

# Anatomical and morphological traits, bone mineral profile, and health status of laying pheasants (*Phasianus colchicus* L.) fed diets containing *Hermetia illucens* meal or dried larvae

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### Summary

In recent years, insect protein has become a popular alternative to plant protein in poultry nutrition. Therefore, it is justified to investigate how diets supplemented with such protein affect various bird species, including wild ones. This study presents the results of a feeding experiment conducted under aviary conditions on five groups of common pheasant (*Phasianus colchicus* L.) hens during the laying period. The birds were divided into one control group and four experimental groups, in which plant protein was replaced at 50% or 100% with black soldier fly (*Hermetia illucens*) meal or whole dried larvae (BSFL). Dietary supplementation with animal-derived components affected muscle group proportions and body weight, but statistically significant stimulation was noted only for skeletal development, as indicated by selected bone lengths. The most beneficial effect in this regard was observed with 50% supplementation of dried BSFL. Conversely, the total replacement of soybean meal (SBM) showed no beneficial effect on the traits under assessment. Analysis of bone micro- and macro-mineral content revealed no significant differences. Nor did the substitution of SBM with *Hermetia illucens* material affect internal organ mass. Regardless of the type or level of supplementation, no significant impact on disease incidence or overall bird health was observed. These results indicate that dietary supplementation with *Hermetia illucens* larvae protein is viable in intensive pheasant breeding, provided it does not exceed the optimal level of 50%.

**Keywords:** common pheasant, hens, black soldier fly meal and larvae, muscles, bones

In recent years, there has been an increasing search for alternative sources of feed protein, driven by global population growth and the need to intensify animal production. To meet the serious challenge posed by the growing demand for protein feed, new solutions are being sought across origin, production volume, and technological processing, in accordance with the principles of sustainable agriculture and with carbon

footprint in mind. Therefore, insect larvae are undoubtedly an interesting alternative source of protein and energy (33). By far the most popular are black soldier fly (*Hermetia illucens*) meal (BSFM) and whole dried larvae (BSFL). These insects are characterized by a high protein content of 40-60%, a fat content of 10-30%, and, importantly, the ability to convert organic waste (25, 32, 35).

Insect larvae are mainly introduced into feed used in poultry nutrition, especially broilers and laying hens, where a positive effect on growth and productivity has been observed, with more efficient feed utilization (10, 17). Studies in this field have also shown that dietary strategies involving a partial or complete replacement of plant protein with insect-derived protein can positively influence production performance, including egg yield and chick rearing outcomes, while simultaneously enhancing immune responses and promoting a more favorable intestinal microflora (24, 26, 38).

Innovative nutritional solutions have also been implemented in pheasant farming, both for repopulation and for egg or meat production. Although pheasants are not native to Europe, having been introduced to Central Europe between 500 and 800 AD, they can be considered a so-called naturalized species (3, 9). To date, local populations are so numerous that they are classified as game birds and are hunted every year.

Nevertheless, as part of the hunting management of this species, annual repopulation measures are carried out using birds bred in closed breeding farms to maintain population stability. Therefore, an adequate quantity and quality of material for restocking is needed every year. Birds bred for restocking should be of good quality not only in terms of their individual condition, but also in terms of their ability to adapt to the natural environment after release (9). For many years, nutritional solutions have been sought to influence production capabilities to optimize the breeding of these birds. The basic solutions are dietary strategies based on various additives to optimize breeding, mainly reproduction and weight gain, the chemical composition of meat and eggs, and, in the case of pheasants intended for repopulation, anatomical and morphological characteristics as well (2, 11, 12, 14). In recent years, attention has also been drawn to the possibility of replacing plant protein with insect protein, mainly *Hermetia illucens* meal (BSFM), in various proportions. Initial nutritional experiments have demonstrated a wide range of applications for insect meal in pheasant nutrition (7, 8, 30).

The study aimed to determine the effect of replacing 50 or 100% of post-extraction soybean meal with black soldier fly (*Hermetia illucens*) products, in the form of meal (BSFM) or whole-dried larvae (BSFL), on the anatomical and morphological characteristics and mineral composition of the bones of pheasant laying hens (*Phasianus colchicus* L.). The occurrence of potential diseases was also assessed depending on the type of protein feed in the birds' diet.

## Material and methods

**Ethical approval.** The Local Ethics Committee on Animal Experimentation of the University of Life Sciences in Lublin, Poland, approved the experimental procedures used throughout this study (Resolution No. 8/2024 of 29 January 2024).

**Birds and housing.** The research was conducted at an experimental farm in Stara Kiszewa, Pomerania, Poland (18°10'E, 53°59'N). The research material consisted of pheasant hens forming the so-called basic flock kept in five feeding groups in a closed system. The pheasants were kept in aviaries measuring 72 m<sup>2</sup> and 4 meters high, with one aviary per group (control + 4 experimental). The aviaries were made of flexible mesh, which allowed the birds to fly around without injury while preventing them from escaping. Feed was provided in feeders and water in nipple drinkers *ad libitum*. The feed was provided as a granulated mixture. Its components and chemical composition are presented in Table 1. The diet's nutritional value was similar to the recommendations of the National Research Council [NRC, 1994] (29). The experimental feed was introduced 4 weeks before the start of the laying period and continued throughout the laying period. The control group's diet consisted of plant ingredients commonly used in pheasant nutrition. In the other four groups, SBM was substituted with *Hermetia illucens* in the form of either BSFM (group II – 50% HM; group III – 100% HM) or BSFL (group IV – 50% HL; group V – 100% HL). The insect larvae (BSF) were fed a mixture of plant-based by-products, supplied by HiProMine S.A. from Robakowo (Poland). The feed included a mineral and vitamin premix comprising (in 1 kg diet) Mn 60 mg, I 1 mg, Fe 50 mg, Zn 100 mg, Cu 12 mg, Se 0.2 mg, vit. A 10,000 IU, vit. D3 2,500 IU, vit. E 50 mg, vit. K3 2 mg, vit. B1 1.5 mg, vit. B2 4.5 mg, vit. B6 3 mg, vit. B12 0.015 mg, biotin 0.1 mg, folic acid 0.8 mg, nicotinic acid 20 mg, pantothenic acid 12 mg, and choline 300 mg. Each group (control and experimental) contained 10 hens and one rooster.

**Data collection and sampling.** After the end of the laying period, the hens were slaughtered. After stunning, the birds were decapitated. The carcasses and internal organs were then dissected, and measurements of individual muscle groups, bones, and organs were taken. The weight of muscles and internal organs was assessed on a laboratory scale (AXIS ATA 520, Poland) with an accuracy of 1 g, and bone length was measured with a caliper to 0.1 mm. Before measurement, the bones were thoroughly cleaned of muscle and connective tissue residues with blunt dissection tools. Body weight, breast muscle weight, thigh muscle weight, and lower leg muscle weight were measured, and the weight of the heart, liver, gizzard, and intestines was determined. The length of the femur, lower leg, upper arm, ulna, and sternum were measured.

**Chemical analyses of feed and bones.** The nutritive value, including dry matter, crude protein, crude fiber, ether extract, and crude ash, of the diets and insects (meal and whole larvae) was analyzed according to AOAC procedures [2012] (1). The content of Ca, Na, Mg, Fe, Zn, and Cu in the feed and bone (femur and tibia) samples was determined by the AAS flame technique (AAS Unicam 939, Shimadzu Corp., Tokyo, Japan) after preheating at 550°C, according to methods adopted by AOAC [2012]. The total P content in the feed was determined colorimetrically with a Helios Alpha UV-VIS spectrophotometer (Spectronic Unicam, Leeds, United Kingdom).

Tab. 1. Ingredients (g kg<sup>-1</sup>) of pheasant hens' diet and the nutritive value of the diet, meal (BSFM) and whole insect larvae (BSFL)

Item	Diets					<i>Hermetia illucens</i>	
	Control	50% HM	100% HM	50% HL	100% HL	Meal	Larvae
<b>Ingredients of the diet</b>							
Corn	281.50	307.96	334.41	306.47	331.42		
Wheat	252.0	252.0	252.0	252.0	252.0		
Soybean meal	220.0	110.0	0.0	110.0	0.0		
Pea seeds meal	50.0	50.0	50.0	50.0	50.0		
Linseed meal	40.0	40.0	40.0	40.0	40.0		
Soya oil	20.0	10.0	0.0	10.0	0.0		
Sunflower meal	80.0	80.0	80.0	80.0	80.0		
Sorghum	50.0	50.0	50.0	50.0	50.0		
Black soldier fly meal (BSFM)	0.0	93.5	187.0			1000.0	
Black soldier fly larvae (BSFL)				95.0	190.0		1000.0
Salt	3.0	3.0	3.0	3.0	3.0		
Mineral-vitamin premix*	2.5	2.5	2.5	2.5	2.5		
DL-methionine	0.30	0.32	0.34	0.31	0.33		
L-lysine chloride	0.70	0.72	0.75	0.72	0.75		
<b>Value analyzed</b>							
Dry matter	894.5	897.2	901.4	891.5	889.3	912.4	908.6
Crude protein	189.7	189.2	190.3	187.4	186.8	40.6	39.8
Crude fat	33.9	33.6	33.5	33.9	34.2	31.4	31.9
Crude fiber	49.7	47.5	46.2	48.4	47.5	0.21	0.26
Crude ash	69.2	69.5	69.7	70.5	71.2	5.83	5.91
Calcium	25.9	25.6	25.2	26.1	26.3	25.4	25.7
Total phosphorus	6.92	6.87	6.81	6.83	6.59	5.68	5.61
Sodium	1.58	1.71	1.83	1.69	1.81	3.71	3.68
Magnesium	0.32	0.39	0.45	0.39	0.46	2.87	2.91
Iron	0.045	0.057	0.075	0.058	0.074	0.34	0.33
Copper	0.008	0.011	0.016	0.011	0.017	0.027	0.026
Zinc	0.061	0.084	0.095	0.083	0.094	0.98	0.95

**Anatomopathological studies.** For anatomopathological studies, sections of internal organs, including lungs, heart, liver, spleen, and kidneys, were used. Tissue fragments of approximately 0.5-1 cm<sup>3</sup> were collected using sterile surgical instruments immediately after slaughter and evisceration of the birds. Each specimen was immediately placed in 4% neutral buffered formalin in a tissue to fixative ratio of at least 1 : 10 to ensure proper fixative penetration. The samples were fixed at room temperature for 24 hours. The containers were then sealed and transported to a histopathology laboratory in accordance with biosafety guidelines. In the laboratory, the tissues were subjected to a standard histological procedure. The material was dehydrated in increasing concentrations of ethyl alcohol (70%, 80%, 96%, 100%). After clearing in xylene, the material was embedded in paraffin at 56-60°C. Using a sled microtome, 4 µm thick tissue sections were cut from the paraffin blocks. The sections were mounted on microscope slides, stretched in a water bath at 40-45°C, and then dried in an incubator. The sections were stained with HE according

to the standard procedure. The preparations were deparaffinized in xylene and then rehydrated through a series of alcohols of decreasing concentration to distilled water. Cell nuclei were stained with Mayer's hematoxylin for several minutes, after which the preparations were rinsed in water and differentiated by brief exposure to acidic alcohol (1% HCl in 70% ethanol, Sigma-Aldrich). Differentiation was performed in a slightly alkaline environment using ammonia water. Contrast staining of the cytoplasm and extranuclear structures was achieved by immersing the preparations in a 1% eosin solution. After staining, the preparations were dehydrated in increasing concentrations of alcohol, examined in xylene, and sealed. During staining, the cell nuclei acquired an intense blue-violet color, while the cytoplasm and extranuclear structures acquired a pink color, allowing detailed analysis of tissue structure (18, 27).

The finished preparations were analyzed using a light microscope (Olympus BX 43, Japan) at magnifications of 100×, 200×, and 400×. Microscopic evaluation was performed to assess overall tissue architecture and pathological

changes. Photographic documentation was performed using a digital camera mounted on a microscope and dedicated image-acquisition software.

**Statistical analysis.** Statistical analysis was performed using Statistica 13.3 (TIBCO Software, Inc., Palo Alto, CA, USA). Normality and homogeneity of variance were assessed by the Shapiro-Wilk test. To determine the significance of differences between groups, a one-way analysis of variance was performed, and significance was evaluated by Tukey's test, with  $p < 0.05$  as the significance threshold.

The relationship between the presence of disease signs in pheasant carcasses and carcass weight was assessed by the nonparametric Mann-Whitney U test, comparing carcass weight values with and without disease signs. The study also evaluated the relationship between the presence of disease signs and membership of an experimental group using the chi-square test for independence. In addition, a logistic regression model was fitted, with the dependent variable indicating disease presence (0 – healthy, 1 – sick) and the independent variables being body weight (continuous) and experimental group (categorical). The significance level was set at  $\alpha = 0.05$ .

## Results and discussion

An assessment of the effects of insect meal and dried larvae in diets (Tab. 2) revealed a slight increase in body weight in pheasants receiving the insect material compared with the control group, but these differences were not statistically significant. The highest growth in average body weight (over 7% compared to the control group) was observed in the 50 HL group. Still, the complete replacement of soy did not provide any additional benefits. A similar trend was observed in muscle mass, which, along with the skeleton, forms the basis of body weight. The three muscle groups analyzed showed slightly higher weights in almost all cases, but the effect of supplementation was also

statistically insignificant. The addition of dried insect larvae, especially 50 HL, had a better effect on muscle development than insect meal.

No clear and significant changes were observed in the weight of internal organs (heart, liver, gizzard, and intestines) as a result of dietary supplementation. The weight of organs relative to body weight varied slightly across the feeding groups. Heart weight ranged from 0.4% to 0.5%, liver weight ranged from 2.1% to 2.4%, and gizzard weight in the four feeding groups was 1.8% in the 100 HM group and 1.9% in the other groups. However, an interesting finding was a more than an approx. 6% decrease in average liver weight in the 100 HL group (complete supplementation with dried insect larvae) compared with the control group, but these differences were not statistically significant.

The effect of supplementation with *Hermetia illucens* meal and dried larvae was most evident in bone length (ulna, femur, and lower leg) and was statistically significant. The greatest differences occurred in the groups with the addition of dried insects.

Dietary supplementation with various levels of insect meal or whole dried larvae replacing post-extraction soybean meal did not significantly affect the chemical composition of leg bones or the content of the bioelements assessed (Tab. 3). No significant differences were found between the feeding groups and the control group. The most crucial variation was found in the copper content of both the femur and tibia. Still, these differences were not statistically significant.

The analysis of interdependence between the morphological characteristics of pheasants revealed numerous significant positive correlations (Tab. 4). The strongest correlation was the one between body weight and breast muscle weight ( $r = 0.924$ ). Moderately

Tab. 2. Body weight and post-slaughter characteristics of pheasants (mean  $\pm$  SD)

Trait	Control	50 HM	100 HM	50 HL	100 HL	p-value
Body weight (g)	921.43 $\pm$ 103.05	963.00 $\pm$ 120.38	926.43 $\pm$ 87.75	990.57 $\pm$ 49.88	960.71 $\pm$ 178.19	0.7879
Lower leg muscle weight (g)	28.87 $\pm$ 5.27	29.01 $\pm$ 2.05	29.19 $\pm$ 3.24	30.51 $\pm$ 4.19	29.64 $\pm$ 6.79	0.9636
Breast muscle weight (g)	198.29 $\pm$ 23.95	200.57 $\pm$ 17.23	198.57 $\pm$ 18.14	208.14 $\pm$ 12.35	203.43 $\pm$ 30.20	0.9015
Thigh muscle weight (g)	41.57 $\pm$ 9.10	40.91 $\pm$ 4.11	41.70 $\pm$ 4.78	44.93 $\pm$ 5.63	42.47 $\pm$ 9.32	0.8392
Heart weight (g)	4.17 $\pm$ 0.69	4.19 $\pm$ 0.79	4.49 $\pm$ 0.77	4.37 $\pm$ 0.73	4.43 $\pm$ 0.77	0.9052
Liver weight (g)	22.39 $\pm$ 3.55	21.51 $\pm$ 2.99	21.37 $\pm$ 2.66	21.21 $\pm$ 2.20	20.93 $\pm$ 6.47	0.9659
Gizzard weight (g)	16.74 $\pm$ 2.03	17.33 $\pm$ 2.11	17.21 $\pm$ 2.41	17.96 $\pm$ 0.99	16.84 $\pm$ 2.27	0.8107
Intestinal weight (g)	45.31 $\pm$ 10.78	46.54 $\pm$ 18.27	45.56 $\pm$ 5.96	44.86 $\pm$ 5.82	44.76 $\pm$ 6.66	0.9979
Humerus length (g)	7.11 $\pm$ 0.39	7.21 $\pm$ 0.45	7.43 $\pm$ 0.31	7.51 $\pm$ 0.36	7.39 $\pm$ 0.29	0.2541
Ulna length (mm)	6.31 <sup>a</sup> $\pm$ 0.36	6.70 <sup>ab</sup> $\pm$ 0.36	6.83 <sup>b</sup> $\pm$ 0.10	7.06 <sup>b</sup> $\pm$ 0.17	6.84 <sup>b</sup> $\pm$ 0.18	0.0002*
Femur length (mm)	7.49 <sup>a</sup> $\pm$ 0.27	7.80 <sup>b</sup> $\pm$ 0.15	7.89 <sup>b</sup> $\pm$ 0.16	7.96 <sup>b</sup> $\pm$ 0.11	7.70 <sup>ab</sup> $\pm$ 0.14	0.0002*
Tibiotarsus length (mm)	10.07 <sup>a</sup> $\pm$ 0.48	10.34 <sup>a</sup> $\pm$ 0.20	10.36 <sup>a</sup> $\pm$ 0.26	10.47 <sup>a</sup> $\pm$ 0.26	10.56 <sup>b</sup> $\pm$ 0.20	0.0544*
Sternum length (mm)	9.59 $\pm$ 0.56	9.76 $\pm$ 0.68	9.87 $\pm$ 0.47	9.74 $\pm$ 0.70	10.11 $\pm$ 0.52	0.5557

Explanations: \* – significant for  $p \leq 0.05$ ; One-Way ANOVA; <sup>a, b</sup> – means marked with different letters differ significantly between groups

Tab. 3. Mineral content in femur and tibia bones of pheasant layers (mean  $\pm$  SD)

Item	Control	50 HM	100 HM	50 HL	100 HL	p-value
<b>Femur</b>						
Dry matter (%)	81.23 $\pm$ 1.29	81.27 $\pm$ 1.23	81.31 $\pm$ 1.22	81.21 $\pm$ 1.22	81.37 $\pm$ 1.34	0.893
Crude ash (%)	46.12 $\pm$ 3.11	46.19 $\pm$ 3.23	46.45 $\pm$ 3.08	46.25 $\pm$ 3.18	46.43 $\pm$ 3.21	0.911
Calcium (%)	23.78 $\pm$ 1.21	23.73 $\pm$ 1.16	23.67 $\pm$ 1.23	23.79 $\pm$ 1.25	23.81 $\pm$ 1.24	0.894
Phosphorus (%)	9.76 $\pm$ 0.36	9.68 $\pm$ 0.35	9.63 $\pm$ 0.37	9.77 $\pm$ 0.34	9.79 $\pm$ 0.36	0.906
Sodium (g/kg)	6.31 $\pm$ 0.17	6.52 $\pm$ 0.21	6.83 $\pm$ 0.18	6.49 $\pm$ 0.16	6.77 $\pm$ 0.19	0.453
Magnesium (g/kg)	3.52 $\pm$ 0.09	3.88 $\pm$ 0.11	3.95 $\pm$ 0.12	3.75 $\pm$ 0.09	3.87 $\pm$ 0.09	0.317
Iron (mg/kg)	129.3 $\pm$ 8.78	131.5 $\pm$ 8.83	132.6 $\pm$ 8.81	130.9 $\pm$ 8.37	133.4 $\pm$ 8.74	0.673
Copper (mg/kg)	14.92 $\pm$ 0.31	16.94 $\pm$ 0.34	17.89 $\pm$ 0.33	17.05 $\pm$ 0.35	18.94 $\pm$ 0.33	0.201
Zinc (mg/g)	264.4 $\pm$ 16.1	269.3 $\pm$ 16.2	271.2 $\pm$ 17.3	266.8 $\pm$ 15.5	269.8 $\pm$ 16.4	0.672
<b>Tibia</b>						
Dry matter (%)	85.82 $\pm$ 2.32	86.12 $\pm$ 2.31	85.98 $\pm$ 2.34	85.92 $\pm$ 2.24	86.08 $\pm$ 2.32	0.902
Crude ash (%)	51.24 $\pm$ 3.42	51.31 $\pm$ 3.12	51.43 $\pm$ 3.14	52.02 $\pm$ 3.42	52.06 $\pm$ 3.42	0.319
Calcium (%)	25.56 $\pm$ 1.45	25.45 $\pm$ 1.41	25.39 $\pm$ 1.32	25.37 $\pm$ 1.34	25.46 $\pm$ 1.48	0.787
Phosphorus (%)	9.94 $\pm$ 0.43	9.85 $\pm$ 0.42	9.84 $\pm$ 0.45	9.89 $\pm$ 0.41	9.91 $\pm$ 0.47	0.892
Sodium (g/kg)	6.59 $\pm$ 0.21	6.59 $\pm$ 0.21	6.59 $\pm$ 0.21	6.59 $\pm$ 0.21	6.59 $\pm$ 0.21	0.965
Magnesium (g/kg)	3.94 $\pm$ 0.11	3.99 $\pm$ 0.14	4.04 $\pm$ 0.13	3.95 $\pm$ 0.12	3.98 $\pm$ 0.15	0.806
Iron (mg/kg)	102.4 $\pm$ 9.22	106.5 $\pm$ 9.04	108.7 $\pm$ 9.12	105.8 $\pm$ 9.02	107.5 $\pm$ 8.94	0.917
Copper (mg/kg)	16.94 $\pm$ 0.28	17.71 $\pm$ 0.23	17.92 $\pm$ 0.24	17.49 $\pm$ 0.21	17.66 $\pm$ 0.28	0.358
Zinc (mg/g)	215.8 $\pm$ 15.8	218.6 $\pm$ 16.9	219.4 $\pm$ 15.2	216.9 $\pm$ 16.3	218.8 $\pm$ 13.3	0.762

Tab. 4. Pearson correlation coefficients between selected biometric parameters

Item	Body weight	Pectoral muscle mass	Humerus length	Lower leg muscle mass	Ulna bone length	Femur length
Breast muscle mass	0.924	ns	ns	0.717	ns	ns
Lower leg muscle mass	0.743	ns	ns	ns	ns	ns
Thigh muscle mass	0.465	0.464	ns	0.446	ns	ns
Tibiotarsus length	0.451	0.462	0.426	ns	0.631	0.581
Thigh bone length	0.450	0.474	0.432	ns	0.709	ns
Sternum length	0.401	0.441		0.497	ns	ns
Heart mass	0.396	0.436	0.350	ns	0.345	ns
Ulna bone length	0.392	0.399	0.686	ns	ns	ns

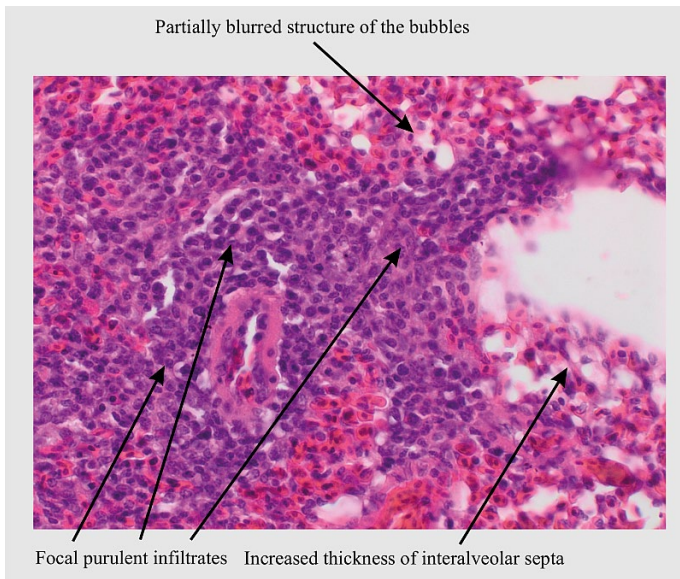
Explanation: ns – not statistically significant

strong correlations were also found between breast muscle mass and lower leg muscle mass ( $r = 0.717$ ) and between body weight and lower leg muscle mass ( $r = 0.743$ ). The relationships between limb bone lengths indicate proportional skeletal development. In addition, there were significant positive correlations between heart mass and body mass ( $r = 0.396$ ) and pectoral muscle mass ( $r = 0.436$ ). The results indicate that adding insects and insect meal to the diet does not disrupt the natural relationships between the development of individual tissues and may even support the harmonious development of the musculoskeletal and circulatory systems.

Gross and histopathological examinations revealed changes in some internal organs. In each organ evaluated, isolated cases of congestion were considered post-

mortem artifacts. Extensive suppurative inflammation was noted in some lungs (Fig. 1), including 3 cases in the control group, 1 case in the HM 50 group, 2 cases in the HM 100 group, none in the HL 50 group, and 1 case in the HL 100 group.

These pulmonary changes were characterized by focal loss of standard alveolar architecture in areas with intense cellular infiltration, predominantly neutrophils, with minor involvement of lymphocytes and macrophages. The alveolar lumens contained cellular exudate and necrotic debris with localized fibrin deposits. Inter-alveolar septa were frequently thickened, congested, and infiltrated by inflammatory cells. In focal areas, tissue degradation led to the formation of small cavities filled with purulent material, corresponding to microabscesses.



**Fig. 1. Diffuse suppurative pneumonia with neutrophilic infiltration in the alveolar lumens and partial loss of alveolar architecture. Interalveolar septa were thickened, and foci of suppuration were observed. Stained with HE. Magnification: 400×**

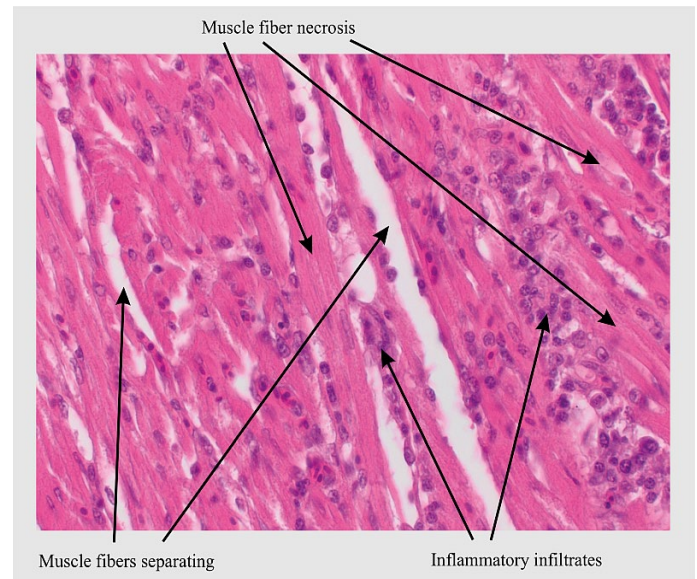
In one hen from the HL 100 group, numerous inflammatory infiltrates were present within the myocardium, composed mainly of lymphocytes and heterophils, with occasional macrophages (Fig. 2). The intramuscular infiltrates separated and damaged cardiac muscle fibers. Some cardiomyocytes exhibited signs of degeneration and necrosis, including enlargement, increased cytoplasmic acidophilia, and pyknotic or disappearing nuclei. Small foci of intercellular fibrosis were also observed. These changes are consistent with focal lymphocytic-heterophilic myocarditis.

No other pathological changes were observed in the organs. Differences in body weight between healthy and affected hens were not statistically significant (Mann-Whitney test,  $U = 133.0$ ;  $p = 0.54$ )

Analysis of the relationship between disease occurrence and membership of an experimental group showed no significant association ( $\chi^2 = 1.61$ ;  $df = 4$ ;  $p = 0.81$ ). The incidence of disease was similar in all five experimental groups.

In the logistic regression model, neither of the variables (body weight and experimental group) had a significant effect on the probability of disease signs ( $\beta = 0.0015$ ;  $p = 0.65$ ).

The results of supplementing the diet of laying pheasants with *Hermetia illucens* meal or whole dried larvae indicate that, regardless of the supplement and its level, there were no significant differences in the anatomical and morphological structure of the laying hens' skeleton. However, in the same nutritional study (31), which assessed the physicochemical and strength parameters of bones, it was found that a partial substitution of plant protein (soybean meal) with insect protein improved bone condition and quality. In poultry



**Fig. 2. Focal myocarditis. Inflammatory infiltrates within the myocardium, composed predominantly of lymphocytes and heterophils, separate the cardiac muscle fibers. Some cardiomyocytes exhibit signs of degeneration and necrosis. Stained with HE. Magnification: 400×**

nutrition, it is also reasonable to feed birds dried insect larvae, which they readily accept. The use of this type of fodder in breeding also corresponds to the behavioral needs of chickens. This fact is significant, especially in aviary breeding conditions for birds intended for release into the wild, where their natural food base includes insect-derived food (6, 37). Continuing to use a protein supplement is also justified after the birds have been released into the environment to reduce mortality and strengthen their immunity during periods of food shortage (5). Modifying the feed mixtures used in poultry nutrition by replacing plant protein with insect protein (*Hermetia illucens*) does not have a negative impact on the health of broilers (19). This is confirmed by studies (8) showing that supplementation with *Hermetia illucens* meal improves pheasant chick survival and egg fatty acid ratios. A positive effect of supplementation was also observed in male Ross broilers (22), whose slaughter yield increased without any negative impact on the animals' health. In a feeding experiment in which plant protein in the diet of chickens was replaced with 10% of *Hermetia illucens* and *Bombyx mori* meal, higher slaughter weight was observed with more efficient feed utilization. Including 10% of these insect meals in the diet affected the content of some essential amino acids and the proportion of omega-3 fatty acids in the meat. A similar phenomenon was also observed in laying hens. Nevertheless, it should be emphasized that a complete substitution of plant protein with insect protein reduces feed intake and may negatively affect muscle mass gains (16, 26).

The statistically significant differences in the lengths of the ulna, femur, and tibiotarsus indicate that diet affects the length of individual bones. The femur length

in the experimental groups was similar to that reported by Kokoszyński et al. (20), who showed that this value depended on the age of pheasants at slaughter.

When comparing the weight of internal organs, no significant differences were found, except for a decrease in liver weight. On the other hand, a comparison of the ratio of internal organ weight to body weight with the results reported by Wegner et al. (36) for farm-raised pheasants showed that, for the heart, these proportions were similar. The results of our study showed that, regardless of the proportion of animal protein and its form in the feed, the birds were characterized by a lower liver weight and a higher stomach weight compared to the control group. Studies on quails (28) also found no significant differences in the weight of internal organs, except for an increase in gizzard weight (linear increase, assumed to be due to the presence of chitin).

The mineral content of pheasant femur and tibia varies depending on age, sex, and diet, with calcium and phosphorus being the main minerals in bone tissue. Studies on broiler chickens show that bone mineralization increases with age, suggesting a similar pattern in pheasants. The tibia is usually the heaviest and contains the most ash and calcium, while males typically have larger and heavier bones than females (13). In other studies, the proportions of individual elements in hen bones varied by breed, with significant differences between genotypes observed in most cases (21).

Our results indicate that the use of modified feeds with different levels of *Hermetia illucens* animal protein in the form of meal (BSFM) or larvae (BSFL) had no significant impact on bird health. In the cases presented, few inflammatory changes were found in both the respiratory and cardiovascular systems, including pneumonia and focal myocarditis. Similar observations have been described in the literature on wild and farmed birds. Studies conducted on wild pheasant chicks in Germany found cases of suppurative to necrotizing pneumonia, often of bacterial or parasitic etiology (*Syngamus trachea*), as well as a relationship between the general condition of the birds and the severity of inflammatory changes (23). Studies of parrots (Psittacidae) have shown that inflammatory changes in the heart may be bacterial in origin, accompanied by endocarditis and pericarditis (15). Cases of myocarditis in pheasants are poorly documented, with only isolated reports indicating focal myocarditis in chicks (23), which suggests that this process may also occur in this species, possibly in the course of systemic or toxic infections.

The literature emphasizes that modifying feed composition by a partial replacement of plant protein with animal protein yields mixed results. It can lead to improved gut health and have an indirect impact on growth and feed utilization (4). For most of them, the effects on body condition and weight are consequences

of improved intestinal barrier integrity, reduced oxidative stress, or reduced inflammatory responses (34). The results indicate that the feeds discussed, based on a partial or complete replacement of plant protein with animal protein, are safe for health, as they do not increase the incidence of diseases or cause side effects, such as weight loss.

The present research and analyses have shown that insect meal or whole-dried larvae can be an alternative and valuable ingredient in laying pheasant feed, replacing post-extraction soybean meal. The partial replacement of soybean meal with dried insects (50%) yielded the best results in terms of skeletal structure and individual muscle groups. All experimental groups showed increased body weight and bone length compared to the control group. On the other hand, the complete replacement (100%) of soybean meal with either meal or dried insects did not significantly affect the characteristics analyzed. Dietary supplementation did not significantly affect disease incidence or the birds' health status. Therefore, a 50% addition of dried insect larvae to the diet of pheasants, which are the main flock on farms intended for fattening and release into the natural environment, seems optimal.

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