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PARTIAL AND TOTAL REPLACEMENT OF FISHMEAL WITH INSECT MEALS IN COMMON CARP AND AFRICAN CATFISH FEEDS

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1. BACKGROUND OF THE STUDY, OBJECTIVES

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 will fill this gap and relieve pressure on capture fisheries, which have been gradually declining. However, the feed, particularly its aquaculture protein source, is another challenging aspect. Fishmeal (FM) 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. The increased demand for FM, especially in the commercial fish diet industry, overfishing pressure, finite nature, and climate change has resulted in a supply shortage with affiliated price enforced researchers, farmers, and stakeholders to find dietary alternative protein sources. In aquaculture to remain sustainable, various efforts have been exerted to find alternative protein sources to replace FM in aqua feeds like plant origin (soybean meal, vegetable, and nut meals, vegetable meal concentrates), animal origin (trimmings, processed animal products and by-products), or single cell protein sources (algae, yeasts or bacteria). Plant proteins still present disadvantages such as anti-nutritional factors, high levels of fiber and non-starch polysaccharides, inadequate fatty acid (FA) and amino acid profile, and low palatability. likewise, animal products and by-products are not well balanced in nutritional compositions and those single-cell proteins are high in cellulose contents and difficult to harvest. Moreover, either plant or animal-origin proteins are associated with high land and water resource utilization as well as competition from food for humans and feed for animal sectors.

In the current study replacement of fishmeal with black soldier fly and mealworm meal was studied with the objectives of evaluating production parameters, apparent digestibility, fillet yield, and flesh quality of African catfish and production parameters, fillet yield, and flesh quality in common carp. The results revealed that either partial or total replacement of fishmeal with black soldier fly and mealworm meal did not adversely affect the production parameter, apparent digestibility, and fillet yield or flesh quality of African catfish and common carp respectively. But long-term inclusion of 100 kg^{-1} of MW might affect the survival rate of common carp.

2. MATERIALS AND METHODS

The experiment was conducted on two fish species (common carp and African catfish) using two insect species meals (mealworm and black soldier fly) as fishmeal replacements. All experiments were carried out at the Hungarian University of Agriculture and Life Science Kaposvár Campus, in the Department of Aquaculture using recirculating Aquaculture system (RAS) in 250 L volume capacity tanks. A total of five experiments were carried out. In the first experiment, the different inclusion levels of black soldier fly meal (BSF) in the common carp diet were studied at the substitution level of fishmeal (FM) (120gkg-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 with the substitution level of FM (100 gkg-1) in the diet 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, and apparent digestibility coefficient. Lastly, in the fourth and fifth experiments BSF, MW, and 1:1 combination of BSF and MW were studied on African catfish and common carp for fillet yield and flesh quality.

Common procedures for all experiments

All fish used for overall experiments were reared in the Hungarian University of Agriculture and life science recirculating aquaculture system (RAS, Kaposvár). Also, all experiments were carried out at the Hungarian University of Agriculture and life science using recirculating aquaculture system (RAS). The study design applied was a complete randomized design (CRD). The experimental diets were set as iso-nitrogenous and iso-energetic. For experiments I and II the feed was pelleted by hand, while for experiments III, IV, and V it was extruded. Water parameters were checked regularly. Dissolved oxygen and temperature were measured daily by portable dissolved oxygen meter HI9147, HANNA instrument Woonsocket R1 USA, made in Europe (Romania). Nitrogen forms: NO_3^-, NO_2^-, NH_4^+, and pH are measured by (PF-12 plus photometer, MACHEREY NAGEL MN) every week. At the beginning and the end of the experiment, the individual weight and length of the fish were measured by Shimadzu scales, Japan accuracy ±1g) and length (±0.5cm) respectively. In all experiments, fish were kept in 250L volume capacity tanks connected to recirculating system containing a drum filter (Trome Belgium) moving bed biofilter,

and sump. All tanks were aerated with a radial blower and air stone (3±0.5 L/min./tank). The total biomass was measured to adjust the daily feed portions every week in Experiments 1 and 2, every two weeks in experiment 3, and every three weeks in experiment 4 and 5 by a portable measuring scale (MMX, China- accuracy ±5g). 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.

2.1 Experimental Procedures and tank management (Experiment I)

2.1.1 Diet formulation

The experiment was designed to investigate the effect of total and partial replacement of FM with BSF in common carp feed. Therefore, 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^{-1} FM 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.

2.1.2 Fish Stocking, feeding, and tank management

Experimental fish (n=180 w_0=35.2±6.01g) were randomly distributed to three groups (control, BSF50, and BSF100) in triplication (20 fish per tank). The fish were 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, 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: 4.9±0.5 mg L^{-1} measured daily, NH_4^+: 0.50±0.02 mg L^{-1}, NO_3^-: 20.5±0.19 mg L^{-1}, NO_2^-: 0.14±0.02 mg L_{-1}, and pH: 7.1±0.2. The length of the experiment was 8 weeks.

2.1.3 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 -20ºC

2.2 Experimental procedures and tank management (Experiment II)

2.2.1 Diet formulation

The experiment was designed to investigate the effect of total and partial replacement of FM with MW in carp feed. Therefore, the following diets were formulated: yellow mealworm meal originated from Berg and Schmidt Pte. Ltd Singapure; in dried and processed form and imported by Hecron-Agro Kft. Hungary. The basal diet was set to 35.2% crude protein and 6.7% crude fat and contained 10% fishmeal. 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. The experimental diets were prepared manually by mixing dried ingredients with oil, and slightly warm water, and carboxymethyl cellulose (CMC) was used as the binder. The homogenized and moisturized ingredients were then pelleted using a minced grinder and dried with cold ventilation for 48 hrs.

2.2.2 Fish Stocking, feeding, and tank management

Experimental fish (n:135 w_0= 97.54g ± 15.0) were randomly distributed to three groups (control, MW50 and MW100) in triplication (15 fish per tank). The fish were 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, NH4+, 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^{-1} measured daily, NH_4^+: 0.50±0.02 mg L^{-1}, NO_3^-: 19.0±0.3 mg L^{-1}, NO_2^-: 0.14±0.02 mg L^{-1}, and pH: 7.2±0.2. The duration of the experiment was 6 weeks.

2.2.3 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 ºC 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.

2.3 Experimental Procedures and tank management (Experiment III)

2.3.1 Diet formulation

The experiment was designed to evaluate the effect of total and partial replacement of FM with BSF, MW, BSF MW 1:1 combination. Accordingly, seven experimental diets including the control diet were prepared. The basal diet was set to 44.97% ±0.45crude protein and 8.62±0.16% crude fat, it contained 200 gkg^{-1} fishmeal 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 by 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).

2.3.2 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, NH4+, NO3-, NO2-, 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-1 measured daily, NH4+: 0.50±0.02 mg L-1, NO3-: 28.5±0.19 mg L-1, NO2-: 0.16±0.02 mg L-l, and pH: 7.1±0.2 measured on a two-weekly basis.

2.3.3 Sample collection

2.3.3.1 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.

2.3.3.2 Fecal samples collection

After six weeks of the feeding experiment, the fish was sacrificed to collect feces from the intestine (Cho et al., 1982) 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 feces were collected from the posterior part of the intestine according to (Cho et al., 1982). Fecal samples from the same experimental group tanks were pooled on a Petri dish, frozen and lyophilized.

2.3.3.3 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. 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.

2.3.4 Blood Serum Biochemistry Analysis

2.3.4.1 Total protein assay

The total protein (TP) concentration of the serum was determined by a colorimetric assay based on the biuret reaction, using a protein diagnostical reagent kit (Fluitest TP, Analyticon Biotechnologies AG, Germany), according to the manufacturer's instructions.

2.3.4.2 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.

2.3.4.5 Measuring other biochemical parameters

Serum samples were stored at -20 °C defrost at room temperature and homogenize by a vortex mixer at a speed of 1800 rounds per minute, then 70 µL samples 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).

2.4 Experimental procedures and tank management (Experiments IV and V)

All procedures and methods are similar for both experiments (IV and V) except for sample size (n=92) fish for African catfish and n=36 common carp), the experimental period was 25 weeks in the case of African catfish and 13 weeks in the case of common carp. The fatty acid analysis was not done for common carp. Experiments IV and V were set after evaluation of the 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±40.27) African catfish were randomly distributed to four tanks (23 fish/tank) fed 1.75% biomass weight. To avoid feed wastage and minimize handling stress, the withdrawal of feeding one day before and two days after measurement was applied, and the fish was kept for about 25 experimental periods. 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±40.21) common carp (9 fish /tank) were also randomly distributed to four diet groups and fed 1.75% biomass. One day before and after measurement was feed withdrawal 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), NO_3^- (min-max: 20-44.8 mg/l), 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).

2.4.1 Diet formulation (Experiments IV and V)

The control diet containing 20% dietary fishmeal protein was replaced with 50% (10%) dietary protein to evaluate the long-term effect on fillet quality and the yield on African catfish and common carp. The feed was formulated in four isonitrogenous and iso-lipid varieties (control, BS50, MW50, and BSFMW50)

2.4.2 Sample collection

At the end of the experiment, six fish from each group were sacrificed by manual stunning method following the administration of anesthesia (2-phenoxyethanol, Sigma Aldrich, Schnelldorf, Germany). After measuring body weights and standard lengths, fish were dissected, internal organs removed, the head removed, skin removed, and fillets removed from the bone by the sharp knife. 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 postmortem (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 thawing at room temperature after 2 days. To determine drip loss, fillet samples (50 g) was sealed in a bag and hung over firige at 4°Cover night and cautiously opened and wiped then measured.

2.5 Chemical analysis (experiments I, II, III, IV and V)

2.5.1 The chemical composition of test ingredients, feeds, and faces

The chemical composition of test ingredients, feeds, and feces was analyzed by standard methods of the AOAC (2000) Crude protein (CP) was determined by the 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_2SO_4 and 10 mL 30% H_2O_2, afterwards the generated ammonium sulphate was distilled off by using 2% H3BO3. The CP was calculated as N × 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_2SO_4 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 an ADF solution prepared from N-cetyl-trimethyl-ammonium bromide dissolved in 0.5M H_2SO_4 (100 g/5 L) and a few drops of the 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).

2.5.2 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 N HCl 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).

2.5.3. Titan, calcium, and phosphorus

Titan, calcium, and phosphorus content were 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 (Sorisole, Italy) microwave apparatus. The concentrations of elements 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, and added with 0.1% titan oxide was 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

2.5.4 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 NaOCH3 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. The calculation of the atherogenic index and thrombogenicity index was based on Ali (2021).

2.6 Calculations.

Growth parameters were compared by calculation of:

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

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

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

$SGR = 100 \times (\ln W_f - \ln W_i) / t$

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:

FCR = offered feed (g) / (final weight of fish (g) – the initial weight of fish (g)

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

PER = weight gain (g) / protein intake (g)

The survival of fish (S %) was calculated as S = 100 x (number of fish at the beginning of the experiment/number of fish at the end of the experiment) (%)

The body condition indices were calculated by the following formulas:

The hepatosomatic index (HSI%): HSI =LW/BW*100

LW=liver weight (g)

BW body= weight (g)

Viscero-somatic index (VSI%): VSI =VW/BW x 100

VW= visceral weight(g)

BW= body weight(g)

Condition factor (k) = BW / TL^3 x 100

BW = t body weight (g) and TL = total length (cm) respectively

Relative gut length (RGL%) RGL = GL/BL x 100

Apparent digestibility coefficient was calculated by the formula:

ADC % = 100 - (%TiO2 in feed * % nutrient in faeces / %TiO2 in Faeces %nutrient in feed) * 100

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

Fillet physical parameters are calculated as follows

DL (%) = [(raw fillet weight (g) − raw fillet weight after 24h (g)) / raw fillet weight (g)] × 100

CL (%) = [(raw fillet weight (g) − cooked fillet weight (g)) / raw fillet weight (g)] × 100

TL (%) = [(raw fillet weight (g) − thawed fillet weight (g)) / raw fillet weight (g)] × 100

DL=drip loss

CL= cooking loss

TL =thawing loss

Fillet yield (%) = fillet(g)/BW(g)*100

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

2.7 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). All efforts were made to minimize the fish's suffering.

2.8 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.

3. RESULTS

3.1 Experiment I. Effect of different replacement levels of FM with BSF in common carp diet.

3.1.1 Production parameters

Replacement of black soldier fly meal in the practical diets of common carp was found to be an excellent alternative protein source and significantly improved ($p < 0.05$ ANOVA) final body weight (FBW), specific growth rate (SGR), the protein efficiency ratio (PER) of Common carp. The highest value for FBW (64.58±15g) was recorded in total (120 gkg-1) replacement. The lowest FBW (59.8±14.02g) was observed in the control group 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. This implies that there is a chance to increase the replacement of fishmeal with black soldier fly to some extent.

3.1.2. Survival rate and biometric indices

Survival rate (SR), which also was not significantly affected by dietary FM replacement, and all fish survived in all diet groups. Among somatic indices of common carp fed different inclusion levels of black soldier fly meal, only viscero somatic index (VSI) exhibited significantly higher ($p < 0.05$ ANOVA) results in the control group. The hepatosomatic index HSI ranged between 2.17 to 2.72% which is slightly higher than the recommended ranges for fish (1 to 2%) and it can be associated with the gonad developmental stage since HSI increases at early ripening and decreases at late phase). In the present study, HIS was not significantly different ($p > 0.05$ ANOVA) between diet groups

3.2 **Experiment II.** Effect of mealworm meal on common carp

3.2.1 Production parameters

Replacement of fishmeal with mealworm did not significantly differ between diet groups in terms of the FBW, SGR, and FCR. However, the highest values for FBW (134.7 ± 30.42) were recorded at partial (MW50) FM replacement with MW compared to FBW at control (132.1 ± 34.39) and, at 100% replacement level (130.9 ± 26.62) respectively. The feed utilization parameters (FCR, PER) of common carp fed MW showed decreasing tendency at a total replacement rate.

3.2.2 Survival rate and biometric indices

Survival rate (SR), which also was not significantly affected by dietary FM replacement, and all fish survived in all diet groups. Somatic indices like, viscero somatic index (VSI), hepatosomatic index (HSI), and condition factor (CF) were not significantly affected by partial or total replacement of FM with MW.

3.2.3 Proximate composition of eviscerated body

The proximate composition of the eviscerated carp was not significantly affected by the replacement level of FM with MW except for crude fat content which was significantly higher ($p < 0.05$ ANOVA) at a replacement rate of 50%. The difference in terms of crude fat was not significantly different between MW50 and MW100%. While the significant difference was

observed between control (12.56 ± 1.09%) which was the lowest and M50% (15.20 ± 0.60%) (highest).

3.3 **Experiment III.** Effect of different replacement levels of FM with BSF and MW in African catfish diet

3.3.1 Production parameters

There were no significant differences ($p > 0.05$) in terms of FBW, SGR, FCR, 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. However, the decreasing tendency was observed at 100% ($200gkg^{-1}$) and the least result was found in mealworm groups.

3.3.2 Somatic indices

Condition factor, total length, and hepatosomatic indices were not significantly affected by the dietary replacement level of fishmeal by BSF and MW as well as the 1:1 combination of BSF and MW.

3.3.3 Apparent digestibility

Higher apparent digestibility coefficients (> 91%) compared to the control diet in diets containing different levels of BSF and MW meals, while lower values were found when the insects were used simultaneously in the diet.

3.3.4 **Blood plasma Biochemistry**

All the plasma biochemical indices analyzed were not significantly influenced by the diet, except for cholesterol and phosphate. The cholesterol level increased significantly ($p < 0.05$) in fish fed with different inclusion levels of MW. Phosphatase levels decreased significantly in the BSF50 and MW100 treatments compared to the control group.

3.3.5 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 differed in BSF100, MW50, MW100, BSFMW50, and BSFMW100 treatments.

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

3.4.1 Fillet yield, physical characteristics of the flesh

The highest final body weight at 100 gkg^{-1} replacement of fishmeal with black soldier fly meal. The $_{P}H$ value showed slightly lower records at 24hrs postmortem. fillet yield was not significantly affected by the partial replacement of fishmeal with BSF and BSFMW. The physical parameters of fillet (cooking loss, dripping loss, and thawing loss) were not affected by either diet nor sex differences.

3.4.2 Survival rate and biometric indices

From the present investigation, the highest mortality was observed at 100gkg-1 replacement rate of mealworm meal with fishmeal. However, the reason for mortality was not known, feed ingredients might be one reason as survival rate reduction with increasing levels of MW in different fish species was reported. For instance, Tubin et al. (2020) reported the survival rate of Nile Tilapia (*Oreochromis niloticus*) juveniles significantly ($p < 0.05$ ANOVA) 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 by

mealworm in the practical diet of European perch. The biometric parameters were not significantly different between different diet groups.

3.4.3 Proximate quality parameters

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 BSF.

3. 5 Experiment V. Effect of partial replacement of FM with BSF and MW in African catfish diet on fillet yield and flesh quality

3.5.1 Slaughter values, fillet physical properties

The slaughter values (eviscerated, trunk with skin, a trunk without skin, and skinned fillet) were not significantly ($p > 0.05$) different between treatments and control. the eviscerated weight ranged from 81.25% to 91.67% while, the range reported for African catfish was 90.4 to 90.9% (FAO, 2015). The difference might be due to the final weight difference. Cooking and dripping loss were not ($p > 0.05$) different among the different diet groups and ranged between 17-23% and 2.78-3.9% respectively. The $_{P}$H 24 hrs post mortem showed significant ($p < 0.05$) highest at the fish fed black soldierly meal at 100gkg^{-1} inclusion this might relate to antemortem stress of the fish and as black soldier fly meal is enriched with lauric fatty acid that has stress resistance and immune system builder resulting the highest pH recorded.

3.5.2 Fillet proximate composition

In this study, the proximate composition of African catfish fed partially replaced fishmeal with BSF, MW, and 1:1 combination (BSFMW) was not affected, as compared to the control group. The crude protein content ranged from 18.05 (in the control group) to 18.82% (in BSF50).

3.5.3 Fillet fatty acid profile

The partial replacement of fishmeal with BSF, MW, and BSFMW did not lead to a 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 is an indicator of the suitability of partial replacement for human health. 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. The lauric acid (C12:0) proportion was significantly increased in the BSF diet group. In our study, the atherogenic index was significantly higher ($p < 0.05$ ANOVA) at partial replacement of fishmeal with black soldier fly meal. This might be due to the high lauric acid proportion of black soldier fly meal. Interestingly, lipid health indicators atherogenicity and thrombogenicity indices were within the normal range (below 1%). 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. However, either the decreasing tendency of n-3 or the increasing tendency of total saturated FA was not statistically significant. 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).

4. CONCLUSION 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 35.9g 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 factors 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. This absence of negative effect on growth performance and biometric indices, and significant improvement of 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 97g) 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. However, the simultaneous replacement (BSFMW) worsened the ADC of the diet. Based on this fact, BSF and MW can successfully replace FM ($200 gkg^{-1}$) totally or partially in the practical diet of African catfish without significant adverse effects on nutrient utilization and growth performance of African catfish. However, further investigation should be done on nutrient ADC of the simultaneous inclusion of 1:1 combination (BSFMW) on the practical diet of African catfish.

In the case of survival, some mortality was observed in all groups except BSF50. Concerning 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 juvelin African catfish.

In experiment IV, black soldier fly and 1:1 combination feed at a rate of 100 gkg^{-1} 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 mortality of 100% was observed at an inclusion rate of 100 gkg^{-1} 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^{-1} 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.

In general, we would like to conclude African catfish that as the digestibility, growth, biometric indices, fillet yield, and quality was not affected, it would be soundful to replace 200 gkg^{-1} FM after enrichment of the diet with EFA, particularly the marine source.

5. NEW SCIENTIFIC RESULTS

1. Total (120 gkg^{-1}) replacement of fishmeal with black soldier fly meal significantly improved growth performance and feed utilization of juvenile common carp.
2. Total (100 gkg^{-1}) or partial (50 gkg^{-1}) replacement of fishmeal with mealworm meal did not significantly affect growth performance, survival, and proximate quality of juvenile common carp.
3. Total (200 gkg^{-1}) 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. It could reduce the mortality, (100% survival) without a significant decline in growth feed utilization and changes in the fatty acid profile of the liver.

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 combination in African catfish fillet.
6. Partial (50%) replacement of dietary fishmeal with mealworm and black soldier fly meal does not change the chemical composition of filet except for partially replaced fishmeal with black soldier fly meal, which exhibited significantly highest ash content in common carp fillet**.**
7. Partial (100 gkg^{-1}) replacement of fishmeal with black soldier fly and 1:1 combination is suitable for common carp feed without negative changes in fillet yield, physical, and proximate quality of flesh.
8. Partial (100 gkg^{-1}) replacement of fishmeal with black soldier fly mealworm, and a 1:1 combination of both is suitable for the African catfish diet, without negative changes in fillet yield, and chemical and physical quality of flesh.

6. PUBLICATIONS

6.1 Peer-reviewed paper published in the foreign scientific journal

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.
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
3. **Gebremichael**, A, Sándor Z.J, Kucska B. (2022). Does dietary inclusion of defatted yellow mealworm (*Tenebrio molitor*) affect the growth and body composition of juvenile common carp (Cyprinus carpio)? South African Journal of Animal Science 52 (4),444-451

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

6.2 Abstract in foreign scientific journals

1. Askale Gebremichael*, Balázs Kucska1 (2021): Effect of total or partial replacing of fishmeal with black soldier fly (*Hermetia illucens*) and mealworm (*teneberio Molitor*) meal on growth performance and body condition indices of common carp (*Cyprinus carpio*) international symposium animal science day 29th international conference Goddolo 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 indices of African catfish *(Clarias gariepinus)* 13th annual conference of the Ethiopian Fisheries and Aquatic Sciences Association (EFASA) Addis Ababa Ethiopia May 13-14, 2022.

6.3 Conference abstracts published in Hungarian

1. Gebremichael Askale; Stettner, Gabriella; Varga, Dániel Kucska, Balázs Alternative feherjeforras -lisztkukac (teneberio molitor) alkalmazasa harcsa (silurus glanis) takramanyokban ISBN: 9789632699615 Journal Article/Conference paper in journal (Journal Article)/Scientific [32224291] [Approved]
2. Askale, Gerbremichael; Yarsmin, Yunuis Zeebone; Omeralfaroug, Ali; Müller, Tamás ; Kucska, Balázs A rovarliszt takarmányba történő részleges bevonásának hatása az Afrikai harcsa (Clarias gariepinus) ikrások reprodukciós paramétereireHALÁSZATFEJLESZTÉS 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]

4. Askale, Gebremichael; Kucska, Balázs; Stettner, Gabriella; Varga, Dániel; Ardó, László; Szűcs, Anita; Nagy, Zoltán; Jakabné, Sándor ZsuzsannaA halliszt teljes és részleges helyettesítése rovarliszttel az afrikai harcsa ivadék (Clarias gariepinus) takarmányában HALÁSZATFEJLESZTÉS 38 pp. 70-72. 3 p. (2021)

7. OTHER PUBLICATIONS NOT RELATED TO THE TOPIC OF THE DISSERTATION

7.1 Peer-reviewed paper published in the foreign scientific journal

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.
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.
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

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

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

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She graduated with MSC in tropical animal production and health from Addis Ababa University

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She served as a board member of four organizations (Bonga University, kaffa development association, Gogiba Omo-Micro Finance, Kaffecho intellectuals association) till she departs for Hungary.

She was promoted to assistance professorship in June 2018.

She joined Ph.D. study program in the Department of applied fish biology by the sponsor of the Hungarian government (SH) in the 2019/2020 academic year

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