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Insect meal as alternative protein ingredient in broiler feed

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Abstract

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Insects are a potential alternative source of nutrients for the livestock husbandry in response to increasing demands and high price of conventional feeds. The aim of this study was to evaluate the inclusion of 10% insect meals from Black soldier fly – BSF (*Hermetia illucens L.-HI*) and from Silkworm – SW (*Bombyx mori*), as alternative source of protein in diets of broiler chickens on productive traits, contents of amino acids, fatty acids and minerals in their meat and broilers' health. A total of 180 Ross day-old male broiler chickens with uniform live body weight divided in 3 groups C – control; BSFd – fed 10% deffated larvae meal instead of part of soybean meal; SW – fed 10% silkworm pupae meal instead of part of soybean meal) with 3 replicates, 20 birds/replicate were studied throughout 49 days.

At the end of the experiment, statistically significantly higher live weight was found out in the SW group and BSFd group: 3278.67 g and 3140.67 g, respectively compared to control chickens (2925.33 g; P = 0.001). Again, the experimental groups were outlined with better weight gain, feed conversion ratio and slaughter traits. The dietary inclusion of 10% insect meals (BSF and SW) influence the content of some essential amino acids (EAA) and omega-3 fatty acids proportion in meat. The blood plasma lysozyme, APCA and beta-lysin in broiler chickens were not negatively influences in the groups supplemented with insect meals. The results from the study confirmed that SW and BSFd meals may be a good alternative to soybean meal in broiler chickens nutrition as they improved productive traits.

Keywords: Black soldier fly (*Hermetia illucens*); Silkworm (*Bombyx mori*); insect-based diets; broilers; feeding with insects

Introduction

The increased human population and higher living standards worldwide during the last years has resulted in shortage and increased prices of food resources. Therefore, alternative protein sources for human and animal nutrition turned out to be of increasing significance for global economy.

Insects are among alternative protein sources for poultry feeds due to comparable fat (30-40% on dry matter basis – DM) and protein content (40–60% DM) as compared to fishmeal (FM) or soybean meal (SBM) (Makkar et al., 2014). The replacement of conventional protein sources, e.g. SBM and FM by insect-based ingredients may result in more efficient utilisation of natural resources and lower greenhouse gas emissions (van Zanten et al., 2014).

Chickens with outdoor access catch insects at all stages of their life and consume them voluntarily, which shows that they are evolutionary adapted to insects as a part of their natural diet (Bovera et al., 2015). The available literature confirms that the complete or partial substitution of FM with insect meal in poultry nutrition is feasible. In particular, no negative effect on growth performance of chickens fed insect-based diets have been reported, yet most reports described similar or even better growth performance in chickens compared to those fed either SBM or SBM + FM. Moreover, the digestibility of nutrients was not worsened or it was better when insect-based poultry feeds were used in comparison to FM (Khatun et al., 2003; 2005; Okah & Onwujiariri, 2012). A number of researchers have utilised defatted *Hermetia illucens L.*(*HI*) meal in poultry nutrition, with inconsistent results. Schiavone et al. (2017) found out the defatted HI meals may be an excellent source of energy and digestible protein for broilers. Others (Fagoonee, 1983; Khatun et al., 2003; Konwar et al., 2008; Sheikh & Sapcota, 2010) have carried out experiments with replacement of conventional dietary protein sources with silkworm meal in the feed of broilers reporting a positive effect on productive performance.

On the basis of available literature data for possible total or partial replacement of SBM with insect meals in poultry feeds, the present research aimed to evaluate the effects of 10% insect meals from silkworm (SW) and defatted Black soldier fly larvae (BSFd), as alternative source of protein in diets of broiler chickens on productive traits, amino acids, lipid and mineral content of meat and broilers' health.

Materials and Methods

Birds and husbandry

The present study was conducted in the Experimental farm of the Agricultural Institute – Stara Zagora with 180 Ross day-old male broiler chickens with uniform live body weight divided in 3 groups C – control; BSFd – fed 10% deffated larvae meal instead of part of soybean meal; SW – fed 10% silkworm pupae meal instead of part of soybean meal) with 3 replicates, 20 birds/replicate; throughout 49 days).

Three diets were formulated: soybean meal was the main protein source for control group, and for the two experimental groups, a part of soybean meal was replaced with 10% deffated larvae meal (BSFd) or 10% silkworm pupae meal (SW). Diets were isoprotein and isoenergy and formulated according to requirements for nutrition of the hybrid. The composition of feeds used in this study is presented in Table 1

All diets were in mashed form. Each group was placed to a clean floor pen, in a brooder ring for the first seven days, with equal floor space, one feeder, one drinker and one heating lamp for each group. The litter (wood shavings) was covered with paper during the first week to prevent the poults

Table 1. Composition of diets

Ingredients, %	Starter			Grower			Finisher		
	C	BSFd	SW	C	BSFd	SW	C	BSFd	SW
Corn	20.00	28.00	20.00	26.30	20.15	25.00	31.00	31.00	36.08
Wheat	32.00	22.85	40.79	28.00	34.80	39.33	29.38	29.86	34.00
Soybean meal	35.00	23.20	20.00	27.70	15.40	13.00	21.25	9.30	7.00
Sunflower meal	5.00	5.00	5.00	7.00	7.00	7.00	7.00	7.00	7.00
BSFd	–	10.00	–	–	10.00	–	–	10.00	–
SW	–	–	10.00	–	–	10.00	–	–	10.00
Sunflower oil	4.01	6.81	1.00	7.30	8.75	2.70	7.70	8.95	3.00
Methionine	0.39	0.37	–	0.39	0.38	–	0.40	0.39	–
Lysine	0.29	0.54	–	0.27	0.55	–	0.28	0.55	–
Salt	0.20	0.20	0.20	0.25	0.25	0.25	0.30	0.30	0.30
Limestone	0.60	0.52	0.60	0.46	0.52	0.55	0.49	0.55	0.52
Dicalcium phosphate	2.01	2.01	1.91	1.83	1.70	1.67	1.70	1.60	1.60
Premix	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
Salgard	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Nutritional value									
Metabolizable energy, Kcal/kg	3025	3024	3025	3177	3178	3177	3213	3213	3212
Crude protein, %	22.53	22.23	22.22	20.27	20.28	20.26	18.00	18.00	18.00
Fat, %	5.83	9.58	5.51	9.20	11.36	7.31	9.71	11.80	7.85
Crude fiber, %	4.67	3.90	4.11	4.49	3.84	4.00	4.16	3.48	3.64
Ut. Phosphorus, %	0.49	0.50	0.50	0.45	0.45	0.45	0.42	0.42	0.43
Calcium, %	1.03	1.02	1.02	0.90	0.90	0.90	0.85	0.86	0.85
Lizine, %	1.52	1.49	1.49	1.32	1.33	1.32	1.16	1.16	1.16
Methionine, %	0.74	0.73	0.74	0.71	0.71	0.71	0.69	0.70	0.69

from eating the litter. Birds had ad libitum access to feed and water and lighting was provided continuous.Feed intake was measured based on residual feed deduction from the total supplied feed. Body weight (BW) and feed intake (FI) were recorded by treatment group, and FCR (feed conversion ratio) per group were then calculated for the total experimental period.

Insect meals

The BSF meal (defatted) used in the study was produced and provided by 'NASEKOMO', Bulgaria. The spent silkworm pupae (chrysalis, *Bombix mori* L.) were obtained from the Scientific Center of Sericulture, Vratsa, Bulgaria. Chemical analyses of insect meals (SW, BSFd) 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 analyses were performed using HPLC Waters AccQ Tag Method. Insect meal analyses are present in Table 2.

Slaughter procedures and carcass evaluation

At 49 days of age, slaughter analysis was performed on six chickens from each group with live weight equal to group average. After 12-hour fasting, birds were stunned and slaughter in line with the requirements of Ordinance 22 of 14.12.2005 of the Ministry of Agriculture and Food to reduce suffering of animals during slaughter or killing to a minimum. The cleaned carcass without the neck and edible offal was cut after 24-hour cooling at 0-4^{0}C. At slaughter, edible offal was taken away after removal of legs, feathers and the head. The following slaughter traits were evaluated after evisceration and weighing of internal organs: grill weight (g); carcass yield (%); breast (g); thighs (g); wings (g); gizzard (%); liver (%); abdominal fat (%).

Table 2. Chemical composition of insect meals

Parameters	BSFd	SW
Fat, %	7.79	24.50
Protein, %	56.16	57.14
Moisture , %	1.03	11.50
Gross energy, kcal/kg	2090	5831
Calcium, %	0.84	0.55
Phosphorus,%	0.67	0.75
Amino acid content, %		
Valine	4.79	5.60
Isoleucine	4.93	6.90
Leucine	1.00	7.24
Lysine	8.04	3.83
Methionine	1.88	3.70
Methionine+Cystine	11.22	4.65
Threonine	5.19	5.20
Phenylalanine+Tyrosine	7.91	10.70
Arginine	7.16	4.50
Glycine	2.71	4.70
Histidine	11.25	2.90

Meat quality

Breast and leg meat samples were collected to determine their amino acid profile, fatty acid composition, mineral composition (Fe, Ca, Mg, P), and cholesterol level. The laboratory analyses were carried out in the officially accredited food laboratory Alimenti (D & V Consult Ltd.), Plovdiv, Bulgaria.

Blood sampling serum

At the end of the experiment, six birds per group were randomly selected to collect blood samples aseptically from vena ulnaris in sterile heparinised vacutainers (FLmedical, Italy) for analyses. 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).

Statistical analysis of results

Data were statistically analysed by one-way analysis of variance (ANOVA) using SPSS v. 19 software, according to the model:

$$Yij = \mu + CPi + e\text{ij},$$

where Y – single observation,

μ – general mean,

CP – effect of protein source,

e – random error.

When factor effect was significant, the least significant difference post-hoc test (LSD-test) was also performed at P < 0.05.

Results

Productive performance

The results for average live weight of broiler chickens on experimental days 1, 10, 28 and 49 are shown in Table 3. Data demonstrated no significant differences between live weight on days 1 and 10 in studied groups (P > 0.05). The live weight at 28 days of age was significantly higher (P =

0.036) in group SW – 1341.13 g compared to group BSFd – 1248.40 g and to control group (C) – 1204.53 g. At the end of the trial (day 49), the tendency towards significantly higher live weight in SW and BSFd chickens (3278.67 g and 3140.67 g, respectively) vs controls (2925.33 g; P = 0.001) was preserved.

Table 3. Live weight of broiler chickens, g

Parameters	Groups			SEM	*P*-value
	C	BSFd	SW		
1 days	44.13	43.93	44.00	0.83	0.985
10 days	231.60	237.67	239.40	3.19	0.269
28 days	1204.53^{b}	1248.40^{ab}	1341.13^{a}	28.23	0.036
49 days	2925.33^{b}	3140.67^{a}	3278.67^{a}	40.28	0.002

^{a,b} – different superscripts within a row indicate statistically significant differences at P < 0.05

Table 4. Weight gain, feed intake and feed conversion ratio in broiler chickens

Parameters	Groups			SEM	P-value
	C	BSFd	SW		
1–10 days					
ADG, g	187.47	193.73	195.40	2.75	0.179
FI, g	261.74	271.46	280.32	5.84	0.160
FCR, kg/kg	1.39	1.40	1.43	0.02	0.504
11–28 days					
ADG, g	972.93	1010.73	1101.73	30.35	0.058
FI, g	1803.68	1853.33	1926.10	47.35	0.262
FCR, kg/kg	1.86^{a}	1.83^{a}	1.7 ^{b}	0.02	0.004
29–49 days					
ADG, g	1720.80^{b}	1892.27^{a}	1937.53^{a}	20.70	0.001
FI, g	3835.05^{b}	4012.00^{ab}	4222.00^{a}	76.93	0.033
FCR, kg/kg	2.23^{a}	2.12^{b}	2.18^{ab}	0.02	0.031
1–49 days					
ADG, g	2911.07^{b}	3096.73^{a}	3234.67^{a}	41.12	0.004
FI, g	5900.47^{b}	6239.61^{a}	6428.42^{a}	60.90	0.002
FCR, kg/kg	2.02	2.02	1.99	0.02	0.602

^{a,b} – different superscripts within a row indicate statistically significant differences at P < 0.05

ADG – average daily gain; FI – feed intake; FCR – feed conversion ratio

The weight gain (g), feed intake (g) and feed conversion ratio (kg/kg) from days 1-10, days 11-28, days 29-49 and for the entire experimental period (days 1-49) are shown in Table 4. The highest weight gain during the first 10 days of life was shown by chickens fed SW meal – 195.40 g, followed by the group fed BSFd – 193.73 g whereas controls exhibited the lowest weight gain – 187.47 g (P > 0.05).

During that period, no statistically significant between-group differences regarding feed intake and feed conversion ratio were present. During the period from the 11th to the 28th day, weight gain and feed intake did not differ among the groups. Considerably better feed conversion ratio (P = 0.004) was demonstrated in the group fed silkworm meal SW – 1.7 kg/kg as compared both to control group (1.86 kg/kg) and to BSFd group (1.83 kg/kg). Better weight gain and feed conversion ratio vs controls was observed in both experimental groups from the 29th to the 49th day (P < 0.05). In general, for the entire 49-day duration of the trial, the highest weight gain was exhibited by the chickens from the SW group – 3234.67 g, followed by birds from the BSFd group – 3096.73 g; the worse results regarding this parameter were those of controls – 2911.07 g (P = 0.004).

Slaughter analysis

The studied slaughter parameters in this experiment are presented in Table 5. Experimental groups fed BSFd and SW insect meals had statistically significantly higher slaughter weight and grill weight compared to controls (P < 0.001). For slaughter yield, experimental groups were superior to the control one, although insignificantly (P = 0.104).

The most valuable part of chicken carcass, e.g. breast, had the highest weight in both experimental groups – 781.67 g for BSFd and 742.67 g for SW and at the same time, lowest

Table 5. Slaughter analysis in broiler chickens

Parameters	Groups			SEM	P-value
	C	BSFd	SW		
Slaughter weigh, g	2933.33^{b}	3141.67^{a}	3270.00^{a}	60.35	0.004
Carcass weight, g	1952.33^{b}	2119.83^{a}	2233.00^{a}	46.65	0.003
Carcass yield, %	66.56	67.50	68.25	0.52	0.104
Breast *, g	644.17^{b}	742.67^{a}	781.67^{a}	16.25	0.000
Thigh *, g	683.33^{a}	721.00^{ab}	759.33^{b}	15.89	0.014
Wings , g	195.33	190.83	200.83	4.28	0.284
Gizzard, %	1.42^{a}	1.33^{a}	1.11^{b}	0.06	0.007
Liver, %	2.02	1.96	1.86	0.09	0.455
Abdominal fat, %	1.31	1.02	1.02	0.15	0.335

^{a,b} – different superscripts within a row indicate statistically significant differences at P < 0.05

* – weight of breast and thighs with bone and skin

weight in controls – 644.17 g (P < 0.001). Thighs weight was statistically significantly higher in chickens from SW and BSFd groups than in controls (P < 0.01). It should be noted that the percentage of the gizzard was the highest in control chickens (1.42%) that received neither BSFd nor SW (P = 0.007). The weight of wings and abdominal fat percentage from grill weight showed insignificant differences among groups (P > 0.05).

Amino acid, fatty acid and mineral contents in broiler meat

Table 6 presents the content of studied amino acids (AA) in breast and thigh muscles of control and insect meal-fed chickens (BSFd and SW). Statistically significant between-group differences in breast muscle were observed with respect to isoleucine, leucine and lysine. Higher isoleucine content 1.67 µg/kg and 1.28 µg/kg was observed in breast meat of controls and SW group respectively, compared to BSFd group – 0.49 µg/kg; P = 0.034. The content of leucine, lysin, phenylalanine and histidine were also higher in control and SW-fed chickens and the lowest in BSFd chickens (P < 0.05). For the other AA in breast muscle, no relevant differences among the groups were found out. For thigh meat, statistically significant differences among the group were demonstrated only for lysine and tryptophan content.

Table 6. Amino acid content of breast and thigh meat, µg/kg

Amino acids	Groups			SEM	P-value
	C	BSFd	SW		
Breast					
Valine*	2.03	2.27	1.51	0.18	0.058
Glutamine***	2.25	1.47	1.90	0.20	0.089
Isoleucine*	1.67 [a]	0.49 [b]	1.28 [ab]	0.24	0.034
Leucine*	3.34 [a]	0.98 [b]	2.56 [ab]	0.48	0.033
Lysine*	3.32 [a]	1.01 [b]	3.65 [a]	0.60	0.040
Methionine*	0.76	0.02	0.49	0.16	0.054
Serine***	0.66	0.06	0.51	0.15	0.067
Threonine*	0.91	0.28	0.70	0.14	0.066
Tryptophan*	0.19	0.07	0.14	0.03	0.066
Phenylalanine*	2.59 [a]	0.87 [b]	2.10 [a]	0.32	0.021
Histidine**	0.52 [b]	0.28 [b]	0.84 [a]	0.09	0.012
Cysteine ***	< 0.05	< 0.05	< 0.05	–	–
∑EAA	15.33 [a]	6.29 [b]	13.27 [ab]	2.08	0.049
Thigh					
Valine *	2.47	2.71	2.02	0.21	0.140
Glutamine ***	4.39	4.32	5.04	0.28	0.207
Isoleucine *	0.52	0.40	0.63	0.06	0.115
Leucine *	1.03	0.81	1.26	0.13	0.116
Lysine *	1.00 [b]	0.88 [b]	1.78 [a]	0.20	0.039
Methionine *	0.05	0.05	0.15	0.03	0.053
Serine ***	0.14	0.12	0.27	0.05	0.157
Threonine *	0.38	0.33	0.49	0.05	0.171
Tryptophane *	0.06 [b]	0.06 [b]	0.09 [a]	0.01	0.029
Phenylalanine *	0.90	0.74	1.11	0.09	0.072
Histidine **	0.26	0.27	0.41	0.04	0.064
Cysteine ***	< 0.05	< 0.05	< 0.05	–	–
∑ EAA	6.66	6.25	7.94	0.69	0.269

[a,b] – different superscripts within a row indicate statistically significant differences at P < 0.05

*valine, isoleucine, leucine, lysine, methionine, threonine, tryptophan, phenylalanine – essential AA for humans; **histidine – essential AA for growing children; ***glutamine, serine, cysteine – non-essential AA for humans

Lysine concentrations were the highest in thigh muscle of SW-fed birds – 1.78 µg/kg and lower in controls (1.00 µg/kg) and BSFd birds (0.88 µg/kg); P = 0.039. Again, the content of the amino acid tryptophan was the highest in SW-fed chickens – 0.09 µg/kg and equally reduced in control and BSFd-fed birds: 0.06 µg/kg; P = 0.029.

Linoleic, linolenic, arachidonic, eicosapentaenoic and docosahexaenoic fatty acids and cholesterol content in breast and thigh muscles of studied groups of chickens are listed in Table 7.

Table 7. Fatty acid and cholesterol content of breast and thigh meat, %

Parameters	Groups			SEM	P–value
	C	BSFd	SW		
Breast					
C 18:2 (n–6)LA	0.16	0.18	0.17	0.02	0.788
C 18:3 (n–3) ALA	0.01[b]	0.01[b]	0.06[a]	0.01	0.022
C 20:4 (n–6)ARA	0.006	0.004	< 0.001	–	–
C 20:5 (n–3)EPA	< 0.001	< 0.001	< 0.001	–	–
C 22:6 (n–3)DHA	< 0.001	< 0.001	< 0.001	–	–
Cholesterol	< 0.20	< 0.20	< 0.20	–	–
Thigh					
C 18:2 (n–6)	0.72	0.75	0.60	0.05	0.148
C 18:3 (n–3)	0.03	0.04	0.20	0.05	0.378
C 20:4 (n–6)	0.01	0.01	0.01	–	–
C 20:5 (n–3)	< 0.001	< 0.001	< 0.001	–	–
C 22:6 (n–3)	< 0.001	< 0.001	< 0.001	–	–

[a,b] – different superscripts within a row indicate statistically significant differences at P < 0.05

LA – linoleic acid; ALA-C 18:3 (n-3) alpha-linolenic acid; ARA – arachidonic acid; EPA-Eicosapentaenoic acid; DHA – Docosahexaenoic acid

The content of the main omega-3 amino acid – linolenic acid, was the highest in the breast meat of SW-fed birds – 0.06% which was statistically significantly different from respective percentages of both control and BSFd birds -0.01%; P < 0.05. The other studied fatty acids in breast and thigh meat of chickens from the three groups were not significantly different.

Table 8. Macromineral and trace mineral content of breast and thigh meat, %

Parameters	Groups			SEM	P-value
	C	BSFd	SW		
Breast					
Fe	3.08	3.79	3.90	0.32	0.225
Ca	< 0.50	< 0.50	< 0.50	–	–
Mg	281.24	239.67	241.16	13.66	0.126
P	2180.94	2047	2012.47	66.40	0.245
Thigh					
Fe	11.26	6.98	8.35	2.73	0.430
Ca	< 0.50	< 0.50	< 0.50	–	–
Mg	204.30	208.51	207.56	12.22	0.968
P	1825.53	1837.75	1818.63	79.95	0.985

a,b – different superscripts within a row indicate statistically significant differences at $P < 0.05$

The mineral content of breast and thigh meat is presented in Table 8. There were no consistent differences in iron, magnesium and phosphorus content of breast and thigh meat between control and both experimental groups ($P > 0.05$).

The minimum value of the macromineral calcium in both breast and thigh muscles in all groups of chickens should be noted: < 0.050%.

Immune response

The lysozyme, APCA and beta-lysin concentrations in the blood plasma of experimental broiler chickens are presented in Table 9.

Table 9. Blood plasma lysozyme, APCA and beta-lysin in broiler chickens

Parameters	Groups			SEM	P-value
	C	BSFd	SW		
Lysozyme, μg/mL	0.58	0.45	0.35	0.06	0.075
APCA (CH_{50})	577.62	593.74	558.35	16.02	0.322
Beta lysin, %	18.67	17.79	31.44	8.11	0.433

Blood lysozyme was the highest in control broilers – 0.58 mg/L, but insignificantly ($P > 0.05$). This parameter was with reduced levels in BSFd-fed and SW-fed birds: 0.45 mg/L and 0.35 mg/L respectively ($P > 0.05$). As APCA values (CH50) were concerned, differences among the groups were inconsistent. Beta-lysins (%) demonstrated the highest average value in SW-fed group SW – 31.44%, 18.67% in control group and 17.79% in BSFd-fed group, but again, differences were insignificant ($P = 0.433$).

Discussion

Productive performance

The results for live weight in this study revealed significant differences among the groups at 28 and 49 days of age (end of experiment). Previous reports having evaluating the possibility for inclusion of insect fat in the diets of broiler chickens yielded inconsistent results with respect to productive performance traits and meat quality. In them, live weight differences resulted probably from different FA profile of insect meals (Danieli et al., 2019; Kawasaki et al., 2019). Schiavone et al. (2017) and Sypniewski et al. (2020) used fat from BSF larvae for partial or complete replacement of soy oil (SO) and observed no negative influence in both broiler chickens and in turkey poults.

In this study, the best weight gain and feed conversion ratio were observed in the group fed SW meal, followed by the BSFd group. Having compared the effect of three dietary fat sources: corn oil, coconut oil and BSF larvae oil in broiler chickens, Kim et al. (2020) did not report any effect on BW, ADG or ADF at the age of 15 and 30 days, yet reported reduced FCR in the groups supplemented with coconut oil and BSF larvae compared to the group fed corn oil. The authors attributed the improved FCR to effects of medium-chain FA which improved the digestibility and utilisation of nutrients. As the use of *Tenebrio molitor* (TM) oil was concerned, researchers observed that it reduced ADFI and FCR and had no effect on ADG when used as substitute of SO in broiler diets (Kierynczyk et al., 2018). The authors also observed that the use of 5% TM or *Zophobas morio* fat for complete replacement of SO in a 28-day trial did not influence growth performance and feeding efficiency in broiler chickens, but showed differences in ADG among the treatment s for intermediate periods (days 14 –21 and days 21–28). Similar conclusions on growth performance of domestic fowl were made by Benzertiha et al. (2019) in an experiment on broiler chickens in whose diet palm oil and poultry fat were entirely replaced with TM oil. These studies confirmed the possibility for inclusion of insect fat in broiler chickens' diet as alternative dietary fat source.

Literature data suggested that the improved productivity of broilers resulting from replacement of fish meal (FM) or soybean meal (SBM) with silkworm pupae (SWP) may be associated with the higher content of essential amino acids (EAA), minerals, energy, nutrients, digestibility and increased rate of protein accumulation (Khatun et al., 2003; Ullah et al., 2017a). Furthermore, Fagoonee (1983) affirmed that SWP contain growth promoting factor; a hormone involved in protein synthesis and tissue formation, although this fact was not reported since then.

Meat amino acid, fatty acid and mineral contents

Zhou & Han (2006) reported that SWP protein contained 18 known AA, including all EAA and sulfur-containing AA. Longvah et al. (2011) reported that in prepupae and pupae, AA were 99 and 100, respectively, and that leucine was the limiting AA. Cullere et al. (2016) reported that the most prevalent EAA were valine (val) and leucine (leu), whereas defatted BSF larvae were rich in alanine (alan) and glutamic acid (glu). The concentrations of AAs in defatted BSF larvae meal differs from full-fat BSF larvae meal reported by De Marco et al. (2015) with regard to EAAs and contents of lysine (lys), methionine (meth), arginine (arg) and histidine (hist). The present study demonstrated higher levels of EAA for men – lysine and phenylalanine in breast meat of chickens fed SW meal and histidine, an EAA for growing children in comparison to the other studied groups of chickens. The levels of EAA for men lysine and tryptophan in the thigh meat of SW-fed chickens were again statistically significantly higher vs control birds.

The concentrations of n-3 PUFA (polyunsaturated fatty acids) in animal tissues depend mainly on dietary fatty acid (FA) composition. The dietary disbalance of the n-6:n-3 PUFA ratio may have an impact on human health, especially in case of high n-6:n-3 PUFA ratio in modern diets compared to recommendation of n-6:n-3 PUFA from almost 3:1 to 1:1 (Ibrahim et al., 2018). Our study has shown that feed composition has an influence on n-6 and n-3 profile of meat, as well as on saturated FA content. The SW meal contains n-3 FAs, in particular б-linolenic acid (36.3%) as main ingredient (Tomotake et al., 2010). Kwon et al. (2012) also reported that the FA composition of SW fat revealed a high ratio of essential (б-linolenic + linoleic acids; 49.0%) and non-essential FAs (19.9% oleic acid, 2.5% palmitoleic acid, 19.7% palmitic acid; 8.6% stearic acid and 0.3% eicosapentaenoic acid).

Arango Gutierrez et al. (2004) reported that Black soldier fly larvae (*Hermetia illucens*) were rich in calcium (Ca; 5–8% DM) and phosphorus (P; 0.6–1.5% DM), but other minerals were in lower amounts: potassium (K; 0.69% DM), sodium (Na; 0.13% DM), magnesium (Mg; 0.39% CB), iron (Fe; 0.14% DM), manganese (Mn; 246 mg/kg DM), zinc (Zn; 108 mg/kg DM) and copper (Cu; 6.0 mg/kg DM) whereas Maurer et al. (2016) reported very low levels of Ca in BSF larvae meal (0.80 and 0.98%). These contradictory results may depend on the stage of development of the insect (prepupal stage). No statistically significant difference in iron, magnesium and phosphorus content in breast and thigh meat were found out among the controls and BSFd and SW groups in this study, whereas the Ca concentration in breast and thigh meat in all groups was < 0.050%. In a previous study of ours, Lalev et al. (2021) observed that calcium concentrations were under the limit of detection of the analytical method. In the breast meat of studied turkeys, differences were found out only for turkeys fed BSFw, expressed in reduced levels of magnesium (279.40 mg/kg vs control level of 412.22 mg/kg), although statistically insignificant (P = 0.075). In thigh meat, substantially higher iron concentrations were found out in turkeys fed BSFd and BSFw (13.99 and 16.79 respectively) than in controls – 9.48 and in the SW group – 9.26 (P = 0.029). Magnesium reduction was also observed in the thigh meat of these groups (185.26 and 174.81 vs control level of 284.82 and vs SW group level of 224.08) (P = 0.078).

Immune response

This experiment showed no consistent differences between the groups for lysozyme, APCA and beta-lysins concentrations in blood plasma in studied broiler chickens. The exoskeleton of insects (chitin polysaccharide) and its derivatives may act as alternative to antibiotics when fed to animals. Previous studies have confirmed the antimicrobial potential of chitin, chitosan and their derivatives (Suzuki et al., 1984; Sudarshan et al., 1992; Shahidi et al., 1999). Chitosan contains antioxidants and exhibits antimicrobial activity against bacteria, moulds and yeasts (Cutter, 2006; Portes et al.,2009). Primo et al. (2018) reported that lysozymes are enzymes that degrade the cellular wall and interrupted the life cycle of bacteria through cleavage of the bond between N-acetylglucosamine and N-acetylmuramyl-(pentapeptide) carbohydrates. Thus, the addition of insect ingredients to the diet of broiler chickens will increased serum lysozyme concentrations and improve the resistance of birds to infectious diseases. It is known that APCA (CH50) is the main humoral means for control of viruses, virus-infected cells, Gram-negative bacteria, tumour cells etc. (Sotirov et al., 1998; Yotova et al., 2004; Bozakova et al., 2017). Beta-lysins are biologically active substances of pituitary origin, a kind of bactericidal system against spore-forming microflora, and also are involved in the maintenance of immunostructural homeostasis. Increased serum beta-lysin activity was reported in the beginning of the lay in hens in support of the hypothesis for the involvement of beta-lysins in stress situations for the organism (Karakolev & Nikolov, 2015). In this study, beta-lysins were increased in the SW-fed group compared to both control and BSFd groups. In a previous study of ours with turkeys, blood lysozyme concentration did not vary considerably among the tested groups despite the slightly higher levels in the group fed BSFd. The differences in APCA were neither consistent (Lalev et al., 2020).

Conclusions

On the basis of obtained results in this study, it could be concluded that the inclusion of 10% SW and BSFd meal in the diet of broiler chickens improved their productive performance. A higher final live weight, better daily weight gain, better feed conversion and slaughter traits were found out. The study demonstrated that the inclusion of insect meals influenced the amino acid and fatty acid profile of meat, with no negative effect on chickens immunity and health. The findings suggested that SW and BSFd meals may be a good alternative to soybean meal in broiler chicken nutrition.

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Competing interests

The authors declare that they have no competing interests.

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