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RESEARCH ARTICLE

Partial or complete replacement of soybean meal with black soldier fly larvae meal improves feed efficiency in laying hens between 22 to 30 weeks of age

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Abstract

The European Commission recently authorised the inclusion of insect meal in poultry feed. Black soldier fly larvae meal (BSF) has comparable nutritional value to soybean meal (SBM) and higher calcium content, making it an attractive alternative protein source for laying hens While a few studies have explored this objective, inconsistent results have been reported, likely due to variations in hen age, breed, husbandry systems, and sunflower meal composition across studies, making it difficult to draw clear conclusions. We hypothesised that partial or complete replacement of SBM with BSF has no adverse effects on egg production and quality in laying hens at the initiation of laying (22-30 weeks of age). This study consisted of three dietary treatments and nine replicates per treatment. Diets were a control diet, and two diets containing 5% (BSF5%) and 10% (BSF10%). The experimental unit was a pen (100 × 200 × 200 cm) of 14 Brown Nick laying hens resembling an aviary setting. We found that complete replacement of SBM with BSF10% in the diet of laying hens improved feed efficiency attributed to a lower feed intake in comparison to laying hens fed the BSF5% or the control diets, while production performance, body weight, and egg quality were maintained and the colour of the egg yolk increased. Therefore, replacing SBM with up to 10% BSF in brown laying hens in aviary pens at the initiation of laying had no adverse effects on production performance, chemical and physical characteristics, and sensory attributes of eggs. Maintaining production and egg quality with a lower intake of nutrients requires further investigation.

Keywords

aviary – brown laying hen – egg quality – egg sensory – *Hermetia illucens*

1 Introduction

The human population is expected to reach 9.7 billion by 2050 with projections indicating a demand for approximately 2 billion tonnes of animal-origin protein sources by 2050 (Yitbarek, 2019). Eggs play a crucial role in ensuring global food security due to their cost-effectiveness and high nutritional value. The efficient conversion of feed to eggs in laying hens, coupled with the contribution of chicken manure as a fertiliser for

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crop production, underscores their significance (Mottet and Tempio, 2017). Forecasts suggest that global egg production will reach 102 million tonnes by 2050, necessitating approximately 214 million tonnes of feed to meet this growing demand (Yitbarek, 2019). However, the environmental impact of egg production is significantly influenced by feed use. Soybean meal (SBM) serves as the predominant protein source in laying hens' diets, and the environmental impact of soybean production, marked by carbon emissions, soil erosion, eutrophication, and acidification, poses challenges. The increasing demand for soybeans from Latin America has led to deforestation (Hunter, 2019). In the EU, only 7.5% of the demand for soy products is produced domestically and 75% of the total import is from Latin America (IDH, 2023). Efforts are ongoing to reduce the reliance on SBM in poultry nutrition by exploring alternative protein sources.

Insects, in particular black soldier fly larvae, have recently received more attention as a sustainable locally produced source of protein and fat for feed and food. Rearing black soldier fly larvae on agri-food residuals and including the larvae in poultry feed in exchange for SBM may reduce the nutrient waste and environmental impact of poultry products. The European Commission recently authorized the use of insect proteins in poultry diets (EC, 2021). A recent review on the use of black soldier fly meal (BSF) in laying hen feed, conducted by Dörper *et al.* (2021), suggests that a partial replacement of SBM with 10% BSF does not adversely affect the production performance of laying hens. The comparable nutritional value of BSF to SBM, along with its higher calcium content, make it an attractive option for substituting SBM in laying hen feed. However, it is difficult to draw definitive conclusions due to the limited number of published studies. Additionally, the majority of studies have been conducted on laying hens kept in small cages, with variations in the breed and age of hens, discrepancies in the nutritional composition of BSF, and differences in feed formulations across studies. These factors complicate drawing a clear conclusion (Rezaei Far *et al.*, 2024).

This study aimed to examine the effects of partial and complete replacement of SBM as the main protein source of the feed with two inclusion levels of BSF (5% and 10%) on laying performance, egg quality, and sensory characteristics of the eggs in laying hens at the beginning of laying. Our hypothesis was including 5 or 10% BSF in laying hen feed does not negatively affect the production performance of laying hens in an experimental setting closer to the aviary system.

2 Material and methods

The experiment was executed at Carus, the animal experiment facility of Wageningen University & Research (Wageningen, the Netherlands) following Dutch legislation and regulations for animal experiments. The project was approved by the Central Authority for Scientific Procedures on Animals (CCD) and the experimental protocol was approved by Wageningen University's Animal Welfare body, with application number AVD40100202010104.

This study included three dietary treatments, the control diet, and two diets containing increasing levels of BSF inclusion, namely 5% (BSF5%) and 10% (BSF10%). According to the literature, principle parameters of performance (e.g. FCR) (Clark *et al.*, 2019) and egg quality (e.g. breaking strength) (Sirri *et al.*, 2018) were selected for power analysis. Based on the dataset from the laying hen studies of our previous research (unpublished data), nine replicates of our experimental unit with 14 laying hens were required to identify significant differences between the three treatments with the type I error probability of 0.05 and power of 0.95. Each dietary treatment was replicated nine times. The experimental unit was a pen (100 × 200 × 200 cm) of 14 brown laying hens resulting into a total of 27 pens. Each pen was equipped with a pan feeder, nipple drinkers, two laying nests, and enriched with wood shavings on the floor, perches, and pecking stones. Pens were distributed over three rooms having the same environmental conditions. The experimental design was a randomized complete block design, and each block contained three pens. Pens were randomly assigned to one of the three treatments.

Diets

Diets were formulated and produced at ForFarmers (Lochem, the Netherlands). Hermetia Baruth GmbH (Baruth/Mark, Germany) produced and provided BSF which was analysed for nutritional composition before feed formulation (Supplementary Table S1). The control diet represented the conventional laying hen feed meeting the nutritional requirements of laying hens in the Netherlands (CVB, 2016). Soybean meal (SBM) was replaced partially and fully in the diets with 50 (BSF5%) and 100 (BSF10%) g/kg BSF, respectively. Diets were formulated as isocaloric and isonitrogenous providing similar content of most limiting digestible amino acids (Table 1).

Diets were analysed for dry matter (ISO 6496), ash (ISO 5984), crude fat (ISO 6492), crude fibre (ISO 6865),

TABLE 1 Ingredient composition, calculated and analysed nutrient content of the control diet, and diets with partial (BSF5%) or complete (BSF10%) substitution of soybean meal with black soldier fly larvae meal (BSF)

	Control	BSF5%	BSF10%
Wheat	15.00	15.00	15.00
Maize	45.62	45.94	43.75
Rapeseed	3.00	3.00	3.00
Soybean meal 48%	13.48	6.92	0.00
Sunflower seed meal HiPro	10.00	10.00	10.00
Wheat middlings	0.00	2.39	7.00
Poultry fat	2.32	1.82	1.88
BSF	0.00	5.00	10.00
Limestone	7.90	7.90	7.90
Calcium carbonate	1.18	0.61	0.09
Monocalcium phosphate	0.39	0.23	0.05
Sodium chloride	0.27	0.21	0.14
Sodium bicarbonate	0.14	0.19	0.25
Lysine 50%	0.07	0.07	0.07
Lysine HCL	0.00	0.05	0.11
Methionine DL	0.11	0.13	0.16
L-Isoleucine	0.00	0.00	0.01
L-Threonine 98	0.00	0.01	0.03
Tryptophan 98	0.00	0.01	0.04
Premix[a]	0.53	0.53	0.53
Calculated (chemically analysed) content of nutrients and standardised ileal digestible amino acids (g/kg)[b]			
Crude ash	126.1 (125.8)	119.4 (128.0)	113.5 (103.7)
Crude fibre	40.9 (36.7)	42.7 (41.7)	45.7 (51.1)
Crude protein	165.0 (160.9)	165.0 (162.2)	165.0 (169.9)
Ether Extracts	51.2 (50.3)	50.7 (49.5)	55.6 (54.6)
Starch	377.5 (364.1)	383.6 (379.8)	378.3 (371.3)
Calcium	37.0 (39.7)	37.0 (38.8)	37.0 (32.0)
Available P (Total P)	3.2 (4.9)	3.2 (4.9)	3.2 (5.2)
Sodium	1.5 (1.6)	1.5 (1.6)	1.5 (1.6)
Chloride	2.2 (2.1)	2.2 (2.4)	2.2 (2.3)
Lysine	6.9 (8.0)	6.9 (8.3)	6.9 (8.3)
Methionine	3.8 (3.8)	4.0 (3.9)	4.2 (4.4)
Methionine+Cysteine	6.2 (6.6)	6.2 (6.4)	6.2 (6.9)
Threonine	5.0 (6.1)	5.0 (6.0)	5.0 (6.4)
Tryptophan	1.7 (2.1)	1.5 (2.3)	1.5 (2.8)
Isoleucine	5.9 (6.8)	5.7 (6.4)	5.5 (7.6)
Valine	6.8 (7.9)	7.0 (8.0)	7.3 (8.6)
Available metabolisable (Gross) energy (Kcal/kg)	2800 (3588)	2800 (3626)	2800 (3805)

[a]Including vitamins, minerals, phytase, and xylanase as follows per kilogram of complete diet: vitamin A = 9,000 IU; vitamin D_3 = 2,500 IU; vitamin E = 15 IU; vitamin K_3 = 2.0 mg; vitamin B_1 = 1.0 mg; vitamin B_2 = 5.0 mg; vitamin B_6 = 3.0 mg; vitamin B_{12} = 0.03 mg; niacinamide = 40 mg; D-pantothenic acid = 15.2 mg; folic acid = 0.5 mg; biotin = 0.25 mg; iron = 15.0 mg (as $FeSO_4{\cdot}7H_2O$); iron = 5 mg (as Fe(II)chelate); copper = 12 mg (as $CuSO_4{\cdot}5H_2O$); manganese = 10.0 mg (as Mn chelate); manganese = 50.0 mg (as MnO); zinc = 80 mg (as $ZnSO_4$); iodine = 1.0 mg (as CaI); selenium = 0.2 mg (as $Na_2SeO_3{\cdot}5H_2O$); endo-1,4-bètaxylanase = 1500 EPU, (supplied by Trouw Nutrition Benelux, Putten, the Netherlands). [b]CVB (2018).

starch (ISO 6493), Ca (ISO 6869), P (ISO 6941), Na (ISO 6869), Cl (ISO 6495). Nitrogen was determined by the Kjeldahl method (ISO 5983), and the gross energy by the bomb calorimeter method (ISO9831). Amino acids contents were determined by ion exchange chromatography in an Amino Acid Analyzer (Evonik, 2010).

Animal and housing

The experiment was conducted with 378 laying hens (Brown Nick) obtained from a commercial farm at 19 weeks of age. The climate control and lighting program was applied according to the standard operating protocol of the experimental facility. In brief, temperature was kept around 21 °C, and 10 hours of continuous light for the first week while the light period extended for one hour weekly until reaching 14 hours of light, whereafter hours of light per day were kept constant.

Upon arrival, hens were individually weighed and randomly assigned to 27 floor pens in three rooms. All the hens were fed with a commercial pre-lay diet for the first three weeks. The experimental period started at 22 weeks of age by feeding the hens with the assigned dietary treatments and finished at 30 weeks of age. Hens had access to feed and water *ad libitum*.

The health status of the hens was monitored daily throughout the experiment. Every day at 10.00 in the morning, eggs were collected to record the egg production and to calculate the laying rate of each pen. Every week on the same day at 11.00 in the morning, feed intake was recorded. Two days a week, all the eggs from each pen were weighed. These data were used to calculate egg mass and feed efficiency. At the start and the end of the trial, hens were individually weighed and the evolution in body weight was calculated at the pen level.

Physical and chemical quality of eggs

Ten eggs from each pen were randomly collected in one day at week four and week eight of the experiment and used to assess the physical characteristics of the egg at the Institute for Egg Quality Management (IKE, Amersfoort, the Netherlands). Physical characteristics included egg weight and egg components weight (digital Kern scale, 440-47 N, Kern & Sohn GmbH – Waagen, Balingen Germany), Shell colour (reflectometer TSS, York, UK), length and width by calliper, shell strength (Futura Egg Shell Tester, Broeringtech, Lohne (Oldenburg), Germany), Haugh Unit and albumen height gauge (QCD Range TSS, York, UK), yolk colour Roche (R-G-B sensor, Broeringtech, Lohne (Oldenburg), Germany), shell thickness by a micrometre (Broeringtech, Lohne (Oldenburg), Germany).

Over two consecutive days, at week four and week eight of the experiment, 20 eggs from each pen were randomly collected for chemical analysis. Eggs were analysed at the DISAFA laboratory (Turin, Italy). The yolk and albumen of the eggs were separated. Subsequently, yolk and albumen from five eggs per pen were pooled, freeze-dried, and ground before chemical analysis. In detail, albumen was analysed for DM, and CP (AOAC, 2000). Yolk was analysed for DM, CP, and ash (AOAC, 2000), and for EE (AOAC, 2003). Ash content was not analysed in albumen due to the technical limitations of weighing the albumen residue after heating in the stove. Additionally, ether extract content was not determined in albumen due to its absence of fat.

Sensory evaluation of eggs

At week eight of the experiment, 14 eggs from each pen of the control treatment and seven eggs from each pen of the other two dietary treatments, BSF5% and BSF10%, were collected for sensory evaluation. Sensory evaluation was executed at Sense Test (Porto, Portugal). Eggs were heated in boiling water for 10 minutes and cooled down under tap water. Boiled eggs were deshelled, cut longitudinally into halves, and presented on white porcelain dishes coded with a three-digit random number. Each panellist received a set of four samples, two samples from the control treatment and one sample from the other two treatments (BSF5% and BSF10%). To compensate for eventual carry-over effects a monadic sequential presentation was followed, with their order previously balanced according to MacFie *et al.* (1989). Sensory evaluation was executed in individual tasting booths in a special room equipped following ISO 8589:2007 – sensory analysis – general guidance for the design of test rooms. In addition to the implementation of informed consent, Sense Test ensures the protection and confidentiality of data through the authorization 2063/2009 of the National Data Protection Commission and following EU Regulation (EU 2016/679), as well as an internal code of conduct certified according to ISO 9001:2015-Quality management systems.

The panel was recruited from the sensory evaluation company Sense Test's consumer database (Vila Nova de Gaia, Portugal). It consisted of 67% women and 33% men, with an average age of 44 years (19-64 years old). All participants were regular consumers of eggs, consuming at least two times per week and were selected based on their willingness to try products containing edible insects (Ribeiro *et al.*, 2022). A total of 120 naïve consumers evaluated overall liking using a 9-point hedonic scale, ranging from 1 = "dislike extremely" to 9 =

TABLE 2 The list of the four sensory dimensions consisted of 24 sensory attributes. All attributes were rated as absent or present and if present, rated using a 5-point structured scale (from 1 = "slightly applicable" to 5 = "very applicable")

Appearance	Odour	Texture	Taste
Large-sized yolk	Characteristic egg	Consistent egg white	Astringent
Orange colour of the yolk	Fresh	Consistent yolk	Characteristic egg white
Small-sized yolk	Sulphur/sulfuric	Crumbled yolk	Characteristic yolk
White colour of the egg white		Hard egg white	Fresh
Yellow colour of the yolk		Rubbery texture of egg white	Intense egg white
		Soft/smooth egg white	Intense yolk
		Soft/smooth yolk	Persistent
		Yolk dryness	Sulphur / sulfuric

"like extremely" (Peryam and Pilgrim, 1957). For each sample, overall liking evaluation was followed by the evaluation of the sensory profile, through the Rate-All-That-Apply (RATA) methodology. Table 2 contains the list of terms included in the RATA, which were selected based on bibliographic research and previous studies of the lab (non-published data). Attributes were organized by sensory dimension: appearance (5), odour (3), texture (8) and taste (8) to reduce the cognitive effort required by the participants. During the session, individual attributes within each sensory dimension were presented in a randomized order, different for each participant and each product, while the order of the sensory dimensions was fixed (Ares *et al.*, 2014b; Baião *et al.*, 2022). Panellists were asked to check the terms they considered applicable and to rate the intensity of each selected attribute using a 5-point structured scale (from 1 = "slightly applicable" to 5 = "very applicable") (Ares *et al.*, 2014a; Meyners *et al.*, 2016).

Statistical analysis
The statistical analysis of the data was performed by using SAS (version 9.4). Response variables of the performance and physical characteristics of the eggs were analysed with a linear mixed model (Proc GLIMMIX), with dietary treatments (Control, BSF5%, BSF10%) having a fixed effect, and block as a random effect, and pen as the experimental unit. As the laboratory reported the chemical compositions of the eggs of each pen within the treatments without indicating the pen number, the chemical compositions of the eggs were analysed by a generalised linear model (Proc GLM) with dietary treatment as the fixed effect. Analysis of the RATA data, following a mixed-effect two-way ANOVA, with panellist as a random effect and dietary treatments as a fixed effect, for each attribute. Pairwise comparisons of the treatment groups were performed using Tukey's post hoc test. The level of significance was set to 0.05. Data are presented as least-square means and pooled SEM unless stated otherwise.

3 Results

Diet composition
The nutritional composition of the diets is reported in Table 1. There was a slight deviation between the calculated and analysed content of Ca, as in BSF10% dietary treatment Ca content was lower than expected (32.0 vs 37.0 g/kg as DM). There were no other major deviations between the calculated and analysed content of nutrients in each dietary treatment or among the three dietary treatments.

Performance
Over the experimental period, diet had no effects on body weight, laying rate, average egg weight, or egg mass (Table 3). Replacing SBM with BSF was associated with lower feed intake ($P = 0.0090$), which improved the feed conversion ratio ($P = 0.0127$). Laying hens fed BSF10% diet had a lower FCR in comparison to the control diet ($P = 0.0104$; 2.23 vs 2.33).

The effects of including BSF in the diet of laying hens on feed intake (Table 4) were already observed in the first four weeks of the experimental period (~4 g/hen/day, $P = 0.0728$) and laying hens in BSF10% treatment showed a tendency for a lower feed intake compared with the laying hens in the control treatment ($P = 0.0855$; 119 vs 124 g/day). The effects of treatment on the feed intake became stronger between weeks 5 to 8 of the experiment ($P = 0.0035$) as the contrast increased in the average feed intake between the control and BSF10% treatments ($P = 0.0026$, 124.1 vs 118.2 g/day/bird) and between the control and BSF5% treatments ($P = 0.0820$, 124.1 vs 120.7 g/day/bird).

TABLE 3 Production performance parameters and body weight of laying hens fed a control diet or diets with partial (BSF5%) or complete (BSF10%) substitution of soybean meal with black soldier fly larvae meal (BSF) between 22 to 30 weeks of age

	Control	BSF5%	BSF10%	SEM[1]	*P*-value[2]
Laying rate (%)	93.8	93.4	95.1	0.72	0.2499
Average egg weight (g)	56.7	56.2	56.0	0.31004	0.2707
Average daily feed intake (g/day)	123.8[a]	120.3[ab]	118.8[b]	1.0235	**0.0090**
Egg mass (g)	53.22	52.49	53.27	0.5447	0.5377
Feed conversion ratio (g/g)	2.329[a]	2.293[ab]	2.231[b]	0.02063	**0.0127**
Discarded eggs (%)	2.8	2.8	2.3	0.34	0.5515
Body weight (g)[3]	510.6	501.9	504.9	12.57	0.8871

[a-b] Values in the row, not sharing a common superscript differ ($P < 0.05$).

1 Pooled standard error of the mean.

2 Model established *P*-values for the fixed effects of dietary treatment, and the random effects of block are depicted.

3 Body weight evolution represents the differences between the average body weight at the start (week 19 of age) and the end of the experiment (week 30 of age). Laying hens in all the dietary treatments fed similar commercial laying hen feed between week 19 to week 22 of the age.

TABLE 4 Production performance parameters of laying hens after feeding a control diet or diets with partial (BSF5%) or complete (BSF10%) substitution of soybean meal with black soldier fly larvae meal (BSF) for week 1-4 or week 5-8 between 22 to 30 weeks of age

	LR	EW	FI	EM	FCR
Week 1-4					
Control	93.0	54.4	123.6	50.5	2.448
BSF5%	91.8	53.6	119.95	49.2	2.446
BSF10%	94.5	53.4	119.4	50.5	2.364
SEM	1.23	0.3807	1.303	0.7927	0.0465
P-value	0.3815	0.2016	0.0728	0.4072	0.2305
Week 5-8					
Control	94.6%	59.1	124.1[a]	55.9	2.220[a]
BSF5%	93.6%	58.8	120.7ab	55.1	2.195ab
BSF10%	95.6%	58.6	118.2b	56.1	2.110b
SEM	0.96	0.32	1.03	0.71	0.0246
P-value	0.3319	0.5778	0.0035	0.5629	0.0152

LR% = laying rate, EW = average egg weight (g), FI = average daily feed intake (g/bird/day), EM = egg mass (g), and FCR = feed conversion ratio.

Egg quality

According to Table 5, diet had no effect on the content of DM and CP in the albumen. Diet did not affect the chemical composition of yolk (Table 6).

In general, replacing SBM with BSF in the diet of laying hens between 22 to 30 weeks of age had limited effects on the physical characteristics of the eggs. In week four, the absolute ($P = 0.0355$) and relative ($P = 0.0742$) weights of albumen were lower in the eggs of laying hens fed BSF10% than the eggs of laying hens fed BSF5% diets while laying hens fed BSF10% produced eggs with a high relative weight of yolk (Figure 1). The average weight of the eggs was higher in BSF5% than in BSF10% treatments ($P = 0.0677$, 57.0 vs 56.1 g), which was not different from the control treatments. According to Table 7, the contrast in the average egg weight of BSF5% and BSF10% treatments was associated with a lower surface area of the eggs in BSF10% than in BSF5% ($P = 0.067$, 68.96 vs 68.16). According to Table 8, the differences in the physical characteristics of the eggs between BSF5% and BSF10% treatments were no longer observed at week eight. The only significant effect of diet on eggs in week eight was the highest yolk

TABLE 5 The content of dry matter (DM%) and crude protein (CP%) of albumen in eggs from the laying hens after four weeks and after eight weeks of feeding a control diet or diets with partial (BSF5%) or complete (BSF10%) substitution of soybean meal with black soldier fly larvae meal (BSF)

	DM	CP
Week 4		
Control	12.56	91.26
BSF5%	12.57	91.20
BSF10%	12.59	91.84
SEM	0.08011	0.8349
P-value	0.9841	0.8372
Week 8		
Control	12.51	91.80
BSF5%	12.57	91.86
BSF10%	12.52	92.79
SEM	0.05735	0.7275
P-value	0.7695	0.5586

colour in the laying hens fed BSF10% diet ($P < 0.0001$) among the treatments.

Sensory profiling and overall liking

In general, samples were well liked by consumers, with positive overall liking scores (all mean scores above 7.5, in the upper half of the positive side of the scale). No significant differences ($P > 0.05$) were found between samples for overall liking (Table 9).

Analysis of the RATA data indicated significant differences in five of the eggs' sensory attributes (Figure 2). According to Figure 3, consumers perceived a larger yolk and a softer egg white in eggs of the hens fed with diets containing BSF, while there were no significant differences in attributes related to the odour and taste of the eggs (Figure 2).

4 Discussion

We found that partial or complete replacement of SBM with 5% or 10% of BSF had no negative effects on production performance, chemical and physical characteristics, and sensory attributes of eggs in laying hens between 22 to 30 weeks of age. In fact, including 10% BSF in the diet of laying hens improved feed efficiency, driven by a lower feed intake in comparison to laying hens fed the BSF5% or the control diets. The lower feed intake is associated with a lower intake of nutrients and ingredients such as amino acids, Ca for eggshell formation and carotenoids for egg yolk colour. However, laying hens in BSF10% maintained production performance, body weight, and egg quality, while the colour of the egg yolk increased. Replacing SBM with 5% or 10% BSF had no negative effects on the sensory attributes of the eggs at the week eight of the experiment, while consumers perceived a larger yolk size but egg white with lower consistency in laying hens fed BSF5% or BSF10% diets.

The average daily feed intake was lower in laying hens fed BSF10% compared with the control diet ($P = 0.008$; 123.8 vs 118.8 g/day), with no negative effects on the production performance. In most of the studies in laying hens, replacing SBM with BSF did not affect feed intake (Park *et al.*, 2021, Kawasaki *et al.*, 2019; Bovera *et al.*, 2018, Maurer *et al.*, 2016) even in higher inclusion levels than the BSF inclusion levels in our study, i.e. 15% (Zawisza *et al.*, 2023; Heuel *et al.*, 2021, 2022). In one study, replacing SBM with 7.5% BSF in the diet of laying hens was associated with a higher feed intake compared with the laying hens fed the control diet (Mwaniki *et al.*, 2018). In a few cases, feed intake declined in laying hens fed diets containing BSF compared with the control diets (Patterson *et al.*, 2021; Marono *et al.*, 2017). Feed intake declined (98.6 vs 92.4 g/hen/day) in laying hens fed a diet including 24% BSF (Patterson *et al.*, 2021) or 17% BSF (125 vs 108 g/day/hen) with negative effects on the egg weight (Marono *et al.*, 2017). In contrast to our study, in which there was no significant effect of diet on egg weight, the lower feed intake declined the average egg weight in both studies by 5% (Patterson *et al.*, 2021) or by 3% (Marono *et al.*, 2017) in comparison to the average egg weight of the laying hens fed the control diets. Besides that, the lower feed intake negatively affected the body weight evolution and egg production in both studies (Patterson *et al.*, 2021; Marono *et al.*, 2017), suggesting that the nutrient intake of laying hens was lower than their requirements.

The decline in the content of arginine and tryptophan by replacing SBM with 24% BSF in comparison to the recommendation of the breeding company was suggested as the reason for the impaired egg production (Patterson *et al.*, 2021). Although dietary treatments were formulated to meet the requirements of laying hens by replacing SBM with BSF (Patterson *et al.*, 2021, Marono *et al.*, 2017), the expected variations in the digestible amino acid content of the insect meal (Matin *et al.*, 2021) could result in an overestimation of BSF nutritional value. Such overestimation in digestible amino acid content may adversely impact the production performance. It is expected that laying hens increase their feed intake when limiting nutrients are

TABLE 6 The content of dry matter (DM%), ash, crude protein (CP%), and ether extract (EE%) of egg yolk from the laying hens after four weeks and after eight weeks of feeding a control diet or diets with partial (BSF5%) or complete (BSF10%) substitution of soybean meal with black soldier fly larvae meal (BSF)

	DM	Ash	CP	EE
Week 4				
Control	48.03	3.98	34.03	55.45
BSF5%	47.41	3.87	34.57	55.32
BSF10%	47.74	3.75	33.96	55.57
SEM	0.2616	0.1132	0.1967	0.1898
P-value	0.2517	0.3542	0.0690	0.6407
Week 8				
Control	41.11	3.49	34.14	56.79
BSF5%	40.73	3.54	34.09	56.66
BSF10%	41.57	3.54	34.26	56.79
SEM	0.5146	0.01819	0.1483	0.1114
P-value	0.5147	0.0730	0.7197	0.6546

TABLE 7 The physical characteristics of the eggs from the laying hens after four weeks of feeding a control diet, or diets with partial (BSF5%) or complete (BSF10%) substitution of soybean meal with black soldier fly larvae meal (BSF). The standard error means of the model are provided in parentheses

	Control	BSF5%	BSF10%	*P*-value
Average egg weight (g)	56.38 (0.301)	57.02 (0.286)	56.07 (0.319)	0.0713
Shape index[1]	79.14 (0.325)	78.29 (0.315)	78.61 (0.342)	0.0805
Shell colour	19.96 (0.368)	19.30 (0.351)	19.08 (0.391)	0.2269
Breaking strength (N)	58.57 (1.097)	59.46 (1.053)	59.42 (1.155)	0.7814
Shell thickness (mm)	0.411 (0.0029)	0.412 (0.0028)	0.418 (0.0031)	0.1500
Shell weight (g)	6.19 (0.052)	6.22 (0.050)	6.19 (0.055)	0.8610
Surface area[2]	68.41 (0.258)	68.96 (0.245)	68.16 (0.273)	0.0698
SWUSA[3]	90.6 (0.84)	90.3 (0.82)	90.7 (0.88)	0.8933
Haugh unit	82.39 (0.891)	83.20 (0.854)	80.98 (0.951)	0.2048
Yolk colour (Roche)	10.71 (0.121)	10.52 (0.115)	10.75 (0.128)	0.3408
Yolk weight (g)	15.05 (0.161)	15.05 (0.155)	15.34 (0.170)	0.3089
Albumen weights (g)	35.2 (0.36)	35.7 (0.34)	34.6 (0.37)	0.0465

1 Shell index = egg length/egg width.
2 Surface area (cm^2) = 3.9782. Egg weight $(g)^{0.7056}$ (Carter, 1975).
3 Shell weight per unit surface area (mg/cm^2) = shell weight (mg) / surface area (cm^2).

deficient in the feed. However, Heuel *et al.* (2021, 2022) failed to stimulate the compensatory rise in feed intake when the content of lysine and methionine, as the most limiting amino acids, were deficient in the diet of laying hens by replacing SBM with 15% BSF and Lohmann Brown laying hens maintained the production performance between 28 to 36 (Heuel *et al.*, 2021) and 40 to 47 (Heuel *et al.*, 2022) weeks of age. Although SBM was replaced completely in BSF10% dietary treatment in our study, all diets contained sunflower seed meal (100 g/kg) and rapeseed meal (30 g/kg) as protein sources. Insect meal production is in an early stage and the variability in the nutritional value of BSF is currently a constraint for feed producers (Gasco *et al.*, 2023a). Therefore, providing a portion of protein with other protein sources in diets with a complete replacement of SBM with BSF might prevent the negative effects on performance. For example, by alleviating the effects of the potential variations in the content of digestible amino acids in BSF. In our study, lower FI was not associated with negative effects on egg production or body weight evolution, indicating that the nutritional requirements were met

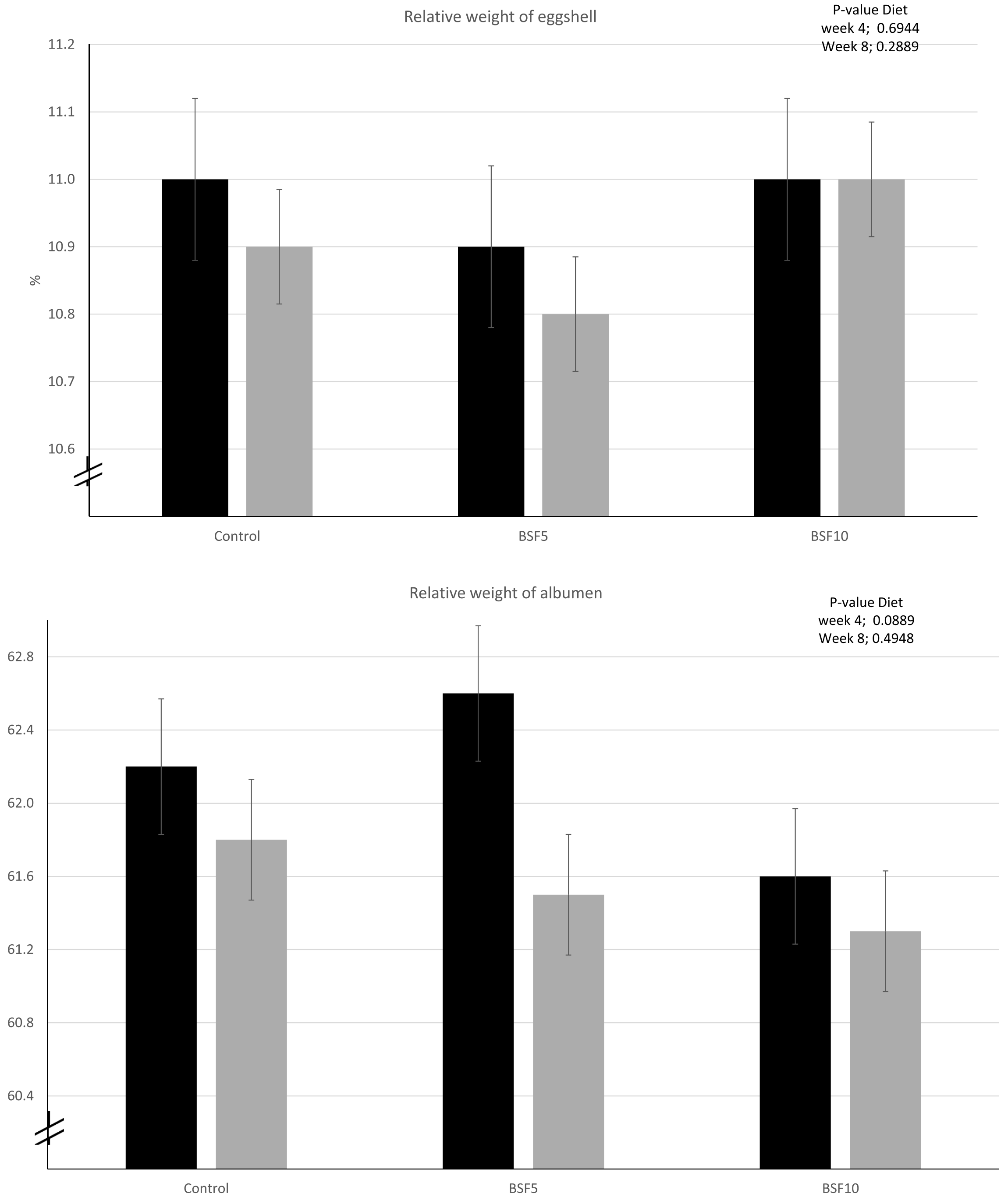

FIGURE 1 The relative weight of egg components (eggshell, albumen, yolk) in the eggs of laying hens after 4 weeks (black bars) or after 8 weeks (grey bars) of feeding a control diet or diets with partial (BSF5%) or complete (BSF10%) substitution of soybean meal with black soldier fly larvae meal (BSF) between 22 to 30 weeks of age.

in laying hens in BSF10% treatment despite the lower FI.

In comparison to the control treatment, the average feed intake (g/bird/day) was significantly lower in laying hens fed BSF5% and BSF10% diets. Lower feed intake, along with no significant changes in egg mass, resulted in the improvement of the feed conversion ratio (FCR) for 0.04 units in BSF5% and for 0.10 units in BSF10% dietary treatments. Complete replacement of SBM with BSF was associated with an impaired FCR in laying hens (Mwaniki *et al.*, 2018, 2020). In contrast, including up to 15% BSF had no effects on feed intake and feed efficiency in laying hens between 25-37 weeks of age (Zawisza *et al.*, 2023; Bovera *et al.*, 2018), while improvements in FCR have not been observed in the existing studies. Heuel *et al.* (2021) observed a tendency (*P*-value 0.063) for a positive effect of replacing SBM with BSF on feed efficiency, however, this effect was predominantly influ-

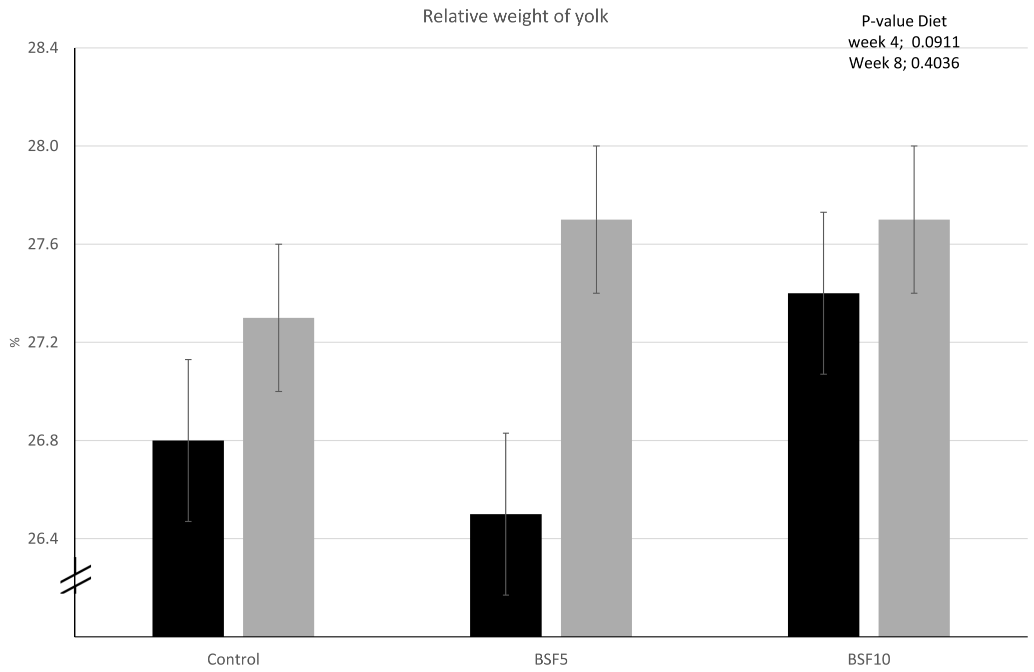

FIGURE 1 (Continued.)

TABLE 8 The physical characteristics of the eggs from the laying hens after eight weeks of feeding a control diet or diets with partial (BSF5%) or complete (BSF10%) substitution of soybean meal with black soldier fly larvae meal (BSF). The standard error means of the model are provided in parentheses

	Control	5%	10%	*P*-value
Average egg weight (g)	59.8 (0.42)	60.0 (0.40)	59.2 (0.44)	0.2688
Shape index[1]	78.50 (0.281)	78.02 (0.267)	78.01 (0.298)	0.3743
Shell colour	15.59 (0.282)	15.19 (0.270)	15.60 (0.298)	0.4616
Breaking strength (N)	57.69 (0.864)	56.83 (0.815)	58.00 (0.923)	0.6304
Shell thickness (mm)	0.413 (0.0022)	0.410 (0.0021)	0.416 (0.0023)	0.1829
Shell weight (g)	6.53 (0.051)	6.47 (0.049)	6.48 (0.054)	0.6701
Surface area[2]	71.30 (0.351)	71.52 (0.337)	70.76 (0.372)	0.2594
SWUSA[3]	91.6 (0.63)	90.6 (0.60)	91.5 (0.67)	0.4238
Haugh unit	76.97 (0.689)	77.55 (0.662)	77.23 (0.729)	0.8020
Yolk colour (Roche)	9.5[b] (0.10)	9.3[b] (0.10)	10.2[a] (0.10)	<0.0001
Yolk weight (g)	16.2 (0.15)	16.6 (0.14)	16.4 (0.16)	0.3238
Albumen weights (g)	36.9 (0.42)	37.0 (0.41)	36.3 (0.44)	0.4157

1 Shell index = egg length/egg width.
2 Surface area (cm^2) = 3.9782. Egg weight $(g)^{0.7056}$ (Carter, 1975).
3 Shell weight per unit surface area (mg/cm^2) = shell weight (mg) / surface area (cm^2).

TABLE 9 The mean overall liking scores of the eggs from the laying hens fed a control diet or diets with partial (BSF5%) or complete (BSF10%) substitution of soybean meal with black soldier fly larvae meal (BSF) for eight weeks between 22 to 30 weeks of age. Each panellist consumed duplicate samples from the control dietary treatment to evaluate the internal variability

Overall liking	Control (1)	Control (2)	BSF5%	BSF10%
Mean (± SD)	7.6 ± 0.9	7.8 ± 0.9	7.7 ± 0.9	7.7 ± 0.9
% Positive responses (6-9 overall liking)	98%	100%	100%	100%

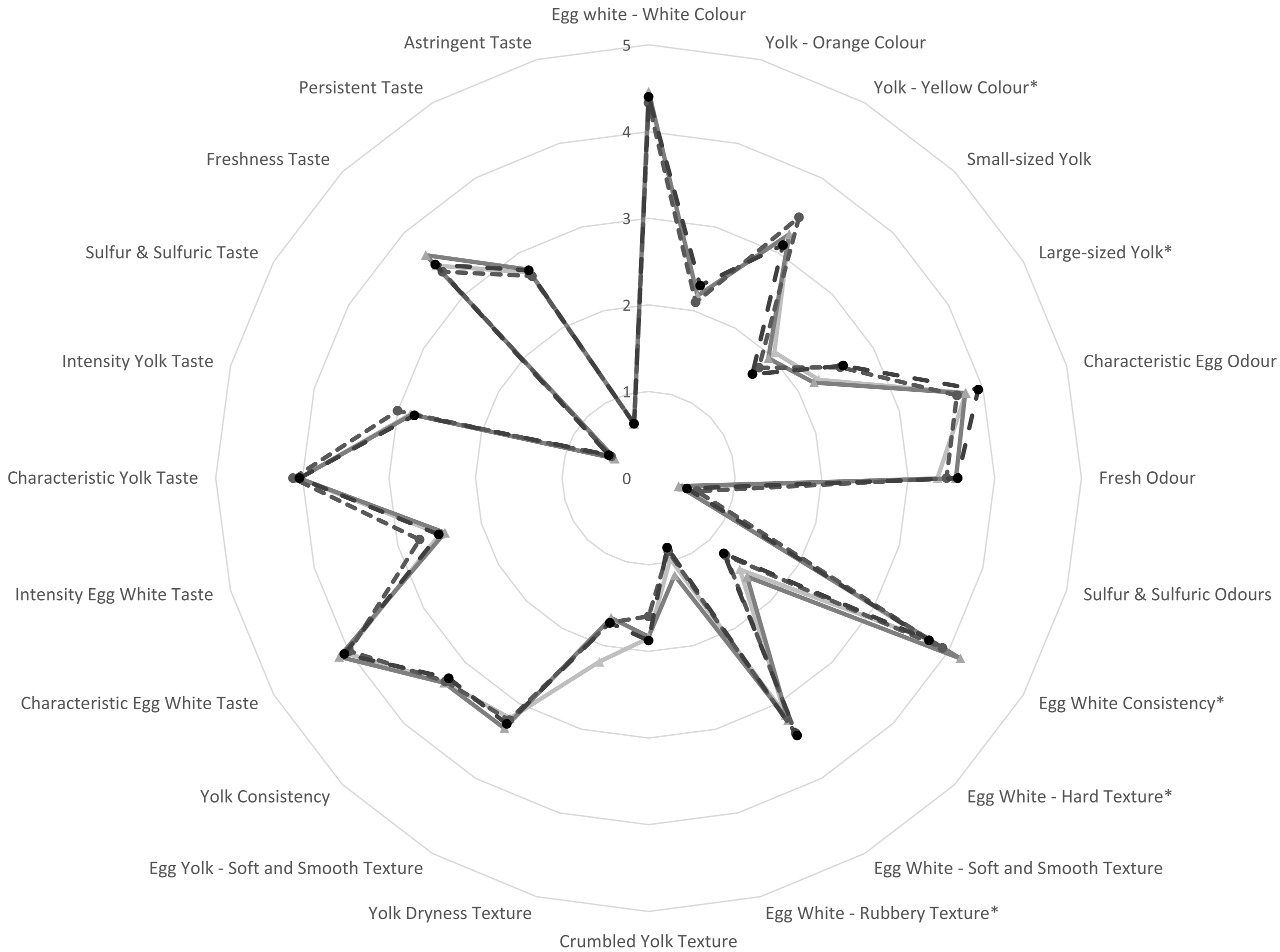

FIGURE 2 Mean scores of the Rate-All-That-Apply attributes by a panel of consumers (n = 120 consumers) after evaluating eggs from the laying hens fed a control diet in duplicates (grey lines with triangles) or diets with partial (BSF5%, Grey dash with circle) or complete (BSF10%, black dash with circle) substitution of soybean meal with black soldier fly larvae meal (BSF) for eight weeks between 22 to 30 weeks of age. The discriminating attributes (*) indicate significant effects of the diet as the fixed effects.

enced by an increase in egg weight and the consequent rise in egg mass of the laying hens fed BSF rather than a lower feed intake. Few studies have proposed explanations when replacing SBM with BSF was associated with a decline in feed intake in laying hens. The dark colour of BSF or its specific flavour could decline the palatability of the feed (Marono *et al.*, 2017). The dark colour could indicate heat damage and a consequent decline in the bioavailability of amino acids (Patterson *et al.*, 2021). The content of the most limiting amino acids in feed, lysine and methionine+cysteine, was lower in our study in comparison with the other two studies in which replacing SBM with BSF negatively affected the feed intake, egg production and body weight evolution (Patterson *et al.*, 2021; Marono *et al.*, 2017) indicating that the lower feed intake in our study was not related to a lower amino acids availability. A possible explanation for the improvement in feed efficiency in our study might be the health promoting effects of BSF bioactive compounds, i.e. lauric acid and chitin, on the intestine of laying hens (Borrelli *et al.*, 2017). The content of lauric acid was about 12 times higher in the BSF10% dietary treatment in comparison to the control feed, with potentially positive effects on intestinal health. Despite this intriguing finding, further study is needed to identify the cause of the improved feed efficiency by replacing SBM with 10% BSF in the diet of laying hens at the onset of lay.

Replacing SBM with 5% or 10% BSF had limited effects on the physical and chemical characteristics of the eggs in the laying hens between 22 to 30 weeks of age. In four weeks after feeding the test diets, the relative weight of yolk in BSF10% treatments showed a tendency to be higher than in BSF5% treatment. While the positive effects of including BSF on relative or absolute weight of yolk has been observed before (Secci *et al.*, 2018, 2020), this effect was no longer significant in our study at week eight of the experiment. In fact, in

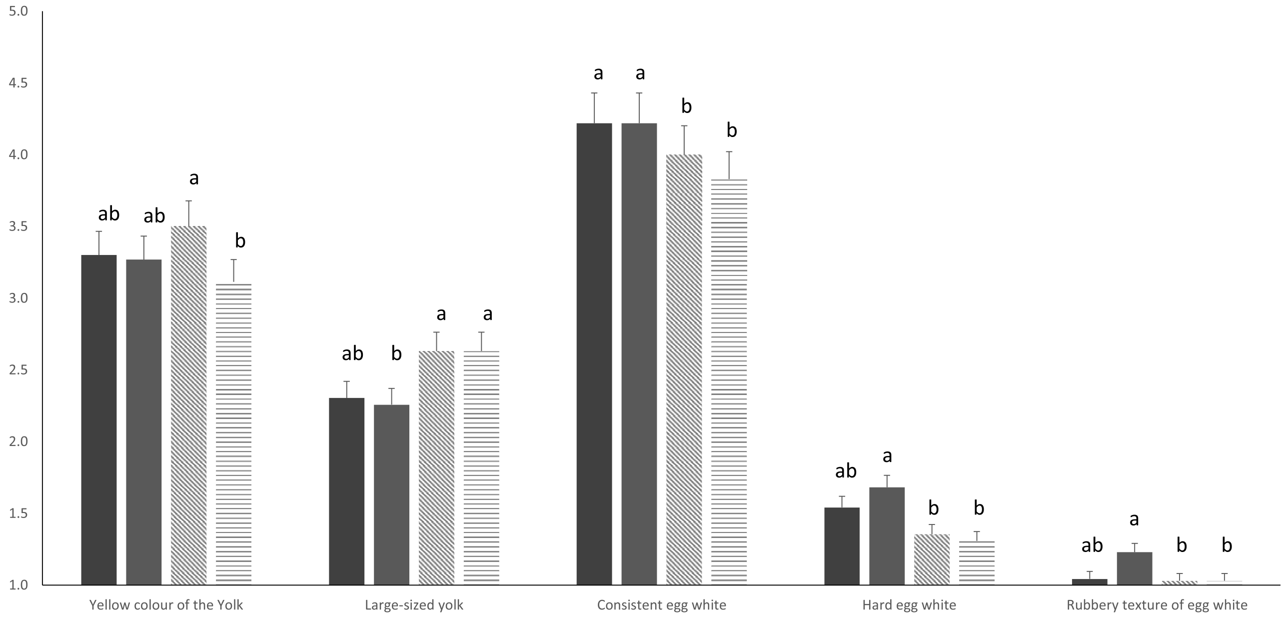

FIGURE 3 Mean scores and standard deviation of the discriminating Rate-All-That-Apply attributes (n = 120 consumers), with the indication of the significant differences between samples by attribute. Samples were eggs from the laying hens fed a control diet (black and grey bars) or diets with partial (BSF5%, diagonal striped bars) or complete (BSF10%, horizontal striped bars) substitution of soybean meal with black soldier fly larvae meal (BSF) for eight weeks between 22 to 30 weeks of age. Each panellist evaluated samples from the control dietary treatment in duplicate to evaluate the internal variability.

week eight, the only significant effect was the highest yolk colour in eggs of BSF10% treatment. The lack of negative effects of replacing SBM with BSF in laying hen feed on egg quality has been observed in other studies (Heuel *et al.*, 2021, 2022; Secci *et al.*, 2018; Mwaniki *et al.*, 2018, 2020; Patterson *et al.*, 2021). Maintaining the egg quality by replacing SBM with BSF while feed intake was reduced indicates meeting the nutritional requirement of the laying hens in our study and the comparable nutritional value of BSF as an alternative protein source and SBM as the most common protein source in poultry feed.

Rise in yolk colour has been a common finding in studies on the application of BSF in laying hen feed (Heuel *et al.*, 2021, 2022; Patterson *et al.*, 2021; Park *et al.*, 2021; Mwaniki *et al.*, 2020; Secci *et al.*, 2018). In other studies, including BSF up to 7.5% in the diets of Shaver White hens between 19 to 27 weeks did not affect yolk colour (Mwaniki *et al.*, 2018). However, increasing BSF content up to 15% in the same laying hens between 28 to 43 weeks of age increased yolk colour (Mwaniki *et al.*, 2020). The intake of dietary carotenoids by laying hens determines egg yolk colour. Carotenoids are provided in feed by the inclusion of common ingredients such as maize and specific feed additives such as synthetic or plant-based carotenoids (Grashorn, 2016). In fact, replacing SBM with 17% BSF increased the content of carotenoids in feed associated with a higher concentration of total carotenoids in the egg yolk and redder yolk colour in comparison to the egg yolks of laying hens in the control treatment (Secci *et al.*, 2018). Black Soldier fly larvae effectively accumulate carotenoids from their feed (Leni *et al.*, 2022), making BSF a valuable source of carotenoids for laying hens (Borel *et al.*, 2021). All the diets in our study contained a feed additive providing canthaxanthin 3 mg/kg feed (RoodMix 0.1%, Trouw Nutrition Benelux, Putten, the Netherlands). However, the lower average daily feed intake in BSF10% compared with BSF5% and the control treatments was associated with a lower intake of feed components such as the sources of carotenoids including the feed additive and maize. This is similar to the study of Secci *et al.* (2018) in which both diets contained a feed additive providing carotenoids (Canthaxanthin 8 mg/kg) while egg yolk colour was stronger in the BSF treatment group despite the lower feed intake compared with the control treatment group. The higher yolk colour of the eggs in the BSF10% treatment group in our study indicates the positive effect of replacing SBM with BSF meal.

In addition to lower feed intake in the BSF10% treatment, the results of the feed analysis indicated a 20% lower dietary Ca content in this treatment than in the other two treatments (Table 2). The average intake of Ca in laying hens in the BSF10% treatment was 3.8 g/day/bird which was lower than the average daily intake of Ca by the laying hens in the control diet (4.9

g/day Ca). Despite this, there were no significant effects of diet on eggshell quality in our study. A possible explanation for this might be that the average Ca intake of 3.8 g/day is marginally meeting the recommendation of the breeding company (Brown Nick, 2020). Another possible explanation for this is the positive effects of including BSF on the eggshell quality (Mwaniki *et al.*, 2018) and Ca metabolism in laying hens (Kawasaki *et al.*, 2019; Zawisza *et al.*, 2023; Marono *et al.*, 2017). The primary hypothesis explaining the positive effects of including BSF in feed on Ca metabolism and eggshell quality in laying hens is attributed to the rise in volatile fatty acid (VFA) content in the intestine due to the fermentation of BSF chitin fraction. Including BSF in the diet of laying hens in exchange with SBM was associated with a rise in caecal VFA content (Cutrignelli *et al.*, 2018) which could enhance dietary Ca absorption (Metzler-Zebeli *et al.*, 2010). Further study with more focus on the effects of including BSF in laying hen feed on Ca metabolism is therefore suggested.

The sensory attributes of the eggs affect the consumers' acceptance. In our study, diet did not affect the overall liking scores of the eggs, while consumers perceived a larger yolk and softer egg white in eggs of the laying hens fed with BSF in comparison to the eggs of the control treatments. Only very few studies have focused on the effects of including BSF in laying hens' feed on the sensory attributes of the eggs (Heuel *et al.*, 2022; Bejaei and Chang, 2020; Al Qazzaz *et al.*, 2016). In an early study, including 1% or 5% BSF in the diet of laying hens was associated with improvements in the appearance, texture, taste, and the general acceptance of the boiled eggs by an untrained panel of consumers, while odour was not affected significantly (Al Qazzaz *et al.*, 2016). Complete replacement of SBM with 15% BSF in the diet of laying hens did not affect the scrambled egg odour assessed by a trained sensory panel (Heuel *et al.*, 2022) or the appearance, texture, and taste of the boiled eggs (Bejaei and Cheng, 2020). These data must be interpreted with caution because of the differences in study designs. The sensory attributes of laying hen eggs are influenced by several factors, including their genetics, production system, and diets (Shaviklo, 2023). The positive effects of including BSF in the diet of laying hens on sensory attributes of the eggs were observed in a study in which BSF replaced fish meal (Al Qazzaz *et al.*, 2016) in contrast to our study and the other two existing studies in which BSF replaced SBM (Heuel *et al.*, 2022; Bejaei and Chang, 2020). In addition, the genetics and production systems were different among studies. For example, Al Qazzaz *et al.* (2016) conducted a study on Arabian village chicken, while the other studies were conducted on commercial brown layer strains (Heuel *et al.*, 2022; Bejaei and Chang, 2020). Besides that, different methodologies for sensory evaluation were applied in the studies. Despite these limitations, the outcomes of our study were consistent with the few published studies suggesting that consumers do not perceive differences when including BSF in laying hens feed.

Like the majority of the studies in the inclusion of BSF in laying hen feed, a limitation of our study can be the eight weeks of the experimental period which is a short period in the production life of the laying hens. Our study included the initiation of the laying and around the peak of production, while most of the published studies addressed laying hens at a later age when production is established. However, the outcome of our study indicating that laying hens maintain production performance and egg quality when BSF is included up to 10% in the diet of laying hens is in line with the outcome of a study continued for 52 weeks with no negative effects on production performance and egg quality (Zhao *et al.*, 2022). Another limitation is the existence of other protein sources, sunflower seed, and rapeseed meals, in the dietary treatments when BSF partially or completely replaced SBM. Due to low production and high costs, BSF remains relatively expensive and less competitive than conventional protein sources such as SBM limiting its application to high inclusion levels in poultry feed (Gasco *et al.*, 2023b). Despite this, the bioactive compounds in BSF, known for their health-promoting effects, are gaining attention, positioning BSF as a potential health supplement rather than a primary protein source, allowing its inclusion despite its high price (Veldkamp *et al.*, 2022). Our data cannot reveal the cause of lower feed intake or improved feed efficiency in laying hens in BSF10% dietary treatment; here, we presented hypotheses based on existing evidence encouraging future study.

Taken together, our findings indicate that a complete replacement of SBM with 10% BSF had no adverse effects on production performance, chemical and physical characteristics, or sensory attributes of eggs. Additionally, it improved feed efficiency in laying hens between 22 to 30 weeks of age. This study provides further evidence of the possibility of substituting SBM with 5% and 10% BSF in the diet of laying hens at the onset of laying. Future research is warranted to assess the impact of including BSF meal in feed throughout the entire production life of the laying hens.

Supplementary material

Supplementary material is available online at: https://doi.org/10.6084/m9.figshare.27266739

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