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Partial replacement of soybean meal with partially defatted Black Soldier Fly (*Hermetia illucens* L.) larvae meal did not change dietary metabolizable energy and growth performance of broilers

Частичното заместване на соев шрот с частично обезмаслено брашно от ларви на черна войнишка муха (*Hermetia illucens* L.) не влияе върху обменната енергия на фуража и растежа на пилета бройлери

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ABSTRACT

The experiment aimed to assess the impact of partial replacement of dietary soyabean meal (SBM) with defatted Black Soldier Fly Larvae meal (BSFLM), at 0, 40, 80 and 120 g/kg, as main protein sources in isocaloric and isonitrogenic diets on daily feed intake (FI), weigh gain (WG) and feed conversion ratio (FCR) of female Ross 308 broiler chickens from 13 to 31 days of age. Dietary apparent metabolizable energy (AME) and dry matter retention (DMR) coefficient were also determined. Replacement of SBM with BSFLM did not change (P>0.05) AME and DMR. There were no changes (P>0.05) in the intake of AME. Daily FI, WG and FCR of birds were not affected (P>0.05) by the diets. Dietary lysine and methionine contents were similar between diets, as lysine was slightly greater than calculated. However, BSFLM inclusion led to increased dietary nitrogen. This study suggests that the lack of balance in dietary nutrients, particularly protein and amino acids, when replacing SBM with insect meals, may be the reason for the observed discrepancies in published reports. Research on feeding higher levels of dietary BSFLM during different growing phases and for longer periods is warranted. Overall, BSFLM is a promising ingredient in poultry nutrition.

Keywords: black soldier fly larvae meal, insect meal, broilers, metabolizable energy, growth performance

АБСТРАКТ

Експериментът имаше за цел да оцени въздействието на частичната замяна на диетично соево брашно (SBM) с обезмаслено брашно от ларви на черна войнишка муха (BSFLM), в количества 0, 40, 80 и 120 g/kg, като основни източници на протеин в изокалорични и изонитрогенни диети върху дневния прием на храна (FI), наддаването на тегло (WG) и коефициента на конверсия на храна (FCR) на женски бройлери Ross 308 от 13 до 31-дневна възраст. Определени бяха също така диетичната видима метаболизируема енергия (AME) и коефициентът на задържане на сухо вещество (DMR). Замяната на SBM с BSFLM доведе до увеличение на AME (P<0,001; L<0,001) и DMR (P=0,033; L=0,006) по линеен модел, но приемът на AME само имаше тенденция (P=0,084) да се увеличава. Дневният FI, WG и FCR на птиците не бяха засегнати (P>0,05). Съдържанието на лизин и метионин в диетата беше сходно и в очакваните граници, въпреки че високото съдържание на BSFLM доведе до повишен суров протеин в диетата. Това проучване показва, че балансът на аминокиселините в диетата е важен фактор в диетичните формули, когато традиционните източници на протеин, напр. SBM, се заменят с насекомни брашна. Необходими са изследвания върху храненето с по-високи нива на BSFLM в диетата по време на различни фази на растеж и за по-дълги периоди. Като цяло, BSFLM е обещаваща съставка в храненето на птици.

Ключови думи: брашно от ларви на черна муха войник (BSFLM), брашно от насекоми; пилета бройлери, обменна енергия, ефективност на растежа

INTRODUCTION

Crude protein (CP) is an important nutrient for poultry that helps maintain high welfare, growth and vital physiological processes (Abdulla et al., 2016; Yang et al., 2018). Modern broilers require over 20% high quality (well-balanced in amino acids) CP during the starter and growing phases (Aviagen, 2022). Soybean meal (SBM) is widely used in poultry diets, but its cost constantly increases, which reflects on the cost of production (Józefiak et al., 2016; Abdulla et al., 2021). In addition, soybean production is increasingly associated with a change of land use (e.g., deforestation), resulting in a negative impact on the environment (Grossi et al., 2022). Although various laboratories are doing research on replacing SBM with more available, less expensive locally produced alternative protein sources, there are still limits on the suitability of their amino acid profiles, nutrient availability and yield (Whiting et al., 2019; Karkelanov et al., 2021; Watts et al., 2021). Recent research suggests that insects are a promising, high-quality, sustainable source of dietary protein for poultry (Józefiak and Engberg, 2017; Chobanova et al., 2024; Solecka and Drazbo, 2024). However, the information on inclusion rates of BSFLM in literature is contradictive: there were no responses on growth performance variables in quail fed 0%, 10% and 15% of BSFLM at the expense of SBM (Cullere et al., 2016); broiler chickens fed 5% (Popova et al., 2021) or 10% BSFLM (Lalev et al., 2022) grew faster compared to control; feeding 16% BSFLM to broiler chickens led to reduce growth and feed efficiency (Chobanova et al., 2023); analyzing published data, Moula and Detilleux (2019) concluded that feeding over 10% dietary insect meal to poultry is detrimental to their growth performance.

In general, the insect meals are rich in protein and amino acids (AA), but contain chitin, which is considered an antinutrient for poultry that may reduce dietary energy and protein availability (Lokman et al., 2019). However, research with laying hens (Marono et al., 2017; Bovera et al., 2018) and pigs (Spranghers et al., 2018; Biasato et al., 2019) suggested that AA and CP balance of the diets may also be a reason for the observed differences in BSFLM research. Yordanova et al. (2025) reported no differences in growth performance in pigs where up to 120 g/kg of SBM was replaced with BSFLM and the diets were well balanced for amino acid content. Thus, suggesting that not only chitin content in BSFLM is the reason for the reported results in the literature.

In view of the above-mentioned, more updated information on the influence of dietary insect meal on broiler growth performance is needed. Thus, this study aimed to assess the impact of partial replacement of dietary SBM with defatted BSFLM, at 0, 40, 80 and 120 g/kg, in isocaloric and isonitrogenic well balanced on amino acids diets on daily feed intake (FI), weigh gain (WG) and feed conversion ratio (FCR) of female Ross 308 broiler chickens from 13 to 31 days of age. Dietary apparent metabolizable energy (AME) and dry matter retention (DMR) coefficient were also determined.

MATERIAL AND METHODS

Insect meal sample and experimental diets

The insect meal sample used in this experiment was from larvae of the Black Soldier Fly (*Hermetia illucens* L.; Diptera: Stratiomyidae) and was purchased from HexaflyTM (Navan, Co. Meath, Ireland). The same BSFLM sample was used in previous research with pigs (Yordanova et al., 2025; Table 1). To produce the BSFLM, the company followed EC regulations (Regulation, E.C. No 1069/2009) as previously described (Chobanova et al., 2023).

Table 1. Proximate, carbohydrate, mineral and amino acid composition of Black Soldier Fly larvae meal (as fed basis)[1]

Proximate and carbohydrate composition (g/kg)		Indispensable amino acids (g/kg)	
Dry matter	972.7	Arginine	21.36
Gross energy (MJ/kg)	21.15	Histidine	17.21
Crude protein (N x 5.60)	454	Isoleucine	20.43
Crude fat	171	Leucine	33.31
Ash	122	Lysine	32.61
Acid detergent fibres	79.5	Methionine	9.08
Acid detergent lignin	20.4	Phenylalanine	20.75
Chitin	59.1	Threonine	18.92
Minerals		Valine	29.21
Calcium (g/kg)	39.7	Tryptophane	7.68
Magnesium (g/kg)	4.0	Indispensable amino acids (g/kg)	
Phosphorus (g/kg)	11.9	Alanine	30.38
Potassium (g/kg)	13.5	Aspartic acid	47.41
Sulphur (g/kg)	4.3	Cystine	3.32
Copper (mg/kg)	23.9	Glycine	25.09
Manganese (mg/kg)	132.3	Glutamic acid	46.89
Sodium (mg/kg)	77.0	Proline	24.93
Zink (mg/kg)	120.7	Serine	19.54
		Tyrosine	30.36

[1] Yordanova et al. (2025)

Four isocaloric and isonitrogenic diets, following breeders' recommendation (Aviagen Ltd, Edinburgh, UK), were formulated to have about 13.00 MJ/kg AME and 211 g/kg CP (Table 2). The diets were based on maize, wheat, sunflower meal and SBM, and contained graded levels of BSFLM, primarily at the expense of SBM as follows; the control diet did not contain BSFLM (T0); diet contained 40 g/kg BSFLM (T40); diet contained 80 g/kg BSFLM (T80); diet containing 120 g/kg BSFLM (T120). The diets were fed *ad libitum* in a meal form.

Ethical Statement

The study was designed in compliance with the guidelines of the European and Bulgarian legislation regarding the protection of animals used for experimental and other scientific purposes (EC 2010), put into law in Bulgaria with Regulation 20/2012. The experiment was conducted at the poultry research facility of Trakia University (Stara Zagora 6000, Bulgaria) and approved by the University Research Ethics Committee.

Table 2. Dietary composition (g/kg, as fed)

Ingredients (g/kg) / Diets	T0	T40	T80	T120
Maize	200.0	200.0	200.0	215.0
Wheat	380.0	395.0	400.0	400.0
Sunflower meal (35% CP)	40.0	40.0	40.0	40.0
Soybean meal (46% CP)	290.0	245.0	205.0	163.0
BSFLM[1]	0.0	40.0	80.0	120.0
L Lysine	3.0	2.85	2.7	2.4
DL Methionine	2.5	2.35	1.9	1.9
L Threonine	1.0	1.0	0.9	0.7
Salt	2.5	2.5	2.5	2.5
Sodium bicarbonate	2.5	2.5	2.5	2.5
Monocalcium phosphate	7.0	7.0	7.0	7.0
Limestone	15.5	14.8	14.5	13.5
Choline chloride	2.0	2.0	2.0	2.0
Sunflower oil	52.0	43.0	39.0	27.5
Premix[2] (Melo Vit 4S)	2.0	2.0	2.0	2.0
Total	1000.0	1000.00	1000.00	1000.00
Calculated values				
Metabolizable energy (MJ/kg)	12.95	12.99	13.03	13.03
Crude protein[3] (g/kg)	210.5	210.6	210.3	210.9
Digestible Lysine (g/kg)	11.9	11.8	11.8	11.8
Digestible Methionine + Cysteine (g/kg)	8.4	8.4	8.1	8.3
Ca (g/kg)	8.4	8.8	8.9	8.9
P (g/kg)	5.8	5.9	5.9	5.9

[1] Black Soldier fly larvae meal.

[2] Supplied the following amounts per kilogram premix (MeloVit 4S 0,2%): 6 000 000 IU of vitamin A; 2 500 000 IU of vitamin D3; 45 000 mg of vitamin E as tocopherol acetate; 2 000 mg of vitamin K3; 2 000 mg vitamin B1; 32 500 mg vitamin B3 (niacin); 10 000 mg vitamin B5 (calcium D-pantothenate); 1 100 000 µg folic acid; 125 000 µg biotin; 10 000 µg vitamin B12; Fe (Fe-II-sulphate) 12 500 mg; Zn (Zn oxide) 45 000 mg; Mn (Mn oxide) 60 000 mg; Cu (Cu-II-sulphate) 7 500 mg; Se (sodium selenite) 150 mg; I (calcium iodide) 500 mg.

[3] The crude protein for the vegetable protein sources was obtained as N × 6.25 and as N × 5.6 for the Black Soldier Fly larvae meal.

Birds and experimental design

About 120, day-old Ross 308 female broiler chickens were obtained from a commercial hatchery (Martivo - Rumen Kirchev Ltd., Sliven, Bulgaria) and raised in a single-floor pen until 13 days of age. Birds were fed proprietary commercial broiler feed. At 13 days of age, the chickens were allocated to 24 wire-mesh floor pens, 5 birds in each. Standard rearing conditions for broiler chickens (Aviagen Ltd., Edinburgh, UK) were maintained during the entire study period. The room where the birds were housed was environmentally controlled with a starting temperature of 32 °C, which was reduced slightly every day until day 21, when the temperature was 21 °C. The room had an automatic lighting schedule, with the light: dark ratio decreasing from 23:1 hours at the day-old, to 18:6 hours for seven-day-old birds. Each diet was fed to 6 pens following randomisation (pen was the experimental unit). Birds and feed were weighed at the start, at 13 d old, and at the end of the study, at 32 d old, at which point FI, WG and FCR were determined. Excreta were collected for the last four days, from 29 to 32 d age, oven-dried at 60 °C, milled and used for the determination of dietary AME and DMR. Feed intake was also determined for the same time.

Chemical composition, dietary AME and DMR determination

Laboratory analyses of BSFLM and diets, including dry matter (DM), minerals, N and crude fat, were determined following standard procedures (AOAC, 2000), as described elsewhere (Whiting et al., 2017; Schiavone et al., 2017; Pirgozliev et al., 2021). Crude protein in BSFLM was calculated as 5.60 × N (Janssen et al., 2017). The gross energy in feed and excreta was determined with an isoperibol bomb calorimeter (Pirgozliev et al., 2006). Amino acids in the BSFLM were determined by SSNIFF Spezialdiaten GmbH (Soest, Germany) according to the EC directives 2000/45/EC for tryptophan (OJEC, 2000), and EC/98/64 (L 257/16) for the other amino acids (OJEC, 1998). Fibre analyses on BSFLM were performed following the method of Van Soest et al. (1991). The content of chitin in BSFLM was calculated as described by Hanh et al. (2018). Dietary AME was determined following the standard total collection procedure (Hill and Anderson, 1958). Dry matter retention coefficients of diets were determined as previously described (Watts et al., 2020).

Statistical analysis

Statistical analyses were performed using GenStat (23rd edition) statistical software (IACR Rothamsted, Hertfordshire, UK). The data were analysed by ANOVA. Orthogonal polynomial contrasts were used to compare treatment differences for the linear relationship with increasing level of BSFLM. Data are expressed as means and their pooled standard errors (SEM). Results were considered significant at $P<0.05$.

RESULTS AND DISCUSSION

The analysed chemical composition of the same BSFLM sample was already published (Yordanova et al., 2025), but for clarity of the reader, it is also presented in Table 1. The BSFLM sample contained 454 g/kg CP and, due to the mild defatting process, was relatively rich in fat, 171 g/kg. The sample contained 59.1 g/kg chitin, 39.7 g/kg calcium and 11.9 g/kg total phosphorus. Leucine, lysine and valine were the highest indispensable AA, and aspartic and glutamic acids were the main dispensable AA. The BSFLM chemical composition was within expected values (Bovera et al., 2018; Cullere et al., 2016; Mahmoud et al., 2023; Chobanova et al., 2023). However, variations in fat, protein, minerals and chitin content of BSFL are recognised and mostly depend on larvae age and rearing substrate (Makkar et al., 2014; Józefiak and Engberg, 2017; Kieronczyk et al., 2022). The acid detergent fibre (ADF) content of BSFLM is usually associated with the chitin content of insects. To increase the accuracy of chitin determination, ADF can be further adjusted for AA (Finke, 2007) or acid detergent lignin (Hahn et al., 2018) contents. The chitin content in the reported study agreed with previous reports (Schiavone et al., 2017; Chobanova et al., 2023), although variation may be expected depending on the method of chitin calculation selected.

The calculated CP content in experimental diets (Table 2) differs from the determined values (Table 3). Compared to calculated values, determined dietary N and CP, respectively, were linearly increasing with the increase of dietary BSFLM. Since chitin contains 3.0% to 6.8% non-protein nitrogen (NPN), a N-to-CP conversion factor of 5.6 for BSFLM was used to avoid dietary protein overestimation (Janssen et al., 2017).

Table 3. Analysed composition of poultry diets used in the experiment (as fed basis)[1]

Ingredients / Diets	T0	T40	T80	T120
Gross energy (MJ/kg)	16.96	17.16	17.34	17.57
Dry matter (g/kg)	909.9	902.5	906.3	917.7
N (g/kg)	32.07	34.90	37.35	38.29
Crude protein (g/kg)	201.9	217.2	231.5	236.3
Crude fat (g/kg)	70.4	65.6	68.3	56.4
Indispensable amino acids (g/kg)				
Arginine	15.4	13.93	10.78	12.39
Histidine	7.16	7.3	7.44	7.05
Isoleucine	9.34	8.89	8.21	8.68
Leucine	17.05	12.67	15.02	15.78
Lysine	15.76	14.85	13.88	14.41
Methionine	5.00	4.54	4.48	4.72
Phenylalanine	11.24	10.22	9.52	9.99
Threonine	10.4	9.92	9.08	9.75
Tryptophan	4.42	5.09	4.61	5.45
Valine	10.83	10.66	9.89	11.31
Dispensable amino acids (g/kg)				
Alanine	9.78	9.81	9.63	11.05
Aspartic acid	22.37	20.65	18.81	20.38
Cystine	3.79	3.06	2.9	2.94
Glutamic acid	46.21	41.13	38.58	38.6
Proline	13.48	12.68	12.8	13.38
Tyrosine	8.13	7.97	7.29	8.46
Serine	10.63	10.22	9.33	10.02
Glycine	10.75	9.73	8.83	9.77
Total indispensable amino acids (g/kg)	106.60	98.07	91.91	99.53
Total dispensable amino acids (g/kg)	125.14	115.25	109.17	114.6
Total amino acids (g/kg)	231.74	213.32	201.08	214.13

[1] Analyzed in technical duplicates

However, research by Hanh et al. (2018) showed that the chitin content of insect samples may differ when measured by different methods, i.e. acetyl, ADF or ADF-ADL method. Thus, suggesting that the N-to-CP conversion factor of 5.6 may be further adjusted/ reduced, which may lead to changes/ equalising the CP content in the diets. Leeson et al. (1996) reported that dietary CP dilution may reduce carcass weight and breast meat yield in male broilers when applied for 14 days feeding period. Although diets with more BSFLM had greater CP, there was no difference in growth performance variables in our study (P>0.05). The lack of difference in growth performance variables of birds fed different diets suggests that dietary CP level may have been overestimated, i.e. there was more NPN in BSFLM-containing diets than the assumed values. However, Selle et al. (2007) and Oliveira et al. (2013) reported that additional dietary lysine improved broilers' growth and feed efficiency. Thus, suggesting that despite the observed differences in dietary CP, the relatively high dietary lysine content in our study may also have prevented differences in performance.

The lack of differences in growth performance variables (P>0.05) coincided with a lack of differences (P>0.05) in AME, AME intake and DMR (overall nutrient retention) coefficients (Table 4). The overall dietary AME was 12.96 MJ/kg, which is close to the calculated one (Table 2) and supports the accuracy in dietary formulation. The overall final body weight of the birds was 1532 g vs 1795 g expected by breeders. This reduction of 15% may be due to feeding mash instead of pelleted diets and rearing birds in small groups in cages (Pirgozliev et al., 2016). Suboptimal levels of specific dietary amino acids, including arginine, methionine, and cysteine, may have played a contributory role. However, it was not considered to be detrimental to the experimental objectives. Changes in dietary chitin level may also be a factor in this study. The results of this study showed that it is possible to at least partially replace dietary SBM with BSFLM for broilers when diets are properly balanced in energy and amino acids. The value of the N-to-CP conversion factor for BSFLM needs further investigation. In addition, research involving longer feeding periods over different feeding phases is warranted for a better understanding of the long-term suitability of feeding BSFLM to broilers.

Table 4. The effect of Black Soldier Fly larvae meal inclusion in feed on growth performance, metabolizable energy and dry matter retention coefficients in broiler chickens

Diets[1], Variables[5]	T0	T40	T80	T120	SEM[2]	*P*-value[3]	L[4]
Start weight (g/bird)	505	503	517	502	6.8	0.392	0.881
End weight (g/bird)	1501	1556	1570	1501	51.6	0.693	0.948
FI7 (g/bird/day)	94.9	95.5	98.1	94.7	2.29	0.693	0.846
WG (g/bird/day)	55.3	55.4	58.5	55.5	1.93	0.602	0.670
FCR (kg:kg)	1.721	1.729	1.685	1.708	0.0413	0.884	0.660
AME (MJ/kg)	12.80	12.86	13.09	13.11	0.201	0.619	0.216
AME intake (MJ/day)	1.22	1.23	1.29	1.24	0.0412	0.671	0.489
DMR (kg:kg)	0.766	0.761	0.760	0.745	0.0112	0.614	0.229
Excreta moisture (kg)	0.666	0.687	0.665	0.681	0.0087	0.224	0.537

[1] T0, T40, T80, T120: diets contain 0, 40, 80 and 120 g/kg of Black Soldier Fly larvae meal in replacement of soybean meal, respectively; [2] SEM: pooled standard error of means; [3] *P*-value: Fisher's probability; [4] L: orthogonal polynomial contrast for linear response.
[5] Variables: Start weight: weight of the birds at the beginning of the experiment at 13 days of age; End weight: weight of the birds at the end of the experiment at 31 days of age; FI: daily feed intake; WG: daily weight gain; FCR: feed conversion ratio; AME: dietary apparent metabolisable energy; AME intake: daily intake of apparent metabolisable energy; DMR: total tract dry matter retention coefficient; Excreta moisture: water content of fresh excreta.

CONCLUSIONS

Feeding BSFLM 120 g/kg dietary BSFLM in replacement of SBM to growing broilers did not change dietary metabolizable energy, overall nutrient retention or productive performance variables. The accurate determination of the chitin content in BSFLM needs further investigation. Further research involving longer feeding periods may allow a better understanding of the long-term suitability of feeding BSFLM for poultry production.

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