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Does dietary inclusion of defatted yellow mealworm (*Tenebrio molitor*) affect growth and body composition of juvenile common carp (*Cyprinus carpio*)?

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Abstract

The objective of this study was to evaluate the effects of total and partial replacement of fishmeal with defatted yellow mealworm (*Tenebrio molitor*) meal on one-summer-old common carp (*Cyprinus carpio*) with an initial bodyweight of 97.54 g ± 51.0 g. Fish were kept in an experimental recirculating aquaculture system (9 x 250 L tanks) and fed with two experimental diets formulated with mealworm meal replacements and one control, which had 100 g/kg fishmeal without mealworm meal (MWM0). In the first treatment, 50% of fishmeal was replaced with mealworm meal (MWM50). In the second treatment, fishmeal was replaced totally with mealworm meal (MWM100). Fish were fed three times per day at 3.0% of fish biomass. After six weeks of the experimental period, growth performance, nutrient utilization, body composition, and biometric indices were compared. The results revealed that the growth performance of common carp was not affected significantly by the inclusion level of mealworm meal. However, the highest weight gain was observed in MWM100, where specific growth rate was 0.76 ± 0.10 g/day. Crude fat content of the fish body differed significantly between experimental groups and the control. This investigation demonstrated that MWM could be used as an alternative dietary protein source to replace fishmeal without adverse effects on the growth performance of one-summer-old common carp.

Keywords: aquafeed, dietary protein sources, insect meal

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Introduction

Common carp (*Cyprinus carpio*) is considered a significant species for commercial aquaculture in Asia and some European countries as it has high adaptive capability to the environment and food (Rahman, 1996; Soltani *et al*., 2010; Manjappa *et al*., 2011). Feed in aquaculture accounts for 50–70% of production costs (Rana *et al*., 2009). Feeding technology in the leading carp producer countries is based mainly on formulated feeds. Until recently fish meal was one of the main dietary protein sources.

Overfishing pressure, the finite nature of fishmeal, climate change, and increased demand, especially in the commercial fish production industry, have resulted in a supply shortage, with the concomitant price increase in fish meal as an optimal protein source in fish diet (Rana *et al.,* 2009; Oliva-Teles *et al*., 2015; Golden et al., 2016; Hua *et al.,* 2019). It is vital to reduce the use of fish meal in fish diets by replacing it with local sustainable protein sources or, as in the poultry sub-sector, with an amino acid supplement instead of crude protein (Hafez & Attia, 2020) to minimize production costs and maximize the profitability of the fish farming sub-sector.

Insect meals are considered one of the most promising, sustainable, novel protein sources in aquaculture feed. As part of the natural diet of many fish species, insect meals have favourable nutritional composition, especially in protein content and amino acid profile, and their production leaves a small ecological footprint (need less land and water) and is eco-friendly (Oonincx & de Boer, 2012; Magalhães *et al*., 2017; Hua *et al*., 2019). Mealworm (*Tenebrio molitor*) is a widely used and approved food and animal feed by the European Union (EU) (Schiavone *et al.,* 2014; Shafique *et al*., 2021; Bovera *et al*., 2015; Bovera *et al*., 2016; Tran *et al*., 2022). Based on experimental data, MWM seems suitable for partial or total replacement of fish meal in aqua feeds for a variety of marine and freshwater fish species in terms of growth performance and nutrient utilization (Ng *et al*., 2001; Belforti *et al*., 2015; Henry *et al*., 2015; Gasco *et al*., 2016; Iaconisi *et al*.,

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2019; Hoffman *et al*., 2020; Basto *et al*., 2021). The replacement level of MWM has been studied for various fish species and 75% is recommended for yellow catfish *(Pelteobagrus fulvidraco*) (Su *et al.,* 2017); 67–100% for rainbow trout *(Oncorhynchus mykiss)* (Belforti *et al*., 2015; Rema *et al*., 2019; Chemello *et al*., 2020; Jeong *et al*., 2020); 32% for mandarin fish *(Siniperca scherzeri*) (Sankian *et al*., 2018), 36% for European sea bass (*Dicentrarchus labrax* L) (Gasco *et al*., 2016), 38% for rockfish *(Sebastes schlegeli)* (Khosravi *et al*., 2018), and 100% for red seabream (*Pagrus major*) (Ido *et al*., 2019). However, there are few studies of MWM inclusion level for cyprinid species such as the common carp. However, data are available for the utilization of mealworm oil in mirror carp diet (Xu *et al.,* 2020). Mealworm meal as a fishmeal replacement in the diet of ornamental cyprinids was assessed by Mamuad *et al*. (2021), who confirmed that 30% *Ptecticus tenebrifer* (Walker) and 30% MWM increased the length of common carp, but the result was not conclusive because the amounts of both insects were increased from 0–18% and 0–12%.

Evaluating the effect of replacement with insects in aqua feeds would be important in determining the optimum inclusion level of scarce resources and monitoring the health status and effect on performance (Henry *et al*., 2015; Chemello *et al*., 2020; Tran *et al*., 2022). Therefore, the aim of the current study was to investigate the effect of partial or total replacement of fish meal with MWM on growth performance, feed efficiency, and body composition of one-summer-old common carp.

Materials and Methods

The experiments were approved ethically by the institutional Animal Care and Use Committee of the Research Institute of Aquaculture and Fisheries (licence number BE/25/4302-3/2017).

The basal diet was set at 35.2% crude protein and 6.7% crude fat and contained 10% fish meal (Table 1). In Treatment 1, 50% of fish meal was replaced with MWM (MWM50), and in Treatment 2, fish meal was totally replaced by MWM (MWM100). The diets were isonitrogenous and isoenergetic. Yellow MWM was imported by Hecron-Agro Kft., Hungary (Table 2), from Berg & Schmidt Pty Ltd, Singapore in dried and processed form.

The diets were prepared manually by mixing dried ingredients with oil and slightly warm water. Carboxymethyl cellulose was used as binder. The homogenized and moisturized ingredients were then pelleted with a grinder and dried with cold ventilation for 48 hours.

Table 1 Formulation (%) and proximate composition (%, wet weight) of control and experimental diets in a nutritional trial with common carp juveniles

Ingredients (%) / Diets	**MWM0**	**MWM50**	**MWM100**
Fish meal[1] (CP 60)	10.0	5.0	0.0
Corn[2]	45.0	45.5	46.0
Poultry by-product conc.[3] (CP 67.5)	20.5	21.0	21.0
Mealworm meal[4]	0.0	5.0	10.0
Soy protein conc.[5] (CP 65)	19.0	18.0	18.0
Sunflower oil[6]	2.0	2.0	1.5
Fish oil[7]	2.0	2.0	2.0
Vitamin/mineral premix[8]	0.5	0.5	0.5
Carboxymethyl cellulose	1.0	1.0	1.0
Proximate composition			
Dry matter	10.1	10.2	10.3
Crude protein	35.2	35.4	35.4
Crude fat	6.7	6.9	7.1
Crude ash	6.4	5.6	4.9
Gross energy (kJ g^{-1})	19.5	19.7	19.8

[1] Europrotein Ltd, Verőce, Hungary
[2] Agroszász Ltd, Szászvár, Hungary
[3] Europrotein Ltd, Verőce, Hungary
[4] Berg & Schmidt Pty Ltd, Singapore
[5] Sojaprotein Becej, Serbia
[6] Bunge Hungary Co., Martfű, Hungary
[7] Europrotein Ltd, Verőce, Hungary
[8] Cargill Ltd, Hungary
MWM0: 0% mealworm meal, MWM50: 50% mealworm meal, MWM100: 100% mealworm meal

Table 2 Composition of mealworm meal (dry weight, %)

Ingredients	**%**
Dry matter	91.96 ± 0.30
Crude protein	56.56 ± 0.14
Crude fat	6.20 ± 0.17
Crude ash	7.01 ± 0.05
Crude fibre	2.02 ± 0.33
Acid detergent fibre (ADF)	27.69 ± 0.12
Chitin	5.81 ± 2.08
ΣEAA	**27.62**
ΣAA	**52.61**

*Protein was calculated by applying a nitrogen to protein conversion factor of Kp = 4.76 (Janssen *et a*l., 2017); ΣEAA: sum of essential amino acids, ΣAA: sum of total amino acids

The six-week experiment was carried out at the Department of Applied Fish Biology, Hungarian University of Agriculture and Life Science, Kaposvári Campus, with a recirculating aquaculture system (RAS).

A completely randomized design was used to set up the experiment. The fish were stocked in an experimental RAS facility with 250 L tanks. All tanks were connected and contained a drum filter, moving bed biofilter, and aeration. Experimental fish (n: 135) (w_0: 97.54 ± 15.0 g) were distributed randomly to the three groups (MWM0, MWM50, MWM100) in triplicate (15 fish per tank) and acclimatized for one week with experimental feed before the nutritional trial. The fish were fed manually three times a day at 3% of bodyweight. Water parameters were checked regularly, dissolved oxygen was measured daily and kept close to saturation, and water temperature was kept at 24.0 ± 0.5 °C. Water parameters such as ammonium, nitrite, nitrate, and pH were measured weekly. Fish weight and length were determined individually before and at the end of the trial, and tank biomass was measured weekly to adjust the daily feed portions for fish.

The proximate composition of the MWM, feeds, and whole fish body were analysed using standard methods of the AOAC (1998) (Tables 1 and 2). The Kjeldahl method was used for protein analysis with conversion factors of N × 6.25 for fish and N × 4.76 for mealworm diets. Soxhlet extraction was used for crude fat content analysis with petroleum ether (40–60 °C) as solvent. Ash was determined by measuring 2 g ground and homogenized samples in a crucible and burning in a muffle furnace set at 550 ºC for three hours. Moisture content was established with the oven set at 105 ºC for four hours. Amino acid content was analysed with an amino acid analyser (Waters Acquity UPLC H-Class with AccQ UPLC BEH C18 2.1 × 100 mm), 1.7 µm column and AccQ Tag Ultra Eluent A, B, and water in gradient mode. The chitin was assessed as the difference between ash-free acid detergent fibre (ADF) and protein linked to ADF (chitin% = ADF% - acid detergent insoluble protein%) according to Finke (2007) and Marono *et al.* (2015). Acid detergent fibre was determined on a Gerhardt Fibretherm FT12 apparatus with the protocol provided by the manufacturer.

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

These parameters were calculated at the end of the trial to determine growth performance and nutrient utilization:

Relative growth rate (RGR) = 100 x $\frac{Wf-Wi}{Wi}$ (%) (Wannigamma *et al*., 1985)

Specific growth rate (SGR) = 100 x $\frac{ln\,Wf-\ln Wi}{t}$ (g/day) (Duman, 2020)

where wf = final average weight at the end of the experiment (g); wi = initial average weight at the beginning of the experiment (g); t = experimental time in days.

Weight gain (WG) = wf - wi (g)
where wf = final total biomass (g); wi = initial total biomass (g)

Protein efficiency ratio (PER) = $\frac{weight\ gain\ (g)}{protein\ intake\ (g)}$ (Abdel-Tawab, 2012)

Feed conversion ratio (FCR) = $\frac{Weight\ of\ feed\ given\ (g)}{weight\ gain\ (g)}$ (Duman, 2020)

Survival rate (SR, %) = 100 x $\frac{Number\ of\ fish\ survived}{Total\ number\ of\ fish\ stocked}$ (Duman, 2020)

Biometric and post-harvest indices were calculated as:

Hepatosomatic index: (HSI, %) =100 × LW/FBW (%) (Chemello et al., 2020)
Viscera somatic index: VSI (VSI, %) =100 × VW/FBW (%) (Chemello *et a*l., 2020)
Condition factor = 100 × total FBW/FTL^3 (g/cm^3) (Chemello *et al.,* 2020)
Relative gut length (RGL, %) = 100 × GL/FTL (Chemello *et al.*, 2020)
where VW = visceral weight (g)
LW = liver weight (g)
FBW = final total bodyweight (g)
FTL = final total length (cm)
GL = gut length (cm)
Gross energy (GE, kJ/g or MJ/kg) =17.2 × TC + 23.6 × protein + 39.5 × lipid,
where total carbohydrate (TC, %) = 100 – (crude protein% + crude fat% + crude fibre% + crude ash%) (Halver & Hardy, 2002).

The data was analysed with Rcmdr version 4.0.2 with one-way ANOVA at confidence interval level 95% considering P <0.05 significance (Gebremichael *et al.*, 2021).

Results and Discussion

All diets were highly palatable to common carp juveniles, which accepted the feed voluntarily throughout the experiment. All fish were active and appeared healthy. The growth parameters did not differ significantly between MWM50 and MWM100 and the control, but the highest values of FBW, SGR, and weight gain were obtained with MWM50 (Table 3). The feed utilization (FCR, PER) parameters showed a decreasing tendency with rising inclusion level of MWM. This finding is in agreement with the reports of Mamuad *et al.* (2021). Growth performance showed a decreasing pattern as the replacement level of fishmeal with MWM increased.

The better growth performance in MWM50 than MWM100 might be because the high dietary chitin content of MWM impaired the digestibility of other nutrients; most fish have limited chitinolytic action (Lindsay et al., 1984; Ng *et al.*, 2001; Krogdahl *et al*., 2005; Kroeckel *et al*., 2012; Abro *et al*., 2014). Chitin contributed to increased bulk, reduced faecal retention time, and lowered enzyme accessibility to substrates (Zhang *et a*l., 2014).

Table 3 Effect of replacing dietary protein of fish meal with mealworm meal on growth performance of common carp juveniles

Parameters	**Treatments**			***P*-value**
	MWM0	**MWM50**	**MWM100**	
IBW (g)	97.44 ± 14.13	97.62 ± 15.71	97.55 ± 15.62	0.99
FBW (g)	132.1 ± 34.39	134.7 ± 30.42	130.9 ± 26.62	0.83
WG (g)	568.33 ± 59.34	556.33 ± 94.51	501.60 ± 46.11	0.49
RGR (%)	35.56 ± 4.46	37.98 ± 6.05	34.23 ± 3.17	0.63
FBL (cm)	18.32 ± 1.36	18.45 ± 1.28	18.54 ± 1.32	0.73
IBL (cm)	17.12 ± 0.91	17.14 ± 0.92	17.36 ± 0.94	0.41
SGR (%/day)	0.72 ± 0.07	0.76 ± 0.10	0.70 ± 0.05	0.64
SR (%)	100	100	100	0.37
FCR (g/g)	3.35 ± 0.33	3.40 ± 0.48	3.68 ± 0.37	0.56
PER (g/g)	0.85 ± 0.08	0.84 ± 0.11	0.77 ± 0.07	0.52

Mean ± standard deviation. MWM: mealworm meal, MWM0: 0% mealworm meal, MWM50: 50% mealworm meal, MWM100: 100% mealworm meal, IBW: initial bodyweight, FBW: final bodyweight, RGR: relative growth rate, FBL: final body length, IBL: initial body length, SGR: specific growth rate, SR: survival rate, FCR: feed conversion rate, PER: protein efficiency ratio, WG: weight gain

Studies with silkworm meal by Nandeesha *et al*. (2000) and Rahman *et al.* (1996) confirmed that partly replacing fish meal in common carp diets did not affect performance. In Iaconisi *et al*. (2019) and Piccolo *et al.* (2017), full-fat MWM was replaced by up to 49% and 74% without negative effects on growth performance and FCR in 18- and 23-week feeding experiments with red sea bream and gilthead sea bream. Total replacement of fish meal with defatted MWM did not affect FCR and increased growth performance after four weeks of feeding of red sea bream (Ido *et al*., 2019).

In another study, MWM replaced fishmeal successfully in practical diets of Nile tilapia *(Oreochromis niloticus)* juveniles (Tubin *et al*., 2020). The authors observed greater growth (P <0.05) at the highest inclusion level MWM. Su *et al.* (2017) stated that after five weeks of feeding, total replacement of fishmeal with MWM did not affect the growth performance of yellow catfish *(Pelteobagrus fulvidraco).* Moreover, Chemello *et al*. (2020) reported up to 100% replacement of fishmeal with MWM without negative influence on the performance of rainbow trout. Similarly, Gebremichael *et al*. (2021) reported improved FCR and PER of common carp fed at 100% replacement of fishmeal with black soldier fly meal. These discrepancies might be the result of variations in the nutritional properties of mealworm larvae reared on different substrates (Jajić *et al.,* 2019; Liu *et al*., 2020), particularly in chitin content, which could affect the crude protein digestibility of MWM (Marono *et al*., 2015); in lack of micronutrients and essential amino acids (Nogales-Merida *et al*., 2018); in low digestibility (Adesulu & Mustapha, 2000); and in the processing methods of mealworm (Tschirner & Simon 2015; Huang *et al*., 2019), although the size of the fish could contribute to growth and nutrient utilization results.

Biometric indices indicate the physiological condition of specimens based on fat accumulation, gonadal development, general wellbeing, and adaptation to the environment (Nikolsky, 1963). The condition factor (K) expresses the ratio between fish bodyweight and length, reflecting the interactions between biotic and abiotic variables in its physiological condition (Le Cren, 1951). In this study, biometric parameters such as total body length, bodyweight, hepatosomatic index (HSI), and condition factor were not affected significantly by partial or total replacement of fish meal with MWM (Table 4). This result agrees with the findings of Gebremichael *et al*. (2021) and Hoffmann *et al*. (2020), who reported that HSI and condition factor of common carp and sea trout larvae *(Salmo trutta)* were not affected significantly by partial or total replacement of fish meal with black soldier fly meal and MWM, respectively.

Similarly, Sankian *et al.* (2018) confirmed that there were no significant differences in the survival rate, condition factor, HSI, and VSI values among mandarin fish fed a diet in which up to 30% fish meal was replaced with MWM. Chemello *et al*. (2020) reported the absence of significant differences among treatments for condition factor and somatic indices of rainbow trout fed up to 100% replacement of fish meal with MWM, whereas Gasco *et al*. (2014) observed that the lowest hepatosomatic indices were observed in rainbow trout fed 25% and 50% yellow mealworm replacement diets. These differences might be because of the age of the fish, the gonadal maturity stage, and processing methods of MWM (Chemello et al., 2020; Rizzo & Bazzoli, 2020).

Table 4 Effect of replacing dietary protein of fish meal with mealworm meal on body condition of common carp juveniles

Parameters	**Treatments**			**P-value**
	MWM0	**MWM50**	**MWM100**	
LW (g)	4.43 ± 1.56	6.03 ± 1.73	5.24 ± 1.28	0.23
GL (cm)	34.75 ± 4.12	37.01 ± 4.19	36.93 ± 3.40	0.53
BLW (g)	0.23 ± 0.11	0.24 ± 0.10	0.28 ± 0.10	0.76
KW (g)	0.62 ± 0.16	0.78 ± 0.19	0.88 ± 0.42	0.29
CF (g/cm^3)	2.17 ± 0.42	2.24 ± 0.13	2.02 ± 0.18	0.40
HSI (%)	0.03 ± 0.00	0.04 ± 0.00	0.04 ± 0.00	0.05
VSI (%)	0.03 ± 0.00	0.04 ± 0.00	0.04 ± 0.00	0.17
RGL (cm)	1.86 ± 0.18	1.92 ± 0.19	1.95 ± 0.20	0.72

Mean ± standard deviation, MWM: mealworm meal, MWM0: 0% mealworm meal, MWM50: 50% mealworm meal, MWM100: 100% mealworm meal, LW: liver weight, GL: gut length, BLW: bile weight, KW: kidney weight, CF: condition factor, HIS: hepatosomatic index, VSI: viscera somatic index, RGL: relative gut length

The proximate composition of the eviscerated carp was not affected significantly by inclusion level of MWM, except that crude fat content was substantially higher at MWM50 and MWM100% (Table 5). Tubin *et al.* (2020) also observed a rise in crude fat content of Nile tilapia as the level of MWM replacement increased (P <0.05). Rema *et al*. (2019) observed no significant difference in whole body composition of rainbow trout fed various levels of MWM, which agreed with the findings of Iaconisi *et al.* (2018). There were no differences in the proximate composition of fillets (raw and cooked), whereas fatty acid profile was affected strongly by the diet containing insect meal. Ng *et al*. (2001) reported differences, although non-significant, in the lipid composition of African catfish fillets with various inclusion levels of MWM.

Table 5 Effect of replacing dietary protein of fish meal with mealworm meal on meat proximate composition of common carp juveniles

Parameters	Inclusion levels			P-value
	MWM0	MWM50	MWM100	
Moisture (%)	70.50 ± 1.27	68.33 ± 0.55	69.40 ± 1.47	0.15
Crude protein (%)	13.80 ± 0.72	13.76 ± 0.05	13.80 ± 0.17	0.99
Crude fat (%)	12.56 ± 1.09	15.20 ± 0.60	14.03 ± 1.20	0.04
Ash (%)	1.93 ± 0.20	1.86 ± 0.25	1.76 ± 0.05	0.59

MWM0: 0% mealworm meal, MWM50: 50% mealworm meal, MWM100: 100% mealworm meal

Because of variations among fish and insect species, their rearing conditions, processing methods of insect meal, duration of trials, and water temperature, the direct comparison of results among studies would be difficult and the optimal fish meal replacement levels could vary (Osimani *et al.,* 2016; Iaconisi *et al.,* 2019; Jeong *et al.,* 2020). In addition, standardization of insect protein production is central to assuring the correct formulation of diets for fish and other targeted animal species.

Conclusions

Mealworm meal in the diet of common carp juveniles did not significantly affect growth performance, feed utilization, or biometric indices up to six weeks of feeding. The body composition of the fish might indicate differences in nutrient utilization. Thus, further investigation should be undertaken on nutrient digestibility, chitinolytic enzyme activity, and chitin level in mealworm diets for common carp juveniles. The current results indicated that in the longer term MWM50 in carp diet should be optimal.

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Authors' contributions

AG: data collection, analysis and interpretation, drafting manuscript; ZJS: chemical analysis of the diet and mealworm meal, revision of the manuscript; BK: conception and design of the experiment, revision of the manuscript

Conflict of interest

The authors declare that there is no conflict of interest concerning this manuscript

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