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Effects of insect- and probiotic-based diets on turkeys' production, health, and immune parameters

Mitko Lalev[1], Nadya Mincheva[1], Magdalena Oblakova[1], Pavlina Hristakieva[1], Ivelina Ivanova[1], Atanas Atanassov[2] and Adelina Petrova[3]

[1]*Agricultural Academy, Agricultural Institute, 6000 Stara Zagora, Bulgaria*
[2]*Joint Genomic Center, Sofia University, "Dragan Tzankov" 8, 1164 Sofia, Bulgaria*
[3]*'Nasekomo' AD, Business Park Sofia Building 2, 1715 Sofia, Bulgaria*
[*]*Corresponding author:* mtlalev@abv.bg

Abstract

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Replacement of soybean with locally produced insect meals and products into poultry diets will deliver a model with potential for improvement of the economic sustainability in the poultry industry. The study aimed to elucidate the effects of 10% inclusion of insect meals from Black soldier fly (*Hermetia illucens*) and Silkworm (*Bombyx mori*) on turkeys' growth, carcass traits and health parameters. A total of 75 Hybrid commercial female turkeys at 56 days of age were individually weighed to make uniform groups and assigned to five dietary treatments: Control (soybean meal),Silkworm meal (SW), Silkworm meal with probiotic mix 'Zoovit' (Swpro), Black soldier fly defatted (BSFd) and Black soldier fly whole larvae (BSFw) meals. The experiment lasted for 74 days, from 56-130 days of a turkey's age.

Thenew diets had a positive impact on turkeys' growth and production parameters. Overall, BSF meals had a superior impact on turkeys than the SW, with the strongest responses observed at 2 weeks post-insect feeding (LW ~7-9% increase from control; FCR, 2.45 vs.3 control). Carcass composition traits were not affected, except for the gizzard weight, which was smaller in turkeys fed with the insect-based diets. The physiological andimmune status of turkeys from all groups did not vary significantly, except for glucose decreases in SWpro fed group (possible probiotics effects) and uric acid increases in SW fed group (high uric acid levels in SW pupae meal).

Overall, the study demonstrated insect species-specific diet effects on turkeys' responses. Both BSF meal formulations (defatted-whole larvae) produced similar trends on turkeys' production. The combination of insect meal and probiotics improved turkeys' physiological status but had no effect on the production parameters.

Keywords: Black soldier fly (*Hermetiaillucens*); Silkworm (*Bombyx mori*); insect- probiotic-based diets; turkeys, feeding

Introduction

Insects are a natural food of birds, and in the wild they are the main protein source for many animals. The superior nutritional quality of the insect meal is determined not only by the amount of proteins and fats, but also by their balanced spectrum of essential amino acids (Veldkamp & Bosch, 2015; Van Huis & Tomberlin, 2017), a key factor in poultry nutrition ensuring rapid growth and better production parameters (Makkar et al., 2014; Józefiak et al., 2016). In addition, insects are rich in a wide range of health-promoting components such as chitin, lauric acid, and antimicrobial peptides (Otvos, 2000; Skřivanová et al., 2005; Lieberman et al., 2006; Khempaka et al., 2011; Harikrishnan et al., 2012; Chernysh et al., 2015; Józefiak & Engberg, 2017; Karlsen et al., 2017; Tonk & Vilcinskas, 2017; Belghit et al., 2018; Gasco et al., 2018) that can impact birds' growth and welfare. Thus, such chemical complexity of the insect meal may provide many advantages for poultry feeding. To date, several insect species such as Black soldier fly (Schiavone et al., 2019; Pasotto et al., 2020), Silkworm (Khatun et al., 2003; Sheikh et al., 2005; Sharmila, 2008; Ijaiya & Eko, 2009; Ullah et al., 2017), grasshoppers (Sun et al., 2012) , house flies (Radulović et al., 2018), and mealworms (Bovera et al., 2015; Elahi et al., 2020), have been investigated as a potentialfeed for poultry and livestock. Although results on the effects of insect meals on poultry production parameters vary, positive trends are emerging.

Manufacturing of insect-based products for animal feed will address the growing trend of feed shortage and cost increases, worldwide. EU, including Bulgaria, is highly dependent on soybean market price unpredictability and trends, as 80% of raw protein materials required for animal feed in EU are imported. Replacement of soybean with locally produced insect meals and products will deliver a model with potential for improvement of the economic sustainability in the poultry industry. Black soldier fly (BSF) is one of the few insect species currently used in insect farming for mass production. Its potential is underlined by many features of its biology, including the ability to feed and digest any type of organic waste and survive in harsh environments (Bruno et al., 2019). Such features have defied BSF as one of the most important insects for bioconversion. Among the four different life-stages (egg, larva, pupa, adult), only larvae feed actively, and this stage is employed for mass-production. In contrast, the Silkworm (SW) is a part of the silk production process, and the pupal stage is used as a nutrient source. While the SW feeds on mulberry leaves only, the BSF can use different organic substrates, including spent grain. Such differences will ultimately dictate the specificity of the chemical composition of the insect meals.

In parallel to the main objective, several other aims were forming the core of the project. One aim focused on investigating the combination of the probiotic mix Zoovit ('LBLacBas'-Bulgaria) and SW meal to understand how the interactions between the two components affect turkeys' growth, production, and health parameters. Furthermore, the study assessed the effects of different BSF meal fractions on turkey performance by inclusion of BSF defatted meal (BSFd) and BSF whole larvae meal (BSFw).

Material and Methods

Insect meals

The BSF meals (whole larvae and defatted) used in the study (Table 1) were produced and provided by 'NASEKOMO', Bulgaria. The spent silkworm pupae (chrysalis, *Bombix mori* L) were obtained from the Scientific Center on Sericulture, Vratsa, Bulgaria. Chemical analyses of all insect meals (SW, BSFd, BSFw) were carried out at the *University of Food Technologies* in *Plovdiv*, Bulgaria. Moisture and fat contents were determined according to the Association of Official Analytical Chemists methods 925.09 and 922.06, respectively (Association of Official Analytical Chemists [AOAC] 2006). Crude protein was determined by the Kjeldahl method (984.13). Amino acid analysis was performed using HPLC Waters AccQ Tag Method.

Table 1. Chemical composition of insect meals

Parameters	SWM	BSFd	BSFw
Fat content, %	24.50	7.79	13.48
Protein content, %	57.14	56.16	44.76
Moisture content, %	11.50	1.03	0.83
Gross energy, kcal/kg	5831	2090	2325
Calcium, %	0.55	0.84	0.18
Phosphorus, %	0.75	0.67	0.43
Amino acid content, %			
Valine	5.60	4.79	4.35
Isoleucine	6.90	4.93	4.00
Leucine	7.24	1.00	0.82
Lysine	3.83	8.04	6.92
Methionine	3.70	1.88	6.41
Methionine+Cystine	4.65	11.22	13.32
Threonine	5.20	5.19	4.32
Phenylalanine+Tyrosine	10.70	7.91	5.75
Arginine	4.50	7.16	5.81
Glycine	4.70	2.71	1.94
Histidine	2.90	11.25	10.90

Birds and husbandry

All procedures, including the use of birds, management and care, were in compliance with the European Council Directive regulations on the protection of animals used for experimental and other scientific purposes (2010/63/EU), and national protocol № 20 from 01.11.2012.

The present study was conducted at the Poultry farm of the Agricultural Institute, Stara Zagora, Bulgaria. A total of 75 Hybrid commercialfemale turkeys at 56 day of age were individually weighedto make uniform groups (P>0.05) and were assigned to five dietary treatments (3 replications per treatment). The experiment lasted for 74 days, from 56-130 days of turkey's age. The birds in each group were reared in a floor pen, divided in three sections, covered with wood shav-

Table 2. Composition of the experimental diets

Ingredients, %	Feeding periods															
	56-78 days				79-94 days				95-114 days				115-130 days			
	C	SWM	BSFd	BSFw	C	SWM	BSFd	BSFw	C	SWM	BSFd	BSFw	C	SWM	BSFd	BSFw
Wheat	28.00	55.00	19.00	15.00	34.00	51.04	29.00	20.00	11.00	28.00	27.00	20.00	21.00	39.00	29.00	22.00
Corn	21.80	7.79	33.74	34.11	20.34	17.00	28.43	33.73	48.87	45.59	37.68	40.57	45.26	40.87	41.20	44.04
Soybean meal	33.77	16.00	21.00	24.57	28.00	11.00	15.00	19.00	23.00	6.00	8.00	12.00	17.00	-	3.00	7.00
Sunflower meal	7.00	7.00	7.00	7.00	7.00	7.00	7.00	7.00	7.00	7.00	7.00	7.00	7.00	7.00	7.00	7.00
SWM	-	10.00	-	-	-	10.00	-	-	-	10.00	-	-	-	10.00	-	-
BSFd	-	-	10.00	-	-	-	10.00	-	-	-	10.00	-	-	-	10.00	-
BSFw	-	-	-	10.00	-	-	-	10.00	-	-	-	10.00	-	-	-	10.00
Sunflower oil	5.00	-	5.10	5.10	6.50	-	6.50	6.30	6.60	-	7.00	7.00	6.50	-	6.80	6.80
Dicalcium phosphate	2.62	2.18	2.45	2.60	2.36	1.95	2.13	2.30	2.01	1.62	1.70	1.83	1.76	1.36	1.50	1.65
Limestone	0.55	0.92	0.58	0.61	0.51	0.86	0.61	0.61	0.40	0.75	0.58	0.62	0.43	0.77	0.55	0.58
Methionine	0.22	-	0.18	-	0.19	-	0.16	-	0.13	-	0.11	-	0.10	-	0.07	-
Lysine	0.29	0.36	0.20	0.26	0.30	0.35	0.21	0.26	0.19	0.24	0.13	0.18	0.15	0.20	0.08	0.13
Salt	0.25	0.25	0.25	0.25	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30
Salgard	0.20	0.20	0.20	0.20	0.20	0.20	0.40	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Premix,	0.20	0.20	0.20	0.20	0.20	0.20	0.16	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Optizyme	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Calculated nutritive value																
Metabolizable energy (Kcal/kg)	2951	3045	2953	2952	3104	3120	3102	3103	3217	3216	3216	3217	3250	3253	3253	3252
Crude protein, %	22.75	22.64	22.73	22.71	20.80	20.82	20.82	20.83	18.38	18.36	18.32	18.36	16.62	16.62	16.67	16.71
Fat, %	6.84	4.06	7.08	7.84	8.30	4.28	8.36	9.02	9.00	4.87	9.07	9.87	8.86	4.81	8.97	9.77
Crude fiber, %	4.85	4.30	4.21	4.37	4.60	4.02	3.94	4.09	4.14	3.60	3.56	3.72	3.90	3.36	3.32	3.48
Phosphorus, %	0.95	0.88	0.91	0.92	0.88	0.82	0.83	0.85	0.79	0.74	0.73	0.74	0.74	0.68	0.68	0.69
Calcium, %	1.21	1.20	1.20	1.20	1.09	1.09	1.09	1.09	0.92	0.92	0.92	0.92	0.83	0.83	0.83	0.83
Lysine, %	1.50	1.51	1.50	1.51	1.36	1.37	1.36	1.36	1.11	1.11	1.10	1.10	0.92	0.92	0.93	0.93
Methionine, %	0.58	0.64	0.57	0.59	0.52	0.62	0.52	0.56	0.44	0.60	0.44	0.53	0.39	0.57	0.38	0.51
Methionine+cysteine,%	0.94	1.00	0.75	0.60	0.85	0.95	0.66	0.55	0.75	0.92	0.55	0.48	0.68	0.86	0.47	0.44
Threonine, %	0.85	1.11	0.93	0.88	0.77	1.04	0.84	0.80	0.69	0.96	0.74	0.69	0.61	0.88	0.70	0.62

ing litter under the identical management conditions e.g., temperature, relative humidity, light, ventilation, and floor space. The floor section dimensions were 1 x 2 meters which provided 0.40 square meters of space to each bird.Feed and waterwere provided on an*ad libitum* basis. Five diets were set up, where in the control group turkeys were fed with a standard soybean-based diet (SBM), and in the treatment groups 10% of insect meals wereincluded as follow: SW – 10% silkworm meal, SWpro – 10% silkworm meal supplemented with probiotic Zoovit in the amount of 0.05%, containing *L. bulgaricus, L. acidophilus, S. termophilus, L. lactis, Propioni bacterium* ($0.1x10^9$ CFU/ per gram), BSFd – 10% defatted BSF larvae meal, BSFw – 10% BSF whole larvae meal. The diets were formulated to meet nutritional requirements of birds according to the nutrition recommendation for Hybrid commercial turkeys (2019). The feeding program covered four feeding periods adjusted to the age and developmental stage of the birds. The composition and nutritional characteristics of experimental diets are reported in Table 2.

Growth performance

Body weight and feed intake of turkeys were recorded at 56, 78, 94, 114 and 130 days of age per replicate to estimate growth parameters, such as, average daily gain (ADG), average daily feed intake (ADFI) and feed conversion ratio (FCR) on a cumulative basis.Mortality was monitored daily during the whole experimental period.

Blood sampling, serum biochemical parameters and evaluation of immune response of turkeys

At the end of the experiment, sixbirds per group were randomly selected to collect blood samples aseptically from *vena ulnaris*in sterile heparinised vacutainers (FLmedical, Italy) for analyses of Aspartate aminotransferase (AST), Alanine aminotransferase (ALT), Gama-glutamyltransferase (GGT), total protein, creatinine, uric acid, glucose, triglyceridesand cholesterol to evaluate liver and kidney function. Within 30 min of blood collection, blood samples were centrifuged at 1500 × g for 10 min. Plasma was harvested and stored at –20°C until further analysis. All biochemical analyses were assayed on an automated biochemical analyzer BS–120 (Mindray, China). To evaluate the effect of the insect meals on turkeys' immune responses two factors were analyzed: the serum lysozyme concentrations, measured by the method of Lie et al. (1985), and the alternative pathway of complement activation (APCA) analyzed by the method of Sotirov (1991).

Carcass analysis

At 130 d of age, three birds from each dietary treatment were chosen on the basis of the average body weight and were slaughtered *in-house*, following 12 hours of fasting. The objective of fasting is to reduce carcass contamination during processing. Birds were individually weighted just before slaughter for the final body weight determination. After evisceration, the hot carcasses were weighed to evaluate dressing yield expressed as a percentage of slaughter body weight. Thereafter, the carcasses were cut into parts. The yields of breast (including the skin and bone), legs (including the thigh and drumstick with skin), wings and back were determined relative to carcass weight. The relative internal organ weights (liver, heart and gizzard) and abdominal fat content were determined with respect to the carcass weight.

Statistical analysis

Results were analyzed by one-way analysis of variance (ANOVA)using STATISTICA software ver. 10 (Statsoft, Inc., 2011), according to the following model: $Yij = \mu + CPi + eij$, in which Y is the single observation, µis the general mean, CP is the effect of protein source (i = SBM; SW; BSFd; BSFw) and *e* is the error. Normality and homoscedasticity tests were run on data before the means were tested and the significance was set to $P<0.05$. For parametric analysis, Fisher's least significant difference (LSD) test was used to compare the mean group differences, while for nonparametric analysiswas used the Kruskal-Wallis test. The results were expressed as a mean, pooled standard error of the mean,and the threshold for significance was set at $P<0.05$.

Results

Insect meals

All chemical analyses of the insect meals are shown in Table 1. As expected, the chemical profiles of SW, BSFd, BSFw meals very. While the protein content in SW and BSFd were similar (~56-57% from DM), the BSFw had lower value (~45% from DM). Lipid analyses for BSFd and BSFw meals were 7.79% and 13.48% respectively, while for SW meal this was 24.5% from DM. The moisture content was also higher in SW meal (11.5% from DM) than the BSFd (0.83% from DM) and BSFw meals (1.03% from DM). On energy level, SW meal showed the highest calories values (5831 kcal/kg) compared to BSFd and BSFw meals (2090 kcal/kg, 2325 kcal/kg). The amounts of calcium and phosphorus were determined as well, indicating that the BSFw meal was with the lowest values (Ca-0.18%; P-0.43% from DM). The amino acids profiles of all insect meals differ, as expected. It must be noted that among the three insect meals, BSFw had the highest score of Methionine+Lysine and Methionine+Cystine (Table 1), a key factor in promoting rapid growth.

Table 3. Turkey live weight (g) over the experimental period

Age, days	Groups					SEM	*P*-value
	Control	SW	SWpro	BSFd	BSFw		
56	2646.67	2563.30	2602.67	2532	2517.33	20.85	0.286
78	4973.33 b	5260.71 a	5326.67 a	5337.5 a	5420 a	47.58	0.005
94	6766.67 b	7040 a	7093.33 a	7062.5 a	7140 a	42.16	0.012
114	8446.67 b	8713.3 b,c	8717.8 a,b	9126.7 a	9086.67 a,c	84.35	0.021
130	9873.33 b	10273 a,c	10389 a	10072 b,c	10247 a	56.67	0.008

a,b,c- means with different letters in the row represent significant differences at P< 0.05
SW – silkworm meal; SWpro – silkworm meal +probiotic; BSFd – Black solder fly defatted meal; BSFw – Black solder fly whole larvae meal; SEM- standard error of the mean

Turkeys' performance

Prior to the experimental time, turkeys were fed with the same starter diet. The 10% inclusion of insect meals was introduced at day 56 of turkeys' age where small weight variations between the control and treatment groups occurred but did not differ significantly (Table 3; $P = 0.286$). No turkey deaths were observed from days 56 to 130.

Overall, all insect- and probiotic-based diets had a positive effect on turkeys' live weight (LW), with the strongest observed in the first period (56-78 days) with an average of 6-9% weight increase (Figure 1) between insect fed groups and the control group (Table 3; $P = 0.005$). BSFw fed group had the highest responseof ~9%. This trend was not followed in the second period (56-94 days), where the difference in weight gain between the control and treatment groups was about 4-5% (Figure 1), but statistically significant (Table 3; $P = 0.012$). In the third period (56-114), a noteworthy weight gain of about 8% was observed only in BSFd and BSFw fed groups (Figure 1) and not in SW and SWpro fed groups, compared to the control group (Table 3; $P = 0.021$). In the final period (56-130), a significant increase of weight (~3-5%), compared to the control, was recorded in SW, SWpro, and BSFw fed groups (Table 3; $P = 0.008$), with the highest observed in SWpro (5.51%).

The daily average gain recordings (Table 4) followed a similar pattern. Weekly recordings of daily feed intake show no significant differences in feed intake in the first period (56-78 days), following the introduction of the insect meals (Table 4; $P = 0.478$). The feed intake in the second period (56-94 days), however, was characterized with a significant increase (7.4 %-10.6%; Table 4; $P = 0.041$) in SW, SWpro and BSFw groups compared to the control, with highest in SW group. In the third period (56-114) no appreciable differences were observed. In contrast, in the last period (56-130), the consumption of feed significantly increased in SW and SWpro fed groups (10.6%, 9.4%; Table 4; $P = 0.049$), and not in BSFd and BSFw fed groups, compared to the control.

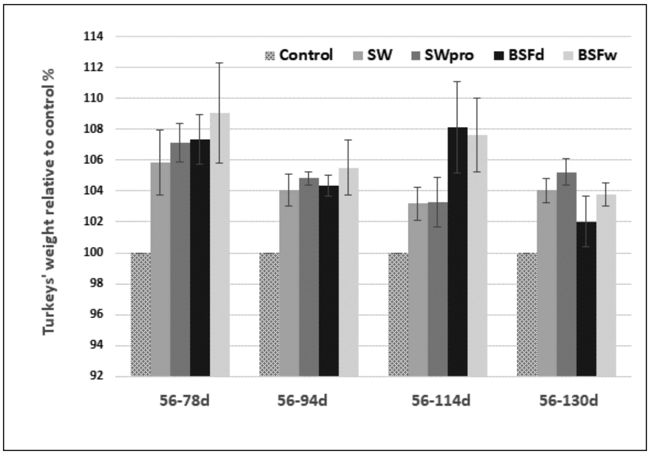

Figure 1. Turkeys' live weight relative to the control group

Overall, feed conversion ratio (FCR) analyses confirm that turkeys fed with BSFd and BSFw meals exhibited a better (than SW and SWpro) and significant FCR over the period of 56-114 days ($P = 0.000$). In the first period (56-78 day), the FCR values for BSFd and BSFw were the most impressive, 2.46 and 2.45 respectively, compared to the control 3.00 (Table 4; $P = 0.000$). The differences in SW and SWpro were less notable (Table 4), with the exception of the first period, where FCR values were 2.74 and 2.65 respectively (Table 4; $P = 0.000$). In the last period, none of insect and probiotic fed turkeys exhibit significantly different FCR values from that of the control.

Carcass composition

Inclusion of insect- and probiotic-based meals had no effects on turkeys' carcass composition (Table 5), with one exception that of a gizzard weight of turkeys from SWpro, BSFd and BSFw fed groups. While in the control fed group the gizzard-weight was 1.72% from the carcass weight (CW), in SWpro,

Table 4. Turkeys' performance parameters following inclusion of insect- and probiotic-based diets

Parameters	Groups					SEM	*P*-value
	Control	SW	SWpro	BSFd	BSFw		
56-78 d							
ADG (g/day)	105.76 b	122.65 b	123.82 a,b	127.55 a,b	131.94 a	2.57	0.000
DFI (g/day)	316	336.33	327.83	313.17	324	4.25	0.478
FCR (g/g)	3.00 a	2.74 b	2.65 b	2.46 c	2.45 c	0.05	0.000
56-94 d							
ADG (g/day)	108.42 b	117.83 a	118.18 a	119.23 a	121.65 a	1.42	0.008
DFI (g/day)	311 b	344 a	337 a,c	318 b,c	334.12 a,c	4.23	0.041
FCR (g/g)	2.87 a	2.92 a	2.85 a	2.67 c	2.75 b	0.03	0.000
56-114 d							
ADG (g/day)	100 b	106.03 b	105.43 b	113.71 a	113.26 a	1.58	0.003
DFI (g/day)	313	320	317.67	321.67	318	2.64	0.910
FCR (g/g)	3.13 a	3.02 b	3.01 b	2.83 c	2.81 c	0.03	0.000
56-130 d							
ADG (g/day)	97.66 b	104.19 a	105.22 a	101.89 a	104.45 a	0.89	0.014
DFI (g/day)	319 b	353 a	349 a	336 a,b	331 a,b	4.31	0.049
FCR (g/g)	3.27 a,b	3.39 a	3.32 a	3.30 a	3.17 b	0.02	0.029

a,b,c- means with different letters in the row represent significant differences at $P< 0.05$

SW – silkworm meal; SWpro – silkworm meal +probiotic; BSFd – Black solder fly defatted meal; BSFw – Black solder fly whole larvae meal; ADG – average daily gain; DFI- daily feed intake; FCR – feed conversion ratio SEM – standard error of the mean.

Table 5. Carcass composition of turkeys fed with insect- and probiotic-based diets

Parameters	Groups					SEM	*P*-value
	Control	SW	SWpro	BSFd	BSFw		
Slaughter weight, g (SW)	9 733 b	10 133 a,b	10 433 a	9 933 b,c	10 267 a,c	81.65	0.023
Carcass weight, g (CW)	7 575	7 578	7 893	7 632	7 659	59.08	0.460
Carcass yield, % SW	77.83	74.77	75.66	76.85	74.59	0.45	0.084
Breast, % CW	37.02	38.86	38.46	39.02	38.70	0.36	0.434
Legs *, % CW	28.71	29.95	30.29	30.46	29.40	0.30	0.385
Wings *, % CW	11.34	11.55	11.42	11.29	11.55	0.12	0.957
Back, % CW	21.97	19.24	19.66	19.09	20.41	0.41	0.137
Gizzard, % CW	1.72 a	1.52 a,b	1.38 b	1.37 b	1.34 b	0.05	0.033
Liver, % CW	1.52	1.48	1.45	1.48	1.39	0.03	0.760
Heart, % CW	0.47	0.46	0.45	0.42	0.48	0.01	0.659
Abdominal fat, % CW	1.50	1.24	1.88	1.95	2.90	0.20	0.058

a,b,c- means with different letters in the row represent significant differences at $P<0.05$; SEM: standard error of the mean.

SW – silkworm meal; SWpro – silkworm meal +probiotic; BSFd – Black solder fly defatted meal; BSFw – Black solder fly whole larvae meal; *With bone and skin; SEM – standard error of the mean

BSFd and BSFw fed groups they were significantly smaller-1.38%, 1.37% and 1.34% respectively (Table 5; $P = 0.033$). Two other trends must be noted. The carcass weight was expected to follow similar tendency as in pre-slaughter live weight (SW). However, despite the higher carcass weight in SWpro, BSFd and BSFw fed groups compared to the control, no statistical significance was observed (Table 5; $P = 0.460$). It must be noted, as well, that the BSFw had 2.90% abdominal fat content, while in the control this was 1.50% (Table 5; $P = 0.058$), which is on the borderline of the statistical significance.

Blood biochemistry

Several factors in turkeys' blood have been analyzed to indicate the effects of insect- and probiotic-based diets on turkeys' metabolic status (Table 6). Overall, recorded data suggests no significant (Table 6; $P>0.05$) changes among the different dietary groups except for glucose and uric acid levels. While the glucose levels in the control and other treatment groups were in the range of 16.75-17.17 mmol/L, in the SWpro group these were significantly lower – 15.85 mmol/L (Table 6; $P = 0.012$). In terms of uric acid, analysis showed a

Table 6. Biochemical parameters in the blood plasma of turkeys fed with insect- and probiotic-based diets

Parameters	Groups					SEM	*P*-value
	Control	SW	SWpro	BSFd	BSFw		
Glucose, mmol/L	16.75 a	16.92 a	15.85 b	17.13 a	17.17 a	0.14	0.012
Total protein, g/L	43.33	46.65	43.50	41.58	39.92	0.82	0.104
Creatinine, µmol/L	37.33	37.00	35.83	36.83	33.83	0.47	0.115
Uric acid, µmol/L	320.00 b	433.83 a	284.5 b	248.50 b	327.50 b	18.48	0.012
Cholesterol, mmol/L	2.97	3.13	2.90	2.79	2.56	0.07	0.135
Triglyceride, mmol/L	1.25	1.96	0.89	1.25	1.20	0.12	0.058
AST, U/L	532.33	529.17	519.17	547.00	526.67	12.44	0.974
ALT, U/L	12.33	10.17	12.83	13.83	11.00	0.58	0.297
GGT, U/L	4.17	4.83	3.50	4.50	3.50	0.38	0.770

a,b – means with different letters in the row represent significant differences at $P< 0.05$; SEM: standard error of the mean
SW – silkworm meal; SWpro – silkworm meal +probiotic; BSFd – Black solder fly defatted meal; BSFw – Black solder fly whole larvae meal; AST – Aspartate aminotransferase; ALT – Alanine aminotransferase; GGT – Gama-glutamyltransferase; SEM – standard error of the mean

significant increaseof 36% in SW fed group (433.83 µmol/L; *P* = 0.012) compared to the control fed group (320.00 µmol/L). In contrast, turkeys from BSFd group exhibited a lower level of uric acid (248.50 µmol/L) compared to the control. The next two factors that were measured were the levels of serum protein and creatinine, which among different treatment groups were not affected (P>0.05). The level of the cholesterol remained similar for all groups and ranged between 2.56-3.13 mmol/L. Insect-based diets did not significantly affect the levels of serum triglycerides, as well. However, while in the control, in BSFd and BSFw fed groups the range was 1.20-1.25 mmol/L,in SW and SWpro fed groups these were 1.96 and 0.89 mmol/L (Table 6; *P* = 0.058).

As a liver function biomarker, three enzymes – Aspartate aminotransferase (AST), Alanine aminotransferase (ALT), and Gama-glutamyltransferase (GGT) were analyzed, and among all groups no significant differences were found (Table 6; *P* = 0.974, *P* = 0.297, *P* = 0.770 respectively). The values obtained for the biochemical indices were within the normal ranges for turkeys given by Wilkanowska (2015).

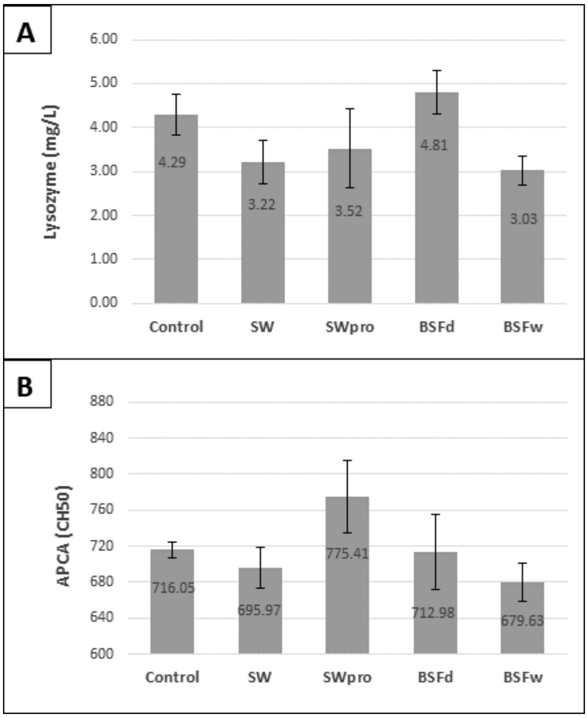

Figure 2. Analyses of turkeys' innate immunity factors
All new diets had no significant effects on turkeys' (A) – Lysozyme (P = 0.174) and (B) – Alternative pathway of complement activation (APCA), (P = 0.322) responses

Innate immunity factors

Two components of innate immunity were analyzed – Lysozyme and Alternative pathway of complement activation (APCA). For both factors no appreciable differences among all groups were recorded (Figure 2).

Discussion

Growth performance

Overall, inclusion of insect meals, including SWpro had a positive and statistically significant impact on turkeys' LW, ADG and FCR. Importantly, each examined period demonstrated specific interactions between the new diets and turkeys' growth responses (Figure 1; Tables 3 and 4). The important points that must be noted are: LW, ADG and FCR values varied over time, but the strongest responses were observed in the first period (56-78 day) of the experiment. Insect fed groups of turkeys had an increase of ~6-

9% in LW than the control group, with better responses in BSFd (7.35%) and BSFw (9.05%) fed groups (Figure 1; Table 3; *P* = 0.008). In contrast, the final periodwas characterized with only ~2-5%increase of LW, with most differences observed in SWpro (5.24%) fed group.The SW and SWpro fed groups tended to have more constant LW response over time (~3-5% increase difference compared to the control). In contrast, BSFd and BSFw had two LW response 'peaks' at 78d with 7-9%, and 114d with ~8%. Turkeys fed with SW and probiotic mix did not exhibit any specific and different trends in LW than those from the other treatment groups, suggesting that the Zoovit probiotic mix had no effect on turkeys' LW.

On the bases of ADG, again, both BSF meals had similar effects on turkeys, but differ from SW meals. For the entire experimental period (56-130d), BSFd and BSFw meals had statistically significant impacts on turkeys' rate of weight gain per day (Table 4; *P* = 0.000, *P* = 0.008, *P* = 0.003; *P* = 0.014), with one exception in the first period, where in the BSFd group the ADG was not statistically different from the control (127.55 vs.105.76 g/day). In contrast, the SW and SWpro fed groups followed different patterns, where a statistical significance was observed only at 94d and 130d periods (Table 4). To better understand turkeys' growth parameters, DFI recordings were carried out over the experimental time (56-130 d). Once these were included in the analytical model, important trends were beginning to emerge. The first period (56-78 d) was characterized with no statistical differences in DFI among all groups, suggesting that inclusion of insect meals and probiotics did not affect the feed intake either way (increase or decrease). However, for the rest of the period 78-130 d, turkeys fed with SW and SWpro meals ingested more food (~8-11% relative to the control), as suggested by DFI values (Table 4; *P* = 0.041; *P* = 0.049). Thus, SW meals modulated turkeys' feeding behavior by stimulating feeding, while BSF meals did not have this effect with one exception for BSFw group at 94 d. When these results are put into context of LW and ADG, it can be suggested that BSF meal effects on LW and ADG are the result of qualitative mechanisms such as improved assimilation of nutrients and growth promoting factors in BSF meals. These are reflected in greater FCR values:BSFd and BSFw fed groups exhibited the most positive response with FCR 2.46 and 2.45 respectively, while the FCR in the control fed group was 3 (Table 4; *P* = 0.000). This positive trend continued to 114d. FCR values in SW and SWpro groups were closer to that of the control, except for the first period (SW-2.74, SWpro-2.65). Thus, both insect species – BSF and SW had a positive impact on turkeys' performance parameters, but in a species-specific manner. The positive effects of insect meals have been demonstrated by an array of studies. For instance, poultry fed with partial inclusion of SW have similar production parameters as those fed with fish meal only (FM), (Dutta et al., 2003; Khatun et al., 2003; Ijaiya & Eko, 2009), suggesting that the nutritional quality of the SW meal is similar to that of FM. Replacement of the expensive FM with SW meal will not only reduce the cost of feed, but importantly, will improve poultry health as often FM is treated with pesticides. Many investigations in Europe and around the world on BSF effects on poultry also demonstrated positive trends (Schiavone et al., 2017; Dabbou et al., 2018; Schiavone et al., 2019).

Analysis on carcass composition added an additional level of understanding of the effects of the new diets on turkey performance. Overall, all insect meals, including SWpro, did not affect the carcass composition, such as carcass weight (CW), carcass yield (CY) and the commercial portions (i.e. breast, thigh, drumstick, wing and back), (Table 5; *P*>0.05). However, the only part from the carcass that differs between the control and treatment groups was empty gizzard (Table 5; *P* = 0.033). The reduction of gizzard weight might be related to the reduced muscular activity of the gizzard. The insect mealsare not known to have no effect on gizzard weight in other studies (Sheikh et al., 2005; Sharmila, 2008; Uushona, 2015), although Kareem et al. (2018) also observed similar effectsin broiler chickens fed with 10% BSF larvae meal.

Other studies investigating the effects of insect meals on poultry reported varying results on LW, CW, CY, which is expected, as different concentrations of insect meals are included in different growth stages, including different experimental set-ups and number of analyzed birds. For instance, Schiavone et al. (2019) reported small, but significant increase of ~ -0.4-0.8% for chickens fed with 5% and 10% BSF defatted meal. Importantly, the study showed that 15% inclusion of BSF defatted meal resulted in decrease of LW and CW, suggesting that the 10% is the maximum concentration that can have positive effects on chicken performance. In another study, which investigated the effects of BSF on broiler quails (Cullere et al., 2016), no difference was shown between the control and treatment groups for many parameters, including LW, CW, CD, FCR, and palatability. However, inclusions of BSF meals in the diets were introduced only for 18 days (from 10d old to 28d old). In contrast, our study demonstrated the highest responses (LW, FCR) were in the first two weeks, following the introduction of BSF and SW meals into turkeys' diet. Therefore, these studies point out the importance of detailed investigations to understand the dynamics of interactions between diets and poultry performance parameters and establish economically viable strategy.

The second part of this study was to establish the effects of insect- and probiotic-based diets on turkey's overall health and physiological status by measuring several biochemical blood parameters (Table 6). Blood analyses showed that parameters such as total protein, creatinine, cholesterol and triglycerides among all groups did not vary significantly, suggesting that the insect meals and the probiotic mix did not have any adverse effects on turkeys' metabolic homeostasis. From all analyzed parameters, only two were affected significantly, the uric acid and glucose. The levels of uric acid in SW fed group were significantly higher compared to the control (433.83 μmol/L vs. the control 320 μmol/L; Table 6, $P = 0.012$) and other treatment groups. This can be explained by the higher content of uric acid in SW meal that was previously reported by He et al. (2019). The uric acid is the end product of the purine metabolism, and foods rich in purines may affect kidney functions by not eliminating the uric acid efficiently. An additional and very important mechanism for eliminating the uric acid is its metabolization by the gut microflora (Sorensen 1965). Interestingly, turkeys fed with SW meal and the probiotic mix Zoovit exhibited significantly lower level of uric acid (284.5 μmol/L), suggesting the importunate of the interactions between the microbiota and intestinal uric acid metabolism and excretion that could potentially modulate serum uric acid levels. Further investigations on the role of Zoovit in regulating uric acid levels will elaborate on the current findings, as it may offer an alternative therapeutic strategy for lowering the levels of uric acid in humans with gout condition. The role of Zoovit in turkeys' metabolism was also noted in lowering the levels of blood glucose (Table 6). Turkeys fed with SWpro diet contained significantly lower level of blood glucose (15.85 mmol/L; $P = 0.012$) than the control (16.75 mmol/L) and other treatment groups (SW = 16.92 mmol/L, BSFd = 17.13 mmol/L, BSFw = 17.17 mmol/L). Several reports demonstrated the antidiabetic effects on probiotics (Honda et al., 2012; Kim et al., 2013; Widodo et al., 2019). However, it is important to emphasize that not all lactobacilli can modulate blood glucose levels, even though they convert glucose to lactic acid. For instance, it has been shown that *Lactobacillus rhamnosus* can modulate serum glucose levels in mice, but this was not observed for *Lactobacillus bulgaricus* (Honda et al., 2012). The mode of action of probiotics is complex (Naydenov et al., 2012; Jadhav et al., 2015), and the present study indicates that the Zoovit formulation works well with a combination of SW meal, resulting in positive impacts on turkeys' health. Further indication for this is the fact that the levels of triglycerides in SWpro fed group (0.89 mmol/L) were half from those in SW fed group (1.96 mmol/L) and lower than the control, BSFd and BSFw (1.25, 1.25, 1.20 mmol/L respectively), although this was on the border of the statistical significance (Table 6; $P = 0.058$).

All insect- and probiotic-based diets did not affect liver function, as differences in concentrations of the analyzed enzymes (AST, ALT, GGT) among all groups did not vary significantly (Table 6; $P = 0.974$, $P = 0.297$, $P = 0.770$ respectively). Based on this, and the above-mentioned results, it can be concluded that inclusion of 10% of SW, SWpro, BSFd, BSFw meals in turkeys' diet does not have negative impact on turkeys' metabolic homeostasis and liver function. Similar results are reported by many studies, investigating the effects in ducks (Gariglio et al., 2018), broiler chickens (Dabbou et al., 2018), or laying hens (Marono, 2017).

It's becoming more evident that insects are a rich source of bioactive molecules with diverse pharmacological properties, including anti-cancer, anti-tumor, anti-viral, and anti-microbial activities (Imamura et al., 1999; Akiyama et al., 2000; Chernysh et al., 2002; Schuhmann et al., 2003; Yang et al., 2004). Such products may hold enormous potential for improvements of health in humans and livestock, including poultry. Several bioactive molecules are candidates for such functions, but it appears that the group on polysaccharides is one with the leading role. Chitin, for instance, which is an important part of insect exoskeleton, modulates the innate immune system (Lee et al., 2008). An addition of 1% chitin or chitosan to the diet stimulates immune response and improves disease resistance in the kelp grouper, *Epinephelus bruneus,* against infections of the protozoan parasite, *Philasterides dicentrarchi* (Harikrishnan et al., 2012). Feeding shrimp chitin to broilers inhibits the growth of the foodborne pathogens *Escherichia coli* and *Salmonella* in the intestine (Khempaka et al., 2011). Chitin is digested by acidic chitinases in the gut, and the resulting molecules can be used as a substrate for the probiotic bacteria. In fish experiments, chitin was able to reduce pathogen growth by enhancing the growth of beneficial intestinal microbiota (prebiotic activities) with positive effects on performance and health (Karlsen et al., 2017). To test if insect meals influence the immune responses of turkeys, two parameters were measured – the lysozyme concentration and the alternative pathway of complement activation (*APCA*), a primary humoral factor of the innate immunity (Sotirov et al., 2001). Lysozyme is an enzyme that is secreted by macrophages and polymorphonuclear leukocytes, whose primary function is to destroy glucosidic bonds in the cell walls of bacteria during phagocytosis. Analyses on Lysozyme concentrations in this study demonstrated that the enzyme levels did not vary significantly between all groups, although a small increase in BSFd fed group was observed. No appreciable differences were obtained for APCA, as well. Further investigations will elaborate more on the immunomodulatory role of insect meals, but for now, it can be concluded that insect- and probiotic-based diets had no effects on the primary immune responses.

Conclusion

In summary, the study demonstrated that insect-based diets had positive effects on turkey production parameters, with clear insect species-specific dietary effects. Over the experimental period BSF meals demonstrated superior effects than the SW or SWpro meals on turkeys' production parameters. Both BSF meals (defatted-whole larvae) produced similar trends on turkeys' responses. The new diets had no major effects on turkeys' physiology, carcass composition and immune responses. A combination of insect meal and probiotic improved turkeys' physiological status but had no effect on the production parameters.

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