbányi, Béla; Kucska, Balázs Különböző hormonbejuttatási módszerek hatása afrikai harcsa indukált szaporítása során In: Biró, Janka (eds.) Halászatfejlesztés 37: A XLIV. Halászati Tudományos Tanácskozás kiadványa Szarvas, Hungary: Nemzeti Agrárkutatási és Innovációs Központ Halászati Kutatóintézet (2020) pp. 51-52, 2 p. Chapter in Book/Abstract (Chapter in Book)/Scientific [31614161] [Validated]
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### 13.4 Other publications not related to the subject of dissertation

1. Kucska, B., Quyen, N. N., Szabó, T., Gebremichael, A., Alebachew, G. W., Bógó, B., ... and Müller, T. (2022). The effects of different hormone administration methods on propagation successes in African catfish (*Clarias gariepinus*). Aquaculture Reports, 26, 101311(Q1, IF=3.385)

2. Abiyu Tadele, Askale Gebremichael and Teshome Gemechu, 2020. Impact of hybrid/exotic chicken breed distribution on performances of indigenous chicken in South Western Ethiopia. *Asian J. Poult. Sci.*, 14: 6-16. (Q4)
3. Gebremichael A and Fantahun T (2019): Assessments of the fishery, challenges and opportunities of Denbi reservoir in Bench maji Zone, Southwester part of Ethiopia. *International Journal of fisheries and aquaculture* 11(1),7-12
4. Gebremichael A and Fantahun T (2019): Physico-chemical Properties of Denbi Reservoir for FishProduction in Bench Maji Zone, Ethiopia *chemistry and materials research* 11(5)
5. Askale G and Mekonnen H (2018): dairy cattle husbandry practices and major constraints in Tello district, Ethiopia. *International journal of sustainable development research* 4(4),47-54
6. Gebremichael A (2019): Cattle milk production, processing and marketing situations of smallholder farmers in Tello district Kaffa ethiopa *African journal of agricultural research* 14(18), 806-812
7. Tegegn Fantahun\* and Askale G/Michael (2017): Goat production system and breeding practices in pastoral and mixed crop livestock production system in south western part of Ethiopia. *Agric. Biol. J. N. Am.*, 2017, 8(3): 67-71

#### **14. CURRICULUM VITEA (CV)**

Askale Gebremichael was born in Kaffa on 9th of January 1985 in Ethiopia.

She graduated BSC in Animal and range science from Hawassa university since 2008

She started working as assistance graduate in Samara and Mitan-Tepi university in 2008

She graduated in MSC in tropical animal production and health (TAPH) (Dairy cattle stream) from Addis Ababa university

She worked as lecturer and vice dean of college of Agriculture and Natural Resources

She served as board member of four organizations (Bonga University, kaffa development association, Gogiba omo-micro finance, Kaffecho intellectuals association) till she departs to Hungary.

She promoted to assistance professorship from June 2018.

She joined PhD study program in the department of applied fish biology by the sponsor of Hungarian government (SH) in 2019/2020 academic year

She is married and mother of 3 delightful children (2 boys and 1 girl)