sciendo

J Vet Res 67, 197-207, 2023
DOI:10.2478/jvetres-2023-0022

The impact of full-fat *Hermetia illucens* larvae meal on the health and immune system function of broiler chickens

Bartłomiej Tykałowski[1]✉, Andrzej Koncicki[1], Joanna Kowalczyk[1], Marcin Śmiałek[1], Tadeusz Bakuła[2], Daria Murawska[3], Wiesław Sobotka[4], Tomasz Stenzel[1]

[1]Department of Poultry Diseases, Faculty of Veterinary Medicine,
[2]Department of Veterinary Prevention and Feed Hygiene, Faculty of Veterinary Medicine,
[3]Department of Commodity Science and Animal Improvement, Faculty of Animal Bioengineering,
[4]Department of Animal Nutrition and Feed Science, Faculty of Animal Bioengineering,
University of Warmia and Mazury, 10-719 Olsztyn, Poland
bartlomiej.tykalowski@uwm.edu.pl

Received: November 22, 2022 *Accepted: April 12, 2023*

Abstract

Introduction: Alternative protein sources have recently been attracting growing interest as potential components of livestock nutrition. This study evaluated the effect on broiler health of replacing the soybean protein component of poultry feed with processed insect protein from farmed *Hermetia illucens* (HI) larvae. **Material and Methods:** A total of 384 male broiler chicks were divided into four diet groups (eight pens/treatment and 12 birds/pen) and reared to the 42nd day of life (dol). Each treatment group received a starter diet until the 14th dol, then a grower diet until the 35th and finally a finisher diet until the 42nd. The soybean meal in standard diets was replaced with full-fat HI larvae meal in the following amounts: 0% for the control group HI-0, 50% for group HI-50, 75% for group HI-75, and 100% for group HI-100. At 1 dol, chicks were vaccinated against Marek's disease, coccidiosis, Newcastle disease and infectious bronchitis and at 7 dol against avian metapneumovirus infections using live-attenuated vaccines. Blood and spleen samples were collected at three and six weeks of age and analysed using ELISA, flow cytometry, haematology and biochemistry. **Results:** On the 42nd dol, as the content of larvae meal in the chickens' diets increased, the birds' body weights decreased significantly. The substitution of the protein source had no effect on the haematological markers. In chicks that received larvae meal, there was a decrease in creatine kinase activity and phosphorus levels and an increase in calcium and uric acid levels in serum. Raising the proportion of full-fat HI larvae meal in the diet raised the percentage of T $CD3^+CD8a^+$ cells and lowered that of T $CD3^+CD4^+$ cells in both sample types. Chickens fed larvae meal had significantly lower post-vaccination anti-infectious bronchitis virus antibody titres. **Conclusion:** The poorer production results and impaired health in experimental birds may indicate lower than 50% protein substitution with full-fat HI larvae meal to be optimal.

Keywords: broiler chickens, health status, humoral and cell-mediated immunity, *Hermetia illucens*, processed insect protein.

Introduction

With global warming and environment destruction by the expansion of industry, the land area intended for food production is successively shrinking (11, 43). This situation urges the search for alternative food sources, with the most attention being paid to insects, which are perceived as food of the future (32). This claim is justified because insects have high contents of protein, fat, carbohydrates, fibre, minerals, amino acids and fatty acids, and offer an excellent production efficiency compared to other conventional food groups (21, 51). It is also worth emphasising that edible insects represent a source of many bioactive substances (29). These include chitin, which – as a polysaccharide – accounts for 5–20% of the dry weight of insects and may play an important role as dietary fibre (30). In addition, insects provide multiple peptides exhibiting antibacterial, antifungal and antiprotozoal activity (7, 39); therefore, they can mitigate the growing problem of drug resistance of microorganisms (6, 34). Furthermore, they contain peptides that inhibit the angiotensin-converting enzyme, thereby reducing blood pressure (46, 48), as well as antioxidant enzymes (4) and endopeptidases effective in

© 2023 B. Tykałowski et al. This is an open access article distributed under the Creative Commons Attribution-NonCommercial-NoDerivs license (http://creativecommons.org/licenses/by-nc-nd/3.0/)

the treatment of coeliac disease (12). Nevertheless, as van Huis and Oonincx (43) claimed, the nutritional value of insects varies depending on their diet, stage of development, sex, species or the environment they inhabit.

While edible insects are included in the multifaceted strategy of ensuring global food security and environmental sustainability of food production (where their merits are low greenhouse gas emissions, high feed conversion efficiency, low land use, and their ability to transform low-value organic side streams into high-value protein products) (42), their high nutritional value, minimal space requirements, and low environmental impact also make them an attractive option as an animal feedstuff (5, 45). In addition, insect-based animal feeds are particularly attractive in terms of price, especially because the cost of standard feeds accounts for around 70% of livestock production costs today (44). This motivates more and more researchers to address the use of insects as a protein-rich component of feed mixtures for poultry, pigs, and fish (3, 17–19, 27, 33, 35, 37, 45, 47, 49).

Although approximately 2,000 insect species are consumed in at least 113 countries (50), the most thoroughly analysed species that are potentially suitable for mass production for feedstuff purposes are only two, *Hermetia illucens* (HI) and *Tenebrio molitor* (11). This is because processed insect protein from HI larvae has been shown to be a practical source of protein in feed mixtures for broiler chickens, replacing up to 4% of fishmeal (3), and been shown not to suppress feed intake, feed conversion efficiency, egg production, hen health or immune status when added to a fodder for laying hens as the only source of protein, replacing soybean meal (27). Research by Ramos-Elorduy *et al.* (33) has also shown that a 5–10% addition of processed insect protein from *Tenebrio molitor* larvae could effectively replace 20–31% of soybean meal in a feed mixture for broiler chickens, and a study by Wang *et al.* (49) has demonstrated that this lifecycle stage of the insect replacing fishmeal in a feed mixture for laying hens increased egg production by 2.4%.

The literature data indicate that further research is necessary to more fully evaluate the possibility of deploying insects in animal nutrition and to assess the impact of such a diet on animal health. Therefore, this study was undertaken feeding broiler chickens a diet containing different proportions of full-fat HI larvae meal as substitutes for the protein of genetically modified soybean meal to assess its impact on their health and selected elements of their humoral and cellular immunity.

Material and Methods

Birds and housing. The current study was performed in the poultry house of the Department of Commodity Science and Animal Improvement of the University of Warmia and Mazury (Olsztyn, Poland). A total of 384 male Ross 308 broiler chicks obtained from a commercial hatchery were randomly distributed into four dietary treatment groups (eight pens/treatment and 12 birds/pen) and reared to the 42nd day of life (dol). Each pen was 1.10 m × 1.25 m (width × length), was equipped with a feeder and an automatic drinker, and provided pellets from straw as litter. During the first 3 weeks, the pens were heated by specialised electric radiators to maintain the temperature recommended for standard breeding practices consistent with the Ross Broiler Management Handbook (2). Fodder and water were provided *ad libitum* throughout the experiment. At the hatchery, one-day-old chickens were vaccinated against Marek's disease and coccidiosis. Then, upon delivery to the poultry house, they were vaccinated against Newcastle disease (ND) and infectious bronchitis (IB) and on the 7th dol the chickens were vaccinated against the diseases caused by avian metapneumoviruses (aMPV) using live-attenuated Nobilis Ma5 + Clone 30 (Merck, Rahway, NJ, USA) and Poulvac TRT (Zoetis, Parsippany, NJ, USA) vaccines applied individually in the dose recommended by the producers with a dropper at one drop (0.05 mL) per bird into one eye.

Diets and feeding programme. The diets were split into three phases for each treatment group: a starter diet (1–14 days), a grower diet (15–35 days) and a finisher diet (36–42 days) with the composition matched to the nutritional demands of broiler chickens. The protein from genetically modified soybean meal in standard diets was replaced with full-fat HI larvae meal in the following amounts: 50% for group HI-50, 75% for group HI-75, and 100% for group HI-100. Broiler chickens from group HI-0 were fed a diet without HI larvae meal and served as the controls. The composition of diets is presented in Table 1.

The dietary formulae in Table 1 were used to produce experimental feed mixture pellets at the Gorzyń Experimental Department of Animal Feeding, Poznań University of Life Sciences (Poznań, Poland). The full-fat HI larvae meal was obtained from a commercial producer (HiProMine S.A., Robakowo, Poland). The composition and energy value of full-fat HI larvae meal are shown in Table 2.

Clinical and anatomopathological examination of chickens. Body weight gains and feed conversion ratio (FCR), the number of culled birds and the mortality rate were registered throughout the experiment. Dead birds were subjected to anatomopathological examinations.

Sample collection. Blood samples were collected from the wing vein into sterile tubes without anticoagulant for serological (n = 23) and biochemical (n = 10) analyses as well as into sterile tubes containing ethylenediaminetetraacetic acid K2 anticoagulant for haematological (n = 5) and flow cytometry analyses (n = 8). For haematological, biochemical, serological and cytometric analyses, blood was sampled on the 21st and 42nd dol. The birds were sacrificed on the 42nd dol,

and spleens were collected from eight birds (n = 8) from each group. These were used together with the blood samples for isolation of mononuclear cells and determination of the sizes of the $CD3^+CD4^+$, $CD3^+CD8a^+$ and $CD4^+CD8a^+$ T cell subpopulations and the $CD3^-Bu\text{-}1^+$ B cell subpopulation by flow cytometry.

Table 1. Ingredient composition and nutrient content of complete diets (g/kg, as-fed basis) with or without full-fat *Hermetia illucens* (HI) larvae meal fed to broiler chickens at 1–14, 15–35 and 36–42 days of life (dol)

Item	Diet											
	Starter (1–14 dol)				Grower (15–35 dol)				Finisher (36–42 dol)			
	HI-0	HI-50	HI-75	HI-100	HI-0	HI-50	HI-75	HI-100	HI-0	HI-50	HI-75	HI-100
Substitution[1] (%)	0	50	75	100	0	50	75	100	0	50	75	100
Ingredients												
Corn	300.0	300.0	300.0	300.0	300.0	300.0	300.0	300.0	200.0	200.0	200.0	200.0
Wheat	288.0	303.6	288.1	274.6	315.6	330.3	319.0	311.2	467.6	494.6	489.6	484.1
Soybean meal	340.0	170.0	85.0	0.0	300.0	150.0	75.0	0.0	240.0	120.0	60.0	0.0
Soybean oil	30.0	0.0	0.0	0.0	45.0	25.0	30.0	25.0	55.0	35.0	30.0	25.0
HI larvae meal	0.0	200.0	300.0	400.0	0.0	170.0	250.0	340.0	0.0	130.0	200.0	270.0
Monocalcium phosphate	15.0	4.0	4.0	3.0	13.0	4.0	4.0	3.0	12.0	3.0	3.0	3.0
Fodder chalk	12.5	6.0	6.0	4.0	12.5	6.0	6.0	4.0	12.5	4.0	4.0	4.0
Sodium bicarbonate	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5
Fodder salt	2.0	0.5	0.5	0.5	2.0	1.0	0.0	0.0	2.0	0.5	0.0	0.0
L-Lysine HCl	2.5	4.2	4.5	6.0	2.5	3.5	5.7	6.5	1.8	3.0	3.5	4.0
DL-Methionine	2.4	2.8	3.0	3.0	1.6	1.7	1.9	1.9	1.2	1.5	1.5	1.5
L-Threonine	0.2	1.5	1.5	1.5	0.4	1.0	1.0	1.0	0.5	1.0	1.0	1.0
Choline chloride	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.9
Mineral-vitamin premix[2]	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
Calculated nutrient content												
AME_N, kcal/kg	3,000	3,000	3,000	3,000	3,120	3,120	3,120	3,120	3,180	3,180	3,180	3,180
Total protein[4]	233.6	238.1	237.8	237.2	213.8	218.2	214.9	216.3	196.1	192.5	194.5	195.0
Lysine[3]	13.5	13.5	13.5	13.5	11.7	11.8	11.8	11.8	10.0	10.0	10.0	10.0
Methionine[3]	6.3	6.4	6.7	6.7	5.9	6.0	6.0	6.0	4.7	4.9	5.0	5.0
Met+ Cys[3]	10.1	10.2	10.2	10.3	9.5	9.6	9.6	9.6	7.5	7.7	7.7	7.7
Threonine[3]	8.2	8.2	8.4	8.4	8.2	8.4	8.4	8.4	6.8	7.0	7.0	7.1
Tryptophan[3]	2.3	2.3	2.3	2.3	2.2	2.1	2.2	2.1	2.1	2.0	2.0	2.0
Crude fibre[4]	28.8	32.4	31.3	34.2	27.8	35.3	35.6	35.8	27.8	30.5	31.7	31.2
Crude fat[4]	50.3	74.0	93.5	131.5	64.9	75.6	105.7	125.7	75.8	82.2	104.3	119.1
Total calcium[3]	10.0	10.0	9.9	9.9	9.4	9.4	9.3	9.3	8.8	8.8	8.6	8.6
Available phosphate[3]	4.8	4.9	4.9	4.9	4.5	4.6	4.6	4.6	4.0	4.1	4.1	4.1
Na[3]	1.7	1.7	1.8	1.8	1.7	1.6	1.6	1.6	1.7	1.6	1.6	1.6

[1] – Substitution of proteins from genetically modified soybean meal with processed insect protein derived from farmed *Hermetia illucens* (HI) larvae

[2] – Premix composition on days 1–35: 2,400,000 IU of vitamin A; 600,000 IU of vitamin D_3; 10,000 IU of vitamin E; 600 mg of vitamin K_3; 400 mg of vitamin B_1; 1,400 mg of vitamin B_2; 6,000 mg of niacin (B_3); 2,800 mg of pantothenic acid; 800 mg of vitamin B_6; 5,000 µg of vitamin B_{12}; 30,000 µg of biotin; 80,000 mg of choline chloride; 300 mg of folic acid; 14,000 mg of iron; 20,000 mg of manganese; 2,400 mg of copper; 12,000 mg of zinc; 200 mg of iodine; 80 mg of cobalt; 50 mg of selenium; 5,000 mg of antioxidant; 240 g of calcium; 14,000 mg of salinomycin coccidiostat. Premix composition on days 36–42: 1,800,000 IU of vitamin A; 600,000 IU of vitamin D_3; 7,500 IU of vitamin E; 300 mg of vitamin K_3; 300 mg of vitamin B_1; 1,000 mg of vitamin B_2; 4,000 mg of niacin (B_3); 2,800 mg of pantothenic acid; 600 mg of vitamin B_6; 4,000 µg B_{12}; 24,000 µg of biotin; 80,000 mg of choline chloride; 300 mg of folic acid; 14,000 mg of iron; 20,000 mg of manganese; 2,400 mg of copper; 12,000 mg of zinc; 200 mg of iodine; 80 mg of cobalt; 50 mg of selenium; 5,000 mg of antioxidant; 340 g of calcium

[3] – Calculated according to Polish feedstuff analysis tables (40)

[4] – Values analytically evaluated according to standard methods (1)

DL – dextro- and levorotatory; L – levorotatory; AME_N – Apparent metabolisable energy corrected to zero nitrogen balance; Met – methionine; Cys – cysteine

Table 2. Ingredient composition and energy value of full-fat *Hermetia illucens* larvae meal

Ingredients	%
Dry matter	93.20
Crude protein	40.76
Crude fat	29.38
Crude fibre	6.65
Crude ash	6.94
Ca	1.38
Na	0.23
Total P	0.78
Energy value	MJ/kg
Gross energy	24.81
AME_N	18.16

AME_N – Apparent metabolisable energy corrected to zero nitrogen balance

Haematological analysis. The blood samples were analysed for red blood cell count, haemoglobin concentration, packed cell volume, white blood cell count, and percentage composition of leukocytes (by leukogram) – including percentages of lymphocytes, monocytes and granulocytes (heterophils, eosinophils and basophils). Haematological analyses were performed in accordance with the methodology described by Krasnodębska-Depta and Koncicki (23).

Biochemical analysis. Blood serum samples were evaluated for levels of total protein and activity of selected enzymes, including alanine aminotransferase, aspartate aminotransferase (AST), alkaline phosphatase, lactate dehydrogenase (LDH) and creatine kinase (CK) as well as for total concentrations of calcium (CALC), phosphorus (PHOS) and uric acid (UA). The tests were performed using a VetTest Chemistry Analyzer (IDEXX Laboratories, Westbrook, MN, USA) according to the manufacturer's instructions.

Serological analysis. Vaccine-induced immunoglobulin Y titres of anti-ND, anti-IB and anti-aMPV antibodies were evaluated with the use of a commercial ELISA kit (IDEXX Laboratories, Westbrook, MN, USA) according to the manufacturer's recommendations. The ELISA assay was performed with an Eppendorf epMotion 5075 LH automated pipetting system (Eppendorf, Hamburg, Germany), a BioTek Elx405 washer and Elx800 absorbance microplate reader (Agilent, Santa Clara, CA, USA) and a KBF 115 constant climate chamber (Binder, Tuttlingen, Germany).

Isolation of mononuclear cells and flow cytometry. Mononuclear cells from the blood and spleens were isolated according to a previously described procedure (22). The cells were counted, and their viability was evaluated using the Vi–Cell XR cell counter (Beckman Coulter, Brea, CA, USA). Viable mononuclear cells (1×10^6) were stained with Pacific Blue–conjugated Mouse Anti–Chicken CD3-PACBLU clone CT-3, fluorescein–conjugated Mouse Anti–Chicken CD4-FITC clone CT-4, Alexa Fluor 647–conjugated Mouse Anti–Chicken CD8a-AF647 clone CT-8, and phycoerythrin–conjugated Mouse Anti–Chicken Bu-1–PE clone AV-20 (Southern Biotech, Birmingham, AL, USA). Data were acquired using a FACSAria II digital flow cytometer (Becton Dickinson, Franklin Lakes, NJ, USA) in the FACSDiva 8.0 environment (Becton Dickinson). A total of 100,000 events were recorded for each sample. The immunophenotype and sizes of subpopulations of T $CD3^+CD4^+$, $CD3^+CD8a^+$ lymphocytes, double-positive cells ($CD4^+CD8a^+$), and B cells ($CD3^-Bu\text{-}1^+$) were analysed using FlowJo V10 software (Becton Dickinson). Fluorescence minus one controls for Pacific Blue, FITC, Alexa Fluor 647 and PE fluorochromes were used to determine the cut–off point between background fluorescence and positive populations.

Statistical analysis. All calculations were made using Statistica 13.1 software (StatSoft, Kraków, Poland). The significance of differences in values of the measured parameters between the investigated groups was analysed with Student's *t*-test for independent variables and post-hoc Tukey's test. Differences were considered statistically significant at $P \leq 0.05$.

Results

Health of broiler chickens. The production parameters for the broiler chickens are presented in Table 3.

On the 42 dol, the birds differed significantly by treatment group in terms of body weights, especially in the group fed the diet with the highest content of insect larvae meal. As observed at this time point (experiment end), the average group body weights of broiler chickens were significantly lower in correlation with the increasing content of full-fat HI larvae meal in the diet group by group, and only reached 2,375 g in group HI-100 compared to 3,046 g in group HI-0. The body weights of birds from groups HI-50 and HI-75 were significantly lower than those from group HI-0 and significantly higher than those from group HI-100. Also the FCR evaluated for birds from group HI-100 was significantly higher than the FCR of birds from the other groups and was 1.77 *vs* 1.63 in HI-0. In groups HI-50 and HI-75 FCR, it was 1.59 and was lower than in group HI-0 despite the statistically significant body weight differences between the HI meal-fed birds and the control group birds. The culling and mortality rate reached 28.2% in group HI-100 but was only 3.15% in group HI-0. The high number of culled and dead birds in group HI-100 was due to growth inhibition and gizzards becoming filled with pellets of the litter on which the birds were raised.

Haematological parameters. The haematological parameters are summarised in Table 4 (for 21-day-old chickens) and in Table 5 (for 42-day-old chickens).

The substitution of soybean meal with the full-fat HI larvae meal had no effect on the values of the haematological markers evaluated in either 21-day-old or 42-day-old chickens, regardless of the dietary inclusion level of larvae meal.

Biochemical parameters. The results of the biochemical analyses are set out in Table 6 (for 21-day-old chickens) and Table 7 (for 42-day-old chickens).

The levels of soybean meal substitution with full-fat HI larvae meal had no significant effect on the majority of the analysed parameters in 21-day-old chickens. In all groups that received full-fat HI larvae meal, there was a significant decrease in CK activity and PHOS levels compared to the control group. In addition, the HI-100 group chickens showed a statistically significant increase in serum CALC and UA levels compared to the control group (HI-0).

A statistically significant increase in AST, CK and LDH activity and UA level was found in 42-day-old chicks that had received full-fat HI larvae meal compared to control birds. In addition, there was a statistically significant increase in CALC and decrease in PHOS levels in the HI-75 and HI-100 groups compared to the control chickens from group HI-0.

Serological parameters. The results of serological analyses are provided in Table 8.

On the 42nd of life, the broiler chickens fed a diet with the addition of full-fat HI larvae meal had highly significantly lower titres of anti-infectious bronchitis virus (IBV) antibodies compared to the control birds (HI-0).

Immunological parameters. Tables 9 and 10 present the T and B cell subpopulations in blood and spleen samples of broiler chickens.

On the 21st dol, the percentage of T cells which were $CD3^+CD4^+$ was significantly higher in the blood samples of group HI-75 than in those of HI-0 and HI-50. The size of the $CD3^+CD8a^+$ T cell subpopulations was significantly lower in group HI-100 in comparison with HI-50. The full-fat HI larvae meal had no significant effect on the size of the T and B cell subpopulations in blood samples collected from birds on day 42 of life.

No significant differences between treatment groups were found in the percentages of immune cells which were $CD4^+CD8a^+$ T cells and $CD3^-Bu\text{-}1^+$ B cells in blood and spleen samples. On the 42nd dol, the percentage of T cells which were $CD3^+CD4^+$ was significantly lower in the spleen samples of group HI-100 than in those of HI-0 and HI-50. In general, the increasing proportion of full-fat HI larvae meal in the diet increased the percentage of $CD3^+CD8a^+$ T cells and decreased that of $CD3^+CD4^+$ T cells in both blood and spleen samples in 42-day-old chickens.

Table 3. Production parameters of broiler chickens fed diets with different levels of full-fat *Hermetia illucens* (HI) larvae meal

Parameter	Age (days)	Group				SEM	P-value
		HI-0	HI-50	HI-75	HI-100		
BW (g)	42	3046.0[a]	2727.0[b]	2504.5[b]	2378.0[c]	39.80	<0.001
FCR (kg/kg)	1–42	1.63[a]	1.59[a]	1.59[a]	1.77[b]	0.068	0.009
CM (%)	1 – 42	3.15[a]	5.20[b]	7.20[c]	28.20[d]	0.62	<0.001

BW – body weight; FCR – feed conversion ratio; CM–culling and mortality
HI-0 – group that received fodder without the addition of full-fat HI larvae meal; HI-50 – group that received fodder with proteins from soybean meal and full-fat HI larvae meal in a 50:50 ratio; HI-75 – group that received fodder with proteins from soybean meal and full-fat HI larvae meal in a 25:75 ratio; HI-100 – group that received fodder exclusively with proteins from full-fat HI larvae meal
SEM – standard error of the mean
[a–d] – mean values within a row (dietary treatment) not followed by a common superscript are significantly different by Tukey's test ($P < 0.05$)

Table 4. Mean values (± standard deviation) of haematological parameters in blood samples of 21-day-old broiler chickens (n = 5)

Group	Parameter				Leukogram (%)				
	RBC (10^{12}/L)	WBC (10^9/L)	Hb (g/dL)	PCV (%)	Lymphocytes	Granulocytes			Monocytes
						Heterophils	Eosinophils	Basophils	
HI-0	1.92 (±0.42)	29.60 (±24.00)	9.34 (±1.25)	25.40 (±3.43)	53.60 (±12.36)	39.60 (±11.06)	0.60 (±0.45)	1.20 (±0.45)	5.20 (±3.70)
HI-50	1.56 (±0.54)	26.70 (±14.75)	9.1 (±0.34)	25.60 (±0.55)	57.40 (±9.94)	37.00 (±10.91)	0.80 (±1.09)	2.20 (±2.86)	3.00 (±1.41)
HI-75	1.22 (±0.26)	18.2 (±7.16)	9.38 (±0.58)	26.60 (±0.89)	60.20 (±6.94)	34.00 (±9.77)	0.80 (±1.30)	1.80 (±1.09)	3.20 (±3.11)
HI-100	1.58 (±0.40)	20.90 (±9.15)	8.82 (±1.35)	24.20 (±2.95)	55.80 (±10.01)	37.80 (±11.63)	0.90 (±1.31)	1.60 (±0.89)	4.80 (±2.39)

HI-0 – group that received fodder without the addition of full-fat HI larvae meal; HI-50 – group that received fodder with proteins from soybean meal and full-fat HI larvae meal in a 50:50 ratio; HI-75 – group that received fodder with proteins from soybean meal and full-fat HI larvae meal in a 25:75 ratio; HI-100 – group that received fodder exclusively with proteins from full-fat HI larvae meal
RBC – red blood cells; WBC – white blood cells; Hb – haemoglobin; PCV – packed cell volume

Table 5. Mean values (± standard deviation) of haematological parameters in blood samples of 42-day-old broiler chickens (n = 5)

Group	Parameter				Leukogram (%)				
	RBC (10^{12}/L	WBC (10^{9}/L)	Hb (g/dL)	PCV (%)	Lymphocytes	Granulocytes			Monocytes
						Heterophils	Eosinophils	Basophils	
HI-0	2.02 (±0.43)	10.40 (±4.80)	11.26 (±0.89)	30.80 (±1.30)	26.30 (±13.92)	77.40 (±12.99)	0.40 (±0.42)	0.20 (±0.40)	4.20 (±2.97)
HI-50	1.40 (±0.35)	17.60 (±3.36)	9.84 (±0.69)	26.40 (±1.95)	34.00 (±11.91)	62.20 (±11.61)	0.30 (±0.26)	0.10 (±0.25)	3.8 (±2.77)
HI-75	1.48 (±0.16)	11.70 (±4.98)	9.48 (±0.67)	25.60 (±1.67)	29.60 (±13.06)	73.40 (±12.54)	0.30 (±0.67)	0.20 (±0.33)	4.6 (±3.21)
HI-100	1.34 (±0.13)	17.50 (±9.28)	10.28 (±1.06)	27.80 (±2.49)	25.30 (±19.51)	42.80 (±41.91)	0.50 (±1.09)	0.30 (±0.89)	3.8 (±2.48)

HI-0 – group that received fodder without the addition of full-fat HI larvae meal; HI-50 – group that received fodder with proteins from soybean meal and full-fat HI larvae meal in a 50:50 ratio; HI-75 – group that received fodder with proteins from soybean meal and full-fat HI larvae meal in a 25:75 ratio; HI-100 – group that received fodder exclusively with proteins from full-fat HI larvae meal
RBC – red blood cells; WBC – white blood cells; Hb – haemoglobin; PCV – packed cell volume

Table 6. Mean values (± standard deviation) of biochemical parameters in blood serum samples of 21-day-old broiler chickens (n = 10)

Group	Parameter								
	ALT (U/L)	AST (U/L)	ALP (U/L)	TP (g/dL)	CK (U/L)	LDH (U/L)	CALC (mg/dL)	PHOS (mg/dL)	UA (mg/dL)
HI-0	9.80 (±4.78)	239.00 (±114.00)	29,881.00 (±36,308.00)	2.60 (±0.19)	4,966.00^{a} (±2,563.00)	1,780.00 (±133.00)	11.43^{a} (±0.60)	7.81^{a} (±0.78)	6.75^{a} (±1.45)
HI-50	9.21 (±5.16)	177.00 (±27.00)	39,223.00 (±48,949.00)	2.69 (±0.26)	2,058.00^{b} (±542.00)	1,502.00 (±699.00)	12.27^{ab} (±1.70)	4.45^{b} (±0.86)	8.80^{ab} (±3.02)
HI-75	8.69 (±7.64)	163.00 (±9.00)	33,367.00 (±34,329.00)	2.71 (±0.23)	2,104.00^{b} (±699.00)	1,419.00 (±542.00)	12.33^{ab} (±1.30)	6.06^{b} (±1.29)	8.41^{ab} (±6.03)
HI-100	8.93 (±6.78)	169.00 (±15.00)	19,175.00 (±23,075.00)	2.64 (±0.19)	2,021.00^{b} (±1,330.00)	1,588.00 (±541.00)	12.58^{b} (±0.90)	6.13^{b} (±1.15)	10.10^{b} (±3.64)

HI-0 – group that received fodder without the addition of full-fat HI larvae meal; HI-50 – group that received fodder with proteins from soybean meal and full-fat HI larvae meal in a 50:50 ratio; HI-75 – group that received fodder with proteins from soybean meal and full-fat HI larvae meal in a 25:75 ratio; HI-100 – group that received fodder exclusively with proteins from full-fat HI larvae meal
ALT – alanine aminotransferase; AST – aspartate aminotransferase; ALP – alkaline phosphatase; TP – total protein; CK – creatine kinase; LDH – lactate dehydrogenase; CALC – calcium; PHOS – phosphorus; UA – uric acid
$^{a–b}$ – mean values in a column not followed by a common superscript are significantly different at $P \leq 0.05$

Table 7. Mean values (± standard deviation) of biochemical parameters in blood serum samples of 42-day-old broiler chickens (n = 10)

Group	Parameter								
	ALT (U/L)	AST (U/L)	ALP (U/L)	TP (g/dL)	CK (U/L)	LDH (U/L)	CALC (mg/dL)	PHOS (mg/dL)	UA (mg/dL)
HI-0	14.50 (±7.42)	428.00^{a} (±69.00)	9,020.00 ±4,594.00	3.01 ±0.29	28,086.00^{a} ±7,286.00	4,679.00^{a} ±981.00	11.14^{a} ±0.18	7.09^{a} ±0.85	4.60^{a} ±1.97
HI-50	11.70 (±8.16)	338.00^{b} ±61.00	5,668.00 ±2,398.00	3.16 ±0.20	15,321.00^{b} ±5,732.00	2,843.00^{b} ±434.00	11.35^{ab} ±0.63	6.49^{ab} ±0.94	6.66^{b} ±1.68
HI-75	12.30 (±7.16)	265.00^{c} ±30.00	6,662.00 ±2,795.00	3.27 ±0.27	13,469.00^{b} ±3,085.00	2,206.00^{c} ±459.00	12.61^{b} ±0.42	5.73^{b} ±0.54	6.70^{b} ±1.36
HI-100	10.70 (±6.29)	278.00^{bc} ±51.00	5,575.00 ±2,288.00	3.21 ±0.12	14,805.00^{b} ±5,483.00	2,280.00^{c} ±322.00	11.95^{b} ±0.99	5.80^{b} ±0.85	6.78^{b} ±2.68

HI-0 – group that received fodder without the addition of full-fat HI larvae meal; HI-50 – group that received fodder with proteins from soybean meal and full-fat HI larvae meal in a 50:50 ratio; HI-75 – group that received fodder with proteins from soybean meal and full-fat HI larvae meal in a 25:75 ratio; HI-100 – group that received fodder exclusively with proteins from full-fat HI larvae meal
ALT – alanine aminotransferase; AST – aspartate aminotransferase; ALP – alkaline phosphatase; TP – total protein; CK – creatine kinase; LDH – lactate dehydrogenase; CALC – calcium; PHOS – phosphorus; UA – uric acid
$^{a–c}$ – mean values in a column not followed by a common superscript are significantly different at $P \leq 0.05$

Table 8. Mean values of post-vaccination antibody titres in serum samples of broiler chickens on the 21st and 42nd days of life (n = 23)

Group	Titre age of birds (days)					
	aMPV		NDV		IBV	
	21	42	21	42	21	42
HI-0	9	5	213	197	172	2,147[a]
HI-50	5	1	287	108	140	877[b]
HI-75	26	27	209	88	169	866[b]
HI-100	5	25	368	117	133	918[b]

HI-0 – group that received fodder without the addition of full-fat HI larvae meal; HI-50 – group that received fodder with proteins from soybean meal and full-fat HI larvae meal in a 50:50 ratio; HI-75 – group that received fodder with proteins from soybean meal and full-fat HI larvae meal in a 25:75 ratio; HI-100 – group that received fodder exclusively with proteins from full-fat HI larvae meal
aMPV – avian metapneumovirus; NDV – Newcastle disease virus; IBV – infectious bronchitis virus;
[a–b] – mean values in a column not followed by a common superscript are significantly different at $P \leq 0.05$

Table 9. Results of cytometric analysis of blood samples of broiler chickens on the 21st and 42nd days of life (n = 8)

Group	Mean size of particular T and B cell subpopulations as percentages of gated lymphocytes (± standard deviation) age of birds (days)							
	$CD3^+CD4^+$		$CD3^+CD8a^+$		$CD4^+CD8a^+$		$CD3^-Bu\text{-}1^+$	
	21	42	21	42	21	42	21	42
HI-0	4.81[a] (±0.82)	9.59 (±3.88)	3.70[ab] (±1.36)	2.12 (±0.69)	0.55 (±0.25)	0.27 (±0.13)	5.2 (±0.55)	5.19 (±0.63)
HI-50	5.09[a] (±0.89)	7.22 (±2.00)	3.95[a] (±1.06)	2.20 (±2.28)	0.39 (±0.11)	0.22 (±0.11)	5.49 (±0.42)	5.08 (±0.32)
HI-75	6.60[b] (±1.17)	7.58 (±3.61)	3.68[ab] (±1.07)	2.99 (±1.82)	0.45 (±0.28)	0.21 (±0.10)	5.19 (±1.00)	5.24 (±0.56)
HI-100	5.67[ab] (±1.42)	6.59 (±1.12)	2.34[b] (±1.04)	3.27 (±1.76)	0.46 (±0.23)	0.24 (±0.10)	5.09 (±0.56)	5.58 (±0.39)

HI-0 – group that received fodder without the addition of full-fat HI larvae meal; HI-50 – group that received fodder with proteins from soybean meal and full-fat HI larvae meal in a 50:50 ratio; HI-75 – group that received fodder with proteins from soybean meal and full-fat HI larvae meal in a 25:75 ratio; HI-100 – group that received fodder exclusively with proteins from full-fat HI larvae meal
[a–b] – mean values in a column not followed by a common superscript are significantly different at $P \leq 0.05$

Table 10. Results of cytometric analysis of spleen samples of broiler chickens on the 42nd day of life (n = 8)

Group	Mean size of particular T and B cell subpopulations as percentages of gated lymphocytes (± standard deviation)			
	$CD3^+CD4^+$	$CD3^+CD8a^+$	$CD4^+CD8a^+$	$CD3^-Bu\text{-}1^+$
HI-0	30.91[a] ±7.36	49.14 ±7.20	2.16 ±0.74	13.92 ±0.84
HI-50	25.18[a] ±6.37	51.14 ±5.34	2.17 ±0.48	14.86 ±2.72
HI-75	23.06[ab] ±4.12	55.48 ±4.00	1.81 ±0.57	13.03 ±1.63
HI-100	22.70[b] ±4.95	55.98 ±5.47	1.95 ±0.75	14.51 ±3.33

HI-0 – group that received fodder without the addition of full-fat HI larvae meal; HI-50 – group that received fodder with proteins from soybean meal and full-fat HI larvae meal in a 50:50 ratio; HI-75 – group that received fodder with proteins from soybean meal and full-fat HI larvae meal in a 25:75 ratio; HI-100 – group that received fodder exclusively with proteins from full-fat HI larvae meal
[a–b] – mean values in a column not followed by a common superscript are significantly different at $P \leq 0.05$

Discussion

Insects are receiving much attention these days as a proposition for a food of the future (32). Interest in edible insects increased sharply when the Food and Agriculture Organization (FAO) began promoting them as a viable dietary option for humans (44). They have also drawn growing interest as an attractive source of animal feedstuff (5, 45), especially in response to Commission Regulation (EU) 2017/893 of 24 May 2017 on the possibility of using insect protein in feeding strategies for aquaculture and to EU Regulation No. 2021/1372 allowing the use of processed animal proteins derived from farmed insects in fodders for poultry and pigs (13).

In response to the greater awareness of the exploitability of insect protein, numerous studies have been conducted for many years on the economic effects of feeding poultry with diets with different inclusion levels of this protein as fishmeal or soybean meal replacement (3, 27, 33, 49). Józefiak *et al.* (18) showed that a 0.2% addition of full-fat *Hermetia illucens* and *Tenebrio molitor* meal to fodders for chickens increased feed intake and improved the microbiota of their gastrointestinal tract. However, on the aspects of poultry health and immune system function, to the best of the authors' knowledge there were no studies prior to the present research on the impact of feed mixtures with insect meal.

The analysis of the production results from the present study indicates a progressive negative impact of the increasing content of insect protein in the feed mixtures on the body weight gains, FCR values, and bird mortality. This seems to be in contradiction to the results of previous insect meal feed studies conducted on various species of birds including broiler chickens, in which the meal yielded a beneficial effect on the production performance of birds (9, 20). Earlier studies with laying hens demonstrated better body weight gains and better FCR in the groups receiving feed mixtures with the addition of partly de-fatted HI larvae meal (28, 31). In the study by Marono *et al.* (27) with laying hens, although a more favourable feed conversion rate was noted in the experimental group than in the control group, other production parameters such as laying percentage, feed intake and average egg weight were significantly lower. Interestingly, in the case of studies by Schiavone *et al.* (37), replacing 50% and 100% of soybean oil with fat from HI larvae had no effect on the growth or body weight of the birds. Similarly, Sypniewski *et al.* (41) found no effect of soybean meal substitution with insect protein on the production performance of turkeys. It seems that the influence of the insect component on the growth and development of birds is not clear or obvious. These differences in findings may depend on the species and utility type of birds, the period of feeding them with mixtures containing the insect component, the content of chitin in the diet and its potential adverse impact on protein digestibility, the content of insect protein in the diet, the process of preparation of feed components, in particular larvae peeling, and even its form and colours, as confirmed by previous studies (9, 36). Some authors suggested that daily feed intake could be affected by fodder colour, because HI meal is dark brown in colour and darker than soybean meal, and therefore is less willingly consumed by birds. Perhaps only purified protein and insect fat should be used for poultry feed production.

The results of haematological analyses show that the diet with insect protein addition had no significant influence on evaluated parameters, which is consistent with the findings reported by Schiavone *et al.* (38) and Li *et al.* (25). However, a decrease in the number of peripheral white blood cells was observed in 21-day-old chicks, which corresponds to the results obtained by de Souza Vilela *et al.* (10). The percentages of lymphocytes, granulocytes and monocytes remained normal both in the control group and in the experimental groups, which leads to the conclusion that full-fat HI larvae meal had no effect on the white blood cell profile. The leukogram results obtained correspond with the findings of Marono *et al.* (27).

Based on the selected biochemical parameters, it is possible to determine the impact of the diet on liver, kidney, bone and muscle functions in birds (15). Although there were statistically significant differences between the groups in the levels of CALC, PHOS, and UA and the activities of AST, CK, and LDH, the values of these parameters were still within the reference ranges for this poultry species (23). The presented results correspond to the observations of other authors. Marono *et al.* (27) noted a significant increase in calcium and a decrease in serum phosphorus levels in laying hens fed fodders with HI meal. It is not easy to explain the significantly higher levels of CALC in blood of broiler chickens from the HI-75 and HI-100 groups compared to the HI-0 group, because the fodders used in the experiment contained similar levels of calcium. Uric acid is the major poultry nitrogenous waste product and its level in serum reflects protein catabolism (15). Our study showed that increasing levels of HI larvae meal increased serum UA levels in chickens. These results are inconsistent with those obtained by Marono *et al.* (27): those authors did not show any effect of HI larvae meal on UA levels in the serum of laying hens.

When assessing the post-vaccination response, it should be noted that out of the three vaccinations given to the broiler chickens as part of the experiment, the one against infectious bronchitis should elicit the strongest immune response, because IBV antigens are characterised by the highest immunogenicity. This is reflected in the results of serological tests: the six-week-old birds showed more clearly marked IBV seroconversion than the three-week-old birds. As our observations show, feeding birds a diet in which the standard protein component was replaced by HI larvae protein significantly impaired the effectiveness and efficacy of

vaccination against IBV, as manifested by the statistically significantly lower titres of post-vaccination antibodies in six-week-old birds from these groups. This situation is most likely due to lower daily feed intake, which resulted in poorer body weight gains and deterioration of the general condition of birds in these groups, which translated into low production results and debilitated immune systems. Unfortunately, so far there has been no similar research on the development of post-vaccination immunity in birds fed with insect protein supplemented diets. The only available reports on the protein obtained from HI larvae and the efficacy of vaccination concern fish and indicate a complete lack of its influence on the level of anti-infectious pancreatic necrosis virus specific antibodies (26). On the other hand, the results of the present study correspond with findings of other authors concerning the impact of deficient feeds on post-vaccination immunity in poultry, *e.g.* with the research by Chen *et al.* (8), in which birds fed a diet low in lysine had lower titres of anti-NDV antibodies, which confirms the effect of the feed provided to birds on the functioning of their immune systems.

The cytometric analysis of blood and spleen samples conducted in the present study showed no effect of the protein component on the size of the B cell subpopulation, but indicated an increase in the percentage of T cells which were $CD3^+CD8a^+$ and a decrease in the percentage of them which were $CD3^+CD4^+$. These results contradict the research of Lee *et al.* (24), where the $CD3^+CD4^+$ subpopulation of spleen T lymphocytes significantly increased with the addition of increasing levels (1, 2 or 3%) of HI larvae protein in fodder. On the other hand, de Souza Vilela *et al.* (10) observed not only a general decrease in the percentage of white blood cells in haematological analyses, but also a decrease in the percentage of $CD3^+CD8^+$ jejunal intraepithelial T lymphocytes in chickens fed fodder supplemented with 20% full-fat HI larvae meal. According to the observations of Huo *et al.* (16), the fatty acid profile in feed materials has a substantial impact on the formation of immune mechanisms and the ratio of $CD4^+$ to $CD8^+$ T cells. *Hermetia illucens* larvae have a high content of lauric acid, which, in addition to its antibacterial properties, elicits an immunomodulatory effect (14).

One conclusion of the present study results is that full-fat HI larvae meal used as a protein and substitute for genetically-modified soybean meal has a negative effect on the health of broiler chickens. The low production results and high mortality in experimental birds may indicate the need to use feeds with a lower insect component than those tested in this study or to use only purified insect proteins.

Conflict of Interests Statement: The authors declare that there is no conflict of interests regarding the publication of this article.

Financial Disclosure Statement: The research was funded by the "Strategy for Using Insects as Alternative Protein Sources for Animal Feed and Prospects for Future Production in Poland" project GOSPOSTRATEG 1/385141/16/NCBiR/2018. Publication was co-financed by the Ministry of Education and Science as part of the programme entitled "Regional Initiative of Excellence" for the years 2019–2023, project no. 010/RID/2018/19, amount of funding 12,000,000 PLN.

Animal Rights Statement: The experiment was carried out under strict observance of the guidelines of the Local Ethics Committee on Animal Experimentation of the University of Warmia and Mazury in Olsztyn (Authorisation No. LKE.065.22.2019). The researchers made every effort to minimise the suffering of birds.

References

1. Association of Official Analytical Chemists: Official Methods of Analysis, 21st Edition, edited by G.W. Latimer (Jr), AOAC International, Rockville, 2019. www.aoac.org/official-methods-of-analysis-21st-edition-2019.
2. Aviagen: *Ross Broiler Management Handbook, 2018*, Aviagen, Huntsville, AL, 2018, https://en.aviagen.com/assets/Tech_Center/Ross_Broiler/Ross-BroilerHandbook2018-EN.pdf.
3. Awoniyi T.A.M., Aletor V.A., Aina J.M.: Performance of broiler-chickens fed on maggot meal in place of fishmeal. Int J Poult Sci 2003, 2, 271–274, doi:10.3923/ijps.2003.271.274.
4. Barbehenn R.V.: Gut-based antioxidant enzymes in a polyphagous and a graminivorous grasshopper. J Chem Ecol 2002, 28, 1329–1347, doi: 10.1023/A:1016288201110.
5. Belluco S., Losasso C., Maggioletti M., Alonzi C.C., Paoletti M.G., Ricci A.: Edible insects in a food safety and nutritional perspective: A critical review. Compr Rev Food Sci Food Saf 2013, 12, 296–313, doi: 10.1111/1541-4337.12014.
6. Bulet P., Hetru C., Dimarcq J.L., Hoffmann D.: Antimicrobial peptides in insects; structure and function. Dev Comp Immunol 1999, 23, 329–344, doi: 10.1016/s0145-305x(99)00015-4.
7. Chae J., Kurokawa K., So Y., Hwang H.O., Kim M., Park J., Jo Y., Lee Y.S., Lee B.L.: Purification and characterization of tenecin 4, a new anti-Gram-negative bacterial peptide, from the beetle *Tenebrio molitor*. Dev Comp Immunol 2011, 36, 540–546, doi: 10.1016/j.dci.2011.09.010.
8. Chen C., Sander J.E., Dale N.M.: The Effect of Dietary Lysine Deficiency on the Immune Response to Newcastle Disease Vaccination in Chickens. Avian Dis 2003, 47, 1346–1351, doi: 10.1637/7008.
9. Dabbou S., Gai F., Biasato I., Capucchio M.T., Biasibetti E., Dezzutto D., Meneguz M., Placha I., Gasco L., Schavione A.: Black soldier fly defatted meal as a dietary protein source for broiler chickens: Effects on growth performance, blood traits, gut morphology and histological features. J Anim Sci Biotechnol 2018, 9, 49, doi: 10.1186/s40104-018-0266-9.
10. de Souza Vilela J., Andronicos N.M., Kolakshyapati M., Hilliar M., Sibanda T.Z., Andrew N.R., Swick R.A., Wilkinson S., Ruhnke I.: Black soldier fly larvae in broiler diets improve broiler performance and modulate the immune system. Anim Nutr 2021, 7, 695–706, doi: 10.1016/j.aninu.2020.08.014.
11. Dobermann D., Swift J.A., Field L.M.: Opportunities and hurdles of edible insects for food and feed. Nutr Bull 2017, 42, 293–308, doi: 10.1111/nbu.12291.
12. Elpidina E.N., Goptar I.A.: Digestive peptidases in *Tenebrio molitor* and possibility of use to treat celiac disease. Entomol Res 2007, 37, 139–147, doi: 10.1111/j.1748-5967.2007.00103.x.
13. European Commission: Commission Regulation (EU) 2017/893 of 24 May 2017 amending Annexes I and IV to Regulation (EC) No. 999/2001 of the European Parliament and of the Council and

Annexes X, XIV and XV to Commission Regulation (EU) No. 142/2011 as regards the provisions on processed animal protein. OJEU L 2017 138, 60, 25/05/2017, 92–116.
14. Fortuoso B.F., dos Reis J.H., Gebert R.R., Barreta M., Griss L.G., Casagrande R.A., de Cristo T.G., Santiani F., Campigotto G., Rampazzo L., Stefani L.M., Boiago M.M., Lopes L.Q., Santos R.C.V., Baldissera M.D., Zanette R.A., Tomasi T., Da Silva A.S.: Glycerol monolaurate in the diet of broiler chickens replacing conventional antimicrobials: impact on health, performance and meat quality. Microb Pathog 2019, 129, 161–167, doi: 10.1016/j.micpath.2019.02.005.
15. Harr K.E.: Clinical chemistry of companion avian species: A review. Vet Clin Path 2002, 31, 140–151.
16. Huo W., Li M., Wang J., Wang Z., Huang Y., Chen W.: On growth performance, nutrient digestibility, blood T lymphocyte subsets, and cardiac antioxidant status of broilers. Anim Nutr 2019, 5, 68–73, doi: 10.1016/j.aninu.2018.04.004.
17. Hwangbo J., Hong E.C., Jang A., Kang H.K., Oh J.S., Kim B.W., Park B.S.: Utilization of house fly-maggots, a feed supplement in the production of broiler chickens. J Environ Biol 2009, 30, 609–614.
18. Józefiak A., Kierończyk B., Rawski M., Mazurkiewicz J., Benzertiha A., Gobbi P., Nogales-Mérida S., Świątkiewicz S., Józefiak D.: Full-fat insect meals as feed additive – the effect on broiler chicken growth performance and gastrointestinal tract microbiota. J Anim Feed Sci 2018, 27, 131–139, doi: 10.22358/jafs/91967/2018.
19. Józefiak A., Nogales-Mérida S., Mikołajczak Z., Rawski M., Kierończyk B., Mazurkiewicz J.: The utilization of full-fat insect meal in rainbow trout (*Oncorhynchus mykiss*) nutrition: The effects on growth performance, intestinal microbiota and gastrointestinal tract histomorphology. Ann Anim Sci 2019, 19, 747–765, doi: 10.2478/aoas-2019-0020.
20. Khan S., Khan R.U., Alam W., Sultan A.: Evaluating the nutritive profile of three insect meals and their effects to replace soya bean in broiler diet. J Anim Physiol Anim Nutr 2018, 102, 662–668, doi: 10.1111/jpn.12809.
21. Köhler R., Kariuki L., Lambert C., Biesalski H.K.: Protein, amino acid and mineral composition of some edible insects from Thailand. J Asia Pac Entomol 2019, 22, 372–378, doi: 10.1016/j.aspen.2019.02.002.
22. Koncicki A., Tykałowski B., Stenzel T., Śmiałek M., Pestka D.: Effect of infection of turkeys with haemorrhagic enteritis adenovirus isolate on the selected parameters of cellular immunity and the course of colibacillosis. Pol J Vet Sci 2012, 15, 215–220, doi: 10.2478/v10181-011-0136-2.
23. Krasnodębska-Depta A., Koncicki A.: Diagnostyka hematologiczna i biochemiczna (Haematological and Biochemical Diagnosis – in Polish). In: *Choroby drobiu Wydanie III*, edited by M. Mazurkiewicz, A. Wieliczko, Wydawnictwo Uniwersytetu Przyrodniczego we Wrocławiu, Wrocław, 2019, pp. 889–909.
24. Lee J., Kim Y.M., Park Y.K., Yang Y.C., Jung B.G., Lee B.J.: Black soldier fly (*Hermetia illucens*) larvae enhances immune activities and increases survivability of broiler chicks against experimental infection of *Salmonella* Gallinarum. J Vet Med Sci 2018, 80, 736–740, doi: 10.1292/jvms.17-0236.
25. Li S., Ji H., Zhang B., Tian J., Zhou J., Yu H.: Influence of black soldier fly (*Hermetia illucens)* larvae oil on growth performance, body composition, tissue fatty acid composition and lipid deposition in juvenile Jian carp (*Cyprinus carpio* var. Jian). Aquaculture 2016, 465, 43–52, doi: 10.1016/j.aquaculture. 2016.08.020.
26. Li Y., Kortner T.M., Chikwati E.M., Munang'andu H.M., Lock E., Krogdahl Å.: Gut health and vaccination response in pre-smolt Atlantic salmon (*Salmo salar*) fed black soldier fly (*Hermetia illucens*) larvae meal. Fish Shellfish Immunol, 2019, 86, 1106–1113, doi: 10.1016/j.fsi.2018.12.057.
27. Marono S., Loponte R., Lombardi P., Vassalotti G., Pero M.E., Russo F., Gasco L., Parisi G., Piccolo G., Nizza S., Di Meo C., Attia Y.A., Bovera F.: Productive performance and blood profiles of laying hens fed *Hermetia illucens* larvae meal as total replacement of soybean meal from 24 to 45 weeks of age. Poult Sci 2017, 96, 1783–1790, doi: 10.3382/ps/pew461.
28. Maurer V., Holinger M., Amsler Z., Früh B., Wohlfahrt J., Stamer A., Leiber F.: Replacement of soybean cake by *Hermetia illucens* meal in diets for layers. J Insects Food Feed 2016, 2, 89–90, doi: 10.3920/JIFF2015.0071.
29. Mlcek J., Rop O., Borkovcova M., Bednarova M.: A comprehensive look at the possibilities of edible insects as food in Europe – A Review. Pol J Food Nutr Sci 2014, 64, 147–157, doi: 10.2478/v10222-012-0099-8.
30. Muzzarelli R.A.A., Boudrant J., Meyer D., Manno N., Demarchis M., Paoletti M.G.: Current views on fungal chitin/chitosan, human chitinases, food preservation, glucans, pectins and inulin: A tribute to Henri Braconnot, precursor of the carbohydrate polymers science, on the chitin bicentennial. Carbohydr Polym 2012, 87, 995–1012, doi: 10.1016/j.carbpol.2011.09.063.
31. Oluokun J.A.: Upgrading the nutritive value of full-fat soyabeans meal for broiler production with either fishmeal or black soldier fly larvae meal (*Hermetia illucens*). Niger J Anim Sci, 2000, 3, 51–61, doi: 10.4314/tjas.v3i2.49768.
32. Patel S., Suleria H.A.R., Rauf A.: Edible insects as innovative foods: Nutritional and functional assessments. Trends Food Sci Technol 2019, 86, 352–359, doi: 10.1016/j.tifs.2019.02.033.
33. Ramos-Elorduy J., González E.A., Hernández A.R., Pino J.M.: Use of *Tenebrio molitor* (Coleoptera: Tenebrionidae) to recycle organic wastes and as feed for broiler chickens. J Econ Entomol 2002, 95, 214–220, doi: 10.1603/0022-0493-95.1.214.
34. Rossi L.M., Rangasamy P., Zhang J., Qiu X.Q., Wu G.Y.: Research advances in the development of peptide antibiotics. J Pharm Sci 2008, 97, 1060–1070, doi: 10.1002/jps.21053.
35. Rumpold B.A., Schluter O.K.: Nutritional composition and safety aspects of edible insects. Mol Nutr Food Res 2013, 57, 802–823, doi: 10.1002/mnfr.201200735.
36. Sánchez-Muros M.J., Barroso F.G., Manzano-Agugliaro F.: Insect meal as renewable source of food for animal feeding: a review. J Clean Prod 2014, 65, 16–27, doi: 10.1016/j.jclepro.2013.11.068.
37. Schiavone A., Cullere M., De Marco M., Meneguz M., Biasato I., Bergagna S., Dezzutto D., Gai F., Dabbou S., Gasco L., Zotte A.D.: Partial or total replacement of soybean oil by black soldier fly larvae (*Hermetia illucens* L.) fat in broiler diets: Effect on growth performances, feed-choice, blood traits, carcass characteristics and meat quality. Ital J Anim Sci 2017, 16, 93–100, doi: 10.1080/1828051X.2016.1249968.
38. Schiavone A., Dabbou S., De Marco M., Cullere M., Biasato I., Biasibetti E., Capucchio M.T., Bergagna S., Dezzutto D., Meneguz M., Gai F., Dalle Zotte A., Gasco L.: Black soldier fly larva fat inclusion in finisher broiler chicken diet as an alternative fat source. Animal 2018, 12, 2032–2039, doi: 10.1017/ S1751731117003743.
39. Schröder J.: Clinical significance of epithelial peptide antibiotics. BioDrugs 1999, 11, 293–300, doi: 10.2165/00063030-199911050-00001.
40. Smulikowska S., Rutkowski A.: *Zalecenia Żywieniowe i Wartość Pokarmowa Pasz dla Drobiu, Wydanie IV* (Recommended Allowances and Nutritive Value of Feedstuffs. Poultry Feeding Standards, Fourth Edition – in Polish). The Kielanowski Institute of Animal Physiology and Nutrition, Polish Academy of Sciences, Jabłonna, 2005.
41. Sypniewski J., Kierończyk B., Benzertiha A., Mikołajczak Z., Pruszyńska-Oszmałek E., Kołodziejski P., Sassek M., Rawski M., Czekała W., Józefiak D.: Replacement of soybean oil by Hermetia illucens fat in turkey nutrition: effect on performance, digestibility, microbial community, immune and physiological status and final product quality. Br Poult Sci 2020, 61, 294–302, doi: 10.1080/00071668.2020.1716302.
42. van Huis A.: Edible insects contributing to food security? Agric Food Secur 2015, 4, 20, doi: 10.1186/s40066-015-0041-5.
43. van Huis, A., Oonincx D.G.A.B.: The environmental sustainability of insects as food and feed. A review. Agron Sustain Dev 2017, 37, 43, doi: 10.1007/s13593-017-0452-8.

44. van Huis A., van Itterbeeck J., Klunder H., Mertens E., Halloran A., Muir G., Vantomme P.: FAO Forestry Paper 171, Edible insects: Future prospects for food and feed security. Food and Agriculture Organization of the United Nations, Rome, 2013.
45. Veldkamp T., Bosch G.: Insects: A protein-rich feed ingredient in pig and poultry diets. Anim Front 2015, 5, 45–50, doi: 10.2527/af.2015-0019.
46. Vercruysse L., Smagghe G., Herregods G., van Camp J.: ACE inhibitory activity in enzymatic hydrolysates of insect protein. J Agric Food Chem 2005, 53, 5207–5211, doi: 10.1021/jf050337q.
47. Wang L., Li J., Jin J.N., Zhu F., Roffeis M., Zhang X.Z.: A comprehensive evaluation of replacing fishmeal with housefly (*Musca domestica*) maggot meal in the diet of Nile tilapia (*Oreochromis niloticus*): Growth performance, flesh quality, innate immunity and water environment. Aquac Nutr 2017, 23, 983–993, doi: 10.1111/anu.12466.
48. Wang W., Shen S., Chen Q., Tanga B., He G., Ruan H., Das U.N.: Hydrolyzates of silkworm pupae (*Bombyx mori*) Protein is a new source of Angiotensin I-converting enzyme inhibitory peptides. Curr Pharm Biotechnol 2008, 9, 307–314, doi: 10.2174/138920108785161578.
49. Wang Y., Chen Y., Li X., Xia J., Du Q., Zhi C.: Study on the rearing larvae of *Tenebrio molitor* Linne and the effects of its processing and utilizing. Acta Agriculturae Universitatis Henansis 1996, 30, 288–292.
50. Yen A.L.: Edible insects: Traditional knowledge or western phobia? Entomol Res 2009, 39, 289–298, doi: 10.1111/j.1748-5967.2009.00239.x.
51. Zielińska E., Baraniak B., Karaś M., Rybczyńska K., Jakubczyk A.: Selected species of edible insects as a source of nutrient composition. Food Res Int 2015, 77, 460–466, doi: 10.1016/j.foodres.2015.09.008.