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SHORT COMMUNICATION

Inclusion of *Tenebrio molitor* meal in diets for free-range hens does not affect egg sensory quality

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Abstract

The incorporation of insect-derived products into poultry diets has received increasing attention as a sustainable alternative to conventional protein sources. This study evaluated the effects of replacing soybean meal and vegetable oil with whole *Tenebrio molitor* larvae meal on egg quality and sensory attributes in free-range laying hens. A total of 110 Lohmann Brown hens housed in 10 pens were assigned to two dietary treatments for 16 weeks: a control diet based on cereals, soybean meal, and sunflower oil, or an experimental diet in which *T. molitor* meal was included at 80 g/kg, partially replacing soybean meal and vegetable oil. Egg quality parameters, including Haugh units, yolk colour and the incidence of blood spots in the yolk and meat stains in albumen, were assessed at the end of the experimental period. Sensory evaluation was performed by 57 untrained panellists using the discriminative triangle test methodology (ISO 4120:2021) to assess whether there were noticeable sensory differences between the eggs from the two experimental groups. No significant differences were detected between treatments in yolk colour, blood spots, or meat stains ($P > 0.10$), although Haugh units tended to be lower in eggs from hens fed the *T. molitor* diet compared with the control ($P = 0.071$). Results from the sensory analysis indicated that panellists could not reliably distinguish between eggs from the two experimental groups. In conclusion, the partial substitution of soybean meal and vegetable oil with whole *T. molitor* larvae meal did not negatively affect egg quality or sensory attributes. These findings support the potential use of insect meals as sustainable feed ingredients in laying hen nutrition without compromising consumer acceptance.

Keywords

egg quality – insect meal – protein source – sensory characteristics

1 Introduction

The use of insects as a source of nutrients has received increasing attention in recent decades (van Huis and Tomberlin, 2017). However, the high costs of insect-derived products cannot yet compete with those of conventional protein sources unless considered within a circular economy framework (Gasco *et al.*, 2023). In addition, although their commercial implementation as food remains limited by low consumer acceptance in societies unaccustomed to entomophagy, the use of insect-derived products in fish, poultry and swine nutrition is generally well accepted (van Huis *et al.*, 2024).

Insect meal is a novel protein source proposed to replace soybean meal in livestock diets, and its use has been evaluated in different species (Rossi *et al.*, 2025).

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However, the limited number of studies assessing the sensory characteristics of poultry products from birds fed insect-based diets may constrain their market viability in terms of consumer acceptance (Okaiyeto *et al.*, 2024; Shaviklo, 2023). Therefore, the sensory quality of eggs from free-range hens fed whole *T. molitor* larvae meal in substitution of soybean meal and vegetable oil was compared using the triangle test methodology to identify potential sensory differences.

2 Material and methods

Bird husbandry and experimental diets

The procedures used in this research were approved by the Animal Ethics Committee of the University of Zaragoza (procedure PI69/20) and complied with the Spanish Policy for Animal Protection (RD 53/2013), in accordance with the EU Directive 2010/63 on the protection of animals used for experimental and other scientific purposes. A total of 110 Lohmann Brown laying hens at 17 weeks of age were randomly allocated in 10 free-range pens (2.5 m^2 × 3.5 m height indoor area, 3 m^2 outdoor area) for 16 weeks. Throughout the experimental period, indoor environmental conditions and the lighting program were adjusted according to commercial practices (Lohmann, 2018). Outdoor minimum and maximum environmental temperatures ranged from 3 to 12 °C and 13 to 24 °C, respectively. Feed and water were provided *ad libitum*, and birds had unrestricted access to outdoor areas.

The feeding program consisted of a single diet provided from 17 to 33 weeks of age. Hens received either a control commercial diet, based on cereals, soybean meal and sunflower oil, or a diet in which whole *T. molitor* larvae meal (Iberinsect, Reus, Spain) was included at 80 g/kg to partially replace soybean meal and sunflower oil. Both diets were formulated to be isoenergetic and isonitrogenous, providing 2.730 MCal AMEn/kg and 7.4 g/kg digestible Lys (FEDNA, 2018). To avoid nutritional deficiencies, feed formulation ensured that all other nutrients, including essential amino acids, minerals and vitamins, met or exceed bird requirements. Each treatment was replicated in 5 pens. The ingredient composition of the experimental diets and the analysed nutrient content of diets and whole *T. molitor* larvae meal are presented in Table 1.

Laboratory analysis and egg quality measurements

Representative samples of the *T. molitor* meal experimental diets were analysed by duplicate for dry matter, total ash, crude protein (as N × 6.25) and ether extract by AOAC (2005) procedures (refs. 934.01, 942.05, 976.05 and 2003.05, respectively). Neutral detergent fiber was determined with an Ankom 200 Fiber Analyzer (Ankom Technology, Fairport, NY, USA) using α–amylase and sodium sulphite (Mertens, 2002), and results expressed exclusive of residual ash. Total starch content was measured enzymatically with a commercial kit (Total Starch Assay Kit K-TSTA 07/11; Megazyme, Bray, Ireland) from samples ground to 0.5 mm.

At the end of the experimental period, 8 eggs per pen were randomly collected and immediately analysed for Haugh Units, the presence of meat stains in the albumen and blood spots in the yolk using an Egg Quality Meter (Technical Services and Supplies, York, UK), and yolk colour by visually assessment (DSM yolk colour fan).

Sample preparation and sensory evaluation: triangle test procedure

Batches of 8 eggs laid 3 days before the sensory evaluation were randomly selected according to treatment and stored at room temperature. Tests were carried out in two sub-groups (26 and 31 assessors) with a total of 9 sub-sessions (3–9 assessors per sub-session). Two hours before each sub-session, eggs were beaten for 30 s, poured into silicone moulds, and cooked in a convection oven (Rational SCC 61, Italia) at 80 °C and 100% humidity for 15 min. After cooling, samples were cut into 1 × 1 cm cubes and presented on ceramic plates. Each plate contained three coded samples (triad), two identical and one different, following the six possible combinations (ABA, AAB, BAA, BAB, BBA, ABB).

Sensory analysis followed the European Standard ISO 4120:2021 (triangle test method). The panel comprised 57 untrained volunteers (13 males, 40 females, aged 20-60 years) from University staff. Panellists received written and verbal instructions as specified in the standard and performed the test individually in a standardised sensory room. Assessors were blinded to treatments and received randomized coded triads. Each assessor indicated which sample they believed was different, and the number of correct identifications of the different sample was recorded.

Statistical analysis

Results on the effects of dietary treatment on egg quality measurements were analysed by one-way ANOVA using the GLM procedure (SAS version 9.4), considering the pen as the experimental unit (n = 5). Differences were

TABLE 1 Ingredient composition of the experimental diets and chemical analysis of diets and whole *T. molitor* larvae meal

	Control diet	*T. molitor* diet	*T. molitor* meal
Ingredients (g/kg)			
Maize	303.5	328.5	
Wheat	250	250	
Barley	30	30	
Sunflower meal	84.5	84.5	
Soybean meal, 47% crude protein	204.5	124.5	
T. molitor larvae meal	0	80	
Palm oil	21	0	
Sunflower oil	4	0	
Calcium carbonate (coarse)	84	84	
Dicalcium phosphate	9.5	9.5	
Sodium chloride	3	3	
Vitamin-mineral mixture[1]	4	4	
DL methionine	2	2	
Analysed chemical composition (g/kg dry matter)			
Organic matter	886	880	959
Crude protein (N × 6.25)	175	169	411
Ether extract	44	34	365
Neutral detergent fibre	122	107	63
Starch	416	490	—

Per kg feed: Mn, 100 mg; Zn, 85 mg; I, 1.52 mg; Se, 0.30 mg; Vit A, 7500 IU; Vit D3, 3000 IU; Vit B1, 10 mg; 6-phytase, 667 FYT; endo-b-xylanase, 1100; endo- b-glucanase, 1500; sepiolite, 0.15 g; lutein, 6.72 mg; zeaxanthine, 0.84 mg; cantaxanthine, 2.23 mg.

considered significant at $P \leq 0.05$, and trends were considered at $P \leq 0.10$.

Sensory evaluation results from the triangle test were analysed by calculating the minimum number of correct responses required to confirm perceptible differences as defined in ISO 4120:2021. For the discriminative test, statistical sensitivity was set at $\alpha = 0.05$ (Type I error: false detection of a difference), $\beta = 0.01$ (Type II error: failure to detect an existing difference) and pd = 40% (proportion of assessors expected to perceive a true sensory difference), following standard recommendations by Lawless and Heymann (2010).

3 Results

The effects of dietary treatments on egg quality measurements are presented in Table 2. No treatment differences were detected for the presence of meat stains in the albumen or blood spots in the yolk ($P > 0.10$). Yolk colour was not affected by diet, whereas Haugh Units tended to be higher in eggs from hens fed the control diet compared to *T. molitor* diet ($P = 0.071$).

Sensory analysis encompasses a set of techniques for the accurate assessment of human responses to foods and for tracing potential sources of bias related to fraud, brand identity and perceived quality. The results of the sensory analysis obtained from the triangle test are presented in Table 3. Among the 57 panellists asked to identify the different sample in the triad, 19 correct responses (33%) and 38 incorrect responses (66%) were recorded. The triangle test method indicates that a proportion of correct responses exceeding the expected level constitutes evidence of a perceptible difference between products (Lawless and Heymann, 2010). According to the sensitivity test (ISO 4120:2021), the minimum number of correct responses required to confirm a perceptible difference between the two products ($\alpha = 0.05$) was 24.8 out of 57. Since the number of correct responses (19) did not reach this threshold, no significant sensory differences were detected between eggs from the control and *T. molitor* groups.

TABLE 2 Effects of the diet fed to the hens on egg measurements at 16 weeks of experiment

	Control	*Tenebrio*	SEM (n = 5)	*P*-value
Meat stains in albumen (%)	62.5	40.8	14.5	0.323
Haugh units	100.6	95.1	1.866	0.071
Blood spots in yolk (%)	62.5	37.5	14.1	0.245
Yolk colour	6.625	6.925	0.119	0.112

TABLE 3 Results on the sensory analysis of eggs evaluated using the triangle test (n = 57)

	Number of responses	Proportion of responses (%)
Correct	19	33.3
Incorrect	38	66.7

Assessors denoted the sample in the triad they consider different, and the number of correct/incorrect responses was recorded. The minimum number of correct responses required to confirm a perceptible difference, according to the sensitivity test (ISO 4120:2021) was 24.8 out of 57.

4 Discussion

Information on the effects of including insect-based products in laying hen diets on egg quality parameters is scarce and mainly limited to the use of *Hermetia illucens* larvae (Al-Qazzaz *et al.*, 2016; Maurer *et al.*, 2016; Mwaniki *et al.*, 2018). In our study, yolk colour, as well as the presence of meat stains in the albumen and blood spots in the yolk, were similar between eggs from hens fed the control or *T. molitor* diets. Ko *et al.* (2020) reported a linear increase in yolk colour with the inclusion of 3% defatted *T. molitor* larvae meal replacing soybean meal in the diet of brown laying hens. In contrast, Dublecz *et al.* (2025) found that the inclusion of 10 and 15% of larvae meal from *Alphitobius diaperinus* (Tenebrionidae) in diets for brown laying hens as a replacement for extracted soybean meal and corn oil did not affect yolk colour, in agreement with the current results. On the other hand, eggs from hens fed the *T. molitor* diet in the present study tended to have lower Haugh Units compared with those from hens fed the control diet, despite values obtained were well above the thresholds (>90) considered optimal by industry standards (USDA, 2020). Therefore, the trend observed is of limited practical relevance, consistent with the absence of any effect on Haugh Units following the inclusion of *T. molitor* in laying hen diets reported by Ko *et al.* (2020).

Previous studies evaluating the sensory attributes of broiler meat from chickens fed *T. molitor* meals reported limited differences on consumer preference and meat quality traits compared with conventional protein sources (Bovera *et al.*, 2016; Khan *et al.*, 2018; Shaviklo *et al.*, 2021). The effects of including insect-derived products in laying hen diets on the sensory properties of eggs have also been investigated. For example, the inclusion of defatted (Al-Qazzaz *et al.*, 2016) or whole (Bejaei and Cheng, 2020) *H. illucens* larval meal in laying hen diets showed positive or no effects, respectively, on egg sensory parameters such as appearance, texture, taste, flavour and overall acceptance. However, no studies have evaluated the effects of including *T. molitor* larvae meal in laying hen diets on egg sensory properties. In laying quails, Dalle Zotte *et al.* (2024) reported that dietary supplementation with live *T. molitor* larvae did not affect overall quality traits, chemical composition or storage stability of the eggs, but decreased overall flavour, sulphur and greasy-oily intensities in a descriptive sensory analysis. In this respect, Ko *et al.* (2020) and Dublecz *et al.* (2025) observed that the inclusion of defatted *T. molitor* larvae meal altered the fatty acid profile in the egg yolks, which in turn could potentially influence consumer sensory acceptance. In this respect, the sensory evaluation of boiled eggs from hens fed *A. diaperinus* meal revealed higher scores for quality and overall acceptance than of eggs from hens fed a commercial diet, with the insect-containing diet producing a specific volatile compound profile characterized by reduced intensity of dimethyl sulphide, ethyl acetate and acetaldehyde (Dublecz *et al.*, 2025). In our study, participants in the triangle test were unable to reliably distinguish between eggs from hens fed the control or *T. molitor* diets. Therefore, any differences in sensory properties resulting from the dietary inclusion of whole *T. molitor* larvae meal were too small to have practical relevance for consumer perception.

In conclusion, the inclusion of whole *T. molitor* larvae meal partially replacing soybean meal and vegetable oil in laying hen diets did not compromise egg quality or sensory attributes. The lack of perceptible differences in yolk colour, albumen and yolk defects and sensory characteristics between control and insect-based diets suggests that *T. molitor* meal can be included without adversely affecting consumer-relevant egg traits. These findings support the suitability of using insect meals as sustainable protein and lipid sources in poultry production.

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