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Effect of a dietary inclusion of full-fat or defatted silkworm pupa meal on the nutrient digestibility and faecal microbiome of fattening quails

A. Dalle Zotte [a,*], Y. Singh [a], A. Squartini [b], P. Stevanato [b], S. Cappellozza [c], A. Kovitvadhi [d], S. Subaneg [a,d], D. Bertelli [e], M. Cullere [a]

[a] Department of Animal Medicine, Production and Health, MAPS, University of Padova, Agripolis, Viale dell'Università 16, 35020 Legnaro, Padova, Italy
[b] Department of Agronomy, Food, Natural Resources, Animals and Environment, DAFNAE, University of Padova, Agripolis, Viale dell'Università 16, 35020 Legnaro, Padova, Italy
[c] Council for Agricultural Research and Economics, Research Centre for Agriculture and Environment (CREA – AA), Sericulture Laboratory of Padua, Via Eulero 6a, 35143, Padova, Italy
[d] Department of Physiology, Faculty of Veterinary Medicine, Kasetsart University, 10900 Bangkok, Thailand
[e] Department of Life Sciences, University of Modena and Reggio Emilia, Via Campi 103, 41125 Modena, Italy

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ABSTRACT

Silkworm (*Bombyx mori* L.) pupae are a by-product derived from silk production, which is often treated as waste and thus discarded: this can cause serious environmental problems and a loss of nutrients. Silkworm pupae are a rich source of protein and lipids, and the resulting protein meal can provide promising outcomes as livestock feed, notably for monogastric species. However, one possible issue that needs to be considered is the possible implication of the 1-Deoxynojirimycin (**1-DNJ**), a bio-compound of the silkworm that impairs glucose absorption, in poultry nutrition. Therefore, the present study evaluated the effect of the dietary inclusion of full-fat or defatted silkworm pupa meal (**SWM**) on the apparent digestibility of nutrients, feed choice and faecal microbiome in meat-producing quails. For the digestibility trial, a total of thirty-three 27-day-old Japanese quails (*Coturnix coturnix japonica*) were individually housed in digestibility cages and received three experimental diets: a control diet (control, commercial feed for fattening quails), and two other diets containing the 12.5% of either a full-fat SWM (**SWM-FULL**) or a defatted SWM (**SWM-DEF**). Subsequently, twenty-seven 33-day-old quails were simultaneously provided with Control, SWM-FULL and SWM-DEF diets for a 10-day feed choice trial. The results of the digestibility trial showed that the DM intake and excreta production were higher in both SWM groups than in the Control one ($P < 0.001$). The apparent digestibility of DM, organic matter, CP, ether extract, starch and energy was lower in both SWM groups than in the control group ($P < 0.001$), suggesting the possible implication of chitin and 1-DNJ. The feed choice test showed that quails preferred the Control diet ($P < 0.001$). From the microbiome analysis of the excreta, families such as Streptococcaceae ($P < 0.05$), Rikenellaceae and Eubacteriaceae ($P < 0.01$) and taxa at species level such as *Lactobacillus delbrueckii* ($P < 0.05$), *Aneurinibacillus thermoaerophilus* and *Bacillus thermoamylovorans* ($P < 0.01$) scored higher in SWM-FULL quails than in SWM-DEF and Control treatments. The present study demonstrated that a successful dietary inclusion of SWM for fattening quails needs to overcome the digestive criticalities caused by the of presence specific bio-compounds, namely chitin and 1-DNJ.

Implications

Due to the rapid development of the poultry industry, the demand for conventional feed ingredients (i.e. soybean meal and oil) has also increased, causing rising costs and further environmental pressure. In this sense, the search for sustainable alternatives has led to a growing interest for protein-rich insect species as novel ingredients for poultry rations. This study suggested that silkworm pupa meal could partially replace conventional ingredients in the diet for fattening quails, upon prior removal of anti-nutritional compounds which are naturally present in the silkworm pupa.

Introduction

Scarcity of animal protein intake has been reported as a critical bottleneck for populations of developing countries where it continues to be an issue of concern. To satisfy the growing meat demand (+58% being expected in the period 2010–2050), an increase in the livestock production will surely be required (Food and Agriculture Organization of the United Nations (FAO), 2013). However, under the present conditions, this would lead to an excessive use of non-renewable resources causing a non-sustainable environmental pressure. In parallel, the rapid

* Corresponding author.
E-mail address: antonella.dallezotte@unipd.it (A. Dalle Zotte).

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expansion in the demand of soybean meal/oil is bound to increase their prices, resulting in an estimated >30% rise in meat prices in the period 2000–2050 (Food and Agriculture Organization of the United Nations (FAO), 2010). The above-mentioned scenario will contribute to an expected number of 821 million undernourished people in the whole world (Food and Agriculture Organization of the United Nations (FAO), 2013).

Poultry production is one of the most convenient ways to increase protein supply. Among poultry species, the Japanese quail (*Coturnix coturnix japonica*) is a small, rustic and nutritious bird with a short production interval, qualifying as an interesting option to face the increasing animal protein demand (Cullere et al., 2018). Specific rations for poultry species are typically grain-based and must be supplemented with sufficient amounts of protein and essential amino acids, to fulfil the animal growth requirements. In this perspective, insects can be a promising and sustainable feed ingredient to satisfy the nutritional needs of poultry species, partially or fully replacing conventional feedstuffs (Cullere et al., 2016). Among the possible candidates, silkworm pupae can be a rich source of nutrients, including high-quality protein and lipids, intended for human consumption and/or for livestock feeding (Makkar et al., 2014).

Bombyx mori is a Lepidopteran species, which is reared for silk production and used by the textile industry. Pupa, resulting by larval metamorphosis, contributes for about 60% of the dry weight of the final cocoon harvest and represents a major by-product of sericulture. Pupae are exploited as food in certain regions of the world. However, they are often discarded, leading to a waste of potential nutrients and to a further environmental problem (Wang et al., 2010).

From the nutritional point of view, silkworm pupa meal has a CP content ranging from 52 to 80% on a DM basis. It is also a notable source of healthy lipids, as omega-3 can account for up to 35–40% of its total fatty acids (FAs) (Makkar et al., 2014). Among the bio-compounds of the silkworm, 1-Deoxynojirimycin (**1-DNJ**) is accumulated upon larval consumption of mulberry leaves, which are their natural feeding substrate (Ryu, 1997). The 1-DNJ, also known as duvoglustat or moranolin, is a potential intestinal α-glucosidase inhibitor, which exerts its biological effect by competitively inhibiting specific glycosidase enzymes as it mimics the enzymes' normal carbohydrate substrates such as D-glucose and D-mannose (Vichasilp et al., 2016). Therefore, as 1-DNJ can inhibit enzymes involved in glycogenolysis, glycoprotein processing and saccharide hydrolysis, the possible use of silkworm pupa into poultry diets needs to be carefully evaluated. Silkworm pupae are also reported to contain chitin, accounting for about 3–4% of the DM (Makkar et al., 2014), which is a polysaccharide with structural function in many organisms, including insects (exoskeleton). Previous research on chickens showed that chitin can act as prebiotic by improving the immune response of birds (Bovera et al., 2015) and increasing the caecal production of butyric acid, primal energy source for enterocytes, which would improve tissue oxygenation, nutrient transport and absorption (Khempaka et al., 2011). Conversely, other studies showed that chitin can worsen nutrients absorption in chickens (Razdan and Pettersson, 1994; Bovera et al., 2015). Based on the above-mentioned considerations, the present research tested the effect of a dietary supplementation with silkworm (*Bombyx mori* L.) pupa meal on the apparent digestibility of nutrients, feed choice and faecal microbiome of meat-producing quails.

Material and methods

Insect

Dried silkworm (*Bombyx mori* L.) cocoons were provided by the farmers belonging to the "Rete Bachicoltura Setica", network in Veneto region (Italy). This farmers' network obtains silkworm polyhybrid eggs from the CREA – AA (Padova, Italy). Cocoons were cut to extract silkworm pupa. Subsequently, half of the collected amount of silkworm pupae was defatted by INNOVHUB (Divisione Olio e Grassi, Stazioni Sperimentali per l'Industria S.r.l., Milano, Italy). Both defatted and full-fat silkworm pupae were ground at 4000 g for 10 s with a Retsch Grindomix GM 200 mill (Retsch GmbH & Co, Haan, Germany) and used to manufacture the experimental diets.

Experimental design

The study was approved by the Ethical Committee of the University of Padova (Protocol number: EC2018/87). The trial was performed at the Japanese quail (*Coturnix coturnix japonica*) farm located in the Padova province (Italy) with which the Department of Animal Medicine, Production and Health – MAPS (University of Padova) has an established scientific agreement. Here, a separated room was dedicated to the trial. Three experimental diets were formulated: a control diet (Control), similar to the fattening diet used in commercial quail farming, and two diets in which 12.5% of either full-fat (**FULL**) or defatted (**DEF**) silkworm pupa meal (**SWM**) was incorporated. Full-fat SWM and defatted SWM mainly replaced soybean meal and soybean oil. All diets were set up to meet the energy and nutrient requirements of Japanese quails and to be as much isoenergy and isonitrogenous as possible (National Research Council, Subcommittee on Poultry Nutrition (NRC), 1994). The ingredients, chemical composition and energy content of the experimental diets are shown in Tables 1 and 2. Experimental diets were used for digestibility and feed choice trials. The environmental conditions of the room were monitored: the average temperature and relative humidity were 18.4 °C and 68.5%, respectively, and the adopted photoperiod was 16 light:8 dark.

Digestibility trial

A total of forty-two 16-day-old quails of both sexes (21 males and 21 females) were individually weighed and assigned to the three dietary treatments (Control, SWM-FULL and SWM-DEF), featuring 14 quails/each. In the three dietary groups, quails had a similar initial live weight (**LW**) and SD (151 ± 9.98 g). After being individually housed in digestibility cages, quails were subjected to 11 days of adaptation to the experimental diets. During this period, the commercial diet was gradually replaced with the experimental diets and individual feed intake was measured.

At the end of the adaptation period, quails (27 days old) were weighed again and selected (quails exhibiting feed waste were excluded), within the dietary group, for the *in vivo* digestibility trial. The

Table 1
Ingredients of the experimental diets (g/kg as fed) for fattening quails.

	Experimental diets		
	Control	SWM-FULL	SWM-DEF
Corn meal	471	431	469
Wheat meal	63.7	183	152
Soybean meal	406	236	205
Silkworm meal	0.00	125	125
Soybean oil	30.7	0.00	22.5
Calcium carbonate	12.1	14.6	14.8
Dicalcium phosphate	9.40	6.00	6.50
NaCl	2.70	2.70	2.70
L-Lysine	0.80	0.00	0.00
DL-Methionine	1.80	0.00	0.00
Vitamin–mineral premix[1]	2.50	2.50	2.50

FULL and DEF correspond to 12.5% inclusion with full-fat (FULL) and defatted (DEF) Silkworm (*Bombyx mori* L.) pupa meal (SWM), respectively.

[1] Vitamin and mineral premix provided the following per kg of diet: vitamin A, 20 000 IU; vitamin D3, 6 000 IU; vitamin E (α-tocopherol acetate), 90 mg; vitamin K3, 7 mg; vitamin B1, 3.5 mg; vitamin B2, 16 mg; niacinamide, 100 mg; vitamin B6, 8 mg; Vitamin B12, 0.04 mg; biotin, 0.4 mg; folic acid, 2.5 mg; Ca-pantothenate, 27.78 mg; Fe, 80 mg; Mn, 200 mg; Cu, 50 mg; Zn, 200 mg; Ca-iodate, 2 mg/kg; Se, 0.4 mg; E 1604 Endo 14, 2 200 U; Endo 1, 3 000 FTU; sepiolite, 175 mg.

Table 2
Chemical composition (g/kg as fed), 1-Deoxynojirimycin (1-DNJ; μg/g) and gross energy contents (MJ/kg as fed) of the full-fat and defatted (*Bombyx mori* L.) pupa meal (FULL and DEF, respectively) and of the experimental diets (SWM-FULL and SWM-DEF, respectively) for fattening quails.

	SWM		Experimental diets		
	FULL	DEF	Control	SWM-FULL	SWM-DEF
DM	944	947	895	894	901
CP	539	667	223	225	228
Ether extract	291	94.9	465	548	534
Crude fibre	–	–	35.9	23.1	21.7
Starch	–	–	396	439	444
Ash	50.1	66.5	54.3	50.2	51.3
Chitin	28.7	34.0	0.00	0.89	1.40
1-DNJ[1]	–	–	ND	1.07	1.23
Gross energy	25.2	21.9	17.4	17.3	17.0

FULL and DEF are diets corresponding to 12.5% inclusion with full-fat (FULL) and defatted (DEF) silkworm (*Bombyx mori* L.) pupa meal (SWM), respectively.
[1] Analysed on diets only.

latter included 11 quails/treatment, ensuring a homogenous LW among experimental groups and sex balance.

After 8 h fasting, quails were fed with the experimental diets for 3 days and subsequently fasted for 8 h. During this period, feed intake and excreta production were accurately determined. Specifically, excreta was daily collected from each cage, cleaned from feed residues and feathers, weighed and stored at +4 °C until the end of the digestibility trial. Afterwards, the excreta of each bird was freeze-dried, ground and stored at +4 °C until subsequent chemical analyses.

At the end of the digestibility trial, quails were fed again with the experimental diets and, after 24 h, individual excreta was collected from five birds/treatment and immediately subjected to microbiome analysis.

Feed choice trial

After excreta collection for microbiome analysis, the three experimental diets were simultaneously offered *ad libitum* to twenty-seven 33-day-old quails. After 3 days of adaptation, a 10-day feed choice trial was carried out, during which each feeder was filled to guarantee the *ad libitum* daily feed intake. Within the cage, the three feeders were placed in complete randomized order and their position was changed every 3 days. At the end of the trial, the feed intake from each feeder was determined on the cage basis (single quail). Feed choice was expressed as % of total feed intake.

Chemical analyses of silkworm pupa meal, diets and excreta

Chemical analyses of the two types of silkworm pupa meal (SWM-FULL and SWM-DEF), experimental diets and freeze-dried excreta were carried out in accordance with the Association of Official Analytical Chemists (AOAC) (2000) methods to determine DM (method no. 934.01), CP (method no. 2001.11), crude fibre (method no. 978.10), ash (method no. 967.05) and starch (amyloglucosidase-α-amylase, method no. 996.11) contents. The ether extract (EE) was determined after acid hydrolysis (EC, 1998). The CP content of excreta was corrected for uric acid content, which was analysed according to the procedure described by Fievez et al. (2001), with the subsequent modifications reported by Cullere et al. (2016). Gross energy was measured with an adiabatic bomb calorimeter (International Organization for Standardization (ISO), 1998).

Fatty acid profile determination of SWM-FULL, SWM-DEF pupa meal and of the three experimental diets was determined as descried by Cullere et al. (2018), with petroleum ether as solvent used for lipid extraction. Results (Table 3) were expressed as % of the total detected fatty acid methyl esters (FAME).

Table 3
Fatty acid profile (% of total fatty acid methyl esters) of the full-fat (FULL) and defatted (DEF) (*Bombyx mori* L.) pupa meal and of the experimental diets for fattening quails.

	SWM		Experimental diets		
	FULL	DEF	Control	SWM-FULL	SWM-DEF
C14:0	–	–	0.09	0.12	0.09
C15:0	0.17	–	0.00	0.08	0.06
C16:0	24.2	23.7	11.7	19.1	14.9
C18:0	5.45	5.89	3.09	3.84	3.32
C20:0	–	–	0.12	0.10	0.15
C23:0	0.59	0.00	–	–	–
Total SFA	30.4	29.6	15.0	23.3	18.5
C16:1	1.04	1.37	0.13	0.69	0.36
C18:1 *n*-9	31.2	29.3	23.4	29.6	25.0
C18:1 *n*-11	–	–	1.07	0.29	0.82
C24:1 *n*-9	0.54	–	–	–	–
Total MUFA	32.8	30.7	24.6	30.6	26.1
C18:2 *n*-6	6.16	6.18	53.9	21.4	41.6
C22:2 *n*-6	0.06	–	–	–	–
C18:3 *n*-6	–	–	0.00	0.07	0.02
C18:3 *n*-3	29.5	31.5	4.48	23.5	12.1
C20:5 *n*-3	–	–	0.24	0.08	0.20
Total PUFA	35.7	37.7	58.6	45.0	54.0
Total *n*-6	6.22	6.18	53.9	21.4	41.6
Total *n*-3	29.5	31.5	4.72	23.6	12.3
n-6/*n*-3	0.21	0.20	11.4	0.91	3.38
Identified fatty acids, %	98.9	98.0	98.2	98.9	98.6

SFA = saturated fatty acids; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids. FULL and DEF correspond to 12.5% inclusion with full-fat (FULL) and defatted (DEF) silkworm (*Bombyx mori* L.) pupa meal (SWM), respectively.

Determination of bioactive compounds

The two pupa meals, the experimental diets and the freeze dried excreta samples were analysed in triplicate to determine the chitin content according to the method described by Zhang and Zhu (2006) with the modifications provided by Woods et al. (2019).

The three experimental diets were also analysed for the quantification of 1-DNJ. The 1-DNJ extraction and quantification were performed applying the methods developed by Wang et al. (2011) and Vichasilp et al. (2016), with the following modification. Two grams of each experimental diet was extracted with 20 ml of solution water:ethanol (50:50) for 2 h, under magnetic stirring and at room temperature. The extracts were then combined, the final volume was adjusted to 50 ml and stored at 4 °C until analysis. The extraction procedure was repeated twice for each sample. Before injection in an ultra-high pressure liquid chromatography (UHPLC) system, extracts were filtered using a 0.22 μm cellulose acetate filter (GVS, Bologna, Italy) and properly diluted with acetonitrile. A Thermo Scientific Dionex Ultimate 3000 UHPLC system (Thermo Scientific, Waltham, MA, USA) coupled with a high resolution Q Exactive mass spectrometer (Thermo Scientific, Bremen, Germany) was used. The capillary temperature was set at 270 °C and the following N_2 flows (arbitrary units) were used: sheath gas 40, auxiliary gas 30 and sweep gas 3. The tandem MS/MS parameters were optimized with standard 1-DNJ under positive ion electrospray ionization. A hydrophilic interaction liquid chromatography (HILIC) column DNJ (Cortecs UPLC HILIC, 1.6 μm, 2.1 × 100 mm) (Waters, Milford, MA, USA) was used. The 1-DNJ was eluted with a binary gradient consisting of ammonium formate 20 mM in water (solvent A) and acetonitrile (solvent B). The gradient profile was as follows: 0–10 min, 10% A; 11–16 min, 50% A; 16–27 min, 10% A. Flow rate was adjusted to 0.3 ml/min, and the column temperature was maintained at 30 °C. The 1-DNJ was detected by MS/MS with parallel reaction monitoring, and the amount was quantified through the calibration curve built with the pure standard compound (Sigma-Aldrich Co. LLC., Saint Louis, USA) at different concentrations (0.051–6.67 ppm). The method showed good linearity ($r^2 > 0.9995$) within the selected range of concentrations. The 1-DNJ content was expressed as μg/g diet.

Faecal microbiome analysis

The excreta of 15 quails ($n = 5$/treatment) was the source for total DNA isolation. In parallel, the experimental diets were also analysed to verify the possible overlapping of the microbiome of the excreta and that of the feed.

Deoxyribonucleic acid extraction was carried out upon homogenizing 100 mg of freshly collected material using a Tissue Lyser (Qiagen, Hilden, Germany) for 5 min at 30 Hz in a 2 ml Eppendorf tube with 300 µl of RTL buffer (Qiagen). Samples were centrifuged for 5 min at 6000 g to collect the supernatant. Deoxyribonucleic acid purification was performed with a Biosprint 96 suite, using the MagAttract HMW DNA Kit (both from Qiagen) as recommended by the manufacturer. Deoxyribonucleic acid was quantified with a Qubit 3.0 Fluorometer (Thermo Fisher Scientific, Waltham, MA, USA) using the Qubit™ DNA HS Assay Kit Fluorometer (Thermo Fisher Scientific).

For the DNA Sequencing and subsequent bioinformatics data analysis, a 16S Ion Metagenomics Kit (Thermo Fisher Scientific) was used to amplify seven hypervariable regions of the 16S rDNA genes. Library preparation was carried out using the standard protocol recommended by the manufacturer. Sequencing was performed with Ion™ Torrent S5 System with 520 chip using 850 flows. Data analysis was conducted with a Torrent Suit (Thermo Fisher Scientific) with default parameters for the 16S target sequencing. Ion Reporter cloud software (version 5.12) was adopted to process 16S metagenomic data.

Statistical analysis

Experimental data on apparent digestibility of nutrients, nutritive value of diets and feed choice were subjected to a one-way ANOVA with the experimental diet (Control, SWM-FULL and SWM-DEF) as a fixed effect, following the GLM procedures of the SAS 9.1.3 statistical analysis software for Windows (Statistical Analysis Software for Windows (SAS), 2008). Least-square means were obtained using the Bonferroni test, and the significance was calculated at a 5% confidence level.

Molecular data regarding bacterial species compositional differences across the different treatments were analysed using the Calypso online software tool (Zakrzewski et al., 2017). The relative abundances of taxa were normalized by applying the total sum of squares scaling normalization followed by square root transformation. Significant differences among treatments were assessed by the non-parametric Kruskal–Wallis test. Pairwise comparisons for differences between individual treatments were performed by the Wilcoxon rank test.

Results

Chemical composition and fatty acids profile of silkworm pupa meal and experimental diets

Results presented in Tables 1 and 2 showed that SWM is a rich source of protein (53.9 and 66.7% as fed for SWM-FULL and SWM-DEF, respectively) and lipids (29.1 and 9.49% as fed for SWM-FULL and SWM-DEF, respectively). Among lipids, a remarkable proportion of polyunsaturated fatty acids were highlighted, whose average value in SWM-FULL and SWM-DEF was 36.7% of total FAME. The latter was mainly attributable to the α-linolenic acid (C18:3 *n*-3), averaging 30.5% of total FAME. As a result, the diets SWM-FULL and SWM-DEF showed a healthier fatty acid profile compared to the Control one, highlighting a reduction of the *n*-6/*n*-3 ratio: 11.4, 0.91 and 3.38 for Control, SWM-FULL and SWM-DEF diets, respectively. Experimental diets with the SWM meal showed also a certain chitin (0.89 and 1.40 g/kg as fed, for SWM-FULL and SWM-DEF diets, respectively) and 1-DNJ (1.07 and 1.23 µg/g as fed, for SWM-FULL and SWM-DEF diets, respectively) contents, which were not detected in the Control diet.

Table 4
Effect of dietary inclusion of 0% (Control), 12.5% SWM-FULL (full-fat) and 12.5% SWM-DEF (defatted) silkworm pupa meal (*Bombyx mori* L.) on the fattening quail nutrients apparent digestibility.

	Experimental diets			RSD	*P*-value
	Control	SWM-FULL	SWM-DEF		
No. of quails	11	11	11		
Average live weight (LW) (g)	165	168	165	11.1	0.8179
DM intake (g)	87.4^{B}	93.4^{AB}	98.3^{A}	5.89	0.0006
DM intake (g/100 g LW)	53.0^{B}	55.7^{AB}	59.6^{A}	4.05	0.0025
Excreta (g DM)	34.1^{B}	45.5^{A}	51.7^{A}	6.39	<0.0001
Apparent digestibility (%):					
DM	61.0^{A}	51.5^{B}	47.7^{B}	4.64	<0.0001
Organic matter	63.9^{A}	53.8^{B}	49.9^{B}	4.73	<0.0001
CP	69.8^{a}	70.2^{a}	65.9^{b}	3.41	0.0117
Ether extract	88.2^{A}	88.2^{A}	82.1^{B}	2.85	<0.0001
Starch	90.3^{A}	68.6^{B}	62.1^{B}	6.86	<0.0001
Chitin	–	48.1	89.5	9.80	<0.0001
Energy	68.0^{A}	59.8^{Ba}	55.6^{Bb}	3.90	<0.0001

FULL and DEF correspond to 12.5% inclusion with full-fat (FULL) and defatted (DEF) silkworm (*Bombyx mori* L.) pupa meal (SWM), respectively.
$^{a–b}$ Means within a row with different superscript letters differ at $P < 0.05$.
$^{A–C}$ Means within a row with different superscript letters differ at $P < 0.01$.

Nutrient apparent digestibility, nutritive value and feed choice

Results, shown in Table 4, highlighted a remarkable effect of the experimental diets on the apparent digestibility of nutrients. The DM intake was higher in the SWM-DEF treatment compared to the Control one, with SWM-FULL being intermediate (87.4 vs 93.4 vs 98.3 g for Control, SWM-FULL and SWM-DEF, respectively; $P < 0.001$). Quails of the SWM-FULL and SWM-DEF groups exhibited a higher excreta production compared to the Control group (45.5 g and 51.7 vs 34.1 g for SWM-FULL, SWM-DEF and Control, respectively; $P < 0.001$).

In general, results showed that the dietary inclusion of both SWM provided the absolute worst digestibility outcomes for DM ($P < 0.001$), organic matter ($P < 0.001$) and starch ($P < 0.001$) compared to Control diet, whereas SWM-DEF exhibited significantly lower digestibility of CP ($P = 0.0117$) and ether extract ($P < 0.001$) compared to SWM-FULL and Control diets. The latter two diets led to comparable results for CP and ether extract. Chitin digestibility was significantly higher in SWM-DEF quails compared to SWM-FULL ones (89.5 vs 48.1%, respectively; $P < 0.0001$). Energy digestibility showed the following trend: Control > SWM-FULL > SWM-DEF (68.0 vs 59.8 vs 55.6%, respectively; $P < 0.0001$). Consequently, the dietary treatment affected the nutritive value of diets (Table 5): Control quails showed the highest metabolisable energy (ME), followed by the SWM-FULL group and SWM-DEF one, the latter exhibiting the lowest value (13.3 vs 11.6 vs 10.5 MJ/kg DM for Control, SWM-FULL and SWM-DEF, respectively; $P < 0.0001$). As a consequence, the opposite trend was observed for the MP/ME ratio (11.7 vs 13.6 vs 14.4 for Control, SWM-FULL and SWM-DEF, respectively; $P < 0.0001$).

Growing quails disliked the dietary inclusion of either SWM-FULL or SWM-DEF, therefore determining the following feed choice pattern: Control > SWM-FULL and SWM-DEF (61.3 vs 17.2 and 21.5%, respectively; $P < 0.0001$).

Faecal microbiome

The 16S bacterial amplicon community sequencing yielded a total of 1 489 527 annotated sequences assigned at family rank and a total of 408 748 assigned at species level. Sixty-two different families were encountered. The most abundant one in all three treatments resulted that of the Leuconostocaceae, with the highest abundance in the Control. The second was Streptococcaceae, with a maximum in the SWM-FULL diet with a statistically significant difference over the other two diet

Table 5
Effect of dietary inclusion of 0% (Control), 12.5% SWM-FULL (full-fat) and 12.5% SWM-DEF (defatted) silkworm pupa meal (*Bombyx mori* L.) on the nutritive value of diets and feed choice of fattening quails.

	Experimental diets			RSD	*P*-value
	Control	SWM-FULL	SWM-DEF		
No. of quails	11	11	11		
Nutritive value					
Metabolizable protein (**MP**; g/kg feed)	156	158	150	7.69	0.0778
Metabolizable energy (**ME**; MJ/kg DM)	13.3^{A}	11.6^{B}	10.5^{C}	0.74	<0.0001
MP/ME ratio	11.7^{C}	13.6^{Bb}	14.4^{Aa}	0.69	<0.0001
Feed choice trial[1]:					
Feed intake (%)	61.3^{A}	17.2^{B}	21.5^{B}	15.1	<0.0001

FULL and DEF correspond to 12.5% inclusion with full-fat (FULL) and defatted (DEF) silkworm (*Bombyx mori* L.) pupa meal (SWM), respectively.
[a–b] Means within a row with different superscript letters differ at $P < 0.05$.
[A–C] Means within a row with different superscript letters differ at $P < 0.01$.
[1] On $n = 27$ quails.

Table 6
Effect of dietary inclusion of 0% (Control), 12.5% SWM-FULL (full-fat) and 12.5% SWM-DEF (defatted) silkworm pupa meal (*Bombyx mori* L.) on the most relevant bacterial families and species found on droppings of fattening quails. The mean abundance of each taxon is reported (%). Criteria for inclusion in this table were abundance > 1% in at least one of the treatments or significant difference ($P < 0.05$) among treatments.

	Experimental diets			*P*-value[1]
	Control	SWM-FULL	SWM-DEF	
No. of quails	5	5	5	
Total no. of families observed	52	43	38	
Leuconostocaceae	65.52	24.65	46.25	0.17
Streptococcaceae	10.14	50.80	8.88	0.024
Sphingomonadaceae	6.87	7.11	13.61	0.46
Rikenellaceae	0.0084	2.59	0.0057	0.0059
Oxalobacteraceae	1.57	1.14	2.53	0.62
Rhodobacteraceae	2.59	1.79	0.33	0.97
Gloeobacteraceae	4.80	5.02	0.51	0.86
Pseudonocardiaceae	1.63	1.95	5.61	0.21
Bradyrhizobiaceae	1.19	0.29	2.57	0.37
Eubacteriaceae	0.0052	0.53	0.0147	0.0057
Planococcaceae	0.12	0.47	1.11	0.14
Cellulomonadaceae	2.23	0.14	1.13	0.71
Burkholderiaceae	0.90	0.67	6.82	0.48
Lactobacillaceae	0.11	0.02	2.42	0.83
Ruminococcaceae	0.02	1.20	0.65	0.96
Enterobacteriaceae	0.00	0.00	3.85	0.29
Total no. of taxa identified at species level	60	51	59	
Lactobacillus salivarius	38.75	64.57	32.50	0.31
Burkholderia fungorum	21.30	9.81	18.71	0.52
Ruminococcus gnavus	0.94	8.05	0.00	0.53
Herbaspirillum huttiense	4.90	3.06	7.96	0.24
Enterococcus cecorum	0.00	0.00	8.87	0.29
Aneurinibacillus thermoaerophilus	0.00	5.51	0.00	0.0022
Burkholderia caledonica	3.81	1.77	3.11	0.59
Novosphingobium sediminicola	1.25	0.80	2.11	0.77
Streptococcus pasteurianus	0.07	0.00	2.46	0.36
Bifidobacterium saeculare	1.19	0.95	1.05	0.34
Escherichia coli	14.94	0.56	4.59	0.32
Bifidobacterium thermacidophilum	0.00	0.00	3.24	0.29
Lactobacillus delbrueckii	0.00	1.43	0.07	0.042
Bacillus thermoamylovorans	0.00	1.10	0.00	0.0022
Phenylobacterium sp.	0.70	0.55	1.01	0.96
Mucilaginibacter sp.	0.70	0.05	2.68	0.25
Enterococcus columbae	0.00	0.00	1.07	0.29
Faecalibacterium prausnitzii	1.06	0.11	0.09	0.88

FULL and DEF correspond to 12.5% inclusion with full-fat (FULL) and defatted (DEF) silkworm (*Bombyx mori* L.) pupa meal (SWM), respectively.
[1] The *P*-values refer to a significant difference ($P < 0.05$) stemming from a non-parametric Spearman's rank test of the data. The relative abundances of taxa were normalized by applying a total sum scaling (TSS) normalization followed by square route transformation. Statistical significance of differences was assessed by the non-parametric Kruskal–Wallis test.

regimes; the third was Sphingomonadaceae, widely present in the three diet type groups. Other diet-related differences were the Rikenellaceae (Phylum Bacteroidetes) and Eubacteraceae (Phylum Firmicutes, Order Clostridiales), both enriched in the SWM-FULL diet with a $P < 0.01$ difference from the Control diet and a $P < 0.05$ difference from the SWM-DEF one.

At species level, a total of 109 cases were counted. The instances were in the *Lactobacillus* genus with *L. salivarius*, accounting between 32 and 64% of the reads. The second, ranging from 9 to 21% and also present across the three diet regimes, was the gram-negative *Burkholderia fungorum*. The data showing the means observed for each experimental diet are shown in Table 6. Marked differences arose from the comparison of each group receiving a different diet. In particular, three species had a statistically significant increase (Table 6, Fig. 1), positively related to silkworm content in the meal and namely *Aneurinibacillus thermoaerophilus*, more abundant in the SWM-FULL diet compared to the SWM-DEF one ($P < 0.05$) and, even more strongly, to the Control ($P < 0.01$). The second was *Bacillus thermoamylovorans* with the same above-mentioned significances, respectively, and the third was *Lactobacillus delbrueckii*, which showed a significant increase ($P < 0.05$) only between the SWM-FULL and the Control groups. Conversely, a major presence in the faeces of quails fed on the Control diet was *Escherichia coli*, which dropped from 15% (Control-fed quails) to 4.65% (SWM-DEF diet-fed quails), and to a mere 0.56% in the SWM-FULL diet-fed quails.

Regarding the bacteria that increased under the SWM diets, the above-mentioned first two species were featured also as the main taxa resulting from the sequencing data of the three fed substrates. *A. thermoaerophilus* was the dominant taxon at species level (79.6%) of the SWM-FULL diet and a main one in the SWM-DEF diet (28%) too, while it was not detectable in the Control diet. *Bacillus thermoamylovorans* was also SWM-FULL diet related being the second most abundant taxon (16.9%), and absent from both the SWM-DEF and the Control diets. At family level, instead, Rikenellaceae accounted for 79% of the taxa in the SWM-FULL diet, for 0.09% in the SWM-DEF one, while it was zero in the Control diet.

Discussion

The nutritional and fatty acid profiles of the SWM-FULL and SWM-DEF were in line with literature findings (Makkar et al., 2014; Ullah et al., 2018; Miah et al., 2020), further confirming the potential of this ingredient in feed formulations and to provide healthy food for humans. As for the chitin content, SWM values of the present study (3.0–3.6% DM) confirm the range 3–4% DM proposed by Makkar et al. (2014).

In the present study, the higher DM intake and the higher excreta production observed in the SWM groups may either be due to the presence of chitin, which was reported to reduce the digestibility of nutrients in chickens (Makkar et al., 2014; Ullah et al., 2017), and/or to the bioactive substance 1-DNJ, which limits the digestion of essential nutrients (Gao et al., 2016). In fact, while the apparent digestibility of DM, organic matter, CP, ether extract and starch in the Control group was similar to that usually reported in the literature for quails, values of SWM-FULL and SWM-DEF groups were lower than those reported in common findings (Ullah et al., 2018). The impaired apparent digestibility of nutrients in SWM-fed quails led to a reduction in the nutritive value of the diet, as the ME was below the recommended threshold value which was not sufficient to ensure the optimum 2900 Kcal ME/kg feed (National Research Council, Subcommittee on Poultry Nutrition (NRC), 1994). This could possibly explain the results of the feed choice test.

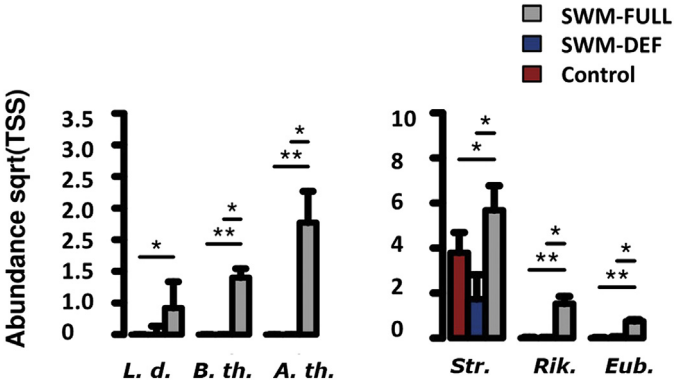

Fig. 1. Rank test plot based on the pairwise comparisons by Wilcoxon rank test of the taxa of fattening quails' droppings that resulted significantly different across the different dietary treatments. Compared data were transformed by square root (sqrt) abundance (TSS: total sum of squares). Left panel: Species data; Right panel: Family data. *L. d.*: *Lactobacillus delbrueckii*; *B. th.*: *Bacillus thermoamylovorans*; *A. th.*: *Aneurinibacillus thermoaerophilus*; *Str.*: *Streptococaceae; Rik.*: *Rikenellaceae; Eub.*: *Eubacteriaceae.* Significant differences are marked by *: $P < 0.05$, **: $P < 0.01$. The order of treatments is, from left to right, Control, SWM-FULL (full-fat) SWM-DEF (defatted), respectively. FULL and DEF correspond to 12.5% inclusion with full-fat (FULL) and defatted (DEF) silkworm (*Bombyx mori* L.) pupa meal (SWM), respectively.

Previous research showed that chitinolytic activity is an intrinsic property of the gastrointestinal tract of the chicken, which was reported to digest chitin in the range 67–92% (Han et al., 1997). Results of the present study are coherent with such range, which was expected, as quails are also occasional insectivorous birds. Despite chitin can be partly digested by the quail, the deacetylation of chitin leads to the formation of chitosan which is reported to slow down nutrient degradation by forming complexes with feed nutrients, mostly protein and lipids, ultimately lowering their digestibility (Jayanegara et al., 2020). The negative effect of chitin on nutrients digestibility has also been reported when diets are supplemented with other insect species, for instance, the black soldier fly (Cullere et al., 2016) and the yellow mealworm larvae (Bovera et al., 2015). Recent research discovered that the acidic chitinase mRNA expression level, and thus the chitinolytic activity of the enzyme, is strongly related to the dietary group (carnivore, herbivore, omnivore) of the considered animal species (Tabata et al., 2018). Therefore, a possible explanation of the remarkable different chitin digestibility in SWM-FULL and SWM-DEF treatments could be related to the absolute chitin content of the diets. Hypothetically, a higher dietary chitin content could have increased the magnitude of the acidic chitinase mRNA expression, thus determining different chitin digestibility. However, the possible association of the dietary inclusion level of chitin with the acidic chitinase mRNA expression remains to be investigated.

Among the biological activities of the 1-DNJ, it is reported to be an effective antihyperglycaemic and anti-obesity. Specifically, it is an effective inhibitor of α-glucosidase enzymes, responsible for the conversion of starch into the monosaccharides which are then absorbed in the intestine (Gao et al., 2016). Previous literature indicated that the 1-DNJ content of silkworm pupae ranges from 75 to 105 mg/100 g (Vichasilp et al., 2016), but the present experiment seemed to indicate that a dietary content of 0.107 mg/100 g of 1-DNJ (SWM-DEF) was sufficient to negatively affect starch digestibility in growing quails. Moreover, 1-DNJ also plays a regulatory role in fat deposition and metabolism, which could inhibit FA synthesis and enhance FA oxidation, resulting in the reduction of lipids accumulation (Tsuduki et al., 2013). This could partly justify the lowest ether extract digestibility observed in the SWM-DEF group, which had the highest dietary content of 1-DNJ.

A possible way to solve the above-mentioned drawbacks would be the partial chitin removal through high-pressure processing, which would also disrupt the bond between some chitin-bound proteins, ultimately improving their quality (Rumpold and Schlüter, 2013), and the extraction of 1-DNJ. Once extracted, 1-DNJ could be exploited for other purposes, that is, to develop functional foods to lower postprandial glucose (Vichasilp et al., 2016).

Since the 1-DNJ seems to be the responsible of the drop in starch digestibility, an unclaimed amount of starch remains available for microbial consumption. Such material would not necessarily be exhausted by bacterial metabolism, as testified by its presence in the faecal material, leading to the reduced digestibility result. Nevertheless, starch explains the observed shifts in amylolytic microbial guilds. In fact, among the taxa that significantly increased in the SWM diets, there is the Rikenellaceae family whose growth showed to be stimulated in raw potato starch-fed mice (Bang et al., 2019). Furthermore, an inhibited starch assimilation by the animal can be seen as turning a metabolizable substrate into one equivalent to the resistant starch (RS) category (Berry, 1986), which is in line with the significant increase of the Eubacteriaceae. The latter is a family reported in the human microbiome and showed the strongest increase in response to a diet enriched in resistant starch (Upadhyaya et al., 2016). Also at species level, the amylolytic attitude of enhanced taxa is sometimes implied in their name (*Bacillus thermoamylovorans*) or, as for the *Lactobacillus* group, known as a specific biochemical proficiency (Gänzle and Follador, 2012). As aforementioned, both SWM diets had equally impaired starch apparent digestibility. However, the differences between the two types of SWM diets in each of these taxa responses, and the fact that SWM-FULL induced the most extreme changes, could be explained by the different starch sources. In fact, the SWM-DEF diet contained considerably lower amounts of wheat meal and soybean meal, replaced by corn meal, compared to the SWM-FULL one.

Overall, findings of the present study indicated that the dietary supplementation of SWM can negatively affect nutrients digestibility of growing quails, mainly due to the presence of chitin and 1-DNJ. In addition, the analysis of faecal microbiome can be considered an effective tool to interpret digestibility data in livestock production. Further research is needed to assess the digestibility of nutrients and microbiome composition in livestock species after the removal of chitin and 1-DNJ from silkworm pupa meal, aiming to assess the relative contribution of each bio-compound in affecting poultry digestion.

Ethics approval

The study protocol was approved by the Ethical Committee for Animal Experimentation of the University of Padova (Ethical approval number 147882). All the animals were handled in compliance with the principles stated by the EC Directive 86/609/EEC regarding the protection of animals used for experimental and other scientific purpose.

Data and model availability statement

None of the data were deposited in an official repository. The data sets analysed in the current study are available from the corresponding author upon request.

Author ORCIDs

A. Dalle Zotte: https://orcid.org/0000-0001-9214-9504.
Y. Singh: https://orcid.org/0000-0003-3811-4372.
A. Squartini: https://orcid.org/0000-0002-1961-1165.
P. Stevanato: https://orcid.org/0000-0002-6577-6956.
S. Cappellozza: https://orcid.org/0000-0002-7703-188X.
A. Kovitvadhi: https://orcid.org/0000-0002-0769-5234.
S. Subaneg: none.
D. Bertelli: https://orcid.org/0000-0002-6227-7369.
M. Cullere: https://orcid.org/0000-0001-9409-8202.

Author contributions

Antonella Dalle Zotte: Conceptualization, funding acquisition, investigation, project administration, resources, supervision, writing – original draft, writing – review & editing. Yazavinder Singh: Data curation, formal analysis, software, visualization, writing – review & editing. Andrea Squartini: Formal analysis, investigation, methodology, software, validation, writing – original draft, writing – review & editing. Piergiorgio Stevanato: Data curation, formal analysis, methodology, validation. Silvia Cappellozza: Funding acquisition, investigation, writing – review & editing. Attawit Kovitvadhi: Funding acquisition, resources. Sirawit Subaneg: Formal analysis. Davide Bertelli: Formal analysis. Marco Cullere: Data curation, investigation, methodology, writing – original draft, writing – review & editing.

Declaration of interest

None.

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