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Laying hen performance, feed economy, egg quality and yolk fatty acid profiles from laying hens fed live black soldier fly larvae

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Abstract: The black soldier fly (*Hermetia illucens*) serves as an alternative source of protein in poultry production. However, there is little available information on feeding live black soldier fly larvae (LBSFL) to hens. The present study filled this gap by testing the effect of dietary LBSFL on laying hen performance, feed economy, egg quality, and yolk fatty acid composition. In total, one hundred forty-four 25-week-old Charoen Pokphand Brown laying hens were assigned to four groups. Experimental birds were provided a basal diet supplemented with 0.0 (T1), 10.0 (T2), 20.0 (T3), or 30.0 (T4) g/kg LBSFL. Our results indicated that no effects on body weight gain, egg yield, egg weight, or egg mass ($P > 0.05$) were observed in any of the dietary LBSFL groups. The addition of 10.0 g/kg LBSFL decreased feed intake, feed conversion ratio, and feed cost ($P < 0.05$), contrary to two higher doses of LBSFL. Similarly, the egg quality parameters were not influenced ($P > 0.05$) by the dietary LBSFL except for the egg yolk colour which significantly decreased with increasing LBSFL levels. Furthermore, the values for lightness (L*), redness (a*), and yellowness (b*) of the egg yolk were different ($P < 0.05$) between the four treatments. Paler egg yolk was found in the 20.0 and 30.0 g/kg LBSFL groups compared to the control group ($P < 0.05$). Significantly lower deposition of C18:3n6 was found for 30.0 g/kg, whereas there was a significant decrease in deposition of C22:6n3 with all LBSFL groups ($P < 0.05$). C20:3n6 levels, on the other hand, increased significantly in the 20.0 g/kg LBSFL group ($P < 0.05$). Thus, feeding LBSFL has no potential to increase the proportion of anti-inflammatory n-3 fatty acids. From an economic point of view, it is advantageous to feed the hens a dose of 10 g/kg LBSFL.

Keywords: C18:3n6 fatty acid; C20:3n6 fatty acid; hens; insect; yolk colour

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Global warming and climate change have resulted in an increase in feed and energy costs in feed manufacturing for poultry (Abd El-Hack et al. 2020). With the cost of largely imported feed taking up to 60% of the total cost of poultry production, some farms have been pressed into increasing the amount of native ingredients they use in their feed and supplementing protein with insects (Ramanee 2020). MacLeod et al. (2013) reported that the feed production accounts for 606 million tons of CO_2/year emissions generated in the chicken meat and egg production industries. Locally produced larvae fed on organic waste present a large potential source for reducing CO_2 emissions in the meat and egg supply chains. Consequently, there has been recent research interest in the identification and utilization of insects in poultry diets. Insects have played an important role in nutrition, as they are available in large quantities of good quality, are highly digestible, and can feed on waste biomass that can be transformed into a high-value food and feed resource. In addition, this is not a disease vector (Makkar et al. 2014).

The most valuable insect species considered for use in poultry feeds include *Musca domestica* L. (house fly) (Biasato et al. 2017), *Tenebrio molitor* (mealworm) (Loponte et al. 2019), *Hermetia illucens* L. (black soldier fly, BSF) (Liu et al. 2021). In particular, BSF has recently been used in layer feed (Tahamtani et al. 2021). Feed containing full-fat dried BSF larvae has been shown to enhance the feed conversion ratio and egg production and increase the egg weight and eggshell thickness (Kawasaki et al. 2019). In another study, older laying hens were found to have lower feather damage when fed live BSF larvae (Star et al. 2020). Based on these findings with laying hens, it could be possible that BSF larvae may be adopted as a potential animal feed. Other studies that used both dried and live BSF larvae were able to demonstrate that all of the larvae were consumed by chickens. Furthermore, while BSF commonly contains a higher amount of fat (up to approximately 40%) and is rich in saturated fatty acids (SFAs), it is known for its antimicrobial activity against gram positive bacteria (Caligiani et al. 2019) because some of the organic acids have higher efficacy than short-chain fatty acids. SFAs have the ability to cross the semipermeable membrane of bacteria in undissociated and fat-soluble forms, which enter the cytoplasm of the bacterial cell and suppresses cytoplasmic enzymes and nutrient transport, resulting in cellular death (Bergsson et al. 2001). BSF could be added to animal feed to beneficially enhance the antioxidant ability and antibacterial activity (Chu et al. 2020).

Several studies have reported that using defatted BSF larvae (Mwaniki et al. 2020), full-fatted larvae (Chu et al. 2020), dried BSF larvae (Liu et al. 2021), and live BSF larvae (Tahamtani et al. 2021) as feed can increase the performance and quality of eggs produced by layer hens. Marono et al. (2017) reported that the utilization of BSF was evaluated in hens during the middle and late laying periods, but there is limited knowledge available for layer chickens during the starter period. In addition, the present study represents the continuation and completion of a previous trial (Tahamtani et al. 2021) in which the feed consumption and egg quality of laying hens fed live black soldier fly larvae (LBSFL) from 18 to 31 weeks of age were investigated, but without analysis of the feed cost and yolk fatty acid composition. As a result, more effort is required to provide the requisite scientific data on LBSFL for layer hens at various stages of production. The present research aimed to fill this gap by examining the effects of LBSFL on laying hen performance, feed economy, egg quality, and yolk fatty acid composition of laying hens fed LBSFL from 25 to 37 weeks of age.

MATERIAL AND METHODS

Animal trials and experimental diets

This research was conducted at the Animal Farm, Faculty of Natural Resources and Agro-Industry, Kasetsart University Chalermphrakiat Sakon Nakhon province campus, Sakon Nakhon, Thailand. Seventeen-week-old Charoen Pokphand Brown laying hens were purchased from a Charoen Pokphand Group Company in Bangkok, Thailand. All birds were placed in an evaporative cooling system house and fed a diet (crude protein, 18%; metabolizable energy, 2 850 kcal/kg) during the pre-experimental period. At the age of 25 weeks, 144 Charoen Pokphand Brown laying hens with similar laying uniformity were assigned into four dietary treatment groups with 12 replications (three birds/replication). The room was equipped with 60 cages (40 × 40 × 36 cm), each containing three birds. Each pen was supplied with a water trough and a feeder.

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During the experimental periods, the photoperiod was set at 16-hours constant light, and the birds had ad libitum access to water. Laying hens in the control group were provided with a maize-soybean meal basal diet without supplement (NRC 1994; Table 1). The remaining three groups were given the same basal diet and supplemented with 10, 20, or 30 g/kg LBSFL.

LBSFL supplement was produced by the Non Sala Organic Waste Management Center, Sakon Nakhon province, Thailand. The production of the larvae was carried out at home from November 2021 to February 2022, with an average room temperature of 22 °C and a relative humidity of 75%. The ingredients of the LBSFL feed are presented in Table 2. The proximate contents (dry matter, crude protein, crude fat, crude fibre, ash, total calcium, and total phosphorus) of the LBSFL and experimental diets were determined as described by AOAC (1990). The gross energy content was determined using bomb calorimetry.

An analysis of the dry matter content of the LBSFL was performed prior to the start of the experiment. There was on average 32% dry matter, which was used to calculate the daily portion of larvae. The total estimated dry matter intake of 100 g/day was allocated to the hens in the 10 g/kg (3.10 g larvae/hen/day), 20 g/kg (6.20 g larvae/hen/day), and 30 g/kg (9.30 g larvae/hen/day) treatments for the duration of the study.

Table 1. Ingredients and chemical composition of the diet[1]

Ingredients (g/kg)	Diet without live black soldier fly larvae
Corn	552.20
Defatted rice bran oil	60.00
Soybean meal	229.80
Fish meal	50.00
Rice bran oil	15.00
Oyster shell	78.50
Dicalcium phosphate	4.50
Salt	3.50
DL-methionine	1.50
Vitamin-mineral premix[2]	5.00
Analysed nutrient content (g/kg)	
Crude protein	175.00
Crude fiber	37.60
Crude fat	82.00
Calcium	35.10
Available phosphorus	3.50
Lysine	9.60
Methionine	7.50
Metabolizable energy (MJ/kg)	11.93

[1]Other experimental diets were supplemented with 10.0 g/kg, 20.0 g/kg or 30.0 g/kg live black soldier fly larvae
[2]Vitamin-mineral premix provided per kg of diet: vitamin A 10 000 IU, cholecalciferol 2 000 IU, vitamin E 0.25 IU, vitamin K_3 2 mg, vitamin B_{12} 10 µg, choline 250 mg, folacin 1 mg, niacin 30 mg, pantothenic acid 10 mg, pyridoxine 3 mg, riboflavin 6 mg, thiamine 2 mg, ethoxyquin 125 mg, choline 1 500 mg, copper 10 mg, iron 60 mg, iodine 0.5 mg, iodine 0.5 mg, manganese 40 mg, zinc 50 mg, selenium 0.2 mg, preservative 6.54 mg, feed supplement 26 mg

Determination of amino acids and fatty acids in black soldier fly larvae

The amino acids in LBSFL were analysed using liquid chromatography-tandem mass spectrometry (LC-MS/MS) as described by Anthony et al. (2014). The fatty acids in LBSFL were measured using a gas chromatograph (Shimadzu International Trading Co., Ltd, Kyoto, Japan) as described by Wang et al. (2019). The amino acid and fatty acid composition of LBSFL was identified at the Institute of Food Research and Product Development (IFRPD), Bangkok, Thailand (October 17, 2021, No. 650644).

Assessment of egg performance and egg quality

All data were recorded during the 12-week experiment to evaluate the egg performance and egg quality of the laying hens fed different levels of dietary LBSFL. Eggs were collected from each experimental pen twice a day (8:00 AM and 5:00 PM) and expressed as the number of eggs per one replication.

Table 2. Ingredients of live black soldier larvae feed

Ingredients (g/kg)	
Soybean meal	588.30
Mixed fruits peel	147.10
Molasses	44.10
Effective microorganism	73.50
Water	73.50
Rice bran	73.50

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Collected eggs were weighed and recorded daily, while the remaining feed was measured weekly. Daily feed intake, feed conversion ratio (FCR), egg yield, egg weight, egg mass, FCR of feed and FCR of eggs were calculated on the basis of recorded data. The FCR was computed using the equation:

$$\text{FCR} = \text{feed intake (g)/egg mass (g)} \quad (1)$$

The egg mass was computed using the equation:

$$\text{Egg mass} = \text{egg yield (\%)} \times \text{egg weight} \quad (2)$$

The feed cost per 1 kg of egg was computed using the equation:

$$\text{Feed cost/1 kg of egg} = \text{FCR} \times \text{price of 1 kg feed} \quad (3)$$

The feed cost per egg was computed using the equation:

$$\text{Feed cost/egg} = \text{feed cost per 1 kg of egg/} \text{/numbers of eggs in 1 kg} \quad (4)$$

At 37 weeks of age, 30 eggs per treatment were evaluated to measure egg quality. The following parameters were assessed: eggshell breaking strength, measured using a breaking strength measuring device (ORKA Food Technology LLC, Utah, USA); eggshell thickness, measured using a digital Mitutoyo absolute thickness gauge (Mitutoyo Corp., Stockholm, Sweden) and calculated as the mean of three measurements from the equator of the egg; egg weight, Haugh units, and egg yolk colour, measured using an egg multi-tester instrument (Tohoku Rhythm Co., Ltd., Tohoku, Japan). The shape index was computed using the equation:

$$\text{Shape index} = \text{(width of egg/length of egg)} \times 100 \quad (5)$$

The yolk index was computed using the equation:

$$\text{Yolk index} = \text{(height of egg yolk/} \text{/width of egg yolk)} \times 100 \quad (6)$$

The albumen index was computed using the equation:

$$\text{Albumen index} = \text{height of albumen/} \text{/(width of albumen +} \text{+ length of albumen)} \times 100 \quad (7)$$

The egg yolk, albumen, and eggshell were weighed using a digital precision scale (0.01 g) and expressed as a ratio. A CR-310 Chroma Meter (Konica Minolta, Tokyo, Japan) was used to measure the lightness (L*), redness (a*), and yellowness (b*) of egg yolk colour.

Determination of fatty acids in egg yolk

Twelve egg yolks per treatment were pooled, and the fatty acid composition in the egg yolk was analysed (Institute of Food Research and Product Development; February 9, 2022; No. 65-0463, Bangkok, Thailand).

Statistical analysis

Egg performance, feed cost, egg quality, and egg yolk fatty acid profiles were analysed using one-way analysis of variance (one-way ANOVA) in the Statistical Package for the Social Sciences (SPSS), v17.0 (SPSS Inc., Chicago, IL, USA). All results were presented as means of groups and the combined standard error of the mean (SEM). Differences between the treatments were appraised using Duncan's multiple range test, and $P < 0.05$ was considered significant.

RESULTS AND DISCUSSION

The chemical components of LBSFL and the experimental diets are reported in Table 3. The amino acid and fatty acid composition of LBSFL is shown in Table 4 and 5. The major AAs and FAs in LBSFL were glutamic acid (1 215 mg/100 g) and lauric acid (22.36 mg/100 g total fatty acids). In addition, no cholesterol was detected in LBSFL. These findings are in accordance with those made by Secci et al. (2018), who noted that lauric acid (32.34 g/100 g total fatty acids) is reported to be the most abundant in LBSFL. The body weight gain, egg yield, egg weight, and egg mass were unaffected by dietary treatments ($P > 0.05$; Table 6). These results were similar to Star et al. (2020), who reported that the egg weight was not affected by the addition of LBSFL up to 10% in a diet consisting of maize and soybean.

In this experiment, all birds were healthy and there was no mortality, which was consistent with

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Table 3. Chemical components of live black soldier fly larvae (LBSFL) and experimental diets

Item	LBSFL	LBSFL in diet (g/kg)			
		0	10.0	20.0	30.0
Dry matter (%)	74.00	90.00	88.60	88.29	87.99
Crude protein (%)	49.27	18.32	18.83	19.20	19.66
Crude fat (%)	21.35	4.99	5.07	5.19	5.27
Crude fiber (%)	10.37	6.10	6.26	6.38	6.71
Ash (%)	9.70	14.39	14.47	14.53	15.10
Metabolizable energy (MJ/kg)	19.20	13.40	13.53	13.79	13.84
Total calcium (%)	0.42	6.83	7.85	8.38	8.52
Total phosphorus (%)	0.99	0.56	0.59	0.63	0.65

the results of Kawasaki et al. (2019). A decrease in feed intake combined with a decrease in feed conversion ratio led to a decreased feed cost during the experiment. Interestingly, our intention was to confirm that the 10 g/kg LBSFL group decreased the feed cost by 0.06 baht/egg in comparison with the 0 g/kg LBSFL group, resulting in economic savings for the egg industry. De Marco et al. (2015) reported that the digestibility values of crude protein, ether extract, gross energy, dry matter, and organic matter in BSF larvae feed were 51%, 99%, 69%, 53%, and 66%, respectively. These results were in agreement with the findings reported by Liu et al. (2021) and Tahamtani et al. (2021), who reported negative effects of *Hermetia illucens* larvae feeding on FCR. Additionally, some studies have shown that the dietary incorporation of 12 g LBSFL per hen per day reduced their feed intake and FCR in older laying

Table 4. Amino acid components of live black soldier fly larvae

Amino acid (mg/100 g)	
Aspartic acid	919.45
Glutamic acid	1 215.00
Serine	434.23
Threonine	446.31
Histidine	255.51
Glycine	677.69
Alanine	1 087.00
Arginine	630.80
Tyrosine	497.69
Valine	557.76
Methionine	135.90
Cystine	325.73
Isoleucine	441.59
Phenylalanine	821.89
Tryptophan	7.03
Leucine	820.05
Lysine	610.58
Proline	1 144.00

Table 5. Fatty acid components of live black soldier fly larvae

Fatty acid (g/100 g total fatty acid)	
C10:0	0.36
C12:0	22.36
C14:0	6.12
C15:0	0.14
C16:0	19.86
C17:0	0.22
C18:0	4.17
C20:0	0.89
C21:0	0.33
C22:0	0.30
C14:1	0.13
C16:1	2.22
C18:1n-9	18.57
C18:1n-11	0.23
C18:2n-6	22.25
C18:3n-3	1.87
C20:1n-11	0.23
Omega 3	1.87
Omega 6	22.25
Omega 9	18.57

Fatty acids C4:0, C6:0, C8:0, C11:0, C13:0, C23:0, C24:0, C15:1n10, C17:1n10, C18:1n9t, C22:1n9, C24:1n9, C18:2n6t, C18:3n6, C20:2, C20:3n6, C20:3n3, C20:4n6, C22:2, C20:5n3, C22:6n3, and cholesterol were not detected

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Table 6. Egg performance of laying hens aged 25–37 weeks fed dietary live black solider fly larvae (LBSFL)

Item	LBSFL in diet (g/kg)				Pooled SEM	*P*-value
	0	10.0	20.0	30.0		
Body weight gain (g)	250	280	270	300	20.00	0.12
Daily feed intake (g)	124.93^{ab}	122.25^{b}	127.41^{a}	127.26^{a}	2.80	0.01
FCR	2.17^{ab}	2.11^{b}	2.26^{a}	2.29^{a}	0.31	0.01
Egg yield (%)	95.12	95.05	94.10	92.73	0.93	0.68
Egg weight (g)	60.67	61.21	59.88	60.48	1.24	0.48
Egg mass (g)	57.65	58.16	56.31	56.01	1.40	0.23
Feed cost (baht/1 kg egg)	31.46^{ab}	30.54^{b}	32.82^{a}	33.26^{a}	1.25	0.01
Feed cost (baht/egg)	1.89^{ab}	1.83^{b}	1.97^{a}	2.00^{a}	0.96	0.01
Mortality (%)	0	0	0	0	–	–

FCR = feed conversion ratio; SEM = standard error of the mean

^{a,b}Different lowercase superscripts in the same row indicate significant value at $P < 0.05$ (n = 15 samples per treatment)

hens (Star et al. 2020). As expected, the hens in the present study showed great interest in the live larvae, demonstrating that supplementation of LBSFL in a maize-soybean meal diet for laying hens had positive effects on palatability. Furthermore, based on anecdotal observations made at feeding time, the hens in the 10 g/kg LBSFL group would finish their portion of live larvae in approximately 1 minute. This is highly relevant in the effort to decrease the consumption of basal diet. The feed ingredients and nutrients can affect the intestinal function of the host (Van der Aar et al. 2017). Chu et al. (2020) demonstrated that the fat derived from BSF is rich in medium-chain fatty acids that can have a positive effect on increasing the energy availability and nutrient absorption in the intestine, thus promoting the FCR.

The different LBSFL levels had no significant effect on the egg quality parameters, such as shell breaking strength, shell thickness, egg weight, Haugh unit, shape index, yolk index, albumen index, yolk ratio, albumen ratio, and shell ratio ($P > 0.05$; Table 7). These results were in agreement with the results from Liu et al. (2021) and

Table 7. Egg quality of laying hens aged 25–37weeks fed dietary live black solider fly larvae (LBSFL)

Item	LBSFL in diet (g/kg)				Pooled SEM	*P*-value
	0	10.0	20.0	30.0		
Shell breaking strength (N)	52.12	51.20	51.50	52.30	1.08	0.90
Shell thickness (mm)	0.39	0.38	0.38	0.39	0.02	0.48
Egg weight (g)	63.38	62.15	62.03	62.89	1.02	0.43
Haugh units	85.67	81.68	82.29	83.49	3.01	0.42
Shape index	77.87	76.95	77.73	77.40	0.25	0.48
Egg yolk index	40.11^{a}	39.77^{a}	40.79^{a}	37.73^{b}	0.35	0.004
Albumen index	40.61	40.70	40.78	40.79	0.78	0.38
Egg yolk ratio (%)	25.88	26.26	26.75	26.38	1.56	0.21
Albumen ratio (%)	64.05	63.53	63.46	63.42	1.00	0.62
Shell ratio (%)	10.07	10.21	9.79	10.20	0.64	0.42
Egg yolk color	11.56^{a}	11.28^{b}	10.90^{c}	10.94^{c}	1.00	0.000 1
Lightness (L*)	42.07^{c}	44.91^{b}	46.44^{a}	47.33^{a}	0.70	0.000 1
Redness (a*)	21.05^{a}	19.01^{cb}	18.31^{c}	19.69^{b}	0.12	0.000 4
Yellowness (b*)	53.38^{a}	51.25^{ab}	49.40^{b}	51.81^{ab}	0.84	0.000 1

a* = red (+) to green (–) axis; b* = yellow (+) to blue (–) axis; L* = as lightness ranging from black (0) to white (100); SEM = standard error of the mean

$^{a–c}$Different lowercase superscripts in the same row indicate significant value at $P < 0.01$ (n = 30 samples per treatment)

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Tahamtani et al. (2021), who demonstrated that BSF larvae had no adverse effects on shell breaking strength, shell thickness, egg weight, shell weight, yolk diameter, shape index, albumen index, and Haugh unit in laying hens. In the present study, adding 20 and 30 g/kg of LBSFL to the diet significantly decreased the egg yolk colour and increased the lightness (L*) of the egg yolk during the whole experimental period. This finding was in agreement with Star et al. (2020) and Tahamtani et al. (2021), who reported that when LBSFL were used *ad libitum*, the egg yolk colour was significantly paler, suggesting that LBSFL have less pigment. On the other hand, Heuel et al. (2021) and Patterson et al. (2021) showed that *Hermetia illucens* larvae meal in the diet significantly increased the egg yolk colour. Some researchers reported that BSF larvae were rich in carotenoids, ß-carotene, and lutein.

The egg yolk colour is affected by the dietary content of carotenoids, xanthophyll, lutein, and zeaxanthin (Nys 2011). According to Secci et al. (2018), insects do not contain carotene or xanthophyll. The disagreement between the results from other trials using the same insect species could be ascribed to the well documented variability of the rearing substrate utilized for larvae production (Loponte et al. 2019). This outcome might have resulted from a low content of xanthophyll in LBSFL. According to the diet composition in the present study, the addition of LBSFL impacted the egg yolk colour and may be useful in specialty markets.

The different LBSFL levels had significant effects on the fatty acid composition of egg yolk, such as C18:3n6, C20:3n6 and C22:6n3 (Table 8). Singh and Sachan (2010) reported that the fatty acid composition of yolk lipids could be altered depending upon the diet of the layer birds. Eggs produced by hens receiving conventional feed tend to be relatively high in omega-6 fatty acids. Similarly, omega-6 fatty acids (C18:3n6, C20:3n6 and C22:6n3) were influenced ($P < 0.05$) by the dietary LBSFL. A highly significant ($P < 0.01$) reduction in deposition of C18:3n6 occurred in the 30.0 g/kg LBSFL group, whereas a significant ($P < 0.05$) reduction in deposition of C22:6n3 occurred in all LBSFL groups. However, the deposition of C20:3n6 significantly ($P < 0.05$) increased at the 20.0 g/kg LBSFL level. Present-day poultry eggs are depleted of essential nutrients because of intensive and industrialized poultry farming (Khan et al. 2017). It has been recognized that the yolk fatty acid composition should be assessed when using a diet with high fat content (Bouvarel et al. 2011). Bejaei and Cheng (2020) found that cis-monounsaturated fatty acids and saturated fatty acids slightly increased, but cis-polyunsaturated fatty acids, omega-3 polyunsaturated fatty acids, and omega-6 polyunsaturated fatty acids were slightly lower with the addition of 10% and 18% full-fatted dried black soldier fly larvae to the laying hen diet. The fatty acid content of the insect could be affected by the types and sources of the materials fed to the larvae. This hypothesis is related to Patterson et al. (2021), who reported that black soldier fly larvae can contain upwards of 28% crude fat and are rich in essential fatty acids. Consequently, it appears that the use of black soldier fly larvae may be a novel choice to increase the amount of essential fatty acids for the enhancement of designer eggs. These results indicate that dietary LBSFL have no stimulating effect on the proportion of anti-inflammatory n-3 fatty acids. Further studies are required to determine if it is practical to introduce LBSFL into the entire production cycle of laying hens.

CONCLUSION

The results of the present study show that the dietary supplementation of live black soldier fly larvae did not significantly alter the proportion of the C18:3n6 and C20:3n6 n-3 content. From an economic point of view, live black soldier fly larvae could be used in laying hen diet at 10 g/kg.

Conflict of interest

The authors declare no conflict of interest.

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Table 8. Fatty acid components of egg yolk from hens aged 25–37 weeks fed dietary live black solider fly larvae (LBSFL)

Fatty acid (g/100 g fatty acid)	LBSFL in diet (g/kg)				Pooled SEM	*P*-value
	0	10.0	20.0	30.0		
Total SFA	33.42	33.95	34.03	33.67	0.05	0.58
C14:0	0.36	0.36	0.38	0.36	0.05	0.88
C16:0	25.38	25.71	25.76	25.36	0.53	0.55
C17:0	0.17	0.17	0.17	0.17	0.01	0.95
C18:0	7.76	7.71	7.72	7.78	0.04	0.98
Total MUFA	47.39	46.43	46.57	47.10	0.06	0.38
C16:1	2.59	2.64	2.59	2.48	0.03	0.66
C18:1n9c	43.90	43.57	43.76	44.40	0.06	0.35
C20:1n11	0.22	0.22	0.22	0.23	0.01	0.65
Total PUFA	19.14	19.60	19.41	19.21	0.06	0.76
C20:2	0.14	0.16	0.17	0.17	0.01	0.57
C18:2n6c	16.18	16.20	15.95	15.84	0.06	0.77
C18:3n3	0.34	0.35	0.34	0.34	0.03	0.83
C18:3n6	0.10^{a}	0.10^{a}	0.10^{a}	0.09^{b}	0.01	0.01
C20:3n6	0.13^{b}	0.12^{b}	0.19^{a}	0.12^{b}	0.02	0.03
C20:4n6	2.08	2.03	2.03	2.03	0.02	0.60
C22:6n3	0.66^{a}	0.64^{b}	0.62^{b}	0.62^{b}	0.01	0.02
Fat	30.94	30.75	30.73	30.64	0.54	0.99
Omega 3	1.01	0.98	0.96	0.96	0.01	0.07
Omega 6	18.48	18.47	18.27	18.08	0.06	0.73
Omega 9	43.90	43.57	43.75	44.59	0.07	0.21
Cholesterol	1 135	1 104	1 086	1 075	0.47	0.07
Omega 6 : omega 3	18.29	18.79	18.77	18.83	0.05	0.11
AI	0.41	0.48	0.42	0.48	0.01	0.62
TI	1.01	0.84	1.06	1.08	0.01	0.66

AI = atherogenicity index = (C12:0 + 4 × C14:0 + C16:0)/[total MUFA + total (n-6) + total (n-3)]; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids; SEM = standard error of the mean; TI = thrombogenicity index = (C14:0 + C16:0 + C18:0)/[(0.5 × total MUFA) + 0.5 × (n-6) + 3 × (n-3)/(n-6)]

^{a,b}different lowercase superscripts in same row indicate significant value at $P < 0.01$

Fatty acids C4:0, C6:0, C8:0, C10:0, C11:0, C12:0, C13:0, C15:0, C20:0, C21:0, C22:0, C23:0, C24:0, C14:1, C15:1n10, C17:1n10, C18:1n9t, C22:1n9, C24:1n9, C18:2n6t, C20:3n3, C22:2 and C20:5n3 were not detected

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