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Egg fatty acid profiles and potential health risk from defatted insect meal in laying hens' diets

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RESEARCH ARTICLE

Abstract

Insects, a staple feed for wild birds and free ranging poultry, have a relatively high protein quality and are a promising feed for commercial poultry. Replacing soybean meal with insect derived feeds potentially reduces dependency on feed imports, increasing the sustainability of egg production – but only if maintaining or enhancing their nutritional quality. This study investigated egg fatty acid (FA) profiles from replacing soyabean meal with *Hermetia illucens* (black soldier fly) meal (HIM) for laying hens. A three-week trial with 30 organic Lohman Selected Leghorn hens between 64-74 weeks old was repeated with four flocks at the end of their first laying cycle. In all replicate trials, ten birds were randomly allocated to each of three diets: (1) control with 360 g soybean/kg and no HIM; (2) H12 with 120 g HIM and 156 g soybean/kg; and (3) H24 with 240 g HIM/kg and no soybean. Complete replacement of soya (H24) increased saturated fatty acid (SFA) and monounsaturated fatty acids (MUFA) and decreased total polyunsaturated fatty acids (PUFA), omega-6 (n-6) and omega-3 (n-3) PUFA concentrations in eggs. The intermediate H12 diet (replacing 33% soya) gave similar n-3 and MUFA concentrations to control eggs but significantly increased SFA and reduced total PUFA. However, birds moderated the transfer of high intakes of potentially damaging C12:0 and C14:0 into eggs and although differences in eggs were highly significant and great (relative to very low levels in control eggs) concentrations were substantially lower than in insect meal itself and some commonly consumed foods.

Keywords: *Hermetia illucens*, soyabean replacement, egg composition, fatty acids, health risk

1. Introduction

The widespread use of soya in livestock diets is questioned since its production is linked to rain forests destruction and these land-use changes carry a heavy greenhouse gas liability when considering the environmental cost of food production (Tallentire *et al.*, 2018). Feeding soya in Europe also has the additional burden of relying on imports and associated food-miles from non-European countries (Tallentire *et al.*, 2018). There is also concern over the inefficiency of feeding soya to animals, since its conversion into eggs, rather than direct consumption, results in 60% less available protein (Wilkinson and Lee, 2018). Consequently, there is increasing desire to identify alternative protein feeds for poultry and egg production which maintain high production standards without a negative impact on food security or quality.

Insects and other invertebrates are staple in the diet of many wild birds and free ranging poultry (Sun *et al.*, 2013) and contribute directly to human's diet in some cultures (Van Huis, 2013). They are useful feeds since they are high in energy and protein, potentially meeting amino acid requirements for humans and other monogastric animals,

as well as being rich in several micronutrients like copper, iron, magnesium, manganese, phosphorous, selenium, and zinc as well as riboflavin, pantothenic acid and biotin (Rumpold and Schlüter, 2013). They also have the added attraction of minimal land use (Salomone *et al.*, 2017) and the ability to utilise waste and by-products from food and farming (including biogas digestate) in their production (Heuel *et al.*, 2019; Salomone *et al.*, 2017; Spranghers *et al.*, 2017), further enhancing the sustainability of their use as livestock feeds. Various insect species such as mealworm and locust have been investigated as feed ingredients for fish and poultry and preliminary work suggests black soldier fly larva (*Hermetia illucens,* HI) show promise (Dewi Apri and Komalasari, 2020; Heuel *et al.*, 2019; Józefiak *et al.*, 2016; Maurer *et al.*, 2015). There has been a report of a commercial farm in UK supplementing layer diets with live HI larvae (James, 2022).

However, replacing soya with insects to reduce the environmental footprint of animal production may not necessarily enhance sustainability if nutritional quality is compromised and consumer health or safety are at risk. The composition of insect meal does vary, depending on substrate used in their production (Barroso *et al.*, 2017; Heuel *et al.*, 2019; Rumpold and Schlüter, 2013) and there is worrying documentation of bioaccumulation of contaminants such as cadmium and arsenic by insects (Hare, 1992). Also, black soldier fly larvae are high in lipids, which appears to be dominated by saturated fatty acids (SFA) particularly medium chain lauric (LAU, C12:0), myristic (MYR, C14:0) and palmitic (PAL C16:0) acids (Barroso *et al.*, 2017; Heuel *et al.*, 2021; Spranghers *et al.*, 2017). This, along with their low content of omega-3 polyunsaturated fatty acids (n-3), could have a negative impact on consumer health if transferred to animal products, since both have been associated with greater risk of heart disease (Calder, 2015; Wang and Hu, 2017), although this has been questioned (Praagman *et al.*, 2019). Previous studies reported that including HI meal in animal diets does affect the fatty acid profiles (FA) in poultry meat (Schiavone *et al.*, 2017) eggs (Heuel *et al.*, 2021; Park *et al.*, 2021) and fish (St-Hilaire *et al.*, 2007; Zhou *et al.*, 2018), although another study by Secci *et al.* (2018) reported little difference in egg FA profiles. This study aimed to quantify differences for nutritionally relevant fatty acids in eggs from replacing soyabean meal with insect meal in layer diets, and identified associations between feed ingredients, individual fatty acid intakes by hens and egg fatty acid profiles.

2. Materials and methods

Experimental set up

This study was part of a Swiss feeding trial investigating performance of organic Lohman Selected Leghorn classic white layers, described by Maurer *et al.* (2015), outlining the experimental procedures and preparation of the partially defatted *Hermetia illucens* (black soldier fly) meal (HIM). Briefly, four replicates of a 3-week feeding trial were conducted, each with 30 birds purchased from different commercial flocks at the end of their lay period, between 64-74 weeks old. In all four replicate flocks, hens were randomly distributed, with 10 birds allocated to each of three feeding groups. They were adapted to experimental diets for one week, ahead of 3-weeks' recording. During the trials, hens had access to a covered outdoor area but not to pasture or soil so, whilst herbage or wild insect consumption cannot be totally ruled-out, it is likely to be insignificant compared to levels of HIM in feed. All animal-related procedures complied with the Swiss animal welfare act, the animal welfare ordinance, and the animal experimentation ordinance, registered as experiment no. 75645.

Feed formulation and composition

Mixed isoenergetic feeds with three levels of HIM were produced by a commercial feed manufacturer (all components, except HIM, were certified as organic): (1) control diet containing 360 g soyabean/kg feed and no HIM; (2) diet containing 120 g HIM and 156 g soyabean meal/kg feed (H12); and (3) diet containing 240 g HIM and no soyabean (H24)/kg feed. The HIM was produced with *Hermetia illucens* larvae fed for 10 days initially on the control poultry feed then 2-3 weeks on wheat-based by-products from pasta production. Preparation of HIM is explained fully by Maurer *et al.* (2015),with larvae harvested pre-pupal and the dried meal pressed to partly reduce fat to 11%, leaving a protein content of 59% CP. The formulation and chemical composition of the 3 diets offered are presented in Table 1.

Collection of eggs and feed samples

Ten eggs were collected from each flock on the last day of their feeding trial. Yolks were separated, placed in sterile plastic containers, kept frozen at -20 °C before being transported on ice to Newcastle University, where they were stored at -20 °C until analysis. Three replicate samples of the diets were also collected from each batch and delivered along with egg yolks.

Fatty acid analysis of feed and eggs

Chemicals and analytical standards for fatty acid analysis of lipids

Hexane (≥99.9%) and toluene (≥99.5%) were purchased from Sigma-Aldrich (Gillingham, UK). Methanol (≥99.8%), chloroform (≥99.8%), 2,2,4-trimethylpentane (isooctane; ≥99.5%), boron trifluoride 12% in methanol, acetyl chloride (≥98.0%), sodium chloride (≥99.9%), potassium chloride and sodium hydroxide pellets were purchased from Thermo-

Fischer Scientific Ltd. (Loughborough, UK). Analytical standard with 52 FA methyl esters (GLC463) was purchased from Nu-Chek Prep Inc. (Elysian, MN, USA).

Egg fatty acid analysis

Egg yolks were thawed at room temperature, thoroughly homogenised before lipid extraction and gravimetric quantification, as described by Folch *et al.* (1957). Preparation of fatty acid methyl esters (FAME) from the extracted lipid was done as per Joseph and Ackman (1992). Briefly, 50 mg of the extracted lipid samples went through a series of solvent additions with nitrogen purging and vortexing before and after each addition. Initially 1.5 ml of methanolic 0.5 N NaOH was added, and samples heated at 100 °C for 5 min, returned to room temperature before adding 2 ml of 12% boron trifluoride (BF_3) in methanol. Samples were heated at 100 °C for 30 min then left to cool for 5 min before 1 ml of isooctane and 5 ml of saturated NaCl added before centrifuging for 5 min at 2,000 rpm and the upper layer transferred into a new glass tube. This step was repeated, and samples were dried down at room temperature, under a stream of nitrogen. Tubes were rinsed with 1 ml of hexane and 0.4 ml transferred to a sealed amber vial and stored at -20 °C until GC analysis.

Quantifying FAMEs was carried out by gas chromatography (GC) (Shimadzu, GC-2014, Kyoto, Japan) fitted with a flame ionisation detector (FID) and an Agilent CP-Sil 88 column (100 m × 0.25 mm ID × 0.20 µm film thickness). Purified helium was the carrier gas with a pressure of 210 kPa and a column flow of 1 ml/min. 1 µl of sample was injected to the column by an auto injector (Shimadzu, AOC-20i) using a split injection mode (ratio of 50), an injector temperature of 255 °C with a FID temperature of 260 °C. Samples were injected at an initial column temperature of 70 °C, held for 1 min before being raised to 100 °C at a rate of 5 °C/min, held for 2 min and then raised to 160 °C at a rate of 10 °C/min and held for 71 min. Finally, the temperature was increased to 240 °C at a rate of 5 °C/min and then held for 29 min leading to a final gradient profile with a 131 min total runtime per sample. Identification of peaks was achieved by comparing retention times with those of a 52 FAME standard but also by using already identified peaks by published papers (Stergiadis *et al.*, 2015) using the same GC conditions. Peaks were integrated using the GC Solution Shimadzu software and quantification was based on peak areas of individual FA, expressed as a percentage of the total peak area for known quantified fatty acids.

Feed fatty acid analysis

Lipids were extracted from feeds with petroleum ether under controlled conditions using the Soxhlet method (Ministry of Agriculture, 1973) for 6 h. Fifty mg of the extracted lipid was transferred in a glass tube and the same procedure was followed as described by Butler *et al.* (2011). Feed fatty acid methyl esters (FAMEs) were analysed as described for the yolks.

Calculated dietary feed and fatty acid intakes by hens

The specification and FA profiles of the mixed diets were used to estimate dietary intakes of individual feedstuffs, FA and FA groups (g/hen/day), using the following equations with recorded feed intakes (Maurer *et al.*, 2015), (although this makes no allowance for lipid constituents other than the determined FAs):

$$\text{Feedstuff intake (g)} = \text{Total feed intake (g)} \times \frac{\text{Inclusion of feed ingredient (g/100 g feed)}}{100}$$

$$\text{FA intake (g)} = \text{Total feed intake (g)} \times \frac{\text{Feed lipid content (g/100 g feed)}}{100} \times \%\ \text{of} \frac{\text{FA (g/100 g total FA in feed)}}{100}$$

Statistical analysis

Analyses of variance (ANOVA), derived from linear mixed-effects models, were performed in R statistical environment (R Development Core team, 2009) using diet (Control, H12, H24) and trial/flock (1-4) as main, fixed factors and egg replicate as a random factor. Differences in FA profile between the three different diets and the four different rounds were assessed by one way ANOVA using diet and round as fixed factors, respectively. Pairwise comparisons of means (P<0.05) were performed using post-hoc Tukey's honestly significant difference test.

Four different redundancy analysis (RDA) were conducted in Canoco (Ter Braak, 1998) to assess the relationships between feed and fatty acid intake and egg composition. The relative proportion of individual fatty acid in eggs were computed against: (1) the proportion of each feed ingredients in the diet; (2) the intake of feed ingredients by the hens; (3) the fatty acid profile in the diet; and (4) fatty acid intake by hens.

3. Results

General

Results on laying performance were described by Maurer *et al.* (2015), who reported similar feed intakes (134-159 g/bird/day) and production across all three diets, with egg output between 79-84% per day for all treatments, as expected for hens in the late stage of their laying cycle.

Diet fatty acid composition and estimated intake

Slight (non-significant) differences in feed consumption between the 3 diets means that FA content for each diet is not directly reflected in actual FA intakes by the different experimental groups (Table 2). The higher feed intakes recorded by birds on the H12 diet (+13% relative to the

Table 1. Feed formulation and nutrient concentrations of mixed diets (adjusted from Maurer *et al.*, 2015).

Feed constituents (g/kg fresh matter)	Control	H12[a]	H24[b]
Defatted *Hermetia* meal	0	120	240
Soybean meal	360	156	0
Corn/maize	350	409	343
Wheat	0	46	146
Wheat bran	31	22	21
Mixed bran	22	0	0
Sunflower cake	26	81	82
Alfalfa/lucerne meal	31	31	30
Grass meal	21	22	21
Granulated cereals	41	0	0
Mineral, limestone, vitamins	118	113	117
Nutrient concentrations (g/kg dry matter)			
Crude fat	45.0	57.2	64.4
Metabolisable energy (MJ/kg)	11.3	11.3	11.3
Crude protein	200	203	214
Methionine	3.2	3.6	3.9
Lysine	12.0	10.3	10.1
Crude ash	140	134	137
Calcium	40.0	40.8	42.6
Phosphorus	5.3	5.2	5.3
Sodium	2.0	1.9	2.2
Chloride	2.2	2.3	2.8
Vitamin content[c] (mg/kg feed)			
Vitamin A	3.6	4.0	4.6
Vitamin E	60	65	75
Vitamin D	0.058	0.065	0.075

[a] Feed with *Hermetia* meal at 12%.

[b] Feed with *Hermetia* meal at 24%.

[c] Vitamin content were omitted in the original publication but provided subsequently.

other diets) influenced FA intake patterns and all results presented here and discussed are based on FA intake by the hens although a comparison of FA profiles for the 3 diets is presented in supplementary Table S1.

There were no differences in diet FA content or intakes between the 4 trials or flocks but the different diets significantly changed dietary supply and intake of most FA and groups (Table 2). Major differences were seen for C12:0, C14:0 and eicosapentanoic acid (EPA) intakes, which were all very low from the control diets but increased substantially and incrementally (between 12- and 200-fold higher) for both H12 and H24 diets (P<0.001). These increases were responsible for higher intake of total SFA (almost doubled in the HI24 diet) and long chain n-3 (lc n-3, between 2.5 to 4-fold higher than control intakes for H12 and H24, respectively). Although significant, the pattern of change for other nutritionally relevant FA and groups were smaller and less clear cut. They did not appear to increase or decrease incrementally with HIM feeding, except for C18:0 intake and the ratio of n-6:n-3, both of which were reduced in line with HIM inclusion.

Egg fatty acid composition

The total lipid content and egg FA profiles from birds on the 3 experimental diets are presented in Table 3. Total lipids did not differ between diets or flocks; oleic acid (OA, c9 C18:1) was dominant (~37% of total) followed by palmitic (PA, C16:0 at 27%) and linoleic (LA, c9,12 C18:2 at 16%) acids. Together these contributed between 77-83% all FA and whilst the profiles were similar over the 4 flocks, the different diets significantly influenced the relative proportion of many nutritionally relevant FA in eggs.

Introducing insect meal raised total SFA in eggs by 6 and 9% at H12 and H24 compared with control eggs, although concentrations of C12:0 and C14:0 were increased substantially more than this (with almost a 50- and 70-fold increase for C12:0 and 5- and 7-fold increase in C14:0, for H12 and H24, respectively, all P<0.001). Palmitic acid dominates SFA and although higher (P<0.001) from insect meal (but only by 6-8%), its concentration in eggs did not differ between H12 and H24. Stearic acid (C18:0) on the other hand was slightly lower (P<0.001, 8-9% less than control) in eggs from insect meal fed hens, with similar levels from both experimental diets.

Oleic acid, the main MUFA, was high in all eggs and only differed slightly (P=0.004) between the 2 insect meal diets – H24 eggs were 4% higher than from H12 and neither differed from control eggs. Total MUFA were 7% higher in eggs from H24 diets than control eggs.

The concentrations of total and most individual PUFA in eggs were lower with increasing insect meal, except for EPA, which increased. We see a similar pattern and magnitude of differences for the main PUFA, LA, total PUFA and n-6 concentrations, as well as arachidonic acid. These all fell between 12-17% in eggs from H12 and further still to 26-29% less from H24 diets, compared with control eggs, with each incremental drop being significant (P<0.001).

Differences for n-3 were less consistent; these were generally low and the influence of diet was more variable. For alpha-linolenic acid (ALA c9,12,15 C18:3), concentrations fell, following the incremental increase in insect meal; 15% lower than control eggs from H12 and 37% less from H24 (P<0.001). Total n-3, differences were only significant between control and H24 eggs, with the latter 17% lower (P<0.001).

Table 2. Estimated dietary fatty acid (FA) and total lipid intakes by birds (means ± standard error of means in g of FA per hen per day) from control diet and diets with *Hermetia* meal at 12% (H12) or 24% (H24) and differences relative to control diet.[1]

Diet FA	Control n=4	H12 n=4	H24 n=4	*P*-value	H12 ± to control[2]	H24 ± to control[2]
C12:0	0.01±0.002^{c}	1.79±0.11^{b}	2.40±0.04^{a}	<0.001	14,601%	19,696%
C14:0	0.01±0.00^{c}	0.25±0.013^{b}	0.33±0.01^{a}	<0.001	2,699%	3,606%
C16:0	0.88±0.03^{b}	0.96±0.01^{a}	0.83±0.01^{b}	0.001	9%	-5%
C18:0	0.29±0.02^{a}	0.17±0.004^{b}	0.11±0.002^{c}	<0.001	-41%	-60%
OA	1.59±0.08^{a}	1.60±0.03^{a}	1.35±0.01^{b}	0.015	1%	-15%
LA	2.08±0.119^{a}	2.29±0.10^{a}	1.47±0.07^{b}	0.003	10%	-29%
ALA	0.10±0.01^{b}+	0.14±0.002^{a}	0.10± 0.002^{b}	0.015	45%	6%
EPA	0.001±0.00^{c}	0.008±0.00^{b}	0.010±0.00^{a}	<0.001	1,276%	1,961%
DHA	0.002±0.00^{b}	0.001±0.00^{b}	0.001±0.00^{a}	0.002	-57%	-66%
FA groups and ratios						
SFA	1.28±0.05^{c}	3.24±0.13^{b}	3.76±0.06^{a}	<0.001	154%	194%
MUFA	1.68±0.09^{ab}	1.75±0.03^{a}	1.50±0.012^{b}	0.029	4%	-11%
PUFA	2.23±0.12^{a}	2.47±0.10^{a}	1.61±0.07^{b}	0.002	11%	-28%
n-3	0.10±0.01^{b}	0.15±0.003^{a}	0.12±0.003^{b}	0.012	49%	14%
n-6	2.08±0.12^{a}	2.29±0.10^{a}	1.48±0.07^{b}	0.003	10%	-29%
n-6:n-3	20.99±2.32^{a}	15.05±0.92^{ab}	12.67±0.84^{b}	0.035	-28%	-40%
EPA+DHA	0.002±0.00^{c}	0.009±0.001^{b}	0.01±0.001^{a}	<0.001	265%	424%
Sum FA	5.19	7.46	6.87			

[1] Means with different superscripts are significantly different (P<0.05) according to Tukeys's honestly significant difference test. ALA = α-linolenic acid; DHA = docosahexaenoic acid; EPA = eicosapentanoic acid; LA = linoleic acid; MUFA = monounsaturated FA (c9C14:1, t9C16:1, c9C16:1, c9C17:1, t6+t7+t8C18:1, t9C18:1, t11C18:1, c9C18:1 (OA), c11C18:1, c13C18:1, c5C20:1, c8C20:1, c15C24:1; OA= oleic acid; PUFA = polyunsaturated FA (t10t14C18:2, t8c13C18:2, c9t12C18:2, t9c12C18:2, ctmix10,14+12,16C18:2, c9c12C18:2 (LA), c9c15C18:2, c6c9c12C18:3(GLA),c 9c12c15C18:3 (ALA), conjugated linoleic acid (CLA), t11c13C18:2, unknown CLA(t,t), unknown CLA(t,t), c9c13c15C18:3, c11c14C20:2, c9c11c15C18:3, c8c11c14C20:3, c11c14c17C20:3; SFA = saturated FA(C12+C11:1, C14:0+9C13:1, C15:0, C16:0, C17:0, C18:0, C20:0, C22:0, C24:0).
[2] Differences expressed as percentage of values for control diet.

The greater depression in n-6 relative to n-3 means the ratio of these 2 groups were lower in eggs from insect meal diets by 10 and 12% for H12 and H24 diets, respectively compared with control, with no difference between the two insect meal diets (P<0.001).

Associations between feed and fatty acids in layer feeds and egg composition

Differences in egg composition were substantially less than the impact on diet composition or fatty acid intakes. This particularly applies to C12:0 and C14:0 with intakes from H12 and H24 almost 150 and 200 TIMES higher (respectively) than layers on the control diet, yet eggs show a 49- and 69-fold increase (respectively), compared with control. This apparent moderating influence on egg composition is reinforced by RDA results, indicating only 16% of variation in egg composition is explained by feed intakes (Figure 1A and Table 4) or 35% by hens' fatty acid intake (Figure 1B). RDA biplots 1 and 2 (diet composition vs feed intakes) were very similar, as were those for 3 and 4 (FA composition vs intakes) so, as with Table 2, only plots for feed and FA intakes are presented. Eigene values indicate axis 1 explains most variation, 84% (for feed ingredients) and 82-83% (for FA results) of the totals. For feedstuffs intake (Figure 1A) only HIM intake proves to have a significant (P<0.002) influence over egg composition and is associated with the appearance of C14:0, C12:0, C16:0 and EPA in eggs – all negative along the dominant axis 1, diminishing in the order presented. The total yolk lipids appears under weak influence of dietary feed ingredients since it has low coordinates for both axes, as do DPA and OA concentration.

Figure 1B depicts the relationship between FA intakes and egg composition, with significant drivers as c9 C16:1, ALA, C18:0, LA and C12:0 intakes (P<0.05 for all). Both intakes and egg concentrations for LA and ALA have the strongest positive values, almost sitting on axis 1, closely followed by OA, C18:0, C16:0 and DHA (which is very low across the board) with stronger positive values on axis 2 as well. In the opposite direction with strong negative values for axis 1 and axis 2, intakes of C12:0 and C14:0 are closely associated with their concentrations in eggs. However, whilst the appearance of both these clusters of FA in eggs are closely associated with the ordination of

Table 3. Means ± standard error of means and ANOVA *P*-values for the effect of diet on layer performance, yolk weight (g) and lipid content (%) and fatty acid (FA) profile (g/100 g total FA) of eggs and differences relative to control diet.[1]

	Control n=28	H12 n=31	H24 n=31	*P*-value	H12 ± to control[2]	H24 ± to control[2]
Yolk weight (g)[3]	19.2±0.30	18.6±0.23	18.6±0.23	0.15		
Eggs/day[1]	0.79±0.044	0.84±0.084	0.83±0.032	0.79		
Lipid content %	32.5±0.87	35.1±0.81	33.7±1.01	0.13		
Lipid in eggs (g/day)[4]	6.2	6.5	6.3			
Individual FA (g/100 g total FA)						
C12:0	0.004±0.00^{c}	0.20±0.01^{b}	0.28±0.02^{a}	<0.001	4,900%	6,900%
C14:0	0.23±0.01^{c}	1.42±0.07^{b}	1.87±0.10^{a}	<0.001	517%	713%
C16:0	25.71±0.18^{b}	27.34±0.17^{a}	27.66±0.2^{a}	<0.001	6%	8%
C18:0	8.91±0.14^{a}	8.17±0.08^{b}	8.11±0.13^{b}	<0.001	-8%	-9%
c9 C16:1	1.89±0.07^{c}	2.89±0.10^{b}	3.3±0.12^{a}	<0.001	53%	75%
OA	37.23±0.46^{ab}	36.78±0.31^{b}	38.17±0.43^{a}	0.004	-1%	3%
LA	18.26±0.62^{a}	15.59±0.38^{b}	13.04±0.44^{c}	<0.001	-15%	-29%
ALA	0.54±0.05^{a}	0.46±0.02^{b}	0.34±0.02^{c}	<0.001	-15%	-37%
AA	2.02±0.03^{a}	1.78±0.03^{b}	1.7±0.04^{c}	<0.001	-12%	-16%
EPA	0.01±0.001^{c}	0.02±0.001^{b}	0.03±0.001^{a}	<0.001	100%	200%
DPA	0.08±0.003^{ab}	0.09±0.003^{a}	0.08±0.003^{b}	0.031	13%	0%
DHA	0.82±0.02^{a}	0.80±0.02^{ab}	0.74±0.02^{b}	0.004	-2%	-10%
FA groups (g/100 g total FA) and ratios						
SFA	35.16±0.23^{c}	37.39±0.18^{b}	38.18±0.18^{a}	<0.001	6%	9%
MUFA	41.62±0.5^{b}	42.4±0.35^{b}	44.40±0.43^{a}	<0.001	2%	7%
PUFA	22.93±0.67^{a}	19.9±0.4^{b}	17.07±0.47^{c}	<0.001	-13%	-26%
n-3	1.59±0.07^{a}	1.50±0.03^{a}	1.32±0.04^{b}	<0.001	-6%	-17%
n-6	21.24±0.61^{a}	18.26±0.38^{b}	15.58±0.45^{c}	<0.001	-14%	-27%
n-3>18C	0.93±0.03^{a}	0.92±0.02^{ab}	0.86±0.03^{b}	0.012	-1%	-8%
n-6:n-3 ratio	13.58±0.26^{a}	12.25±0.27^{b}	11.92±0.37^{b}	<0.001	-10%	-12%
Total lipid[3]	5.2	7.5	6.9			

[1] Means with different superscripts are significantly different (*P*<0.05) according to Tukeys' honestly significant difference test. AA = arachidonic acid; ALA = α-linolenic acid; DHA = docosahexaenoic acid; DPA = Docosapentaenoic acid; EPA = eicosapentanoic acid; LA = linoleic acid; MUFA = monounsaturated FA (c9C14:1, t9C16:1, c9C16:1, c9C17:1, t6+t7+t8C18:1, t9C18:1, t11C18:1, c9C18:1 (OA), c11C18:1, c13C18:1, c5C20:1, c8C20:1, c15C24:1; OA= oleic acid; SFA = saturated FA(C12+C11:1, C14:0+9C13:1, C15:0, C16:0, C17:0, C18:0, C20:0, C22:0, C24:0); PUFA = polyunsaturated FA (t10t14C18:2, t8c13C18:2, c9t12C18:2, t9c12C18:2, ctmix10,14+12,16C18:2, c9c12C18:2 (LA), c9c15C18:2, c6c9c12C18:3(GLA),c 9c12c15C18:3 (ALA), conjugated linoleic acid (CLA), t11c13C18:2, unknown CLA(t,t), unknown CLA(t,t), c9c13c15C18:3, c11c14C20:2, c9c11c15C18:3, c8c11c14C20:3, c11c14c17C20:3.

[2] Differences expressed as percentage of values for control diet.

[3] Reported by Maurer *et al.* (2015).

[4] Calculated from mean performance and lipid content.

their respective intakes, this does not apply to other FA. The most divergent example from this 3rd group is C16:0; in eggs (negative for both axis 1 and 2) it appears in direct opposition to its intake by the birds, with positive values for axis 1 and 2. Oleic acid in eggs (the most abundant single FA) is also poorly linked to its consumption; OA intake is closely clustered alongside intakes of C16:0, C18:0 and DHA, all with positive values on both axis and distant from their respective appearances in eggs.

4. Discussion

This study reports considerable differences in egg FA when expeller soyabean meal is replaced by defatted HIM in layer diets, in contrast to the consistent egg output in this trial, reported by Maurer *et al.* (2015). That said, comparing feed or fatty acid intake with egg composition indicates hens' incredible ability to attempt to maintain egg composition (presumably relating to chick viability) against the challenges of drastic changes to their diet. Introducing defatted HIM substantially raised total diet lipid, especially

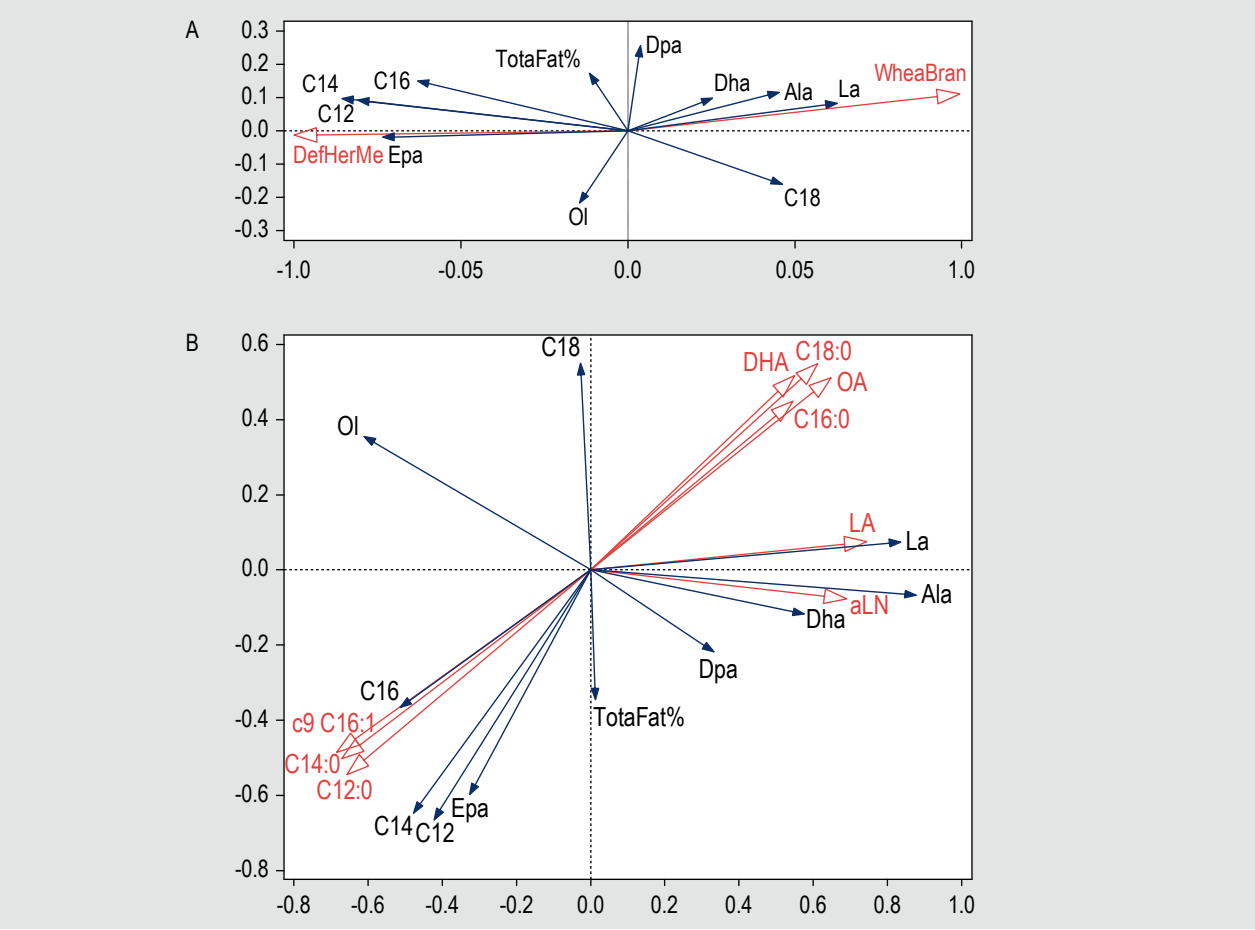

Figure 1. Biplot from redundancy analysis showing the relationship between intakes of (A) feedstuffs and (B) fatty acid and egg fatty acid profiles (expressed as g/100 g total fatty acids). Intakes (drivers) shown in red: (A) DefHerMe = defatted *Hermetia illucens* meal; WheaBran = Wheat bran; (B) C12:0 = lauric acid; C14:0 = myristic acid; C16:0 = palmitic acid; c9 C16:1 = palmitoleic acid; C18:0 = stearic acid; OA = oleic acid; LA = linoleic acid; aLN = alpha-linolenic acid; DHA = docosahexaenoic acid. Responses i.e. Egg fatty acid concentrations (responses in 1A and 1B) shown in black: TotalFat = yolk lipid %; C12 = lauric acid; C14 = myristic acid; C16 = palmitic acid; C18 = stearic acid; Ol = oleic acid; La = linoleic acid; Ala = alpha-linolenic acid; Epa = eicosapentanoic acid; Dpa = docosapentaenoic acid; Dha = docosahexaenoic acid.

Table 4. Details from redundancy analysis shown in Figure 1; significant drivers and abbreviations.[1]

Driver name	% variation explained	*P*-value
Figure 1A feedstuffs intakes		
Defatted *Hermetia illucens* meal	13.9	0.002
Wheat bran	*2.5*	0.084
Figure 1B fatty acid intakes		
c9 C16:1	13.7	0.002
aLN	7.6	0.002
C18:0	6.2	0.004
C12:0	*2.6*	0.054
LA	*3.3*	0.10

[1] aLN = alpha-linolenic acid; C12:0 = lauric acid; C18:0 = stearic acid; c9 C16:1 = palmitoleic acid; LA = linoleic acid.

C12:0 and C14:0, yet despite this, the total lipid content of the eggs was generally unaffected. This is in line with Secci *et al.* (2018) and Heuel *et al.* (2021) who also reported feeding HIM had no effects on total lipid content of the eggs, despite the quantity and source of dietary lipid. It is also interesting to note similar yolk weights reported by Maurer *et al.* (2015) for this trial, further demonstrating the resilient buffering capacity with respect to egg composition under various dietary treatments.

Diet fatty acid intakes

Although the main differences between treatments here was the inclusion of HIM and associated reduction in soyabean meal, experimental diets also differed slightly in wheat and sunflower meal content (aiming for consistent protein levels) which influences FA supply and potentially their passage into eggs. The trial was conducted under

organic standards (except for HIM) using expeller soyabean and sunflower meals, with higher residual oil, compared with chemically extracted feeds, destined for non-organic livestock. Hence, FA profile of residual soyabean and sunflower oils might influence egg composition, considering the 4.5% lipid (likely to be soya oil) in the control diet. Irrespective of HIM, replacing some soyabean meal with an extra 55-56 kg sunflower meal per tonne will reduce C16:0, SFA and n-3 and increase LA and n-6 content (Glasser *et al.*, 2008; Oliveira *et al.*, 2010) (as well as the ratio of n-6:n-3). However, this is will not contribute towards the major increases seen for C12:0, C14:0 and SFA in experimental diets, relative to the control. These increases are common with Heuel *et al.* (2021) (the only other report on FA intakes when HIM replaces soyabean meal), as is the reduction in C18:0 intake although other differences here are at odds to that study. Intakes EPA were generally low from all diets in both studies, however, here they were increased in HIM diets, unlike reduced consumption reported by Heuel *et al.* (2021). There are also discrepancies in the ratios of n-6:n-3 – which were incrementally reduced by the HIM diets from the very high value (~21:1) for the control diet in this study. In contrast, the control diet reported by Heuel *et al.* (2021) had a much lower ratio, at 9.4:1, which increased with HIM or HI oil – to levels comparable to our H24 diet. The FA in insect meal are known to vary depending on the growth medium (Barroso *et al.*, 2017), possibly explaining some discrepancies when comparing results across studies. However, since wheat based pasta waste, as used in this study, is generally low in lipid and (like most grains and seeds) is dominated by n-6 rather than n-3 FA (Butler, 2014), this (along with residual soyabean oil) might explain the relatively high n-6 intake from all diets here, although not the higher (or less low might be more accurate) EPA supply from the HIM diets.

Egg fatty acid composition and comparison with other studies

Results in this trial show similarities but also differences with findings from 4 other studies reporting egg FA profiles after full or partial replacement of soyabean meal with HIM in layer diets (Bejaei and Cheng, 2020; Heuel *et al.*, 2021; Park *et al.*, 2021; Secci *et al.*, 2018). At 120 g and 240 g HIM per kg feed, inclusion rates in this study (matching the high protein content of the soyabean control diet) were higher than the others in this comparison, although intakes of HIM lipid by hens were possibly lower than treatments adding an extra 20 g HI oil/kg feed reported by Heuel *et al.* (2021). Unfortunately, comparing results across all 5 studies is challenging since FA concentrations (or output) in eggs are not reported with consistent units. However, comparing egg composition from experimental diets relative to the control hens, within the 5 studies, shows interesting similarities and differences (Table S2). Perhaps it ought not to be surprising that the level and statistical significance of differences appears to depend on the inclusion and consumption of HIM or oil by the hens. The 20 g HIM/kg diet reported by Park *et al.* (2021) makes little difference to yolk composition, nor does their 40 g HIM/kg diet. It is also worth noting Bejaei and Cheng (2020) unfortunately give no indication of statistical significance of differences in FA identified in their study (with 100 and 180 g HIM/kg diet). Also, despite a relative high rate of HIM feeding (at 170 g/kg diet) Secci *et al.* (2018) report few significant differences in egg composition, possibly due to the inclusion and influence of 'vegetable oil' in both control and HIM diets. Interestingly, the trial reported by Bejaei and Cheng (2020) included soyabean oil across all diets which again could mask the impact of lipids of insect origin on egg composition.

That said, the most striking and consistent differences across these 5 studies are the elevation in C12:0 and C14:0 output in eggs from HIM (as in this study) although C12:0 was only reported in 3 of the papers. However, despite higher levels of these medium chain SFA, other SFA were either unchanged or in the case of stearic acid (SA, C18:0) reduced in eggs from HIM diets. Consequently, only Heuel *et al.* (2021) reports total SFA output to be significantly higher (between 8-15% more in eggs from the 4 diets containing HIM and/or extracted oil) than control eggs – as in this study.

Other differences common across some studies (including this work) were lower total PUFA in eggs from HIM (on average 19% less across all results presented in Table S2). This was more marked for ALA (32% lower) and n-3 (22% lower) but LA (18% lower) and n-6 (19% lower) were also less than in control eggs. Total long chain n-3 (lc n-3) and their individual contributions in eggs were low across all studies, with minor differences from feeding HIM (significant in some cases) although their content in insect meal could be enhanced by enriching the growth media for its production (Barroso *et al.*, 2017).

Comparing differences in dietary fatty acid intake vs egg output

Higher intakes and output in eggs of medium chain SFA, C12:0 and C14:0 (and total SFA) from HIM in layer diets (Heuel *et al.*, 2021) were confirmed here although, as with that study, we found differences in intake (Table 2) to be much greater than their transfer into eggs (Table 3). The RDA results support the theory of hens' selective secretion of FA into eggs, despite the close association in the biplot between C12:0 and C14:0 intakes with their appearance in egg, which give no indication of major discrepancies between the magnitude of changes in dietary intakes vs egg composition. On the other hand, the apparent weak relationship between feedstuffs and FA intakes on total egg lipids and concentrations of some FA (C18:0, OA

individual and total lc n-3) and the ratio of n-6:n-3, suggests an attempt to maintain egg composition, irrespective of diet. Presumably the birds' resilient ability to control egg composition (up to a point) is aimed at consistent early nutrition for developing embryos in fertilised eggs. There may not be evidence in avian species, but mammals prioritise functional PUFA for reproductive advantage (McKeegan and Sturmey, 2012) and Burdge and Calder (2005) identified greater ability for lc n-3 synthesis in women (compared with men), speculating on its role for neonate viability.

Consequences for consumer health

Despite indications of antimicrobial activity from C12:0 and its potential to enhance gut health in pigs and poultry (Gasco *et al.*, 2018; Veldkamp *et al.*, 2021) (especially with suboptimum hygiene and husbandry), there is on-going debate about a negative influence of C12:0, along with C14:0, over human health. These medium chain FA are thought to elevate circulating low density lipoprotein (LPL) cholesterol and promote blood coagulation, insulin resistance and inflammation (Calder, 2015; Wang and Hu, 2017). However, some arguments might question if the increases in eggs seen here represents a threat to health and perhaps suggests the need for further investigation. The relative proportions of LPL and high density lipoprotein cholesterol (LDL:HDL) is a global lipid maker predicting cardiovascular risk, with the preference to reduce the proportion of LDL (Wang and Hu, 2017). Although C14:0, C12:0 and C16:0 all are reported to raise LDL and total cholesterol (in this order of potency) (Calder, 2015), C12:0 (the FA with the greatest increase in eggs) has also been reported to raise HDL cholesterol, significantly increasing its proportion relative to LDL (Wang and Hu, 2017). Also, further evidence challenging or questioning the health risk from C12:0 and C14:0 comes from a European prospective investigation with 75,000 participants in UK and Denmark, reporting an inverse or neutral relationship between their intake and incidence of myocardial infarction during the 18 year study (Praagman *et al.*, 2019).

Secondly, although the greatest difference in birds' intake (relative to control diets) was for C12:0 and C14:0 and eggs from HIM feeding were substantially and significantly higher than controls, the actual levels (<0.3 and <2% of total FA, respectively) are not particularly high, compared to other common foods. Relative increases are large due to the very low levels in control eggs (hence the 'big' increases) yet the highest inclusion of HIM only 'adds' 17 mg C12:0 and 103 mg C14:0 acids to each egg, giving 58 and 385 mg per 100 g edible egg (respectively). To put this into context, these are substantially lower than many foods listed in the UK food composition reference base (McCance and Widdowson, 2015). With respect to C12:0, 'fruit and fibre breakfast cereal' (11-779), 'chocolate covered ice cream' (12-384), 'instant cup soup' (17-653) and 'Thai chicken curry' (19-465) are all quoted between 30-160 times higher than H24 eggs; corresponding values for C14:0 are 2-9 times higher than these eggs. Another interesting comparison considers the composition of insect meal relative to the eggs produced from its consumption by hens. Using the HI composition reported by Secci *et al.* (2018) and assuming comparable lipid intakes, H24 eggs in this study would supply less than 1% of the C12:0 and 25% of C14:0 than direct consumption of insect meal.

5. Conclusions

This study adds to the evidence of laying hens' ability to control egg composition despite variation in diet, in this case with respect to lipid content and composition. Replacing some or all soyabean meal with defatted insect meal at 120 g and 240 g per kg diet increased dietary fat, particularly the proportion and intake of medium chain fatty acid and total SFA, at the expense of the MUFA and PUFA (both n-3 and n-6). These differences were reflected to some extent in egg FA profiles, with more C12:0, C14:0 and SFA appearing in eggs. However, differences in egg composition, relative to control eggs, were minor compared to changes in intake and although C12:0 and C14:0 and SFA were significantly increased in eggs, they were not particularly high and may or may not challenge consumer health. Based on the FA profiles, this study suggests replacing soyabean meal with HIM in layer diets is likely to have a 'less negative' impact on public health, than direct consumption of insect meal.

Supplementary material

Supplementary material can be found online at https://doi.org/10.3920/JIFF2022.0027

Table S1. Diet fatty acid (FA) profile (mean concentrations ± standard error of means in g/100 g total FA) of control diet, diet with *Hermetia* meal at 12% (H12 and, diet with *Hermetia* meal at 24% (H24). Percentage difference to control diet.

Table S2. Comparison of egg fatty acid profiles reported from 5 trials, partially of fully replacing soya bean meal in layer diets with black soldier meal (HI/HIM).

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Conflict of interest

None of the authors are aware of any conflict in interest associated with this paper or its publication.

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