# Black soldier fly larvae meal and fat can completely replace soybean cake and oil in diets for laying hens

M. Heuel,[*] C. Sandrock,[†] F. Leiber,[†] A. Mathys,[‡] M. Gold,[‡,§] C. Zurbrügg,[§] I. D. M. Gangnat,[*] M. Kreuzer,[*] and M. Terranova[#,1]

*ETH Zurich, Institute of Agricultural Sciences, 8092 Zurich, Switzerland; †Research Institute of Organic Agriculture (FiBL), Departement of Livestock Science, 5070 Frick, Switzerland; ‡ETH Zurich, Laboratory of Sustainable Food Processing, 8092 Zurich, Switzerland; §Eawag: Swiss Federal Institute of Aquatic Science and Technology, Department Sanitation, Water and Solid Waste for Development (Sandec), 8600 Dübendorf, Switzerland; and #ETH Zurich, AgroVet-Strickhof, 8315 Lindau, Switzerland

**ABSTRACT** Currently, there is a great interest in finding alternative protein and energy sources to replace soybean-based feeds in poultry diets. The main objective of the present study was to completely replace soybean in layer diets with defatted meal and fat from black soldier fly larvae without adverse effects. For this purpose, $5 \times 10$ Lohmann Brown Classic hens were fed either a soybean-based diet or diets based on defatted black soldier fly larvae meal and fat from 2 producers (1 commercial, 1 small-scale) operating with different rearing substrates, temperatures, and larvae processing methods (10 hens/diet). The data obtained included nutrient composition of larvae meals and diets, amino acid digestibility (6 hens/diet), and metabolizability, performance and egg quality (all 10 hens/diet). In addition, the acceptance of the 4 larvae-based diets was tested against the soybean-based diet in a 6-day choice feeding situation (10 hens/treatment). The nutritional value of the larvae-based diets was equivalent to the soybean-based diet in hens with a laying performance of 98%. Although average feed intake was not significantly different over the 7 experimental weeks, the diets based on larvae feeds from the small-scale production appeared to be slightly less accepted in a choice situation than the soy-based diet and those with larvae from commercial origin. This was more likely the effect of the larvae fat rather than that of the larvae protein meal. In addition, the commercial larvae material was superior to that from the small-scale production concerning supply with digestible sulfur-containing amino acids (548 vs. 511 mg/day) and lysine (792 vs. 693 mg/day), egg weight (67 vs. 63 g), daily egg mass (66 vs. 61 g/day) and, in tendency, feed efficiency. The results indicate that soybean-based feeds can be replaced completely by black soldier fly meal and fat in diets of high-performing layers. However, because of nutritional differences between the larvae materials of different origin the quality of the larvae has to be closely monitored before being used.

**Key words:** *Hermetia illucens*, defatted larvae meal, layer, feed acceptance, performance

## INTRODUCTION

Globally, soybean meal and oil are major protein and fat sources in poultry nutrition. The use of these feeds as diet ingredients is controversially discussed due to potentially adverse environmental effects of its cultivation such as deforestation, greenhouse gas emissions or a high water expenditure (Ercin et al., 2012;

Mungkung et al., 2013). For organic diets, even higher amounts of organic soybean-based feed ingredients (here: soybean cake produced without use of solvent) are needed to be able to cover the requirements of the birds because supplementation with synthetic amino acids is prohibited (Früh et al., 2015). In addition, the global population expansion will be associated with an increased consumption of animal source foods requiring protein to feed livestock in several regions (Alexandratos and Bruinsma, 2012).

In response to this situation, the current research concentrates on alternative protein sources more sustainable than soybean which in addition do not compete with human food production. A particularly great potential is seen in insects (van Huis, 2013; Makkar et al.,

2014; Veldkamp and Bosch, 2015; Smetana et al., 2019). In particular, the black soldier fly larvae (**BSFL**, *Hermetia illucens*) are promising (Gold et al., 2018) as they are rich in CP (370–630 g/kg DM), energy (70–390 g fat/kg DM), have a favorable amino acid profile and can be reared on various substrates like food industry side streams (Diener et al., 2009; Tschirner and Simon, 2015; Barragan-Fonseca et al., 2017; Gold et al., 2020). In Europe, BSFL are currently only allowed in aquaculture and pet nutrition, but their approval as a component in poultry feed is awaited in the near future. At the same time, differently from other regions in the world, the substrates permitted in Europe to rear the insects are quite restricted (EFSA Scientific Committee, 2015; Schweizer Bundesrat, 2018) and include some that could be used directly as foods or feeds, for example, corn, wheat or rice mixtures, but also former food products and preconsumer waste (Pinotti et al., 2019). It is important to investigate the efficiency of using preconsumer waste as a rearing substrate, as BSFL otherwise might become an unnecessary intermediate stage of production namely when fed with feed or food. A large influence on the nutritional composition of the larvae and their products can be expected from the rearing substrate, the instar at harvest (before or after the start of transformation to prepupae; Bosch et al., 2014), the efficiency of fat removal, but also from a number of other operational parameters during rearing or processing of the larvae (Gold et al., 2018; Ottoboni et al., 2018; Gasco et al., 2020). So far, research on the use of BSFL in layer diets focused on the defatted BSFL meal as a protein source (Maurer et al., 2016; Marono et al., 2017; Secci et al., 2018; Mwaniki et al., 2020). The fat resulting from the defatting process occurring in large amounts is currently mostly transferred to industry like biodiesel production (Manzano-Agugliaro et al., 2012; Leong et al., 2016; Li et al., 2015). However, this is a major loss of feed energy, which is avoidable, because BSFL fat has been demonstrated to be without adverse effect in broiler and turkey nutrition (Surendra et al., 2016; Schiavone et al., 2017a; Sypniewski et al., 2020). Such studies in layers are missing. In case it has a sufficiently high nutritional value, this excess fat could replace edible plant oils in the hen's diet.

In the present study, the following hypotheses were tested. 1) The nutritional value of defatted BSFL meals and fats is comparable with that of soybean cake and oil and thus may be used as complete substitutes. 2) The addition of insect material to the laying hen's diet does not impair their feed acceptance. 3) Variation in the composition of defatted BSFL meals and fats affects their substitution value.

## MATERIALS AND METHODS

### Animals and Housing Conditions

The experiment was approved by the Cantonal Veterinary Office of Zurich, Switzerland (license number ZH221/17). It was carried out at the research station AgroVet-Strickhof, Lindau, Switzerland. Fifty 28-week-old Lohmann Brown Classic hens, purchased from Burgmer Geflügelzucht, Weinfelden, Switzerland, were housed individually at 20°C, 40 to 45% humidity and with an artificial light regime of 14L:10D. Their cages had a size of 80 × 80 × 80 cm and were enriched with meshed floor, nest, perch, and a box filled with fine sawdust. The mealy diets and water were provided at ad libitum access with 1 feeding trough and 2 nipple drinkers per cage, respectively. To allow the hens an easy access, feed was refilled before trough fill got less than half of initial. During the entire experimental periods, the health status of the birds was visually controlled daily. No hen died during the experiment or showed signs of sickness and therefore all hens completed the experiment.

### Larvae-Based Feeds

The experimental diets consisted of formulations of defatted BSFL meal and fat from 2 production systems (A, B) varying in rearing (e.g., larvae density, feeding rate, environmental parameters), harvesting and post-harvest processing parameters (e.g., killing, drying, and defatting method). Larvae meal and fat A were purchased from InnovaFeed (Paris, France). According to the producer, the BSFL were reared on a substrate consisting of wheat bran (air dry) and solubles from wheat distillery (about 20% dry matter). They stated that larvae were harvested before the prepupal stage, killed by heat shock, dried, and mechanically defatted with an industrial press. More details on the production of larvae materials A were not disclosed by the company. Larvae meal and fat B were produced exclusively for the experiment by FiBL, Frick, Switzerland, during a period lasting from spring 2017 to spring 2018 in a small-scale research operation. This production was designed to mimic the potential and challenges of an on-farm BSFL production based on locally available preconsumer waste and production side streams. The BSFL were reared on a substrate consisting of 40% fruit and vegetable raw waste, and 30% each of spent brewer's grains and pasta production discard (off-specification batches of precooked spaetzle, gnocchi and vegetarian variants of tortellini and ravioli). While the composition of the 2 latter components was quite constant over the production cycle, the composition of fruit and vegetable waste of substrate B varied in its components. About one quarter always consisted of unpeeled banana. However, proportions of apples, pears, stone fruits, exotic fruits, root vegetables, cabbages, solanaceous vegetables, and members of the leek family varied seasonally. Ingredients for the BSFL feeding substrate B were obtained once a week, mixed, and ground to few mm particle size in a shredder (Biospüler F-650, WEREC AG, Switzerland). This composition allowed to closely approach a target DM content of 22% (22 ± 3% were confirmed by repeated analyses). Every week new containers each containing 70,000 BSFL of 5 to 7 d of age were initiated at 27°C and 40% relative humidity and provided with 4 lump feedings during the first 10 d at a

total rate of 950 mg fresh feed per larvae. The BSFL were harvested on day 14, when the proportion of prepupae had reached levels ≥50%. After the separation from the residue (substrate, frass, and larvae exoskeleton) by sieving through a self-made 2-deck with vibrating screens (sizes of 7 and 15 mm), the larvae of each batch were sacrificed by freezing, dried in a drying chamber at 60°C for 30 to 36 h (TSW 700 ED, Salvis Instruments, Switzerland) and defatted by a modified commercial oil press (KK 20 F Universal, Screw Press, Reut, Germany) as described in detail in Leiber et al. (2017). All larvae materials were stored at −20°C until being thawed, blended across weekly production batches, and mixed into the diets.

## Formulation of Diets

Five experimental diets were tested which differed in the ingredients used as main protein and fat source (Table 1). The control diet (diet **SS**), containing soybean cake and soybean oil as protein and fat sources, was formulated to meet the requirements stated by the breeder (Lohmann Tierzucht, 2016) for this hen type, specified as 2,720 kcal ME/kg diet (11.4 MJ ME/kg diet) and 170 g protein/kg (when assuming 110 g ADFI). The 4 larvae-based diets were composed of defatted meal A and soybean oil (diet **AS**), defatted meal A and fat A (diet **AA**), defatted meal A and fat B (diet **AB**), and defatted meal B (diet **BB**). Owing to the high amount of residual fat in meal B, no extra supplementation of fat B was necessary. Apart from the BSFL meal and fat, and the soybean oil, all components used were certified organic. No synthetic amino acids and no yolk coloring pigments were added to the diets.

Assuming that the larvae fat was isocaloric to soybean oil (see, e.g., Sypniewski et al., 2020), and considering that the diets were isonitrogenous, the 5 diets were assumed to be quite similar in ME content. However, these assumptions may not be totally correct, and diet BB was richer in ether extract than the other diets. Celite was added to the diets as an indigestible marker to determine apparent digestibility and metabolizability. All feeds were mixed as per the current feed production standards. For this purpose, all mealy feed ingredients were first accurately weighed proportionately to the recipes and mixed to homogeneity for 10 min in a 100 kg single shaft feed mixer (Gericke AG, Zurich, Switzerland). Afterward the respective portions of either soybean oil or larvae fat, liquefied in advance by heating to 25°C, were distributed on top of this mixture. This was followed by another mixing of 15 min duration.

The diets offered intentionally had only a marginal supply of limiting amino acids to exhibit differences between the BSFL meals and the soybean cake, if there are any. The breeder's recommendations (Lohmann Tierzucht, 2016) were met for Lys, but not for Met, especially in the soybean-based diet. When applying the recommendations given by the National Research Council (1994) for this type of hens, in all diets supply was substantially below assumed requirements. However, we could expect the hens would well cope with this theoretical Met deficiency based on previous results obtained in the same facilities (Gangnat et al., 2020).

## Experimental Design

For the first part of the experiment, the hens were allocated in a complete randomized design to 1 of the 5

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

| Diet[1] | SS | AS | AA | AB | BB |
|---|---|---|---|---|---|
| Soybean cake | 150 | – | – | – | – |
| Soybean oil | 30 | 20 | – | – | – |
| Defatted larvae meal A | – | 150 | 150 | 150 | – |
| Defatted larvae meal B | – | – | – | – | 150 |
| Larvae fat A | – | – | 20 | – | – |
| Larvae fat B | – | – | – | 20 | – |
| Wheat | 300 | 240 | 240 | 240 | 305 |
| Corn | 180 | 205 | 205 | 205 | 190 |
| Wheat boll meal | 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 |
| Calcium carbonate | 27 | 27 | 27 | 27 | 27 |
| Limestone grit | 70 | 70 | 70 | 70 | 70 |
| 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[2] | 2.0 | 2.0 | 2.0 | 2.0 | 2.0 |
| Celite[3] | 16 | 16 | 16 | 16 | 16 |

[1]SS = control, soybean cake and soybean oil; AS = larvae meal A and soybean oil; AA = larvae meal A and larvae fat A; AB = larvae meal A and larvae fat B; BB = larvae meal B rich in larvae fat B.

[2]Contains 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 IE; 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.

[3]No. 545, acid-washed diatomaceous earth (Schneider Dämmtechnik, Winterthur, Switzerland).

different diets mentioned above (n = 10 hens per diet). Also the spatial position of the hens receiving the same diet was randomized within the bird house. After 1 wk of adaption to the experimental diets, 7 wk of experiment followed. This experimental period was complemented by a choice feeding test, based on experimental designs from several studies (e.g., Salem et al., 1994; Loetscher et al., 2014) with 40 birds which had not received the control diet (SS) in the 7 wk before (n = 10 per choice feeding group). Each hen was offered the choice between the control diet and 1 of the 4 larvae-based diet. In this respect, the 10 hens per choice feeding group were randomly allocated to a larvae-based diet yet unknown to them. Diets were served in equal amounts and in 2 separate adjoining troughs during 6 d, a period after which differences in the feed acceptance should have been fully established. After 2 d the troughs were exchanged to avoid position effects. Both troughs were refilled regularly to the same filling level.

### Data and Sample Collection

During the experiment, ADFI and BW were measured weekly and individually adding up to 7 measurements per hen over the entire experiment. The eggs from each hen were collected and weighed daily. A total of 6 eggs per hen were collected from day 38 to day 43 (week 6) for quality assessments. Four from them were used to determine various inner and outer quality traits. Afterward yolks and albumens were separated. The materials from the 4 eggs per hen were frozen at $-20°C$, lyophilized (Beta 1-16 Christ, Osterode am Harz, Germany) and homogenized to a fine powder with a standard kitchen mortar (Haldenwanger, Berlin, Germany). The 2 additional eggs were used to determine yolk and albumen heights. Excreta samples were collected daily from day 44 to day 48 (week 7) from each hen, weighed and frozen at $-20°C$ immediately after collection. After being lyophilized they were ground to 0.75 mm with a centrifugal mill (ZM 1, Retsch GmbH, Haan, Germany). Diet samples were taken twice, on day 1 and day 29, and samples of the soybean cake and the 2 BSFL meals were taken once. All feed items were milled to 0.5 mm with a centrifugal mill (ZM 1, Retsch GmbH, Haan, Germany).

### Laboratory Analyses

All sample analyses were performed in duplicate. Feed items, lyophilized excreta, and eggs were analyzed following the standard procedures (AOAC, 1997). Dry matter and total ash were analyzed by a thermogravimetric device (model TGA-701; Leco, St. Joseph, MI; AOAC index no. 942.05). Organic matter was calculated as the difference between DM and ash. Nitrogen (**N**) was measured with a C/N-Analyzer (model TruMacCrude CN, Leco, St. Joseph, MI; AOAC index no. 968.06). The CP was calculated as 6.25 × N, except for the BSFL meals, where CP was set to 4.76 × N following Janssen et al. (2017). Ether extract (**EE**) was determined in feed items and yolks with a Soxhlet extraction system

(B-811, Büchi, Flawil, Switzerland; AOAC index no. 963.15). Crude fiber content of the BSFL meals was measured on a Fibertherm FT 12 (Gerhardt, Königswinter, Germany; AOAC index no. 973.18). Chitin in BSFL meals and complete diets was determined according to Black and Schwartz (1950). Briefly, the samples were treated subsequently with diluted acid and alkaline solutions to remove all constituents expect for chitin and cellulose. The content of chitin (containing 68.9 g N/kg) was then calculated from the N content of this residue. Acid insoluble ash was determined in diets and excreta according to Vogtmann et al. (1975). Calcium, phosphorus and magnesium were quantified in the feed items by an automatic photometer (Cobas Mira, Roche Diagnostics Corporation, IN) subsequent to overnight incineration at 550°C. Sodium was analyzed in accordance with AOAC method 2011.14 with an inductively coupled plasma optical emission spectrometer (Varian 720-ES; autosampler CETAC ASX-520 HS, Varian, Palo Alto, CA). Argentometric titration was used to determine chloride (Autotitration-system, Metrohm Schweiz, Zofingen, Switzerland). The amino acid contents of feed items and selected amino acids in the excreta of groups SS, AA, and BB were analyzed by HPLC (Alliance 2690; Waters Corporation, Milford, MA) as described in the study by Gangnat et al. (2020). Briefly, 400 mg of sample was hydrolyzed with 25 mL of 6 M phenolic hydrochloric acid/L for 24 h at 110°C. Afterward, the solution was diluted and adjusted to pH 2.2 with citric acid buffer solution and subjected to HPLC analysis with ninhydrin post-column derivatization. For methionine (**Met**) and cysteine (**Cys**), the same hydrolysis procedure was used, but samples were oxidized in advance with 4 mL performic acid for 16 h at 0°C to form Met sulfone and cysteic acid. For tryptophan, samples were hydrolyzed with barium hydroxide and water at 110°C for 20 h. During HPLC analysis with fluorimetric detection (excitation: 280 nm, emission: 353 nm) $\alpha$-methyl tryptophan was added as internal standard. Gross energy was measured in feed items and excreta by combustion with a bomb calorimeter (Calorimeter System C7000 and Cooling System C7002, IKA-Werke, Staufen, Germany). Color of feed items and egg yolks was determined in 3 replicates each with the chromameter CR-300 (Konica Minolta, Ramsey, NJ) applying the L* a* b* color space.

Egg shell strength was measured with an electronic hardness tester (type PTB 301, Pharma Test, Hainburg, Germany). Shell thickness was determined on the flat pole of the egg using a thickness gauge (Bruetsch Ruegger, Urdorf, Switzerland). The whole eggs and, after separation, yolks were weighed. Egg shell weight was recorded after cleaning from the membrane and drying for 24 h at 40°C. Albumen weight was calculated as the differences between egg weight and shell plus yolk weight. Yolk and albumen heights were measured with the help of an analog precision dial gauge (Käfer Messuhrenfabrik, GmbH & Co. KG, Villingen-Schwenningen, Germany) and the latter was used to calculate the Haugh units, a measure for processing quality of the

albumen, as $100 \times \log$ (albumen height $- 1.7 \times$ egg weight$^{0.37}$ + 7.6) (Haugh, 1937).

### Calculations and Statistical Analyses

Coefficients of apparent amino acid digestibility as well as metabolizability of energy and N were calculated as outlined by Vukić Vranješ et al. (1994):

$$\text{Digestibility or metabolizability} = 1 - \frac{\text{Nutrient in excreta(g/d)} \times \text{indicator in feed(g/d)}}{\text{Nutrient in feed(g/d)} \times \text{indicator in excreta(g/d)}}$$

Data measured daily, weekly, or in more than 1 egg or repeatedly in feed items were combined to 1 value per hen or per feed item. Data were subjected to ANOVA with the MIXED procedure of SAS version 9.4 (SAS Institute, Cary, NC) with Tukey-Kramer adjustment for multiple comparisons. Diet was considered as fixed effect, individual hen as experimental unit. For the feed acceptance assessment, the ratios of intake of the BSFL-based diets in relation to total ADFI from both diets offered were calculated. The significance of the deviation from 50% of total intake (i.e., no choice) was determined by ANOVA with the MIXED procedure, and the Tukey-Kramer correction was applied for multiple comparisons among these deviation means. Normal distribution and homogeneity of variance were controlled visually. Results are given as least square means and SEM. Effects were considered significant at $P < 0.05$, and trend was established at $P < 0.10$.

## RESULTS

### Larvae Materials and Experimental Diets

The CP content of BSFL meal A was highest, followed by soybean cake and BSFL meal B (Table 2). The intended CP content of about 170 g/kg DM was reached in all diets except for BB with only 158 g/kg DM. The EE content of the defatted BSFL meal B was higher by 55 and 70% than that of BSFL meal A and the soybean cake, respectively. Even though no BSFL fat B had been added, diet BB had the highest EE content of all diets, but differences among diets in the other nutrients analyzed were not substantial. The crude fiber content of the 2 BSFL meals was quite similar with 143 and 129 g/kg DM for BSFL meals A and B, respectively. The chitin content of the 2 BSFL meals was comparable at about 70 g/kg DM. Larvae meal A was richest in phosphorus, calcium, and magnesium, whereas BSFL meal B and the soybean cake were more similar in phosphorus and magnesium, but not in calcium. Contents of sodium and chloride were quite similar between the 2 BSFL meals. The proportions of the limiting amino acids differed to some extent between the BSFL meals and the soybean cake. The protein of the BSFL meals contained more Met and less Cys than the soybean cake, resulting in quite similar proportions of total sulfur containing amino acids. Lysine (**Lys**) contents were similar. The proportions of the other amino acids resembled each other more between the BSFL meals than in relation to the soybean cake. In the complete diets, the contents of the limiting amino acids varied with slightly more Met and less Cys in all the larvae-based diets, and slightly more Lys particularly in diets with BSFL meal A (AS and AA). The BSFL meals were around 2 times darker (lower L* value) than the soybean cake, and BSFL meal B was most red (high a*). The soybean cake was most yellow, followed by BSFL meal A and B. Accordingly, the larvae-based diets were slightly darker, less yellow and slightly redder than diet SS.

### Effects of the Larvae-Based Diets on Performance and Egg Quality

There were only few diet effects on the performance of the laying hens (Table 3). At quite similar total ADFI, hens provided with diet SS consumed 39 mg/day less ($P = 0.009$) Met than the AS hens, with all other groups being intermediate. Intakes of digestible Met did not differ significantly between groups SS, AA, and BB, whereas, after the lower Cys intake, BB hens consumed less digestible total sulfur-containing amino acids compared with SS hens. The Lys intake was higher with AS and AA compared with BB ($P = 0.009$ and $P = 0.011$, respectively), with SS and AB being intermediate. The intake of digestible lysine was 13% lower ($P = 0.005$) with the diet BB than with SS and AA. The average BW of the hens from the different groups was similar at 1.94 kg. Laying performance was close to 100% and did not differ significantly. As eggs of group AS were heavier (+9%; $P = 0.010$) than those from group BB, daily egg mass also differed (+11%; $P = 0.003$) between these 2 groups. There was a trend ($P = 0.063$) for differences in feed efficiency toward being more favorable in AS and AA than in the other diets. The apparent digestibility of Met was not different among diets, but that of Cys and Lys was lower ($P < 0.001$) in BB compared with SS and AA. The metabolizability of N and energy, as well as the measured ME content, were highest ($P < 0.001$) in group AS. Diet BB exhibited the lowest N metabolizability, whereas there were no diet differences in energy metabolizability and ME content apart from those to AS. There was a trend ($P = 0.051$) toward differences in the N utilization of the hens, where diet BB seemed to be most favorable. In all diets, the actual intake of CP, Met, and total sulfur-containing amino acids was clearly below requirements assumed for laying hens at peak performance and eating 120 g feed/day (except in SS for total sulfur-containing amino acids). This was not the case for lysine. Across all diets, CP and Met were supplied in amounts being 17 and 10% below assumed requirements, respectively. Diet SS was

particularly low in Met compared with the requirements, whereas the same diet provided enough total sulfur-containing amino acids (Met + Cys).

In most egg quality traits, the diet had no significant effect (Table 4). This includes shell stability and egg composition of yolk, albumen, and shell as well as albumen composition and most yolk composition traits. In addition, albumen quality, described by the Haugh units, and yolk height did not differ among the groups. However, compared with diet SS, feeding diet BB resulted in a clearly redder yolk ($P = 0.011$). In addition, BB yolks contained more ($P = 0.025$) EE than those from group AB.

### Acceptance of the Larvae-Based Diets in Comparison With the Soybean-Based Control Diet

During the 6 d of choice feeding, the selection of diets AS and AA did not differ from that of diet SS (Figure 1). In case of AA vs. SS, the median was almost at 50% selection for both diets. Selection of the larvae-based diet was lower ($P < 0.05$) when containing BSFL material B (diets AB and BB). The hens that had the choice between AB and SS ate 30.5 g/day g of AB (27%) and 79.9 g/day of SS. The corresponding amounts for diet BB were 28.5 g/day (25.6%) and 85.5 g/day for SS. When comparing the proportions of ADFI selected from the different larvae-based diets, the hens having the choice between AA and SS selected proportionally more of the larvae-based diets than those that received diet BB and SS instead, but there was only a trend ($P < 0.10$) for a diet effect (data not shown).

### DISCUSSION

The purpose of the present study was to compare the nutritional value of diets based on differently composed products of BSFL to diets based on soybean cake and oil to determine their potential for substitution. The evaluation comprised a detailed analysis of diet composition, acceptance and intake, as well as digestion of and supply with amino acids, performance of the hens, and possible side-effects on egg quality.

### Nutritional Value of the BSFL Protein Meal Compared With the Soybean Cake

Across the 2 BSFL meals, contents of CP, Lys, and many other amino acids were similar to the soybean cake, but BSFL meals were richer in Met and poorer in Cys. They were similar in proportions of Met and Lys in total amino acids compared with those reported by Veldkamp et al. (2012) and Makkar et al. (2014). Methionine and Lys are known as the first and second limiting amino acids in layers (Toride, 2004). Yet, this empirical knowledge is based on diets including soybean meal, an ingredient containing a sufficiently high Cys content. With BSFL-based proteins, part of the extra Met would

have to be used to compensate for the lower Cys levels (efficiency: 0.81 g Cys/g Met; Baker, 2009). Contents of total S-containing amino acids were higher in both BSFL meals than in the soybean cake, but lower in the diets. This indicates that Cys supply has to be closely monitored in BSFL-based diets, especially as this amino acid was also less digestible compared with that of the soybean-based diet. However, it is additionally important to mention here that feeding BSFL material can lead to a change in intestinal morphology, which reduces nutrient digestibility and absorption (Dabbou et al., 2018). This seems to be especially a problem at higher supplementation levels of 15% in the total diet. However, gut morphology was not examined in the present study. Therefore, this is only an assumption to explain the poorer Cys digestibility. By contrast, the metabolizability of nitrogen was not generally different between larvae-based diets compared to that with soybean cake. Obviously, the chitin in the larvae did not cause a depression in these variables.

The results obtained in the hens showed a similar ADFI across the diets based on BSFL meals compared with the soybean cake diet. The lower acceptance of the diets with BSFL material B was rather the effect of the BSFL fat B than that of BSFL meal B because the reduced acceptance also occurred in diet AB (discussed in more detail below). Also studies carried out with broilers showed that differences between diets based on soybean- and larvae-based protein meals in acceptance might be small. Cullere et al. (2016) found a trend for the birds to prefer a diet containing BSFL meal compared with a soybean-based control diet whereas in the study of Schiavone et al. (2017a) broilers did not clearly differentiate in acceptance between a BSFL-based diet and a soybean-based diet. In the present study, there were no general differences between the BSFL meal–based diets and the soybean cake diet in performance measured as BW, laying percentage, egg mass, and N utilization, and this at a very high performance level. This indicates that, at the supply with nutrient and energy by the diets provided, the diets were in general widely equivalent and soybean could be totally substituted as a protein source in such diets. Effects on performance of replacing soybean-based feeds by BSFL meal–based feeds reported by other authors were variable. A lack of effect was also described by Maurer et al. (2016) for Lohmann Selected Leghorn hens, but this was measured only within a 3-week feeding period. Another study, which was more similar to the present experiment, reported that using BSFL meal improved feed efficiency from 2.17 to 1.97 g feed/g egg (a tendency also found in the present study) in Lohmann Brown Classic hens over a feeding period of 21 wk (Marono et al., 2017). However, concomitantly, other performance traits such as laying percentage were lowered in that study. The authors explained the latter by a lower feed intake which they assumed to be related to the darker color of the BSFL-containing diet compared with the control diet. However, BSFL-containing diets do not necessarily have a clearly darker color as the

**Table 2.** Analyzed chemical composition and color of the soybean cake, the 2 larvae meals and the 5 experimental diets (g/kg DM unless stated otherwise).

| Item | Soybean Cake | Larvae meal[1] A | Larvae meal[1] B | Diet[2] SS | Diet[2] AS | Diet[2] AA | Diet[2] AB | Diet[2] BB |
|---|---|---|---|---|---|---|---|---|
| DM | 929 | 952 | 937 | 906 | 907 | 908 | 907 | 902 |
| Organic matter | 871 | 867 | 884 | 766 | 760 | 766 | 744 | 764 |
| Nitrogen | 70.8 | 96.7 | 79.9 | 27.2 | 30.3 | 30.5 | 30.0 | 28.2 |
| CP[3] | 443 | 460 | 380 | 170 | 168 | 169 | 166 | 158 |
| Ether extract | 90.3 | 133 | 299 | 65.7 | 66.6 | 64.6 | 62.0 | 72.5 |
| Chitin | – | 74.4 | 69.5 | – | 10.2 | 9.3 | 10.6 | 8.7 |
| Calcium | 2.0 | 16.4 | 9.5 | 36.4 | 43.7 | 38.8 | 45.8 | 36.7 |
| Phosphorus | 7.1 | 12.1 | 6.8 | 7.5 | 8.9 | 8.0 | 8.3 | 7.3 |
| Magnesium | 0.45 | 0.62 | 0.55 | 1.24 | 1.36 | 1.26 | 1.33 | 1.06 |
| Sodium | n.a.[4] | 1.30 | 1.42 | 1.77 | 1.87 | 1.76 | 1.98 | 1.89 |
| Chloride | n.a. | 3.40 | 4.13 | 1.99 | 2.43 | 2.42 | 1.98 | 2.55 |
| Amino acids | | | | | | | | |
|   Methionine | 5.59 | 9.52 | 7.90 | 2.98 | 3.31 | 3.30 | 3.19 | 3.33 |
|   Methionine + cysteine | 10.86 | 13.69 | 11.06 | 6.18 | 5.84 | 5.95 | 5.73 | 5.88 |
|   Lysine | 26.15 | 32.07 | 23.62 | 8.16 | 8.38 | 8.59 | 8.16 | 8.09 |
| Amino acids (g/kg total amino acids) | | | | | | | | |
|   Alanine | 42.8 | 74.4 | 81.9 | 47.0 | 62.4 | 63.1 | 65.1 | 64.8 |
|   Arginine | 75.2 | 52.1 | 49.6 | 68.5 | 59.1 | 59.9 | 29.5 | 57.6 |
|   Asparagine/aspartic acid | 112.6 | 101.1 | 96.5 | 90.7 | 86.7 | 86.7 | 88.4 | 83.5 |
|   Cysteine | 12.3 | 7.9 | 7.5 | 19.5 | 15.1 | 15.3 | 15.8 | 14.9 |
|   Glutamine/glutamic acid | 183.4 | 119.3 | 117.3 | 212.2 | 177.3 | 176.6 | 187.1 | 182.6 |
|   Glycine | 42.3 | 60.1 | 61.8 | 47.7 | 55.2 | 54.8 | 57.6 | 55.1 |
|   Histidine | 26.4 | 32.9 | 29.9 | 26.9 | 29.5 | 30.0 | 30.8 | 28.5 |
|   Isoleucine | 46.3 | 48.9 | 49.9 | 43.0 | 42.7 | 42.7 | 43.9 | 42.7 |
|   Leucine | 76.2 | 73.5 | 74.4 | 77.9 | 76.8 | 76.5 | 80.2 | 76.4 |
|   Lysine | 61.1 | 60.6 | 55.7 | 49.7 | 49.9 | 49.7 | 50.7 | 47.3 |
|   Methionine | 13.1 | 18.0 | 18.6 | 18.1 | 19.7 | 19.1 | 19.8 | 19.4 |
|   Phenylalanine | 51.8 | 44.7 | 44.0 | 49.7 | 47.3 | 46.6 | 48.7 | 47.3 |
|   Proline | 51.1 | 61.1 | 67.6 | 66.5 | 70.3 | 68.2 | 69.9 | 73.8 |
|   Serine | 49.5 | 44.9 | 45.7 | 47.7 | 44.6 | 45.3 | 46.6 | 45.3 |
|   Threonine | 38.5 | 43.1 | 43.9 | 37.6 | 39.4 | 39.5 | 39.8 | 38.9 |
|   Tryptophan | 13.1 | 17.3 | 17.1 | 14.1 | 15.1 | 15.3 | 15.1 | 15.5 |
|   Tyrosine | 34.2 | 71.9 | 68.5 | 32.9 | 49.2 | 49.7 | 49.3 | 46.0 |
|   Valine | 48.3 | 68.1 | 69.9 | 50.4 | 59.8 | 60.6 | 61.7 | 60.2 |
| Gross energy (kcal/kg DM) | 5,019 | 5,378 | 6,071 | 4,111 | 4,135 | 4,135 | 4,039 | 4,183 |
| Color[5] | | | | | | | | |
|   L* | 78.7 | 42.9 | 30.4 | 69.7 | 62.0 | 66.1 | 65.5 | 69.3 |
|   a* | −2.12 | 5.37 | 6.31 | −0.91 | 0.28 | 0.58 | −0.34 | −1.00 |
|   b* | 27.9 | 17.7 | 9.0 | 19.1 | 13.8 | 15.3 | 14.6 | 15.9 |

[1]defatted larvae meal B was produced on (g/kg) fruit and vegetables raw waste (with seasonal variations), 400; brewer's grain, 300; pasta production waste, 300.Defatted larvae meal A was produced on wheat bran and solubles from wheat distillery;

[2]SS = control, soybean cake and soybean oil; AS = larvae meal A and soybean oil; AA = larvae meal A and larvae fat A; AB = larvae meal A and larvae fat B; BB = larvae meal B rich in larvae fat B.

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

[4]Not analyzed.

[5]L* is defined as lightness ranging from black (0) to white (100), a* as red (+) to green (−) axis and b* as yellow (+) to blue (−) axis.

results of the present study showed. Mwaniki et al. (2020), by contrast, described that a complete replacement of soybean meal by BSFL meal impaired feed efficiency from 1.91 to 2.02 g feed/g egg. The authors attributed that to the lower egg mass produced by the BSFL-fed hens at unchanged ADFI.

Most of the external and internal egg quality traits considered in the present study, such as shell breaking strength, shell thickness, Haugh units, and egg composition, were resistant against the exchange of the soybean cake by the BSFL meals. This is important for the implementation of the BSFL meals in feeding practice. Only few studies examined the influence of BSFL meal on general egg quality, and these reports (Maurer et al., 2016; Secci et al., 2018; Mwaniki et al., 2020) mostly confirm

our results. Regarding shell stability, shell thickness, and Haugh units, the results of Mwaniki et al. (2020) are consistent with our findings, even if they differ slightly due to differences in measurement techniques. Secci et al. (2018) found significant effects, and Maurer et al. (2016) observed a trend toward a lower albumen proportion when replacing soybean-based feeds by BSFL meal. No such effect was found in the present study, also not in protein content of the albumen. Secci et al. (2018) and Mwaniki et al. (2020) described that feeding BSFL meal intensifies the color of the yolks. Also in the present study, the color of the egg yolks produced from the BSFL-based diets appeared to shift toward more red and yellow coloration compared with the eggs from the soybean cake based diet. Secci et al.

**Table 3.** Performance of the laying hens fed the experimental diets for 49 d (n = 10 per treatment; n = 6 per treatment for the digestibility of the amino acids).

| Diet[1] | SS | AS | AA | AB | BB | SEM | P-value |
|---|---|---|---|---|---|---|---|
| Daily intake | | | | | | | |
| Total diet (g as fed) | 122 | 123 | 120 | 121 | 114 | 2.8 | 0.199 |
| Methionine (Met; mg) | 330[b] | 369[a] | 359[a,b] | 350[a,b] | 343[a,b] | 8.0 | 0.013 |
| Digestible Met (mg) | 287 | n.a.[2] | 310 | n.a. | 297 | 8.1 | 0.150 |
| Met + cysteine (Cys; mg) | 685[a] | 653[a,b] | 647[a,b] | 628[a,b] | 606[b] | 14.9 | 0.008 |
| Digestible Met + Cys (mg) | 605[a] | n.a. | 548[a,b] | n.a. | 511[b] | 15.7 | 0.003 |
| Lysine (Lys; mg) | 906[a,b] | 937[a] | 935[a] | 894[a,b] | 835[b] | 20.7 | 0.007 |
| Digestible Lys (mg) | 795[a] | n.a. | 792[a] | n.a. | 693[b] | 20.9 | 0.005 |
| BW (kg) | 1.93 | 1.94 | 1.97 | 1.96 | 1.89 | 0.052 | 0.797 |
| Laying performance (%) | 98.6 | 99.2 | 98.8 | 97.9 | 96.5 | 0.93 | 0.306 |
| Egg weight (g) | 65.0[a,b] | 69.2[a] | 67.0[a,b] | 65.5[a,b] | 63.2[b] | 1.24 | 0.018 |
| Egg mass (g/day) | 64.1[a,b] | 68.6[a] | 66.2[a,b] | 64.1[a,b] | 61.0[b] | 1.40 | 0.007 |
| Feed efficiency (g of feed/g of egg) | 1.91 | 1.79 | 1.81 | 1.89 | 1.88 | 0.314 | 0.063 |
| Apparent digestibility (%)[3] | | | | | | | |
| Methionine | 84.2 | n.a. | 85.9 | n.a. | 84.1 | 0.61 | 0.078 |
| Cysteine | 86.9[a] | n.a. | 82.3[b] | n.a. | 79.3[c] | 0.64 | <0.001 |
| Lysine | 85.1[a] | n.a. | 84.3[a] | n.a. | 80.7[b] | 0.50 | <0.001 |
| Metabolizability (%) | | | | | | | |
| Nitrogen | 49.8[a,b] | 53.1[a] | 45.9[b] | 46.7[b] | 40.9[c] | 1.09 | <0.001 |
| Energy | 76.6[b] | 79.6[a] | 76.6[b] | 77.5[b] | 75.9[b] | 0.46 | <0.001 |
| ME (kcal/kg feed DM) | 3,153[b] | 3,272[a] | 3,153[b] | 3,129[b] | 3,177[b] | 19.1 | <0.001 |
| Nitrogen utilization[4] (%) | 35.1 | 34.0 | 33.9 | 33.7 | 36.7 | 0.77 | 0.051 |
| Supply over requirements[5] (%) | | | | | | | |
| CP | −14.4 | −18.3 | −18.5 | −16.3 | −17.6 | 1.27 | 0.146 |
| Methionine | −14.7[b] | −10.0[a,b] | −9.9[a,b] | −9.7[a,b] | −7.2[a] | 1.40 | 0.009 |
| Methionine + cysteine | 1.0[a] | −9.0[b] | −7.4[b] | −7.7[b] | −6.6[b] | 1.46 | <0.001 |
| Lysine | 16.2 | 13.5 | 16.5 | 14.4 | 12.1 | 1.77 | 0.389 |

[a-c]Least square means within a row with no common superscript are significantly different (P < 0.05).
[1]SS = control, soybean cake and soybean oil; AS = larvae meal A and soybean oil; AA = larvae meal A and larvae fat A; AB = larvae meal A and larvae fat B; BB = larvae meal B rich in larvae fat B.
[2]Not analyzed.
[3]Calculated as outlined by Vukić Vranješ et al. (1994) for indicator techniques.
[4]Nitrogen excretion along with the egg in relation to nitrogen intake.
[5]Requirements of the experimental animals were calculated according to NRC (1994), and then related to the actual supply per hen.

(2018) showed that yolks produced by the groups fed BSFL-based diets contained more total carotenoids, lutein, zeaxanthin, and β-carotene than the yolks of a soybean control group. These compounds are major egg yolk colorants (Grashorn, 2016). However, concerning the more intensive yellow coloration of the yolks in

**Table 4.** Effect of the insect feeding on egg quality traits (n = 10 per treatment).

| Diet[1] | SS | AS | AA | AB | BB | SEM | P-value |
|---|---|---|---|---|---|---|---|
| Shell breaking strength (N) | 53.1 | 53.2 | 51.5 | 54.3 | 51.7 | 2.308 | 0.905 |
| Shell thickness (mm) | 0.43 | 0.43 | 0.45 | 0.44 | 0.42 | 0.012 | 0.580 |
| Yolk color[2] | | | | | | | |
| L* | 63.6 | 63.8 | 63.8 | 65.0 | 63.7 | 0.70 | 0.607 |
| a* | −6.75[b] | −6.57[a,b] | −6.40[a,b] | −6.36[a,b] | −6.17[a] | 0.120 | 0.018 |
| b* | 37.9 | 38.2 | 38.1 | 39.9 | 40.5 | 0.84 | 0.103 |
| Egg composition (g/kg) | | | | | | | |
| Yolk | 255 | 250 | 253 | 260 | 260 | 4.4 | 0.474 |
| Albumen | 634 | 642 | 641 | 630 | 631 | 6.0 | 0.337 |
| Shell | 111 | 108 | 106 | 109 | 109 | 1.7 | 0.509 |
| Yolk composition (g/kg wet weight) | | | | | | | |
| DM | 503 | 506 | 505 | 500 | 507 | 2.3 | 0.323 |
| Total ash | 26.8 | 25.8 | 26.4 | 26.7 | 26.3 | 0.40 | 0.308 |
| Protein | 163 | 163 | 162 | 161 | 160 | 1.2 | 0.423 |
| Ether extract | 265[a,b] | 268[a,b] | 266[a,b] | 263[b] | 272[a] | 1.9 | 0.031 |
| Albumen composition (g/kg wet weight) | | | | | | | |
| DM | 124 | 129 | 129 | 129 | 130 | 2.8 | 0.584 |
| Total ash | 7.22 | 7.49 | 7.58 | 6.98 | 7.31 | 0.331 | 0.729 |
| Protein | 102 | 107 | 107 | 107 | 108 | 2.5 | 0.522 |
| Haugh units | 88.7 | 92.5 | 92.6 | 92.3 | 92.5 | 2.43 | 0.739 |
| Yolk height (mm) | 18.2 | 18.8 | 19.2 | 19.2 | 18.6 | 0.34 | 0.154 |

[a-b]Least square means within a row with no common superscript are significantly different (P < 0.05).
[1]SS = control, soybean cake and soybean oil; AS = larvae meal A and soybean oil; AA = larvae meal A and larvae fat A; AB = larvae meal A and larvae fat B; BB = larvae meal B rich in larvae fat B.
[2]L* is defined as lightness ranging from black (0) to white (100), a* as red (+) to green (−) axis and b* as yellow (+) to blue (−) axis.
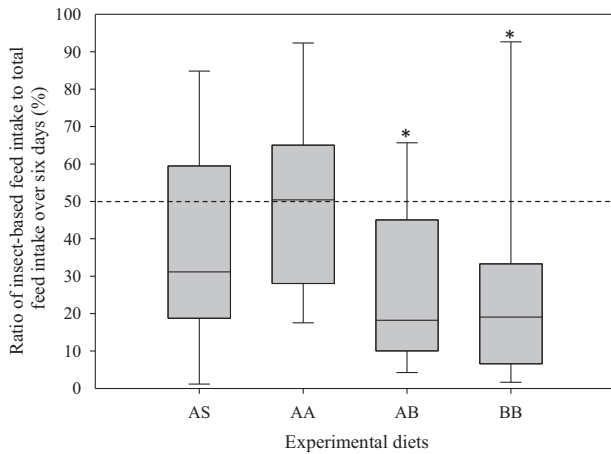
**Figure 1.** Box plots of the ratios (%) of the intake from the 4 larvae-based diets (AS, containing defatted meal A and soybean oil; AA, containing defatted meal A and fat A; AB, containing defatted meal A and fat B; BB, containing defatted meal B) in relation to total feed intake over the 6 d of choice feeding (n = 10 per diet combination). The difference to 100% represents the amount eaten from the control diet (SS). The dotted horizontal line drawn at 50% indicates the value expected when both of the feeds available for selection were consumed to the same amount and, therefore, no choice was made. Data points are least square means, and error bars reflect SE. Means marked with an asterisk are different from the 50% mark at $P < 0.05$.

the BSFL-based diets of the present study, also the slightly higher proportion of corn than the soybean-based diet might have played a role, as corn grains also contain lutein and zeaxanthin.

The 2 BSFL meals obtained with 2 different production methods clearly differed in the proportion of residual fat (higher in B than A). This was also the cause for the differences in contents of CP and limiting amino acids. In addition, there were differences in apparent Cys and Lys digestibility between diets AA and BB which might have resulted, among others, from a possibly too high fat extraction temperature applied in BSFL material B. Otherwise, the proportions, especially those of the limiting amino acids (sulfur-containing and lysine), and apparent Met digestibility were quite similar in the diets containing the 2 BSFL meal origins. This points toward quite a similar protein quality across substantially differing BSFL composition. It has to be stated, although, that the apparent amino acid digestibility measured in the hens may not reflect true bioavailability due to bias caused by microbial amino acid degradation and synthesis in the gut, as well as endogenous excretion. In contrast to Hossain and Blair (2007), the slightly lower chitin content of diet BB compared with the diets containing BSFL meal A did not result in a higher apparent amino acid digestibility protein and N metabolizability. To a limited degree, there were also differences in performance when feeding diets based on BSFL from the 2 production methods. The daily egg mass was lower with diet BB compared with the diets based on BSFL material A, mostly because the eggs were smaller. Although laying percentage did not differ significantly, the between-hen variation was twice as high with diet BB (SD: 4.6%) than with of all other diets

(SD: 2.2%). This points toward greater individual differences in performance responses of the hens receiving BB.

## Energetic Value of the BSFL Fat Compared With the Soybean Oil

The present results indicate that both origins of BSFL fat and the soybean oil were isocaloric to the hens when included into complete diets. This has also been supposed earlier by Schiavone et al. (2017b) based on a comparison of defatted and partially defatted BSFL meals, and by Sypniewski et al. (2020) when replacing soybean oil completely by BSFL fat. Energy metabolizability was highest with diet AS. However, as the control diet SS also contained soybean oil, it is not possible to associate this effect found with AS exclusively to the dietary fat source. Acceptance of the diets containing soybean oil and BSFL fat A was similar. However, hens given the choice selected against the diets containing BSFL fat B, although in the no-choice situation the voluntary intake of diets AB and BB was not significantly lower than that of the diets containing no BSFL fat B. The latter indicates that there was no detrimental acceptance problem with BSFL fat B. There could have been several reasons for the lower acceptance of diets containing BFSL material B. The color of the feed can have a major impact on feed acceptance, and light feed is preferred (Ferket and Gernat, 2006; Rierson, 2011). However, even though BSFL meal B was the darkest of the varied feed items, diet BB was only slightly darker than the other diets (even though the color of the egg yolks shifted toward a more intensive red coloration). The lack of clear color differences among the diets points toward smell differences, making BSFL fat B to be less favorably perceived by the hens than BSFL fat A. This could either have been caused by problematic substrate items (e.g., high residual fiber content, secondary phytochemicals) or by the mode of processing, especially an elevated processing temperature, which may have led to oxidation products.

The variation in lipid source had no effect on performance in the present study. Concerning egg quality, only the EE content of the yolks was modified; this in a way that yolks from diet BB were richer in EE than those from diet AB. This could have been the result of the higher dietary EE content (Grashorn, 2016). The results of Secci et al. (2018) indicate that the effect on yolk EE was not caused by BSFL-based feeds in the diet as such. In their study, the total lipid content of the yolk was lower with BSFL-based feeds than in a control group, even though the EE content of their BSFL-based diet had also been slightly higher. With regard to the tendency to a more intensive red coloration of the yolks of hens fed diet BB compared with the other diets, it has to be considered that dietary fat is also a decisive factor for carotenoid absorption (Grashorn, 2016). Therefore, the high EE content of diet BB could have contributed to this observation. A full assessment of the nutritional value of the BSFL fat needs to include

information about whether or not its medium-chain saturated fatty acids, where BSFL fat is characteristically rich in (Barragan-Fonseca et al., 2017), is transferred to the egg yolk lipids. These fatty acids are considered unfavorable in terms of human health. This question and the one about the occurrence of differences between materials from different origin in this respect are still open.

## CONCLUSION

The results of the present study showed that the substitution value of BSFL-based feeds is high enough to completely replace soybean-based feeds in high yielding hens even in organic diets where no synthetic amino acids can be used. This confirms hypothesis 1 and the statement includes amino acid supply and digestibility, performance, and egg quality. Partially confirming hypothesis 2, the acceptance of the commercially produced BSFL material was as high as that of the soybean-based feeds whereas acceptance of the material from the small-scale production was reduced when offered in a choice situation. This appears to have been exclusively resulted from the BSFL fat and not from the BSFL protein meal. However, the lack of a significant depression of feed intake in a no-choice situation nevertheless indicates a sufficiently high acceptance of this material. Overall, the variations between the 2 origins were small and thus did not to result in differences in their substitution value thus disproving hypothesis 3. Further studies are needed to compare larvae-based feeds at truly limiting dietary amino acid supply and to clarify effects on egg yolk fat content and fatty acid profile.

## ACKNOWLEDGMENTS

## DISCLOSURES

The authors declare that they have no conflicts of interest.

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