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Effect of inclusion insect meals in feed on performance, blood biochemical and immune parameters of layer hens

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Abstract

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The utilization of sustainable, ecologically friendly and high-quality substitutes of soybean meal for feeding poultry becomes increasingly popular. With this regard, the present study investigated the effect of two types of insect meals – from black soldier fly (*Hermetia illucens*) larvae and silkworm (*Bombyx mori*) pupae, as source of protein in diets of layer hens on productivity, blood biochemical and immune parameters. A total of 180 hens, 34 weeks of age, from the egg type line T (Rhode Island Red), were distributed in three groups, each with three replicates, 20 hens/replicate. The control group received standard diet with soybean meal, and the two experimental groups were feed rations containing 7% of tested insect meals: black soldier fly meal (BSF), or silkworm pupae meal (SW) throughout 13 weeks.

The live weight of birds, feed intake, egg production, egg weight and feed conversion ratio did not differ among the groups ($P > 0.05$). Blood biochemical profile and immune indexes (serum lysozyme activity and alternative pathway of complement activation) were neither affected ($P > 0.05$).

On the basis of obtained results, it may be concluded that the tested meals from black soldier fly larvae (*Hermetia illucens*) and silkworm pupae (*Bombyx mori*), could be successfully used as alternative protein source in the rations of layer hens at dietary levels of 7% without adverse effects on egg production, physiological and immunological status of birds.

Keywords: Black soldier fly (*Hermetia illucens*); Silkworm (*Bombyx mori*); hens; performance; blood profiles

Introduction

Poultry farming is one of most rapidly developing livestock branches, and the demands for products of animal origin is continuously increasing. The shortcoming and higher costs of ingredients used in compound feeds, along with ever more important issue of environmental protection encourages research on alternative sources of dietary protein. Feed is the most expensive element of total production costs – approximately 70-75%, out of which about 15% are protein costs (Köse & Öztürk, 2017). Birds are not capable to synthesize some essential amino acids and in order to attain peak productivity, these amino acids should be available in feeds. The poultry industry depends mainly on available plant protein sources as soybean meal due to its excellent amino acid profile and higher digestibility (Willis, 2003; Veldkamp et al., 2012). The interest to insects as a protein source in poultry diets raises continuously (van Huis et al., 2013; Makkar et al., 2014; Pal & Roy, 2014), because of their nutritional value and ecological production (Oonincx et al., 2010), taking into account the fact that they are part of the “natural” diet of birds (Bovera et al., 2016). Insect meals are rich in protein, fats and essential amino acids (Veldkamp & Bosch, 2015; van Huis & Tomberlin, 2017). So far, research studies were mainly focused on black soldier fly (Pasotto et al., 2020), silkworms (Ullah et al., 2017; Kim et al. 2020), grasshoppers (Brah et al., 2017), house fly (Radulović et al., 2018) and yellow meal-worms (Kasri & Purwanti, 2021), as potential dietary protein sources for birds.

Different insects are able to convert organic wastes into valuable protein with beneficial amino acid profile for domestic poultry (Veldkamp et al., 2012; Makar et al., 2014). Among them, the black soldier fly (*Hermetia illucens*) has shown the greatest potential for large-scale production (Rumpold et al., 2018). Unlike it, the silkworm pupae that consume only mulberry leaves, is a waste product in the production of silk that may be another non-traditional source of protein for poultry after appropriate processing.

The high nutritional value of insects and their potential sustainability as a feed source in poultry nutrition become increasingly popular. However, research studies are necessary for the safe introduction of insect protein in poultry feeds. That is why the purpose of the present experiment was to evaluate the possibility of using black soldier fly (BSF) larvae and silkworm pupae (SW), as alternative dietary protein source for layer hens and their effect on productivity, blood biochemical and immune parameters of birds.

Materials and Methods

Birds and husbandry

All procedures, related to management and care of used birds were compliant with European Council Directive (2010/63/EU), on the protection of animals used for scientific purposes and the National Protocol (№ 20 from 01.11.2012).

The present study was conducted in the Poultry farm of the Agricultural Institute, Stara Zagora. A total of 180 laying hens, 34-wk-old, from the egg type line T (Rhode Island Red) with uniform live body weight and egg production (P>0.05) were divided into three groups with three replications (20 hens/replicate) in a 13-week trial, including a one-week adaptation period. The birds were reared on the floor, on deep permanent litter, placed in 9 equal boxes 2x2 m of size, equipped with perches and nests. Feed and water were provided *ad libitum,* in a linear feeder providing at least 10 cm per hen and continuous drinking troughs providing at least 2.5 cm per hen, respectively. Optimum microclimatic parameters were provided: air temperature 15-20°C and relative humidity 60-70%, light day duration 16 hours achieved with additional artificial lighting at optimum light intensity of 3 W/m^2.

Insect meals and diets

The Black solder fly (*Hermetia illucens*) larvae used in the study was produced and provided by a commercial manufacturer NASEKOMO. The nutrient composition is as follows: crude protein – 44.76%; crude fat – 33.52%; Ca – 0.18%; P – 0.43%; methionine – 6.41%; lysine – 6.92%.

The silkworm pupae (chrysalis, *Bombix mori*), was obtained from the Scientific Center of Sericulture – Vratsa and contain crude protein – 57.14%; crude fat – 24.50%; Ca -0.55 %; P – 0.75 %; methionine – 3.70 %; lysine – 3.83%.

Three diets were formulated: control, with soybean meal as a main protein source and two experimental, which included tested insect meals: 7 % black soldier fly larvae (BSF) or 7 % silkworm pupae meal (SW). Diets were isonutritional and formulated according to requirements for layer hens as per NRC (1994) recommendations. Feeding regimen was done according to the age and stage of development of birds. The components and nutritional composition of diets for control and experimental groups are shown in Table 1 and amino acid composition of diets – in Table 2.

Table 1. Composition and nutrient content of the diets, %

Ingredients	Diets		
	Control	BSF	SW
Wheat	34.74	31.58	42.00
Corn	21.00	27.00	22.98
Sunflower meal	15.00	15.00	15.00
Soybean meal	13.00	6.76	1.53
Silkworm pupae meal	0.00	0.00	7.00
Black soldier fly larvae	0.00	7.00	0.00
Sunflower oil	5.00	1.72	0.30
Lysine	0.05	0.13	0.08
Methionine	0.08	0.03	0.01
Salt	0.30	0.30	0.30
Dicalcium phosphate	1.43	1.17	1.20
Limestone	8.70	8.61	8.90
Vitamin-mineral premix	0.20	0.20	0.20
Optizym	0.10	0.10	0.10
Mycotox	0.20	0.20	0.20
Salgard	0.20	0.20	0.20
Nutritional composition:			
Dry matter, %	89.79	90.03	89.09
ME, kcal/kg	2807	2807	2808
Crude protein, %	17.00	17.00	17.00
Crude fibre, %	4.857	4.646	4.465
Lysine, %	0.808	0.808	0.808
Methionine, %	0.386	0.381	0.387
Ca, %	3.720	3.722	3.721
Total P, %	0.711	0.709	0.681
Avaible P, %	0.383	0.382	0.386

BSF – Black solder fly larvae meal; SW- Silkworm meal

Productive traits

The hens' live weight was recorded at the beginning and at the end of the trial. Feed intake was measured monthly per replicate. Egg number and egg weight were recorded daily to determine laying rate and average egg weight for each replicate. Feed conversion ratio was calculated by using the documented data of feed intake, egg number and egg weight.

Table 2. Amino acid composition of the diets, %

Amino acids	Diets		
	Control	BSF	SW
Aspartic acid	13.48	5.54	16.87
Serine	4.26	7.91	5.81
Glutamic acid	6.76	9.07	10.66
Glycine	3.99	1.17	0.35
Histidine	12.55	14.29	10.06
Arginine	9.95	7.89	5.54
Threonine	3.61	4.29	4.38
Alanine	8.56	8.29	8.42
Proline	7.06	6.55	5.47
Cysteine	0.17	0.19	1.55
Tyrosine	6.23	8.20	7.41
Valine	3.42	3.54	2.63
Methionine	1.41	1.98	1.78
Lysine	8.18	9.75	10.37
Isoleucine	3.60	3.84	3.08
Leucine	0.95	0.93	0.80
Phenylalanine	5.80	6.57	4.83
Essential	63.07	61.20	66.60
Non-essential	36.92	38.79	33.42

BSF – Black solder fly larvae meal; SW- Silkworm meal

Blood sampling and analysis of serum biochemical parameters and immune response of hens

At 47 weeks of age, blood samples were collected from the wing vein *(v. cutanea ulnaris*) of six birds per group into sterile tubes. The serum was separated by centrifugation for 10 min and stored at −20°C, until the analysis was performed. All biochemical traits of the blood serum — total protein (TP, g/L), albumin (ALB, g/L), uric acid (UA, μmol/L), creatinine (Crea, μmol/L), cholesterol (Chol, mmol/L), triglycerides (TG, mmol/L), glucose (Glu, mmol/L), aspartate aminotransferase (AST, U/L), alanine aminotransferase (ALT, U/L), gamma glutamyl-transferase (GGT, U/L), calcium (Ca, mmol/L), inorganic phosphorus (P, mmol/L) and magnesium (Mg, mmol/L) were determined, using an automatic biochemical analyzer (Mindray BS-120, China). Globulin concentration was estimated as the difference between total protein and albumin. Then, the albumin to globulin ratio was calculated.

To evaluate humoral non-specific immunity of hens, blood serum was analyzed for determination of lysozyme concentrations following the method described by Lie (1985). The other factor of humoral immunity – alternative pathway of complement activation (APCA) was determined according to method of Sotirov (1991).

Statistical analysis

The statistical analysis was performed using SPSS software (ver.19.0). Data were tested by one-way ANOVA with main effect of diet, followed by LSD post-hoc test. Differences were considered to be statistically significant at $P < 0.05$, and a trend to difference was set at $P < 0.10$.

Results

During the experiment, no mortality was recorded. Daily feed intake, live weight of hens at the end of the trial, egg laying intensity, egg weight, total egg mass and feed conversion ratio did not differ statistically significantly ($P > 0.05$) as demonstrated by Table 3.

The results from blood serum biochemical analysis of layer hens are presented in Table 4. The used insect meals did not cause substantial changes ($P > 0.05$). Blood glucose was not consistently influenced yet a trend towards increased concentrations was observed when BSF meal was used as a dietary protein source ($P = 0.063$). Although, the parameters of liver functions were not significantly affected, the reduced activity of ALT (1.8 times) in the group fed BSF larvae should be noted, whereas the activities of gamma-glutamyl transferase (GGT) increased, when the rations of hens were supplemented with silkworm pupae meal and BSF larvae in comparison with those fed the control diet ($P > 0.05$).

The results from analysis of innate immunity humoral factors (lysozyme and complement) are shown on Figure 1 and Figure 2. The tested insect meals at dietary level 7% did not result in change of serum lysozyme activity. The lyso-

Table 3. Productive performance of hens fed insect-based diets

Parameters	Group			SEM	*P*-value
	Control	BSF	SW		
Live weight, g					
- initial	1592.21	1626.37	1607.36	41.76	0.853
- final	1787.03	1846.41	1901.43	100.41	0.745
Egg weight, g	58.24	57.36	56.01	0.76	0.261
Laying rate, %	67.33	71.34	70.49	2.61	0.580
Egg mass, g	39.18	40.93	39.47	1.46	0.696
Feed intake, g	129.20	127.85	124.50	2.32	0.458
FCR, g/g	3.31	3.13	3.16	0.13	0.168

BSF – Black solder fly larvae meal; SW- Silkworm meal SEM- standard error of the mean

Table 4. Biochemical profile of hens fed insect-based diets

Parameters	Group			SEM	*P*-value
	Control	BSF	SW		
Total protein, g/L	55.62	53.27	59.00	3.33	0.491
Albumin, g/L	22.97	23.77	24.38	1.01	0.621
A/G ratio	0.73	0.82	0.71	0.05	0.229
Uric acid, µmol/L	274.67	299.83	303.00	45.54	0.891
Creatinine, **µmol/L**	36.00	34.67	35.33	1.82	0.876
Cholesterol, mmol/L	2.55	2.05	2.65	0.36	0.477
TG, mmol/l	7.80	7.67	8.73	0.99	0.716
AST, U/L	220.50	228.17	215.50	14.51	0.826
ALT, U/L	13.17	7.17	11.00	2.23	0.189
GGT, U/L	8.83	15.33	14.33	2.89	0.261
Glucose, mmol/l	13.13	14.70	13.67	0.44	0.063
Ca, mmol/L	6.38	5.83	6.34	0.35	0.490
inorg P, mmol/L	1.87	1.40	1.59	0.16	0.143
Mg, mmol/L	1.11	1.15	1.18	0.05	0.589

BSF – Black solder fly larvae meal; SW- Silkworm meal; SEM- standard error of the mean; A/G- Albumin to globulin ratio; TG- triglycerides; AST – Aspartate aminotransferase; ALT – Alanine aminotransferase; GGT – Gama-glutamyltransferase

zyme concentrations in birds fed feed with BSF were comparable to those in controls: 1.35 and 1.30 mg/L, respectively.

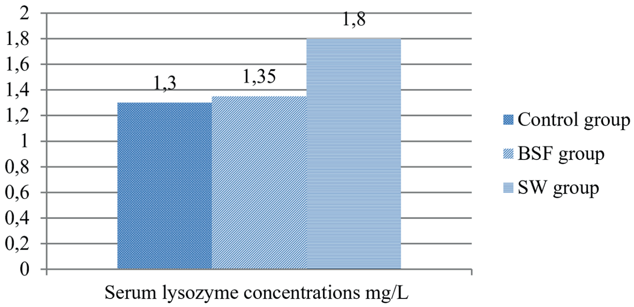

Fig. 1. Serum lysozyme concentrations mg/L in hens fed insect-based diets (BSF – Black soldier fly larvae meal; SW – Silkworm meal)

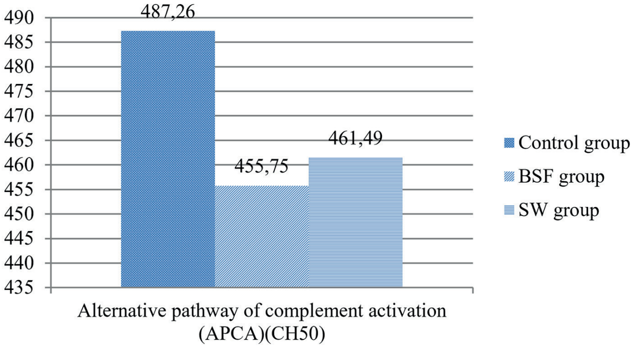

Fig. 2. Alternative pathway of complement activation – APCA – expressed as CH50 (b) in hens fed insect-based diets (BSF – Black soldier fly larvae meal; SW – Silkworm meal)

A relatively weak humoral response with tendency towards increase in serum lysozyme was observed in birds fed silkworm pupae meal – by 27.78% higher compared to control group with insignificant differences. No statistically significant between-group differences in complement haemolytic activity were demonstrated ($P > 0.05$).

Discussion

The present study investigated the effect of replacing soybean meal with silkworm pupae and black soldier fly larvae meals on productive traits, blood biochemical and immune parameters of hens. The effects on productive traits of birds associated with inclusion of BSF, and SW meals were insignificant. The statistical analysis showed that tested meals had no effect on feed intake, confirming the hypothesis for similar taste features. This result was comparable to reports of Ullah et al. (2017), Kawasaki et al. (2019), Kim et al. (2020), who did not found any difference in the amount of feed containing the tested meals, consumed by layer hens. The effect of soybean meal substitution with insect meals on egg weight in available studies is contradictory. In our experiment, the average egg weight was comparable in hens fed the standard ration and rations with BSF larvae and silkworm pupae, in line with findings of Khatun et al. (2005), Ullah et al. (2017) Bovera et al (2018), Kim et al. (2020), Patterson et al. (2021). Other studies established lower egg weight following inclusion of BSF meal in diets (Marono et al., 2017; Bejaei & Cheng, 2020). The possible reason for the observed difference in egg weight in the view of researchers was the lower digestibility of nutrients and the presence of chitin. Our results did not support the findings for higher egg laying rate, when hens were fed rations containing silkworm pupae meal (Khatun et al., 2005; Kim et al., 2020), or BSF meal (Al-Qazzaz et al., 2016). On the other hand, Virk et al. (1980) outlined that egg production declined progressively as the dietary proportion of pupae meal increased. The replacement of soybean meal with silkworm pupae meal did not influence feed conversion ratio, in line with earlier data (Maurer et al.2016; Ullah et al. 2017; Bovera et al. 2018). According to some investigations however, feed conversion has been considerably improved, when silkworm pupae meal was included in the rations of quails and hens (Rahmasari et al., 2014; Kim et al.,2020), as well as when BSF larvae in laying hens' diet (Marono et al., 2017).

Blood biochemical parameter are important for health status of birds (Lumeij, 2008) and are used to evaluate the safety of tested feed components. Plasma proteins play a primary role for maintenance of body homeostasis, while albumin is a source of amino acids for protein synthesis. In the performed study, blood total protein and albumin concentrations were not affected by the inclusion of tested meals in the poultry rations, which presumed a similar bioavailability of dietary protein. A similar conclusion was made by Ullah et al. (2017) and Kim et al. (2020) after feeding silkworm pupae meal to hens. The BSF larvae meal in the diets of guinea fowl and hens did not influence the rate of liver protein synthesis and the levels of analysed serum proteins did not differ (Marono et al., 2017; Wallace et al., 2017). In our study, the albumin to globulin (A/G) ratio was not altered, opposite to results reported by Marono et al. (2017) and Bovera et al. (2018). There were no significant changes (P>0.05) in plasma levels of liver function biomarkers (AST, ALT, GGT).

Data for reduced enzyme activity (AST and ALT) were reported by Kim et al. (2020) in laying hens at substantially lower dietary levels of silkworm meal (up to 0.05%), whereas the partial, or complete replacement of soybean meal had no effect on liver enzymes (Ullah, 2016), as confirmed in the present study as well. The increased blood activity of transaminases (AST, GGT and ALT) indicates their release from hepatocytes following damage (increased permeability of cell membranes, cellular necrosis, or autolytic degradation of hepatocytes). The present study showed a trend towards slight increase in serum glucose concentration in birds fed BSF (P=0.063). Experiments with laying hens have not confirmed any effect from partial, or complete replacement of soybean meal in diets with either pupae meal, or BSF larvae meal (Ullah et al., 2017; Marono et al., 2017), whereas feeding diet with BSF larvae meal to growing turkeys has resulted in increased blood serum glucose levels (Ognik et al., 2020). Total cholesterol and triglycerides in the blood of control and experimental hens did not indicate impaired lipid metabolism following the inclusion of insect meals, in line with other reported data, which confirmed no changes in these blood indices in broiler chickens fed rations with silkworm pupae (Ijaiya & Eko, 2009), or defatted BSF larvae (Dabbou et al., 2018). It is supposed that chitin was bound to bile acids and free fatty acids, when BSF larvae was supplemented to poultry feeds and a favourable reduction of plasma cholesterol and triglyceride levels may be anticipated (Marono et al., 2017; Bovera et al., 2018). Unlike our results, decreased serum cholesterol and triglycerides were reported after inclusion of BSF larvae meal in the feed of hens (Marono et al., 2017; Bovera et al., 2018) and Muscovy ducks (Gariglio et al., 2019).

On the other hand, high dietary levels in the feeds of growing turkeys (10-15%) could probably influence adversely the blood lipid profile of birds as shown by Ognik et al. (2020). Unchanged levels of uric acid and creatinine confirmed a similar rate of protein catabolism and demonstrated normal kidney function. Ullah (2016), Marono et al. (2017), Bovera et al. (2018), Kim et al. (2020), also affirmed lack of any effect from the inclusion of BSF larvae, or pupae meals on birds' protein metabolism. Serum minerals did not change in response to used protein sources. Thus, presented results are somewhat in line with those, reported by Gariglio et al. (2019) and Kawasaki et al. (2019). Higher serum inorganic phosphate was demonstrated in chickens and turkey broilers fed BSF diets (Dabbou et al. 2018; Ognik et al., 2020), as well as in laying hens after complete replacement of soybean meal with BSF larvae meal (Marono et al 2017), which is attributed to greater bioavailability in BSF larvae meal compared to feed components of plant origin (Lee et al., 2016).

The presence of chitin and bioactive compounds in BSF larvae and silkworm pupae suggested that in the role of feed ingredients, they would act as non-specific immunostimulants (Ohta et al., 2014; Anggraeni et al., 2016; Kim & Rhee, 2016; Józefiak & Engberg, 2017). The results about blood immune markers of innate immune response in the present study did not confirm such an effect. Although, the data on effect of insect meals on humoral immune response in birds are scarce, their use as dietary components exhibited positive or neutral effects. The influence of BSF larvae meal added at low dietary levels to compound feed (2% and 3%) on non-specific immune defense was evaluated in broiler chickens experimentally infected with *Salmonella Gallinarum* and a significantly higher serum lysozyme activity was reported (Lee et al., 2018), whereas the study with 10% dietary level showed no effect on humoral factors of innate resistance in turkeys (Lalev et al., 2021). On the basis of above mentioned reports, insect meals may exhibit the greatest effect on humoral immune response at low levels after experimental challenge with a pathogen. A study performed in yellow catfish has confirmed increased serum lysozyme activity when fish meal protein was replaced with BSF meal protein (Xiao et al., 2018). Studies demonstrated that chitin has complex and size-dependent effects on innate and adaptive immune responses (Lee et al., 2008). The results of Benzertiha et al. (2020) confirmed that the addition of a relatively small amount (0.2% and 0.3%) *Tenebrio molitor* and *Zophobas morio* meals to the diet of broiler chickens altered some immune parameters. Pasotto et al. (2020) concluded that black soldier fly nutritional additives had immunostimulating effect on some immune parameters of quails, yet this depended mainly on the substrate used for rearing larvae. Furthermore, the expression of antimicrobial peptides (AMPs) was also associated with the diet of larvae (Vogel et al., 2018). The thermal processing, on the other hand, could influence bioactive compounds and reduce their amount in the end product (Dörper et al., 2021).

Conclusion

The obtained results demonstrated that the tested black soldier fly larvae and silkworm pupae meals could be successfully used as alternative protein source in the rations of layer hens at dietary levels of 7%, without adverse effects on egg production, physiological and immunological status of birds.

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