



THE FIRST REPORT OF THE GROWTH PERFORMANCE AND ENVIRONMENTAL SUSTAINABILITY EFFECTS OF DIETARY INSECT MEAL APPLICATION ON THE JARDINE RIVER TURTLE (*EMYDURA SUBGLOBOSA*)*

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Abstract

This study provides empirical data on the balancing, production, and quality of freshwater turtle diets containing partially defatted black soldier fly larvae *Hermetia illucens* meal (BSFL) as a fish meal (FM) replacement. A 70-day experiment was performed using 27 Jardine River turtle (*Emydura subglobosa*) juveniles (6 months old). Three dietary treatments were applied, namely, CON with 30% fish meal and no BSFL as the control, H75 with 22.5% fish meal and 7.5% BSFL, and H150 with 15% fish meal and 15% BSFL, with 3 replicates per treatment, 3 animals per replicate, and 9 specimens in total per treatment. Post-extrusion tests showed that feed technological parameters are dependent on the BSFL meal proportion in terms of the pellet length expansion rate, volume increase, and water binding capacity. The obtained experimental feeds were well accepted by the animals. During the entire experimental period, no turtle mortality, diet-related issues, or differences in shell development or growth performance were recorded among the treatments. However, the feed intake increased in comparison to CON when 7.5% BSFL meal was used (42.30 g vs. 50.40 g), and a lower feed conversion ratio was observed in the 15% BSFL treatment (1.51 vs. 1.38). For the first time, it was empirically proven that *E. subglobosa* can efficiently utilize BSFL meal for up to 15% of their diet. Moreover, the possibility of an increase in environmental sustainability during turtle husbandry due to a decrease in total marine resource use of 55.8% and a 57.4% decrease in the use of fish meal per kg of body weight gain were recorded.

Key words: herpetoculture, turtle nutrition, black soldier fly (*Hermetia illucens*), fish meal replacement, feed utilization

Reptile nutrition in captivity is still on the edge of “terra incognita” for scientists, breeders, and conservationists. Due to the differences in comparison to mammalian or avian physiology, life history, and longevity in relation to similar-sized endotherms, the lack of effective nutritional strategies results in frequent metabolic disorders in captive reptiles (Rawski et al., 2018 b; Azevedo et al., 2021). However, unequivocally, in many cases, live invertebrates – especially insects – are basic foods consumed by wild reptiles and are the most widely used foods for captive specimens (Luiselli et al., 2011; Oonincx et al., 2017). The popularity of insects reflects the frequent application of this approach for nondomesticated animal nutrition, in which the main tool and goal are to try to meet animal requirements by mimicking natural diets (Oonincx, 2017). However, this approach faces a wide spectrum of issues based mainly on two gaps in knowledge – the first issue is the very fragmented infor-

mation available on reptilian natural diets; in most cases, studies discuss a narrow spectrum of diets in reference to a small area or population studies (Luiselli et al., 2011; Mans and Braun, 2014). The second issue is a lack of empirical knowledge on the nutrient requirements of this group of animals (Rawski et al., 2018 b). As a consequence of inappropriate diet balancing, a high share of reptiles suffer due to nutritional deficiencies or excesses (Rawski et al., 2018 b; Oonincx et al., 2017). However, a number of species are currently subject to captive breeding or trade. Due to the abovementioned facts, it is worth noting that, especially now, during the Anthropocene mass extinction of many wild species, the only chance for survival may be captive breeding. Thus, we should increase our knowledge of captive care, nutrition and breeding of nondomesticated animals. This approach may be useful for establishing and developing safe colonies in captivity.

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It should be emphasized that among more than 350 chelonian taxa, data on feed development and nutritional management are available for only a few in the scientific literature (Zhang et al., 2017; Shang et al., 2022; Wang et al., 2022; Rhodin et al., 2021). Moreover, in the case of animals other than fish, poultry, pigs, and dogs, data on the application of processed insect biomass-derived feed materials are scarce (Rawski et al., 2020; Benzertiha et al., 2019; Håkenåsen et al., 2021; Kierończyk et al., 2018). In the available publications, there are no data for sideneck turtle (*Pelurodira*) species. The only chelonian species on which complete and balanced feeds have been widely tested, based on scientific knowledge, is the Chinese softshell turtle (*P. sinensis*), which was confirmed by a few publications on this topic (Zhang et al., 2017; Shang et al., 2022; Wang et al., 2022; Li et al., 2021).

For a long time, turtle diets were based on live invertebrates and fresh products, including fish byproducts, offal, and various other animal-derived materials. In terms of reintroducing populations to the wild, a reasonable strategy is to support animal fitness with natural diets (Rawski et al., 2018 b). However, for specimens that spend their entire life in captivity, balanced complete diets seem to be the most efficient solution because they meet nutritional requirements and lower the probability of nutritional deficiencies. Thus, these types of formulas and easy-to-use feeds should be developed. Notably, the popularity of reptiles kept as pets and the number of safe colonies for endangered species are increasing. Currently, approximately 1% of households in Poland keep reptiles as pets (Kantar Public, 2014), while in the USA, this share reaches 3% (APPA, 2019). Other large reptile population groups include animals kept and bred by zoos and research units. However, for all of these groups, the sustainability and welfare of captive management must be improved (Azevedo et al., 2021). Thus, to maintain a holistic approach to the environmental sustainability of human activities, alternative feed materials should also be considered, tested, and applied in pet foods for reptiles. Insect-derived feed materials, i.e., pulps and full-fat and defatted meals, are considered one of the most promising dietary nutrient sources for animals (Weththasinghe et al., 2021). Moreover, they contain biologically active compounds, including antimicrobial peptides, chitin, and high concentrations of lauric acid (Veldkamp et al., 2022). Among the species that are considered commercially relevant, *Hermetia illucens* has become the leading species in terms of production scale, product quality, and market availability. The production of insect biomass in recent years has shifted from small-scale laboratories or start-ups to important businesses, especially for pet food and aquaculture. It is estimated that before 2030, the total production of insect protein and fat-containing products will reach 250 thousand tonnes in the EU alone (IPIFF, 2020).

The main aspects of insect-derived material sustainability are the reduction in the use of natural resources, i.e., fish meal and fish oil; bioutilization and bioconver-

sion of food and agricultural byproducts and preconsumer wastes (re-food); and shortening of the supply chains and lowering of CO₂ and greenhouse gas emissions (Van Huis et al., 2017). Thus, unsustainable fish meal and fish oil, which are transported long distances, resulting in fossil fuel consumption during production, may be partially or totally replaced by locally produced and environmentally friendly insect-derived materials. The application of insect-derived materials has been widely studied in terms of aquaculture and farm animals (Veldkamp et al., 2022; Van Huis et al., 2017; Zarantoniello et al., 2020; Caimi et al., 2020).

In the present study, the Jardine River turtle (*Emydura subglobosa*) was selected as a novel model species for the study of turtle nutrition. The use of this species is supported by several of its biological features, including high environmental plasticity, a low level of aggression, high fertility, the ability to work on sibling or half-sibling groups, and popularity in the pet trade (Werneburg et al., 2009; Yu, 2022; Hennig, 2006).

The present study aimed to assess the effects of 25 and 50% fish meal (FM) replacement with partially defatted *H. illucens* (BSFL) meal on the final nutritional composition and physical parameters of the extrudate; shell development, growth performance and feed utilization in 6-month-old *E. subglobosa* juveniles; and the environmental sustainability of experimental feed application. The following research hypotheses were taken into consideration:

1. The use of defatted *H. illucens* meal will be suitable for the formulation and production of extruded, isoproteinous diets for *E. subglobosa* with fish meal replacement rates up to 50%.
2. The applied dietary formulations will not negatively affect the acceptance of the experimental feeds by the turtles.
3. The effects of shell development, growth performance and feed utilization on *E. subglobosa* will not be worse in the experimental treatment groups than in the control group.
4. The environmental sustainability of turtle husbandry will be improved by partial dietary fish meal replacement with *H. illucens* meal.

Material and methods

Ethics statement

This study on live animals was carried out in strict accordance with the recommendations of the act on the protection of animals used for scientific or educational purposes in Poland adopted on 15th January 2015 (Dz. U. 2015. 266) and Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes. According to the mentioned acts, no approval from the Local Ethical Committee for Experiments on Animals in Poznań was required for the protocols described below.

All members of the research staff were trained in animal care and handling. Turtle health and welfare and the environmental conditions in the experimental tanks were checked twice daily by visual observation of animal behaviour, by checking water and land area temperatures, and by checking water flow through filters. After the experiment, all animals were kept in the Laboratory of Inland Fisheries and Aquaculture for further maintenance.

Table 1. Nutritive values and amino acid profiles of fish meal (FM) and partially defatted black soldier fly (*Hermetia illucens*) larvae (BSFL) meal used in experimental diets applied in growth trial with *Emydura subglobosa* juveniles

% of dry matter	Meal	
	FM	BSFL
Crude protein	52.08	53.41
Kp corrected protein level	-	39.13
Crude fat	8.96	12.60
Crude ash	10.15	11.22
Crude fibre	5.12	9.61
Nitrogen free extract	23.68	13.16
Calcium	0.98	1.95
Phosphorus	1.41	1.22
Amino acids (% of crude protein)		
Aspartic acid	4.90	3.55
Glutamic acid	7.99	4.95
Arginine	3.65	2.07
Threonine	1.82	1.69
Alanine	2.54	3.58
Proline	2.88	2.79
Serine	2.43	1.92
Histidine	1.21	1.20
Glycine	3.37	2.57
Cysteine	0.37	0.27
Valine	2.07	2.55
Methionine	0.92	0.76
Isoleucine	1.88	1.82
Leucine	3.37	2.98
Phenylalanine	2.11	1.76
Tyrosine	1.66	2.56
Lysine	2.88	2.14

Feeds preparation

The defatted BSFL meal was produced by HiProMine S.A., Robakowo, Poland, and stored at 4°C before being used for feed preparation. Chemical analyses of the fish meal, defatted BSFL meal and feeds were performed before diet formulation in the chemical laboratory of the Department of Animal Nutrition (Poznań University of Life Sciences) as previously described in detail by Rawski et al. (2020). Analysis of the amino acid composition of the proteins was performed to assess the amount of protein and amino acids, as well as to establish the conversion factor (Kp), which was calculated according to Rawski et al. (2020) (Table 1). In total, three diets were

formulated: 1) a control (CON) diet consisting of 30% FM and 0% BSFL; 2) diet 1 (H75) consisting of 22.5% FM and 7.5% BSFL; and 3) diet 2 (H150) consisting of 15% FM and 15% BSFL. Feeds were prepared by extrusion processing with a single-screw warm extruder (Metalchem S-60 Gliwice, Poland) at the Experimental Station of Feed Production Technology and Aquaculture in Muchocin (Poznań University of Life Sciences). The processing conditions were as follows: 90°C cylinder temperature in the zone of increasing pressure, 110°C cylinder temperature in the zone of high pressure, 120°C head temperature, 52 rpm screw speed and 3 mm nozzle diameter. During postproduction, fish oil was added by vacuum coating (Rollermac BA 15 FR aut. Pomati Group S.R. L, Codogno, Italy). The compositions of the experimental diets and their nutritive values are presented in Tables 2 and 3.

Table 2. Composition of the experimental diets applied in the growth trial with *Emydura subglobosa* juveniles

(%)	Feed		
	CON	H75	H150
Fish meal	30.00	22.50	15.00
BSFL meal	0.00	7.50	15.00
Erythrocyte meal	9.00	9.00	9.00
Soy protein isolate	5.10	7.20	9.20
Wheat gluten	10.00	10.00	10.00
Wheat meal	15.60	13.70	11.90
Corn starch	20.00	20.00	20.00
Fish oil	3.50	3.30	3.10
Lecithin	1.00	1.00	1.00
Mineral premix ¹	1.50	1.50	1.50
Vitamin premix ²	0.10	0.10	0.10
Choline chloride	0.20	0.20	0.20
Carboxymethyl cellulose	2.00	2.00	2.00
Limestone	2.00	2.00	2.00
Vitamin C 1500 mg/kg	0.05	0.05	0.05

¹Polfamix W, BASF Polska Ltd. Kutno, Poland; contains the following (per 1 kg): vitamin A 1000000 IU, vitamin D₃ 200000 IU, vitamin E 1.5 g, vitamin K 0.2 g, vitamin B₁ 0.05 g, vitamin B₂ 0.4 g, vitamin B₁₂ 0.001 g, nicotinic acid 2.5 g, D-calcium pantothenate 1.0 g, chloride 7.5 g, folic acid 0.1 g, methionine 150.0 g, lysine 150.0 g, Fe 2.5 g, Mn 6.5 g, Cu 0.8 g, Co 0.04 g, Zn 4.0 g, and I 0.008 g.

²Vitazol AD₃EC, BIOWET Drwalew, Poland; contains 50 000 IU vitamin A, 5000 IU vitamin D₃, 30.0 mg vitamin E, and 100.0 mg vitamin C per 1 kg. CON: control feed, H75: experimental feed with 7.5% BSFL, H150: experimental feed with 15% BSFL.

Feed physical properties

All feed analyses were performed at a temperature of 21°C and under 60% air-controlled humidity (Toyotomi, YD-C312, Nagoya, Japan). Pellet length was measured using an electronic calliper (Yato, YT-7201, Shanghai, China) with an accuracy of up to 0.01 mm, and the analysis was performed on 20 pellets from each batch. The pellet expansion ratio (ER, %) was calculated as the ratio of the pellet diameter (measured as described above) to the extrusion matrix perforation diameter. The expansion

was calculated according to the formula established by Khater et al. (2014), which was previously used for extruded pellets containing BSFL by Irungu et al. (2018) and Das et al. (1993) for the assessment of the use of other animal-derived feed materials:

$$ER (\%) = \left(\frac{\text{pellet diameter (mm)}}{\text{extruder matrix diameter (mm)}} \right) \times 100$$

Pellet bulk density (PD, g/dm³) analysis was performed using a modified procedure described by Irungu et al. (2018). A total of ten randomly sampled replications of 1000 mL of feed were used.

$$PD (\text{g/dm}^3) = \frac{\text{sample weight (g)}}{\text{container volume filled with sample (dm}^3\text{)}}$$

The sinking velocity (SV, s/100 cm) was measured according to a modified version of the protocol of Das et al. (1993). A 105-cm-high water column was filled to 100 cm with water. A total of twenty randomly selected

pellets per feed batch were individually inserted into the water, and the time taken to reach the bottom was measured with an accuracy of 0.1 s.

$$SV (\text{s/cm}) = \frac{\text{sinking time (s)}}{\text{water column height (cm)}}$$

For a better assessment of feed performance in the water, the increase in volume (VI) and water binding capacity (WBC) were also assessed. For VI, 10 feed samples of 25 mL (initial volume) were placed in a volumetric flask, and the flask was filled with 100 mL of water. After 10 minutes, the volume of the feed (final volume) was measured, and the expansion in water was calculated.

$$VI (\%) = \left(\frac{\text{final sample volume (ml)} - \text{initial sample volume (ml)}}{\text{initial sample volume (ml)}} \right) \times 100$$

Table 3. Proximate chemical analysis of experimental feeds applied in a growth trial with *Emydura subglobosa* juveniles

Nutrient (% dry matter)	Feeds		
	CON	H75	H150
Crude protein	40.02	40.07	40.06
Crude fat	7.49	7.51	7.54
Crude ash	7.44	7.59	7.74
Crude fibre	1.02	1.67	2.32
Nitrogen free extract	31.09	30.58	30.2
Calcium	1.50	1.47	1.44
Phosphorus	0.89	0.79	0.70
Calcium/Phosphorus	1.69	1.86	2.06
Gross energy (MJ/kg)	18.03	17.98	17.94
Gross energy/Crude protein (kJ/g)	45.07	44.86	44.76
Amino acids (% of crude protein)			
Aspartic acid	8.34	8.01	8.42
Glutamic acid	17.00	18.31	18.50
Arginine	5.14	5.24	4.72
Threonine	3.59	3.39	3.43
Alanine	5.81	5.69	4.96
Proline	3.43	3.63	3.67
Serine	4.54	3.87	4.54
Histidine	3.75	3.90	3.65
Glycine	7.46	6.74	5.64
Valine	3.54	4.13	4.16
Methionine	2.20	1.98	1.84
Isoleucine	2.76	2.69	2.43
Leucine	7.28	7.48	7.14
Phenylalanine	4.18	3.94	4.27
Tyrosine	2.24	2.05	1.92
Lysine	3.78	4.35	4.06
Cysteine	0.90	1.18	1.09

CON: control feed, H75: experimental feed with 7.5% BSFL, H150: experimental feed with 15% BSFL.

$$WBC (\%) = \left(\frac{\text{final volume (ml)} - \text{initial volume (ml)}}{\text{initial volume (mm)}} \right) \times 100$$

WBC was calculated as the % of feed mass that was water bonded to the pellets after 10 min in water. For each feed, 10 of 100 g samples (initial sample weight) were placed in a flask, and 500 mL of distilled water was added. After 10 min, the feed was filtered through a 1 mm strainer and weighed (final sample weight) to calculate the proportion of bonded water. Ten replicates of each feed type were analysed, and the following equation was used:

Growth trial

A growth trial was carried out using 27 six-month-old *E. subglobosa* juveniles with an average body weight of 45.7 g (weight ranging from 42.6 to 49.4 g). The turtles were full siblings hatched in the Laboratory of Inland Fisheries and Aquaculture of Poznań University of Life Sciences, they were F2 specimens of Freshwater Turtle Laboratory Line (FTLL) as described in details in Rawski et al. (2024). The turtles were randomly distributed into 9 rectangular tanks (3 tanks per treatment). A total of 9 turtles were used per treatment. The experiment was conducted for 70 days. All animals were identified individually by photo documentation; thus, for morphological measurements, including body weight, there were $n = 9$ replicates per treatment. In terms of feed utilization and environmental sustainability parameters, $n = 3$, and each replicate represented 3 turtles.

The experimental unit was set up in the Laboratory of Inland Fisheries and Aquaculture (Poznań University of Life Sciences); 9 experimental tanks 80×60×40 cm in length were filled with 120 dm³ cm of tap water. A flow-through internal filter was installed in each tank (Aquaszut n750, Suwałki, Poland) with a flow rate of 750 dm³/h. During the entire experimental period, the average daily water temperature was 26.8°C, and the conductivity was 769 µS (HM Digital, Inc., EC-3; Redondo Beach, CA, USA). Each tank was equipped with a land area platform (30×50 cm) over which two 35 W lighting and heating bulbs were installed (Happet, Poznań, Poland); the average surface temperature in the heating spot was 30.8°C. The photoperiod was maintained at 16:8 (light: dark) during the entire experiment, with light provided from 07:00 to 23:00. All the above environmental conditions were applied to avoid the effects of seasonal photoperiods or temperatures on the experiment. These conditions were selected on the basis of more than 10 years of research experience with turtles under laboratory conditions (Rawski et al., 2016, 2018 a). Turtles were hand-fed once daily every day until they reached visual satiation. The animals were weighed and measured every 14 days for 24 h after the last feeding. Feed consumption was controlled daily during the feeding period. If any uneaten feed was observed 10 minutes after the end of feeding, the feed was siphoned, air-dried to a constant weight, and weighed to increase the accuracy of the feed intake measurements.

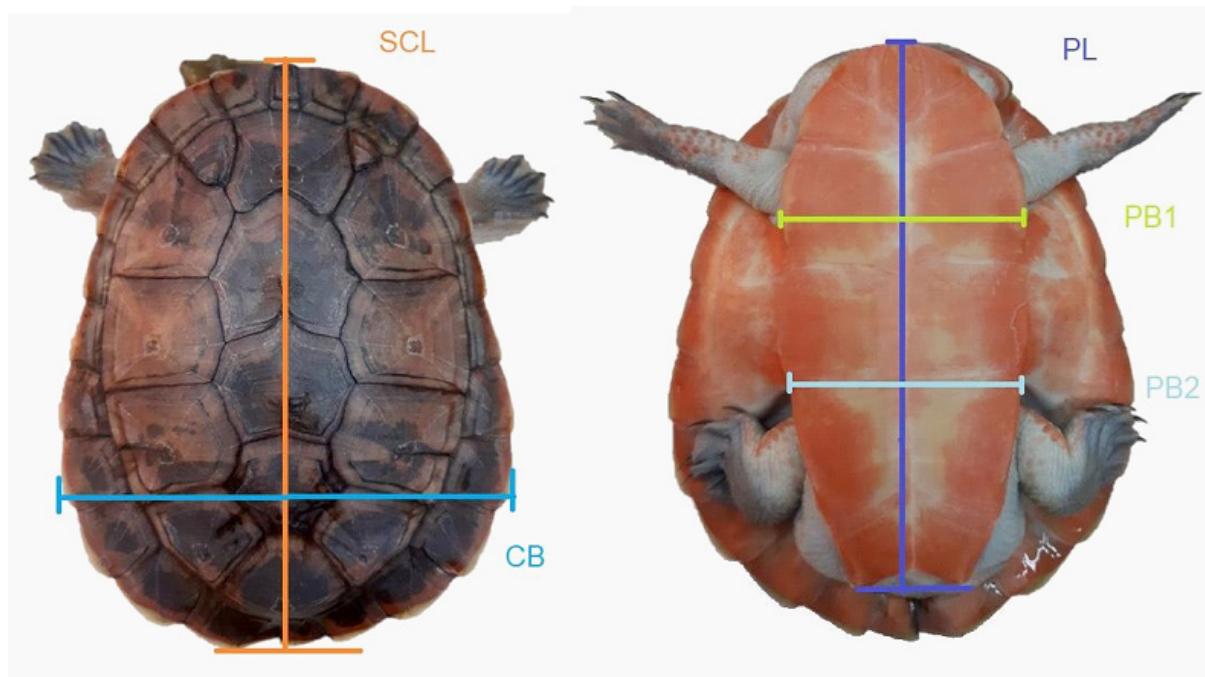


Figure 1. Methodological scheme of the biometric measurements used in the present study. Straight carapace length (SCL), carapace width (CB), plastron length (PL), plastron pectoral width (PB1) and plastron abdominal width (PB2)

Turtle biometry and shell development were assessed via individual shell measurements on a specimen basis ($n=9/\text{treatment}$), and measurements were performed using an electronic calliper (Yato, YT-7201, Shanghai, China) with an accuracy of up to 0.01 mm. The following parameters were measured according to Mitrus (2007): straight carapace length (SCL), carapace width (CB), plastron length (PL), plastron pectoral width (PB1) and plastron abdominal width (PB2). The detailed methodology of each measurement is shown in Figure 1. SCL, CB, PL, and PB1 were measured every 14 days, while PB2 was measured only on the 70th day of the experiment. The growth of the biometric characteristics was calculated as the difference between the final and initial parameter values. The condition index was calculated according to Rawski et al. (2016) using the following formula:

$$\text{Condition index} = \frac{\text{straight carapace length (cm)}}{\text{body weight (g)}}$$

The effects of the diets were evaluated based on turtle individual and biomass weights. For growth performance and diet utilization assessments, the following parameters were calculated: body weight gain (BWG), specific growth rate (SGR), feed intake based on fresh matter (FI), feed conversion ratio (FCR), protein efficiency ratio (PER), and survival rate (SR). The formulas used in the experiment are presented below:

$$\text{SGR (\%/day)} = \frac{\ln \text{final body weight} - \ln \text{initial body weight}}{\text{number of feeding days}}$$

$$\text{FI (g/animal)} = \frac{\text{offered feed} - \text{uneaten feed (g)}}{\text{number of animals}}$$

$$\text{FCR} = \frac{\text{feed intake (g)}}{\text{body weight gain (g)}}$$

$$\text{PER} = \left(\frac{\text{body weight gain (g)}}{\text{feed intake (g)} \times \text{protein level in the diet (\%)}} \right) \times 100$$

$$\text{SR} = \left(\frac{\text{final number of live animals}}{\text{initial number of live animals}} \right) \times 100$$

Sustainability assessment

All the parameters were calculated based on raw data for growth performance and feed utilization parameters to provide enough data for statistical assessment. For each treatment, 3 replicates were used. The relative usage values of marine-derived feed ingredients – fish meal (FMU) and fish oil (FOU) – were calculated according to the following formulas:

$$\text{FMU } \frac{\text{g}}{1000 \text{ g}} \text{ of BWG} = \left(\frac{\text{fish meal share in the diet (g/1 kg)} \times \text{feed intake (g)}}{\text{body weight gain (g)}} \right) \times 100$$

$$\text{FOU } \frac{\text{g}}{1000 \text{ g}} \text{ of BWG} = \left(\frac{\text{fish oil share in the diet (g/1 kg)} \times \text{feed intake (g)}}{\text{body weight gain (g)}} \right) \times 100$$

Marine resource use (MRU) was calculated as the sum of FMU and FOU. For the calculation of wild fish use (WFU), the following formula was used:

$$\text{WFU } \frac{\text{g}}{1000 \text{ g}} \text{ of BWG} = (\text{FMU} \times 4.44) + (\text{FOU} \times 20)$$

where 4.44 is based on the yield of fish meal from wild fish and 20 is based on the yield of fish oil from wild fish (50 g/1000 g) (Jackson, 2009).

Statistical analysis

All the obtained data for feed physical properties and acceptance, growth performance, and feed utilization analysis were tested for a normal distribution using the Kolmogorov–Smirnov test. Analysis of variance homogeneity was conducted using Bartlett's test. The significance of differences between groups was determined with Duncan's multiple range test at a significance level of $P \leq 0.05$. The calculations were performed using SAS software. The following general model was used:

$$Y_i = \mu + \alpha_i + \delta_{ij}$$

where Y_i was the observed dependent variable, μ was the overall mean, α_i was the effect of insect inclusion, and δ_{ij} was the random error.

Results

FM and BSFL meal had similar crude protein levels (52.08 vs. 53.41%). However, the application of nitrogen to the protein conversion factor (Kp) resulted in a protein level of 39.13% in the BSFL meal (Table 1). Due to the absence of chitin, Kp was not applied to the FM. The use of partially defatted *H. illucens* meal did not affect the isoprotein (40.02–40.06%) or isoenergetic (17.94–18.03 MJ/kg of gross energy) character of feeds used in the experiment. The gross energy/crude protein ratio of the experimental diets (44.76 to 45.05 kJ/g) was not affected by diet composition (Table 3). No issues in terms of feed production were observed. However, the physical characteristics of the feed particles significantly affected the pellet expansion, length, volume increase, and water binding capacity (Table 4). Pellet expansion increased while length decreased in the H150 feed compared with

the CON and H75 feeds. The highest volume increase and water binding capacity were observed in H75, while the lowest value was found in the H150 treatment. Pellet density and sinking velocity were not affected by BSFL replacement.

No animal mortality was recorded during the experiment. Final turtle biometrical characteristics were not significantly affected by the addition of BSFL to the diet (Table 5). However, numerically greater values of straight and curved carapace length, carapace width, plastron length and width, shell height, body weight, and condition index were observed in the H75 and H150 treatment groups than in the CON group.

The same trend was observed for the shell development parameters (Figure 2), which included straight carapace length growth, carapace width growth, plastron length growth and plastron width growth. In terms of growth performance and feed utilization parameters (Figure 3), feed intake was greater in the H75 treatment group than in the CON group (50.4 g vs. 42.3 g), while the feed conversion ratio was lower in the H150 group than in the CON and H75 groups (1.38 vs. 1.51). Body weight gain, the specific growth ratio and the protein efficiency ratio were not significantly affected, with the numerically highest values occurring in the H75 and H150 groups.

The final results of all the environmental sustainability parameters except for fish oil use/1000 g of production were affected by BSFL meal use (Figure 4). The final fish meal use was the highest (502 g/1000 g of turtle body weight gain) in the control treatment, while in H150, it was lowered to 205 g/1000 g. The yield for H75 was 341 g/1000 g, which was not significantly different from that of the other treatments. Similar results were recorded in the period from the 1st to 14th day of the experiment, while in the periods to the 28th, 42nd, and 56th days, H75 showed a significant decrease of fish meal use in comparison to that in the control group. In the periods from the 1st to 28th and 42nd days in the H150 treatment group, fish meal utilization was the most efficient among the treatments. Marine resource use (MRU) was affected in all the experimental periods. The final MRU was the highest in the CON treatment (561 g/1000 g) and the lowest (248 g/1000 g) in the H150 group. In the H75 group, MRU significantly decreased during the period from the 1st to the 28th day, while the H150 group had the lowest MRU in all periods. Wild fish use decreased during all experimental periods in the H150 diet and H75 diet during the period from the 1st to the 42nd day. During the remaining periods, wild fish use in H75 did not differ from that in the CON or H150 treatments, as shown by the differences between them.

Table 4. Physical properties of the experimental feeds applied in the growth trial with *Emydura subglobosa* juveniles

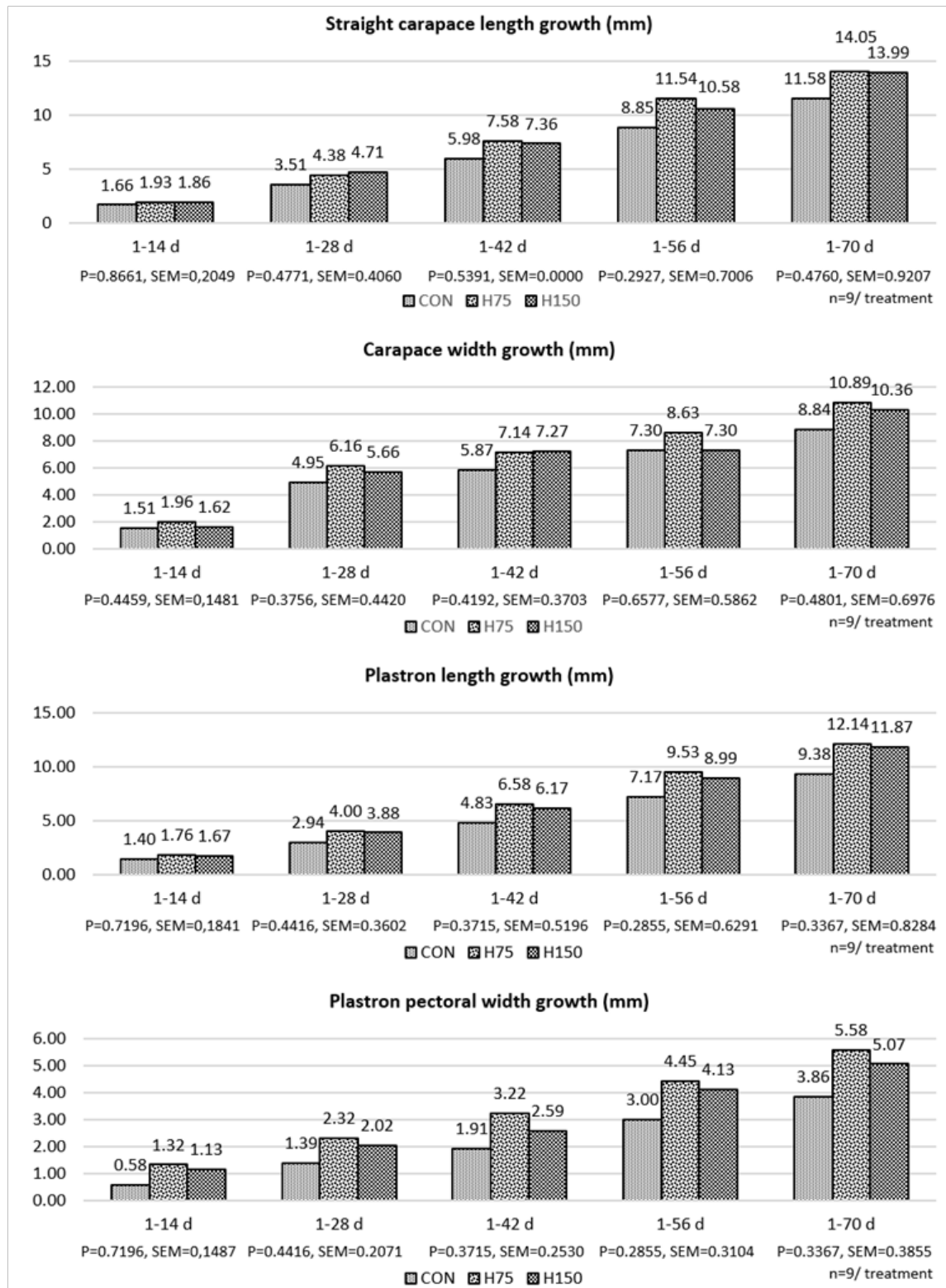
Item	CON	H75	H150	SEM	P
Pellet length (mm)	5.0 a	5.8 a	2.9 b	0.2273	≤ 0.001
Pellet density (g/dm ³)	510.0	516.0	503.0	0.4145	0.458
Pellet expansion rate (%)	41.2 b	43.8 b	86.3 a	3.3546	≤ 0.001
Sinking velocity (s/100 cm)	14.3	14.7	15.2	0.2414	0.344
Volume increase (%)	152.0 b	178.0 a	94.0 c	9.8012	≤ 0.001
Water binding capacity (%)	407.0 b	459.0 a	307.0 c	16.900	≤ 0.001

CON: control feed, H75: experimental feed with 7.5% BSFL, H150: experimental feed with 15% BSFL; different letters (a, b, c) indicate differences among treatments ($P \leq 0.05$). n=20/treatment for pellet length, pellet expansion rate and sinking velocity; n=10/treatment for pellet density, volume increase and water binding capacity.

Table 5. Biometric characteristics of *Emydura subglobosa* juveniles shell on the 70th day of the experiment on *Hermetia illucens* partially defatted meal application

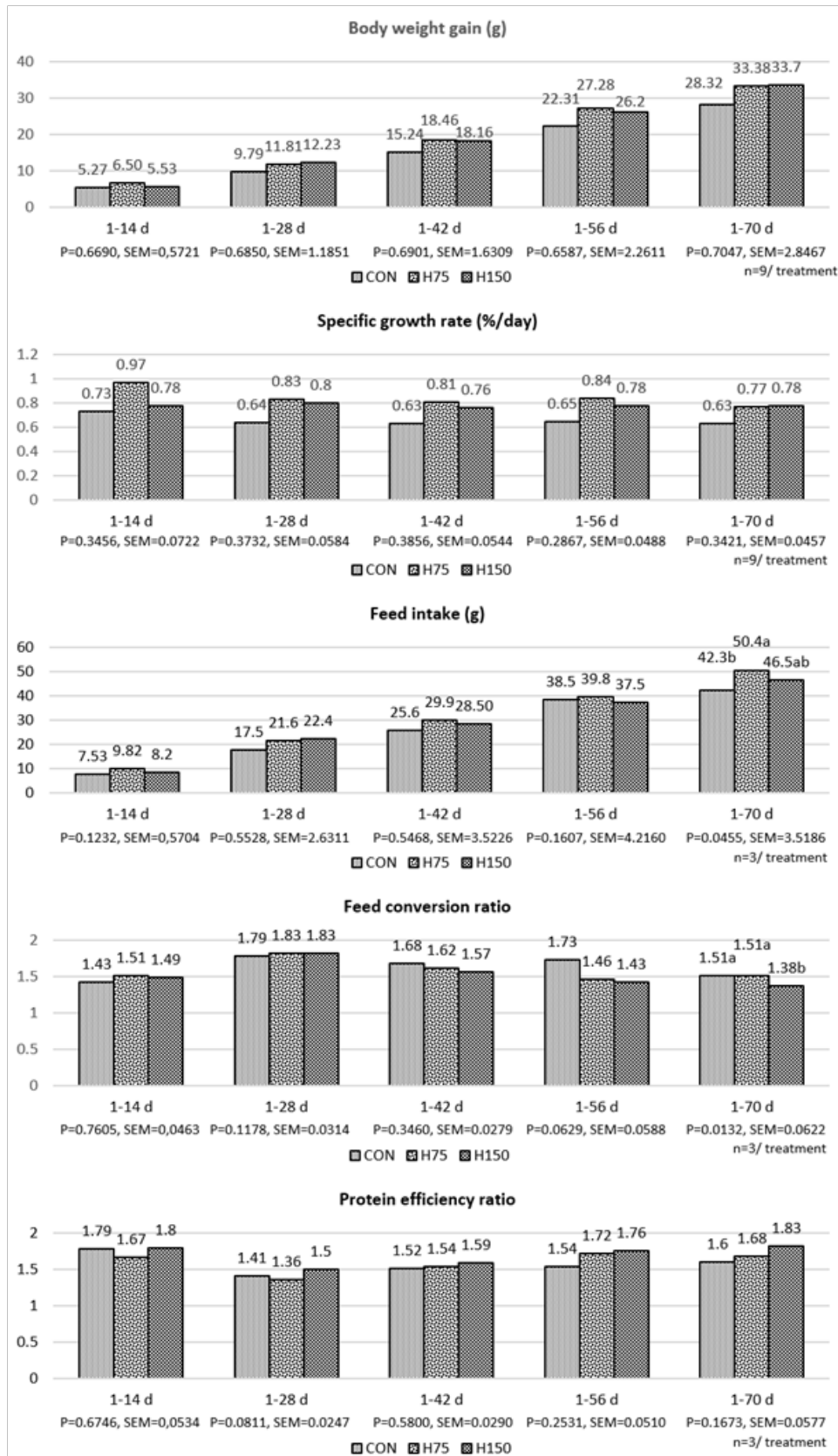
Item	CON	H75	H150	SEM	P
Straight carapace length (mm)	80.9	83.2	82.5	2.165	0.8287
Carapace width (mm)	69.9	71.9	71.2	1.658	0.8070
Plastron length (mm)	67.9	70.2	69.3	2.115	0.8071
Plastron pectoral width (mm)	30.0	30.9	30.7	1.149	0.7957
Plastron abdominal width (mm)	28.6	29.7	29.6	0.985	0.6924
Body weight (g)	74.9	79.7	79.4	1.368	0.8907
Condition index	0.90	0.95	0.94	0.061	0.8277

CON: control feed, H75: experimental feed with 7.5% BSFL, H150: experimental feed with 15% BSFL; n=9/treatment.



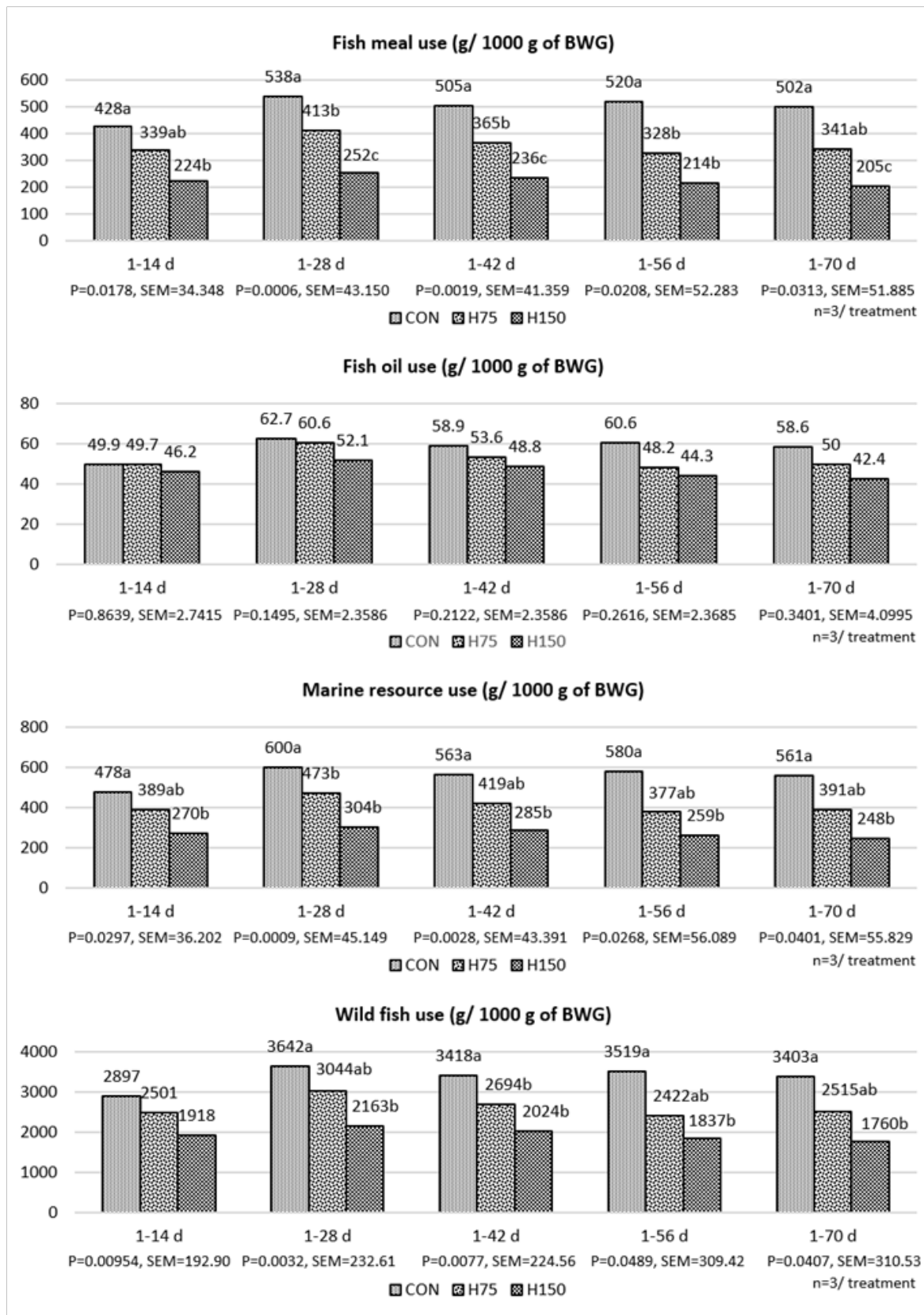
CON: control feed, H75: experimental feed with 7.5% BSFL, H150: experimental feed with 15% BSFL.

Figure 2. Shell growth and development of *Emydura subglobosa* juveniles during a 70-day growth experiment on *Hermetia illucens* partially defatted meal application.



CON: control feed; H75: experimental feed containing 7.5% BSFL; H150: experimental feed containing 15% BSFL. Different letters (a, b, c) indicate differences among treatments ($P \leq 0.05$).

Figure 3. Growth performance and feed utilization in *Emydura subglobosa* juveniles during a 70-day growth experiment on *Hermetia illucens* partially defatted meal application



CON: control feed; H75: experimental feed containing 7.5% BSFL; H150: experimental feed containing 15% BSFL. Different letters (a, b, c) indicate differences among treatments ($P \leq 0.05$).

Figure 4. Environmental sustainability parameters calculated on the basis of the growth performance of *Emydura subglobosa* juveniles during a 70-day growth experiment on *Hermetia illucens* partially defatted meal application

Discussion

For properly balanced insect meal-containing diets, it should be emphasized that the concept of crude protein expressed as the content of nitrogen multiplied by the 6.25 conversion factor should be corrected. In the case of insect meals, the presence of chitin in exoskeletons may explain the difference between crude and true protein levels (Janssen et al., 2017; Boulos et al., 2020). The application of nitrogen to protein conversion factor (Kp) based on amino acid content seems to be the proper technique for diet balancing to avoid amino acid deficiencies or overestimation of crude protein. According to the scientific literature, the Kp for insect meals varies from 4.76 to 5.60 (Janssen et al., 2017). Thus, the experimental feeds were developed taking the abovementioned facts into account and based on earlier literature (Rawski et al., 2018 b; Li et al., 2021). Despite the similar levels of crude protein in FM and BSFL, as previously established, a nitrogen-to-protein conversion factor was applied (Rawski et al., 2020). The results showed a lower Kp of 4.58 and a 39.15% content of Kp-corrected protein in BSFM. This observation may be justified by using partially defatted insect meal in the present study, in which the relative share of chitin could increase due to the fat extraction process. This difference may also be caused by the stage of *H. illucens* development (instar) at the time of collection. However, the key technical point of the study was confirming the possibility of proper balancing of the BSFL-containing diet and aquafeed production by the extrusion technique. These positive results are in agreement with those of studies on a wide spectrum of fish species showing the high usability of BSFL for animal nutrition (Zarantoniello et al., 2020; Stejskal et al., 2020; Li et al., 2021; Veldkamp et al., 2022). It should be emphasized that protein content correction causes further changes in the diet-balancing approach. In the case of a 1:1 weight-to-weight ratio of fish meal replacement with BSFL, which is characterized by lower protein levels after Kp application, an additional source of protein is needed to maintain iso-protein in the diet and maintain its biological value. Another strategy that may and should be applied when additional protein sources are unwanted is the replacement of fish meal with a proportionally greater amount of BSFL. In the present study, the first approach was applied. As a consequence of the 1:1 FM replacement with BSFL, soy protein isolate increased from 5.1% to 7.2% for a diet containing 7.5% BSFL and to 9.2% for a diet containing 15% BSFL. According to our knowledge, based on the scientific literature and experience, the feeds produced for experimental purposes met the nutritional requirements of freshwater turtles (Rawski et al., 2018 b), which is supported by the results of growth performance and feed utilization; however, further studies in terms of digestibility and metabolic effects should be conducted.

Importantly, all aquafeeds should undergo post-production quality assessment, which is one of the key

determinants of feed quality and application perspectives from a practical point of view (Weththasinghe et al., 2020; Khater et al., 2018; Irungu et al., 2018; Das et al., 1993). Physical property analysis of the experimental feeds showed that for the applied technology, the best results were obtained with 7.5% BSFL, which reflected a 25% replacement of fish meal. Pellet expansion increased in the 15% BSFL treatment group (50% FM replacement). However, this positive reception effect should be balanced by increased postproduction breaking vulnerability, which is shown by significantly decreased pellet length. The increase in water and water binding capacity decreased when 15% BSFL was applied, which also indicated its decreased technological quality in comparison to that of the control and 7.5% BSFL containing feeds. These changes may be caused by the presence of a 36% greater relative basis crude fat level in BSFL than in FM. Consequently, the increased proportion of pre-extrusion fat present in the material to post-extrusion fish oil applied by vacuum coating may be the main reason for the worsening of pellet quality. Another explanation of the observed differences may be the decrease of starch content due to wheat meal reduction among the diets (CON = 15.6%, H75 = 13.7% and H150 = 11.9%). Due to 70% starch concentration in it, the decreasing starch content could also cause lowering in pellet length, volume increase and water binding capacity. However, the lack of differences in sinking velocity indicates that the turtle feeding performance is similar for all the tested feeds. This is supported by improvements in feed intake and the feed conversion ratio observed in the experiment.

The next stage of the experimental work involved a 70-day growth trial with *E. subglobosa* juveniles. The final biometrical characteristics of the turtles did not significantly differ among the treatments in terms of animal development. Similarly, no significant differences in the shell development parameters were observed. The growth performance results also showed a lack of significant differences from the numerical advantages of BSFL application at both dietary inclusion levels. Body weight gain, as well as the specific growth rate recorded in the present study, showed a stable but optimal growth rate comparable to that reported in earlier studies on freshwater turtles (Rawski et al., 2020; Gaikhorst et al., 2011; Rawski et al., 2018 a). Feed intake (1–70 days) was the highest in the group fed a diet containing 7.5% partially defatted BSFL meal. These findings support part of the earlier results suggesting that insect-derived materials may be an attractant (Rawski et al., 2020; Kierończyk et al., 2021; Kowalska et al., 2022). However, in some cases described in the literature, opposite effects were observed, which may be explained by insect meal processing, feed preparation technology or species-specific factors (Caimi et al., 2020). The experimental feeds with 15% BSFL were accepted by turtles at a level similar to both the control fishmeal based feed and the 7.5% BSFL containing diet. Thus, it may be concluded that partially defatted BSFL meal is an attractive feed material for

E. subglobosa. The feed conversion ratio was comparable among the experimental treatments in the first phase of the experiment. However, for the entire trial – days 1 to 70, a significant improvement in the FCR was observed in the group fed a 15% dietary share of BSFL meal. Similar dose-response-improving effects were previously observed in fish and explained by alterations in intestinal microstructure development (Rawski et al., 2021) as well as the presence of prebiotic chitin, antimicrobial peptides and lauric acid in BSFL meal (Veldkamp et al., 2022).

The results of the present study are in partial agreement with those of an earlier 70-day-long study on *P. sinensis* in a similar developmental stage (initial body weight = 40 g), where up to 20% BSFL meal was tested as an FM replacement, lowering its share from 60.0 to 48.0%. Shang et al. (2022) concluded that a 5% partially defatted *H. illucens* meal diet – represented by the incorporation of 57% fish meal and 41.5% BSFL meal – is the optimal dietary inclusion; however, they observed that growth performance worsened when 10%, 15% and 20% BSFL meal was applied. Thus, the results obtained for *E. subglobosa* suggest that this species has a greater ability to utilize partially defatted BSFL meal. The authors suggested that the abovementioned worsening may be caused by chitin-induced lower nutrient absorption (Shang et al., 2022). The difference between the discussed species may be partially supported by the fact that *P. sinensis*, as a species bred and selected in captivity for more than 100 years, could lose the natural ability to digest high amounts of chitin in its farmed population. In contrast, the *E. subglobosa* specimens used in the experiment were from the second captive generation, in which grandparents were captured in the wild; thus, the effects of domestication and selection were likely much weaker. This may be supported by earlier studies on probiotic dietary supplementation in turtles, where non-selected populations showed markedly greater improvements than Chinese softshell turtles (Gaikhorst et al., 2011; Rawski et al., 2016, 2018 a). In the case of another 70-day study in which older (initial body weight = 224 g) softshell turtles were used, higher tolerance to BSFL use was also shown. Li et al. (2021) reported a lack of negative effects of a 10% dietary FM replacement with defatted *H. illucens* meal, lowering its dietary share from 62.0% to 55.8% by the application of 8.5% BSFL. This finding suggests the need for studies on species- or age-specific BSFL meal dose optimization. Moreover, as described in the two abovementioned studies, which were based on high FM diets containing 60% FM, we can conclude that lowering FM levels and the use of diverse compositions of the main sources of protein should be the main strategies for lowering marine resource use.

This is likely the first time that turtle husbandry has been assessed in terms of the sustainability of feeds and growth performance. Due to the dietary application of BSFL meal, the use of FM per kilogram of turtle body weight gain decreased significantly during each experimental period as well as for the entire experiment by up to

57.4%. However, no differences were observed in terms of fish oil use. Our previous studies on fish proved that a high sustainability of BSFL meal use may be reached by using full-fat insect meals due to the reduction in fish oil (Rawski et al., 2021; Mikołajczak et al., 2023 a). Thus, the decision on the level of insect meal defatting should be supported by the main aim of replacement – whether it is the replacement of FM or a general increase in diet sustainability. The marine resource use due to BSFL application decreased by 55.8%, and all the abovementioned actions resulted in a final wild fish use of 48.3% in the 70-day rearing period. Earlier sustainability assessments were performed for a several fish species. In the case of Siberian sturgeon (*Acipenser baerii*), the use of FM and fish oil decreased by up to 61.3 and 95.4%, respectively, with the application of up to 30% full-fat BSFL meal (Rawski et al., 2021). In European perch (*Perca fluviatilis*), Stejskal et al. (2020) showed that the fish-in fish-out ratio decreased by 61.2% with a 60% BSFL dietary share. Recently, in studies by Mikołajczak et al. (2023 a, b), the possibility of wide-spectrum sustainability improvements for the production of Atlantic salmon (*Salmo salar*) fries and presmolts was described.

In the present study, the research hypothesis proposed before the experiment was confirmed at each point by empirically proving that *H. illucens* partially defatted meal was suitable for formulating an isoproteinous and isoenergetic diet and preparing extruded feeds for *E. subglobosa* with partial replacement of fish meal.

Conclusions

This study demonstrated that partially defatted *H. illucens* meal is a suitable novel feed component for extruded aquafeed production. The obtained results for turtle growth performance and feed conversion indicate that BSFL meal is well accepted by *E. subglobosa* and that its components are well utilized. It was empirically proven that the dietary application of *H. illucens* increases the environmental sustainability of *E. subglobosa* husbandry.

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