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ORIGINAL ARTICLE

Transfer of Lauric and Myristic Acid from Black Soldier Fly Larval Lipids to Egg Yolk Lipids of Hens Is Low

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Abstract Implementing insects, such as the black soldier fly larvae (BSFL), as animal feed commonly includes the previous removal of substantial amounts of fat. This fat may represent an as yet underutilized energy source for livestock. However, transfer of lauric and myristic acid, prevalent in BSFL fat and undesired in human nutrition, into animal-source foods like eggs may limit its implementation. To quantify this, a laying hen experiment was performed comprising five different diets (10 hens/diet). These were a control diet with soybean oil and meal and a second diet with soybean oil but with partially defatted BSFL meal as protein source. The other three diets were based on different combinations of partially defatted BSFL meal and fat obtained by two different production methods. Lauric acid made up half of the BSFL fat from both origins. Both BSFL fats also contained substantial amounts of myristic and palmitic acid. However, in the insect-based diets, the net transfer from diet to egg yolk was less than 1% for lauric acid, whereas the net transfer for myristic and palmitic acid was about 30% and 100%, respectively. The net transfer did not vary between BSFL originating from production on different larval feeding substrates. The results illustrate that hens are able to metabolize or elongate very large proportions of ingested lauric acid and myristic acid, which are predominant in the BSFL lipids (together accounting for as much as 37 mol%), such that they collectively account for less than 3.5 mol% of egg yolk fatty acids.

Keywords Dietary fat · Fatty acids · Lipid analysis · Nutrition · Saturated fatty acids

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Abbreviations

ALA	α-linolenic acid (18:3n-3)
BSFL	black soldier fly larvae
DM	dry matter
EE	ether extract
FA	fatty acid(s)
FAME	fatty acid methyl ester
LAU	lauric acid (12:0)
LNA	linoleic acid (18:2n-6)
MCFA	medium-chain saturated fatty acids
MUFA	monounsaturated fatty acids
MYR	myristic acid (14:0)
OLA	oleic acid (18:1n-9)
PAM	palmitic acid (16:0)
PUFA	polyunsaturated fatty acids
SFA	saturated fatty acids
SO	soybean oil
STA	stearic acid (18:0)

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Introduction

Along with the perspective of a globally growing human population and rising demand for animal protein, insects are becoming an increasingly attractive alternative source for livestock feeding (van Huis, 2013; Makkar et al., 2014). Insects offer a possible solution to improve the sustainability of livestock farming as they can be grown on a wide range of substrates (Smetana et al., 2019). In particular, black soldier fly larvae (BSFL), a phylogeographically remarkable representative of the family of *Stratiomyidae* of American origin (Ståhls et al., 2020), are considered promising as they efficiently convert food not used for consumption, animal manure, or other organic waste from side streams into a high-quality insect biomass as a basis for animal feed (Gold et al., 2018; Gold et al., 2020; van Huis et al., 2020). Regarding poultry nutrition, research on the use of BSFL so far has concentrated primarily on its fat-reduced form, the protein meal, and investigations have only recently started with respect to a more wide-spread use of pure BSFL fat or full-fat larvae as feed ingredients (Maurer et al., 2016; Marono et al., 2017; Mwaniki et al., 2020; Bejaei and Cheng, 2020; Kim et al., 2020).

Entire BSFL have a fat content ranging from 7% to >40%, a variation strongly depending on the type of rearing substrate (St-Hilaire et al., 2007; Zheng et al., 2012). In order to obtain protein-rich BSFL meal, a substantial amount of fat is removed by technological processing of the BSFL. The resulting fat is currently mostly considered for non-food purposes, like biodiesel production (Manzano-Agugliaro et al., 2012; Leong et al., 2016). However, as this form of use represents a great loss of potentially valuable feed energy, it is important to determine how its use affects the composition of the final animal product. Studies on the utility and the effects of feeding unprocessed BSFL or the BSFL fat, especially on the fatty acid (FA) profile of the resulting animal-source foods are still scarce. Kim et al. (2020) showed that the inclusion of BSFL oil in the diet of broilers increased the proportions of medium-chain saturated FA (MCFA) in the adipose tissue of the broilers, but had no negative effect on either growth or intestinal health of the birds. Bejaei and Cheng (2020) fed full-fat BSFL to laying hens but did not describe the FA profile of the BSFL and of the diets and did not provide data on individual FA but only on groups of FA in the egg lipids. It is well known that, apart from the amount, the composition of the dietary fat has an influence on the FA profile of the egg yolk (Cruickshank, 1934; Thomsen, 1966; Sell et al., 1968; Milinsk et al., 2003; Beynen, 2004).

The BSFL lipids, like that of other insects (i.e. mealworms or crickets) used as food and feed sources or considered for this purpose by legislation currently, are rich in MCFA, namely lauric acid (12:0, LAU), myristic acid (14:0, MYR), and palmitic acid (16:0; PAM) (Finke, 2002; Makkar et al., 2014). Mainly LAU and PAM are found in high concentration in BSFL (Kroeckel et al., 2012; Li et al., 2011; Oonincx et al., 2015). The LAU may make up even half of all FA in BSFL lipids (Kroeckel et al., 2012). However, from a human nutrition perspective, a high intake of foods rich in MCFA, especially in LAU and MYR, can lead to elevations in total and LDL cholesterol in plasma which may increase the risk of coronary heart diseases (Williams, 2000; Calder, 2015). By contrast, FA like those of the n-3 class, that is, α-linolenic acid (18:3n-3, ALA), eicosapentaenoic acid (20:5n-3), and docosahexaenoic acid (22:6n-3) are considered particularly valuable for human health (Candela et al., 2011), but these FA are low or sometimes even absent in BSFL lipids. Their prevalence could be enhanced (with different efficiency) by feeding BSFL with feed substrates rich in such FA (Spranghers et al., 2017).

The FA profile of the BSFL lipids is affected by the feed substrate they are grown on. For example, the BSFL lipids contained 47% LAU, 15% palmitic acid (16:0, PAM), and 14% oleic acid (18:1, OLA) when reared on chicken feed (Kroeckel et al., 2012). Using cow manure and fish offals as feeding substrate reduced LAU to 34% in BSFL lipids, which then contained 14% PAM and 16% OLA (St-Hilaire et al., 2007). Interestingly, the LAU content in BSFL lipids can apparently be reduced to 29% if a rearing substrate rich in fat, but containing little proportions of LAU is used, or even increased up to 50% if a low-fat substrate containing no LAU is used instead (Oonincx et al., 2015). Considering their uncommon FA profile, BSFL may greatly affect egg FA profile when eaten by hens. However, it is still unclear whether the major proportion of the MCFA of BSFL is secreted through the egg or metabolized by the hen. In the first case, the human health value of the eggs would clearly decrease. Indeed, a substantial increase in MCFA proportion was found in the body lipids when pigs were fed coconut oil and palm kernel oil (Jaturasitha et al., 1996; Kreuzer et al., 1997), a clear sign for a high net transfer and a limited metabolization. Along with this, in these studies, the pork fat was found to get extremely firm. Schäfer et al. (2001) were able to show that, when hens were fed large amounts of conjugated linoleic acids, their yolks showed an unusually pronounced rounding due to the increasing firmness of the yolk lipids. Accordingly, a high transfer of MCFA into the yolk lipids could have a similar effect.

In order to close these gaps in knowledge, an experiment with laying hens was carried out where the influence of BSFL fat on egg yolk FA profile and the apparent transfer of key FA to the egg yolk was investigated. The hypotheses to be tested were: (1) The use of BSFL fat in feed affects the FA profile of the egg yolk lipids. (2) The high

proportion of MCFA in the BSFL lipids substantially elevates these FA in the egg yolk. (3) Variations in BSFL lipid FA profiles due to their origin, as for instance caused by rearing substrate and processing, affect the FA profile of the egg yolks differently. For this purpose, two different origins of BSFL were compared to each other and to diets containing soybean oil (SO). Different from most previous studies, not only fat-reduced protein meals but also the pure BSFL fats were tested. Results on animal performance are described elsewhere (Heuel et al., 2021).

Animals, Materials and Methods

Animals and Housing Conditions

Fifty laying Lohmann Brown Classic hens, obtained from Burgmer Geflügelzucht AG, Weinfelden, Switzerland, were kept individually in 80 × 80 × 80 cm sized compartments. The hens were 28 weeks old at the start of the experiment. The compartments were equipped with nest, perch, meshed floor, and a box filled with fine de-dusted wood shavings, which could be used as a bath. Temperature (20°C) and humidity (40–45%) were controlled by an air condition system. Water and feeds (meal form) were offered at *ad libitum* access with nipple drinkers and troughs, respectively. Once a week, 500 g of feed was provided, and 50–150 g was replenished daily as needed. An artificial light program of 14 h light and 10 h dark was used, based on the breeder's recommendation for the housing system used and the stage of laying of the hens of this type (Lohmann Tierzucht, 2016). All hens finished the experiment and remained healthy as assessed by daily visual monitoring. The experiment was carried out at the research station AgroVet-Strickhof, Lindau, Switzerland and authorized by the Cantonal Veterinary Office of Zurich, Switzerland (license number ZH221/17).

Larval Material, Diet Composition, and Experimental Schedule

The BSFL material was obtained from two different sources. Along with processing, BSFL fat and fat-reduced protein meal had been obtained separately by both producers of materials A and B. Protein meal A and fat A were purchased from a commercial BSFL producing company (InnovaFeed, Paris, France). According to the information disclosed by the producer, the feeding substrate A consisted of >80% of wheat bran and solubles from wheat distillery. The BSFL had been harvested before they became prepupae. After being euthanized by exposure to thermic shock at >70°C, they had been subjected to a two-step process with an industrial scale processing instrument, first a drying step followed by a pressing step to remove and collect most of the fat. Protein meal B and fat B were produced in an experimental unit (FiBL, Frick, Switzerland). Larval feed substrate B consisted of 40% of fruit and vegetable raw waste, and of 30% each of spent brewer's grains and pasta production discard (off-specification batches of pre-cooked spaetzle, gnocchi and vegetarian variants of tortellini and ravioli). While the composition of the latter two components was quite constant over the 1-year production cycle of the BSFL material for the present experiment, the composition of fruit and vegetable waste seasonally varied. Harvest took place when prepupae started to occur. The BSFL were sacrificed by freezing after being cleaned from substrate residues. Larvae were then dried for 30–36 h at 60°C and part of the fat was removed with a modified commercial oil press (KK 20 F Universal, Screw Press, Reut, Germany). A detailed description of the conditions of production of BSFL material B can be obtained elsewhere (Leiber et al., 2017). By using these two BSFL origins, materials were expected to clearly vary due to the different production conditions concerning feed substrates, harvest, and processing of the BSFL.

Ten hens each were allocated to one of five experimental diets in a complete randomized design. One week of adaptation to the diet and housing environment was followed by 7 weeks of the experiment. The experimental diets were mixed in a single-shaft feed mixer (100 kg volume, Gericke AG, Zurich, Switzerland), according to the diet formulations. For this purpose, milled feed ingredients were ordered from local companies and first mixed for about 10 min until being homogeneous. Subsequently, the respective lipids (SO or BSFL fat) were evenly distributed on top of this mixture and mixed for another 15 min to form complete mash diets. All diets differed in the combination of the main protein and fat source (Table 1). The control diet (SS) did not contain BSFL material but was based on soybean cake and SO as main protein and fat sources. This diet was designed to comply with the breeder's recommendations of 11.4 MJ metabolizable energy/kg and 156 g crude protein/kg assuming a feed intake of 120 g/day for the type of hens used (all data on an as-fed basis). For all other diets, it was assumed that the protein and energy value of BSFL protein and fat was equivalent to that of soybean protein and SO. In a second experimental diet (AS), the soybean meal was replaced by partially defatted BSFL protein meal A, still with SO as the main lipid source. This diet was thus characterized by a mix of SO and BSFL fat. Finally, there were three experimental diets based on BSFL products, and soybean products were excluded. These included diet AA based on BSFL protein meal A and fat A, diet AB based on BSFL protein meal A and fat B, and diet BB. The latter was based only on partially defatted BSFL protein meal B. Due to the very high content of residual fat

Table 1 Ingredient composition of the experimental diets (g/kg DM)

Diet[a]	SS	AS	AA	AB	BB
Soybean cake[b]	150	–	–	–	–
Soybean oil	30	20	–	–	–
Larval protein meal A	–	150	150	150	–
Larval protein meal B	–	–	–	–	150
Larval fat A	–	–	20	–	–
Larval fat B	–	–	–	20	–
Wheat	300	240	240	240	305
Corn	180	205	205	205	190
Wheat boll meal[c]	31.6	41.9	41.9	41.9	58.9
Broken rice	20	59	59	59	50
Wheat bran	84.5	97	97	97	79
Sunflower cake	72.8	56	56	56	36
Limestone grit	70	70	70	70	70
Calcium carbonate	27	27	27	27	27
Celite[d]	16	16	16	16	16
Dicalcium phosphate	10	10	10	10	10
Sodium bicarbonate	3.3	3.3	3.3	3.3	3.3
Sodium chloride	2.0	2.0	2.0	2.0	2.0
Choline chloride	0.8	0.8	0.8	0.8	0.8
Vitamin and trace element premix[e]	2.0	2.0	2.0	2.0	2.0

[a]AA, larval protein meal A and larval fat A; AB, larval protein meal A and larval fat B; AS, larval protein meal A and soybean oil; BB, larval protein meal B rich in larval fat B; SS, soybean cake and soybean oil.

[b]Residue from soybean oil production by high-pressure treatment.

[c]By-product flour production from wheat with parts of the endosperm and all bran.

[d]Included in the diets as an indigestible marker for digestibility determination (for results *cf*. Heuel et al., 2021).

[e]Contained per kg: Ca, 86.5 g; P, 0.2 g; Mg, 25 g; Cu, 5 g; Mn, 30 g; J, 400 mg; Zn, 25 g; Fe, 25 g; Se, 100 mg; vitamin A, 5,000,000 IU; vitamin D_3, 1,250,000 IU; vitamin E, 15 g; vitamin K, 1.5 g; vitamin B_1, 1 g; biotin, 250 mg; folic acid, 750 mg; niacin, 20 g; pantothenic acid, 8.2 g.

Table 2 Analyzed contents (g/kg dry matter) of ether extract and crude protein of the soybean cake, the larval protein meals and the five experimental diets

Item	Soybean cake	Larval protein meal[a]		Diet[b]				
		A	B	SS	AS	AA	AB	BB
Ether extract	90.3	133	299	65.7	66.6	64.6	62.0	72.5
Crude protein[c]	443	460	380	170	168	169	166	158

[a]A produced on wheat bran and solubles, B produced on (g/kg) fruit and vegetables raw waste, brewer's grain and pasta production waste.

[b]AA, larval protein meal A and larval fat A; AB, larval protein meal A and larval fat B; AS, larval protein meal A and soybean oil; BB, larval protein meal B rich in larval fat B; SS, soybean cake and soybean oil.

[c]Nitrogen × 4.76 for larval crude protein (Janssen et al., 2017); diets: 150 g/kg of total dietary crude protein from N × 4.76 and N × 6.25 for the remaining crude protein.

(reported as ether extract (EE) in Table 2), no addition of fat B to diet BB was necessary.

Data Collection and Sampling

Egg yield , calculated as total number of eggs laid × 100/ days of the experimental period, and egg weights of the individual hens were determined daily during the 7 weeks of the experiment, while feed intake was measured weekly. Feed samples were collected on days 1 and 29, and samples of soybean cake and oil as well as of the different BSFL materials were obtained once before mixing them into the diets. In week 6 (days 38–43) four eggs per hen were collected to determine the composition of the yolks. Based on

these four eggs per hen the yolk yield per hen was calculated (average yolk weight/100 × egg yield).

Sample Preparation and Laboratory Analysis

Prior to laboratory analysis, complete diets, soybean cake, and BSFL protein meals were ground to 0.5 mm with a centrifugal mill (ZM 1, Retsch GmbH, Haan, Germany). The yolks of the four eggs collected were separated from the albumen, weighed, combined to one sample ($n = 10$ per diet), frozen at −20 °C, lyophilized (Beta 1–16 Christ, Osterode am Harz, Germany) and homogenized with a commercial kitchen mortar to a fine powder. Results on color and composition of the fresh yolks are described in Heuel et al. (2021). Feed items and lyophilized egg yolks were analyzed following the standard procedures (AOAC International, 1997) for DM (AOAC Official Method 942.05; model TGA-701, Leco, St. Joseph, MI, USA) and nitrogen (C/N analyzer (model TruMacCrude CN, Leco, St. Joseph, MI; AOAC Official Method 968.06; feed items only). Crude protein was calculated differently for BSFL and non-BSFL feed items (*cf.* footnote to Table 2). Contents of EE were measured in individual feed ingredients (except pure oils and larval fats) and the homogenized yolk material with a Soxhlet extractor using petrol ether as the solvent (System B-811, Büchi, Flawil, Switzerland; AOAC Official Method 963.15). The FA profiles of SO and larval fats, diets, and yolks were determined by gas chromatography. At first, FA were extracted from all samples with a hexane/isopropanol solution (3:2) in an Accelerated Solvent Extractor (model ASE 200, Dionex Corp., Sunnyvale, CA, USA). Prior to the extraction, triundecanoin (11:0) and butylated hydroxytoluene were added as internal standards. The FA were then derivatized to FA methyl esters (FAME) according to method 2.301 of the International Union of Pure and Applied Chemistry (IUPAC, 1991). This was accomplished by boiling for 3 min with 2 mL 0.5 NaOH and subsequent treatment with 3 mL of a borontrifluoride-methanol solution and 4 mL hexane (Fluka Chemie, Buchs, Switzerland). The FAME were then purified on silica gel and injected into a gas chromatograph (model HP 6890, Hewlett-Packard, Wilmington, PA, USA) equipped with a FID detector. The column used was a CP7421 column (200 m × 0.25 mm × 0.25 μm, Varian Inc., Darmstadt, Germany). The injection volume was set to 1 μL (split ratios of 1:20 and 1:30 for diets and yolks, respectively). Hydrogen served as carrier gas with a flow rate of 1.5 mL/min. The chromatograms were evaluated with the HP ChemStation software (Agilent, Palo Alto, CA, USA), whereby the individual FAME peaks were identified by comparison with the retention times of a 37-component FAME standard (Supelco). The response factors were computed from data obtained with sunflower oil (diet) and pork fat (yolk).

Calculations and Statistical Analysis

As no information about transformation or *de novo* synthesis of FA was available, only the net transfer could be calculated, that is, the net recovery of FA in the eggs of the amounts ingested by the hens. The net transfer of selected FAs was calculated by relating the daily secretion of each FA through the egg yolk to the hen's daily FA intake with the feed:

1. FA secretion with the egg yolk (g/d) = [EE secretion with the egg yolk (g/d) × proportion of FA (mole% of total FAME)]/100
2. FA intake (g/d) = [EE intake (g/d) × proportion of FA (mole% of total FAME)]/100

For this calculation, it was assumed that EE was equivalent to total FA. This might not be totally the case, but at least the same procedure was applied to all samples. Data were statistically analyzed with SAS version 9.4 (SAS Institute, Cary, NC, USA). The Mixed procedure as well as the Tukey–Kramer adjustment for multiple comparisons among means were applied. Control of normal distribution and homogeneity of variance was performed visually. Diet was considered as fixed effect and hen was treated as experimental unit. Data of the same variable measured more than once were combined to one value per hen. Results are given as least square means with standard errors of the mean, and effects at $P < 0.05$ were considered statistically significant. In the discussion, data on the FA profiles had to be occasionally converted from mole% to g/100 g of total FA, in order to allow comparisons with data from the literature.

Results

Fatty Acid Profile of Larval Materials and Complete Diets

The EE content of the BSFL protein meal B was 2.25 times higher compared to the BSFL protein meal A and 3.31 times higher than that of the soybean cake, respectively (Table 2). Although no BSFL fat B had been added, diet BB was still slightly richer in EE compared to the other diets. As intended, the diets were similar in crude protein content.

The FA profiles of the main dietary lipid sources differed in a way that PUFA dominated in the SO, followed by MUFA and SFA (Table 3). In contrast, in the two BSFL fats, the lipid fraction of the SFA predominated, followed

Table 3 Analyzed fatty acid profile (mole% of total identified fatty acids; determined as FAME) and total fatty acid contents of the soybean oil, the two larval fats and the five experimental diets

Item	Soybean	Larval fat[a]		Diet[b]				
	Oil (SO)	A	B	SS	AS	AA	AB	BB
8:0	–	–	–	0.09	0.08	0.07	0.07	0.06
10:0	–	1.29	1.14	0.07	0.50	0.82	0.77	0.82
12:0 (lauric acid, LAU)	–	48.4	45.8	0.51	16.0	30.4	27.6	30.1
14:0 (myristic acid, MYR)	0.10	8.57	9.38	0.26	3.03	5.69	5.40	5.64
iso-14:0	–	–	–	–	0.02	0.02	0.05	0.04
15:0	0.03	0.16	0.20	0.03	0.10	0.13	0.14	0.14
16:0 (palmitic acid, PAM)	11.9	11.2	15.5	12.3	12.9	13.6	13.7	13.5
iso-16:0	–	0.29	0.37	0.04	0.13	0.24	0.23	0.24
17:0	0.09	0.15	0.14	0.07	0.13	0.13	0.14	0.13
18:0 (stearic acid, STA)	3.81	1.61	2.66	3.17	2.48	2.06	2.11	2.05
20:0	0.34	0.07	0.07	0.40	0.22	0.13	0.12	0.13
21:0	–	–	–	0.03	0.04	0.05	0.06	0.05
22:0	0.37	0.02	0.02	0.35	0.23	0.12	0.13	0.12
24:0	0.12	0.01	-	0.16	0.13	0.08	0.09	0.08
12:1	–	0.03	0.03	–	–	–	–	–
14:1	–	0.15	0.25	0.13	0.03	0.13	0.12	0.13
15:1	–	–	–	0.02	0.02	0.02	–	–
16:1	0.09	0.05	0.08	0.08	0.04	0.02	0.06	0.05
16:1n-7	-	1.82	2.35	0.12	0.76	1.36	1.32	1.35
17:1	0.05	0.07	0.10	0.02	0.04	0.04	0.09	0.08
22:1	–	–	–	0.02	0.01	0.02	0.02	0.01
22:2	0.04	–	–	0.03	0.05	0.03	0.01	0.02
18:1n-9	20.8	11.1	13.1	28.9	23.7	19.9	21.5	19.9
18:1n-11	1.43	0.35	0.33	1.22	0.90	0.55	0.59	0.55
*t*10-18:1	–	0.04	0.11	–	–	–	–	–
*t*11-18:1	–	0.04	0.09	–	–	–	–	–
18:2n-6	52.2	12.2	6.49	46.7	34.4	21.9	23.0	22.2
9/*t*11-18:2	–	0.66	0.35	–	0.27	0.33	0.36	0.33
9/11–18:2	–	0.29	0.05	–	0.01	0.01	0.02	0.01
*t*9/*t*11-18:2	–	0.07	0.04	–	0.05	0.08	0.06	0.07
18:3n-3	8.23	1.00	0.87	4.90	3.37	1.72	1.76	1.73
18:n-6	–	–	0.03	–	–	–	–	–
20:1n-9	0.21	0.09	0.06	0.28	0.25	0.21	0.21	0.20
20:1n-7	0.03	0.05	–	0.03	–	–	0.02	0.02
20:2n-6	0.06	–	–	0.03	0.02	0.03	0.03	0.03
20:4n-6	–	0.02	0.17	–	–	0.05	0.05	0.06
20:5n-3	–	0.01	0.07	0.07	0.05	0.05	0.03	0.06
24:1n-9	–	–	–	0.01	0.01	0.03	0.01	0.02
Σ Saturated FA	16.8	71.8	75.2	17.4	35.9	53.2	50.4	52.8
Σ Monounsaturated FA	22.6	13.9	16.7	30.8	25.8	22.3	23.9	22.4
Σ Polyunsaturated FA	60.6	14.3	8.10	51.8	38.2	24.3	25.3	24.5
Σ n-3 FA	8.23	1.02	0.96	4.96	3.43	1.77	1.79	1.76
Σ n-6 FA	52.3	12.2	6.69	46.8	34.4	22.0	23.1	22.3
n-6:n-3 FA ratio	6.35	12.0	6.97	9.43	10.0	12.4	12.9	12.6
Total FA (g/kg as fed)	1000	951	852	58.3	57.0	55.5	52.9	57.8

–, not detected.

[a]A produced on wheat bran and solubles, B produced on (g/kg) fruit and vegetables raw waste, brewer's grain and pasta production waste, 300. Contents of dry matter (DM, g/kg), ether extract (g/kg DM) and gross energy (MJ/kg DM) were 99.7 and 97.9, 100 and 93.4, and 38.1 and 34.5 for larval fats A and B, respectively.

[b]AA, larval protein meal A and larval fat A; AB, larval protein meal A and larval fat B; BB, larval protein meal B rich in larval fat B; AS, larval protein meal A and soybean oil; SS, soybean cake and soybean oil.

by MUFA and PUFA. The proportion of SFA in both BSFL fats was four times higher than in the SO, and LAU was most abundant, followed by PAM, MYR, and stearic acid (18:0, STA). Proportions of MUFA were higher by on average 7% units in SO than in the BSFL fats, and this was mainly mediated by OLA. Proportions of PUFA in the

Table 4 Fatty acid (FA) profile (mole% of total identified fatty acids; determined as FA methyl esters) and total FA contents of the egg yolk lipids (n = 10)

Diet[y]	SS	AS	AA	AB	BB	SEM	*p*-value
10:0	0.02	0.02	0.02	0.01	0.01	0.007	ns
12:0 (lauric acid, LAU)	0.03^{c}	0.19^{b}	0.39^{a}	0.33^{a}	0.41^{a}	0.020	***
14:0 (myristic acid, MYR)	0.34^{d}	1.57^{c}	2.89^{ab}	2.56^{b}	2.96^{a}	0.090	***
15:0	0.08^{c}	0.10^{bc}	0.11^{ab}	0.11^{a}	0.12^{a}	0.004	***
16:0 (palmitic acid, PAM)	24.2^{c}	26.3^{b}	27.4^{a}	27.4^{a}	27.2^{a}	0.21	***
Iso-16:0	1.13^{a}	0.80^{b}	0.71^{b}	0.73^{b}	0.82^{b}	0.033	***
17:0	0.23^{a}	0.22^{a}	0.20^{ab}	0.20^{ab}	0.19^{b}	0.007	**
18:0 (stearic acid, STA)	7.79^{a}	7.17^{b}	6.99^{bc}	6.68^{bc}	6.57^{c}	0.125	***
20:0	0.02	0.02	0.02	0.02	0.02	0.001	ns
14:1	0.06^{c}	0.33^{b}	0.77^{a}	0.75^{a}	0.85^{a}	0.036	***
16:1	1.75^{c}	2.87^{b}	3.91^{a}	4.25^{a}	4.24^{a}	0.144	***
16:1 ×	0.05	0.07	0.08	0.06	0.06	0.013	ns
Aiso-16:1	0.02^{c}	0.09^{b}	0.17^{a}	0.16^{a}	0.19^{a}	0.006	***
17:1	0.07	0.06	0.08	0.06	0.07	0.009	ns
18:1n-9	38.0	37.0	37.5	38.4	37.5	0.39	ns
18:1n-11	1.46^{c}	1.67^{b}	1.92^{a}	2.02^{a}	2.05^{a}	0.036	***
18:1n-13	0.04^{d}	0.12^{c}	0.23^{ab}	0.23^{b}	0.26^{a}	0.007	***
18:2n-6	20.0^{a}	17.0^{b}	12.8^{c}	12.3^{c}	12.6^{c}	0.36	***
9/*t*11-18:2	0.02^{d}	0.14^{c}	0.24^{a}	0.19^{b}	0.16^{c}	0.005	***
18:3n-3	1.07^{a}	0.85^{b}	0.46^{c}	0.46^{c}	0.50^{c}	0.021	***
18:3n-6	0.11^{a}	0.09^{b}	0.08^{c}	0.08^{c}	0.07^{c}	0.003	***
20:1n-7	0.01^{d}	0.02^{c}	0.03^{a}	0.02^{ab}	0.02^{b}	0.001	***
20:n-9	0.17^{c}	0.18^{bc}	0.19^{ab}	0.20^{a}	0.19^{ab}	0.005	***
20:2n-6	0.15^{a}	0.13^{b}	0.09^{c}	0.09^{c}	0.09^{c}	0.005	***
20:3n-6	0.12^{a}	0.11^{ab}	0.09^{c}	0.09^{bc}	0.09^{c}	0.003	***
20:4n-6	1.60^{a}	1.51^{ab}	1.40^{bc}	1.38^{c}	1.37^{c}	0.026	***
20:5n-3	0.01^{ab}	0.01^{ab}	0.01^{c}	0.01^{bc}	0.01^{a}	0.001	**
22:4n-6	0.12	0.13	0.12	0.11	0.12	0.005	ns
22:5n-3	0.15^{ab}	0.16^{a}	0.09^{c}	0.09^{c}	0.12^{bc}	0.008	***
22:5n-6	0.16^{c}	0.24^{b}	0.35^{a}	0.34^{a}	0.36^{a}	0.018	***
22:6n-3	0.98^{a}	0.84^{b}	0.61^{c}	0.59^{c}	0.69^{c}	0.025	***
Σ Saturated FA	33.9^{c}	36.5^{b}	38.9^{a}	38.2^{a}	38.5^{a}	0.22	***
Σ Monounsaturated FA	41.6^{b}	42.3^{b}	44.8^{a}	45.9^{a}	45.2^{a}	0.38	***
Σ Polyunsaturated FA	24.5^{a}	21.2^{b}	16.4^{c}	15.8^{c}	16.2^{c}	0.41	***
Σ n-6 FA	22.3^{a}	19.2^{b}	14.9^{c}	14.4^{c}	14.8^{c}	0.38	***
Σ n-3 A	2.23^{a}	1.85^{b}	1.18^{d}	1.16^{d}	1.32^{c}	0.033	***
n-6:n-3 FA ratio	10.0^{c}	10.4^{c}	12.7^{a}	12.4^{a}	11.2^{b}	0.166	***
Total FA (mg/yolk[z])	4341	4545	4408	4484	4322	173	ns

Within a row, least squares means without a common letter differ ($p < 0.05$).

ns, not significant; SEM, standard error of the mean.

Significant differences are indicated as *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

[y]AA, larval protein meal A and larval fat A; AB, larval protein meal A and larval fat B; AS, larval protein meal A and soybean oil; BB, larval protein meal B rich in larval fat B; SS, soybean cake and soybean oil.

[z]Yolk yields (g/d) were SS = 15.7, AS = 16.3; AA = 16.1, AB = 16.1, BB = 15.3 (ns).

BSFL fats were up to seven times lower compared to that in the SO. Regarding PUFA, proportions of total n-3 FA were higher by 7% units and those of total n-6 FA were higher by >40% units compared to those found in the BSFL fats. This was mainly due to corresponding differences in proportions of ALA and linoleic acid (18:2n-6, LNA) in SO compared to the BSFL fats. The n-6/n-3 FA ratio was slightly lower in SO compared to BSFL fat B. Compared to the great differences to SO, the two BSFL fats were rather similar in their FA profile. Regarding the total SFA, which dominated both BSFL fats with a difference of only 4% units, the proportion of LAU in BSFL fat A was 2.64% units higher than in BSFL fat B. However, the proportions of PAM and MYR were greater by 4.3% and 0.81% units, respectively, in BSFL fat B than A. Similarly, STA was also more abundant in BSFL fat B than in A. The proportion of total MUFA differed only slightly between the two BSFL fats, where the dominant MUFA, OLA, was more prevalent in BSFL fat B than in A. In contrast, BSFL fat A had a higher proportion of PUFA and, concomitantly, higher proportions of n-3 and n-6 FA. The difference in ALA proportion between the two BSFL fats was less than that in LNA proportion, which was higher by nearly 6% units in A. Accordingly, the ratio of n-6/n-3 FA was almost twice as high in A compared to B.

The complete diets reflected the specific FA profiles of the dominating fat sources. Still, dietary SFA and PUFA proportions differed to a lesser degree among the diets than those between SO and BSFL fats. The lipids of the BSFL-based diets had up to 60 times higher LAU proportions (7.07–14.1 g/kg diet) compared to diet SS (0.24 g/kg diet). Due to relatively higher proportions of MYR in the BSFL fats, the corresponding dietary proportions also increased compared to diet SS (by 3% to 5%). In contrast, all diets showed almost the same proportions of PAM (6.61–8.01 g/kg diet). According to the proportions of LNA and ALA in the SO, diet SS showed the highest levels. The differences in the n-6/n-3 FA ratio were less pronounced in the diets compared to the fat sources.

Fatty Acid Profile of the Yolks and Net Fatty Acid Transfer from Diet to Yolk

The yolks varied in their FA profiles, but they did not significantly vary in their total FA contents (mg/yolk) (Table 4). The greatest diet-dependent variations ($P < 0.001$) were observed in proportions of LAU, MYR, PAM, 16:1, STA, LNA, and ALA, and the differences covered a range from 0.4% to 7.4% units. The proportions of SFA in the yolk lipids were higher ($P < 0.05$) in groups AA, AB, and BB compared to those of SS and AS. Yolks from diet AA had the numerically highest SFA proportion, mainly because of corresponding variations in PAM, STA, and MYR. Regarding the SFA, LAU proportion was increased ($P < 0.05$) in the yolks of groups AA, AB, and BB compared to SS and AS, with the lowest proportions found in the yolks of SS. The yolk lipids of groups AA, AB, and BB contained more ($P < 0.05$) MUFA than those of SS and AS, while they were dominated by OLA and 16:1. The PUFA proportions were higher ($P < 0.05$) in the yolk lipids of the SS hens compared to those obtained from the insect-based diets. Diet SS specifically led to greater

Table 5 Intake of ether extract and its secretion with the egg as well as net transfer of selected fatty acids from the experimental diet to the yolk (n = 10)

Diet[y]	SS	AS	AA	AB	BB	SEM	*p*-value
Ether extract (g/day)							
Intake	7.29[ab]	7.45[ab]	7.03[ab]	6.79[b]	7.48[a]	0.168	*
Secretion with the egg	4.15	4.31	4.28	4.24	4.15	0.145	ns
Net transfer (%)							
12:0 (lauric acid, LAU)	3.05[a]	0.68[b]	0.79[b]	0.75[b]	0.75[b]	0.414	***
14:0 (myristic acid, MYR)	73.9[a]	29.9[b]	31.0[b]	29.6[b]	29.1[b]	3.56	***
16:0 (palmitic acid, PAM)[z]	112[ab]	118[ab]	123[ab]	125[a]	112[b]	3.16	*
12:0 + 14:0	27.3[a]	5.34[b]	5.56[b]	5.47[b]	5.22[b]	0.996	***
12:0 + 14:0 + 16:0	107.3[a]	50.8[b]	37.6[c]	40.5[c]	34.4[c]	1.73	***

Within a row, least squares means without a common letter differ ($p < 0.05$).

ns, not significant; SEM, standard error of the mean.

Significant differences are indicated as $*p < 0.05$, $**p < 0.01$, $***p < 0.001$.

[y]AA, larval protein meal A and larval fat A; AB, larval protein meal A and larval fat B; AS, larval protein meal A and soybean oil; BB, larval protein meal B rich in larval fat B; SS, soybean cake and soybean oil.

[z]Transfer of >100% are possible because the hens most likely biosynthesized PAM from other fatty acids.

($P < 0.05$) proportions of n-6 and n-3 FA, as dominated by LNA and ALA, compared to the BSFL-based diets. This resulted in a lower ($P < 0.05$) n-6/n-3 FA ratio compared to groups AA, AB, and BB. Among the BSFL-based diets, the AA and AB yolks had a higher ($P < 0.05$) n-6/n-3 ratio than those from AS and BB.

Along with the highest content of dietary fat (always shown as EE), the hens receiving diet BB consumed most EE ($P < 0.05$ against diet AB; Table 5). The corresponding daily feed DM intakes (g) were 111, 112, 109, 110, and 103 for groups SS, AS, AA, AB, and BB, respectively (data not shown in table). The daily amounts of EE secreted through the egg (yolk) did not differ significantly among groups. The yolk yields were 15.7, 16.3, 16.1, 16.1, and 15.3 g/d for groups SS, AS, AA, AB, and BB, respectively (Table 4). Standardized yolk heights (mm/g yolk) did not differ among treatments and amounted to 1.16, 1.15, 1.18, 1.17, and 1.18 for SS, AS, AA, AB, and BB, respectively (data not shown in table).

When opposing intake with feed and output with the egg yolk, the relatively low net transfer of LAU and MYR into the yolk is evident, whereas the amount of PAM found in the eggs was greater than that eaten by the hens (Fig. 1). Accordingly, the net transfer of most of the selected key FA was affected by the diet (Table 5). Concerning LAU and MYR, the net transfer from the feed to yolk was highest in group SS ($P < 0.05$) and comparably lower in the other groups. Regarding the net transfer of PAM, more PAM was secreted with the egg in all groups than the hens had ingested with the diet. The lowest net PAM transfer was found in the groups SS and BB and the highest in group AB ($P < 0.05$ between these two groups).

Discussion

The effects of partially defatted BSFL protein meal and that of additional BSFL fat, a major energy source, in the diet of laying hens on the FA profile of the yolk remain widely unexplored. In the only study known to the authors (Bejaei and Cheng, 2020) where unprocessed, that is, full-fat BSFL was fed to layers, individual FA in feed and yolk were not specified, and variation caused by BSFL origin, especially the feeding substrate used, was not investigated. Data on the utility of the BSFL fat in livestock would help valorizing the entire BSFL production chain. Even in case only defatted BSFL protein meal is used, this knowledge is important as the meal is typically still rich in residual fat. To start filling this gap of knowledge, we investigated the influence of two differently produced BSFL fats on the FA profile of yolks, with a special focus on the net transfer of

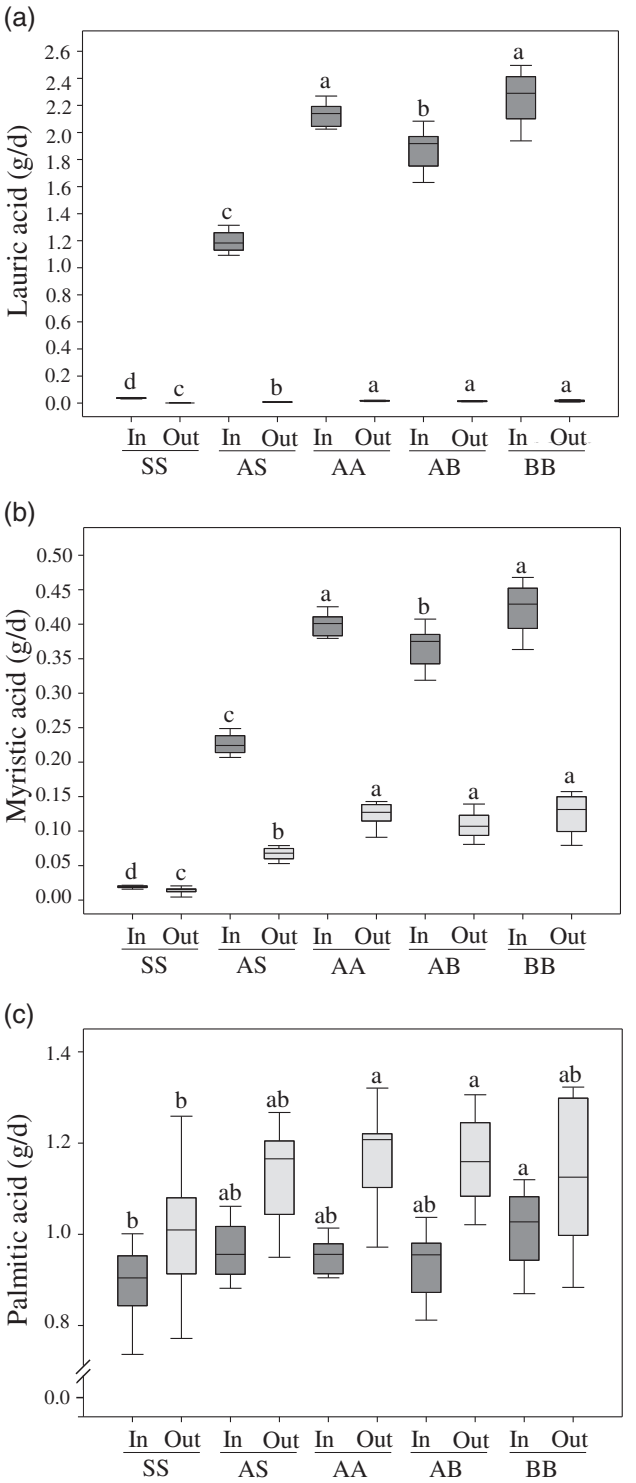

Fig. 1 Boxplots of intake with the feed (In; g/d) and secretion with the egg yolk (Out; g/d) of lauric acid **(a)**, myristic acid **(b)** and palmitic acid **(c)** in the five experimental groups AA, larval protein meal a and larval fat a; AB, larval protein meal a and larval fat B; AS, larval protein meal a and soybean oil; BB, larval protein meal B rich in larval fat B; SS, soybean cake and soybean oil. Boxplots marked with different letters within in or out are significantly different at $p < 0.05$ (n = 10 per treatment)

the MCFA which are prevalent in BSFL, but undesired in human nutrition in excessive amounts.

Differences in Fat Content and Fatty Acid Profile between Larvae of Different Origin

The two BSFL origins were produced differently and varied clearly in fat content, and this also concerning the proportion of residual fat content after processing. However, from the information concerning the rearing substrate available in our study, it is not possible to clearly associate the type of the feeding substrate with BSFL composition as there was confounding with stage of BSFL development at harvest time and fat-removal technique. Even though studies showed that the fat content of BSFL is influenced by the type of rearing substrate (St-Hilaire et al., 2007; Zheng et al., 2012), the timing of the insect harvest could be even more decisive in this respect. Bosch et al. (2014) showed that the fat content of black soldier fly pupae is higher by 7% than that of the larvae. In the present study, the differences between the BSFL of different origin was much lower in the FA profile than in the fat content. In particular, there were almost no differences between the two BSFL fats in terms of SFA and MUFA proportions, but substrate A resulted in higher proportions of PUFA in the BSFL fat A compared to substrate B (mainly dominated by LNA). With respect to MCFA, especially the LAU contents in BSFL fats merely did not differ, while BSFL fat B was slightly richer in MYR and PAM compared to BSFL fat A. Otherwise, the two rearing substrates appear to have been quite similar in their effect on the FA profile of BSFL. Oonincx et al. (2015) also found similarly high LAU proportions of the BSFL fat when the larvae were reared on different substrates. Based on this, these authors assumed that BSFL might be able to convert other FA into LAU. A mutually nonexclusive explanation could be that BSFL tend to primarily synthetize LAU from various available carbohydrates, and both pathways may jointly reflect a species-specific strategy to accumulate energy reserves particularly during the last BSFL instar (prepupae) for the forthcoming, nonfeeding adult stage (Sheppard et al., 2002; Liu et al., 2017). In addition, Oonincx et al. (2015) noted that, compared to LAU proportions, MYR and PAM proportions in BSFL fat are more depending on the rearing substrate.

Similar to the BSFL fat analyzed in the present study, MCFA are also characteristic for other insect species approved as food and feed (in the EU, BSFL are currently only approved for aquatic nutrition). Accordingly, mealworms and crickets are rich in PAM (21–28% of total FA) and relatively high in MYR (4–7% of total FA) (Finke et al., 2002; Makkar et al., 2014). However, they are low in LAU and only half as rich in total SFA (about 30% of total FA) compared to BSFL.

General Influence of the BSFL Fat on the Fatty Acid Profile of the Yolk Lipids

The present results showed that yolk lipids produced with BSFL-based diets are slightly richer in SFA, especially LAU, PAM, STA and MYR, and MUFA, and have lower proportions of PUFA compared to the eggs produced by hens fed the soybean-based control diet. This is consistent with the observations of Bejaei and Cheng (2020) who included dried and crushed but nondefatted BSFL in the diets of laying hens instead of soybean meal and found an increase in SFA and MUFA proportions. In their experiment, this was associated with a decline in the proportion of PUFA in general and in n-3 and n-6 FA in the yolk lipids. Secci et al. (2018), using defatted BSFL protein meal as a substitution for soybean meal and including the same plant oil in their experimental diets, could not find great differences in the FA profile of the eggs. However, this may have been due to the presumably dominating vegetable oil resulting in only small differences in the FA profile of the diets. This remains speculative to some extent, because Secci et al. (2018) did not quantify the content of residual fat content of the BSFL protein meal used. Indeed, the results by Secci et al. (2018) do not coincide with our results with diet AS, which was based on partially defatted BSFL meal and SO, but where egg yolk FA profile still resembled more those found with the other BSFL-based diets than with the BSFL-free control diet. This result was unexpected because fat from the 150 g BSFL protein meal A/kg diet (130 g EE/kg DM) and the SO both contributed about 20 g/kg to the whole diet.

Different from pork fat (Jaturasitha et al., 1996; Kreuzer et al., 1997) and consistent with the unexpectedly low increases in LAU and MYR in the egg yolk lipids found in the present study, feeding of BSFL fat to hens does not seem to have had a clear influence on the firmness of the yolk. This was concluded from the unchanged standardized yolk height, a variable which was found to clearly respond to feeding CLA in the study by Schäfer et al. (2001). A limitation of this conclusion is that the eggs of the present study had not been taken directly from the refrigerator like those of Schäfer et al. (2001).

Fate of Lauric, Myristic, and Palmitic Acid from BSFL Fat in the Metabolism of the Hen

Considering the net transfer of the major MCFA, it is noticeable that especially LAU from the insect-based diets merely does not get incorporated in the yolk lipids. Also for MYR, the net transfer of 30% was quite low, but not so

with PAM. These differences, therefore, seem to be related to FA chain length rather than to the proportion in the dietary fat, once levels in feed are elevated. Otherwise, a higher accumulation would have been expected with LAU than with PAM and especially with MYR. Thomsen (1966), who added 15 g coconut oil to the feed of laying hens, which enriched the diets in LAU (42.1% of total FA) and MYR (16.4% of total FA), also found a low proportion of LAU in the egg yolks (1.3% of total FA in the egg lipids) and a more elevated level of MYR (8.2% of total FA in the egg lipids). Also in his study, the hens had received clearly less MYR than LAU through the feed. This shows that at shorter chain length, more of the MCFA are either partitioned to other functions, especially energy metabolism, in the body of the hen rather than being used for incorporation into the egg lipids or that they were elongated in the hen's metabolism (enterohepatic *de novo* lipogenesis). A low digestibility of the MCFA is unlikely as a major reason for the low recovery of LAU (and MYR) in the egg because Renner and Hill (1961) demonstrated that in laying hens the absorbability of SFA decreases with increasing chain length. By contrast, the net use of PAM in the metabolism of the hen seems to be zero, although this does not exclude that some dietary PAM was used for energetic purpose and *de novo* synthesis of PAM through the enterohepatic lipogenesis occured (Ravindran et al., 2016; Carta et al., 2017). This would explain why the hens secreted even more PAM with the egg yolk than they consumed. Secci et al. (2018) even found that the proportion of PAM in the egg yolk lipids almost doubled compared to the proportions in the experimental diets. Although *de novo* synthesis is possible also for LAU and MYR, it obviously did not happen to a noticeable extent in the present study.

In conclusion, the results of the present study confirm that the inclusion of BSFL fat, either as pure fat or through partially defatted protein meal in the diet of laying hens has a rather small effect on the FA profile of the egg yolk lipids even though it widely differs in composition from the soybean oil (widely disproving hypothesis 1). Furthermore, laying hens seem to be able to modify the larval fat, particularly concerning LAU, and to a lesser extent MYR, likely through chain elongation or metabolization. Thus, the human health value of the lipids is enhanced (disproving hypothesis 2). Since the exact composition of the rearing substrates was not known, it is not possible to draw a firm conclusion about the extent to which the rearing substrate influenced the FA profile of the BSFL. Basically, the FA profiles of both BSFL origins were comparable and thus also did not result in great differences in the egg yolk lipids. Furthermore, the results suggest that entire BSFL could be included in poultry nutrition without the complex defatting process and having to find alternative applications for the excess fat. The present results also allow some predictions about expected FA transfer when other feed insect species are fed to laying hens. Since they may be lower in LAU, there would be mainly a reduction of MYR from the diet into the yolk.

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Conflict of Interest The authors have no conflict of interest to declare.

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