



Integrating hemp cake in organic layer diets: a comprehensive assessment of egg quality, hen behavior, production costs, and life cycle impact

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ABSTRACT

This study evaluated the effects of incorporating 30% locally produced hemp cake into the diet of organically reared laying hens. A total of 280 Hy-Line Brown hens (18 weeks of age) were randomly allocated to two dietary treatments: a commercial control diet based on sunflower cake and soybean seeds as the main protein sources (Control_D), and an experimental diet in which these ingredients were partially replaced with 30% locally produced hemp cake (Hemp_D). Each treatment consisted of two replicate pens of 70 hens, and data were collected over a 16-week experimental period following dietary adaptation. Egg production was recorded daily, whereas egg quality was evaluated at the end of the experimental period. Behavioral time budgets were analyzed using instantaneous scan sampling, while production costs per egg and environmental impact were estimated through feed-cost analysis and life cycle assessment, respectively. The Hemp_D did not affect laying performance, egg weight, or egg mass. Yolk fatty acid composition was significantly modified by hemp cake inclusion, with higher polyunsaturated fatty acids, increased omega-3 content, and a lower n-6/n-3 ratio, whereas egg physical traits and sensory acceptability were largely unaffected. Hen behavior and spatial distribution were not influenced by diet. The inclusion of hemp cake increased feed-related production costs and the CO₂-equivalent environmental impact compared with Control_D. Overall, hemp cake can be included at high levels in organic laying hen diets without impairing performance, behavior, or egg sensory quality, while improving egg nutritional value. However, the environmental and economic outcomes underscore the need for targeted policy measures and continued research aimed at optimizing hemp cultivation practices and reinforcing sustainable, locally based feed supply chains.

1. Introduction

Eggs are a rich source of essential nutrients for humans, particularly high-quality proteins and fats [1]. Like other animal-derived foods, the nutritional quality of eggs can be influenced by the hen diet that can enhance or suppress specific attributes. Although modifying the overall composition of eggs is constrained by their role as reproductive cells, the fatty acid (FA) profile of the yolk, such as the content of omega-3 and other polyunsaturated fatty acids (PUFA), can be more readily manipulated through targeted dietary strategies [2].

Hemp (*Cannabis sativa* L.) is a globally cultivated annual crop, with its cultivation rapidly increasing in the Mediterranean region. Historically used as a source of fiber and medicine [3], hemp has evolved into a versatile crop with applications ranging from oil and seeds rich in PUFA and bioactive compounds to the production of cosmetics and bioethanol [4,5]. Moreover, integrating of hemp into cropping systems is gaining attention as a strategy to reduce dependence on herbicides. Hemp exhibits effective competition against light-dependent weeds, particularly at high planting densities (40–80 plants/m²), making it an attractive option for weed control in organic farming systems [6,7]. Consequently,

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hemp holds the potential to both improve the nutritional quality of eggs and promote environmentally friendly practices in poultry feed production. Indeed, several studies have been carried out in the last decade to evaluate the effect of the administering of hempseed and hemp cake, i.e. the protein-rich solid residue after mechanical oil extraction, on the nutritional properties of foods of animal origin [8]. For laying hens, studies have mainly focused on the use of hemp seeds and oil [9], whereas the use of the residual cake has been less studied [10,11]. Therefore, it may be of interest to further investigate the use of hemp cake in laying hen diets, including the effect of high inclusion levels on egg sensory quality, an area currently lacking sufficient information. Moreover, to the best of our knowledge, no studies have specifically evaluated the effects of hemp supplementation on the behavioral patterns of laying hens. Nevertheless, the hypothesis is supported by the presence of bioactive compounds in hemp that have been associated with stress modulation [12], antioxidant activity [13], and antiparasitic and insecticidal effects [14,15], all of which may potentially influence animal behavioral responses. Finally, the use of hemp cake and its associated environmental implications remain poorly documented within the context of organic egg production where there is a mandatory requirement to incorporate a portion of locally produced feed. In this context, the application of Life Cycle Assessment (LCA) is essential to quantify the environmental implications associated with alternative feed ingredients, particularly when assessing locally produced ingredients that may differ in productivity and production techniques compared with commonly used protein feed ingredients [13,14].

Against this background, this study evaluated the effects of incorporating a high proportion of hemp cake into the diet of organically raised laying hens through an integrated, multidisciplinary approach. Unlike previous studies, which have primarily focused on production performance and egg nutritional quality, the present work integrates productive, nutritional, behavioral, sensory, consumer, economic, and environmental assessments within a single experimental framework, thereby providing a holistic evaluation of the advantages and limitations of hemp cake as an alternative protein source for organic egg production.

2. Materials and methods

The birds were treated humanely according to the principles of the EC Directive 2010/63 on the protection of animals used for experimental and other scientific purposes. The experimental protocol was approved by the Federico II University Institutional Animal Care and Use Committee (Protocol number PG/2021/0100910). All agronomic and livestock management procedures applied in this study complied with the European Union organic production framework.

2.1. Hemp cake

The hemp variety USO31 was grown at farm scale on an organic farm located in the Campania region of southern Italy. The seedbed was prepared by medium-depth ploughing (20 cm) followed by harrowing. Poultry manure was applied after harrowing at a rate of 100 kg N/ha [15], and the soil was then rolled. Sowing took place on May 5, 2020, with 100 seeds per m⁻², equivalent to 20 kg/ha. During the growing cycle, three emergency irrigations of 20 mm each were applied with a rain gun system. At harvest, plant density was 45.9 ± 2.2 plants/m² with a plant height of 124 ± 3.5 cm. The total biomass yield was 4.25 ± 0.5 t/ha, and the seed yield was 0.84 ± 0.09 t/ha, resulting in a harvest index of 20%. Seeds were mechanically harvested using a combine harvester, as described by Faugno et al. [16]. In accordance with organic farming regulations, oil extraction was performed by mechanical cold pressing, which is also recognized to optimize the yield of phenolics and polyphenols in oil [16]. The oil was marketed as a nutritional and functional food, while the hemp cake was delivered to an organic feed manufacturing industry, where it was used to formulate the

experimental diet for the feeding trial.

2.2. Animal, diet and experimental design

The feeding study was carried out on a privately owned organic poultry farm certified under EC Regulation 834/2007 (40°39'N, 15°06'E, 281 m a.s.l.). The farm housed Hy-Line brown laying hens in three sheds, each accommodating 40,000 hens. At the start of a new production cycle, two weeks after the hens were housed, two hundred and eighty 18-week-old hens were randomly chosen from the main flock in one of the sheds. A veterinary examination was conducted to ascertain the health status of the hens, and their weight was recorded. The selected birds were then randomly and evenly allocated to four separate pens within the shed, maintaining a stocking density of 9 hens/m². Each pen was provided with the basic equipment, including an outdoor area (3 m² per hen), a nest/feeding area (0.15 m² per hen), and facilities such as linear feeders with hoppers and drinking troughs.

After a 2-week adaptation period to the new fencing, during which the birds were fed the same commercial organic pre-lay diet (17.5% crude protein) used in the pullet rearing facility, the hens were allocated to two dietary treatments, two pens of 70 hens each per treatment: the organic commercial diet usually used on the farm (Control_D), and the organic diet formulated to contain 30% hemp cake (Hemp_D). This inclusion level was chosen to maximize the use of hemp cake as an alternative protein source under practical organic farming conditions. Details on the chemical composition and nutritional characteristics of the hemp cake and the experimental diets are provided in Tables 1 and 2, respectively.

The diets in mash form were formulated to be isoenergetic and isonitrogenous, in accordance with organic layer nutritional requirements [17] and manually administered by a worker on a semi-ad libitum basis, assuming a daily feed intake of 110–130 g/hen. The study lasted 18 weeks, including a two-week adaptation period to dietary treatments and a 16-week period for data collection and sampling (from 21 to 37 weeks of age). No hens died or fell ill during the study, meaning the birds remained the same throughout the entire experimental period.

2.3. Data recording and sampling procedure

The number and weight of eggs produced in each pen, as well as the laying rate, were recorded daily at 09:00 h. Feed intake of the Control_D and Hemp_D groups was estimated weekly as the difference between feed offered and feed refusals, averaging 116.5 ± 10.9 and 117.1 ± 10.3 g hen/d, respectively.

Diet samples were collected and immediately analyzed for dry matter (DM) content. At the end of the experimental period, samples were pooled by diet and analyzed for ash, crude protein (CP, Kjeldahl method), crude fiber (CF), and ether extract (EE) according to the procedures 920.153, 981.10, 978.10 and 2003.05 of AOAC [18]. Neutral

Table 1

Chemical composition of the hemp cake used in the experimental diet (% of dry matter, unless otherwise stated).

Item	Hemp cake
Dry Matter (DM), % as-fed basis	91.32
Ash	5.23
Crude protein	31.90
Ether extract	8.07
Crude fiber	20.62
Neutral detergent fiber (NDF)	45.74
Acid detergent fiber (ADF)	36.26
Acid detergent lignin (ADL)	13.04
Calcium, g/kg DM	2.91
Phosphorous, g/kg DM	10.51
Metabolizable energy ^a , MJ/kg DM	10.03

^a Estimated according to INRA equation: 3951 + 54.4 ether extract – 88.7 crude fiber – 40.8 ash.

Table 2

Ingredients (g/kg as fed) and chemical composition (% of dry matter, unless otherwise stated) of the experimental diets.

Item	Experimental diets ^a	
	Control_D	Hemp_D
Ingredients		
Corn meal	350	350
Hemp cake		300
Wheat	100	80
Tosted soybean seed	200	50
Calcium carbonate	90	90
Sunflower cake	150	50
Wheat bran	50	20
Corn gluten meal	40	40
Dicalcium phosphate	14	14
Vitamin-mineral premix ^b	6	6
Chemical Composition		
Dry Matter (DM), % as fed	89.21	89.82
Ash	13.41	13.92
Crude protein	18.09	18.23
Ether extract	7.82	7.05
Crude fiber	6.40	10.09
Neutral detergent fiber (NDF)	15.40	20.70
Acid detergent fiber (ADF)	7.40	13.12
Acid detergent lignin (ADL)	2.05	4.41
Calcium, % DM	3.92	3.95
Phosphorous, % DM	0.70	0.79
Lysine, g/kg DM	7.61	6.09
Methionine, g/kg DM	3.33	3.74
Cystein ^c , g/kg DM	3.19	3.31
Metabolizable energy ^c , MJ/kg DM	11.22	10.63

^a Estimated based on the amino acidic content for each dietary ingredient, using the standard values proposed by FEDNA.

^a Control_D = control diet; Hemp_D = diet containing 30% hemp cake.

^b Supplied/kg of feed: vitamin A (retinyl acetate) 8,001 IU; vitamin D₃ (cholecalciferol) 3,000 IU; betaine anhydrous 600 mg; biotin 0.04 mg; choline chloride 333 mg; folic acid 0.81 mg; niacinamide 25.5 mg; calcium D-pantothenate 7.28 mg; vitamin B₁ (thiamine mononitrate) 0.75 mg; vitamin B₂ (riboflavin) 1.60 mg; vitamin B₆ (pyridoxine hydrochloride) 1.60 mg; vitamin B₁₂ (cyanocobalamin) 0.02 mg; vitamin E (all-rac- α -tocopheryl acetate) 18.5 mg; vitamin K₃ (menadione nicotinamide bisulfite) 2.50 mg; copper (copper sulfate pentahydrate) 10.0 mg; iron (ferrous sulfate monohydrate) 44.0 mg; zinc (zinc oxide) 50.0 mg; manganese (manganese oxide) 47.0 mg; iodine (calcium iodate anhydrous) 2.0 mg; selenium (sodium selenite) 0.25 mg; endo-1,4- β -xylanase (EC 3.2.1.8) 1,300 U; endo-1,3(4)- β -glucanase (EC 3.2.1.6) 250 U.

^c Estimated based on the metabolizable energy content of each dietary ingredient according to the INRA equation: $3951 + 54.4 \text{ ether extract} - 88.7 \text{ crude fiber} - 40.8 \text{ ash}$.

detergent fiber (NDF), acid detergent fiber (ADF), and acid detergent lignin (ADL) contents were determined according to the method of Van Soest et al. [19]. Metabolizable energy was estimated using the INRA equation [20]. The hemp cake was analyzed prior to diet formulation for its cannabinoid content and antinutritional compounds (i.e., trypsin inhibitor activity, phytic acid, tannins, total polyphenols, and total saponins). The results are reported in Table S1 (Supplementary Materials).

At the end of the study (week 18), the entire production of the two dietary groups was collected over two consecutive days to determine the chemical and physical characteristics of the eggs as well as the sensory characteristics and consumer acceptability of the eggs.

2.4. Egg quality

Prior to analyses, which were performed within three days after collection, eggs were candled to exclude those with pre-existing shell cracks, dirty shells, or other eggshell abnormalities (e.g. blood spots, calcium speckles, or surface deposits). Additionally, egg specific gravity was determined by immersing the eggs in saltwater solutions of increasing density [21] to exclude old eggs that could have been accidentally collected.

2.4.1. Physical traits of eggs and egg components

The short and long diameters of the eggs ($n = 60$ per each of the two dietary groups, randomly selected) were measured using a digital caliper with a precision of 0.01 mm. Then the eggshell strength was evaluated using an Instron Universal Testing Machine (Model 3345, Instron Inc., Norwood, MA, USA) equipped with a 100 N load cell and a 35 mm compression probe, operating at a crosshead speed of 20 mm/min [22].

Subsequently, eggs were completely cracked onto a flat surface, allowed to stand for 5 min [23], and visually inspected for the presence of blood and meat spots. Yolk height and diameter, as well as thick albumen height, were measured using a tripod screw micrometer (Baxlo Precision, Barcelona, Spain) and a digital caliper, according to Benton and Brake [24]. Yolk color was assessed using the Roche 15-grade color fan (DSM, Heerlen, The Netherlands). The yolk was separated from the albumen using a silicone egg separator, gently rolled on filter paper to remove residual albumen and chalazae, and weighed. Eggshells were carefully washed under running tap water, dried at room temperature for 48 h, and weighed [25]. Albumen weight was calculated as the difference between total egg weight and the combined weight of yolk and shell. Shell thickness was measured using a digital micrometer (Mitutoyo, 0.01–20 mm, Tokyo, Japan) and expressed as the mean of measurements taken at the two poles and the equatorial region. Egg shape index, egg surface area (cm²), eggshell density (mg/cm²), yolk index (%), albumen index (%), and Haugh units, as indicators of egg and egg component quality, were calculated according to the methods described by Paganelli et al. [26].

2.4.2. Chemical and biochemical analyses

The analyses were conducted on 40 samples (yolk and albumen), 20 per dietary group, randomly selected. After separation, the measurements of yolk pH and water activity (a_w) were immediately performed in triplicate on homogenized samples. The pH was measured using a pH meter (Testo 206-pH2, Testo Spa, Milan, Italy), while a_w was determined using an AquaLab Series 4 TE water activity meter in AwE mode (Decagon Devices Inc., Pullman, WA, USA), calibrated at 25 °C with verification standards (a_w 0.984 and 0.760; Decagon, Pullman, WA, USA).

Crude protein of the egg yolk was determined according to the Kjeldahl method. Fat extraction from egg yolk was performed according to Naviglio et al. [27] with modifications. Briefly, egg yolks (5 g) were homogenized with 5 mL of a chloroform/methanol (2:1) solution and 4 mL of a saturated NaCl solution. The mixture was vortexed for 1 min and then centrifuged at 6500 rpm for 10 min. This process was repeated three times. The organic phase was then dried with a rotavapor at 30 °C (Laborota4000-Efficient; Heidolph Instrument, Schwabach, Germany). The extracted lipid phase was then dissolved in hexane, purified with saturated NaCl solution (3 mL) and dried with nitrogen. The total lipid content was determined gravimetrically. The extracted lipid fraction was stored in glass tubes at –20 °C until further analysis.

The FA composition of fat yolk was determined by analyzing the fatty acid methyl esters (FAMES) obtained after transesterification according to the method of Romano et al. [28]. Briefly, a 1% of lipid solution in hexane was prepared and 300 μ L of 2 N KOH in methanol was added to 1 mL of the solution. A total of 1 μ L of the upper layer containing the FAMES was injected into an Agilent Technologies 6890N gas chromatograph equipped with a capillary column (100 m $\times$ 0.25 mm inner diameter, 0.20 μ m film thickness) with a polystationary phase (90% biscyanopropyl/10% cyanopropylphenyl siloxane) (Supelco, Bellefonte, PA, USA), a hydrogen flame ionization detector (FID) and a programmed temperature vaporizer (PTV). The initial oven temperature was set at 140 °C for 5 min, increased at 4 °C min^{–1} to 175 °C and held for 20 min, then increased at 3 °C min^{–1} to 240 °C and held for 20 min. The PTV temperature was 60 °C for 0.1 min, then increased to 260 °C at 500 °C min^{–1} and held for 5 min. The FID detector and injector temperatures were fixed at 260 °C and 120 °C, respectively, and the split ratio was 15:1. Helium was used as the carrier gas at a flow rate of 2 mL min^{–1} and

chromatographic air and hydrogen were used as auxiliary gases. The chromatogram peaks of the samples were identified using the external 37-component standard TM 37 FAME mix (Supelco, Bellefonte, PA, USA). Results were expressed as % w/w.

Nutritional indices, including the atherogenic index (AI), thrombogenic index (TI), and the hypocholesterolemic to hypercholesterolemic ratio (h/H), were calculated according to Chen and Liu [29].

2.5. Sensory analyses of eggs

The sensory profile evaluation was conducted using quantitative descriptive analysis (QDA), while consumer liking was assessed through the Home Use Test (HUT) as detailed in the supplementary material. Briefly, all eggs used for QDA and HUT were stored under identical conditions (4 °C) and for the same duration prior to testing, which was carried out within 7 days of collection. Twenty habitual egg consumers (individuals consuming eggs at least once per week) were recruited by phone and selected according to ISO 8586-1 [30]. A total of 10 panelists (4F+6M, aged 20 ÷ 60) were selected. A consensus list of attributes specific to hard-boiled egg yolk was then developed based on available literature [31,32] and panel leader-moderated evaluations of commercial hard-boiled egg samples. The final sensory profile comprised 10 attributes related to appearance (n = 3), odor/flavor (n = 2), taste (n = 3), and texture (n = 2) (Table 3).

Panelists underwent a 2-h training session on the use of unstructured

Table 3

Sensory attributes and reference standards used for quantitative descriptive analysis (QDA) of boiled egg yolk.

Attributes	Definition	Reference standards	
		Low intensity	High intensity
Yolk Appearance			
Yellow intensity	Yolk color intensity (0 light yellow/100 deep yellow)	Egg boiled for 25 min in already boiling water	Egg boiled for 5 min in already boiling water
Yolk opacity	Visual characteristic that describes the product's inability to reflect light	Egg boiled for 5 min in already boiling water	Egg boiled for 25 min in already boiling water
White intensity	Egg white color intensity (0 chalk white/100 off-white)	Egg boiled for 5 min in already boiling water	Egg boiled for 25 min in already boiling water
Odor	Typical odor of a hard-boiled egg boiled for a long time	Egg boiled for 5 min in already boiling water	Egg boiled for 25 min in already boiling water
Yolk Taste/Flavor			
Bitterness	Fundamental taste associated with caffeine		
Sweetness	Fundamental taste associated with sucrose		
Saltiness	Fundamental taste associated with sodium chloride		
Overall flavor	Typical flavor of a hard-boiled egg boiled for a long time	Egg boiled for 5 min in already boiling water	Egg boiled for 25 min in already boiling water
Yolk texture			
Crumbliness	Ease of a sample breaking into fragments following fork compression	Egg boiled for 25 min in already boiling water	Egg boiled for 10 min in already boiling water
Pastiness	Tendency of the sample to form a dense and compact mass during chewing without melting	Egg boiled for 10 min in already boiling water	Egg boiled for 25 min in already boiling water

100-mm intensity scales, following Stone and Sidel [33]. Reference standards for the low and high intensity levels of each attribute were prepared using eggs cooked for different times (Table 3). The final phase involved the evaluation of the experimental samples. Eggs were boiled for 15 min, starting from immersion in cold water. A QDA questionnaire and a master card were generated to randomly distribute samples among panelists. Boiled eggs from the Control_D and Hemp_D were identified using three-digit numerical codes and randomly assigned to panelists. The presentation order was randomized and panelists were blinded to treatment identity throughout the evaluation. Each panelist evaluated two halves of each sample in individual booths under red light for flavor, taste, and texture assessment, and under white light for appearance evaluation. Each sample was assessed in triplicate during three separate sessions conducted on the same day, with approximately 10 min between sessions. Between samples, panelists rinsed their mouths with plain water. The Smart Sensory Box software (version 2.3.5; Smart Sensory Solution, Sassari, Italy) was employed to manage both training and evaluation sessions. The panelists also evaluated the intensity of the yellow of the raw yolk using samples placed in a photo box. For the consumer liking study, 100 participants were recruited via telephone, evenly distributed across three age groups (18-39, 40-59, and over 60) and balanced for gender. The test adopted a household approach, with samples evaluated in a natural home environment. Participants received two coded eggs (one per treatment), together with instructions for storage, cooking, and evaluation. Eggs were brought to room temperature for at least 2 h, boiled for 15 min, cooled under running water, peeled, and evaluated individually. Overall liking, as well as appearance, texture, and taste/flavor liking, were rated on a 9-point hedonic scale ranging from 1 ("extremely unpleasant") to 9 ("extremely pleasant"), with 5 corresponding to "neither unpleasant nor pleasant".

2.6. Hen behavior study

Time budget analysis was used to comprehensively allocate time to different behavioral categories. In the initial phase of the study, a time budget questionnaire was developed covering categories such as hen posture (standing, crouching), location (nest, high area, ground), general activities (resting, walking, exploring) and feeding behavior (feed intake, pecking frequency, water intake), as detailed in Table S2 (see Supplementary Materials). Activity patterns were selected as indirect indicators of stress. Each behavioral category, as defined in Table S1 (see Supplementary Materials), was consistently applied during observations. Behavioral data were collected using the instantaneous scan sampling method. With this method, at each sample point, the observer records whether a behavior pattern is occurring. The animals were subjected to four observation sessions at one-week intervals. In brief, the same observer conducted all observations. On observation days, to minimize disturbance and allow the animals to acclimate, the observer entered the pen 10 min before the start of behavioral observations and took a position in an unoccupied corner. Then, the stockperson entered the pen and delivered the feed. Pecking frequency (n. of pecks per min) was recorded during the first minute of observation using a continuous recording of one focal animal. Then, five scans were performed at 2.5-min intervals. Behavioral observations were recorded using mutually exclusive categories to ensure that no overlapping behaviors were assigned to the same individual or activity at any given observation point. Due to the large number of animals, attempts to recognize individuals were unsuccessful. Therefore, behavioral variables were expressed as the proportion of subjects observed in each posture, location, or activity category, calculated as the number of animals displaying each category/70 (the total number of animals per pen). Additionally, aggressive and feather pecking were continuously recorded. These variables were expressed as the number of interactions per animal. Subsequently, the observer repeated the same procedure for the other three pens. Once the first observation period (0 to 15 min after feed delivery) ended, a second observation period started for each pen (100 to 115 min

after feed delivery), using the same procedure, except with 10 min of familiarization with the observer and feed delivery. This resulted in two observation periods per observation day and per pen, corresponding to 30 min of observations per pen per day. Each observation period included five scan samples collected at 2.5 min intervals. Across the four observation sessions, a total of 2 h of observations were collected per pen (4 h per treatment and 8 h overall). For the statistical analysis, the value in each cell was the mean of the two pens.

2.7. Production cost

Several studies have compared the costs associated with different feeding systems, and various methods have been proposed to estimate production costs in livestock farming [34–38]. Given this background, the economic analysis focused primarily on cost differences attributable to diet formulation. To this end, a detailed analysis of the farm's balance sheet was conducted to estimate feed-related expenditures associated with laying hen production. This analysis was essential for calculating the total production cost per egg. The balance sheet, which reflects the financial condition of the farm at a specific point in time, provides a structured overview of its assets, liabilities, and net worth (equity). It serves as a financial snapshot, capturing the farm's economic position on a given date [39]. The cost per individual egg was estimated by comparing the standard diet (Control_D) and the alternative experimental diet incorporating hemp cake (Hemp_D). The estimation of elementary production costs, crucial for determining the total production cost per egg, required the development of a tailored questionnaire designed to collect detailed economic data which were gathered through comprehensive face-to-face interviews conducted directly on the farm, supplemented by information extracted from the farm's official financial records.

This methodological approach enabled the classification of production costs based on their relevance to the specific object of analysis, distinguishing between specific costs, which are directly attributable to one production process, and common costs, which cannot be unequivocally linked to a single output and require allocation through appropriate estimation methods. Additionally, these costs were categorized into two main types (i) explicit costs, which consist of actual monetary expenditures incurred by the farm operator during production activities; (ii) implicit costs which are given by the imputed value of internal resources, such as land, capital, and family labor, provided by the entrepreneur, which must be considered despite the absence of direct financial outlays. Specifically, the explicit costs for the farm include the following components: (i) operating costs calculated by multiplying the quantity of each resource used by its price. They include expenses for veterinary services and medications. In organic hen farms, these costs are usually lower than in conventional systems due to the use of natural remedies, homeopathy, and preventive medicine. Antibiotics are only allowed in emergencies, under strict veterinary supervision; (ii) other costs including electricity, fuel, water, phone bills, and taxes. Since they apply to the entire farm and can't be linked to a specific production activity, they were estimated as a percentage of the farm's gross sales. The percentage varies by cost type and was based on data from the Italian FADN (Farm Accountancy Data Network) to ensure consistency with industry standards. Implicit costs were estimated using the opportunity cost approach [40,41] and include: (i) family labor, valued at the same rate as hired labor. Managerial work was estimated at 3% of gross sales; (ii) land capital, based on the average local rental price, using FADN data to reflect the real cost of land use; (iii) depreciation, estimated using the straight-line method for buildings and equipment. For machinery, annual depreciation (Dc) was calculated as

$$Dc = \frac{Nv - Sv}{n}$$

where *Nv* is the new value, *Sv* is the salvage value, and *n* is the expected

lifespan (assumed to be 10 years, with 10% annual depreciation); (iv) working capital, estimated using the average interest rate of ten-year government bonds, representing the opportunity cost of capital. No interest was calculated on working capital in this analysis, as eggs were sold on a daily basis [42]. It was also assumed that the equipment used for feed preparation and distribution would remain unchanged following the dietary shift. Consequently, the primary variable under examination was the cost differential between the Control_D and Hemp_D [43,44].

2.8. Life cycle assessment

Using the Life Cycle Assessment (LCA) approach defined by ISO standards 14040 [45] and 14044 [46], the carbon footprint of the Control_D and Hemp_D was assessed. The carbon footprint, expressed in kg CO₂ eq, was calculated using the OpenLCA software (v. 2.6) and the Agribalyse database (v. 3.0.1). The functional unit (FU) adopted was 1 kg of concentrate diet, focusing strictly on the environmental profile of the two diets. This feed-to-feed comparison was considered the most appropriate basis for the environmental assessment, as the study was not designed to assess long-term production responses and no differences in egg production were observed during the experimental period. The Environmental Product Declaration [47,48] method was applied, and the characterization factors used were 1 for CO₂, 28 for CH₄, and 265 for N₂O [49]. LCA focused on the feed production phase to cut off the environmental effects of administering concentrate feeds supplemented with hemp. Furthermore, the evaluation was restricted to the carbon footprint, as greenhouse gas emissions represent the primary metric driving international policy and stakeholder communication in sustainable animal nutrition. [Supplementary Table S3](#) reports the main ingredients selected from the database. The environmental impact of hemp cake was determined using primary data collected directly during the field production phase and oil extraction (see [Supplementary Table S4](#)). An economic allocation approach was applied to partition the environmental impacts, using reference market prices of €25 per kg for hemp oil and €2 per kg for hemp cake. This approach is methodologically justified in accordance with ISO 14044 [46] guidelines for agricultural co-products when substantial differences in economic value exist among outputs. Because hemp oil represents the primary economic driver of the crushing process, economic allocation ensures that environmental burdens are distributed in a manner that reflects the socio-economic reality of the supply chain. Conversely, a mass-based allocation would disproportionately assign upstream agricultural impacts to hempseed cake based solely on its physical mass, despite its lower economic value. As hempseed cake constitutes the largest fraction of the processed seed, this approach would overestimate its environmental burden and misrepresent its role as a low-value co-product with potential applications in circular feed systems [50].

2.9. Statistics

Statistical analyses were performed using JMP Pro software (version 15; SAS Institute Inc., Cary, NC, USA). Egg production parameters were analyzed using a two-way repeated-measures ANOVA, with dietary treatment, sampling day, and their interaction included as fixed effects. The pen was considered the experimental unit for these analyses. Although the number of pen replicates per treatment was limited (*n* = 2), the experimental design generated 112 observations per treatment throughout the experimental period. A post hoc power analysis (G*Power software version 3.1.9.7; effect size *f* = 0.25, α = 0.05, $1 - \beta$ = 0.95) indicated an actual statistical power of 0.95. Egg physical traits and chemical composition were analyzed using a one-way ANOVA, with dietary treatment as the fixed effect and the individual egg considered the observational unit, as commonly applied in egg quality studies where multiple eggs are sampled from homogeneous groups of hens maintained under the same pen conditions [51].

Due to the lack of individual recordings, behavioral data were analyzed using the day of observation as the experimental unit, with dietary treatment (Control_D and Hemp_D), period of observation (0 to 15 min and 100 to 115 min after feed delivery) and their interaction as factors. Pecking and scratching, as well as aggressive pecking and feather pecking, were so rare that they were excluded from the analysis.

Quantitative Descriptive Analysis data were analyzed by ANOVA, including product, replication, and their interaction as fixed effects. When significant effects were detected, mean comparisons were performed using Student's *t*-test as a post hoc test. Statistical significance was declared at $P < 0.05$, with trends discussed at $P < 0.10$.

3. Results

Both diets were formulated to be isonitrogenous and nearly isoenergetic; however, the high inclusion level of hemp cake (30%) in Hemp_D, which partially replaced heat-treated soybean seeds and sunflower cake in the Control_D, resulted in higher fibrous fractions (CF, NDF, ADF, ADL) a slight reduction in metabolizable energy, and changes in the estimated amino acid supply, with higher sulphur amino acids (methionine + cysteine) and lower lysine supply (Table 2).

Table 4 summarizes productive performance of hens fed the Control_D and Hemp_D diets. The effect of week of observation was consistently significant ($P < 0.05$), reflecting the physiological progression of the hens over the experimental period. No significant differences were detected between dietary treatments for productive traits, including laying rate, average egg weight, egg mass, total egg output, or number of eggs produced.

Similarly, no significant differences were observed between the Control_D and Hemp_D groups for most egg quality traits (Table 5). These included whole egg characteristics, shell traits, albumen traits, and yolk traits. The only parameters affected by diet were yolk color and eggshell strength, which tended to be higher in eggs from the Hemp_D group compared with the Control_D group ($0.05 < P < 0.10$).

By contrast, the fatty acid composition of egg yolk (Table 6) was largely influenced by dietary hemp inclusion. Stearic acid (C18:0, +10.1%, $P < 0.05$), oleic acid (C18:1 n-9, +10.4%, $P < 0.001$), and total MUFA (9.18%, $P < 0.001$) were higher in eggs from hens fed the Control_D diet, whereas linoleic (C18:2 n-6, LA, +23.60%) and alpha linolenic acids (C18:2 n-3, ALA, +94.9%) ($P < 0.0001$), docosahexaenoic acid (C22:6 n-3, DHA, +28.48%) ($P < 0.01$), as well as total PUFA (+21.82%), omega-3 (+83%), and omega-6 fatty acids (19.6%) ($P < 0.001$) were higher in eggs from the Hemp_D diet. Regarding nutritional indices, the Hemp_D diet resulted in a lower n-6/n-3 ratio (−19.81%) $P < 0.0001$ and TI (−8.42%) ($P < 0.01$), while no differences were

Table 4

Production performance of laying hens as affected by dietary treatments (least square mean $\pm$ standard error of mean; pen was the experimental unit, $n = 2$ pens per diet).

Item	Dietary treatments ^a		SEM ^b	P-value	Time	Diet x Time
	Control_D	Hemp_D				
Laying rate, %/day	87.72	88.12	0.29	0.4725	<0.0001	0.7686
Average egg weight, g/egg	58.31	58.24	0.27	0.8017	<0.0001	0.3579
Egg mass, g/hen/day	51.62	51.74	0.09	0.4815	<0.0001	0.7675
Total egg weight, g/day	3614.51	3621.52	7.76	0.5870	<0.0001	0.6732
Number eggs, n/day	61.42	61.62	0.14	0.3118	<0.0001	0.7733

^a Control_D = control diet; Hemp_D = diet containing 30% hemp cake.

^b SEM = standard error of mean.

Table 5

Effects of dietary treatments on physical characteristics of eggs and egg components (least square mean $\pm$ standard error of mean; individual eggs were considered the observational unit, $n = 60$ eggs per diet, except for pH and water activity (aw), for which $n = 20$ eggs per diet).

Item	Dietary treatments ^a		SEM ^b	P-value
	Control_D	Hemp_D		
Whole egg				
Weight, g	64.27	63.79	0.67	0.6120
Shape index, %	78.33	78.91	0.35	0.2358
Shell ratio, %	9.74	9.72	0.10	0.8415
Albumen ratio, %	67.71	68.03	0.30	0.4933
Yolk ratio, %	22.54	22.28	0.26	0.9812
Yolk-albumen index, %	33.44	32.89	0.53	0.4666
Shell				
Weight, g	6.24	6.19	0.08	0.6493
Strength, N	3.63	3.96	0.13	0.0800
Thickness, mm	0.36	0.36	0.01	0.9584
Surface, cm ²	68.94	68.62	0.47	0.6262
Density, mg/cm ²	90.57	90.15	0.93	0.7537
Albumen				
Weight, g	43.47	43.39	0.57	0.9167
Height, mm	8.16	8.26	0.18	0.6791
Haugh units	88.58	89.10	1.08	0.7342
Yolk				
Color score	7.32	7.75	0.17	0.0793
Weight, g	14.42	14.16	0.18	0.3176
Yolk index, %	52.14	51.59	0.73	0.5918
pH	6.07	6.06	0.02	0.5100
aw	0.99	0.99	0.00	0.3079

^a Control_D = control diet; Hemp_D = diet containing 30% hemp cake.

^b SEM = standard error of mean.

Table 6

Effects of dietary treatments on fatty acids composition (% of total fatty acids) and nutritional indices of the yolk (least square mean $\pm$ standard error of mean; individual eggs were considered the observational unit, $n = 20$ eggs per diet).

Item	Dietary treatments ^a		SEM ^b	P-value
	Control_D	Hemp_D		
C 14:0	0.32	0.31	0.02	0.8340
C 16:0	25.70	25.00	0.23	0.3463
C 16:1 n-7	2.52	2.82	0.16	0.1850
C 17:0	0.15	0.16	0.01	0.6308
C 18:0	8.90	8.00	0.27	0.0262
C 18:1 n-9	39.02	34.96	0.68	0.0001
C 18:2 n-6	16.90	20.89	0.61	0.0001
C 18:3 n-3	0.59	1.15	0.06	0.0001
C 20:2 n-6	0.18	0.19	0.09	0.5754
C 20:3 n-6	0.09	0.10	0.05	0.4102
C 20:4 n-6	2.08	1.76	0.12	0.0634
C 22:6 n-3	1.51	1.94	0.11	0.0820
Others	2.00	1.72	0.06	0.0041
Total SFA ^c	35.05	34.49	0.40	0.3239
Total MUFA ^d	41.59	37.77	0.74	0.0080
Total PUFA ^e	21.36	26.02	0.73	0.0001
n-3 ^f	2.10	3.86	0.13	0.0001
n-6 ^g	19.26	22.93	0.63	0.0002
n-6/n-3	9.49	7.61	0.30	0.0001
AI ^h	0.43	0.43	0.06	0.8647
TI ⁱ	0.95	0.87	0.02	0.0120
h/H ^j	2.33	2.32	0.036	0.9431

^a Control_D = control diet; Hemp_D = diet containing 30% hemp cake.

^b SEM = standard error of mean.

^c SFA = saturated fatty acids.

^d MUFA = monosaturated fatty acids.

^e PUFA = polyunsaturated fatty acids.

^f n-3 = n-3 PUFA series.

^g n-6 = n-6 PUFA series.

^h AI = atherogenic index.

ⁱ TI = thrombogenic index.

^j h/H = hypocholesterolemic/hypercholesterolemic ratio.

observed for AI or the h/H ratio ($P > 0.05$).

The results of the QDA and consumer liking for the eggs from Hemp_D and Control_D diet are depicted in Table 7. Preliminary ANOVA results revealed no significant product $\times$ replicate or product $\times$ judge interactions. This consistency across sessions and assessors confirms the efficacy of the training program as well as the panel's reliability. Although most sensory attributes showed numerically lower scores in the Control_D (Table 7), only a statistical trend ($0.05 < P < 0.10$) was observed, with the Hemp_D tending to exhibit higher scores for overall flavor, salty taste, friability, pastiness, and yolk color intensity. In line with these findings, dietary treatments did not influence consumer liking as assessed by untrained evaluators, with mean hedonic scores consistently exceeding the neutral point (5, neither like nor dislike) across all evaluated sensory attributes, including overall liking, appearance, taste/flavor, and texture (Table 8).

Likewise, no significant differences were observed between dietary treatments in the spatial distribution of laying hens or in the frequency of the recorded behavioral activities (Table 9). Hens from both dietary groups showed comparable use of nest, elevated, and floor areas, as well as similar time allocation to postural, ingestive, locomotor, comfort, and exploratory behaviors. A significant effect of the observation period was observed for water intake ($P = 0.04$), whereas trends toward significance ($P < 0.1$) were observed for pecking and preening, which is consistent with the variability typically observed in behavioral data. No interaction between diet and period was detected.

Table 10 presents a comprehensive breakdown of the production cost structure, along with the calculated total production cost per egg.

Under Control_D explicit costs constitute 93% of the total production cost per egg, with feeding expenses accounting for 79% of the total cost. The introduction of the Hemp_D diet does not significantly alter the overall cost structure, as explicit costs increase marginally to 94%, primarily due to the higher cost of feed. Implicit costs, borne by the farm owner, have a comparatively smaller impact on total production costs. Notably, 50% of total implicit costs are attributed to management and administrative work carried out by the farmer's family. The allocation of implicit costs, as presented in Table 10, reveals a discrepancy in interest-related costs between the two dietary treatments. In the case of Hemp_D, a higher amount was estimated for interest expenses. This increase is attributable to the higher procurement cost of feed, which necessitates greater working capital and consequently results in higher interest charges. Overall, the economic analysis indicates that under the Control_D diet, the total production cost of a single egg, including both explicit and implicit costs and thus representing the break-even selling price, is €0.14. Switching to the Hemp_D diet increases production costs, raising the break-even price per egg to €0.17. Thus, the substitution of the Control_D with Hemp_D results in a 36.4% increase in feed cost per egg, rising from €0.11 to €0.15.

An additional objective of the study was to assess the potential

Table 7
Quantitative descriptive analysis (QDA) scores of boiled egg yolk as affected by dietary treatments (least square mean $\pm$ standard error of mean).

Item	Dietary treatments ^a		SEM ^b	P-value
	Control_D	Hemp_D		
Yellow intensity	56.48	64.17	2.85	0.0729
Yolk opacity	51.70	53.70	2.97	0.6537
White intensity	45.23	51.63	3.45	0.2061
Odor	63.40	61.54	2.85	0.6430
Flavour	57.55	65.36	3.04	0.0861
Bitterness	17.57	17.18	1.53	0.8571
Sweetness	14.19	13.71	1.43	0.8139
Saltiness	19.94	22.62	1.05	0.0873
Crumbliness	38.79	48.21	3.53	0.0757
Pastiness	55.47	62.81	2.50	0.0528

^a Control_D = control diet; Hemp_D = diet containing 30% hemp cake.

^b SEM = standard error of mean.

Table 8

Consumer liking scores of eggs across dietary treatments (least square mean $\pm$ standard error of mean).

Item	Dietary treatments ^a		SEM ^b	P-value
	Control_D	Hemp_D		
Overall liking	7.13	7.25	0.12	0.4580
Appearance	7.14	7.13	0.12	0.9531
Taste/flavor	6.82	7.01	0.13	0.3256
Texture	7.02	7.00	0.13	0.8969

^a Control_D = control diet; Hemp_D = diet containing 30% hemp cake.

^b SEM = standard error of mean.

impact of the hemp cake diet on consumer prices. Based on data collected during the on-farm interview, packaging costs for a carton of 10 eggs amount to €1.00. When logistics and distribution costs (+40%) are factored in, the final consumer price would rise to €3.78, compared to €3.36 for a carton of 10 organic eggs from Control_D. Thus, the dietary shift could result in a 12.5% increase in the final retail price.

Finally, from an environmental perspective, the life cycle assessment showed that the Hemp_D had a carbon footprint of 0.97 kg CO₂ eq FU⁻¹, whereas the Control_D exhibited a lower impact of 0.42 kg CO₂ eq FU⁻¹. As shown in Fig. 1, the hemp-based diet resulted in higher CO₂ emissions compared with the control diet based on sunflower cake and soybean seeds, but lower N₂O emissions to air. Fossil methane emissions were similar between the two treatments. A detailed contribution analysis of the main processes involved in CO₂ emissions (Supplementary Table S5) showed that diesel combustion for agronomic practices accounted for more than 50% of total emissions for both diets. Marked differences emerged for electricity use, which contributed 37.6% more in Control_D than in Hemp_D, whereas transport had a substantially greater impact in Control_D compared with Hemp_D (+76.5%). Regarding N₂O emissions (Supplementary Table S6), in Control_D, toasted soybean seeds were the primary source of N₂O emissions (43.8%), followed by corn meal (28.1%). In contrast, in Hemp_D, corn meal was the dominant contributor (61.5%), while hemp cake accounted for only 17.3% of total N₂O emissions.

4. Discussion

Although a large body of literature has investigated the use of hemp seed and oil in laying hen nutrition [52,53], the inclusion of hemp cake at substantial dietary levels remains underexplored [11]. This is despite hemp cake's potential to valorize agro-industrial by-products and reduce competition with human-edible feed resources [54]. A further novelty of the present study is the comprehensive evaluation of hemp dietary treatment, incorporating egg quality, hen behavior, economic performance, and environmental impact through LCA estimation. Despite certain limitations, including the use of a single hemp variety (USO31), a single commercial farm, a 16-week experimental period, and the limited pens per treatment, the results provide a broad assessment of the potential of hemp cake as a high-inclusion alternative protein source for organic laying hen production.

Even if dietary levels of hemp cake may potentially impair nutrient digestibility and consequently animal performance because of its elevated fiber content [55], the presence of antinutritional factors [56], and its relatively lower lysine concentration compared with soybean based protein sources [57], egg production was not adversely affected, even at inclusion rates as high as 30%. The absence of negative effects may be related to their gastrointestinal tracts' ability to adapt functionally and morphologically to moderate increases in dietary fiber, thereby maintaining nutrient utilization [58]. Nevertheless, further studies evaluating nutrient digestibility, gut morphology, and intestinal microbiota are warranted to clarify the mechanisms underlying these adaptive responses. Additionally, the bioactive compounds in hemp, such as polyphenols, cannabinoids, and terpenes, might have supported

Table 9

Effects of dietary treatments on spatial distribution and behavioral activities of laying hens (least square mean $\pm$ standard error of mean; $n = 8$ hens per diet). Unless otherwise stated, data are expressed as a proportion of the animals observed in each category.

Item	Dietary treatments ^a		SEM ^b	P-value		
	Control_D	Hemp_D		Diet	Period	Interaction
Spatial distribution						
Nest	0.04	0.03	0.01	0.8113	0.3099	0.9474
Elevated area	0.60	0.57	0.02	0.1705	0.5451	0.5403
Floor area	0.37	0.40	0.01	0.1153	0.9830	0.5206
Posture						
Standing	0.88	0.89	0.02	0.7731	0.1000	0.4100
Crouching	0.12	0.11	0.02	0.7731	0.1000	0.4100
Behavioral activities						
Feed intake	0.38	0.36	0.02	0.4699	0.4050	0.6258
Water intake	0.10	0.09	0.01	0.1420	0.0407	0.1191
Walking	0.08	0.10	0.02	0.2086	0.6750	0.7928
Inactivity	0.15	0.17	0.01	0.4110	0.5789	0.3248
Oviposition	0.03	0.03	0.01	0.7975	0.5822	0.8463
Exploratory behavior	0.10	0.08	0.01	0.2675	0.2020	0.7767
Preening/Self-grooming	0.09	0.11	0.02	0.5093	0.0950	0.5370
Perching	0.02	0.04	0.01	0.2057	0.5147	0.1438
Dust bathing	0.02	0.02	0.01	0.8200	0.3427	0.1045
Pecking, n/min	57.28	60.00	2.60	0.4789	0.0577	0.5689

^a Control_D = control diet; Hemp_D = diet containing 30% hemp cake.

^b SEM = standard error of mean.

Table 10

Production cost structure and total production cost per egg estimated for the case farm (€/egg).

Item	Dietary treatments ^a	
	Control_D	Hemp_D
Explicit costs	0.13	0.16
Operating costs	0.12	0.15
Feed	0.11	0.15
Wages	0.01	0.01
Other costs	0.0011	0.0011
Depreciation	0.0005	0.0005
Implicit costs	0.01	0.01
Family labor cost	0.002	0.002
Interest on stock capital	0.0006	0.0007
Land use cost	0.001	0.001
Management	0.005	0.005
Total production cost	0.14	0.17

^a Control_D = control diet; Hemp_D = diet containing 30% hemp cake.

systemic health through reported antioxidant and anti-inflammatory

pathways [59], thereby indirectly helping to sustain productive performance. Although hemp-derived ingredients generally contain less lysine than soybean [57], the lack of adverse effects on laying rate, egg weight, and egg mass suggests that lysine availability did not fall below the birds' physiological thresholds under our experimental conditions. This interpretation is consistent with previous observations that productive responses in laying hens depend not only on the concentration of individual amino acids but also on the overall balance and adequacy of the dietary amino acid profile [60]. This is particularly relevant for organic feeding systems, since restrictions of synthetic amino acid supplementation limit precise nutrient optimization, organic producers must rely heavily on the inherent nutritional balance of whole ingredients [61]. These findings indicate that the nutritional challenges associated with hemp cake can be effectively managed through appropriate diet formulation, although longer-term studies are needed to confirm these results throughout the entire laying cycle.

In consistency with performance findings, hens' spatial distribution and behavioral activities were not affected by dietary treatment, providing indirect evidence that high dietary inclusion of hemp cake does not compromise animal health status or behavior [62]. This

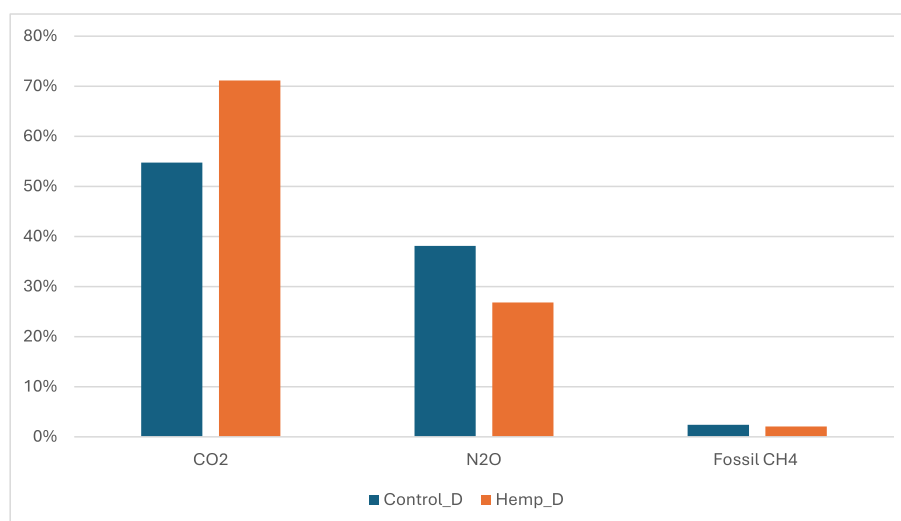


Fig. 1. Percentage contribution of CO₂, N₂O and fossil CH₄ emissions associated with dietary treatments, expresses per kg of diet as functional unit.

interpretation is further supported by the rare occurrence of feather pecking in both groups, as this behavior is widely recognized as a sensitive indicator of behavioral disturbance and social stress in laying hens [63]. Hemp-derived feed ingredients are rich in bioactive compounds and beneficial nutrients, particularly PUFA, but also other bioactive substances [59], which have been reported to exert positive effects on the overall health status and well-being of laying hens [63]. However, these potential nutraceutical effects were not reflected in the behavioral outcomes observed in the present study. This may be because the trial was conducted under organic farming conditions, characterized by greater space allowance and outdoor access, which could have masked subtle dietary effects. Moreover, the relatively short experimental duration and the behavioral observations being limited to the periods immediately before and after feed delivery may have reduced the likelihood of detecting more subtle or long-term behavioral responses. However, the absence of dietary effects on hen behavior indirectly indicates that both experimental diets adequately met the birds' requirements. To date, the relationship between dietary protein sources and hen behavior has received limited attention, with most studies focusing primarily on amino acid composition and essential amino acids [64,65]. The role of hemp-derived ingredients remains unexplored, and the present study contributes additional information in this area.

With regard to egg quality, the absence of dietary effects on egg physical characteristics is consistent with previous literature on hemp cake [66,67]. In line with the statistical tendency observed in the present study, occasional improvements in eggshell strength have been reported for hemp-containing diets [58] whereas a large body of literature didn't find any effect [68]. About yolk color, a tendency toward higher color intensity was observed in the present study using both color fan assessment and sensory analysis. Nevertheless, results reported in the literature remain inconsistent [68], likely due to differences in hemp cake processing, particularly in the extent of lipid and pigment removal. The Hemp_D significantly modified yolk FA composition, resulting in increased PUFA, particularly ALA, LA, and DHA. Similar effects have been reported for whole hemp seed, hemp oil, and hemp cake [11,52,68,69], indicating that the residual oil fraction in the hemp cake remains effective in modulating egg yolk FA composition. The lack of effect on SFA and the decrease in MUFA are also consistent with previous observations [69–72]. The key role in modifying the yolk FA profile is attributable to the well-known high LA and ALA content of hemp. The increase in DHA despite its negligible concentration in hemp ingredients suggests an efficient hepatic conversion of dietary ALA into longer-chain n-3 PUFA, as previously described in laying hens [2]. This response likely reflects the activity of desaturation and elongation pathways, with dietary ALA serving as a precursor for endogenous DHA synthesis [11]. Improvements in nutritional indices, such as a lower TI and a more favorable n-6/n-3 ratio, further support the potential of hemp cake to enhance the nutritional value of eggs [73]. In particular, the n-6/n-3 ratio moved toward the recommended value of 4, despite the higher linoleic acid (LA, n-6) content observed in eggs from the Hemp_D group. From a consumer perspective, the enrichment of eggs with n-3 PUFA, particularly DHA, together with the reduction in the n-6/n-3 ratio, may enhance the nutritional value of eggs by contributing to a greater intake of FA associated with cardiovascular and cognitive health benefits [74].

Hemp cake had only a limited effect on the sensory profile of eggs. Although some sensory descriptors showed trends ($P < 0.10$), these results should be interpreted with caution. The higher PUFA content in hemp-fed eggs may have contributed to the observed tendencies toward increased flavor intensity, saltiness, and textural changes, as PUFA-rich lipids can enhance the formation of volatile compounds during cooking and modulate taste perception [75]. In addition, increased lipid unsaturation can alter yolk protein gelation and microstructure, and this may have contributed to the tendency observed for different crumbliness and mouthfeel [76]. To the best of our knowledge, this is the first study reporting egg sensory properties assessed by both a trained panel and consumer liking following the inclusion of hemp cake in laying hen

diets. Previous studies using hemp seeds reported no or only minor effects on egg sensory attributes, although differences in egg preparation methods make comparisons challenging [77,78]. More recently, Bošković Cabrol et al. [69] conducted a triangle test with untrained panelists, who were able to partially differentiate eggs from diets with or without hemp seed inclusion. Overall, the limited impact of hemp cake on sensory attributes is consistent with the absence of diet-related differences in consumer preferences observed in the present study.

The economic and environmental impact of adding hemp cake to poultry diets really depends on the specific situation, but this study highlights some important observations. Feed is nearly always the biggest expense in livestock production, especially in intensive poultry operations, where it can make up to 70% of all costs - a finding widely reported by the Food and Agriculture Organization [79] and many other empirical studies [80–83]. In our research, the Control_D diet meant that explicit costs accounted for 93% of the cost per egg, with feed alone accounting for 79%. This is higher than what other reports suggest [84], probably because organic rules limit access to the most efficient protein sources. As a result, organic feed usually costs more than conventional feed [85]. In this case, higher costs also resulted from the lack of a supply chain for food-grade hemp. The hemp used was intended for food use, so it had to meet stricter quality and safety standards than those for feed. Although it is not possible to estimate the extent of higher costs due to the lack of market data, we can assume the same patterns apply to other oilseed by-products. For example, sunflower meal is a cheap feed ingredient, but its protein extracts for human consumption are much pricier due to higher required standards [86]. However, our analysis showed that adding the Hemp_D diet does not significantly change the overall cost structure. If we look at the entire supply chain, the final price for consumers would rise by about 12.5%.

Therefore, within a broader market framework, the added value associated with nutritionally enhanced eggs is likely to offset the higher production costs. Empirical evidence from the Italian market indicates a large majority of Italian consumers are willing to pay a statistically significant premium for such enriched products that is totally compatible with the increased production costs due to hemp use (specifically amounting to +0.03 in our study), provided that the product differentiation is correctly communicated [87]. Nevertheless, economic viability remains subject to hemp price fluctuations. It should also be noted that our study relies on human-grade hemp that is expensive. As production chains continue to develop and deliver specialized feed-grade hemp, input costs are expected to consistently decline improving the economic sustainability of hemp-based feeding.

Since the dietary treatments had no effect on egg production, the life cycle assessment was limited to the production of the two diets. The higher carbon footprint observed for the hemp-based feed compared with the sunflower and soybean feed can be primarily attributed to differences in ingredient composition and upstream agricultural processes. Hemp cultivation involves lower yields and less optimized supply chains compared with well-established soybean and sunflower production systems, potentially increasing energy demand per unit of product. In addition, oil extraction efficiency and the relatively small-scale nature of hemp processing could contribute to higher upstream emissions, as reported in previous LCA studies on alternative protein sources for animal feed [88,89]. Consequently, the environmental burdens of the agricultural phase, such as field operations and land management, are concentrated across a smaller volume of output. This aligns with recent life cycle assessments of oilseed crops in Southern Europe [15], which highlight that regional hemp processing, despite its circular economy potential, carries significant environmental burdens during the cultivation and oil extraction phases compared with high-yielding conventional crops. In addition, LCA studies frequently assign higher environmental burdens to imported protein sources, such as soybean, because of long-distance transport and land-use change [90]. However, our results highlight that local sourcing does not automatically guarantee a lower carbon footprint when the alternative crop's agricultural

productivity is substantially lower than that of the baseline ingredients. Therefore, although hempseed cake serves as a valuable tool for local circularity and reduces reliance on imported feed ingredients, its inclusion in laying hen diets introduces an environmental trade-off between local sourcing benefits and total greenhouse gas emissions per kilogram of feed. Despite the higher overall carbon footprint, the hemp-based feed exhibited lower N₂O emissions. Ingredient-level analysis indicated that toasted soybean seeds were the main contributor to N₂O emissions in Control_D. The substantially lower inclusion of toasted soybean in the hemp-based diet (200 vs 50 g/kg) likely influenced this difference. Although soybean does not require synthetic nitrogen fertilization, part of this fixed nitrogen remains in the soil and crop residues and can be transformed into N₂O through nitrification and denitrification processes, still contributing to N₂O emissions. In the present study, hemp was fertilized with poultry manure applied at a rate of 100 kg N ha⁻¹. While manure application also generates N₂O emissions, the moderate nitrogen input rate may have influenced overall emission intensity. This finding is consistent with literature indicating that crops with lower nitrogen inputs can substantially reduce N₂O emissions, one of the most impactful greenhouse gases in agricultural systems [49,91]. Differences in nitrogen form, availability, and soil dynamics could therefore have contributed to the lower N₂O emissions observed for the hemp-based diet.

The similar fossil methane emissions observed for both feeds suggest that CH₄ contributions are largely driven by shared background processes, such as energy production and feed processing, rather than by feed-specific ingredients. This aligns with previous LCA assessments of poultry feeds, where methane plays a secondary role compared with CO₂ and N₂O [92]. Overall, these results highlight the importance of considering individual greenhouse gas contributions when evaluating alternative feed formulations, as increases in total carbon footprint may be partially offset by reductions in more potent gases such as N₂O. Recent studies have observed that the inclusion of by-products in concentrated feed formulations can reduce the carbon footprint per FU [93,94]. From a mitigation perspective, optimization of hemp production practices could further enhance the environmental performance of hemp-based feeds.

5. Conclusion

Under the conditions of the present study, the inclusion of 30% hemp cake did not adversely affect productive performance, hen behavior, or the physical and sensory characteristics of eggs whereas, from a nutritional standpoint, improvements in the fatty acid profile and selected nutritional indices were observed. Nevertheless, these findings should be confirmed in future studies including a greater number of replicate pens and commercial farms to improve their robustness and generalizability. On the other hand, despite these benefits, the hemp-based diet was associated with higher feed-related costs and a greater CO₂-equivalent impact than the control diet, highlighting the current economic and environmental limitations of hemp production. The increased feeding costs, primarily due to the price of hemp cake, could be partially offset through value-based marketing of nutritionally enhanced eggs. A formal sensitivity analysis is needed to assess the robustness of the economic incentives associated with this feeding strategy, particularly once a more established hemp supply chain enables more stable cost and market estimates. Longer-term studies are warranted to confirm the adequacy of lysine supply throughout the laying cycle and to refine recommendations for hemp cake inclusion in diets formulated without synthetic amino acids. Future research should also focus on selecting high-yielding hemp cultivars, with particular attention to the nutritional quality and feed value of the residual hempseed cake, optimizing oil extraction processes, and assessing the regional scaling-up of the supply chain. In addition, future life cycle studies should test alternative allocation methods (e.g., system expansion) and dynamic economic modelling. These developments may help reduce the carbon footprint

per kilogram of feed while enhancing the economic viability of circular, locally sourced organic diets.

CRediT authorship contribution statement

Felicia Masucci: Writing – review & editing, Writing – original draft, Software, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Grazia Scalera:** Writing – review & editing, Methodology, Formal analysis, Data curation. **Francesco Serrapica:** Writing – review & editing, Writing – original draft, Validation, Supervision, Formal analysis, Data curation, Conceptualization. **Ada Braghieri:** Writing – review & editing, Validation, Resources, Methodology, Funding acquisition, Formal analysis, Data curation. **Emilio Sabia:** Writing – review & editing, Methodology, Data curation. **Amelia Maria Riviezz:** Writing – review & editing, Methodology, Formal analysis, Data curation. **Lucia De Luca:** Writing – review & editing, Methodology, Formal analysis, Data curation. **Raffaele Romano:** Validation, Supervision, Resources, Methodology. **Giuseppe De Rosa:** Writing – review & editing, Validation, Supervision, Methodology. **Claudia Lambiase:** Writing – review & editing, Methodology, Formal analysis, Data curation. **Massimo Fagnano:** Writing – review & editing, Validation, Supervision, Resources, Project administration, Funding acquisition. **Nunzio Fiorentino:** Writing – review & editing, Validation, Supervision, Resources. **Gianni Cicia:** Writing – review & editing, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Orlando Cimino:** Writing – review & editing, Methodology, Formal analysis, Data curation. **Carla Cavallo:** Writing – review & editing, Methodology, Formal analysis, Data curation. **Tonia Impegno:** Writing – review & editing, Methodology, Formal analysis, Data curation. **Antonio Di Francia:** Writing – review & editing, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jafr.2026.103150>.

Data availability

Data will be made available on request.

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