Implementation of a metabolizable energy regression model for black soldier fly larvae fat in broiler chicken diets: effect on growth performance, nutrient digestibility, and selected physiological indices

B. Kierończyk[1,*], S.A. Kaczmarek[1], M. Hejdysz[2], P. Szymkowiak[1], M.R. Aslam[1], M. Rawski[3], P.A. Kołodziejski[4], R. Mikuła[1], K. Stuper-Szablewska[5] and D. Józefiak[1,*]

[1] Poznań University of Life Sciences, Department of Animal Nutrition, Wołyńska 33, 60-637 Poznań, Poland
[2] Poznań University of Life Sciences, Department of Animal Breeding and Product Quality Assessment, Złotniki, Słoneczna 1, 62-002 Suchy Las, Poland
[3] Poznań University of Life Sciences, Department of Zoology, Laboratory of Inland Fisheries and Aquaculture, Wojska Polskiego 71c, 60-625 Poznań, Poland
[4] Poznań University of Life Sciences, Department of Animal Physiology, Biochemistry and Biostructure, Wołyńska 35, 60-637 Poznań, Poland
[5] Poznań University of Life Sciences, Department of Chemistry, Faculty of Wood Technology, Wojska Polskiego 75, 60-625 Poznań, Poland

* Corresponding author:
e-mail: bartosz.kieronczyk@up.poznan.pl;
damian.jozefiak@up.poznan.pl

ABSTRACT. This study aimed to evaluate the validity of estimated apparent metabolizable energy (AME) regression models for *Hermetia illucens (*HI) larval fat in broiler chicken diets during various feeding periods. Additionally, it investigated the effects of HI larval fat on selected serum biochemical, immunological, and humoral indices. The experiment involved 300 7-day-old male Ross 308 chicks assigned to two dietary groups (15 replicate pens). The trial lasted 28 days and the following treatments were applied: a basal diet enriched with soybean oil (SO) or larval fat (HI) as the sole source of dietary fat energy, respectively. To obtain an equal energy value of both diets, the AME values for SO and HI were set as follow: 34.2 and 37.54 MJ/kg (7–14 days), 38.4 and 37.72 MJ/kg (15–28 days), and 37.1 and 37.70 MJ/kg (29–35 days), respectively. No differences in body weight gain, feed intake, or feed conversion ratio were observed between the treatments. However, increased coefficients of apparent ileal digestibility (CAID) of crude protein and apparent ileal digestible energy (AIDE), with elevated lipase activity, were recorded in the HI treatment. The inclusion of insect fat led to reduced serum immunoglobulin G and thyroxine (T4) concentrations. Moreover, the HI group showed a decreasing trend in aspartate aminotransferase, free T4, and interleukin 6 levels. In conclusion, the regression models for HI larval fat used in broiler diets proved to be effective. Additionally, insect fat demonstrated superiority over SO in promoting favourable CAID of crude protein and AIDE. Furthermore, HI may contribute to improved liver function and immune status of birds, as well as affect thyroid hormone metabolism.

Introduction

Insect biomass is considered an alternative and environmentally friendly source of dietary protein in poultry diets (Gasco et al., 2023). In addition to high protein content, invertebrates also contain significant amounts of fat. This value ranges from 7.9 to 47% dry matter (Benzertiha et al., 2020), depending on the chemical composition of the rearing substrate, which notably modulates the final larval nutritional value (Kierończyk et al., 2020). Insect dietary fat is a by-product of protein meal produced by various extraction methods, such as solvent systems, mechanical separation and/or pressing, Soxhlet extraction, and supercritical extraction (Kierończyk et al., 2022b). Overall, incorporating insect dietary fat into poultry diets, including those for broilers (Kierończyk et al., 2023), laying hens (Heuel et al., 2021), turkeys (Sypniewski et al., 2020), and pheasants (Flis et al., 2024), has been shown to yield positive results. Growth performance outcomes are comparable to those achieved with commonly used dietary fats, such as soybean oil, palm oil, or poultry fat (Schiavone et al., 2018; Benzertiha et al., 2019). Moreover, studies have neither shown adverse effects on the weight or length of selected gastrointestinal tract (GIT) segments (Kierończyk et al., 2020) nor on favourable nutrient utilisation, particularly for the coefficients of ether extract ileal digestibility (Kierończyk et al., 2022c). The insect fatty acid (FA) profile, in terms of saturated fatty acid (SFA) and unsaturated fatty acid (UFA) levels, is defined between that of animal fats and oils of plant origin (Sosa and Fogliano, 2017; Kierończyk et al., 2023). However, the predominant FAs in *Hermetia illucens* (HI) larvae, a preferred species for insect production in mini-livestock farming, include lauric (C12:0), myristic (C14:0), palmitic (C16:0), palmitoleic (C16:1), oleic (C18:1), and linoleic (C18:2) acids (Kierończyk et al., 2022a) further implementation of meals and fats derived from invertebrates to livestock (poultry and swine). The dominance of SFAs in HI larval fat suggests a potential limitation in metabolizable energy available in the bird GIT. However, previously observed increases in endogenous enzyme activities (Kierończyk et al., 2022c) and changes in selected gut microbiota populations due to the presence of lauric acid might explain the high utility of HI larval fat. To date, only a few studies have proposed an estimate of the apparent metabolizable energy value of HI larval fat for laying hens (7840 kcal apparent metabolizable energy (AME_N/kg) (Patterson et al., 2021) and broiler chickens (9019 kcal AME_N/kg) (Kierończyk et al., 2022). Prior to estimating the energy density of HI

larvae fat, most authors assumed that AME_N corrected to zero nitrogen balance AME_N of insect fat was similar to that of soybean, maize or coconut oils, and that the fat energy density was applied at a 1:1 ratio in poultry diets (Schiavone et al., 2017; Kim et al., 2020a; b). This suppositions were consistent with the results of Schäfer et al. (2023), who calculated the AME_N for broilers using the following equation: $AME_N = 2732.628 + 62.865 \times$ fat inclusion (%). However, the aforementioned studies assumed uniform AME_N levels for each feeding period; thus, the validity of the AME_N regression model proposed by Kierończyk et al. (2022) for HI larval fat in broiler nutrition was only partially evaluated.

Additionally, the physiological response of birds to dietary fat derived from insect biomass mostly had no significant effect on serum biochemical parameters (Schiavone et al., 2017; 2018; Kim et al., 2020b; Dabbou et al., 2021). The exception was liver function, as insect fat was shown to exert a positive effect on cholesterol and triglyceride levels in this organ, as well as on serum cholesterol and alanine transaminase (ALT) concentrations (Kierończyk et al., 2022b,c). A beneficial impact of HI larval fat supplementation was also observed in the immune system of broilers, specifically, a significant reduction of proinflammatory interleukin-6 (IL-6) levels. Moreover, administration of insect larval fat via poultry diets had no influence on the concentrations of hormones, such as insulin and glucagon, which are involved in feed intake and further growth performance (Kierończyk et al., 2022c). In contrast, the activities of endogenous enzymes (lipase and amylase) were shown to raise following complete replacement of soybean oil with *H. illucens* larval fat. Thus far, the number of studies assessing the physiological response of broiler chickens fed diets supplemented with insect biomass are limited. Therefore, the present study, in addition to biochemical and immunological indices, analysed for the first time thyroid hormones in chickens supplemented with dietary HI larval fat. It is well-known that thyroxine and triiodothyronine serve as stress and welfare indicators (Skomorucha and Sosnówka-Czajka, 2017), and they are directly linked through metabolic pathways that modulate feed intake and weight gain (Ghanima et al., 2023). Therefore, the present study aimed to assess the validity of previously estimated metabolizable energy regression models for HI larval fat administered in broiler chicken diets during various feeding periods, and to investigate further effects on selected physiological parameters when soybean oil was fully replaced with HI larval fat.

Material and methods

Ethical statement

According to Polish law (Journal of Laws, 2015, item 266) and EU Directive 2010/63 (European Union, 2010), the experiments conducted in this study did not require approval of the Local Ethics Committee for Experiments on Animals in Poznań. All procedures followed the guidelines for animal experimentation and care of test animals. No measures involving pain or suffering were performed, and all analyses were conducted on post-mortem samples. Sacrificing animals solely for the purpose of accessing their organs or tissues is not considered a procedure.

Birds and housing

A total of 300 7-day-old male Ross 308 chicks (initial weight 174 g ± 7 g) were randomly assigned to 2 dietary treatments using 15 replicate pens with 10 birds per replicate. Broilers were kept for 28 days in 30 floor pens measuring 1 m × 1 m. Fresh wheat straw, with a depth of 7–15 cm, was utilised as litter. The chicken house was equipped with artificial, programmable fluorescent lights, automatic heating, and forced air ventilation, all set up according to the recommendations of the Management Handbook (Aviagen, 2018) and Council Directive (2007/43/EC; European Union, 2007). The lighting program was configured based on guidelines provided in the Council Directive (2007/43/EC). The indoor temperature was set to 32–33 °C at the beginning of the experiment and gradually reduced by 2–3 °C per week. On day 28, the temperature was adjusted to 21 °C, reaching approximately 18 °C by the end of the trial.

Broiler diets

The composition of the basal broiler diet and the fatty acid profile of the experimental dietary fats are shown in Tables 1 and 2. The birds were provided *ad libitum* access to water (2 nipple drinkers per pen) and feed (1 feed hopper per pen) for 28 days (from day 7 to 35). All diets were formulated to meet or exceed the nutritional requirements recommended for broiler chickens by Aviagen (2018) for each feeding period. Starter diets were offered to all birds from 7 to 14 days of age, grower diets from 15 to 28 days of age, and finisher diets were offered from 29 to 35 days of age. No exogenous enzymes such as carbohydrases or proteases were used in the study. Additionally, no ionophore coccidiostat was included in the feed. The following treatments were applied: SO – consisting of

Table 1. Composition and nutritional value of the experimental diets

Ingredients, g/kg	Starter, 7–14 days		Grower, 15–28 days		Finisher, 29–35 days	
	SO	HI	SO	HI	SO	HI
Maize	584	560	640	640	638	640
Soybean meal	338	363	285	284	271	270
Soybean oil	30.0	0.00	29.5	0.00	46.5	0.00
Hermetia illucens larvae fat	0.00	30.0	0.00	30.0	0.00	45.2
Monocalcium phosphate	14.6	14.3	12.8	12.9	13.9	13.9
Vitamin-mineral premix[1]	10.0	10.0	0.00	0.00	0.00	0.00
Limestone	7.80	7.70	15.1	15.1	12.7	12.7
DL-methionine	3.80	3.50	3.60	3.60	3.00	3.00
HCl-lysine	3.10	2.50	3.30	3.40	2.50	2.50
L-valine	2.90	2.40	3.20	3.20	2.50	2.50
Sodium bicarbonate (NaHCO$_3$)	2.40	2.40	2.50	2.50	4.00	4.00
Salt (NaCl)	2.00	2.00	2.00	2.00	2.00	2.00
L-threonine	1.80	1.40	1.70	1.70	1.10	1.10
L-arginine	0.60	0.00	1.40	1.50	0.40	0.40
L-tryptophan	0.00	0.00	0.10	0.10	0.00	0.00
TiO$_2$	–	–	–	–	3.00	3.00
Calculated nutritional value, g/kg (or otherwise noted)						
dry matter	884	884	883	883	885	885
CP	217	224	200	200	190	190
crude fat	58.3	57.7	59.4	59.9	76.2	75.0
crude ash	60.4	65.4	58.6	58.5	57.8	57.8
AME$_N$, MJ/kg	11.19	11.17	11.47	11.47	11.30	11.32
Analyzed nutritive value, g/kg						
gross energy, MJ/kg	17.5	17.0	17.3	17.3	17.4	17.3
dry matter	892	890	890	890	895	894
CP	211	225	200	206	190	199
ether extract	57.7	55.0	55.7	54.7	75.7	71.9

AME$_N$ – apparent metabolizable energy corrected to zero nitrogen balance, CP – crude protein; SO – basal diet enriched with soybean oil, HI – basal diet enriched with *Hermetia illucens* fat; [1] the following ingredients were provided per kg of diet: IU: vit. A 11.166, cholecalciferol 2500; mg: vit. E 80, menadione 2.50, vit. B$_{12}$ 0.02, folic acid 1.2, choline 379, D-pantothenic acid 12.5, riboflavin 7.0, niacin 41.7, thiamine 2.2, biotin 0.18, pyridoxine 4.0, ethoxyquin 0.09, Mn (MnO$_2$) 73, Zn (ZnO) 55, Fe (FeSO$_4$) 45, Cu (CuSO$_4$) 20, I (CaI$_2$O$_6$) 0.6, Se (Na$_2$SeO$_3$) 0.3

the basal diet with soybean oil implemented as the sole source of energy from dietary fat; and HI - comprising the basal diet with *H. illucens* larval fat added as the only source of energy from fat in the diet. The diets were formulated to maintain isonitrogenous and isoenergetic properties. The apparent metabolizable energy (AME) values for soybean oil were adapted from Ravindran et al. (2016), i.e., 34.2 MJ/kg, 38.4 MJ/kg, and 37.1 MJ/kg for the starter (days 7–14), grower (days 15–28) and finisher (days 29–35) periods, respectively. The AME values for

Table 2. Fatty acid profile of soybean oil and *Hermetia illucens* larval fat (g/100 g fatty acids) fed to broiler chickens

Item	SO	HI
Saturated fatty acids		
C8:0, caprylic acid	–	0.06
C10:0, capric acid	–	1.23
C12:0, lauric acid	–	41.1
C14:0, myristic acid	0.10	16.0
C15:0, pentadecanoic acid	0.01	–
C16:0, palmitic acid	11.2	14.8
C17:0, heptadecanoic acid	–	0.15
C18:0, stearic acid	5.00	2.05
C20:0, arachidic acid	0.20	0.10
C21:0, heneicosanoic acid	–	0.21
C22:0, docosanoid acid	0.25	0.04
C24:0, tetracosanoic acid	0.04	–
Monosaturated fatty acids		
C14:1, myristoleic acid	–	0.20
C16:1, palmitoleic acid	0.10	2.18
C17:1, heptadecenoic acid	–	0.09
C18:1, oleic acid	21.1	17.3
C20:1, eicosenoic acid	0.10	–
Polysaturated fatty acids		
C18:2 n-6, linoleic acid	52.3	1.36
C18:3 n-3, linolenic acid	7.50	0.30
C18:3 n-6, γ-linolenic acid	2.10	2.17
C18:4 n-3, parinaric acid	–	0.04
C20:5 n-3, eicosapentaenoic acid	–	0.72
Total	100	100
SFA	55.75	14.3
UFA	44.25	85.7
MUFA	27.55	34.8
PUFA	16.70	50.9

SO – soybean oil, HI – *Hermetia illucens* larvae fat, SFA – saturated fatty acids, UFA – unsaturated fatty acids, MUFA – monounsaturated fatty acids, PUFA – polyunsaturated fatty acids

HI larval fat were calculated in accordance with Kierończyk et al. (2022) and using the following equations: AME = 2654.227 + 63.175 × fat inclusion % (37.54 MJ/kg), AME = 2559.758 + 7.405 × bird age days + 62.989 × fat inclusion % (37.72 MJ/kg), and AME = 2806.513 + 62.032 × fat inclusion % (37.70 MJ/kg) (37.54 MJ/kg), (37.72 MJ/kg), and (37.70 MJ/kg) for the starter (days 7–14), grower (days 15–28) and finisher (days 29–35) periods, respectively. The diets were prepared in mash form. Titanium dioxide (TiO$_2$, 0.3 g/kg) was added to the finisher (days 28–35) diets as an inert marker to calculate the coefficients of apparent ileal digestibility (CAID) of nutrients and apparent ileal digestible energy (AIDE).

Data and sample collection

The following variables were measured: body weight (BW) and feed intake (FI) on days 7, 14, 21, 28, and 35. Additionally, body weight gain (BWG) and the feed conversion ratio (FCR) were calculated. These traits were measured using an NVL6101 laboratory scale (OHAUS, Nanikon, Switzerland) with an accuracy of ± 1 g. To calculate growth performance, the pen was defined as the experimental unit (n = 15). At the end of the experiment (day 35), one randomly selected bird from each replicate (15 birds per treatment, n = 15) was stunned by exposure to an electric field (STZ 6, PPHU KOMA, Słone, Poland), sacrificed by cervical dislocation, and eviscerated to collect the crop, jejunal, and caecal digesta, the following GIT segments: proventriculus, gizzard, duodenum, jejunum, ileum, and caeca, as well as selected internal organs, i.e., heart, pancreas, liver, spleen, bursa of Fabricius, and thymus. The jejunal segment was considered to begin at the end of the duodenum and ending at Meckel's diverticulum. The ileum was defined as the small intestinal segment caudal to Meckel's diverticulum. The selected GIT segments and internal organs were collected to calculate their weights (g/kg BW) and lengths (cm/kg BW) in relation to BW. Before measurements, all organs were rinsed in sterile water, drained, and weighed using a Radwag PS 600/C/2 balance (Radom, Poland) with an accuracy of ± 0.001 g. Their lengths were determined using a standard linear scale with an accuracy of 1 mm. The pH values of the crop, jejunal and caecal digesta were measured immediately after slaughter using a N 6000 BNC ScienceLine Micro pH combination electrode (Schott SI Analytics, Mainz, Germany) and an 1100 H pH meter (VWR International, Leuven, Belgium). The remaining ileal contents were pooled from three birds (n = 5) to estimate the CAID and AIDE and then immediately frozen using dry ice for further chemical analyses. Duodenal digesta from 8 randomly selected birds (n = 8) was collected into 2-ml Eppendorf tubes and placed directly on dry ice for further determination of pancreatic enzyme activity, i.e., trypsin, lipase, and amylase.

Blood serum parameters and pancreatic enzymes

At the end of the experiment (day 35), 8 birds were randomly selected from each treatment (n = 8) for *post mortem* blood sample collection directly after slaughter via decapitation. Subsequently, serum was obtained by centrifugation (Micro 220R, Hettich, Tuttlingen, Germany) at 1000 *g* at 8 °C for 10 min and stored at −20 °C until further analyses. The following biochemical parameters were analysed according to the manufacturers'

instructions of commercially available kits (Pointe Scientific, Warsaw, Poland): glucose, triglyceride (TG), total cholesterol, total protein (TP), albumin, alanine transaminase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), and gamma-glutamyl transferase (GGT) levels. Additionally, the following immune traits were determined in the collected plasma: immunoglobulin A (IgA), immunoglobulin G (IgG), and immunoglobulin Y (IgY), as well as tumour necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6), triiodothyronine (T3), thyroxine (T4), free triiodothyronine (FT3), free thyroxine (FT4), and insulin based on the manufacturer's instructions of commercial kit reagents (Shanghai Sunred Biological Technology Co., Ltd., Shanghai, China or Abnova Corporation, Taipei, Taiwan). Absorbance measurements were conducted using a Synergy 2 multidetection microplate reader (Biotek Instruments, Inc., Winooski, VT, USA). Endogenous enzyme activities were measured using BioVision colorimetric assay kits (Milpitas, CA, USA). Duodenal digesta was homogenised on ice, and Tris-buffered saline was applied to obtain a 20% homogenate. The samples were then centrifuged at 10000 g at 4 °C for 30 min. Subsequently, the supernatants were diluted 100 times using TBS and incubated for 30 min at 37 °C with 1% enterokinase (Sigma-Aldrich, St. Louis, MO, USA), diluted in 0.1 M Tris-HCl with 0.1 M $CaCl_2$, (pH 7.2) to convert trypsinogen. Values are expressed as percentages relative to the SO treatment.

Chemical analyses

Dry matter (DM), crude protein (CP), and ether extract (EE) of the experimental diets and collected ileal contents were analysed following AOAC recommendations (AOAC International, 2005) using methods 934.01, 976.05, and 920.39, respectively. DM content determinations were conducted using a Binder 9010-0333 ED 56 Drying and Heating Chamber (Tuttlingen, Germany). The CP content was analysed using a Kjell-Foss automatic 16210 analyzer (A/S N. Foss Electric, Hillerød, Denmark), and the EE content was determined using a Soxtec System HT-1043 extraction unit (Foss Tecator, Hillerød, Denmark). For TiO_2 analysis, samples were prepared according to the method of Myers et al. (2004), and concentrations were estimated using the procedure described by Short et al. (1996). Gross energy (GE) was determined using a KL 12 Mn adiabatic bomb calorimeter (Precyzja-Bit PPHU, Bydgoszcz, Poland) standardised with benzoic acid (Avantor Performance Materials Poland S.A., Gliwice, Poland).

Calculations

The coefficients of apparent ileal digestibility (CAID) of CP and EE were calculated using the inert marker method, i.e., in relation to titanium dioxide (TiO_2), to determine the nutrient content in the feed and ileal digesta based on the following formula:

$$CAID_{of\ nutrient} = 1 - \left(\left(\frac{TiO_{2\ g/kg\ diet}}{TiO_{2\ g/kg\ digesta}} \right) \times \left(\frac{Nutrient_{g/kg\ digesta}}{Nutrient_{g/kg\ diet}} \right) \right).$$

The apparent ileal digestible energy (AIDE) values of the experimental diets were calculated using the following equation:

$$AIDE = GE_{diet} - \left(GE_{ileal\ digesta} \times \frac{TiO_{2\ diet}}{TiO_{2\ ileal\ digesta}} \right).$$

Statistical analysis

The experiment was conducted using a completely randomised design. The experimental unit was defined as a pen (10 birds per pen, n = 15) for growth performance results, while 15 randomly selected birds (n = 15) were used to measure length and weight of internal organs and selected segments of the GIT. Additionally, the pH values of the crop, jejunal, and caecal digesta were measured in one randomly selected chick per pen (n = 15). Furthermore, the ileal digesta from one bird from each replicate pen was pooled into three samples (n = 5) for CAID and AIDE determinations. For selected physiological parameters, eight randomly chosen birds per treatment were used (n = 8). The Shapiro-Wilk test was applied to assess the normality of data distribution. Student's t test was used to determine the significance of differences between treatments at a significance level of $P < 0.05$. Due to the presence of data with a non-normal distribution, the Mann-Whitney test was employed with the same level of significance. A trend was considered significant at $P < 0.10$. Analyses were performed using RStudio (2022.12.0+353; RStudio, Inc., Boston, MA, USA) and the stats (4.2.2) package.

Results

Growth performance and nutrient digestibility

Growth performance results are summarised in Table 3. No significant ($P > 0.05$) differences were recorded in BWG, FI, or FCR between the treatments during any of the experimental periods. However, increased CAIDs of CP ($P = 0.021$) and AIDE ($P = 0.028$) were noted in the HI treatment (Table 4), while the CAID of EE was not affected ($P = 0.108$). The activities of selected pancreatic

Table 3. Effect of replacing soybean oil with *Hermetia illucens* larval fat on broiler chicken growth performance parameters

Item	Treatments		SEM	*P*-value
	SO	HI		
7–14 days				
BWG, g	304	307	2.40	0.534
FI, g	363	365	2.55	0.770
FCR, g:g	1.20	1.19	0.007	0.655
15–21 days				
BWG, g	524	513	5.77	0.349
FI, g	718	718	7.15	0.992
FCR, g:g	1.37	1.40	0.014	0.327
22–28 days				
BWG, g	606	624	8.05	0.270
FI, g	936	944	10.4	0.727
FCR, g:g	1.56	1.52	0.025	0.418
29–35 days				
BWG, g	901	907	13.0	0.824
FI, g	1384	1416	34.0	0.641
FCR, g:g	1.54	1.56	0.036	0.819
7–35 days				
BWG, g	2332	2308	22.8	0.601
FI, g	3401	3443	43.2	0.638
FCR, g:g	1.46	1.49	0.018	0.362

SO – basal diet enriched with soybean oil, HI – basal diet enriched with *Hermetia illucens* fat; BWG – body weight gain, FI – feed intake, FCR – feed conversion ratio, SEM – standard error of the mean, *P* > 0.05 indicates that data are not significantly different (n = 15)

Table 4. Effect of replacing soybean oil with *Hermetia illucens* larval fat on the coefficients of selected nutrient apparent ileal digestibility and apparent ileal digestible energy (AIDE) in broilers (35 days)

Item	Treatments		SEM	*P*-value
	SO	HI		
Apparent ileal digestibility				
crude protein, %	79.0	82.0	0.71	0.021
ether extract, %	82.2	87.4	1.62	0.108
AIDE, kcal	3072	3227	37.3	0.028
Activity of endogenous enzymes				
trypsin, %	100	113	11.5	0.721
lipase, %	100	137	9.30	0.047
amylase, %	100	99.9	9.06	0.998

SO – basal diet enriched with soybean oil, HI – basal diet enriched with *Hermetia illucens* fat; BW – body weight, SEM – standard error of the mean; *P* < 0.05 indicates that data are significantly different (n = 5)

Table 5. Effect of replacing soybean oil with *Hermetia illucens* larval fat on the length and weight of selected gastrointestinal tract segment of broilers (35 days)

Item	Treatments		SEM	*P*-value
	SO	HI		
Length, cm/kg BW				
duodenum	14.3	14.5	0.23	0.671
jejunum	33.8	35.2	0.63	0.288
ileum	34.6	36.2	0.66	0.257
caecum	7.45	8.02	0.16	0.068
Weight, g/kg BW				
duodenum	0.68	0.68	0.014	0.817
jejunum	1.25	1.28	0.020	0.443
ileum	0.93	0.94	0.015	0.618
caecum	0.33	0.32	0.010	0.810

SO – basal diet enriched with soybean oil, HI – basal diet enriched with *Hermetia illucens* fat; BW – body weight, SEM – standard error of the mean, *P* > 0.05 indicates that data are not significantly different (n = 15)

Table 6. Effect of replacing soybean oil with *Hermetia illucens* larval fat on the weight of selected internal organs of broiler chickens (35 days)

Item	Treatments		SEM	*P*-value
	SO	HI		
Weight, g/kg BW				
proventriculus	0.40	0.38	0.011	0.492
gizzard	1.44	1.35	0.024	0.045
heart	0.57	0.55	0.018	0.514
pancreas	0.23	0.24	0.006	0.538
liver	2.15	2.14	0.039	0.930
spleen	0.10	0.10	0.004	0.911
bursa of Fabricius	0.22	0.20	0.009	0.174
thymus	0.24	0.24	0.009	0.735
bile, ml	0.45	0.49	0.059	0.734

BW – body weight, SEM – standard error of the mean; SO – basal diet enriched with soybean oil, HI – basal diet enriched with *Hermetia illucens* fa, *P* < 0.05 indicates that data are significantly different (n = 15)

Table 7. Effect of replacing soybean oil with *Hermetia illucens* larval fat on crop, ileal, and caecal digesta pH values (35 days)

Item	Treatments		SEM	*P*-value
	SO	HI		
Crop	5.52	5.38	0.104	0.518
Ileum	6.19	6.19	0.065	0.984
Caeca	6.68	6.81	0.065	0.346

SO – basal diet enriched with soybean oil, HI – basal diet enriched with *Hermetia illucens* fat, SEM – standard error of the mean, *P* > 0.05 indicates that data are not significantly different (n = 15)

enzyme remained unchanged (trypsin and amylase, *P* > 0.05), except for lipase (*P* = 0.047), which showed elevated activity in the HI group. The lengths and weights of selected GIT segments and internal organ are presented in Tables 5 and 6. The incorporation of insect dietary fat had no impact (*P* > 0.05) on the organs measured. Nevertheless, the HI treatment reduced gizzard weight (*P* = 0.045), and the length of the caecum tended to increase (*P* = 0.068) when *H. illucens* larval fat was added to the experimental diet.

Digesta pH and selected physiological traits

The experimental factor had no effect (*P* > 0.05) on yield, jejunum or caecal pH (Table 7). Nevertheless, insect fat added to the broiler diet significantly lowered serum IgG and T4 concentrations (Table 8). Moreover, the administration of *H. illucens* larval fat resulted in decreasing trends with respect to AST (*P* = 0.052), FT4 (*P* = 0.062), and IL-6 (*P* = 0.063)

Table 8. Effect of replacing soybean oil with *Hermetia illucens* larval fat in broiler diets on selected biochemical and physiological indices (35 days)

Item	Treatment		SEM	P-value
	SO	HI		
Biochemical serum indices				
glucose, mg/dl	164	164	2.48	0.320
TG, mg/dl	125	136	4.39	0.218
total cholesterol, mg/dl	166	184	7.22	0.494
albumin, g/dl	2.27	2.38	0.051	0.320
TP, g/dl	6.25	6.15	0.143	1.00
ALT, IU/l	5.82	6.72	1.288	0.862
AST, IU/l	69.2	56.8	3.31	0.052
ALP, IU/l	772	861	101.4	0.678
GGT, IU/l	3.32	4.15	0.333	0.225
Immune system traits				
IgA, mg/ml	6.78	4.71	0.690	0.105
IgG, mg/ml	9.47	5.49	0.994	0.021
IgY, µg/ml	1602	1327	300.8	0.505
TNF-α, ng/l	222	167	23.6	0.320
IL-6, ng/l	279	176	27.6	0.063
Selected hormones				
T3, nmol/l	5.39	4.34	0.318	0.098
T4, nmol/l	80.4	53.4	6.83	0.021
FT3, pmol/l	12.0	8.00	1.23	0.130
FT4, pmol/l	34.0	21.6	3.23	0.062
insulin, IU/l	91.0	52.1	13.19	0.156

SO – basal diet enriched with soybean oil, HI – basal diet enriched with *Hermetia illucens* fat; TG – triglyceride, TP – total protein, ALT – alanine transaminase, AST – aspartate aminotransferase, ALP – alkaline phosphatase, GGT – gamma-glutamyl transferase, IgA – immunoglobulin A, IgG – immunoglobulin G, IgY – immunoglobulin Y, TNF-α – tumour necrosis factor-alpha; IL-6 – Interleukin-6, T3 – triiodothyronine, T4 – thyroxine, FT3 – free triiodothyronine, FT4 – free thyroxine, SEM – standard error of the mean, $P < 0.05$ indicates that data are significantly different (n = 8)

levels. No significant ($P > 0.05$) changes were observed in glucose, TG, total cholesterol, albumin, TP, ALT, ALP, GGT, IgA, IgY, T3, FT3, and TNF-α concentrations, or insulin activity.

Discussion

The available literature have reported mainly neutral or positive effects of replacing soybean oil with HI larval fat in poultry diets regarding growth performance (Schiavone et al., 2018; Kim et al., 2020a; b). These results are consistent with the hypothesis that AME levels of both dietary fats are similar. Kierończyk et al. (2022) emphasised, based on estimated regression models for HI larval fat, and the commonly used INRAE-CIRAD-AFZ (2022) nutritive value tables for soybean oil, that the differences between these two dietary fats were negligible, specifically amounting to 14 kcal/kg for 21-day-old birds.

However, due to the need for precise feeding of poultry, the accurate energy density values of oil or fat for each feeding period should be used when formulating the diet. This precision is crucial as fat digestion and energy availability in the broiler gut varies significantly throughout the rearing process, primarily due to limited bile salt excretion in the first week of birds' life, as well as reduced lipase secretion and synthesis of fatty acid-binding protein (Ravindran et al., 2016). Tancharoenrat et al. (2013) demonstrated that the AME of selected fats doubled between the first and third week of broiler age, reaching a plateau thereafter. However, a limitation of the trial by Kierończyk et al. (2022) was the lack of evaluation of fat energy density in 1-week-old chicks. Consequently, using the proposed regression model might lead to energy overestimation during the starter period. Additionally, it should be noted that the AME of insect dietary fat may vary between batches due to the high variable nutritional value of the larvae, as a consequence of the chemical composition of the rearing substrate, with particular emphasis on the initial level of crude fat in the biomass (before extraction), and the final fatty acid profile of *H. illucens* dietary fat. Therefore, the primary objective of the present study was to validate the estimated regression models. Based on the results, which showed no adverse effect on growth performance, nutrient utilisation, AIDE, gastrointestinal tract segment or internal organ measurements, it can be concluded that the regression equations are suitable for application. The comparable or even superior availability of energy in the gut (Kierończyk et al., 2020; 2022b; Sypniewski et al., 2020), as demonstrated in the present study, can be attributed to the similar oleic acid (C18:1) concentration in the FA profile of both soybean oil and HI larval fat. Consequently, this similar C18:1 content potentially results in a comparable increase in lipase, as evidenced by the significant enhancement in the activity of this enzyme observed for HI larval fat administration. These findings align with those of Kierończyk et al. (2022), who reported a more than threefold increase in lipase activity when SO was fully replaced by HI in young turkey diets; however, it should be noted that Sypniewski et al. (2020) did not confirm these findings. Additionally, differences between these dietary fats can also be observed for linoleic acid (C18:2), which has been shown to improve fat digestion (Ravindran et al., 2016); however, the level of linoleic acid in HI fat is relatively low. Despite the potential of oleic and linoleic acids to improve energy availability in the avian gut, their effectiveness is directly influenced by feed intake (Józefiak et al., 2014), but in

the present study, feed ingestion was not affected. Additionally, the concentration of SFAs in HI larval fat may indicate limitations in energy availability in the bird gut. The dominant SFA in *H. illucens* biomass is lauric acid (C12:0), classified as a medium-chain fatty acid (MCFA). The absorption efficiency of MCFAs was shown to exceed that of long-chain fatty acids in the small intestine (Del Alamo et al., 2007). Moreover, MCFAs exert an indirect impact on AME access in birds through bacteriostatic effects, and thus reduced competition for nutrients of the host and endogenous microbiota (Józefiak et al., 2014). The presence of a high lauric acid concentration can reduce the count of lactic acid bacteria (LAB) in ileal digesta (Józefiak et al., 2016), potentially leading to reduced bile salt deconjugation and subsequent impairment of fat emulsification and lipid absorption (Kierończyk et al., 2016). Additionally, improvements in lipase, amylase or even trypsin activities have been documented in the literature (Sypniewski et al., 2020; Kierończyk et al., 2022b). However, these effects varied between experiments and should be further monitored.

Overall, the morphometric measurements of selected GIT segments and internal organs were consistent with the growth performance data. No significant changes between treatments were observed, except for the tendency towards increased length of the caecum and significant reduction in gizzard mass. The enlargement of the caecum may be explained by the high microbial production of SCFAs when HI fat is provided compared to soybean oil (Dabbou et al., 2020). On the other hand, a decrease in the relative weight of the gizzard was reported only by Kim et al. (2020a). This is challenging to explain, as gizzard development is mainly correlated with crude fibre levels and the physical form of the diet. In the present study, the experimental diets differed only in terms of dietary fat source, which is unlikely to affect gizzard mechanical activity. In addition, a properly developed gizzard may positively influence the degree of duodenal reflux, which in turn may improve nutrient digestion. However, no adverse effects of gizzard size were observed in relation to overconsumption, nutrient digestibility limitations or diet AIDE in the HI treatment, as opposed to literature reports (Svihus, 2011). It should be emphasised that both results, i.e., caecal relative length and gizzard mass, are unique, as in most cases, HI fat addition to broiler chicken diets did not result in any morphological changes.

The impact of HI fat on bird serum biochemical parameters appears to be modest, as reported in studies by Schiavone et al. (2017; 2018), Kim et al. (2021), and Kierończyk et al. (2022b). Nevertheless, the results confirm the positive effect of HI fat administration in poultry diets on liver function (Kierończyk et al., 2022b), as evidenced by a tendency toward decreased AST levels. Moreover, the observed decreased IgG and IL-6 concentrations indicated favourable effect of HI fat on the immune status. The present findings are in line with the studies of Sypniewski et al. (2020) and Kierończyk et al. (2022c), who reported a significant reduction in the level of the inflammatory marker IL-6 (mediator of the acute phase response). Nevertheless, decreased IgG concentrations can be comparable to effects observed with the addition of salinomycin to broiler chicken diets (Bortoluzzi et al., 2023) or the incorporation of probiotic preparations in the feed or '*in ovo*' (Oladokun and Adewole, 2023). It is well known that challenged birds typically show elevated immunoglobulin levels in the blood serum (Zhang et al., 2023). Therefore, contrary to the latter findings, the reduced IgG levels observed in the HI treatment suggests that lauric acid may potentially act against pathogenic microbiota and enhance immune function. Surprisingly, for the first time in the literature, changes in the levels of thyroid hormones (T4 and FT4) were found when *H. illucens* larval dietary fat was added to poultry diets. Generally, thyroid hormones play crucial role as regulators of metabolism, particularly in glucose metabolism, including gluconeogenesis and glycogen synthesis, as well as liver storage (Duntas and Brenta, 2018). Elevated levels of both have been frequently associated with improved growth performance (Amer et al., 2023), as T4 plays a key role not only in energy regulation, but also in protein metabolism, along with its ability to increase insulin-like growth factor (IGF-1) levels (Hemmati et al., 2019; Jahanpour et al., 2020). However, instances of reduced serum T4 concentrations have been observed in birds subjected to feed restriction (Ghanima et al., 2023) and chronic heat stress (Beckford et al., 2020). In contrast, lower T4 and FT4 levels were observed in the current study in birds fed the HI diet. Moreover, no adverse effects on growth performance or nutrient utilisation were observed. These findings are consistent with those of Bayraktar et al. (2023), who reported decreased T4 concentrations when *Moringa oleifera* essential oil was incorporated into quail diets. Similarly, the use of feed additives such as genistein or sodium butyrate also reduced T4 concentrations (El-Maaty

et al., 2023; Li et al., 2024). On the other hand, Skomorucha and Sosnówka-Czajka (2017) highlighted the considerable variability in thyroid hormone levels reported by different authors, independent of the experimental factor, such as heat stress exposure. These differences were also observed when HI larval meal was applied to poultry diets. For instance, Nassar et al. (2023) observed an increase in thyroid hormone levels, while Hatab et al. (2020) showed no changes in these parameters. Interestingly, Attivi et al. (2022) documented both non-significant and significant changes depending on the birds' age and HI larval meal inclusion. Thus, the effect of HI administration to poultry diets on serum thyroid hormone levels should be further evaluated to fully elucidate this phenomenon.

Conclusions

The present study indicates the feasibility of employing metabolizable energy regression models for black soldier fly larval fat supplementation in precision feeding of broiler chickens. Additionally, *Hermetia illucens* appears to offer dietary fat with more advantageous qualities compared to soybeans in terms of enhancing the utilisation of crude protein and digestible energy availability in the gut, including elevated pancreatic lipase activity. Moreover, black soldier fly larval fat may support bird liver function and immune status, as well as affect thyroid hormone metabolism.

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Conflict of interest

The Authors declare that there is no conflict of interest.

Data availability statement

All raw data obtained in this study are deposited in an official repository (https://doi.org/10.18150/UVAOTO).

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