Effect of total or partial replacing of fishmeal with black solider fly (*Hermetia illucens*) meal on growth performance and body condition indices of common carp (*Cyprinus carpio*)

Askale Gebremichael, Csaba Hancz, Balázs Kucska

Department of Applied Fish Biology, Hungarian University of Agriculture and Life Sciences, Kaposvári Campus, H-7400 Kaposvár, Guba S. u. 40, Hungary. Corresponding author: A. Gebremichael, onan_ag@yahoo.com

Abstract. A 56-days feeding trial was carried out to evaluate the effects of fish meal (FM) replacement with black soldier fly meal (BSFM) on growth performance, hepatosomatic index and proximate quality of whole common carp (*Cyprinus carpio*). Three experimental diets were made replacing 0% (control group), 50% and 100% of FM protein by BSFM protein. Each diet was randomly assigned to triplicate group of total 180 fish (20 fish per tank) with initial weight of 35.2±6.01 g stocked into 250 L tanks. Fish were fed three times daily to 2.5% of fish biomass. Fish were voluntarily and voraciously feeding on all diets and the survival was 100%. At the end of the experiment, weight gain (WG), specific growth rate (SGR), survival rate (SR), feed conversion ratio (FCR), protein efficiency ratio (PER), condition factor (K), relative gut length (RGL), and hepatosomatic index (HSI) were calculated and found not affected by dietary replacement of FM with BSFM inclusion level, however, viscero-somatic index (VSI) and final body length (FL) exhibited significant differences among the experimental groups at $p < 0.05$. Thus, it is possible a total replacement of FM with BSFM in common carp diet without any adverse effect on growth performance and voluntary feed intake.

Key Words: alternative dietary protein source, insect meal, common carp.

Introduction. In fish farming, nutrition is the most crucial economic factor to take into account due to its contribution of 50 to 70% of the production costs (Rana et al 2009). In formulating nutritive diet for cultured fish, fish meal (FM) is used as the main dietary protein source because of its high nutritional value and favorable palatability (Hardy & Tacon 2002). The increased demand for FM, especially in commercial fish feed industry, and overfishing pressure, have resulted in a supply shortage with concomitant price increase. Main factors of concern in aquaculture are nutritional availability of feed and overall costs of production (Dong et al 1993; Hua et al 2019). To maintain sustainability various efforts had been exerted to find alternative protein sources in aquafeeds like animal origin proteins (trimmings, processed animal products and by-products), plant origin protein sources (soybean meal, vegetable and nut meals, vegetable meal concentrates) and single cell proteins (algae, yeasts or bacteria) (Henry et al 2015; de Jong 2018). Insects are part of the natural diet of many fish species with excellent nutritional profile interims of amino acid balance; in addition, they are ecofriendly, need very limited area of arable land use. Scalability of the production and rapid growth of insect meal processing industries may represent a promising candidate of alternative protein source (Barroso et al 2014; Henry et al 2015; Magalhães et al 2017; Hua et al 2019; Nogales-Merida et al 2019). They are natural food sources for salt or freshwater fish alike (Howe et al 2014; Whitley & Bollens 2014) especially in the juvenile stage (Riddick 2014). Among the different insect species, black soldier fly (BSF) (*Hermetia illucens*) seems to be a very promising species and approved to be used as food and animal feed by European Union (Schiavone et al 2014; Bovera et al 2015; Belghit et al 2019). Nowadays, the novel technology of BSF rearing is becoming increasingly popular around the world. It embraces the concept of circular economy by solving two global problems simultaneously: feed protein deficiency and organic waste utilization (Mertenat et al 2019). BSF larvae can efficiently utilize or recycle a proportion of the fish oils from fish processing leftovers (Barry 2004). It is necessary to maintain a year-round breeding adult colony in a greenhouse with access to full natural light throughout the year (Barry 2004). Common carp (*Cyprinus carpio*) is a very important aquaculture species in many Asian and some European countries and it is the third most frequently introduced species worldwide due to its high adaptive capacity to both environment and food (Soltani et al 2010; Manjappa et al 2011; Rahman et al 2016). Our main objective was to assess the effect of inclusion level of black soldier fly meal (BSFM) to replace FM in common carp feed on performance and whole proximate composition of fish.

Material and Method

Experimental procedures and tank management. The basal diet was set to 38.1% crude protein and 7.5% crude fat containing 120 g kg^{-1} FM (Table 1) and was fed to BSFM free (group 0%). At the first treatment 60 g of FM was replaced with BSFM (group 50%) while in the second treatment all of 120 g FM was replaced by BSFM (group 100%). The experimental diets were set to be iso-nitrogenous and iso-energetic. The fish were stocked into an experimental recirculating aquaculture system (RAS) facility using 250 L tanks. All tanks were connected and operated as a recirculating system containing drum filter, moving bed bio filter and aeration. Experimental fish (n = 180) were randomly distributed to three groups (0%, 50% and 100%) in triplication (20 fish per tank) and acclimatized for one week to the experimental feed prior the actual experimental work. The fish were offered a daily ration of 2.5% of their body weight by hand distribution of feed, three times per day. Water parameters were checked regularly. Dissolved oxygen was measured daily and kept close to the saturation as well the water temperature which was kept at 24.0±0.5°C. Water parameters like ammonium, nitrite, nitrate and pH were measured four times during the experiment. Fish weight and total length were measured individually at the beginning and at the end of the experiment, group weights were measured to adjust the daily feed portions every week. The feeding trial lasted for 8 weeks (June-August 2020).

Preparation of the experimental diet. BSFM originated from a domestic producer in processed form having 23.7% moisture, 52.7% crude protein, 19.9% crude fat and 7.8% crude fiber. The experimental diets were prepared by mixing dry ingredients with fish oil and gelatin adding some hot water, the moist mixtures were then dough pelleted by hand and dried for 48 hrs in air. The proximate composition of BSFM and the experimental diets are shown in Table 1.

Table 1

Inclusion level of ingredients and proximate composition of experimental feeds

Ingredients	*Inclusion levels (g kg^{-1})*		
	0%	*50%*	*100%*
Soya	250	250	250
FM	120	60	0
BSFM	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, BSFM: black solider 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.

Sample collection. At the beginning of the experiment ten fish were killed for somatic measurements and the individual weight of all experimental fish were measured at beginning and end of the experiment. Altogether 18 fish (2 individual per tank, 6 per treatment) were randomly taken and dissected for somatic indexes. Pooled samples of whole fish body per each treatment were sent to Hungarian University of Agriculture and Life Sciences, Central Laboratory Department of Food and Feed Safety, Kaposvár 7400, Guba S.U. 40, Hungary for proximate composition analyses in duplicates. Samples were stored before proximate analysis in fridge under -20ºC.

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:

$$SGR = 100 \times (LnW_f - LnW_i) / t$$

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) – initial weight of fish (g)

Protein efficiency ratio (PER g/g) which 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 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
Viscerosomatic index (VSI%): VSI =VW/BW x 100
Condition factor (k) = BW / TL^3 x 100
VW = visceral weight (g), LW = liver weight (g), BW = total body weight (g) and TL = total length (cm) respectively
Relative gut length (RGL%) RGL = GL/BL x 100
GL = gut length (cm) and BL = total body length (cm)

Statistical analysis. Data were analyzed by Rcmndr software version 4.0.2. One-way ANOVA was used to evaluate treatment effects with Tukey's test for multiple pairwise comparison of treatment means at confidence interval level (CI) of 95% considering p-value < 0.05 significance.

Ethical issues. All procedures involving fish were conducted in line with the Hungarian legislation on experimental animals and were approved by the ethics committee of Hungarian University of Agriculture and Life Sciences Kaposvári Campus. All efforts were made to minimize the fish suffering by using anesthetic (clove oil).

Results. Fish showed similar growth rate in the different treatment groups during the experiment (Figure 1). Fish fed with 100% BSFM diet had slightly better growth than the fish fed on the two other feeds, however these differences in body weight were not significantly different ($p < 0.05$). Similar results were found for specific growth rate (SGR), relative growth rate (RGR) and survival rate (SR) which also were not significantly affected by dietary BSFM levels. As well as the hepatosomatic index (HSI), relative gut length (RGL) and condition factor (k) were not affected by BSFM inclusion level, too. In case of viscero-somatic index (VSI) and final length of carp were significantly different (Tables 2 and 3).

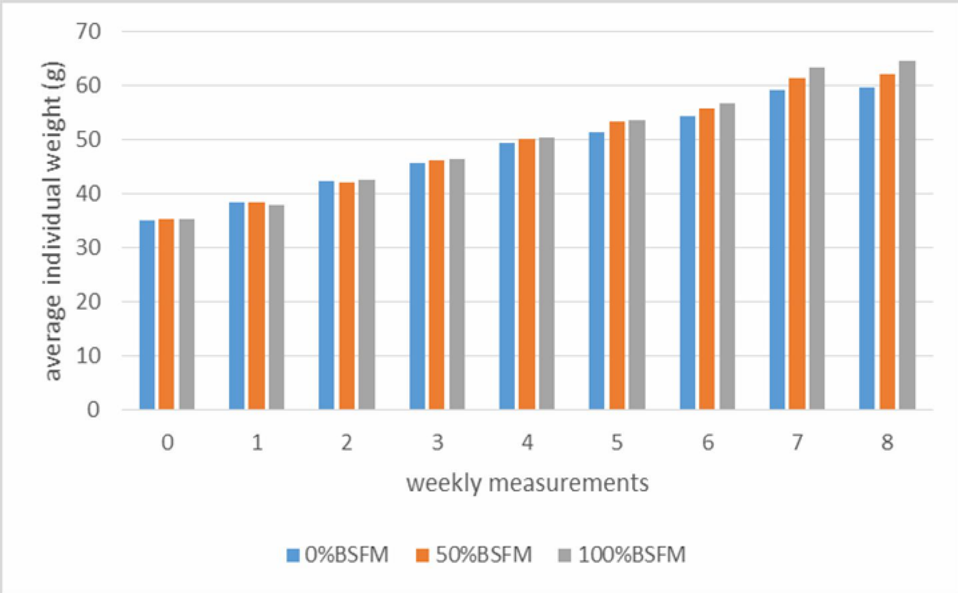

Figure 1. Average weight of fish fed on different inclusion levels of BSFM.

Table 2

Values of growth performance traits* (mean±SD) in the different treatment groups

Parameters	*Inclusion level of BSFM*			*P-value*
	0%	*50%*	*100%*	
IBW (g)	35.2±5.93	35.23±6.10	35.23±6.02	0.999
FBW (g)	59.8±14.02	62.2±14.4	64.58±15.3	0.204
WG (g)	24.6±14.47	26.9±16.74	29.35±16.29	0.264
SGR (%)	4.00±0.23	4.04±0.22	4.07±0.24	0.215
RGR (%)	73.56±44.59	83.35±58.50	88.48±56.22	0.302
FCR	2.10	1.95	1.81	NS
PER	1.24	1.32	1.42	NS
SR (%)	100±0	100±0	100±0	0.37

*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, SR = survival rate, Na = not applicable.

Table 3

Values of body condition indices* (mean±SD) in the different treatment groups

Parameters	*Inclusion level of BSFM*			*P-value*
	0%	*50%*	*100%*	
HSI (%)	2.72±1.00	2.50±0.20	2.17±0.47	0.355
VSI (%)	3.669±0.60[a]	3.01±0.36[b]	3.06±0.39[b]	0.0485
k (g/cm^3)	1.83±0.12	1.75±0.08	1.80±0.16	0.568
TL (cm)	14.81±1.03[a]	15.37±1.25[ab]	15.09±1.16[b]	0.0318
RGL (%)	1.87±0.08	1.69±0.16	1.79±0.18	0.14

*Where: 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 (at $p < 0.05$).

Discussion. The present study revealed that the inclusion level of BSFM in feeds has no significant effect on FBW, SGR, RGR of common carp. These results are in agreement with the findings of Li et al (2017), where also were not found significant differences in FBW and SGR in a similar experiment. Our results are also in agreement with the report of Zhou et al (2018) who found that up to 140 g kg^{-1} BSFM (100% replacement of FM) can be included in diets of Jian carp (*Cyprinus carpio* var. Jian) without unfavorable effects on growth performance. Similar results were reported in Atlantic salmon (*Salmo* *salar*) where dietary replacement of FM by BSF larvae did not result in significant difference on growth performance (Lock et al 2016), while Huda et al (2020) reported that replacement of FM with at inclusion level of 50% gave the best results in African catfish (*Clarias gariepinus*). On the other hand, a study conducted in Nile tilapia (*Oreochromis niloticus*) showed that a total dietary replacement of FM by BSF larvae had significantly reduced WG and SGR, while significantly improved feed intake and FCR (Webster et al 2016). Similarly, Kroeckel et al (2012) have reported that dietary replacement of FM by 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 of fish species needs and developmental stage of BSF of which the insect meal was made (Tschirner & Simon 2015; Liu et al 2017; Smets et al 2020), differences in the substrate on which the BSF has grown on (Spranghers et al 2016; Ewald et al 2020), different processing methods of the meal (Tschirner & Simon 2015; Huang et al 2019). The age and initial weight differences of the experimental unit (fish), water chemistry and health condition of the fish certainly also have significant effects on the efficiency of the FM substitution with insect meals.

Conclusions. This study demonstrated that BSFM is an appropriate dietary protein source for juvenile common carp (35-60 g). FM protein could be replaced even with 100% of BSFM without any adverse effects on growth performance, feed conversion ratio and survival. No significant differences were detected in most of the body indices: hepato-somatic index and condition factor. However, further investigation should be undertaken on nutrient digestibility of BSF larvae meal and its effects on the general fish health.

Acknowledgements. This work was supported by the Ministry of Human Resources (EFOP-3.6.3-VEKOP-16-2017-00005) and Stipendium Hungaricum Scholarship program. We also express our thanks to Grinsect Ltd. Hódmezővásárhely, Hungary for providing the black soldier fly meal.

References

Barroso F. G., de Haro C., Sánchez-Muros M. J., Venegas E., Martínez-Sánchez A., Pérez-Bañón C., 2014 The potential of various insect species for use as food for fish. Aquaculture 422-423:193-201.

Barry T., 2004 Evaluation of the economic, social, and biological feasibility of bio-converting food wastes with the black soldier fly (*Hermetia illucens*). PhD Dissertation, University of Texas, 185 pp.

Belghit I., Liland S., Gjesdal P., Biancarosa I., Menchetti E., Li Y., Waagbo R., Krogdahl A., Lock E. J., 2019 Black soldier fly larvae meal can replace fish meal in diets of sea-water phase Atlantic salmon (*Salmo salar*). Aquaculture 503:609-619.

Bovera F., Piccolo G., Gasco L., Marono S., Loponte R., Vassalotti G., Mastellone V., Lombardi P., Attia Y. A., Nizza A., 2015 Yellow mealworms larvae (*Tenebrio molitor* L.) as a possible alternative to soybean meal in broiler diets. British Poultry Science 56(5):569-575.

de Jong B., 2018 Alternative feed ingredients in a world of volatile fishmeal supply: opportunities and risks. Rabo Research Food and Agribusiness. Available at: https://www.feedproteinvision.com/wp-content/uploads/2018/03/. Accessed: July, 2021.

Dong F. M., Hardy R. W., Haard N. F., Barrows F. T., Rasco B. A., Fairgrieve W. T., Foster I. P., 1993 Chemical composition and protein digestibility of poultry by-product meals for salmonids diets. Aquaculture 116(2-3):149-158.

Ewald N., Vidakovic A., Langeland M., Kiessling A., Sampels S., Lalander C., 2020 Fatty acid composition of black soldier fly larvae (*Hermetia illucens*) - possibilities and limitations for modification through diet. Waste Management 102:40-47.

Hardy R. W., Tacon A. G. J., 2002 Fish meal: historical uses, production trends and future outlook for sustainable supplies. In: Responsible marine aquaculture. Stickney R. R., McVey J. P. (eds), pp. 311-325.
Henry M., Gasco L., Piccolo G., Fountoulaki E., 2015 Review on the use of insects in the diet of farmed fish: past and future. Animal Feed Science and Technology 203:1-22.
Howe E. R., Simenstad C. A., Toft J. D., Cordell J. R., Bollens S. M., 2014 Macro-invertebrate prey availability and fish diet selectivity in relation to environmental variables in natural and restoring north San Francisco Bay tidal marsh channels. San Francisco Estuary Watershed Science 12(1):1-46.
Hua K., Cobcroft J. M., Cole A., Condon K., Jerry D. R., Mangott A., Praeger C., Vucko M. J., Zeng C., Zenger K., Strugnell J. M., 2019 The future of aquatic protein: implications for protein sources in aquaculture diets. One Earth 1(3):316-329.
Huang C., Feng W., Xiong J., et al, 2019 Impact of drying method on the nutritional value of the edible insect protein from black soldier fly (*Hermetia illucens* L.) larvae: amino acid composition, nutritional value evaluation, in vitro digestibility, and thermal properties. European Food Research and Technology 245:11-21.
Huda M. A., Sunarno M. T. D., Nurhudah M., 2020 Potential addition of black soldier fly carcass meal in sangkuriang catfish (*Clarias gariepinus*) feed formulation. AACL Bioflux 13(5):2567-2576.
Kroeckel S., Harjes A. G., Roth I., Katz H., Wuertz S., Susenbeth A., Schulz C., 2012 When a turbot catches a fly: evaluation of a pre-pupae meal of the black soldier fly (*Hermetia illucens*) as fish meal substitute - growth performance and chitin degradation in juvenile turbot (*Psetta maxima*). Aquaculture 364-365:345-352.
Li S., Ji H., Zhang B., Zhou J., Yu H., 2017 Defatted black soldier fly (*Hermetia illucens*) larvae meal in diets for juvenil Jian carp (*Cyprinus carpio* var. Jian): growth performance, antioxidant enzyme activities, digestive enzyme activities, intestine and hepatopancreas histological structure. Aquaculture 477:62-70.
Liu X., Chen X., Wang H., Yang Q., Rehman K., Li W., Cai M., Li Q., Mazza L., Zhang J., Yu Z., Zheng L., 2017 Dynamic changes of nutrient composition throughout the entire life cycle of black soldier fly. PLoS ONE 12(8):e0182601.
Lock E. R., Arsiwalla T., Waagbo R., 2016 Insect larvae meal as an alternative source of nutrients in the diet of Atlantic salmon (*Salmo salar*) postsmolt. Aquaculture Nutrition 22(6):1202-1213.
Magalhães R., Sánchez-López A., Leal R. S., Martínez-Llorens S., Oliva-Teles A., Peres H., 2017 Black soldier fly (*Hermetia illucens*) pre-pupae meal as a fish meal replacement in diets for European seabass (*Dicentrarchus labrax*). Aquaculture 476:79-85.
Manjappa K., Keshavanath P., Gangadhara B., 2011 Influence of sardine oil supplemented fish meal free diets on common carp (*Cyprinus carpio*) growth, carcass composition and digestive enzyme activity. Journal of Fisheries and Aquatic Science 6(6):604-613.
Mertenat A., Diener S., Zurbrügg C., 2019 Black soldier fly biowaste treatment - assessment of global warming potential. Waste Management 84:173-181.
Nogales-Merida S., Gobbi P., Jozefiak D., Mazurkiewicz J., Dudek K., Rawski M., Kieronczy B., Jozefiak A., 2019 Insect meals in fish nutrition. Reviews in Aquaculture 11(4):1080-1103.
Rahman M. M., Noor N. M., Saad S., Yunus K., 2016 Coastal water quality of Tioman Island: effects of human activity and the distance from shoreline. Desalination and Water Treatment 57(1):83-87.
Rana K. J., Siriwardena S., Hasan M. R., 2009 Impact of rising feed ingredient prices on aquafeeds and aquaculture production. FAO Fisheries and Aquaculture Technical Paper No. 541, FAO, Rome, 63 pp.
Riddick E., 2014 Insect protein as a partial replacement for fishmeal in the diets of juvenile fish and crustaceans. In: Mass production of beneficial organisms: invertebrates and entomopathogens. Elsevier, pp. 565-582.

Schiavone A., De Marco M., Rotolo L., Belforti M., Martinez Mirò S., Madrid Sanchez J., Hernandez Ruiperez F., Bianchi C., Sterpone L., Malfatto V., Katz H., Zoccarato I., Gai F., Gasco L., 2014 Nutrient digestibility of *Hermetia illucens* and *Tenebrio molitor* meal in broiler chickens. Proceedings of the 1st International Conference Insects to Feed the World, Wageningen University, Wageningen, The Netherlands. 14-17 May 2014, pp. 73.

Smets R., Verbinnen B., Van De Voorde I., Aerts G., Claes J., Van Der Borght M., 2020 Sequential extraction and characterisation of lipids, proteins, and chitin from black soldier fly (*Hermetia illucens*) larvae, prepupae, and pupae. Waste and Biomass Valorization 11:6455-6466.

Soltani M., Sheikhzadeh N., Ebrahimzadeh-Mousavi H. A., Zargar A., 2010 Effects of *Zataria multiflora* essential oil on innate immune responses of common carp (*Cyprinus carpio*). Journal of Fisheries and Aquatic Science 5(4):191-199.

Spranghers T., Ottoboni M., Klootwijk C., Ovyn A., Deboosere S., De Meulenaer B., Michiels J., Eeckhout M., De Clercq P., De Smet S., 2016 Nutritional composition of black soldier fly (*Hermetia illucens*) prepupae reared on different organic waste substrates. Journal of Science of Food and Agriculture 97(8):2594-2600.

Tschirner M., Simon A., 2015 Influence of different growing substrates and processing on the nutrient composition of black soldier fly larvae destined for animal feed. Journal of Insects as Food and Feed 1(4):249-259.

Webster C. D., Rawles S. D., Koch J. F., Thompson K. R., Kobayashi Y., Gannam A. L., Twibell R. G., Hyde N. M., 2016 Bio-Ag reutilization of distiller's dried grains with solubles (DDGS) as a substrate for black soldier fly larvae, *Hermetia illucens*, along with poultry by-product meal and soybean meal, as total replacement of fish meal in diets for Nile tilapia, *Oreochromis niloticus*. Aquaculture Nutrition 22(5):976-988.

Whitley S., Bollens S., 2014 Fish assemblages across a vegetation gradient in a restoring tidal freshwater wetland: diets and potential for resource competition. Environmental Biology of Fishes 97(6):659-674.

Zhou J., Liu S., Ji H., Yu H., 2018 Effect of replacing dietary fish meal with black soldier fly larvae meal on growth and fatty acid composition of Jian carp (*Cyprinus carpio* var. Jian). Aquaculture Nutrition 24(1):424-433.

Received: 13 April 2021. Accepted: 19 June 2021. Published online: 23 August 2021.

Authors:
Askale Gebremichael, Department of Applied Fish Biology, Hungarian University of Agriculture and Life Sciences, Kaposvári Campus, H-7400 Kaposvár, Guba S. u. 40, Hungary e-mail: onan_ag@yahoo.com
Csaba Hancz, Department of Applied Fish Biology, Hungarian University of Agriculture and Life Sciences, Kaposvári Campus, H-7400 Kaposvár, Guba S. u. 40, Hungary, e-mail: csaba.hancz@gmail.com
Balázs Kucska, Department of Fish Biology, Hungarian University of Agriculture and Life Sciences, Kaposvári Campus, H-7400 Kaposvár, Guba S. u. 40, Hungary, e-mail: kucka.balazs@uni-mate.hu

How to cite this article:
Gebremichael A., Hancz C., Kucska B., 2021 Effect of total or partial replacing of fishmeal with black solider fly (*Hermetia illucens*) meal on growth performance and body condition indices of common carp (*Cyprinus carpio*). AACL Bioflux 14(4):2280-2286.