Poultry Science

Dietary inclusion of defatted silkworm (*Bombyx mori* L.) pupa meal in broiler chickens: phase feeding effects on nutritional and sensory meat quality

Antonella Dalle Zotte [*], Yazavinder Singh [*,1], Eszter Zsedely,[†] Barbara Contiero [*], Bianca Palumbo,[*] and Marco Cullere [*]

[*]*Department of Animal Medicine, Production and Health, University of Padova, Legnaro, Padova 35020, Italy; and* [†]*Department of Animal Science, Szechenyi István University, Győr H-9026, Hungary*

ABSTRACT The present experiment was conducted to test the effect of a 4% defatted silkworm (*Bombyx mori*) pupae meal (**SWM**) incorporation into chickens' diets at different growth phases on meat quality characteristics and sensory traits. Ninety ROSS 308 day-old male broiler chickens were randomly assigned to 3 dietary groups, with 5 replicated pens/diet: the first group received a control (**C**) diet throughout the growing period of 42 d, the second group received a diet with 4% SWM (**SWM1**) during the starter phase (1−10 d) and the C diet up to slaughter, whereas the third group was fed the C diet during the starter phase and 4% SWM during the grower and finisher phases (**SWM2**). Diets were isonitrogenous and isoenergy, and birds had free access to feed and water throughout the experimental trial. At 42 d of age, 15 chickens/treatment were slaughtered at a commercial abattoir. Fatty acid (**FA**) and amino acid (**AA**) profiles and contents of meat, as well as its oxidative status, were determined in both breast and leg meat cuts. Also, a descriptive sensory analysis was performed on breast meat by trained panelists. Results highlighted that the SWM2 treatment increased the *n*-3 proportion and content in both breast and leg meat, thereby improving the omega-6/omega-3 (***n*-6/*n*-3**) ratio in both cuts ($P < 0.001$). However, the dietary treatment had no significant effect on the oxidative status of either breast or leg meat ($P > 0.05$). The SWM had a limited impact on overall sensory traits of breast meat, but it contributed to improve meat tenderness in SWM-fed chickens ($P < 0.01$). Furthermore, SWM1 meat exhibited higher juiciness ($P < 0.05$) and off flavor intensity ($P < 0.05$) compared to the control meat. Overall, the present experiment indicated that defatted SWM holds promise as an alternative ingredient in chicken rations, ensuring satisfactory meat quality. Furthermore, administering SWM during the grower-finisher phase demonstrated beneficial effects on meat healthiness, ultimately enhancing *n*-3 fatty acids content and reducing the *n*-6/*n*-3 ratio.

Key words: *Bombyx mori*, silkworm meal, feed, chicken, meat quality

2024 Poultry Science 103:103812
https://doi.org/10.1016/j.psj.2024.103812

INTRODUCTION

The poultry sector has been experiencing a noticeable global market growth in the last decade, as chicken meat production increased from 90,983,092 t in 2011 to 121,588,359 t in 2021 (FAOSTAT, 2023). This growth has led to a consequent increase in the demand for feed ingredients. This trend, which is also linked to demographic patterns and the consequent increased demand for meat in general (FAO, IFAD, UNICEF, WFP, and WHO, 2021), is pushing the livestock sector in the search for feedstuffs alternatives to conventional ones. It is widely acknowledged that insects are considered a possible solution to increase feedstuffs market diversification, stimulate self-supply in countries that heavily rely on imported feed materials, and potentially improve the sustainability of the sector (van Huis and Oonicx, 2017).

Among the insect species authorized for use as poultry feed in the European Union, the mulberry silkworm (*Bombyx mori*) is included. The mulberry silkworm has been historically farmed and used for silk production, with a history dating back to 5200 years ago in China (Makkar et al., 2014). Once the silk-made cocoon is completed by the pupa, it is collected, boiled, dried or soaked in sodium hydroxide to kill the pupa and prevent it to produce the enzyme that it would use to make a hole in the cocoon, allowing the moth to emerge. Quantitatively speaking, to get about 1 kg of silk, 8 kg of fresh pupae are required (Patil et al., 2013), which can be used as a direct source of food or as fertilizer. However, most of pupae are discarded in open environment which contributes to environmental pollution

Received January 15, 2024.
Accepted April 24, 2024.

[1]Corresponding author: yazavinder.singh@unipd.it

and represents a loss of valuable nutrients (Sheikh et al., 2018). In fact, the meal obtained from spent silkworm pupae has a remarkable protein content (up to 50% on dry matter) and an AA profile comparable to that of fish meal. Additionally, it has a high fat content (about 30% on a dry matter basis) which is rich in polyunsaturated fatty acids (**PUFA**) of the *n*-3 series (Yang et al., 2006; Makkar et al., 2014). These nutritional elements make it an excellent candidate for poultry nutrition, particularly in the perspective of further enhancing product healthiness, with a specific focus on the FA profile of meat. This has been demonstrated in available studies on monogastric species: broiler chickens fed either with 7, 14, or 17% full-fat SWM have been investigated (Miah et al., 2020; Pietras et al., 2021), as well as rabbits fed with 1.3% inclusion of silkworm oil (Dalle Zotte et al., 2022).

However, to date, there is no study evaluating the impact of defatted SWM in chicken diets on meat quality. This is relevant for 2 main reasons: the defatting process 1) lowers the oil content, thereby limiting the potential beneficial effects of SWM *n*-3 FA on improving meat healthiness, and 2) concentrates the amount of chitin and 1-deoxynojirimycin (**1-DNJ**). Chitin is known to potentially worsen protein digestibility and thus absorption (Razdan and Pettersson, 1994). In addition, 1-DNJ, an alkaloid iminosugar (also known as azasugar), is naturally present in silkworm pupae as larvae feed on mulberry leaves containing 1-DNJ. This compound acts as an inhibitor of α-glucosidase enzyme, which is responsible for converting starch into monosaccharides, subsiquently absorbed in the intestine (Gao et al., 2016). In fact, when a 12.5% full-fat and defatted SWM were incorporated into broiler quails' diet, a marked reduction in starch digestibility was observed (Dalle Zotte et al., 2021). These 2 bioactive compounds can affect *in vivo* performance of chickens, which, in turn, could have implications on meat quality traits. In addition, available research on the possible application of SWM in poultry nutrition has not yet considered the incorporation of this emerging feedstuff at different growth stages, which could have relevant productive, qualitative and thus economic implications.

Based on these premises, this research aimed at assessing the impact of a dietary incorporation with 4% defatted SWM into chickens' rations at different feeding phases (starter or grower-finisher) on productive performance, carcass and meat quality traits. The results of the first part of the research project, encompassing productive performance, carcass, and physical meat quality traits, are available in the work by Zsedely et al. (2023). In the present manuscript, insights into chemical meat quality features are presented, including FA, AA, oxidative status, as well as sensory characteristics.

MATERIALS AND METHODS

*Silkworm (*Bombyx mori L.*) Pupae*

Dried silkworm pupae were obtained from a local sericulturist. Subsequently, they were cold pressed to obtain defatted SWM. The defatted SWM was utilized in formulating the experimental diets for broiler chickens.

Animals and Experimental Design

The *in vivo* trial was conducted at the Széchenyi István University (Hungary). All birds were handled according to the principles stated in the European directive 2010/63/EU on the protection of animals used for scientific purposes (European Commission (EC) 2010), and according to the Hungarian legal requirements (32/1999. /III. 31./ and 178/2009. /XII. 29./).

In a phase feeding trial, a total of 90 one-day-old male chicks (ROSS 308) were randomly divided into 3 dietary groups consisting of 5 replicated pens/diet. The first group received a control diet (commercial feed; C) throughout the growing period of 42 d, the second group received a diet including 4% defatted SWM during the starter phase (1−10 d) (SWM1) and the C diet up to slaughter (11−42 d), whereas the third group was fed with the C diet in the starter phase (1−10 d) and the 4% SWM diet up to slaughter (11−42 d) (SWM2). The experimental diets and water were provided *ad libitum* throughout the experiment. Diets were isonitrogenous and isoenergy and were formulated to meet nutrient requirements recommendation for broiler chicken indicated by AVIAGEN ROSS 308 management guide (ROSS Broiler Management Handbook, 2018). A detailed description of the chemical composition, and the energy content of SWM, as well as the ingredients, the chemical composition and the energy content of experimental diets are reported in the paper by Zsedely et al. (2023).

At 42 d of age, n = 45 chickens (n = 15 per treatment; 3 chickens per replicate) were randomly selected, stunned, slaughtered and processed at a commercial abattoir; carcasses were then divided into 2 halves, one of which was transported frozen (-20 °C) to the Department of Animal Medicine, Production and Health (**MAPS**) of the University of Padova, Italy, for meat quality analyses. After overnight thawing at +4 °C, n=10 half carcasses/treatment were dissected, and the breasts and legs were excised. Meat of breasts and legs (after bone removal) was ground (Retsch Grindomix GM 200: 7000 *g* for 10 s), freeze-dried, ground again (7000 *g* for 5 s) and dedicated to fatty acid (**FA**: n = 10 samples/cut/treatment) and amino acid (AA n = 5 samples/cut/treatment) profiles analyses. One week later, following overnight thawing at +4°C, the remaining n = 5 halves of carcasses/treatment were dissected: the leg meat was ground (7000 *g* for 10 s) and promptly utilized for lipid oxidation analysis. From the n = 5 breasts/treatment, 1-cm thick slice/breast was cut from the cranial to the caudal part of the *Pectoralis major* muscle, sternum keel side, ground (7000 *g* for 5 s) and dedicated to lipid oxidation analysis; among the n=5 breasts/treatment, n=3 of them were randomly selected, *vacuum*-sealed in food-grade plastic bags and dedicated to sensory analysis.

Fatty Acid Profiles: Defatted Silkworm Meal, Experimental Diets, and Chicken Breast and Leg Meat

The lipid extraction was performed by Modified Accelerated Solvent Extraction, using different solvents depending on the considered matrixes: for SWM and chicken breast and leg meat samples, lipids were extracted by the binary solvent mixture chloroform: methanol 1:2, whereas for the experimental diets petroleum ether was used as solvent. A complete description of the analytical procedure is provided by Cullere et al. (2022). Samples were trans-methylated using a methanolic solution of H_2SO_4 (4%), in order to determine fatty acid methyl esters (**FAME**). A biphasic separation was obtained by adding 0.5 ml of distilled water and 1.5 mL of *n*-heptane to each sample. FAME were quantified by gas chromatography (Shimadzu GC17A, Nishinokyo-Kuwabara-cho, Nakagyo-ku, Kyoto 604-8511, Japan), equipped with an Omegawax (Sigma-Aldrich Co. LLC., Saint Louis, USA) 250 column (30 m × 0.25 μm × 0.25 μm) and flame ionization detector. Helium was used as carrier gas at a constant flow of 0.8 ml/min. The injector and detector temperatures were both 260 °C. Peaks were identified based on commercially available FAME mixtures (37-Component FAME Mix, Supelco Inc., Bellefonte, PA). The results are expressed as % of total detected FAME. In the case of chicken breast and leg meat, results were also used for the quantitative determination (mg/100 g breast or leg meat) of FA based on the total lipid content of the samples. The FA profile of the SWM and experimental diets are provided in Table 1.

Amino Acid Profile: Defatted Silkworm Meal, Experimental Diets, and Chicken Breast and Leg Meat

The AA composition of the SWM, experimental diets, and chicken breast and leg meat samples was assessed after acid hydrolysis and pre-column derivatization using 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate (**AQC**), separated by RP-HPLC and analyzed by UV detection (Agilent 1260 Infinity, Agilent Technologies, Santa Clara, CA) following an adapted method from European Pharmacopoeia (Council of Europe, 2005), and by using 6M HCl at 105 °C for 24 h to hydrolyze samples. Differently, cysteine was determined by the sum of cysteine and cystine, after reaction with dithiodipropionic acid, producing a mixed disulphide, which then underwent acid hydrolysis. After hydrolysis, samples were neutralized with 8M NaOH, and volume was adjusted and filtered at 0.45 μm. Then, the derivatization step was conducted according to the manufacturer's instructions (AccQ-Tag Ultra Derivatization Kit; Waters, Milford, MA). The obtained results were expressed as g/100 g breast or leg meat (Table 2).

Table 1. Fatty acid profile (% of total FAME) of the defatted silkworm (*Bombyx mori*) pupae meal and experimental diets.

		Experimental diets					
	SWM	C Starter	C Grower	C Finisher	SWM Starter	SWM Grower	SWM Finisher
C6:0	0.00	0.09	0.11	0.20	0.06	0.07	0.07
C8:0	0.00	0.03	0.03	0.04	0.02	0.03	0.02
C12:0	0.05	0.00	0.01	0.01	0.01	0.02	0.01
C14:0	0.14	0.07	0.14	0.11	0.09	0.13	0.11
C15:0	0.03	0.02	0.02	0.02	0.03	0.01	0.18
C16:0	21.6	9.73	9.52	9.92	12.0	11.2	10.7
C17:0	0.08	0.07	0.06	0.06	0.07	0.06	0.06
C18.0	5.75	3.36	3.42	3.65	3.74	3.69	3.56
C20:0	0.40	0.26	0.26	0.30	0.29	0.28	0.27
C22:0	0.19	0.60	0.47	0.56	0.43	0.48	0.45
C24:0	0.09	0.27	0.22	0.26	0.30	0.33	0.34
∑SFA	28.4	14.5	14.2	15.1	17.0	16.3	15.8
C15:1	0.06	0.07	0.07	0.13	0.01	0.06	0.06
C16:1	0.91	0.09	0.10	0.10	0.22	0.21	0.18
C17:1	0.06	0.03	0.03	0.03	0.04	0.03	0.03
C18:1 *n*-9	0.00	31.4	31.9	32.3	30.6	30.9	30.9
C18:1 *n*-11	32.5	0.00	0.00	0.71	0.61	0.66	0.61
C20:1 *n*-9	0.02	0.16	0.09	0.19	0.05	0.06	0.06
C22:1 *n*-9	0.00	0.00	0.05	0.07	0.00	0.00	0.04
∑MUFA	33.6	31.7	32.2	33.6	31.5	31.9	31.8
C18:2 *n*-6	7.88	51.9	51.6	48.8	45.0	47.2	48.4
C18:3 *n*-6	0.12	0.00	0.09	0.11	0.00	0.10	0.11
C18:3 *n*-3	29.6	1.38	1.02	0.90	5.89	3.64	3.31
C20:2 *n*-6	0.04	0.00	0.04	0.04	0.03	0.04	0.03
∑PUFA	37.8	53.2	52.7	49.9	50.9	51.0	51.8
∑*n*-6	8.06	51.9	51.7	49.0	45.0	47.4	48.5
∑*n*-3	29.6	1.38	1.05	0.93	5.89	3.64	3.35
n-6/*n*-3	0.27	37.6	49.2	52.7	7.60	13.0	14.5
Identified	99.7	99.5	99.2	98.6	99.5	99.3	99.5

Abbreviations: C, control; SWM, defatted silkworm meal; SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids.

Table 2. Amino acid content of the defatted silkworm (*Bombyx mori*) pupae meal and experimental diets (g/100 g).

		Experimental diets					
	SWM	C starter	C grower	C finisher	SWM starter	SWM grower	SWM finisher
Essential amino acids							
Arginine	3.29	1.39	1.18	1.04	1.34	1.25	1.09
Histidine	2.01	0.67	0.54	0.50	0.68	0.64	0.55
Isoleucine	2.33	0.86	0.76	0.71	0.91	0.85	0.76
Leucine	3.75	1.59	1.43	1.37	1.68	1.54	1.42
Lysine	3.72	1.35	1.19	1.09	1.60	1.26	1.18
Methionine	1.98	0.54	0.43	0.30	0.55	0.56	0.35
Phenylalanine	2.71	1.04	0.93	0.87	1.09	1.01	0.90
Threonine	2.77	0.88	0.71	0.61	0.90	0.83	0.73
Valine	3.06	0.98	0.88	0.82	1.04	0.98	0.88
Non-essential amino acids							
Alanine	3.07	0.96	0.83	0.80	1.00	0.93	0.87
Aspartic acid	6.09	2.24	1.93	1.74	2.25	2.12	1.86
Cysteine	0.66	0.35	0.32	0.29	0.37	0.33	0.30
Glutamic acid	8.14	4.40	4.00	3.78	4.54	4.20	3.81
Glycine	3.10	0.91	0.78	0.73	0.94	0.88	0.80
Proline	2.82	1.21	1.13	1.12	1.31	1.22	1.14
Serine	2.85	1.11	0.94	0.87	1.14	1.07	0.97
Tryptophan	1.33	0.43	0.39	0.39	0.47	0.43	0.41
Tyrosine	3.53	0.60	0.52	0.46	0.64	0.62	0.54

Abbreviations: C, control; SWM, defatted silkworm meal.

Oxidative Status: Chicken Breast and Leg Meat

Breast and leg meat samples were analyzed for secondary lipid oxidation products (thiobarbituric acid reactive substances − **TBARs**) following the method provided by Botsoglou et al. (1994). Samples absorbance was read at 532 nm with a spectrophotometer (Hitachi U-2000; Hitachi, Mannheim, Germany). The TBARs values were calculated from a standard calibration curve of 1,1,3,3-tetraethoxypropane and values were expressed as mg of malondialdehyde (**MDA**)/kg breast or leg meat.

Sensory Analysis

Breast meat samples were assigned to a descriptive sensory analysis, to detect possible difference among the dietary treatments (C, SWM1, and SWM2). A total of 9 breasts samples (3 breast samples/treatment) were used and a day of analysis was scheduled. Before conducting the sensory evaluations of experimental samples, panelists performed 2 pre-test training sessions of 1 h each to get familiar with the sample type and select appropriate descriptors. Gustative and textural aspects were evaluated. The chicken breast used for the training session was purchased from a local supermarket and was processed, handled and cooked in the same manner of the samples, which were used for the subsequent sensory evaluation. The selected descriptors were odor (general intensity and off-odor intensity), texture (juiciness and tenderness), and flavor (general intensity and off-flavor intensity, *Bombyx mori*/walnut/petfood, liver/blood/metallic). For the selection of the above-mentioned descriptors panelists were asked to taste ground walnut and SWM.

Initially, a random 3-digits code was assigned to each sample for identification. Chicken breasts were cooked in a water bath set at 80 °C; the samples were cooked until the core temperature of the heaviest sample reached 78 °C. Afterwards, the samples were cooled in crushed ice for 30 min to stop cooking reaction. Samples were kept at room temperature for 20 min ad then cut into small pieces which were randomly served to 6 trained panelists. Each panelist evaluated a total of 9 samples (3 samples/treatment). The panel received a list of descriptors to score on balanced 10-point hedonic scale of 150 mm-long: from 1 (the lowest score for each descriptor) to 10 (the highest score for each descriptor) (Table 3). All the evaluations were performed in a room where temperature was 22 °C; unsalted crackers and still water at room temperature were available to panelists.

Statistical Analysis

FA and AA data were subjected to one-way ANOVA with experimental diets (C, SWM1, and SWM2) as a fixed effect following the general linear model (**GLM**) procedure of SAS (SAS® OnDemand for Academics—3.81 Enterprise Edition, SAS Institute Inc., Cary, NC). For sensory traits (odor, texture, and flavor intensities) data were subjected to one-way ANOVA (GLM) to detect any influence on sensory trait scores, therefore considering experimental diets as fixed effect. A chi-square test with Marascuilo (1966) procedure was performed on off-odor and off-flavor intensities (*Bombyx mori*/walnut/petfood and liver/blood/metallic) to detect difference among the treatments. Least square means were obtained, and post-hoc pairwise comparison was performed using the Bonferroni correction. The significance was considered at 5% confidence level.

Table 3. List of sensory attributes and scale anchors.

Attributes	Scores	
	00 mm anchor	150 mm anchor
Odor		
General intensity	Extremely poor	Extremely strong
Off-odor intensity	Extremely poor	Extremely strong
Texture		
Juiciness	Extremely dry	Extremely juicy
Tenderness	Extremely tender	Extremely tough
Aroma		
General intensity	Extremely poor	Extremely strong
Off-flavor intensity	Extremely poor	Extremely strong
Bombyx mori/walnut/petfood	No *Bombyx mori*/walnut/petfood	Extremely *Bombyx mori*/walnut/petfood
Liver/blood/metallic	No liver/blood/metallic	Extremely liver/blood/metallic

RESULTS

Fatty Acid Profile and Contents, and Oxidative Status of Chicken Breast and Leg Meat

The effect of a dietary incorporation with a 4% defatted SWM to broiler chickens at different growth stages on the FA profile (% of total FAME) and oxidative status of breast meat is presented in Table 4. Overall saturated fatty acids (**SFA**), monounsaturated fatty acids (**MUFA**) and PUFA remained unaffected by the SWM inclusion. Despite this, among SFA myristic acid, margaric acid and lignoceric acid (**C14:0, C17:0** and **C24:0**, respectively) differed among groups: myristic acid decreased in SWM1 and SWM2 compared to the C group (0.38 and 0.38 *vs.* 0.41 %, respectively; $P < 0.05$), whereas both margaric and lignoceric acids followed the trend SWM2 > SWM1 > C (C17:0−0.14 > 0.13 > 0.12 %; $P < 0.01$; C24:0−0.41 > 0.29 > 0.20 %; $P < 0.01$). Within the PUFA fraction, γ-linolenic acid (**C18:3 *n*-6**)

Table 4. Effect of the dietary inclusion either with 0% (C) or 4% of defatted silkworm (*Bombyx mori* L.) pupae meal during starter (SWM1) or grower-finisher (SWM2) phases on the fatty acids profile (% of total FAME) and oxidative status (mg MDA/kg meat) of chicken breast meat.

	Experimental diets				
	C	SWM1	SWM2	RSD[1]	*P*-value
N.	10	10	10		
C6:0	0.06	0.08	0.08	0.03	0.2396
C8:0	0.03	0.04	0.06	0.03	0.0720
C12:0	0.01	0.01	0.02	0.02	0.3134
C14:0	0.41[a]	0.38[b]	0.38[b]	0.03	0.0207
C15:0	0.08	0.09	0.11	0.05	0.2308
C16:0	19.9	19.9	20.1	0.61	0.7196
C17:0	0.12[B,b]	0.13[A,b]	0.14[A,a]	0.01	0.0071
C18:0	9.04	9.08	9.44	0.81	0.4985
C20:0	0.25	0.25	0.25	0.03	0.8274
C24:0	0.20[B,b]	0.29[A,b]	0.41[A,a]	0.09	0.0003
∑SFA	30.1	30.2	30.9	1.20	0.2492
C14:1	0.01	0.02	0.04	0.05	0.3504
C15:1	0.12	0.13	0.16	0.03	0.0859
C16:1	2.41	2.40	2.10	0.43	0.1945
C18:1 *n*-9	32.1	32.1	31.4	1.32	0.4369
C18:1 *n*-11	1.70	1.79	1.65	0.18	0.2456
∑MUFA	36.3	36.4	35.4	1.63	0.2895
C18:2 *n*-6	24.6	24.8	24.1	1.51	0.6351
C18:3 *n*-6	0.23[A]	0.22[A]	0.12[B]	0.06	0.0008
C18:3 *n*-3	0.45[B]	0.44[B]	1.36[A]	0.15	<0.0001
C20:2 *n*-6	0.55	0.57	0.63	0.11	0.2122
C20:3 *n*-6	0.56	0.52	0.51	0.10	0.4706
C20:4 *n*-6	3.15	3.11	2.80	0.58	0.3507
∑PUFA	29.8	29.7	29.5	1.74	0.9621
∑*n*-6	29.3	29.2	28.2	1.64	0.2629
∑*n*-3	0.45[B]	0.44[B]	1.36[A]	0.15	<0.0001
n-6/*n*-3	65.3[A]	62.6[A]	21.2[B]	5.57	<0.0001
Identified	96.2	96.3	95.9		
TBARs[2]	0.68	0.68	0.71	0.02	0.0971

Abbreviations: SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids.
[1]Residual standard deviation.
[2]TBARs = thiobarbituric acid-reactive substances (n=5 samples/treatment).
[A,B]Means in the same row with different superscript letters differ for $P < 0.01$ or $P < 0.001$.
[a,b]Means in the same row with different superscript letters differ for $P < 0.05$.

decreased following the trend C = SWM1 > SWM2 ($P < 0.05$), whereas α-linolenic acid (**C18:3 *n*-3, ALA**) increased in SWM2 group compared to C and SWM1 ones (1.36 *vs.* 0.44 and 0.45 % for SWM2, SWM1 and C, respectively; $P < 0.001$). This determined a significant increase of the *n*-3 proportion in the breast meat of chicken fed grower-finisher SWM dies compared to SWM1 and C ones (1.36 *vs.* 0.45 and 0.47 %, $P < 0.0001$), which generated a reduction in the *n*-6/*n*-3 ratio (65.3 and 62.6 *vs.* 21.2 for C, SWM1 and SWM2, respectively; $P < 0.001$). No effects of the dietary treatments on the secondary products of lipid oxidation of breast meat were observed.

The outcomes shown in Table 4 are consistent with the results presented in Table 5, which compares the FAs contents (mg/100 g of meat) of chicken breast meat among the 3 different dietary groups. The overall SFA, MUFA, and PUFA contents did not display any significant difference in the experimental groups with only some FA differing within each main class. In case of SFA, C14:0 was the highest in the C group and the lowest in SWM1 (8.35 *vs.* 6.88 mg/100 g, $P < 0.05$), whereas C24:0 significantly increased in SWM2 group in comparison to both C and SWM1 (7.90 *vs.* 4.07 and 5.26 mg/100 g, respectively; $P < 0.01$). Within MUFA, pentadecanoic acid (**C15:1**) was higher in SWM2 than SWM1, while C showed an intermediate amount (2.97, 2.31, and 2.47 mg/100 g meat, respectively; $P < 0.05$).

Delving into PUFA class, SWM2 showed a lower content of C18:3 *n*-6 than C, with SWM1 being intermediate (4.54, 3.90, 2.49 mg/100 g meat, for C, SWM1 and SWM2, respectively; $P < 0.01$). However, this result did not influence the overall *n*-6 breast meat content, which was similar in all treatment groups. The higher content of ALA (C18:3 *n*-3) was observed in the SWM2 group compared to the SWM1 and C groups (9.11, 7.91, 27.4 mg/100 g of meat for C, SWM1, and SWM2 groups, respectively; $P < 0.001$). Since ALA corresponded to the total amount of *n*-3 FA in the breast meat, the same trend was observed for the total *n*-3 PUFA.

Diversely from the FA profile of breast meat, the experimental diets affected the FA profile of chicken leg meat to greater magnitude (Table 6). The SFA proportion of SWM1 and SWM2 meat was lower than that of the C group (27.9 *vs.* 27.4, 27.1% for C, SWM1, and SWM2 groups, respectively; $P < 0.05$). This was attributable to the variations observed in some singles FA, mainly caprylic acid (**C8:0**) ($P < 0.001$), arachidic acid (**C20:0**) ($P < 0.001$), and C24:0 ($P < 0.001$). Even if total MUFA were similar in all treatment groups, some changes in minor singles MUFA were detected: C15:1 showed the lowest value for SWM2 ($P < 0.001$), whereas the latter displayed the highest proportions of heptadecenoic acid (**C17:1**) (P<0.001), and gondoic acid (**C20:1 *n*-9**) ($P < 0.001$).

Despite some significant changes in the percentage of singles PUFA (C18:3 *n*-6, eicosadienoic acid (**C20:2**

Table 5. Effect of the dietary inclusion either with 0% (C) or 4% of defatted silkworm (*Bombyx mori* L.) pupae meal during starter (SWM1) or grower-finisher (SWM2) phases on the fatty acids content (mg/100 g meat) of chicken breast meat.

	Experimental diets				
	C	SWM1	SWM2	RSD[1]	*P*-value
N.	10	10	10		
C6:0	1.17	1.40	1.45	0.50	0.1461
C8:0	0.63	0.62	1.18	0.55	0.0474
C12:0	0.10	0.17	0.40	0.42	0.2581
C14:0	8.35[a]	6.88[b]	7.44[ab]	1.21	0.0353
C15:0	1.56	1.58	2.15	0.84	0.2219
C16:0	404	354	395	54.0	0.1028
C17:0	2.48	2.30	2.87	0.53	0.0627
C18:0	183	163	185	28.9	0.1808
C20:0	5.16	4.48	4.82	0.81	0.1983
C24:0	4.07[B]	5.26[AB,b]	7.90[A,a]	1.85	0.0003
∑SFA	610	539	609	83.3	0.1122
C14:1	0.18	0.21	0.65	0.76	0.3151
C15:1	2.47[ab]	2.31[b]	2.97[a]	0.49	0.0151
C16:1	49.2	41.7	41.2	8.66	0.0876
C18:1 *n*-9	653	572	621	99.0	0.1999
C18:1 *n*-11	34.6	31.9	32.0	5.01	0.3968
∑MUFA	740	648	698	108	0.1848
C18:2 *n*-6	498	444	481	87.5	0.3919
C18:3 *n*-6	4.54[A]	3.90[AB]	2.49[B]	1.42	0.0101
C18:3 *n*-3	9.11[B]	7.91[B]	27.4[A]	5.00	<0.0001
C20:2 *n*-6	10.9	10.3	12.4	2.29	0.1291
C20:3 *n*-6	11.3	9.29	9.91	2.12	0.1218
C20:4 *n*-6	63.5	55.9	54.5	12.4	0.2360
∑PUFA	601	532	588	99.4	0.2781
∑*n*-6	592	524	561	95.3	0.2961
∑*n*-3	9.11[B]	7.91[B]	27.4[A]	5.01	<0.0001

Abbreviations: SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids.
[1]Residual standard deviation.
[A,B]Means in the same row with different superscript letters differ for $P < 0.01$ or $P < 0.001$.
[a,b]Means in the same row with different superscript letters differ for $P < 0.05$.

Table 6. Effect of the dietary inclusion either with 0% (C) or 4% of defatted silkworm (*Bombyx mori* L.) pupae meal during starter (SWM1) or grower-finisher (SWM2) phases on the fatty acids profile (% of total FAME) and oxidative status (mg MDA/kg meat) of chicken leg meat.

	Experimental diets				
	C	SWM1	SWM2	RSD[1]	*P*-value
N.	10	10	10		
C6:0	0.04	0.04	0.05	0.01	0.5485
C8:0	0.06^{A}	0.05^{B}	0.04^{B}	0.01	<0.0001
C10:0	0.02	0.01	0.02	0.01	0.2617
C12:0	0.04	0.03	0.03	0.01	0.0965
C14:0	0.41	0.41	0.40	0.02	0.6986
C15:0	0.09	0.09	0.09	0.01	0.7048
C16:0	19.3	19.1	18.8	0.68	0.3341
C17:0	0.11^{B,b}	0.12^{A,b}	0.14^{A,a}	0.01	0.0041
C18:0	7.54	7.25	7.21	0.41	0.1649
C20:0	0.21^{A}	0.17^{A}	0.09^{B}	0.04	<0.0001
C22:0	0.00^{B}	0.02^{B}	0.11^{A}	0.03	<0.0001
C24:0	0.14^{A}	0.13^{A}	0.07^{B}	0.03	<0.0001
∑SFA	27.9^{a}	27.4^{b}	27.1^{b}	0.70	0.0312
C14:1	0.09	0.09	0.09	0.01	0.9898
C15:1	0.07^{A}	0.06^{A}	0.04^{B}	0.01	<0.0001
C16:1	3.39	3.31	3.03	0.54	0.3170
C17:1	0.00^{B}	0.02^{B}	0.09^{A}	0.02	<0.0001
C18:1 *n*-9	33.1	32.8	32.6	1.15	0.6760
C18:1 *n*-11	1.45	1.49	1.44	0.27	0.8889
C20:1 *n*-9	0.00^{B,b}	0.08^{B,a}	0.17^{A}	0.06	<0.0001
∑MUFA	38.1	37.9	37.5	1.69	0.7284
C18:2 *n*-6	27.2	28.0	27.2	1.51	0.3998
C18:3 *n*-6	0.25^{A}	0.23^{A}	0.16^{B}	0.03	<0.0001
C18:3 *n*-3	0.52^{B}	0.59^{B}	1.69^{A}	0.10	<0.0001
C20:2 *n*-6	0.27^{B}	0.36^{A}	0.43^{A}	0.08	0.0006
C20:3 *n*-6	0.38^{A}	0.29^{A}	0.07^{B}	0.09	<0.0001
C20:4 *n*-6	2.17	2.08	1.85	0.28	0.0516
C20:3 *n*-3	0.00^{B}	0.00^{B}	0.03^{A}	0.02	0.0010
C20:5 *n*-3	0.05	0.05	0.09	0.04	0.0650
C22:6 *n*-3	0.00^{B}	0.00^{B}	0.08^{A}	0.01	<0.0001
∑PUFA	30.9	31.7	31.6	1.90	0.6094
∑*n*-6	30.3	31.0	29.8	1.81	0.3226
∑*n*-3	0.61^{B}	0.65^{B}	1.85^{A}	0.13	<0.0001
n-6/*n*-3	49.9^{A}	48.1^{A}	16.2^{B}	3.53	<0.0001
Identified	96.9	96.9	96.1		
TBARs[2]	0.70	0.69	0.67	0.03	0.4505

Abbreviations: SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids.
[1]Residual standard deviation.
[2]TBARs = thiobarbituric acid-reactive substances (n=5 samples/treatment).
[A,B]Means in the same row with different superscript letters differ for $P < 0.01$ or $P < 0.001$.
[a,b]Means in the same row with different superscript letters differ for $P < 0.05$.

***n*-6**), and dihomo-γ-linolenic acid (**C20:3 *n*-6**)), this was not enough to generate an appreciable effect on the overall *n*-6 fraction, that showed similar values in all groups. Diversely, *n*-3 proportion increased in SWM2 meat compared to that of SWM1 and C treatments (0.61 and 0.65 *vs.* 1.85 % for C, SWM1 and SWM2 groups, respectively; $P < 0.001$), which determined a reduction in the *n*-6/*n*-3 ratio ($P < 0.001$). This outcome was mainly related to the observed increase in the relative proportion of C18:3 *n*-3 ($P < 0.001$). Despite the variations in the FA profile of meat, its oxidative status was similar in the 3 treatments. The significant modifications highlighted for the FA percentage of leg meat also affected their quantitative presence (Table 7). SWM2 meat had the highest amount of behenic acid (**C22:0**) ($P < 0.001$), but the lowest quantities of C8:0 ($P < 0.01$), C20:0 ($P < 0.001$), and C24:0 ($P < 0.001$) SFA. The dietary inclusion of defatted SWM in the period 11 to 42 d, produced a chicken leg meat richer in C17:1 ($P < 0.001$), and C20:1 *n*-9 ($P < 0.001$) MUFA. However, the greatest influence of the SWM2 dietary treatment was observed on the amount of *n*-3 PUFA: 21.8 and 22.1 *vs.* 60.1 mg/100 g meat for C, SWM1 and SWM2 groups, respectively ($P < 0.001$). This result was associated with elevated levels of ALA ($P < 0.001$), eicosapentaenoic acid (**C20:5 *n*-3, EPA**) (P<0.05), and docosahexaenoic acid (**C22:6 *n*-3, DHA**) ($P < 0.001$) individual PUFA.

Amino Acid Profile of Chicken Breast and Leg Meat

The effect of a 4% dietary inclusion with defatted SWM in the starter (SWM1) or grower-finisher (SWM2) diets of broiler chickens on their meat AA content (g/100 g meet) is presented in Table 8 and Table 9 (for breast and leg meat cuts, respectively). Both breast and leg meat showed similar amounts of essential and non-essential AA in the 3 dietary treatments. Breast meat had an average 9.65 g/100 g meat of essential AA, while non-essential ones accounted for 11.1 g/100 g meat.

Table 7. Effect of the dietary inclusion either with 0% (C) or 4% of defatted silkworm (*Bombyx mori* L.) pupae meal during starter (SWM1) or grower-finisher (SWM1) phases on the fatty acid content (mg/100 g meat) of chicken leg meat.

	Experimental diets				
	C	SWM1	SWM2	RSD[1]	P-value
N.	10	10	10		
C6:0	1.55	1.51	1.55	0.45	0.9757
C8:0	1.99^{A,a}	1.56^{AB,b}	1.32^{B}	0.37	0.0014
C10:0	0.61	0.43	0.68	0.35	0.2972
C12:0	1.33	1.14	0.85	0.46	0.0851
C14:0	14.4	14.2	13.2	3.04	0.6320
C15:0	3.27	3.17	3.09	0.75	0.8684
C16:0	686	657	613	142	0.5164
C17:0	4.06	3.99	4.40	0.98	0.6051
C18:0	269	249	234	54.3	0.3690
C20:0	7.60^{A}	5.97^{AB,a}	3.01^{B,b}	2.29	0.0004
C22:0	0.00^{B}	0.72^{B}	3.69^{A}	0.93	<0.0001
C24:0	5.03^{A}	4.46^{A}	2.11^{B}	1.38	0.0001
∑SFA	995	943	880	202	0.4600
C14:1	3.27	3.17	3.04	0.83	0.8289
C15:1	2.45^{A}	2.16^{AB,a}	1.36^{B}	0.63	0.0018
C16:1	121	114	98.9	30.4	0.2815
C17:1	0.12^{B}	0.79^{B}	2.97^{A}	0.85	<0.0001
C18:1 *n*-9	1173	1134	1062	246	0.5990
C18:1 *n*-11	51.6	51.5	47.4	14.2	0.7496
C20:1 *n*-9	0.00^{B,b}	2.40^{B,a}	5.66^{A}	1.86	<0.0001
∑MUFA	1351	1308	1222	287	0.5942
C18:2 *n*-6	967	962	887	202	0.6155
C18:3 *n*-6	8.75^{A}	7.79^{AB,a}	5.38^{B,b}	1.97	0.0021
C18:3 *n*-3	18.5^{B}	20.3^{B}	55.0^{A}	6.93	<0.0001
C20:2 n-6	9.19^{b}	12.4^{ab}	13.8^{a}	3.31	0.0130
C20:3 *n*-6	13.6^{A}	10.1^{A}	2.53^{B}	4.35	<0.0001
C20:4 *n*-6	77.4	70.5	60.0	16.4	0.0757
C20:3 *n*-3	0.00^{B}	0.00^{B}	0.86^{A}	0.53	0.0011
C20:5 *n*-3	1.55^{b}	1.69^{ab}	3.30^{a}	1.52	0.0280
C22:6 *n*-3	0.00^{B}	0.12^{B}	2.68^{A}	0.37	<0.0001
∑PUFA	1097	1085	1029	230	0.7765
∑*n*-6	1076	1063	969	224	0.5124
∑*n*-3	21.8^{B}	22.1^{B}	60.1^{A}	7.64	<0.0001

Abbreviations: SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids.
[1]Residual standard deviation.
^{A,B}Means in the same row with different superscript letters differ for $P < 0.01$ or $P < 0.001$.
^{a,b}Means in the same row with different superscript letters differ for $P < 0.05$.

Table 8. Effect of the dietary inclusion either with 0% (C) or 4% of defatted silkworm (*Bombyx mori* L.) pupae meal during starter (SWM1) or grower-finisher (SWM2) phases on the amino acid content (g/100 g meat) of chicken breast meat.

	Experimental diets				
	C	SWM1	SWM2	RSD[1]	*P*-value
N.	10	10	10		
Essential amino acids					
Arginine	1.23	1.30	1.28	0.19	0.6852
Histidine	0.67	0.70	0.75	0.20	0.7090
Isoleucine	0.77	0.81	0.81	0.12	0.6797
Leucine	1.57	1.65	1.65	0.25	0.7198
Lysine	2.00	2.15	2.11	0.53	0.8032
Methionine	0.48	0.51	0.49	0.06	0.4622
Phenylalanine	0.86	0.90	0.91	0.08	0.4333
Threonine	0.89	0.94	0.94	0.12	0.5841
Valine	0.82	0.89	0.88	0.10	0.3292
Non-essential amino acids					
Alanine	1.25	1.33	1.32	0.22	0.6885
Aspartic acid	2.08	2.20	2.18	0.44	0.7880
Cysteine	0.16	0.16	0.16	0.03	0.9414
Glutamic acid	3.84	4.09	4.05	1.04	0.8446
Glycine	0.92	1.02	0.96	0.16	0.3793
Proline	0.78	0.84	0.82	0.12	0.5425
Serine	0.87	0.90	0.91	0.20	0.8676
Tryptophan	0.24	0.23	0.24	0.02	0.0552
Tyrosine	0.57	0.60	0.61	0.06	0.3239

[1]Residual standard deviation.

Table 9. Effect of the dietary inclusion either with 0% (C) or 4% of defatted silkworm (*Bombyx mori* L.) pupae meal during starter (SWM1) or grower-finisher (SWM2) phases on the amino acid content (g/100 g meat) of chicken leg meat.

	Experimental diets				
	C	SWM1	SWM2	RSD[1]	*P*-value
N.	5	5	5		
Essential amino acids					
Arginine	1.16	1.17	1.18	0.08	0.8861
Histidine	0.19	0.20	0.21	0.03	0.6074
Isoleucine	0.55	0.56	0.52	0.06	0.5259
Leucine	1.44	1.44	1.44	0.11	0.9985
Lysine	2.05	2.05	2.05	0.17	0.8876
Methionine	0.37	0.37	0.37	0.03	0.9719
Phenylalanine	0.68	0.68	0.69	0.04	0.8579
Threonine	0.81	0.81	0.83	0.06	0.7569
Valine	0.63	0.64	0.62	0.06	0.8459
Non-essential amino acids					
Alanine	1.21	1.23	1.28	0.09	0.4915
Aspartic acid	2.04	2.04	2.15	0.15	0.4205
Cysteine	0.11	0.11	0.11	0.01	0.7024
Glutamic acid	3.85	3.88	4.05	0.28	0.4858
Glycine	0.98	1.04	1.04	0.08	0.4387
Proline	0.82	0.85	0.86	0.06	0.6235
Serine	0.89	0.89	0.94	0.05	0.2764
Tryptophan	0.17	0.17	0.18	0.03	0.9760
Tyrosine	0.47	0.47	0.47	9.04	0.9912

[1]Residual standard deviation.

Essential and non-essential AA in the leg meat accounted for 7.90 and 10.8 g/100 g meat, respectively.

Sensory Analysis

Results of the sensory analysis of chicken breast meat are presented in Table 10 and Table 11. The provision of a 4% defatted SWM to broiler chickens in their starter phase produced a juicier meat compared to that of the C group, with SWM2 one being intermediate (46.4, 67.2, 53.8 mm for C, SWM1 and SWM2 meat, respectively; $P < 0.05$). The same result was detected for the overall off-flavor intensity (44.4, 5.56, 27.8 mm for C, SMW1 and SWM2 meat, respectively; $P < 0.05$). Meat of the SWM1 and SWM2 treatments was scored more tender compared to that of the C ($P < 0.05$). All the other meat sensory attributes as well as specific off-odors and off-flavors were similar in the 3 experimental treatments.

DISCUSSION

One of the interesting aspects about the nutritional profile of silkworm pupa, besides its protein and AA contents, is the FA profile, particularly rich in *n*-3 FA. This clearly emerges from the data presented in Table 1, where SWM displayed a 29.6% *n*-3 PUFA. Among them, α-linolenic acid (C18:3 *n*-3: ALA), known to be an essential FA in mammalian and avian species (Simopoulos, 2009), represented more than the totality of *n*-3 PUFA.

Despite the tested SWM was defatted, a residual quote of lipids was still present (9.49%, as shown by Zsedely et al., 2023) which was sufficient to provide an

Table 10. Effect of the dietary inclusion either with 0% (C) or 4% of defatted silkworm (*Bombyx mori* L.) pupae meal during starter (SWM1) or grower-finisher (SWM1) phases on the sensory traits of chicken breast meat.

	Experimental diets				
	C	SWM1	SWM2	RSD[1]	*P*-value
N.	18	18	18		
Odor					
General intensity	55.3	48.9	52.1	13.1	0.3571
Texture					
Juiciness, mm	46.4[B]	67.2[A]	53.8[AB]	19.6	0.0103
Tenderness, mm	90.2[B,b]	110[A,a]	105[AB,a]	15.4	0.0014
Flavor					
General intensity, mm	59.9	58.6	54.9	12.2	0.4568

Each sensory descriptor was scored on a 150 mm long 10-point scale: from 0 (the lowest score for each descriptor) to 10 (the highest score for each descriptor).
[1]Residual standard deviation.
[A,B]Means in the same row with different superscript letters differ for $P < 0.01$.
[a,b]Means in the same row with different superscript letters differ for $P < 0.05$.

Table 11. Effect of the dietary inclusion either with 0% (C) or 4% of defatted silkworm (*Bombyx mori* L.) pupae meal during starter (SWM1) or grower-finisher (SWM1) phases on the off-odor and off-flavor evaluation on chicken breast meat.

	Experimental diets				
	C	SWM1	SWM2	χ^2	*P*-value
N.	18	18	18		
Odor					
Off-odor intensity, mm	44.4	38.9	16.7	3.50	0.1738
Flavor					
Off-flavor intensity, mm	44.4[b]	5.56[a]	27.8[ab]	7.14	0.0282
Bombyx mori/walnut/petfood, mm	22.2	16.7	33.3	1.42	0.4920
Liver/blood/metallic, mm	38.9	16.7	33.3	2.31	0.3152

Each sensory descriptor was scored on a 150 mm long 10-point scale: from 0 (the lowest score for each descriptor) to 10 (the highest score for each descriptor).
[a,b]Means in the same row with different superscript letters differ for $P < 0.05$.

appreciable improvement of the FA profile of diets (*n*-6/*n*-3 was 37.6, 49.2 and 52.7 in C diets compared to 7.60, 13.0, 14.5 in SWM ones). However, this had an overall negligible effect on the FA composition of the breast and leg meat in the SWM1 group, whereas a significant impact on the meat of the SWM2 treatment was observed. The main purpose of the present research, which represents a novelty in the available literature on the possible use of SWM in chicken nutrition, was to test this emerging feed ingredient at different phase feeding of broiler chicken, namely starter (SWM1) *vs.* grower-finisher (SWM2). This represents a crucial aspect since, on one hand, newly hatched broiler chicks have a poorly developed gastrointestinal tract (immature immune system, low secretory capacity of endogenous enzymes, sensitivity to allergenic compounds of feed, unbalanced microbiota), which make the starter diet particularly critical to allow a correct chicks development, and ensure optimum growth in this and later stages of the production cycle (Gilbert et al., 2007; Kim et al., 2020). In the first part of the study is was highlighted that SWM1 diet did not cause any adverse effect of chicks' growth (Zsedely et al., 2023), thus indicating that a 4% dietary inclusion is an adequate level to prevent the digestive disorders (lower digestibility of protein and starch fractions) that were observed, for example, when higher amounts of defatted SWM (12.5%) were provided to growing broiler quails (Dalle Zotte et al., 2021).

On another hand, the grower-finisher diet needs to allow the full expression of the broiler's growth potential and it can be important to define the FA profile and content of meat (Smink et al., 2010). Upon examination of the SWM2 meat (including both breast and leg meat cuts) it was indirectly evident that the FA profile of the SWM2 diet influenced the FA metabolism, consequently impacting FA deposition in the meat. This was evidenced by modifications in the relative proportions and contents of individual SFA, MUFA and PUFA compared to C and SWM1 treatments, as detailed in the results section.

Despite the commonly asserted effectiveness of diet in shaping the FA profile and content of monogastric animal's meat, it is essential to emphasize that the composition of the animal fat is contingent upon the delicate balance between dietary FA and those synthesized *de novo* from carbohydrates and protein precursors (Villaverde et al., 2006). If the diet satisfies the need for essential FA, the deposition of the PUFA fraction almost entirely relies on the diet. Differently, SFA and MUFA proportions and amounts are strongly affected by the above-mentioned metabolic balance and their deposition in body tissues is negatively correlated with the PUFA deposition (Lopez-Ferrer et al., 2001a, 2001b). Given this, it is important to further consider that the experimental diets maintained comparable absolute fat contents (Zsedely et al., 2023). This similarity may account for the observed effects on specific individual SFA and MUFA due to dietary treatments, whereas the overall amounts of SFA, MUFA and PUFA remained unchanged (Villaverde et al., 2006).

Focusing on *n*-3 PUFA, ALA serves as a precursor for the biosynthesis of long-chain *n*-3 FA. Pertinently, it has been demonstrated that chickens fed diets enriched with ALA exhibit inhibition of hepatic fatty acid synthase activity (Cui et al., 2019), which is a pivotal enzyme in the FA biosynthesis. On the other hand, the activity level of Δ6-desaturase in the chicken's liver is high, therefore making it capable of synthesizing long-chain FA starting from dietary ALA. In fact, both EPA and DHA were detected in the leg meat of SWM2 meat, but not in the breast meat. This cut-dependent difference was probably attributable to the limited lipid content of defatted SWM tested in the present study, as well as to the limited absolute fat content of the breast meat cut which is surely not one of the primary sites in the chickens' body where dietary FA modifications are evident. In fact, chicken breast mainly contains intramuscular fat which is mostly composed by membrane phospholipids which are involved in different metabolic pathways, and, for this reason, their composition is under strict homeostatic control (Villaverde et al., 2006). The present research outcomes are coherent with a previous experiment where the breast meat of chickens fed either with 7 or 14 % full-fat SWM (Miah et al., 2020) showed an ALA increase by a factor of 2.2 and 4.2, respectively. This, however, also led to an increase of EPA and DHA proportions, which was not the case of the present study for the breast meat cut. Once again, the reason for the different outcomes was attributable to

the different lipid amounts of the defatted SWM and full fat SWM in the 2 studies.

An increase in the unsaturation degree of meat degree makes it more prone to oxidative deterioration which can lead to the development of unwanted compounds, responsible for the qualitative deterioration of the food product and/or harmful for consumer's health (Cortinas et al., 2004). Results of the present research indicated that a 4% SWM inclusion in the starter (SWM1) or in the grower-finisher period (SMW2) did not affect the oxidative status of fresh meat, which is coherent with the results observed when SWM oil was included in the diet of growing rabbits as a total replacement of sunflower oil (Dalle Zotte et al., 2022).

Together with FA, AA profile is another crucial nutritional factor that must be carefully evaluated, since AA are involved/play key functions in different physiological pathways (Wu et al., 2014) and directly influence the protein quality of a food product, in this case, meat. Protein quality is measured by considering its efficiency in supporting body protein metabolism, which is affected by the total amount of available nitrogen and on the levels of essential AA. For humans they are histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, and valine (Millward et al., 2008). Chicken meat itself is an excellent source of high-quality protein, which showed to be able to meet or exceed the daily requirements indicated by the World Health Organization (Dalle Zotte et al., 2020). Literature indicates that *Bombyx mori*, together with a significant protein content (Pietras et al., 2021; Zsedely et al., 2023), has an excellent AA profile which is characterized to be particularly rich in methionine (Banday et al., 2023), one of the main limiting AA for poultry nutrition. As a result, together with ensuring normal and satisfactory chickens' growth patterns (Zsedely et al., 2023), meat quality insights of the present study emphasized that the amino acid contents of breast and leg meat obtained from chickens fed with inclusion of SWM at different growth stages were in line with those of the control group. This result found validation in existing literature on the use of SWM as protein source for food-producing animals (Rahimnejad et al., 2019; Kowalska et al., 2020; de Souza Vilela et al., 2021), which further confirms the high nutritional value of its protein fraction and thus suitability as feed ingredient up to certain inclusion thresholds (Sheikh et al., 2018).

Ensuring the acceptance of a food product is a priority for both consumers and the market, as it ensures the sustained purchasing and consumption of the product. The sensory characteristics are pivotal in this regard (Meilgaard et al., 2015). This importance is particularly relevant for novel food products or, as in this instance, when new feedstuffs are exploited as dietary ingredients for food-producing animals. The influence of insect-derived products on the sensory traits of poultry meat has recently been reviewed by Shaviklo (2023). One notable observation is the limited availability of literature specifically focusing on SWM, highlighting the necessity for further research efforts in this domain. Another aspect to consider is the highly heterogeneous impact of insects and the derived products on the sensory profile of poultry meat. This heterogeneity is contingent upon factors such as the tested insect species (which can exhibit diverse sensory attributes), dietary inclusion levels (with certain functional compounds intrinsically present in insects potentially affecting animal performance and, in turn, meat quality attributes), meat cut (with varying chemical composition leading to distinct sensory features) and the sensory evaluation method employed (consumer test or trained panel).

In the context of studying the dietary inclusion of SWM and its impact on meat sensory analysis, current knowledge is still in its infancy. A pivotal aspect requiring attention is the thorough characterization of flavor compounds specific to SWM, analogous to the detailed investigations conducted on the sensory attributes of both raw and cooked *Tenebrio molitor* larvae (Seo et al. (2020). Until now, the available literature on the SWM indicates that its dietary inclusion up to 20% in the diet of broiler chickens for 21 d does not have any discernible impact on the sensory characteristics of breast meat (Mentang et al., 2013; Pietras et al., 2021). This partially aligns with the findings of the current research, as overall breast meat odor and flavor intensities remained unchanged. However, contrary to previous observations, breast meat tenderness improved in SWM-fed chickens. In addition, SWM1 meat exhibited the highest juiciness but also the highest off-flavor intensity. The reason for these findings should however be understood since the result is not attributable to the effects of the dietary treatments on the chemical features of chickens' meat depicted in the present study as well as in the previously published contribution of the same experiment (Zsedely et al., 2023).

CONCLUSIONS

Results of the current study confirmed that the defatted SWM is a suitable alternative feedstuff for broiler chickens, that can be administered at different growth phases without negative effects on meat quality characteristics, including sensory attributes. The phase feeding trial revealed that incorporating this emerging feedstuff during the grower-finisher period can enhance the nutritional quality of chicken meat, particularly by increasing the *n*-3 fatty acids content. This enhancement is attributed to the substantial presence of α-linolenic acid in silkworm pupae. The defatting process, ensuring an approximate 60 g crude protein /100 g product, also retains a considerable residual lipid content (9.49 g/100 g product). Further insights into the sensory characteristics of the silkworm pupa would be crucial to provide key information in order to have a clear view on the observed results on the sensory characteristics of silkworm-fed chickens' meat, as well as to develop more accurate sensory evaluation procedures.

ACKNOWLEDGMENTS

The present research was financially supported by the University of Padova (Italy) funds (BIRD234733/23)

DISCLOSURES

The authors declare no conflicts of interest.

REFERENCES

Banday, M. T., S. Adil, I. U. Sheikh, H. Hamadani, F. I. Qadri, M. E. Sahfi, H. S. A. W. Sait, T. A. Abd El-Mageed, H. M. Salem, A. E. Taha, M. T. El-Saadony, and M. E. Abd El-Hack. 2023. The use of silkworm pupae (*Bombyx mori*) meal as an alternative protein source for poultry. World's Poult. Sci. J. 79:119–134.

Botsoglou, N. A., D. J. Fletouris, G. E. Papageorgiou, V. N. Vassilopoulos, A. J. Mantis, and A. G. Trakatellis. 1994. Rapid, sensitive, and specific thiobarbituric acid method for measuring lipid peroxidation in animal tissue, food, and feedstuff samples. J. Agric. Food Chem. 42:1931–1937.

Cortinas, L., C. Villaverde, J. Galobart, M. D. Baucells, R. Codony, and A. C. Barroeta. 2004. Fatty acid content in chicken thigh and breast as affected by dietary polyunsaturation level. Poult. Sci. 83:1155–1164.

Council of Europe. 2005. European Pharmacopoeia 5.0, 5th rev. ed., 2.2.56. Amino Acid Analysis − Protein Hydrolysis − Methods 1, 5, 7. Pages 87-92 in Directorate for the Quality of Medicines, Council of Europe, Strasbourg, France.

Cui, X., Z. Gou, Q. Fan, L. Li, X. Lin, Y. Wang, S. Jiang, and Z. Jiang. 2019. Effects of dietary perilla seed oil supplementation on lipid metabolism, meat quality, and fatty acid profiles in Yellow-feathered chickens. Poult. Sci. 98:5714–5723.

Cullere, M., Y. Singh, Z. Gerencsér, Z. Matics, S. Cappellozza, and A. Dalle Zotte. 2022. Silkworm (*Bombyx mori* L.) oil in growing rabbit nutrition: effects on meat physicochemical traits, sensory profile and shelf-life. J. Insects as Food Feed. 8:733–741.

Dalle Zotte, A., R. Ricci, M. Cullere, L. Serva, S. Tenti, and G. Marchesini. 2020. Research note: effect of chicken genotype and white striping−wooden breast condition on breast meat proximate composition and amino acid profile. Poult. Sci. 99:1797–1803.

Dalle Zotte, A., Y. Singh, Z.s. Gerencsér, Z.s. Matics, Z.s. Szendrő, S. Cappellozza, and M. Cullere. 2022. Feeding silkworm (*Bombyx mori* L.) oil to growing rabbits improves the fatty acid composition of meat, liver and perirenal fat. Meat Sci 193:108944.

Dalle Zotte, A., Y. Singh, A. Squartini, P. Stevanato, S. Cappellozza, A. Kovitvadhi, S. Subaneg, D. Bertelli, and M. Cullere. 2021. Effect of a dietary inclusion of full-fat or defatted silkworm pupa meal on the nutrient digestibility and faecal microbiome of fattening quails. Animal 15:100112.

de Souza Vilela, J., T. I. Alvarenga, N. R. Andrew, M. McPhee, M. Kolakshyapati, D. L. Hopkins, and I. Ruhnke. 2021. Technological quality, amino acid and fatty acid profile of broiler meat enhanced by dietary inclusion of black soldier fly larvae. Foods 10:297.

European Commission (EC). 2010. Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes (text with EEA relevance). Off. J. Eur. Union L 276:33. Accessed Jan. 2024 http://data.europa.eu/eli/dir/2010/63/2019-06-26.

FAOSTAT. 2023. Food and agriculture data: crops and livestock products. Accessed Nov. 2023. https://www.fao.org/faostat/en/#data/QCL

FAO, IFAD, UNICEF, WFP, and WHO. 2021. The state of food security and nutrition in the world 2021. Transforming food systems for food security, improved nutrition And affordable healthy diets for all. Food and Agriculture Org. (FAO), Rome, Italy.

Gao, K., C. Zheng, T. Wang, H. Zhao, J. Wang, Z. Wang, X. Zhai, Z. Jia, J. Chen, Y. Zhou, and W. Wang. 2016. 1-Deoxynojirimycin: occurrence, extraction, chemistry, oral pharmacokinetics, biological activities and in silico target fishing. Molecules 21:1600.

Gilbert, E. R., H. Li, D. A. Emmerson, K. E. Webb Jr, and E. A. Wong. 2007. Developmental regulation of nutrient transporter and enzyme mRNA abundance in the small intestine of broilers. Poult. Sci. 86:1739–1753.

Kim, E., N. Akhtar, J. Li, Q. Hui, B. Dong, C. Yang, and E. G. Kiarie. 2020. *In ovo* feeding of epidermal growth factor: embryonic expression of intestinal epidermal growth factor receptor and posthatch growth performance and intestinal development in broiler chickens. Poult. Sci. 99:5736–5743.

Kowalska, D., A. Gugołek, and J. Strychalski. 2020. Evaluation of slaughter parameters and meat quality of rabbits fed diets with silkworm pupae and mealworm larvae meals. Ann. Anim. Sci. 20:551–564.

Lopez-Ferrer, S., M. D. Baucells, A. C. Barroeta, and M. A. Grashorn. 2001a. *n*-3 enrichment of chicken meat. 1. Use of very long-chain fatty acids in chicken diets and their influence on meat quality: fish oil. Poult. Sci. 80:741–752.

Lopez-Ferrer, S., M. D. Baucells, A. C. Barroeta, J. Galobart, and M. Grashorn. 2001b. *n*-3 enrichment of chicken meat. 2. Use of precursors of long-chain polyunsaturated fatty acids: linseed oil. Poult. Sci. 80:753–761.

Makkar, H. P., G. Tran, V. Heuzé, and P. Ankers. 2014. State-of-the-art on use of insects as animal feed. Anim. Feed Sci. Technol. 197:1–33.

Marascuilo, L. A. 1966. Large-sample multiple comparisons. Psychol. bull. 65:280.

Meilgaard, M. C., G. V. Civille, and B. T. Caar. 2015. Sensory Evaluation Techniques. 4th ed. CRC Press, London. Accessed Mar. 2023. https://thuvienso.hoasen.edu.vn/bitstream/handle/123456789/8149/Contents.pdf?sequence=4&isAllowed=y.

Mentang, F., O. Jintasataporn, and T. Ohshima. 2013. Fatty acid composition and sensory evaluation of the meat of broilers fed silkworm (*Bombyx mori* L) pupa dietary supplementation. Anim. Prod. 15:24–30. Accessed Jan. 2024 http://animalproduction.net/index.php/JAP/article/view/405.

Miah, M. Y., Y. Singh, M. Cullere, S. Tenti, and A. Dalle Zotte. 2020. Effect of dietary supplementation with full-fat silkworm (*Bombyx mori* L.) chrysalis meal on growth performance and meat quality of Rhode Island Red× Fayoumi crossbred chickens. Ital. J. Anim. Sci. 19:447–456.

Millward, D. J., D. K. Layman, D. Tomé, and G. Schaafsma. 2008. Protein quality assessment: impact of expanding understanding of protein and amino acid needs for optimal health. Am. J. Clin. Nutr. 87:1576S–1581S.

Patil, S. R., S. Amena, A. Vikas, P. Rahul, K. Jagadeesh, and K. Praveen. 2013. Utilization of silkworm litter and pupal waste-an eco-friendly approach for mass production of *Bacillus thuringiensis*. Biores. Technol. 131:545–547.

Pietras, M., S. Orczewska-Dudek, W. Szczurek, and M. Pieszka. 2021. Effect of dietary lupine seeds (*Lupinus luteus* L.) and different insect larvae meals as protein sources in broiler chicken diet on growth performance, carcass, and meat quality. Livest. Sci. 250:104537.

Rahimnejad, S., S. Hu, K. Song, L. Wang, K. Lu, R. Wu, and C. Zhang. 2019. Replacement of fish meal with defatted silkworm (*Bombyx mori* L.) pupae meal in diets for Pacific white shrimp (*Litopenaeus vannamei*). Aquaculture 510:150–159.

Razdan, A., and D. Pettersson. 1994. Effect of chitin and chitosan on nutrient digestibility and plasma lipid concentrations in broiler chickens. Brit. J. Nutr. 72:277–288.

ROSS Broiler Management Handbook. 2018. Accessed Jan. 2023. https://en.aviagen.com/assets/Tech_Center/Ross_Broiler/Ross-BroilerHandbook2018-EN.pdf.

Seo, H., H. R. Kim, and I. H. Cho. 2020. Aroma characteristics of raw and cooked *Tenebrio molitor* larvae (mealworms). Food Sci. Anim. Resour. 40:649–658.

Shaviklo, A. R. 2023. The influence of insect-derived and marine-based diets on sensory quality of poultry meat and egg: a systematic review. J. Food Sci. Technol. 60:1903–1922.

Sheikh, I. U., M. T. Banday, I. A. Baba, S. Adil, S. S. Nissa, B. Zaffer, and K. H. Bulbul. 2018. Utilization of silkworm pupae meal as an alternative source of protein in the diet of livestock and poultry: a review. J. Entomol. Zool. Stud. 6:1010–1016. Accessed Jan. 2024 https://www.entomoljournal.com/archives/2018/vol6issue4/PartR/6-4-78-464.pdf.

Simopoulos, A. P. 2009. Omega-6/omega-3 essential fatty acids: biological effects. World Rev. Nutr. Diet. 99:1–16.

Smink, W., W. J. J. Gerrits, R. Hovenier, M. J. H. Geelen, M. W. A. Verstegen, and A. C. Beynen. 2010. Effect of dietary fat sources on fatty acid deposition and lipid metabolism in broiler chickens. Poult. Sci. 89:2432–2440.

van Huis, A., and D. G. Oonincx. 2017. The environmental sustainability of insects as food and feed. A review. Agron. Sustain. Dev. 37:1–14.

Villaverde, C., M. D. Baucells, L. Cortinas, and A. C. Barroeta. 2006. Effects of dietary concentration and degree of polyunsaturation of dietary fat on endogenous synthesis and deposition of fatty acids in chickens. Br. Poult. Sci. 47:173–179.

Wu, G., F. W. Bazer, Z. Dai, D. Li, J. Wang, and Z. Wu. 2014. Amino acid nutrition in animals: protein synthesis and beyond. Annu. Rev. Anim. Biosci. 2:387–417.

Yang, L. F., S. Siriamornpun, and D. U. O. Li. 2006. Polyunsaturated fatty acid content of edible insects in Thailand. J. Food Lipids. 13:277–285.

Zsedely, E., M. Cullere, G. Takacs, Z. Herman, K. Szalai, Y. Singh, and A. Dalle Zotte. 2023. Dietary inclusion of defatted silkworm (*Bombyx mori* L.) pupa meal for broiler chickens at different ages: growth performance, carcass and meat quality traits. Animals 13:119.