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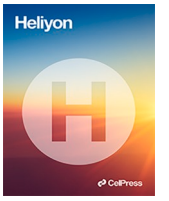

Research article

Lauric acid content in intramuscular fat is a reliable indicator of black soldier fly larvae meal consumption in Muscovy ducks

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ABSTRACT

The present research investigated if intramuscular fatty acid (FA) profile could distinguish meat from ducks fed with black soldier fly larvae meal (BSFLM) during fattening. By using stepwise linear discriminant analysis on FA profiles of 96 meat samples, lauric acid (C12:0) was found to be the best predictor, accurately differentiating samples with only two misclassifications. The Fisher classification functions indicated that breast samples with lauric acid content above 0.222 % in intramuscular fat would be classified as BSFLM-fed. The Fisher classification functions were also effective in other poultry species fed BSFLM, with only two misclassified samples out of 42 samples from a dataset compiled from published papers. Misclassifications were linked to an unexpected lauric acid content in the intramuscular fat. In conclusion, given that BSFLM is the only relevant source of lauric acid in the diet, this fatty acid could identify meat from BSFLM-fed ducks and possibly other poultry species, warranting further exploration of other FA as potential identifiers of BSFLM-fed poultry.

1. Introduction

Over the next decade, poultry meat is expected to account for half of the increased meat production and consumption [1]. Regarding ducks, its global population showed an important increase from 1961 to 2019 (from 193.4 to 1177.4 million heads, respectively), and duck meat production will take a positive role in the future [2].

Dietary protein requirements of poultry are usually met by incorporating in the diet ~20 % of protein supplements, mostly from plant origin [3], which are expensive due to their rising demand and limited supply [4]. Moreover, the efficiency of poultry in converting feed into meat often overlooks its competition for human-edible grains [3,5]. Against this backdrop, the inclusion of insect meals in the diet could benefit the sustainability of the poultry production systems [6,7]. Particularly black soldier fly larvae meal (BSFLM) has attracted great attention in recent years as a sustainable protein source for poultry feeding [8].

Beyond their potential effects on production performance [9,10], the inclusion of insect meals in feeds might affect the nutritional

Abbreviations: BSFLM, black soldier fly larvae meal; CON, control diet; FA, fatty acid.

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and sensory quality of poultry meat and the perception of consumers of such foods [11,12]. Indeed, it has been found that increasing consumers' information on the sustainability and nutritional benefits of using insects in poultry feeds increases both attitude towards and intention to purchase and eat meat products from animals fed those feeds [13,14], which highlights the importance of traceability and transparency in the poultry meat supply chain. Furthermore, meat authentication in terms of dietary background of animals could greatly improve consumers' confidence [15].

The fatty acid (FA) profile of intramuscular fat can characterize feeding practices in several farm species [16]. It has proven to efficiently identify the diets fed to fattening swine, cattle, and sheep by discriminant analysis [17–19]. Discriminant analysis is a multivariate statistical technique that uses observed variables (predictors) to construct a predictive model capable of distinguishing between two or more groups. This model can then be used to classify future observations into one of these predefined groups, based on the predictors [20].

Gkarane et al. [21] showed that discriminant analysis of volatile compounds in chicken meat clearly distinguished the group that was fed BSFLM in their study, but to the best of our knowledge no such separation has been attempted by using meat FA as predictors. In fact, fat composition in insects can change according to sex, development stage, rearing substrates, and processing methods [22,23], but FA profiles are rather specie specific. As for black soldier fly, saturated fatty acids are the main FA in the dry matter with lauric as the most abundant followed by palmitic and myristic acids [24]. Recently, Gariglio et al. [25] reported for the first time the changes of the FA profile in meat from Muscovy ducks (*Cairina moschata domestica*) fed BSFLM. To the best of our knowledge, no other papers have dealt with this topic. Again, from the point of view of consumer's information, it would be interesting to have methods to distinguish meat from ducks fed diets with such alternative protein source. Therefore, we aimed to investigate if and how the intramuscular FA profile could differentiate the meat from Muscovy ducks fed BSFLM during fattening by means of discriminant analysis.

2. Results and discussion

Final body weight (2516 ± 70 g), average daily gain (52 ± 2 g), daily feed intake (120 ± 4 g), feed conversion ratio (2.3 ± 0.1 g/g) and abdominal fat (2.08 ± 0.29 % slaughter weight) did not differ between treatments [25,26].

Univariate test of equality between group means of CON and BSFLM samples (Table 1) showed that nine out of 14 FA differed between treatments ($P < 0.05$). Lauric acid (C12:0) was the only fatty acid selected as predictor in the Fisher classification functions (Table 2). Wilks' lambda test, which checks the linear relationship between the predictors and the grouping variable (i.e., the validity of the analysis) was highly significant ($P < 0.001$). Class means for each group's canonical observation scores were −1.53 (CON) and 0.51 (BSFLM) in breast samples and −1.85 (CON) and 0.62 (BSFLM) in thigh samples, and the Mahalonobis squared distances between groups (4.19 and 6.10 in breast and thigh samples, respectively) were highly significant ($P < 0.001$). The obtained Fisher classification functions incorrectly assigned one breast sample from the BSFLM group into the CON group (2.08 % prediction error rate), whereas all thigh samples were correctly classified. The functions indicated that ~0.222 % and ~0.459 % lauric acid content in the intramuscular fat of the breast and thigh samples, respectively, would be the thresholds to separate the two groups of samples based on the dietary treatment (i.e., samples with a lauric acid content below the threshold would be assigned to the CON group, while samples with a lauric acid content above the threshold would be allocated to the BSFLM group).

Lauric acid content in duck meat has not been usually reported in the literature [27–32]. Nevertheless, the data used in the current work and those available from published research support that lauric acid is a minor FA that exhibits a very low content in the intramuscular fat (less than 0.13 % of total FA) when ducks are fed diets based on conventional ingredients and reared under typical management [25,33,34]. Meanwhile, lauric acid has been consistently found to be the predominant FA in BSFLM fat, making up 44.6 ± 7.4 % of total FA [25,35–40]. This predominance of lauric acid in BSFLM agrees with its high abundance in *Hermetia illucens* larvae

Table 1
Fatty acid composition (mean ± standard deviation, expressed as % of total fatty acids) of the intramuscular fat of breast and thigh from Muscovy ducks, and univariate test of equality between group means of the diet classes (CON: control diet; BSFLM: diet containing black soldier fly larvae meal; N = number of animals in each group).

Fatty acids	Breast			Thigh		
	CON N = 12	BSFLM N = 36	P	CON N = 12	BSFLM N = 36	P
C12:0	0.12 ± 0.04	0.56 ± 0.25	<0.001	0.13 ± 0.03	1.15 ± 0.47	<0.001
C14:0	0.37 ± 0.04	0.51 ± 0.10	<0.001	0.49 ± 0.04	0.7 ± 0.11	<0.001
C16:0	20.62 ± 0.84	20.93 ± 0.87	0.286	20.43 ± 0.83	19.96 ± 0.86	0.109
C18:0	12.97 ± 0.93	11.97 ± 1.14	0.009	9.25 ± 0.79	8.9 ± 0.86	0.222
C16:1 n7c	1.18 ± 0.19	1.23 ± 0.19	0.408	0.40 ± 0.02	0.38 ± 0.03	0.044
C18:1 n9c	23.58 ± 1.37	25.45 ± 2.18	0.008	29.69 ± 1.54	29.74 ± 1.76	0.939
C18:1 n7t	2.35 ± 0.20	2.20 ± 0.25	0.080	0.19 ± 0.03	0.18 ± 0.03	0.413
C18:2 n6	21.07 ± 0.58	21.66 ± 1.36	0.156	24.03 ± 0.97	24.43 ± 1.46	0.380
C18:3 n3	0.98 ± 0.10	1.16 ± 0.16	<0.001	1.53 ± 0.14	1.69 ± 0.13	<0.001
C20:2 n6	0.51 ± 0.06	0.45 ± 0.08	0.041	0.22 ± 0.03	0.22 ± 0.03	0.872
C20:4 n6	8.23 ± 0.97	6.82 ± 1.39	0.002	4.68 ± 0.88	4.23 ± 0.90	0.132
C20:5 n3	0.16 ± 0.04	0.15 ± 0.03	0.358	0.12 ± 0.02	0.11 ± 0.03	0.521
C22:5 n3	0.72 ± 0.13	0.61 ± 0.13	0.014	0.50 ± 0.12	0.45 ± 0.10	0.139
C22:6 n3	0.67 ± 0.10	0.56 ± 0.14	0.013	0.50 ± 0.08	0.44 ± 0.12	0.143

Table 2
Fisher's classification functions.

	Breast		Thigh	
	Function 1 CON	Function 2 BSLFM	Function 1 CON	Function 2 BSLFM
Intercept	−1.534	−3.641	−1.440	−4.202
C12:0	2.520	12.00	0.800	6.818

Samples will be assigned to the group (either control, CON, or black soldier fly larvae meal, BSFLM) based on the function that yields the highest score.

regardless of the rearing substrate [24] except for substrates supplemented with up to 10 % of fats rich in unsaturated FA of 18 atoms of carbon [41]. Therefore, the high discriminating ability of lauric acid in the present work would be explained by its accumulation in the intramuscular fat after BSFLM consumption. Moreover, from the data of Gariglio et al. [25] it can be calculated that increasing the lauric acid content in the dietary fat by 1 % linearly increases the lauric acid content in the intramuscular fat of breast and thigh by ~0.087 and ~0.200 percentage points, respectively.

Table 3
Classification results obtained by applying the Fisher functions to a dataset compiled from the literature.

Author	Poultry (sample)	Treatment[a]	Score in CON[b]	Score in BSFLM	Predicted membership[c]
Altmann et al. [45]	Broilers (thigh)	C†	−1.42	−3.89	CON
		HI	1.07	17.42	BSFLM
Cullere et al. [35]	Quails (breast)	Control†	−1.53	−3.64	CON
		H1	−1.18	−1.96	CON
		H2	−1.20	−2.08	CON
Cullere et al. [36]	Quails (breast)	C†	−0.98	−1.00	CON
		H1	10.01	51.32	BSFLM
		H2	14.82	74.24	BSFLM
Daszkiewicz et al. [40]	Broilers (breast)	0 % PAP–HI†	−1.35	−2.80	CON
		50 % PAP–HI	20.49	101.24	BSFLM
		75 % PAP–HI	28.05	137.24	BSFLM
		100 % PAP–HI	34.25	166.76	BSFLM
de Souza et al. [42]	Broilers (breast)	T1†	0.03	3.77	BSFLM[f]
		T2	8.48	44.02	BSFLM
		T3	16.12	80.38	BSFLM
		T4	26.77	131.10	BSFLM
		T5	33.30	162.22	BSFLM
Heuel et al. [43]	Broilers (breast)	SS†	−1.30	−2.56	CON
		SS–†	−1.23	−2.20	CON
		AA–	17.04	84.80	BSFLM
		AB–	13.82	69.44	BSFLM
		BB–	16.01	79.88	BSFLM
Kim et al. [39]	Broiler[d] (leg)	CON†	−0.94	0.28	BSFLM[f]
		25HILM	−0.73	2.05	BSFLM
		50HILM	−0.74	1.98	BSFLM
Mlaga et al. [46]	Broilers (breast)	T0†	−1.53	−3.64	CON
		T4	1.17	9.20	BSFLM
		T8	2.12	13.76	BSFLM
		T12	5.40	29.36	BSFLM
Popova et al. [38]	Broilers (breast)	CON†	−1.05	−1.36	CON
		T1	1.17	9.20	BSFLM
		T2	4.82	26.60	BSFLM
	Broilers (thigh)	CON†	−1.42	−3.88	CON
		T1	−0.18	6.76	BSFLM
		T2	0.50	12.48	BSFLM
Schiavone et al. [37]	Broilers (breast)	HI0†	−1.53	−3.64	CON
		HI5	−0.70	0.32	BSFLM
		HI10	0.01	3.68	BSFLM
		HI15	1.07	8.72	BSFLM
Secci et al. [47]	Partridges (half carcass)[e]	SBM†	−1.00	−1.12	CON
		HI25	0.69	6.92	BSFLM
		HI50	3.96	22.52	BSFLM

[a] As named in the original paper. The control treatments has been identified with a †.
[b] The scores were calculated with the functions presented in Table 1 and the percentage of lauric acid (C12:0) in intramuscular fat reported by each author.
[c] The membership group was assigned according to the function that yielded the highest score.
[d] Thigh classification functions were used.
[e] Breast classification functions were used.
[f] Indicates a misclassification.

Table 4
Ingredients (g/kg as fed), nutrient composition and fatty acid composition of the experimental diets (adapted from Gariglio et al. [25]).

Ingredients	Starter period (days 3–17)				Grower period (days 18–38)				Finisher period (days 39–50)			
	CON	BSFLM 3 %	BSFLM 6 %	BSFLM 9 %	CON	BSFLM 3 %	BSFLM 6 %	BSFLM 9 %	CON	BSFLM 3 %	BSFLM 6 %	BSFLM 9 %
Maize meal	600	600	600	600	638	638	638	638	670	670	670	670
Soybean meal	212	212	212	212	160	160	160	160	100	100	100	100
HI larva meal	0.00	30.0	60.0	90.0	0.00	30.0	60.0	90.0	0.00	30.0	60.0	90.0
Wheat bran meal	42.5	42.5	42.5	42.5	36.3	36.3	36.3	36.3	66.2	66.2	66.2	66.2
Corn gluten meal	90.0	60.0	30.0	0.0	90.0	60.0	30.0	0.0	90.0	60.0	30.0	0.0
Soybean oil	16.5	16.5	16.5	16.5	28.5	28.5	28.5	28.5	34.5	34.5	34.5	34.5
DL-methionine	2.50	2.50	2.60	2.80	1.70	1.80	1.90	2.20	0.30	0.40	0.50	0.80
L-lysine	3.90	3.90	3.80	3.60	3.90	3.80	3.70	3.40	3.00	2.90	2.80	2.50
Other ingredients[a]	32.6	32.6	32.6	32.6	41.6	41.6	41.6	41.6	36.0	36.0	36.0	36.0
Total	1000	1000	1000	1000	1000	1000	1000	1000	1000	1000	1000	1000
AMEn (MJ/kg)	12.1	12.1	12.1	12.1	12.5	12.5	12.5	12.5	12.8	12.7	12.7	12.7
Nutrient composition (g/kg as fed)												
DM	893	898	901	897	888	892	891	891	887	890	893	889
CP	224	222	227	222	204	201	200	200	179	180	179	179
EE	42.0	43.1	44.1	45.7	55.1	55.3	57.0	58.8	64.8	65.9	67.9	68.5
NDF	113	110	114	111	115	117	112	113	113	117	113	115
ADF	30.4	31.4	33.3	29.6	31.1	31.2	33.0	31.2	30.2	31.2	31.3	30.2
Ash	50.0	53.9	50.5	52.0	69.3	66.9	66.8	71.3	57.7	57.0	61.6	59.6
Fatty acids (% of total fatty acids)												
C12:0	0.07	2.73	5.49	8.11	0.07	2.57	4.82	7.67	0.07	2.07	4.82	6.35
C14:0	0.16	0.60	1.13	1.65	0.14	0.57	0.96	1.46	0.10	0.48	0.99	1.26
C16:0	15.0	13.9	13.7	13.6	14.0	13.7	13.4	13.1	13.8	13.5	13.0	12.7
C18:0	2.83	2.62	2.65	2.53	2.75	2.64	2.58	2.52	2.67	2.63	2.71	2.70
C16:1 n7c	0.15	0.29	0.44	0.57	0.15	0.29	0.39	0.53	0.14	0.26	0.42	0.50
C18:1 n9c	25.8	24.2	23.5	22.4	23.9	22.9	22.2	21.3	23.1	22.8	21.7	21.9
C18:1 n7t	1.10	1.03	1.00	1.03	1.21	1.15	1.12	1.08	1.22	1.16	1.10	1.07
C18:2 n6	49.1	48.8	46.0	44.3	51.5	49.8	48.1	46.1	52.2	50.4	47.8	46.3
C18:3 n3	3.27	3.40	3.35	3.29	4.02	4.13	4.05	4.06	4.48	4.43	5.28	4.98

Abbreviations: BSFLM: Black soldier fly larvae meal; AMEn: apparent metabolizable energy; DM: dry matter; CP: crude protein; EE: ether extract; NDF: neutral detergent fiber; ADF: acid detergent fiber.

[a] Other ingredients (g/kg as fed): dicalcium phosphate (10.0, 13.0, 4.0, for Starter, Grower and Finisher periods, respectively), calcium carbonate (8.0, 14.0, 17.4, for Starter, Grower and Finisher periods, respectively), sodium chloride (2.5 for all periods), sodium bicarbonate (2.0 for all periods), mineral-vitamin premix (5.0 for all periods), choline chloride (0.1 for all periods), Optifos 250 bro (1.0 for all periods), Avizyme 1500 x (1.0 for all periods), titanium dioxide (3.0 for all periods).

The good performance of the Fisher's classification functions to identify the meat from ducks fed BSFLM aimed us to test them in other poultry species (*Gallus gallus domesticus*, *Coturnix coturnix japonica*, and *Alectoris barbara*). A validation dataset was compiled from the results presented in 11 papers published in recent years, which dealt with the effects of dietary BSFLM on FA composition of breast (seven papers), thigh (one paper), breast and thigh (one paper), leg (one paper) and half carcass (one paper) (Table 3). In all the compiled studies, FA in intramuscular fat were determined by gas chromatography and expressed as percentage of total FA. It was found that two CON observations were classified into the BSFLM group [39,42], resulting in an overall prediction error rate of 4.76 %. An examination of the erroneously classified observations revealed that their lauric acid content was largely higher (~2.8 times) than the above-mentioned threshold. Such elevated concentration of lauric acid is difficult to explain, given that the intramuscular fat of broilers fed diets based mainly on cereals and soybean meal, as the offered in the studies of de Souza et al. [42] and Kim et al. [39], typically exhibits minimal lauric acid content [37,43]. This is attributable to the scant presence of lauric acid in such type of diets, as supported by several studies [25,35,37,43,44].

We further tested the reliability of lauric acid content in intramuscular fat as a predictor of meat from birds fed BSFLM by means of an alternative statistical approach. To this purpose, the results of Gariglio et al. [25] were included in the validation dataset to reach a total of 50 observations (15 CON and 35 BSFLM). The new dataset was submitted to logistic regression analysis, weighted by the square root of the number of replicates in each treatment, including lauric acid content as the independent variable. The Hosmer-Lemeshow test indicated an excellent goodness of fit for the logistic regression model (Equation (1)), as shown by a Chi-square value of 2.65 and a P-value of 0.85.

$$P = 1 \,/\, [1 + e^{-(3.3988+7.5842 \times \mathrm{C12:0})}] \quad (1)$$

Where P is the membership probability of belonging to the BSFLM group, expressed as decimal (0–1), e is the base of the natural logarithm, and C12:0 is the percentage of lauric acid in intramuscular fat.

Youden index indicated that the model would achieve optimal sensitivity and specificity, thus minimizing the prediction error, if the cut-off for membership probability was set at P = 0.28, which would correspond to a threshold of ~0.325 % lauric acid in intramuscular fat. At the cut-off, the model resulted in only two false positives and no false negatives. The two observations erroneously assigned to the BSFLM group were the controls in the studies by de Souza et al. [42] and Kim et al. [39], in agreement with the Fisher classification functions. Consequently, the logistic regression model had an overall prediction error rate of 4 %. These results would support the merit of lauric acid content in intramuscular fat to identify the meat from poultry with a BSFLM-based feeding background.

3. Conclusions

Stepwise linear discriminant analysis of the intramuscular FA profile of ducks allowed to obtain Fisher classification functions, with lauric acid as the sole predictor, that accurately identified the meat from ducks fed BSFLM. The functions proved suitable in classifying the meat from other poultry fed BSFLM, suggesting the potential for a broader applicability provided that BSFLM is the only relevant source of lauric acid in the diet. Logistic regression analysis further supported the merit of lauric acid content in intramuscular fat to detect meat from BSFLM-fed birds. Future research with samples obtained under on-farm conditions would be advisable to confirm the potential of lauric acid and/or other FA in the intramuscular fat of poultry meat as distinguishing factors of BSFLM consumption. Establishing these markers could help to trace poultry meat within the food supply chain, facilitating accurate identification of products that come from birds with a BSFLM-based feeding background. This, in turn, would improve transparency and bolster consumers' confidence.

4. Materials and methods

The present study used intramuscular FA profiles of 48 breast and 48 thigh samples obtained from 48 Muscovy ducks fed a control diet without BSFLM (CON group; 12 samples) or a diet containing 3 %, 6 % and 9 % BSFLM (BSFLM group; 36 samples). The data were obtained in one experiment whose results have been published elsewhere [25,26].

Animal husbandry, experimental design, sampling, and analytical methods are described in detail in Gariglio et al. [25,26]. Briefly, a total of 192 three-day-old female Muscovy ducklings (Canedins R71 L White, Grimaud Freres Selection, France) were randomly distributed in 24 pens (eight birds per pen) and allocated to one of four dietary treatments consisting in diets, based on maize and soybean meal (>75 %, as fed), where maize gluten meal was replaced by 0 %, 3 %, 6 % and 9 % of partially defatted BSFLM (Table 4). At 50 days of age, two birds from each replicate (12 birds per treatment) with the closest body weight to the mean weight for their pen were transferred to a commercial processing plant and slaughtered, according to the standard EU regulations. The carcasses were chilled at 4 °C for 24 h. Then the meat from the left breast and left thigh was separated from the bones and cartilage and ground for lipid extraction. Intramuscular fat was extracted using petroleum ether as extraction solvent. The FA in extracted fat were derivatized to methyl esters by acid-catalysed methanolysis using sulphuric acid. The fatty acid methyl esters were identified and quantified by gas chromatography by mean of Agilent 7890A GC System (Agilent Technologies, Santa Clara, CA). For this purpose, the Supelco SP-2560 (Sigma-Aldrich, St. Louis, MO) (75 m × 180 mm internal diameter, 0.14 μm film thickness, flow 0.25 mL/min) and the Agilent J&W HP5ms (3.8 m × 250 μm internal diameter, 0.25 μm film thickness) columns were used, with hydrogen as the carrier (split inlet, heater at 270 °C, mode Pulse Split 25 psi until 0.30 min, split ratio 160:1, 40 mL/min). The temperature of the oven was set to 40 °C (for 2 min), lifted to 170 °C (rate of 50 °C/min and held for 25 min), raised to 250 °C (rate of 2 °C/min, and held for 14 min). The FAME were

identified by comparing the retention times with a standard mixture of 52 FAME (GLC 463, NU-CHEK PREP Elysian, MN). Individual FAME were expressed as percentage of total FA.

All statistical analyses were carried out with SAS OnDemand for Academics (SAS Institute, Cary, NC, USA). The data of intramuscular FA contents in breasts and thighs, as outlined in Table 1, were entered into two separate stepwise linear discriminant analyses to derive Fisher classification functions. The FA to be included as predictors were selected using the STEPDISC procedure (P to enter = 0.15; P to stay = 0.15). Then, in the DISCRIM procedure, the inclusion of BSFLM in the diet was considered as the hypothetical grouping variable (two levels: CON and BSFLM), and the predictor variables were the FA selected in the previous step.

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Ethical approval

Not applicable.

Data availability statement

The data associated with the present study have not been deposited into a publicly available repository. They are available from the corresponding author, [M.G., marta.gariglio@unito.it], upon reasonable request.

CRediT authorship contribution statement

Andrés L. Martínez Marín: Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Conceptualization. **Marta Gariglio:** Writing – review & editing, Investigation, Conceptualization. **Angela Trocino:** Writing – review & editing, Resources, Investigation. **Achille Schiavone:** Writing – review & editing, Resources, Investigation, Funding acquisition, Conceptualization.

Declaration of competing interest

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

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