Fillet yield and flesh quality of common carp (*Cyprinus carpio*) fed with extruded feed containing black soldier fly (*Hermetia illucens*) and mealworm (*Tenebrio molitor*)

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Abstract. In order 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 13 weeks experiment was carried out. A total of 36 fish with initial weight of 209.33±0.07 g were randomly assigned to four groups of diet. The control diet contained 200 g kg^{-1} fishmeal, while experimental diets contained black soldier fly meal 100 g kg^{-1}, mealworm 100 g kg^{-1} and black soldier fly and mealworm 1:1 combination in 100 g kg^{-1}. The fish were fed at 1.75% of their biomass twice per day. After 9 weeks of experimental period, the treatment with mealworm (100 g kg^{-1}) was excluded of the trial due to the high fish mortality. At the end of the experiment (13th week), five fish per group were killed by manual stunning method and measured for slaughter traits and flesh quality. The results revealed that there was no significant difference ($p>0.05$) between various diet groups' interims of profile index, fillet yield, cooking loss, dripping loss and acidification (pH) at 45 minutes and at 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 g kg^{-1} replacement. This study proves that black soldier fly and the mixture of mealworm meal can replace 100 g kg^{-1} of FM without negative effect on flesh yield and quality parameters. Further investigation should be undertaken on optimum g kg^{-1} inclusion level and length of experimental duration of mealworm on common carp.

Key Words: novel protein, quality, somatic index, yield.

Introduction. Common carp, *Cyprinus carpio*, is the third most widely cultivated and commercially important freshwater fish species in the world (FAO 2013). It is being considered as a potential candidate for commercial aquaculture in Asia and some European countries as it has a very high adaptive capability to both environment and food (Soltani et al 2010; Manjappa et al 2011; Rahman 2016). During the period of scarcity of food, *C. carpio* switches to less preferred food and changes its feeding behavior and feeding niche. These peculiar adaptive features make *C. carpio* an economically significant fish not only for monoculture, but also for polyculture ponds (Rahman 2015). In aquaculture, the feed cost accounts about 60-80% of the production cost. *C. carpio* dietary protein requirement is about 32%. Fishmeal has been using as main dietary protein sources in aquafeed, but the supply is very volatile and the price is unaffordable (Oliva-Teles et al 2015; Hua et al 2019). Thus, there is high demand to replace FM with other sustainable protein sources.

Insects have advantages over conventional protein sources and are sustainable (Gasco et al 2020), they reproduce easily, grow fast and have a low feed conversion ratio (FCR) and also a small need of arable land and water (van Huis 2013). Black soldier fly, *Hermetia illucens*, and mealworm, *Tenebrio molitor*, meals are the most novel protein source to replace total and partial portion of FM in practical diet of common carp due to their sustainability (Henry et al 2015) and lower environmental impact, and to their suitability, indicated by the fish interims of nutritional profile and digestibility (Zhou et al

2018), having a positive impact on the growth performance (Gebremichael et al 2021). The nutritional value of the fish muscle may be dependent on several factors, such as fish species, age, sexual maturity, size, oxygen concentration, temperature, photoperiod and pH, that can play an important role in muscle development, as well as on the dietary composition which is the main determinant of the nutritional content of the fish muscle (Suárez et al 2002; Solari 2006; González et al 2009; Varga et al 2010; Videler 2011). Besides, there is scarce information regarding the effect of black soldier fly and mealworm meals on fillet yield and quality attributes. Therefore, this study intended to investigate the replacement of FM with *H. illucens* and *T. molitor* meals on fillet yield, quality and body condition indexes.

Material and Method

Experimental diet. Three experimental and a control diet were formulated (Table 1). The control diet (C) contains 200 g kg^{-1} fishmeal and 50% of fishmeal was replaced and the inclusion level of test ingredients was 10% (100 g kg^{-1}). In the experimental diets, the diet of 100 g kg^{-1} or 10% fish meal was replaced with black soldier fly meal (BS), mealworm meal (MW) and 1:1 mixture of both insect meals (BSMW). The experimental diets were set iso-nitrogenous and iso-energetics (Table 2). MW and BS as originated from Berg and Schmidt Pte. Ltd Singapore in a dried and processed form and imported by Hecron-Agro Kft.

Table 1

Composition of control and experimental feeds

Ingredients (g kg^{-1})	*C*	*BS*	*MW*	*BSMW*
Fish meal (FM)	200	100	100	100
Mealworm meal (MW)	0	0	100	50
Black soldier fly (BS)	0	100	0	50
Soy protein concentrate	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

C-control; BS-black soldier fly; MW-mealworm; BSMW-1:1 mixture of black soldier fly and mealworm meal.

Table 2

Chemical composition of control and experimental feeds

Components	*C*	*BS*	*MW*	*BSMW*
Crude protein (%)	44.35±0.35	45.53±1.09	45.11±0.46	45.97±0.20
Crude fat (%)	7.95±0.05	8.45±0.27	8.29±0.20	8.50±0.06
Crude fibre (%)	1.70±0.28	3.27±0.43	2.97±0.11	2.95±0.66
Crude ash (%)	10.63±0.21	9.38±0.02	8.97±0.01	8.99 ±0.03
Energy (KJ g^{-1})	19.05±0.04	19.94±0.04	19.72±0.00	19.87±0.05
ADF (%)	7.69±0.05	8.62±0.21	10.25±0.05	10.31±0.15

C-control; BS-black soldier fly; MW-mealworm; BSMW-1:1 mixture of black soldier fly and mealworm meal; ADF-acid-detergent fiber.

Experimental set up and the fish. A 13 weeks experiment was carried out in an experimental RAS of the Department of Applied Fish Biology, Hungarian University of Agriculture and Life Science Kaposvar, Hungary. Total of 36 fish with an average initial body weight of 209.33±0.07 g was randomly assigned to four groups of diet (control, MW50, BS50 and BSMW50) and manually fed at 1.75% of their biomass, twice per day, at 8.00 am and 4.00 pm. The fish biomass weight was measured every week to adjust the quantity of feed to the fish requirements. Experimental fish were inspected daily for irregular behavior and mortality. Water quality parameters were monitored daily and the average value recorded during experimental period for temperature, NO_3^-, NO_2^-, dissolved oxygen and pH were: 25.5°C, 26.3±10.66 mg L^{-1}, 0.18±0.04 mg L^{-1}, 4.5±0.4 mg L^{-1} and 7.6±0.12, respectively.

Sample collection. At the end of experiment five fish from each group were sacrificed by manual stunning method (blowing on the head). Length and weight were measured. Fish were dissected, fillet and organs were measured and somatic indices were calculated.

The hepatosomatic index, the viscera somatic index and the condition factor were calculated in accordance with Chemello et al (2020):

$$HSI\ (\%) = (LW/BW) \times 100$$

$$VSI\ (\%) = (VW/BW) \times 100$$

$$K = (TBW\ TL^{-3}) \times 100$$

Where:
HSI - hepatosomatic index;
LW - liver weight (g);
BW - body weight (g);
VSI - viscera somatic index;
VW - visceral weight (g);
K - condition factor;
TBW - total body weight (g);
TL - total length (cm).

The gonado somatic index was calculated using the formula (Muchlisin et al 2010), while the survival rate in accordance with (Duman 2020):

$$GSI\ (\%) = (\text{gonad weight} / \text{body weight}) \times 100$$

$$\text{Survival rate}\ (\%) = 100 \times (\text{number of fish survived}) / (\text{total number of fish stocked})$$

Fillet pH was measured at 45 min and 24 h post mortem, by a Testo 205 precision pH meter (Testo AG, Lenzkirch, Germany). Dripping loss (DL) was determined by the method of Honikel (1998):

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

To determine the cooking loss (CL), fillet samples (100 g) were closed into sealed bags and were 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 following formula was used (Varga et al 2013):

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

The thawing loss (TL) was determined in the same manner, i.e., samples (25 g) were frozen (−20°C) and thawed at room temperature after 2 days. The following formula was used (Varga et al 2013):

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

Proximate analysis. Feed and right side of the fillet was stored in a deep fridge at -20°C until chemical analysis. The proximate composition of *C. carpio* fillet has been analyzed by AOAC (1998) standard methods. After acid digestion, the Kjeldahl method was used for crude protein analysis using a conversion factor of 6.25; a soxhlet apparatus was used for crude fat content analysis. Ash was determined by putting 5 g of grind and homogenized sample in crucible to calibrated muffle furnace set at 550°C for 3 hrs. Moisture content was determined using the electrically heated vacuum oven method of analysis.

Statistical analysis. SAS statistical software was used for the statistical analysis. The first step was to discard the individual data falling outside the double range of standard deviation. ANOVA (GLM, Univariate) with Tukey's "post-hoc" test ($P<0.05$) was used to compare the parameters and to ascertain the between-group differences. Fixed factors were sex and feed.

Results. The fillet yield ranged from 40 to 43%, with the highest average yield at control diet (43.09±1.13) and the lowest (40.38±2.71) at replacement by a combination of mealworm and backslider fly meal. The fillet yield was not significantly affected by the substitution of FM with black soldier fly and a mixture of black soldier fly and mealworm meal (Table 4). A tendency of higher (459.6±199.3) final body weight was observed for the replacement of FM with 100 g kg^{-1} of black soldier fly meal. However, the fillet obtained from the largest live weight was smaller, possibly due to the loss occurred during the filleting. The pH value measured at 45 minutes post mortem showed a slightly higher value at 24 hrs, with the highest value of 7.12±0.11 and the lowest value of 7.04±0.16, for the control and the combination of mealworm with backslider fly meal, respectively. The gonado-somatic index exhibited a significant difference ($p<0.005$) between the diets. This might be related to the gonad maturation stage: the ripe stage gonads exhibit higher gonad weight. From this study, a mortality of 100% for the 100 g kg^{-1} dietary protein replacement of fishmeal with MW was observed and the reason of mortality was unknown.

Fillet proximate composition. Moisture, crude fat and crude protein of common carp fillet was not significantly affected by the replacement level, while the ash content was significantly ($P<0.05$) higher (1.1±0.01) at 50% black soldier fly meal inclusion, compared to the control diet (table 5).

Table 3

Effect of replacing the FM with black soldier fly or mixture of black soldier fly and mealworm feed on the survival rate of *Cyprinus carpio*

Parameter	*Feed*			
	C	*BS*	*BSMW*	*MW*
Survival rate (%)	100	88.8	77.77	0

C-control; BS-black soldier fly; MW-mealworm; BSMW-1:1 mixture of black soldier fly and mealworm meal.

Table 4

Effect of replacing of FM with black soldier fly or mixture of black soldier fly and mealworm feed on flesh quality of *Cyprinus carpio*

Parameters	*Feed*			*P-value*		
	BS	*BSMW*	*C*	*Feed*	*Sex*	*Feed x Sex*
Liveweight (g)	459.6±199.3	405.6±75.1	436.2±77.89	0.81	0.39	0.37
SL (cm)	22.52±3.79	21.40±1.89	22.58±1.69	0.75	0.55	0.41
Profile index	2.39±0.16	2.31±0.12	2.36±0.14	0.74	0.63	0.98
Trunk (g)	272.33±119.32	244.2±46.05	275.39±52.12	0.88	0.49	0.39
Fillet yield (%)	40.38±2.71	40.62±2.62	43.09±1.13	0.23	0.39	0.76

Parameters	Feed			P-value		
	BS	*BSMW*	*C*	*Feed*	*Sex*	*Feed x Sex*
HIS (%)	1.90±0.9	1.60±0.41	1.27±0.48	0.33	0.72	0.56
GSI (%)	2.02±1.38	2.12±1.23	4.13±1.18	0.03	0.66	0.23
VSI (%)	5.93±2.15	5.04±01.27	5.34±0.64	0.76	0.14	0.89
K (g cm^{-3})	3.89±0.47	4.14±0.57	3.78±0.43	0.59	0.96	0.70
pH^{45min}	7.04±0.16	7.05±0.11	7.12±0.11	0.63	0.10	0.86
pH^{24h}	6.48±0.06	6.76±0.17	6.66±0.25	0.15	058	0.58
CL (%)	19.25±1.25	17.97±2.98	17.07±4.50	0.61	0.46	0.96
DL (%)	5.27±2.37	4.43±1.11	4.93±2.30	0.63	0.13	0.45
TL (%)	7.48±0.95	8.30±1.91	7.93±1.12	0.70	0.99	0.49

K-condition factor; HIS-hepatosomatic index; GSI- gonado-somatic index; VSI-visor somatic index; SL-standard length; C-control; BS-black soldier fly; MW-mealworm; BSMW-1:1 mixture of black soldier fly and mealworm meal; CL-cooking loss; DL- Dripping loss; TL-Thawing loss.

Table 5

Proximate composition of *Cyprinus carpio* fillet

Parameters (%)	*BS*	*BSMW*	*C*	*P-value*
Moisture	74.30±1.15	72.46±2.64	70.83±1.55	0.16
Crude protein	17.5±0.20	17.3±0.10	17.66±0.28	0.18
Crude fat	7.03±1.45	9.16±2.73	8.86±0.66	0.36
Ash	1.1 ± 0.01^{b}	1.05 ± 0.05^{ab}	1.00 ± 0.00^{a}	0.02

C-control; BS-black soldier fly; MW-mealworm; BSMW-1:1 mixture of black soldier fly and mealworm meal.

Discussion. The fillet yield obtained from this study was within the range of previous studies on *C. carpio* strains of Hungary, performed by Varga et al (2013), who reported 42 to 45%. From this study we observed highest final body weight at 100 g kg^{-1} replacement of fishmeal with black soldier fly meal. This result is in agreement with the findings of Gebremichael et al (2021), who confirmed the highest final body weight at an inclusion level of 120 g kg^{-1} of black soldier fly meal in the practical diet formulation of *C. carpio*. The pH value showed slightly lower records at 24 hrs post mortem. This result is in line 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 to the fish exposure to an early stress. According to the reports of Poli et al (2005), early stress of the fish before death determines postmortem glycolysis favoring the accumulation of lactic acid in muscles and subsequently decreases the pH value of muscles. On the other hand, the relatively mild pH fall is attributed to the low glycogen concentration in the fish flesh, as compared to mammals (Varga et al 2010). Sex of common carp did not significantly (P>0.05) affect the pH at 45 minutes or 24 hrs.

From this investigation, the highest mortality was observed at 100 g kg^{-1} replacement rate of mealworm feed with fishmeal. However, the reason of mortality was not known; feed ingredients might be one reason, as the survival rate reduction with an increasing level of MW in different fish species was reported. For instance, Tubin et al (2020) reported that the survival rate of Nile tilapia (*Oreochromis niloticus*) juveniles significantly (P<0.05) decreased as MW inclusion increased. At 100% replacement the mortality rate was 46.67%, according to the reports of Coutinho et al (2021); the mortality rate increased as the inclusion level of MW increased in the practical diet of meagre juveniles. Similarly, Gasco et al (2016) observed a significant reduction of the final body weight, weight gain, specific growth rate and feeding rate at a MW inclusion level of 50%. in addition, Tran et al (2022) confirmed the decreasing trend of survival rate as the MW inclusion increases and the significant reduction of growth rate at 75% replacement level of FM by mealworm in practical diet of European perch (*Perca fluviatilis)*.

The reasons of increasing mortality as the replacement level of MW increases might be the more complex chitin-protein matrix in MW (Marono et al 2015) and a lower trypsin susceptibility (Janssen et al 2019), in association with a reduction of nutrient digestibility, which impairs growth and survival (Janssen et al 2019). Another factor might be the processing method of mealworm. As evidences indicated, the release of insects' proteases and phenoloxidases during the grinding of whole- insects for insect meal production negatively affect protein digestibility, solubility and overall quality (Bittner 2006; 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 (g kg^{-1}) and length of the experimental period related to the mealworm feed consequences for the health status of *C. carpio*.

According to Skibniewska et al (2013), the protein content of *C. carpio* is varying from 16.9 to 18.6%. Interestingly, in the present study the protein content was above 17% and was not significantly different (P>0.05) between diet groups. The fat content of *C. carpio* fillets can vary significantly, from 6.3 to 15% (Ljubojević et al 2015). In the present study, the fat content ranged from 7.03 to 9.16% and was not significantly different in all diet groups. In general, the present study revealed that most of proximate quality of *C. carpio* fillet was not affected by the replacement of fishmeal with black soldier fly and mixture of black soldier fly and mealworm feed. This result is in agreement with the previous findings of Rema et al (2019), Iaconisi et al (2018), Tran et al (2022) who observed an absence of significant difference (p>0.05) on the proximate composition of *Oncorhynchus mykiss* and *P. fluviatilis* fed MW as fish meal replacement. Similarly, the proximate composition of *C. carpio* var. Jian, *O. mykiss* and *O. niloticus* was not significantly affected by fishmeal replacement with black soldier fly meal, respectively (Zhou et al 2018; Caimi et al 2021; Devic et al 2018; Sánchez-Muros et al 2016).

Conclusions. This study demonstrates that black soldier fly and mixture of black soldier fly and mealworm feed at a rate of 100 g kg^{-1} of dietary protein can successfully replace FM without any adverse effect on the meat quality and slaughter traits of common carp. A mortality of 100% was observed at an inclusion rate of 100 g kg^{-1}. Further investigations should be performed regarding the long- term inclusion level of mealworm feed in the practical diet formulation of the common carp.

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