A Thesis

for the Degree of Doctor of Philosophy

# **Effects of black soldier fly (*Hermetia illucens*) larvae oil and meal on growth performance, cecal microflora, and meat quality in broiler**

동애등에 유충오일과 유충박의 급여가 육계의 생산성, 맹장 미생물 및 닭고기 품질에 미치는 영향

**August 2021**

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# Effects of black soldier fly (*Hermetia illucens*) larvae oil and meal on growth performance, cecal microflora, and meat quality in broiler chickens

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이 논문을 농학박사 학위논문으로 제출함

2021년 4월

서울대학교 대학원

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지상윤의 농학박사 학위논문을 인준함

2021년 6월

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# Summary

As the needs for animal-origin food have soared with population growth, it became increasingly difficult to secure enough feed resources while food losses have been intensifying in developed countries. Black soldier fly larvae (BSFL) can produce high-quality proteins and fats sustainably, and better yet, convert organic wastes as their diets into feeds. Due to this recycling capability, BSFL have attracted attention as a critical player in the circular economy. The BSFL-derived ingredients can supply macro-nutrients and physiologically active compounds to animals. However, an effective use of BSFL-derived protein and fat as a feed ingredient requires scientific data in target animal species. The present study aimed to address the impact of BSFL oil (BSFLO) and meal (BSFLM) on growth performance, intestinal health issue, and meat quality in broilers.

The first feeding trial evaluated the effects of BSFLO as a partial or total replacement of soybean oil (SBO) on the growth performance and the fatty acid (FA) profile and meat quality of broiler chickens from 1 to 5 week of age. A total of 210 male broiler chickens (Ross 308) at one day of age were randomly allotted to 3 dietary treatments (10 replicates and 7 birds/group): a basal control diet (CON), and two diets in which the soybean oil was replaced by 50% (50 BSFLO) or 100% (100 BSFLO) of BSFLO in the basal diet. The inclusion of BSFLO in diets did not influence the growth performance, physical measurements and chemical traits of leg meat, and sensory analysis of breast meat. However, the relative weight (g/kg) of the gizzard

was reduced by dietary BSFLO at the expense of SBO (14.9, 12.5, and 13.0 for CON, 50 BSFLO, and 100 BSFLO, respectively; $p < 0.05$) The saturated fatty acid proportion (% of total FAME, fatty acid methyl ester) of SFA was increased (27.2%, 27.6%, and 28.7% for CON, 50 BSFLO, and 100 BSFLO, respectively; $p < 0.05$) by BSFLO inclusion and the percentage (% of total fat) of monounsaturated fatty acids was also increased (43.4%, 44.6%, and 48.6% for CON, 50 BSFLO, and 100 BSFLO, respectively; $p < 0.001$). On the contrary, the proportion (% of total FAME) of polyunsaturated fatty acids was decreased (29.5%, 27.8%, and 22.7% for CON, 50 BSFLO, and 100 BSFLO, respectively; $p < 0.001$). The first study confirmed that the replacement of SBO with BSFLO did not have any adverse effect on broilers' growth performance and that BSFLO can be used as a dietary fat source in broiler diets.

The second experiment was performed to examine the effect of BSFLO as an alternative to SBO on the cecal microbiota in broilers. A total of 210 male broiler chickens (Ross 308) at one day of age were randomly allotted to 3 dietary treatments (10 replicates and 7 birds/group) that were same as in the first feeding trial. At the end of the study (d 35), 18 birds (6 broilers/treatment) were randomly selected and slaughtered. The cecal digesta samples were collected to measure the cecal microbiota population. Overall, 235,978 gene sequences were generated, and a total of 4,398 operational taxonomic units were identified in the three groups. At the phylum level, *Firmicutes* were the dominant phylum in all three groups. At the genus level, *Faecalibacterium* was the dominant genera in all treatments. There were no significant differences in relative abundances of all genera between the BSFLO groups and CON. However, genus *Erysipelatoclostridium* was more abundant in the 50 BSFLO than in the CON (p < 0.05). It was concluded that the substitution of the SBO with the BSFLO in broiler diets had no adverse effect on cecal microbiota in broilers.

In the last experiment, the effects of microwave-dried BSFLM on carcass traits, meat quality, FA profiles of abdominal fat and meat, and heavy metal residues in the broiler's meats. A total of 126 male broilers were randomly assigned to three dietary treatment groups (6 replicates and 7 birds/pen): a control diet and two experimental diets in which soybean meal was replaced with 25 or 50% BSFLM. The broilers were slaughtered at day 35; the carcasses were weighed, and breast and leg meats were excised from 12 birds per treatment (2 birds/pen) for meat analysis. For the higher BSFLM diet, the content of saturated FA in the abdominal fat was increased and that of polyunsaturated FA was decreased (p < 0.001); the FA profile of leg meat did not differ between groups. The concentrations of undesirable heavy metals in the BSFLM and leg meat were below maximum permissible levels. However, the carcass weight was decreased (p < 0.001) in the 50% BSFLM group. Microwave-dried BSFLM is a potential ingredient for broiler diets, with up to 25% inclusion showing no detrimental effects on carcass traits, meat quality, FA profiles, and heavy metal residues in the meat.

It was confirmed that BSFLO could be used as an energy source in broilers by replacing SBO without negative impact on their productivity and meat quality. However, even though BSFLO contains a large amount of lauric acid, its effect on the intestinal microflora was not found.

In conclusion, BSFLO can replace the existing energy feed source such as soybean oil with no adverse effects on growth performance, meat quality and health of broilers. On the other hand, BSFLM, microwave-dried and press-defatted, could not fully substitute SBM so its evaluation as a feed ingredient must be preceded to use BSFLM as a feed ingredient. Collectively, the results from the present studies demonstrated that BSFLO and microwaved defatted BSFLM could be an alternative to conventional ingredients such as SBO and SBM in broiler's diet.

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# List of Abbreviations

| | |
|---|---|
| **ADF** | Acid detergent fiber |
| **ADG** | Average daily gain |
| **AMPs** | Antimicrobial peptides |
| **BSF** | Black soldier fly |
| **BSFL** | Black soldier fly larvae |
| **BSFL/P** | Black soldier fly larvae or prepupae |
| **BSFLM** | Black soldier fly larvae meal |
| **BSFLO** | Black soldier fly larvae oil |
| **BSFP** | Black soldier fly prepupae |
| **CP** | Crude protein |
| **DFI** | Daily feed intake |
| **DLP** | Defensin-like peptide |
| **EC** | European commission |
| **EE** | Ether extract |
| **EFSA** | European Food Safety Authority |
| **FA** | Fatty acid |
| **FAME** | Fatty acid methyl esters |
| **FAO** | Food and Agriculture Organization |
| **FBW** | Final body weight |

| | |
|---|---|
| **FCR** | Feed conversion ratio |
| **GIT** | Gastrointestinal tract |
| **IBW** | Initial body weight |
| **IPEC** | Intestinal porcine epithelial cell |
| **LA** | Lauric acid |
| **LCFA** | Long-chain fatty acid |
| **LW** | Live weight |
| **MCFA** | Medium-chain fatty acid |
| **MUFA** | Monounsaturated fatty acid |
| **NDF** | Neutral detergent fiber |
| **PUFA** | Polyunsaturated fatty acid |
| **SBM** | Soybean meal |
| **SBO** | Soybean oil |
| **SFA** | Saturated fatty acid |
| **TBARS** | Thiobarbituric acid-reactive substances |
| **UFA** | Unsaturated fatty acid |
| **VBN** | Volatile basic nitrogen |
| **WHC** | Water holding capacity |

# I. Review of Literature

## 1. The emergence of black soldier fly larvae as a sustainable feed resource

With the world population expected to reach 10 billion in 2050, the global demand for food, especially animal protein, was rapidly increasing. Thus, it is essential to improve the efficiency in animal production, though it was increased by only 1.5% in 2016 compared to 2017 [1]. In addition, the livestock industry has encountered crises including, but not limited to, a lack of arable land and water, climate change, and environmental pollution. A shortage of feed resources become a hot topic to be solved in the feed industry. In the same context, many feed scientists are searching for new protein sources like non-edible grains (e.g., pulses), algae, and insects.

Because insect farming requires smaller space with resources (water, feed, etc.) than livestock, insect proteins have been regarded as a promising alternative to animal-origin food for humans. Humans had a long history of entomophagy where humans have consumed more than 2,000 insect species [2]. *Tenebrio molitor* (mealworm) and *Hermetia illucens* (black soldier fly, BSF) represent edible insect species. In addition, BSF is beneficial for the scale-up of nutrient production because it can convert large quantities of organic waste into biomass in dense populations, eat flexible feed sources, and has a fast life cycle. Therefore, BSF is an eco-friendly player for sustainable protein production in a circular economy.

According to Dossey et al.[3], there were listed 122 companies producing insects as food and feed in their survey, of which 63 were located in Europe. And there were 45 companies in North America and 11 in Asia. Starting with the PROteINSECT project [4], Europe has been preparing for the depletion of food resources through continuous investment in research funds, and is pursuing the goal of replacing animal protein by insect protein of 10% in animal feed and of 20% in human diets in 2025 [5]. In a survey of member companies (64 member companies from 23 countries) conducted by IPIFF (International Platform of Insects for Food and Feed) in 2019 [6], the insect protein production was predicted to be over 2.5 million tons in 2030. But they also forecasted that its production could increase more than 1.5~5 million tons if the feed regulation inappropriate to insects (e.g., prohibition of use of slaughterhouse by-products, catering waste and manure as substrates for insect) were relieved.

Korean MAFRA (Ministry of Agriculture, Food and Rural Affairs) enacted 'Insect Industry Promotion and Support Act [7]' in 2019 to foster the insect industry. According to the statistic report [8] published in 2020 by Korean MAFRA, it was found that the number of BSF farms increased from51 in 2017 to 60 in 2019, and the total sales of their primary products also increased from 8.5 million won to 3 billion won by more than 6 times. Meanwhile, the number of *Tenebrio molitor* rearing farms slightly decreased from 282 in 2017 to 265 in 2019, and the total sales of primary products also increased only slightly from about 2.4 billion won to about 2.8 billion won (Figure 1).

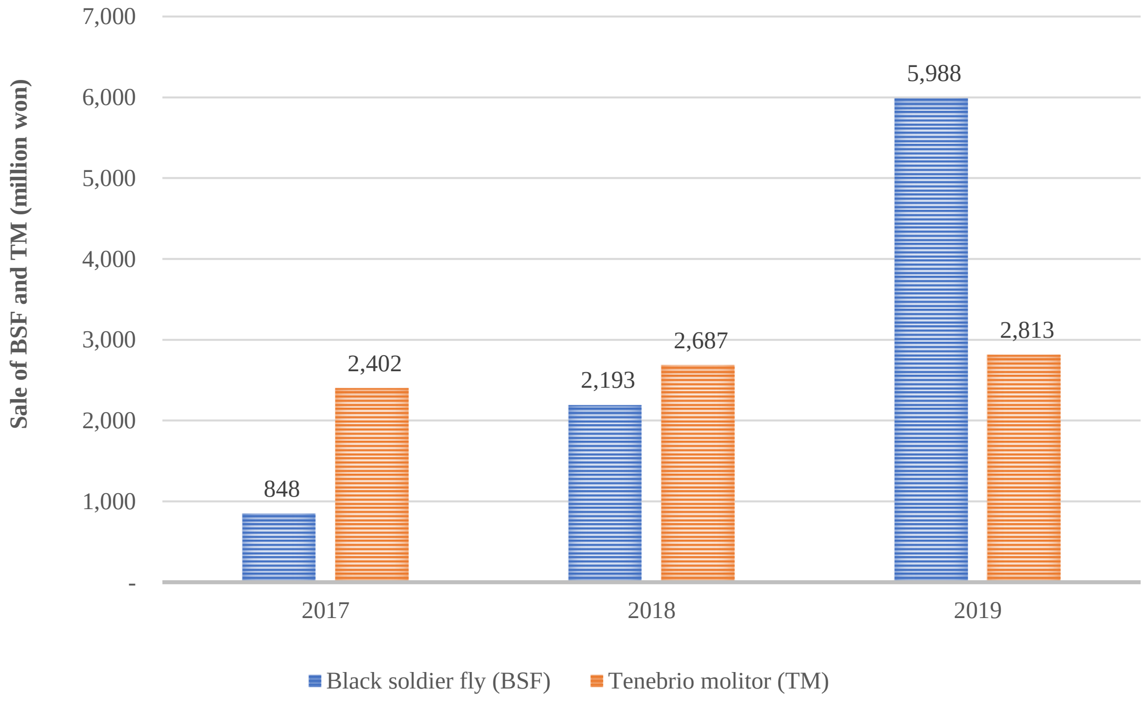


**Figure 1.** Sales of primary products from *Hermetia illucens* and *Tenebrio molitor* for 2017~2019 in Korea.

2. Biological characteristics of black soldier fly

The BSF belongs to the family *Stratiomyidae* and is a very hygienic insect species in ecological and biological respects. The growth stage of BSF can be divided into an egg, larvae, prepupae, and adult, and the lifespan is about two weeks and can vary greatly depending on environmental changes (Figure 2). The adult BSF does not lay eggs in dirty places and cannot ingest any substances due to its non-functional mouth.


**Figure 2.** Changes in the appearance of BSF by growth stages

The biomass of BSF grew more than about 4,000 times in about two weeks. The nutrient composition of BSF dynamically fluctuated during the entire life cycle from eggs to adults. The BSF biomass reached a peak in the prepupae stage and then began to decrease with adulthood [9]. The accumulation of protein and lipid rely on the diet administered to BSF, associated with metabolic adjustment by the fat body. Moreover, the protein-to-fat ratio was strongly affected by the nutrient composition of its food source [10]. These features can facilitate to rear BSF on a large scale, thereby obtaining a large mass of BSF larvae as a feed resource [11]. The BSF larvae (BSFL) is processed into protein meal, oil, and chitin as feed ingredients for animal production (Figure 3).


**Figure 3.** Various types of feed ingredients processed from BSFL

3. Functional properties of BSFL as a feed ingredient resource

In general, insects are highly resistant to bacterial infection [12]. The BSF could reduce foodborne pathogens such as *Escherichia coli* 0157:H7 and *Salmonella enterica* [13]. The antimicrobial activity has been described as the presence of bioactive substances in BSFL [11]. The nutritional components of BSFL can be fractionated into parts, as shown in Figure 4. The main functional substances may be present in antimicrobial peptides (AMPs), functional fatty acids (lauric acid), and chitins.

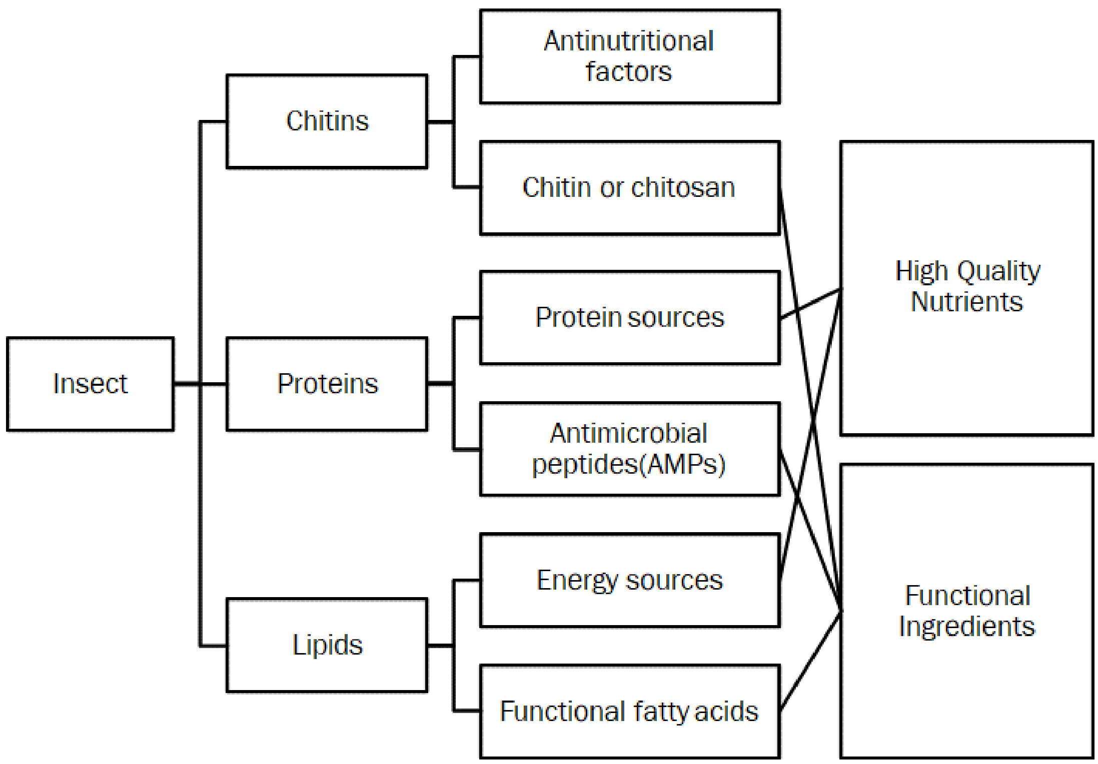


**Figure 4.** Fractionation and usage of nutrients derived from insect

### 3.1. Antimicrobial peptides (AMPs)

Insects have a solid resistance to bacterial infections, which could be partially due to the retention of AMPs [12]. AMPs are the component of the innate immune response discovered in most kinds of organisms and insects. According to the antimicrobial peptide database, about 3,000 AMPs have recently been identified and annotated [14], and insect-derived AMPs accounted for more than 30% of identified ones [14,15]. Therefore, insect proteins have emerged as a prospective source of AMPs in animal production [16].

Vogel et al. [17] found more than 50 AMP genes in *Hermetia illucens*, of which twenty-six were defensins-encoding genes. The sophisticatedly adjusted expressions of these AMP genes resulted in the shift of gut microbial compositions, which could give *Hermetia illucens* the great adaptability for digesting their unusual diets.

Park et al. [18] purified DLP4 from the hemolymph of BSFL challenged with *Staphylococcus aureus* and observed no expression of DLP4 genes throughout the body without immunization. This research group also identified and purified cecropin-like peptide 1 (CLP1), and the immunization was a prerequisite for its gene expression [19]. Lee et al. [20] discovered that an extract from *Lactobacillus casei*–infected BSFL had antimicrobial activity against *Salmonella* species.

Recently, because therapeutic AMPs induced bacteria to develop their resistance like traditional antibiotics, the universal use of AMPs had not been recommended in food products [21]. However, this recommendation was based on studies examining individual or purified AMP. Chernysh et al. [12] indicated that insect antimicrobial peptide complexes did not induce bacterial resistance against AMPs, unlike individual AMP [22]. As the AMP production of BSFL could vary depending on the hygienic environment, further research was needed to breed BSFL for AMP production. Currently, insect- and BSFL-derived AMPs are still not applicable as a feed additive, because it is difficult to be produced due to the higher production cost and the instability of antimicrobial activity in *in vivo* conditions [16,23].

### 3.2. Antimicrobial lipid

Insects contain a large amount of lipid in their fat body as energy reserves for becoming adult insects from larvae without any nutrient ingestion. The BSFL contained more saturated fatty acid than unsaturated one, which was a unique feature in contrast to higher amounts of unsaturated fatty acids in lipid-rich insects such as *Tenebrio molitor*. Especially, lauric acid (C12:0, LA) was dominant in its fatty acid composition of BSF larvae oil (BSFLO). In addition, lauric acids in BSFLO existed in the form of glyceride, with lauric acid monoglyceride (monolaurin) being the most abundant and also in the form of di- or tri-glyceride [24]. And antimicrobial properties of LA were well-mentioned in coconut oil research as its highest resource of MCFAs [25].

**Figure 5.** Photographs of black soldier fly larvae and its oil

Lauric acid was mainly found in vegetable oils such as coconut oil (40~55% of total fatty acids) or palm kernel oil (45~55%), while only small amounts of LA was found at animal fats. For example, concentrations of LA in human breast milk and cow milk were 6.23% and 2.9% of total fat contents, respectively [26]. BSF larvae oil contained a high concentration of up to 30~40% of LA in total lipid [27-30]. In addition, BSFL oil included monolaurin (dodecanoic acid 1-monoglyceride), which has potent antimicrobial activities against microbial pathogens.

Although the mechanism of antibacterial lipids was not completely understood, it was because antimicrobial lipids could inhibit essential functions of bacteria by altering fluidity and metabolic processes of their cell membranes [31]. Lauric acid was known to inhibit the growth of gram-positive bacteria rather than gram-negative ones. Martínez-Vallespín et al. [32] showed that MCFAs including LA influenced the growth rate of *Salmonella enteritidis* and *E. coli*. And they also reported LA could modulate gene expression of IPEC-J2 cells [32].

Although there has been little research on the effects of BSF larvae oil supplementation in pigs and poultry diets, its functions could be deduced from *in vivo* feeding trials with coconut oil or LA. Broilers fed with high level of LA could accumulate more significant amounts of LA in the breast muscles, in which the colonization of *Campylobacter coli* could be hindered with no detrimental effect on meat quality [33]. However, Spranghers et al. [34] suggested the antimicrobial effect of LA in BSF prepupae would be very limited because LA was so rapidly absorbed that it could not arrive at the lower part of the gastrointestinal tract (GIT) in weaned piglets. The same restriction is equally applicable for MCFAs; thus, various processing techniques (e.g., encapsulation) have been evolving to overcome it in the feed industry [35,36].

As well as its antimicrobial effects against gut pathogenic microbes, LA could influence host intestinal cells. Lauric acid affects the immune response of IPEC-J2 cells [32]. Therefore, it was probable for BSFLO to regulate the physiological functions of intestinal cells and the composition of gut microbiota all at once [37-40].

### 3.3. Chitin

Chitin is the second most abundant bio-polymers next to cellulose [41] and is mainly synthesized by crustaceans, mollusk, fungi, and insects. Chitin synthesis and degradation constantly occur during the growth and metamorphosis of insects. Insect chitin forms its exoskeleton and supports the peritrophic matrix on the intestinal epithelium and the epididymal and trachea cuticles [42]. Insect exoskeleton contains about 25~58% of total chitins [43]. Although chitins made up 0.3~5% of insect body weight [44], BSFL or BSFLP held more chitins than other insects. Spranghers et al. [45] reported that the chitin content was 6.7% of BSFLP fed restaurant waste on a dry matter basis.

Chitin and its soluble analog - chitosan, are widely applied in both the food industry and biomedical industries due to its versatile properties such as emulsifiability, biodegradability, antimicrobial activity, and wound healing promotion [46]. However, because chitin exists in nature as a complex with substances other than pure form [47], the effect of chitin on animal health seemed to be differently described by various scientists.

Insect chitin explains the high level of fiber fractions in insect meals [44]. The BSFL has contained 1.76~3.13 folds more chitin than other insects [48], and BSFL or BSFP chitins were analyzed to account for 6.7~9.6% of their body weight on a dry matter basis [45,46,49-52]. Because chitin contains up to 6.89% of nitrogen, the application of protein conversion factor, 6.25, may make an overestimation of crude protein content from the proximate analysis [53]. Therefore, the chitin content must be considered to evaluate the nutrient values of BSFL/P meal. And chitin itself did not impair nutrient absorption [54], but the chitin complex with other substances could disturb the bioavailability of nutrients for animals.

## 4. Safety and regulatory issues for insect-based feed ingredients

The Korean insect industry is targeting a niche in the food and feed market similarly to the market trends in Europe and the United States. The longer history of entomophagy in Asia than that of West tends to make consumers less reluctant to insect as food and feed [55] but many consumers are still concerned about the safety as well as flavor and/or texture of edible and feed insects. EFSA (European Food Safety Authority) carried out risk profiling to identify the biological, chemical and environmental hazards related to farmed insects as food and feed, and compared with those of conventional animal proteins [56]. This risk profile showed that the biological and chemical hazards in insect food and feed would have relevance to the rearing methods, diets for insects, their lifecycle stage, and processing method. Furthermore, the occurrence of microbiological hazards in insect was like that of the other animal proteins, yet more risk assessments are required for the contamination and transfer of chemical hazards from diet to insect.

In small scale farms, farmers often gave raw BSFL to their poultry and pigs without any sterilization step. Muller et al. [57] qualitatively examined risk for the passive transmission of coccidian oocysts or nematode eggs through feeding BSF larvae and prepupae. These parasites or eggs could attach to the larval surface. Therefore, more attentions are required to feed raw BSF to poultry and pigs in field conditions.

The risk profiling results of EFSA [56] could be applicable in that of BSFL produced in Korea. However, since EU currently prohibits food leftover as a food source unlike Korea, the safety evaluation of insects that domestically produced needs to be continuously monitored. In the EU, six insect species were approved as food and feed in 2017 in ‘Regulation (EC) 2017/893’ governing the provisions of processed animal protein [58] and the food sources were restricted to non-animal and some animal by-products such as fish meal, non-ruminant blood by-products, animal-origin di- and tri calcium phosphates, hydrolyzed proteins from non-ruminant, and eggs and egg products. On the other hand, no substances are restricted as food for insects other than manure in Korea.

Insects as foods and feed have been under two laws, ‘Control of Livestock and Fish Feed Act, 2018’ and ‘Insect Industry Promotion and Support act, 2019)’. The former presents quality and safety standards for insects as feed, and the latter provides approved insect species and breeding standards for food and feed. At present, seven insect species are approved as feed ingredients (Table 1).

**Table 1.** Comparison of insect species approved as feed ingredients in EU and Korea.

| Common name | Scientific name | EU [59] | Korea |
|---|---|---|---|
| Black Soldier Fly | *Hermetia illucens* | O | O |
| Common Housefly | *Musca domestica* | O | O |
| Yellow Mealworm | *Tenebrio molitor* | O | O |
| Super Mealworm | *Zophobas morio* | | O |
| Lesser Mealworm | *Alphitobius daiperinus* | O | |
| House cricket | *Acheta domesticus* | O | O |
| Banded cricket | *Gryllodes sigillatus* | O | |
| Field cricket | *Gryllus assimilis* | O | |
| Grasshopper | | | O |
| Silkworm | *Bombyx mori* | | O |

## 5. Experimental studies of feeding BSFLO and BSFLM to broilers

Rabobank forecasted that the poultry feed market will become the third-largest target market for insects, following pet food and aquaculture feed markets [60]. Therefore, a lots of feeding trials using BSFL-derived ingredients have been performed on chickens, but its feed materials tended not to be specified or standardized about manufacturing methods. The defatting process is very common in the insect-based feed production because the defatted insect ingredient is more favorable for optimizing the broiler feed formulation and the preservation of feeds [61]. Both protein and fat fractions of BSFL can be alternatives to the conventional nutrient resources for poultry diets.

The BSFLO can substitute soybean oil partially or fully as energy source for broilers. Cullere et al. [62,63] reported that lauric acid contents in breast of broiler fed BSFLO during finisher stage increased up to 4.61~11.0% of total fatty acid contents in proportion to its dietary inclusion levels (3.43~6.87% of total diet). And they also observed that this dietary BSFLO inclusions decreased the MUFAs in the breast, but not in the leg meat without no adverse effects on growth performance, gut health, and meat quality. As shown in Table 2, the feeding of BSFLO could accumulate lauric acid in the breast or leg meat depending on its intake level with no significant decrease in growth performance.

In an experiment using lauric acid as a feed additive for broiler, a dietary lauric acid could be transferred into breast meat and inhibit growth of *Campylobacter coli* in it. Kim et al. [64] stated that the dietary BSFL oil could improve feed conversion ratio and enhance the incorporation of medium-chain fatty acids into abdominal fat in broiler. They compared corn oil, coconut oil and BSFLO as fat source for broiler's diet, and BSFLO boosted the yellowness of breast meat and modified intestinal SCFAs and serum parameters without any negative impacts on growth performance.

**Table 2.** The change in lauric acid content of abdominal fat and meat derived from BSFLO-fed broiler chickens.

| No. of case | Treatments[1)] | Lauric acid content of experimental diet (% of total FAME[2)]) | Experimental period(days) | Growth performance[3)] | | | Lauric acid content (% of total FAME) of meat | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | DFI | ADG | FCR | Abdominal fat | Breast meat | Leg meat |
| 1 [64] | Corn oil 5% | 0.06 | 1~30 | 71.7 | 45.43 | $1.58^{a}$ | $0.08^{c}$ | | |
| | Coconut oil 5% | 53.98 | | 69.93 | 48.05 | $1.46^{b}$ | $15.43^{a}$ | | |
| | BSFLO 5% | 37.55 | | 71.31 | 48.11 | $1.49^{b}$ | $12.33^{b}$ | | |
| 2 [62,63] | SBO 6.87% | 0 | 21~48 | 208.3 | 107.6 | 2.01 | | $0.01^{c}$ | $0.06^{c}$ |
| | SBO 3.43% + BSFLO 3.43% | 17.3 | | 207.3 | 105.9 | 2.03 | | $4.61^{b}$ | $5.97^{b}$ |
| | BSFLO 6.87% | 34.7 | | 212.4 | 112.8 | 1.95 | | $11.0^{a}$ | $13.2^{a}$ |
| 3 [65] | SBO 5.85% (6.90) * | 0.34(1.06) | 1~35 | 55.1 | 37.1 | 1.48 | | $0.09^{c}$ | |
| | SBO 2.91% (3.45) + BSFLO 2.91% (3.45) | 20.9(21.2) | | 61.2 | 40.4 | 1.51 | | $4.75^{b}$ | |
| | BSFLO 5.85% (6.90) | 37.5(38.1) | | 65.4 | 43.1 | 1.52 | | $8.50^{a}$ | |

1) BSFLO, black soldier fly larvae oil; SBO, soybean oil.
2) Fatty acid methyl ester.
3) DFI, daily feed intake; ADG, average daily gain; FCR, feed conversion ratio.
*Number of parenthesis means the inclusion level of SBO or BSFLO during 22~35 days of feeding.
a~c Means without a common superscript letter differ ($p < 0.05$).

There have been being already carried out feeding trials using BSFLM as a replacer of soybean meal or fish meal together with the rapid growth of insect industry. Most of BSFLM were also produced in a little different manner according to its producer because the manufacturing processes of insect meals were not standardized yet, for this reason, it is necessary to evaluate the bioavailability of BSFL more precisely.

# II. Evaluation of black soldier fly larvae oil (BSFLO) as a dietary fat source in broiler chicken diets

## 1. Introduction

According to FAO global outlook [1], the world population was predicted to reach approximately 9 billion, which could especially increase the demand of animal proteins [66]. The surge of food demands could intensify competition for natural resources among stakeholders in food and feed industry. Soybeans, in particular, would be the main target of these resource competition, which could drive the rise of feed price so strongly that might aggravate the uncertainty of the sustainability in livestock industry [65]. In contradiction, food loss has become a serious social problem in developed countries due to their wasteful food consumption. Therefore the food waste management has been emerging an important topic to be studied [67].

Among the insect species, the black soldier fly (BSF, *Hermetia illucens*) is regarded as a promising species for recycling food waste because of its larvae's ability to convert organic matter such as restaurant waste and manure into highly qualified nutrients [68,69]. The BSF larvae (BSFL) consume organic waste and store protein and fat in their bodies to supply the nutrients for the pupal period and adult stage [69]. Although the contents of protein and fat in the larvae meal and oil can be variable depending on their substrate and manufacturing procedure [67], BSFL can produce high levels of proteins (42%) and fats (35%) on the dry matter basis [68-70].

Insect production mainly focuses on the production of food proteins, which is more economically valuable than fat. However, the insect fat accounts for an irresistible amount in the insect production. Finke and Oonincx [71] reviewed that the insect fat is mainly ( > 50%) composed of unsaturated fatty acid, among which C16:1, C18:1, C18:2, and C18:3 are most prevalent in insects. And the major saturated fatty acid in insect fat are C16:0, and C18:0, which is similar to most terrestrial animals (C16:0 > C18:0). On the contrary, BSFL contain remarkably dominant amounts of saturated fatty acids, especially lauric acid (C12:0) belonging to medium-chain fatty acids (MCFAs). MCFA attracts attention as an energy source that can be used quickly by livestock and material with excellent antibacterial properties. Also, although lauric acid is saturated fat, it is not known to affect cardiovascular health, unlike long-chain saturated fatty acids adversely. Therefore, BSFLO is expected to help improve the productivity of broilers and improve the functionality of broilers.

The quality of BSFL is affected by several factors, of which the processing method has only recently been developed. Research on insect processing has focused on enhancing the efficiency of the fat extraction process, but there are relatively scarce studies on changes of physicochemical properties of insects during processing. With competition among many insect companies, commercial insect processing systems are not well known outside. IPIFF presented an insect processing system as an example, as shown in Figure 6 [72]. This example in Europe showed many differences with the insect processing in Korean insect companies regarding no separation process of liquid and solid parts before drying and defatting. The processing methods of BSFL/P meals can be classified into dry-milling and wet-milling [3], as shown in Table 3. Most of Korean BSFL producers have adopted the dry milling process for BSFL meal production. To produce BSFLM, they first dry raw BSFL/P by using the microwave-drying machine, then heat-press the dried BSFL/P to remove their fat.

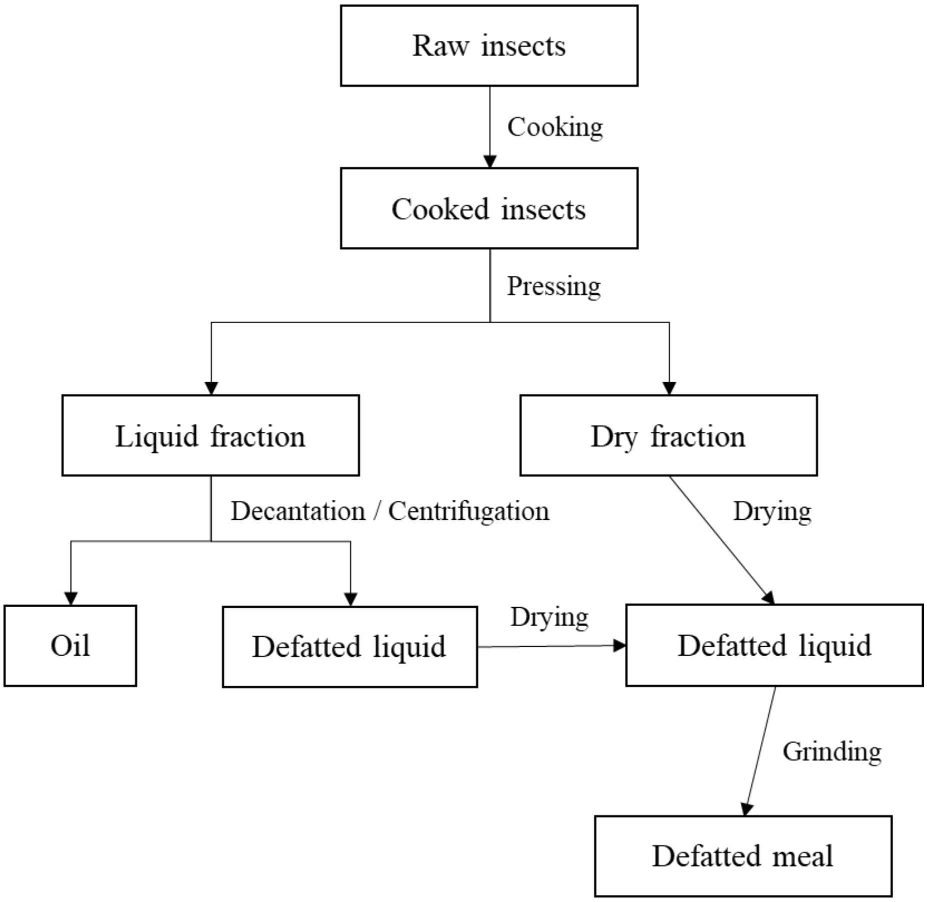


**Figure 6.** Example of insect processing in EU.

**Table 3.** Comparison between dry milling and wet milling of insects.

| | Dry milling | Wet milling |
|---|---|---|
| Definition | • Crushing after drying | • Raw or dried insects are reconstituted with water and heated before and after grinding into pastes, slurries, or wet semi-solids. |
| Advantages | • Inexpensive processing equipment price (in case of drum dryer)<br>• High versatility | • It is easy to transport raw materials for each processing step through pumping in liquid or semi-solid, and various processing methods can be applied.<br>• Easy to remove chitins |
| Disadvantage | • Product quality may deteriorate during the drying process before grinding. | • It is not easy to store due to its large volume and high moisture content.<br>• It is hard to introduce where it is challenging to install related facilities and equipment. |

Recently, the microwave-drying method can make the nutritional quality of BSFL/P meals poor by increasing their particle size due to protein polymerization [73]. Also, during its processing and storage, the endogenous phenoloxidase activity in BSFL/P promotes its melanization to diminish the protein solubility and digestibility [74]. Due to insufficient standardization of research methodology for insects as food and feed, there was not available more detailed information on BSFL used in many studies, including their substrates, processing methods, and various factors. Because of these differences in insect processing between Korean and foreign countries, it is necessary to evaluate the applicability of Korean BSFL/P-derived ingredients as raw material feed for the broiler.

The objectives of this study were to investigate the potential of BSFLO as a replacer of SBO for the broiler's diet. For this, I first examined the effect of dietary BSFLO on the growth performance and meat quality of broilers. Second, it was checked if a large amount of lauric acid content in BSFLO did not affect cecal microbiota negatively in the broiler.

## 2. Effect of black soldier fly larvae oil in diet on growth performances and meat quality of broilers[1]

### 2.1. Materials and methods

*Birds and diets*

The present study was conducted in the poultry facility of the National Institute of Animal Science of South Korea and was approved by the Institutional Animal Care and Use Committee of the Rural Development Administration (No. NIAS-2019-1710). A total of 210 male broiler chicks (Ross 308) at one day of age were randomly allotted to 3 dietary treatments (10 pens/treatment and 7 birds/pen). The poultry house was equipped with an automatic ventilation system. Each pen was equipped with a feeder and an automatic drinker. On day 21, chicks were randomly allocated to individual cages with a feeder and drinker.

The study was performed to evaluate the effects of a partial or total replacement of SBO with BSFLO on broiler chickens using two inclusion levels. The diets were a control diet based on corn and soybean meal (SBM) and 50 and 100 % replacement of SBO with BSFLO. The diets were fed in three phases: starter (day 1 to 7), grower (day 7 to 21), and finisher (day

[1] This chapter comprises a part of the thesis published in Journal of Animal Science and Technology [62(2): p. 187-197] as a partial fulfillment of Sang Yun Ji's Ph.D. program.

21 to 35). All diets were formulated to meet or exceed the NRC [75] requirements (Table 4). Feed and water were provided *ad libitum* throughout the trial.

Table 4. Ingredients and chemical composition of the experimental diets[1)]

| Items[1)] | Starter | | | Grower | | | Finisher | | |
|---|---|---|---|---|---|---|---|---|---|
| | CON | 50BSFLO | 100BSFLO | CON | 50BSFLO | 100BSFLO | CON | 50BSFLO | 100BSFLO |
| Ingredients, % | | | | | | | | | |
| Corn | 53.05 | 53.05 | 53.05 | 59.00 | 59.00 | 59.00 | 61.80 | 61.80 | 61.80 |
| Soybean meal, 44% | 30.00 | 30.00 | 30.00 | 30.00 | 30.00 | 30.00 | 30.00 | 30.00 | 30.00 |
| Corn gluten meal | 6.35 | 6.35 | 6.35 | 4.00 | 4.00 | 4.00 | 1.60 | 1.60 | 1.60 |
| Wheat bran | 3.15 | 3.15 | 3.15 | - | - | - | - | - | - |
| Soybean oil | 3.00 | 1.50 | - | 3.00 | 1.50 | - | 3.00 | 1.50 | - |
| Black soldier fly larvae oil | - | 1.50 | 3.00 | - | 1.50 | 3.00 | - | 1.50 | 3.00 |
| Dicalcium phosphate | 1.75 | 1.75 | 1.75 | 1.50 | 1.50 | 1.50 | 1.45 | 1.45 | 1.45 |
| Limestone | 1.25 | 1.25 | 1.25 | 1.15 | 1.15 | 1.15 | 1.05 | 1.05 | 1.05 |
| Vitamin-mineral premix[2)] | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 |
| L-lysine | 0.40 | 0.40 | 0.40 | 0.25 | 0.25 | 0.25 | 0.10 | 0.10 | 0.10 |
| DL-methionine | 0.20 | 0.20 | 0.20 | 0.20 | 0.20 | 0.20 | 0.15 | 0.15 | 0.15 |
| Salt | 0.35 | 0.35 | 0.35 | 0.40 | 0.40 | 0.40 | 0.35 | 0.35 | 0.35 |
| Calculated composition | | | | | | | | | |
| ME, Kcal/kg | 3,031 | 3,031 | 3,031 | 3,106 | 3,106 | 3,106 | 3,152 | 3,152 | 3,152 |
| Lysine, % | 1.42 | 1.42 | 1.42 | 1.26 | 1.26 | 1.26 | 1.11 | 1.11 | 1.11 |
| Methionine, % | 0.53 | 0.53 | 0.53 | 0.50 | 0.50 | 0.50 | 0.43 | 0.43 | 0.43 |
| Ca, % | 0.96 | 0.96 | 0.96 | 0.91 | 0.91 | 0.91 | 0.86 | 0.86 | 0.86 |
| Total P, % | 0.77 | 0.77 | 0.77 | 0.70 | 0.70 | 0.70 | 0.65 | 0.65 | 0.65 |
| Available P, % | 0.46 | 0.46 | 0.46 | 0.41 | 0.41 | 0.41 | 0.36 | 0.36 | 0.36 |
| Analyzed composition | | | | | | | | | |
| Crude protein, % | 24.63 | 24.48 | 23.28 | 22.15 | 22.77 | 23.14 | 22.71 | 22.03 | 21.37 |
| Crude fat, % | 3.63 | 5.39 | 5.93 | 6.76 | 7.51 | 7.70 | 4.68 | 4.44 | 5.22 |
| NDF, % | 8.95 | 10.51 | 10.27 | 11.00 | 12.49 | 14.43 | 11.83 | 16.39 | 12.55 |
| ADF, % | 3.93 | 4.17 | 3.78 | 3.88 | 3.56 | 3.45 | 4.13 | 4.24 | 3.33 |
| Ash, % | 8.22 | 7.60 | 7.06 | 7.67 | 7.13 | 7.24 | 7.78 | 7.16 | 7.89 |

[1)]CON, control diet; 50 BSFLO, 50% black soldier fly larvae oil diet; 100 BSFLO, 100% black soldier fly larvae oil diet.
[2)]Supplied per kilogram of diet: vitamin A 1,600,000 IU; vitamin $D_3$ 300,000 IU; vitamin E 800 IU; vitamin $K_3$ 132 mg; vitamin B1 97 mg; vitamin B2 500 mg; vitamin B6 200 mg; vitamin B12 1.2 mg; nicotinic acid 2,000 mg; pantothenic acid 800 mg; folic acid 60 mg; choline chloride 35,000 mg; Mn 12,000 mg; Zn 9,000 mg; Fe 4,000 mg; Cu 500 mg; I 250 mg; Co 100 mg; Se 50 mg.

*Growth performances*

Health status and mortality were recorded daily during the whole experimental period. The initial body weight (IBW) and the final body weight (FBW) were recorded on days 1 and 35. Daily feed intake (DFI), average daily gain (ADG), and feed conversion ratio (FCR) were determined for the overall experimental period (1 to 35 days)

*Slaughtering procedure*

Birds were grown to the age of 35 days and then slaughtered. Before slaughter, 33 birds (11 birds/treatment) were selected based on average final live weight. Head, neck, and feet were removed and the length and weight of three sections of the small intestine were measured. The weight of the gizzard, liver, pancreas, spleen, bursa of Fabricius were also immediately weighed and recorded. A total of 33 chicken leg and breast meat samples were individually vacuum sealed and chilled at 4°C to analyze physical measurements, chemical traits, oxidative parameters, and FA profile.

*Physical measurements and chemical traits of chicken leg meat*

Each 5 g of chicken leg meat samples (6 replicates) was homogenized with 15 ml distilled water and then homogenized with a homogenizer (Tissue grinder, 1102-1, Japan) at 10,000 × g for 1 min. The pH values of the homogenates were determined using a pH meter (AM-7, Nihonseiki Kaisha Ltd., Tokyo, Japan). All determinations were performed in triplicate.

The leg meat samples were cooked in a polyethylene plastic bag and heated in a water bath operating at 80°C to an internal temperature of 75°C. After cooking and cooling, the samples were dried and weight to calculate the cooking loss (%). The samples were cut into 1.5 × 1.5 cm sized pieces. Shear force analysis was performed using a TA-HDi Texture Analyzer (Stable Macro System, London, UK). Each block was sheared at a constant speed of 1mm/s. The water holding capacity (WHC) was measured followed by the guide of Kauffman et al. [76]. The Samples were also analyzed for moisture, crude protein (CP), ether extract (EE), and ash [77].

*Thiobarbituric acid-reactive substances (TBARS) and volatile basic nitrogen (VBN) in chicken leg meat*

The thiobarbituric acid-reactive substances (TBARS) and volatile basic nitrogen (VBN) were also measured on d 0, 3, and 6 during the storage period as described by Witte et al. [78]. On each day, 10 g minced leg meat samples (5 replicates) were homogenized with 10 ml of trichloroacetic acid, and the sample volume was adjusted to 50 ml by adding distilled water. The homogenization was performed at 13,500 rpm for 5 min with a homogenizer (Tissue grinder, 1102-1, Japan). After centrifugation, the supernatant was filtered using a filter paper (Whatman No. 1) and 5 ml of the supernatant was added to 5 ml of 2-thiobarbituric acid (0.005 mM). The samples were then mixed and the mixture was heated. Next, the absorbance was measured at 532 nm using a UV/VIS spectrophotometer (OPTI-ZEN 2120UV, Mecasys Co., Ltd., Korea). The result was expressed as mg malonaldehyde (MDA) equivalents/kg sample.

The VBN was analyzed according to Conway [79]. The 10g of leg meat samples (5 replicates) was homogenized for 10 min with 90 ml of distilled water using homogenizer (Tissue grinder, 1102-1, Japan). The homogenate was centrifuged for 10 min at 800 × g, and the supernatant was filtered using a filter paper (Whatman No. 1). Next, 1 ml of 0.01 N boric acid as a VBN absorber was placed in the inner section of a Conway micro diffusion cell. And then, 1 ml of filtrate and 1 ml of 50% potassium carbonate were added to the outer section of the Conway micro diffusion cell. The cell was incubated at 37°C for 120 min and titrated with 0.02N sulfuric acid. The blank test was conducted following the same process without adding 1 ml of 50% potassium carbonate.

*Fatty acid profile of black soldier fly larvae oil and chicken breast meat*

The lipid extraction was performed using chloroform:methanol (1:2) for breast meat samples (6 replicates). The samples were transmethylated using a methanolic solution of $H_2SO_4$ (4%) to determine fatty acid methyl esters (FAME). A biphasic separation was obtained by adding 0.5 ml of distilled water and 1.5 ml of N-Heptane to each sample. The FAME was determined by gas chromatography (Agilent, 7890A series, 2850 Centerville Road Wilmington, DE 19808-1610 USA), equipped with a Hewlett Packard HP-88 capillary column (60 m length, 0.52 mm internal diameter, and 0.20 μm film thickness; J&W Scientific, USA) and a flame ionization detector. The carrier gas was He and the detector temperature was 260°C with the split ration (30:1). The FAs were identified based on a standard FAME mixture (37-Component

FAME Mix, Supelco Inc., Bellefonte, PA, USA). The results were expressed as the percentage (%) of total detected FAME (Table 5).

**Table 5.** Fatty acid profile of the black soldier fly larvae oil (BSFLO) and soybean oil (SBO)

| Fatty acids (% of total FAME[1)]) | BSFLO | SBO[2)] |
|---|---|---|
| C10:0 | 1.76 | - |
| C12:0 (Lauric) | 35.72 | - |
| C14:0 (Myristic) | 5.03 | 0.22 |
| C16:0 (Palmitic) | 13.78 | 11.17 |
| C17:0 | 0.20 | - |
| C18:0 (Stearic) | 2.81 | 3.79 |
| C16:1 | 2.12 | 0.12 |
| C17:1 | 0.18 | - |
| C18:1 n-9 (Oleic) | 18.28 | 23.08 |
| C22:2 | 0.18 | - |
| C18:2 n-6 (Linoleic) | 15.02 | 53.95 |
| C18:3 n-3 (Linolenic) | 1.95 | 7.24 |
| C18:4 n-3 | 0.54 | - |
| C20:3 n-3 | 0.32 | - |
| C20:5 n-3 (EPA) | 0.98 | - |

[1)] Fatty acid methyl ester

[2)] Referred from INRA-CIRAD-AFZ feed tables [80]

*Sensory analysis of chicken breast meat*

Sensory evaluation was conducted by a panel consisting of 6 assessors at the National Institute of Animal Science. For the experiment, a total of 5 breasts per treatment were used and 3 days of analysis were scheduled (d 0, 3, and 6 during the storage period). The breast meats were removed from the refrigerator on each sampling day and used directly. After that, each sample was heated at 80℃ for 8 min, until the core temperature reached 74℃. The samples were put on aluminum trays and served to the assessors. The descriptors were color, flavor, juiciness, firmness, and overall preference and the intensity of the sensory attributes was scored from 1 to 9, with 1 as low intensity and 9 as high intensities.

*Statistical analysis*

Data were analyzed using the PROC GLM of SAS (SAS Inst. Inc., Cary, NC, USA). The experimental unit was the pen for growth performance and the individual bird was used for slaughter traits, oxidative and meat quality parameters, and FA profile. Results are given as mean and standard error of the mean (SEM). Statistical significance and tendency were considered at $p < 0.05$ and $0.05 \leq p < 0.10$, respectively.

### 2.2. Results and discussions

In the present study, partial or total replacement of SBO with BSFLO for broiler chickens has no detrimental effect on growth performance. The inclusion of 100% BSFLO also did not lead to any negative effect on growth performance compared to 50% BSFLO inclusion.

This result shows the possibility of total replacement of SBO by BSFLO. The IBW was the same in all groups and the FBW also did not show differences among dietary treatment groups (Table 6). The DFI, ADG, and FCR were not affected by the inclusion of BSFLO (Table 6). The mortality was not affected by the inclusion of BSFLO (not shown). The existing studies were also reported that the inclusion of BSFLO in a broiler diet did not affect the growth performance [63,65]. Through these results, it is possible to replace 100% of the SBO with the BSFLO in the broiler diet in terms of growth performance.

Like the result of growth performance, the organ weight except the relative weight of gizzard was not affected by the replacement of SBO with BSFLO. Studies of Schiavone et al. [63,65] also have shown no significant differences in carcass traits by the replacement of BSFLO [63,65]. However, the dietary inclusion (150 g/kg) of BSFLM for broiler chickens negatively affect the carcass weight [81]. Dabbou et al. [38] observed that the negative effects on growth performance and carcass trait by 15% inclusion level of partially defatted BSFLM in the broiler diet were caused by reduced villus height: crypt depth. Interestingly, the relative gizzard weight was significantly higher ($p$ = 0.028) in the control group than in other groups (Table 7). A similar result was reported that the weight of gizzard in a control group was heavier than that of other treatments which BSFLM was included in a broiler chicken diet [82].

**Table 6.** Effect of the dietary black soldier fly larvae oil (BSFLO) inclusion level on the growth performance of the broiler chickens[1)]

| Items[2)] | Dietary treatments[3)] | | | SEM | *p*-value |
|---|---|---|---|---|---|
| | CON | 50 BSFLO | 100 BSFLO | | |
| IBW, g (d 1) | 41.60 | 41.71 | 41.20 | 0.16 | 0.104 |
| FBW, g (d 35) | 1739.82 | 1730.88 | 1743.50 | 43.92 | 0.978 |
| DFI, g/d | 79.06 | 81.38 | 79.60 | 1.19 | 0.386 |
| ADG, g/d | 49.71 | 49.45 | 49.81 | 1.25 | 0.978 |
| FCR, g/g | 1.59 | 1.65 | 1.60 | 0.03 | 0.492 |

[1)]Each value is the mean of 10 replicates (7 birds/group).

[2)]IBW, initial body weight; FBW, final body weight; DFI, daily feed intake; ADG, average daily gain; FCR, feed conversion ratio.

[3)]CON, control diet; 50 BSFLO, 50% black soldier fly larvae oil diet; 100 BSFLO, 100% black soldier fly larvae oil diet.

**Table 7.** Effects of the dietary black soldier fly larvae oil inclusion level on the relative length (cm/kg) of the different sections of the digestive tract, relative weight (g/kg) of the digestive track and internal organs of broiler chickens at 35 d of age[1)]

| Items | Dietary treatments[2)] | | | SEM | *p*-value |
|---|---|---|---|---|---|
| | CON | 50 BSFLO | 100 BSFLO | | |
| Live weight (kg) | 1.99 | 1.98 | 2.08 | 0.04 | 0.229 |
| Intestinal sections | | | | | |
| Duodenum length (cm/kg) | 14.32 | 14.44 | 13.34 | 0.55 | 0.330 |
| Jejunum length (cm/kg) | 33.17 | 30.50 | 32.69 | 0.90 | 0.107 |
| Ileum length (cm/kg) | 33.56 | 30.00 | 32.09 | 1.14 | 0.111 |
| Duodenum weight (g/kg) | 6.63 | 6.26 | 6.27 | 0.30 | 0.628 |
| Jejunum weight (g/kg) | 10.91 | 10.41 | 10.41 | 0.45 | 0.665 |
| Ileum weight (g/kg) | 7.61 | 8.16 | 7.68 | 0.33 | 0.456 |
| Internal organs | | | | | |
| Gizzard (g/kg) | 14.85[a] | 12.52[b] | 13.02[b] | 0.59 | 0.028 |
| Liver (g/kg) | 21.67 | 19.94 | 21.19 | 0.85 | 0.349 |
| Pancreas (g/kg) | 2.28 | 2.20 | 2.31 | 0.13 | 0.849 |
| Spleen (g/kg) | 1.14 | 0.95 | 1.09 | 0.08 | 0.271 |
| Bursa of fabricius (g/kg) | 1.80 | 1.66 | 1.42 | 0.29 | 0.655 |

[1)] Each value is the mean of 11 replicates (11 birds/treatment).

[2)] CON, control diet; 50 BSFLO, 50% black soldier fly larvae oil diet; 100 BSFLO, 100% black soldier fly larvae oil diet.

[a,b] Means with different letters within each variable differ ($p < 0.05$).

Moreover, they compared several kinds of BSFLM which are differently defatted in terms of organ yields. The relative weight of the gizzard of broilers fed full-fat BSFLM which was lower than those fed extruded BSFLM. However, the replacement of fish meal with a maggot in a broiler diet, as opposed to BSFLM and BSFLO, increased the weight of gizzard [83]. Overall, it seems that the inclusion of the BSFL by-product may affect the weight of the gizzard and further studies are required to better understand the mechanism.

The results of the physical and chemical traits of chicken leg meat suggested that the use of the BSFLO in the broiler diet was appropriate as an alternative of SBO. The inclusion of BSFLM in terms of carcass weight and dressing percentage had no adverse effect on chicken meat quality [84]. They also investigated the pH, color, and cooking loss of breast meat and the pH was decreased by the inclusion of BSFLM. The low pH value can cause a decrease of WHC and the increase of cooking loss when the value of pH is between 5.2 to 5.5 [84]. However, in the present study, the replacement of BSFLO did not show any adverse effects on the physical measurements of chicken leg meat in Table 8 and the pH value was in a normal range [85]. For this reason, the results of moisture, WHC, and cooking loss which are related to pH value had no significant differences among treatments. However, the more substitution level (24.8 % of SBM and 100% of SBO) of BSFLM in a broiler diet increased the shear force and cooking loss compared to less substitution (16.1 % of SBM and 28.4 % of SBO) of BSFLM [84]. It seems that the high inclusion level of BSFLM may affect the physical measurements of chicken breast meat. On the other hand, the total substitution of the BSFLO did not affect the shear force and cooking loss in the chicken leg meat compared to the partial substitution of the BSFLO in this study (Table 8). The chemical quality such as CP, EE, and ash of chicken leg meat was also not affected by treatment (Table 8). Several studies have also shown the same results that the total replacement of the SBO with BSFLO did not affect the chemical quality of chicken meat [65,81].

**Table 8.** Physical measurements and chemical traits of chicken leg meat as affected by diets containing different levels of black soldier fly larvae oil (BSFLO)[1)]

| Parameters | Treatments[2)] | | | SEM | $p$-value |
|---|---|---|---|---|---|
| | CON | 50 BSFLO | 100 BSFLO | | |
| pH | 5.82 | 5.82 | 5.86 | 0.04 | 0.802 |
| Shear force, kg/0.5 inch$^2$ | 3.33 | 3.29 | 2.79 | 0.55 | 0.745 |
| Cooking loss, % | 15.29 | 18.52 | 15.49 | 1.02 | 0.074 |
| WHC[3)] % | 61.66 | 61.14 | 59.64 | 0.77 | 0.196 |
| Moisture, % | 75.47 | 76.11 | 75.83 | 0.38 | 0.516 |
| Crude protein, % | 22.14 | 21.83 | 21.89 | 0.37 | 0.833 |
| Ether extract, % | 1.36 | 1.18 | 1.17 | 0.19 | 0.754 |
| Ash, % | 1.11 | 1.07 | 1.11 | 0.03 | 0.527 |

[1)] Each value is the mean of 6 replicates (6 birds/treatment).

[2)] CON, control diet; 50 BSFLO, 50% black soldier fly larvae oil diet; 100 BSFLO, 100% black soldier fly larvae oil diet.

[3)] WHC, water holding capacity.

The fatty acid composition of chicken meat has been a major concern for health-conscious consumers [86]. BSFLO is mainly composed of saturated fat, of which lauric acid (C12:0), palmitic acid (C16:0), and oleic acid (C18:1) are predominant fatty acids [67]. Although lauric acid is a saturated one, it functions differently from the other saturated fatty acid inducing cardiovascular disease and hyperlipidemia. Ingestion of lauric acid greatly increases total cholesterol, which is due to the increase of HDL-cholesterol, not LDL-cholesterol [87]. Lauric acid also acts as an antimicrobial agent inhibiting the proliferation of gut pathogens [30,88,89].

This present study showed that the replacement of the BSFLO altered the FA profile of the chicken breast meat (Table 8). The content of the SFA was increased (27.16, 27.58, and 28.72 % in C, 50 BSFLO, and 100 BSFLO, $p = 0.011$) by the supplementation of the BSFLO. However, contrary to the result of the SFA, the unsaturated fatty acid (UFA) was decreased (72.85, 72.42, and 71.29 % in C, 50 BSFLO, and 100 BSFLO, $p = 0.011$) by the total replacement of the BSFLO and the UFA/SFA was also significantly decreased (2.69, 2.63, and 2.48 in C, 50 BSFLO, and 100 BSFLO, $p = 0.013$).

The reason for the change of the FA profile is that the high level of SFA in the BSFLO influences the fraction of PUFA [62]. Schiavone et al. [65] reported that the MUFA was not affected by the BSFLO. On the contrary, in the present study, the fraction of MUFA was increased (43.36, 44.58, and 48.55 % in C, 50 BSFLO, and 100 BSFLO, $p < 0.001$) and this result stems from the desaturation and elongation of SFA such as C14:0, C16:0, and C18:0.

The inclusion of BSFLM in a broiler and laying quails also changed the FA profile in the breast meat and eggs [81,90]. They commonly reported that the concentration of SFA and MUFA was increased and PUFA was decreased. The one thing that should be improved is the negative change of the FA composition of meat when the BLSFM and BSFLO were included. Even though 100 BSFLO showed increased UFA/SFA, there was no significant difference between CON and 50 BSFLO (Table 9). This result means that it seems appropriate to replace with the BSFLO less than 50% in terms of the meat quality.

**Table 9.** Fatty acid profile (% of total FAME) of chicken breast meat as affected by diets containing different levels of black soldier fly larvae oil (BSFLO)[1)]

| Parameters[2)] | Treatments[3)] | | | SEM | $p$-value |
|---|---|---|---|---|---|
| | CON | 50 BSFLO | 100 BSFLO | | |
| C14:0 (Myristic) | 0.55[c] | 1.07[b] | 1.74[a] | 0.08 | <.001 |
| C16:0 (Palmitic) | 20.35[b] | 21.06[ab] | 21.90[a] | 0.28 | 0.005 |
| C18:0 (Stearic) | 6.26[a] | 5.45[b] | 5.08[b] | 0.22 | 0.007 |
| SFA | 27.16[b] | 27.58[b] | 28.72[a] | 0.32 | 0.011 |
| C16:1 n-7 (Palmitoleic) | 3.82[b] | 3.59[b] | 4.78[a] | 0.23 | 0.006 |
| C18:1 n-9 (Oleic) | 39.13[c] | 40.61[b] | 43.32[a] | 0.45 | <.001 |
| C20:1 n-9 (Eicosenoic) | 0.41[b] | 0.38[b] | 0.45[a] | 0.01 | 0.003 |
| MUFA | 43.36[b] | 44.58[b] | 48.55[a] | 0.54 | <.001 |
| C18:2 n-6 (Linoleic) | 26.88[a] | 25.48[a] | 20.90[b] | 0.49 | <.001 |
| C18:3 n-6 (γ-Linolenic) | 0.24[a] | 0.19[b] | 0.17[b] | 0.01 | 0.005 |
| C18:3 n-3 (Linolenic) | 1.78[a] | 1.68[a] | 1.20[b] | 0.04 | <.001 |
| C20:4 n-6 (Arachidonic) | 0.59 | 0.49 | 0.47 | 0.04 | 0.176 |
| PUFA | 29.49[a] | 27.84[b] | 22.74[c] | 0.53 | <.001 |
| UFA | 72.85[a] | 72.42[a] | 71.29[b] | 0.32 | 0.011 |
| UFA/SFA | 2.69[a] | 2.63[a] | 2.48[b] | 0.04 | 0.013 |

[1)] Each value is the mean of 6 replicates (6 birds/treatment).

[2)] SFA, sum of saturated fatty acid; MUFA, sum of monounsaturated fatty acid; PUFA, sum of polyunsaturated fatty acid; UFA, sum of unsaturated fatty acid.

[3)] CON, control diet; 50 BSFLO, 50% black soldier fly larvae oil diet; 100 BSFLO, 100% black soldier fly larvae oil diet.

[a-c] Means with different letters within each variable differ ($p < 0.05$).

The substitution of the BSFLO altered the oxidative status of the chicken leg meat resulting in a decrease of the TBARS value and this result seems to be affected by the decreased proportion of the PUFA. The TBARS and VBN values of all treatments were increased ($p < 0.01$) with longer storage periods (Table 10). However, the TBARS value of the control group was significantly higher ($p < 0.001$) than in other groups on d 0 (Table 10). Even though the FA profile of the leg meat was only measured in the present study, the other studies reported that the replacement of BSFLO in the broiler diet changed the FA profile of both breast meat and leg meat and the composition of SFA was also increased [62,65]. Although the high composition of the PUFA is more valuable for the chicken meat quality, the SFA is less vulnerable to lipid peroxidation than the UFA and TBARS inhibition of SFA was greater than that of UFA [91,92]. On the contrary to my result, Cullere et al. [62] observed that the result of TBARS did not show a significant difference by the inclusion of BSFLO in breast meat.

**Table 10.** Effects of the dietary black soldier fly larvae oil inclusion level on the thiobarbituric acid-reactive substances (TBARS) and volatile basic nitrogen (VBN) of chicken leg meat[1)]

| Items[2)] | Storage (d) | | | SEM | $p$-value |
|---|---|---|---|---|---|
| | 0 | 3 | 6 | | |
| TBARS, mg MDA/kg | | | | | |
| CON | 0.24$^{bx}$ | 0.29$^{b}$ | 0.48$^{a}$ | 0.03 | <0.001 |
| 50 BSFLO | 0.13$^{cy}$ | 0.35$^{b}$ | 0.54$^{a}$ | 0.04 | <0.001 |
| 100 BSFLO | 0.12$^{cy}$ | 0.36$^{b}$ | 0.61$^{a}$ | 0.04 | <0.001 |
| SEM | 0.01 | 0.02 | 0.06 | - | - |
| $p$-value | <.001 | 0.058 | 0.434 | - | - |
| VBN, mg % | | | | | |
| CON | 9.53$^{c}$ | 10.18$^{b}$ | 10.85$^{a}$ | 0.17 | <0.001 |
| 50 BSFLO | 9.33$^{c}$ | 10.15$^{b}$ | 10.93$^{a}$ | 0.23 | 0.001 |
| 100 BSFLO | 9.42$^{b}$ | 9.94$^{b}$ | 10.86$^{a}$ | 0.25 | 0.006 |
| SEM | 0.14 | 0.28 | 0.22 | - | - |
| $p$-value | 0.629 | 0.819 | 0.959 | - | - |

[1)]Each value is the mean of 5 replicates (5 birds/treatment).

[2)]CON, control diet; 50 BSFLO, 50% black soldier fly larvae oil diet; 100 BSFLO, 100% black soldier fly larvae oil diet.

$^{a\text{-}c}$ Different letters within same row differ significantly ($p < 0.01$).

$^{x,y}$ Different letters within same column differ significantly ($p < 0.01$).

The sensory traits of the meat are an important point of view to evaluate the BSFLO, and the sensory profiles of the breast meat derived from the chicken fed the BSFLO showed the meaningful result in the aspect of using the BSFLO as an alternative of the SBO. The breast meat of chickens fed the BSFLO showed similar sensory profiles compared to those fed CON (Table 11) The sensory traits of the chicken meat when the insect meal was included in a diet had also no detrimental effect on sensory analysis of eggs from layers fed BSFLM and of fish fed insect meal [62,70].

**Table 11.** Effects of the dietary black soldier fly larvae oil inclusion level on the sensory evaluation of chicken breast meat[1)]

| Items[2)] | Storage (d) | | |
|---|---|---|---|
| | 0 | 3 | 6 |
| Color | | | |
| CON | 4.77 | 4.22 | 4.44 |
| 50 BSFLO | 4.33 | 4.22 | 4.72 |
| 100 BSFLO | 4.44 | 4.27 | 4.22 |
| SEM | 0.27 | 0.26 | 0.25 |
| Flavor | | | |
| CON | 4.88 | 4.27 | 4.44 |
| 50 BSFLO | 4.83 | 4.66 | 4.50 |
| 100 BSFLO | 4.88 | 4.38 | 4.50 |
| SEM | 0.25 | 0.17 | 0.33 |
| Juiciness | | | |
| CON | 4.16 | 4.22 | 3.88 |
| 50 BSFLO | 3.93 | 4.33 | 4.22 |
| 100 BSFLO | 4.46 | 4.11 | 4.44 |
| SEM | 0.18 | 0.15 | 0.17 |
| Firmness | | | |
| CON | 5.16 | 4.83 | 5.05 |
| 50 BSFLO | 4.44 | 5.00 | 5.00 |
| 100 BSFLO | 5.16 | 4.66 | 4.94 |
| SEM | 0.26 | 0.46 | 0.46 |
| Overall preference | | | |
| CON | 5.00 | 4.69 | 4.44 |
| 50 BSFLO | 4.44 | 4.63 | 4.50 |
| 100 BSFLO | 5.11 | 4.44 | 4.61 |
| SEM | 0.25 | 0.26 | 0.3 |

[1)]Each value is the mean of 5 replicates (5 birds/treatment).

[2)]CON, control diet; 50 BSFLO, 50% black soldier fly larvae oil diet; 100 BSFLO, 100% black soldier fly larvae oil diet.

In conclusion, the present study showed that the partial or full replacement of SBO in the broiler chicken diets by the BSFLO showed comparable results on growth performance, meat quality, and sensory properties to the CON and the BSFLO could be a promising substitute as a dietary fat source in the broiler diet. Therefore, further studies are necessary to improve the drawback that lowering the proportion of the PUFA by improving the FA profiles in the BSFLO as modulating their feed and defatting procedure.

## 3. Effects of black soldier fly larvae oil on cecal microbiota in broilers[2]

### 3.1. Materials and methods

*Sample collection and preparation for microbial analysis*

Birds were grown to the age of 35 days and then slaughtered. Before slaughter, 18 birds (6 birds/treatment) were chosen based on average final live weight. The cecal digesta samples were collected and stored at –80 °C before analysis. The cecal digesta samples (5.0 g) were diluted with Ringer's solution at 1:10 dilution. The samples were homogenized in a Stomacher for 2 min, and 1 ml of the sample solutions were centrifuged at 4°C. The genomic DNA was collected from the pellet part by fast-DNA spin kit (MP Bio Laboratories, USA) and stored at –20 °C before sequencing.

*16S rRNA gene sequencing*

The primers, which produce approximately 400 - 500 bp products, of 341F (5'-CCTACGGGNGGCWGCAG-3') and 805R (5’-GACTACHVGGGTATCTAATCC-3') were used for microbial DNA amplification. The resultant 18 amplicon libraries were sequenced using the MiSeq platform (Illumina, San Diego, USA) at the Macrogen sequencing facility (Macrogen, Inc., Seoul, Korea).

[2] This chapter comprises a part of the thesis published in Korean Journal of Agricultural Science [47(2): 219] as a partial fulfillment of Sang Yun Ji’s Ph.D. program.

–227.

*Statistical analysis*

The data were processed using the SAS version 9.4 GLM procedure and Tukey's multiple range test to determine the differences among treatments. Statistical significance was considered at $p < 0.05$.

## 3.2. Results and discussions

According to the fatty acid profile of BSFLO used in this experiment (Table 9), its ratio of UFA to SFA was 0.63, and SFA accounted for 59.1% of its total fatty acid content. MUFA and PUFA were 20.40% and 16.97% respectively in BSFLO. Also, LA (C12:0) was most abundant SFA species, and its content was 35.72% of total fatty acid content of BSFLO. A typical feature of the BSFLO fatty acid profile is its high lauric acid content [93]. Lauric acid is classified as one of MCFAs, which are composed of 6~12 carbon. Especially, LA is abundant in coconut oil and breast milk [94,95]. Recently, MCFAs have been recognized as an alternative to antibiotic growth promotor because of their antimicrobial and quick energy-providing functions [96].

There were no significant differences in Chao 1 index, Shannon's and Simpson's diversity index among experimental groups (Table 12). In phylum level, major bacterial communities were *Firmicutes*, *Proteobacteria*, and *Bacteriodetes* (Figure 7). *Firmicutes* showed the highest ratio of 90% or more in all three treatments, and their ratios in both treatments with 50% replacement (96.02%) and treatments with 100% replacement (94.45%)

were lower than in the control (97.40%). However, *Proteobacteria* was relatively higher than that of the control group (1.92%) in both treatments (3.19%, 3.03%) fed on BSFLO. A total of 125 were analyzed in bacterial community composition in the genus level, among which 117, 6, 1, and 1 belonged to *Firmicutes*, *Proteobacteria*, *Bacteroidetes*, and *Actinobacteria* in the phylum level, respectively. The ratio was shown for the top 16 genera in Figure 8, at which minor genera (< 1%) was grouped into others. The genus *Faecalibacterium*, which is known as a major microorganism among chicken intestinal microbes, was present in the highest ratio (over 50%) in all three treatments, followed by *Ruminococcus*.

**Table 12.** Number of sequences, operational taxonomic units (OTUs), and diversity estimates of bacteria in cecal digesta[y].

| Index[z] | Treatments | | |
|---|---|---|---|
| | CON | 50 BSFLO | 100 BSFLO |
| No. of Sequences | 13,141 | 14,080 | 12,109 |
| OTUs | 247 | 244 | 242 |
| Chao 1 | 285.48 | 273.86 | 280.18 |
| Shannon | 3.20 | 3.54 | 3.29 |
| Simpson | 0.63 | 0.69 | 0.65 |
| Goods coverage | 0.99 | 0.99 | 0.99 |

CON, control diet; 50 BSFLO, 50% black soldier fly larvae oil diet; 100 BSFLO, 100% black soldier fly larvae oil diet.

[y]Each value is the mean of 6 replicates.

[z]OTUs, operational taxonomic units; Chao 1, richness estimate; Shannon, number and evenness of species; Simpson, measure of diversity.

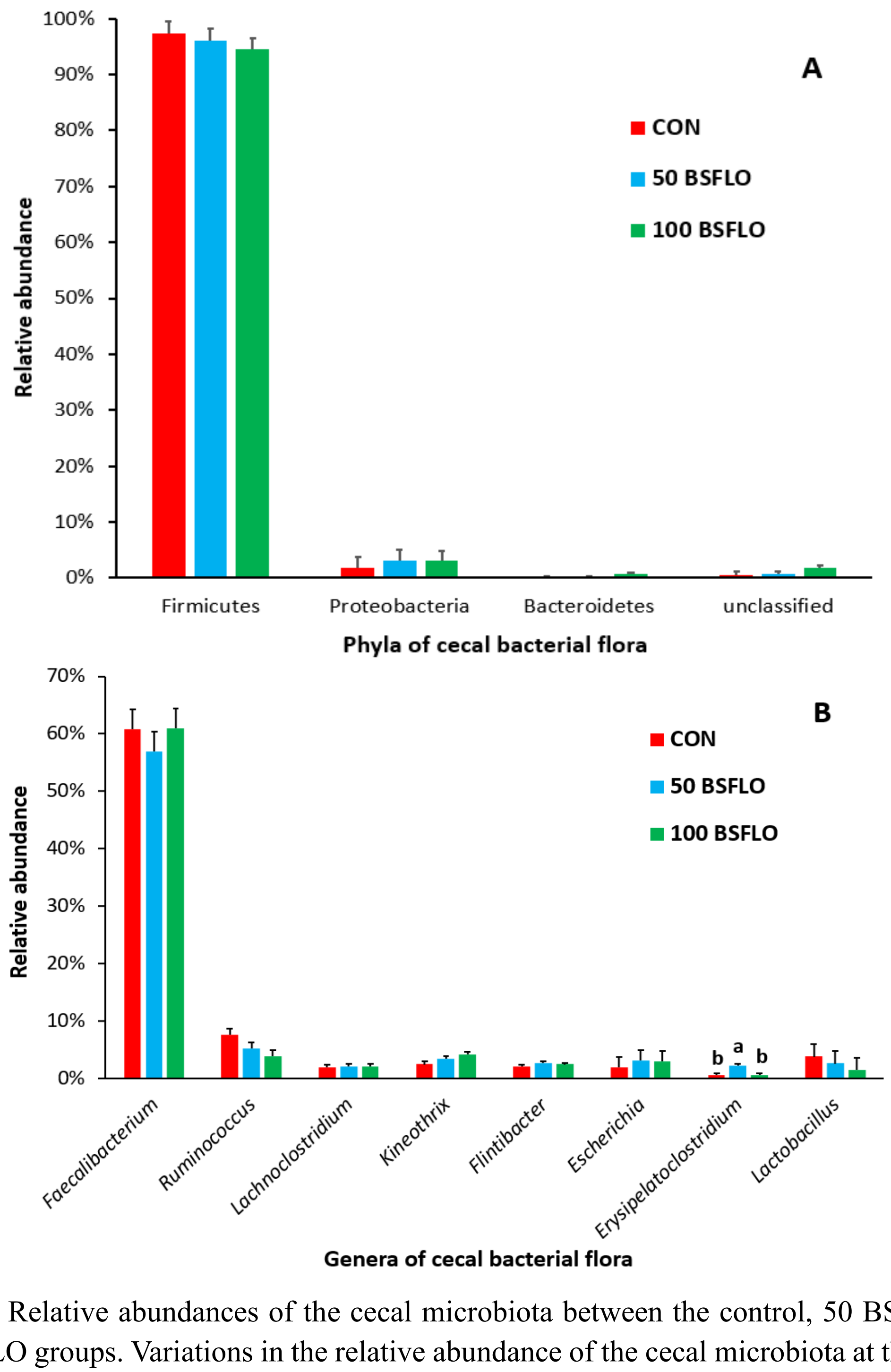


**Figure 7.** Relative abundances of the cecal microbiota between the control, 50 BSFLO, and 100 BSFLO groups. Variations in the relative abundance of the cecal microbiota at the phylum (A) and genus (B) levels. Different letters indicate significant difference ($p < 0.05$).

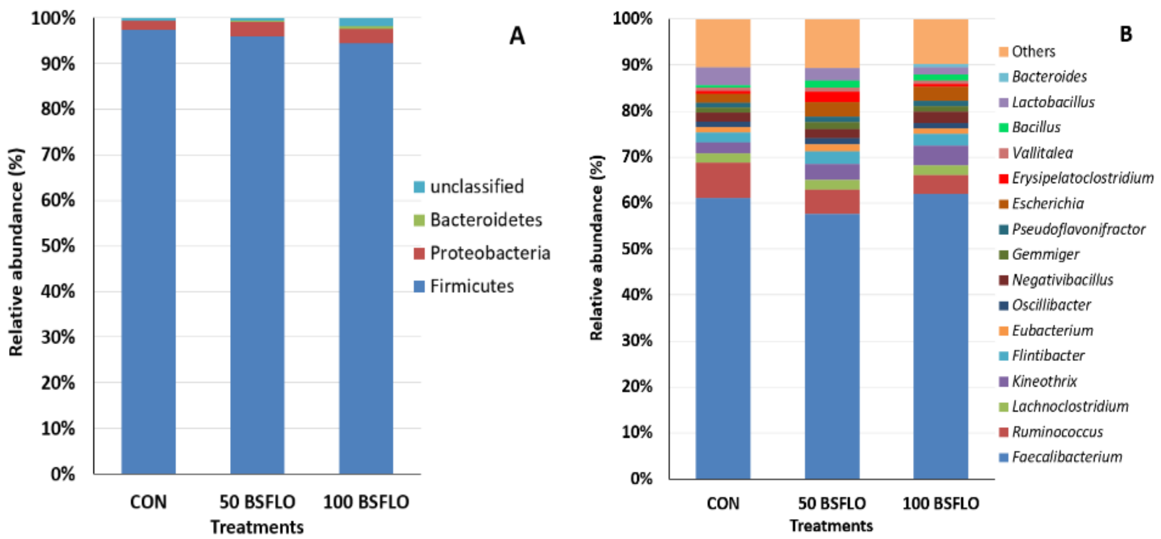


Figure 8. Microbial taxonomic profiles from the cecal digesta of the three dietary treatments at the phylum (A) and genus (B) levels. The taxonomic composition of the cecal microbiota among the treatment groups was compared based on the relative abundance (taxon reads/total reads in the cecal digesta).

The ratio of *Faecalibacterium* was lower in the treatment group (56.89%) than that of the control group (60.75%). The ratio of *Faecalibacterium* was lower in the treatment group (56.89%) than in the control group (60.75%). The genus of *Faecalibacterium* can produce butyrate, which could support enterocytes' development and function, thereby improving the microbiological ecosystem in the gut [97,98]. All treatments with BSFLO (5.22%, 3.92%) had a lower ratio of *Ruminococcus* genus than that of the control (7.66%), and Lactobacillus genus decreased as the replacement ratio increased (3.84%, 2.64%, and 1.45%). *Ruminococcus* genus is a member of the *Ruminococcaceae* family, of which bacteria were known to using cellulose as their substrates and produce SCFA [99,100]. However, the ratio of *Proteobacteria*, including Escherichia genus, was higher in BSFLO-added groups (3.17% and 3.03%) than in the control group (1.91%).

There were no statistical differences in cecal microbial communities among the experiment group at the phylum and genus level. Ratio of *Erysipelatoclostridium* genus was significantly higher in BSFLO 50% group (2.21%) than in control group (0.68%) ($p < 0.05$). Kers et al.[101] reported that *Erysipelatoclostridium* increased in genus level in broiler chicken fed MCFAs, from which LA-rich BSFLO was expected to affect the cecal microbiome. However, PCoA (principal coordinates analysis) analysis did not show a significant difference in microbial composition between treatments (Figure 9).

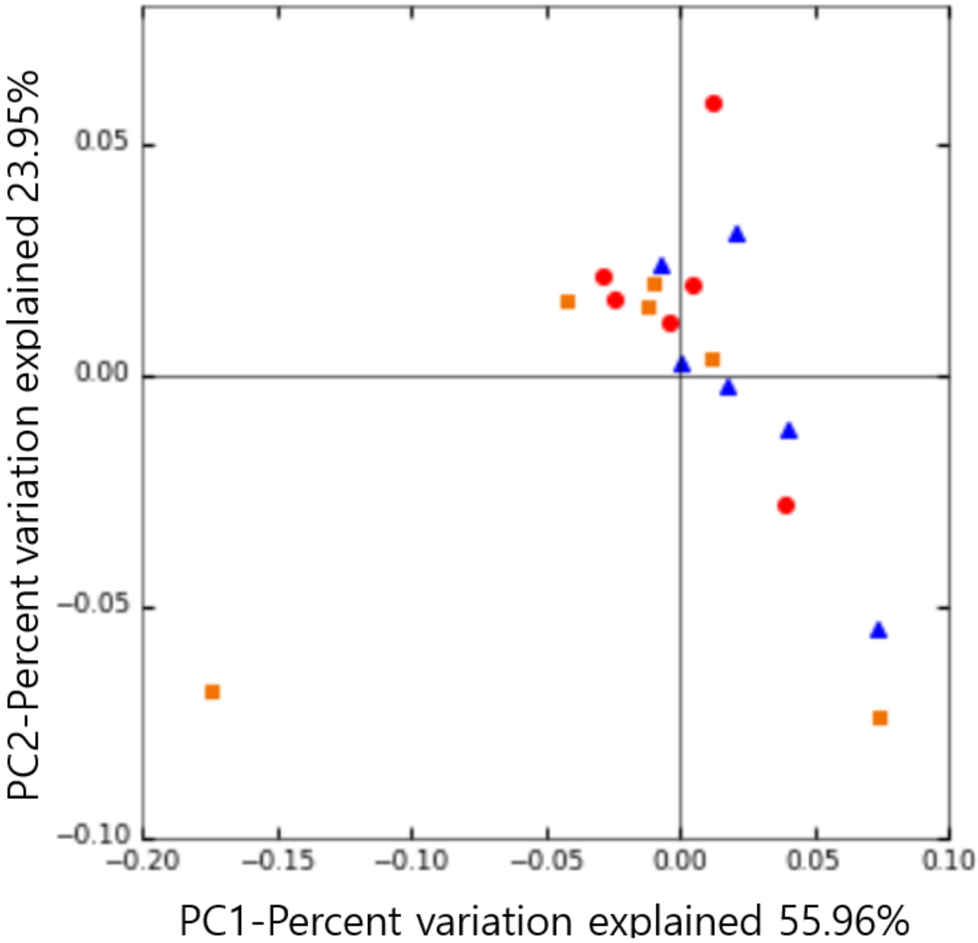


Figure 9. Principal coordinates analysis (PCoA) plots based on weighted UniFrac distance metrics. Individual samples for treatments are designated with the following symbols: CON (red, ○), 50 BSFLO (blue, △), and 100 BSFLO (orange, □).

BSFLO could replace SBO in the diet without any negative effect on the cecal microbiome in broiler chickens. The partial shift in the microbial composition in the cecum is assumed to be due to the difference in the fatty acid composition of soybean oil and BSFLO. MCFAs can be rapidly absorbed into enterocytes in the upper part of the small intestine, compared with LCFA [30,88]. And because LA have antimicrobial activity on Gram-positive bacteria rather than gram-positive one, BSFLO was difficult to make inhibitory action on Gram-negative bacteria like *E. coli*, and Salmonella *spp*. [102,103].

Rapid absorbed MCFAs can promote the development of intestinal villi by supplying energy with intestinal epithelial cells [104]. It is believed that this is the reason why LA-rich BSFLO has a small effect on cecal microflora. It was based on these characteristics that LA-rich BSFLO had little effect on cecal microflora. Replacement of soybean oil by BSFLO in broiler's diet induced shift of cecal microbiomes, but there was no significant difference among experimental groups. As mentioned above, these results were based that BSFLO's LA could not arrive at the cecum by the rapid bioavailability of MCFAs at the upper part of small intestine. To overcome the above problem, attempts have been made to encapsulate MCFAs and deliver them to the target site of the lower digestive tract [104-106]. However, the encapsulation of BSFLO should be considered in terms of fatty acid composition and economic feasibility.

# III. Effects of dietary inclusion level of microwave-dried and press-defatted black soldier fly larvae meal (BSFLM) on carcass traits and meat quality in broilers[3]

## 1. Introduction

Black soldier fly (*Hermetia illucens*) larvae have been considered as a dietary source for animals because of their ability to convert organic waste to edible biomass [45,67]. Furthermore, developing black soldier fly larvae as an animal feed is a potential solution for concomitant problems related to limited food and feed given the increasing world population [107]. Black soldier fly larvae meal (BSFLM) has been evaluated as a valuable protein source for fish and birds [70,108,109]. BSFLM also contains high protein and amino acid contents, which are comparable to those in soybean meal (SBM) [67]. The nutrient composition of BSFLM can be affected by the feeding media and rearing method of the larvae [45,110]. The fatty acid (FA) profiles of BSFLM can be altered by the food sources; however, saturated fatty acid (SFA) levels are typically higher than unsaturated fatty acid (UFA) levels [67]. Despite its potential advantages, toxicity can be caused by the accumulation of heavy metals in the BSFL body via feeding of contaminated media [81,111].

[3] This chapter comprises a part of thesis published in Animals [11(3): 665] as a partial fulfillment of Sang Yun Ji's Ph.D. program.

BSFLM showed satisfactory effects on carcass traits and meat quality except for negative modulation of the FA profile in the meat of broiler quails and chickens because of the different FA composition compared with that of SBM [81,90,109]. Because the SFA content is higher than the UFA content in the BSFLM, the FA profile in meat was altered [81,109]. Moreover, the high content of SFA in the diet may affect the FA profile of abdominal fat because of higher fat deposition compared to polyunsaturated fatty acid (PUFA) [112]. Schiavone et al. [81] also reported that heavy metals and the arsenic level in chicken breast meat of broilers fed the BSFLM did not exceed the maximum allowable limits by the European Union. Moreover, BSFLM contains high levels of chitin, which is indigestible and can decrease protein digestibility [113]. Overall, a low inclusion level (up to 10%) of BSFLM appears to be suitable for broiler diets in terms of the growth performance, intestinal morphology, and FA profile of chicken meat [38,81].

Although BSFL are a good protein source for animals in terms of their nutrient composition, appropriate processing is required to ensure nutrient availability. The nutritional quality of BSFLM is not only altered by substrates but also affected by the drying process, and its digestibility varies depending on the drying method used [73]. The microwave drying method has many advantages compared to conventional drying (60 °C in a drying oven) in terms of efficiency and energy use. Microwave drying transfers thermal energy from the center to the surface of products, whereas the conventional drying method involves the opposite process and is slow [114]. Microwave drying requires less space, is simpler to operate, and has lower operational costs because the walls of the apparatus do not require to be heated [114].

However, whether microwave-dried BSFLM can be used as a protein source for broilers is unclear. In the present study, SBM was substituted with microwave-dried BSFLM as a dietary protein source in broiler diets to evaluate the effects on carcass traits and breast meat quality, abdominal fat and leg meat FA composition, and heavy metal residues in chicken leg meat.

## 2. Materials and methods

*Birds, diets, and experimental design*

This study was approved by the Institutional Animal Care and Use Committee of the Rural Development Administration (No. NIAS-2020-497) and conducted at the poultry facility of the National Institute of Animal Science of South Korea. A total of 126 one-day-old male broiler chicks (Ross 308) were randomly assigned to three dietary treatments (six pens/treatment and seven birds/pen). Three experimental diets (Table 13) were formulated to meet or exceed the nutrient requirements for three phases [17]: starter (days 1–7), grower (days 7–21), and finisher (days 21–35). The control group (CON) was fed a diet based on corn and SBM; in experimental diets, SBM was substituted with 25 or 50% microwave-dried BSFLM (25 and 50 BSFLM diets, respectively). Feed and water were available ad libitum throughout the trial. Cleaned and dehydrated BSFL were dried at 70–80 °C for 30 min using a microwave drying oven; subsequently, they were press-defatted at 45–48 °C using a cold press oil machine (NF-80; Karaerler, Ankara, Turkey). The defatted larvae were pulverized to mix with other ingredients.

The chemical compositions of the experimental diets (Table 13) and the major protein sources (Table 13), excluding chitin, were analyzed using Association of Official Analytical Communities (AOAC) methods [77]. Chitin concentrations in the experimental diets and the BSFLM were calculated as described by Marono et al. [115].

**Table 13.** Ingredients and chemical composition of the experimental diets (as-fed basis).

| Item | Starter | | | Grower | | | Finisher | | |
|---|---|---|---|---|---|---|---|---|---|
| | CON | 25 BSFLM | 50 BSFLM | CON | 25 BSFLM | 50 BSFLM | CON | 25 BSFLM | 50 BSFLM |
| Ingredients, % | | | | | | | | | |
| Corn | 52.69 | 57.21 | 63.15 | 56.03 | 60.89 | 65.72 | 59.32 | 63.10 | 67.86 |
| Soybean meal, 45.8% | 30.00 | 21.50 | 12.00 | 28.00 | 19.00 | 11.00 | 26.00 | 18.50 | 11.00 |
| BSFLM | - | 7.50 | 15.00 | - | 7.00 | 14.00 | - | 6.50 | 13.00 |
| Corn gluten meal | 8.00 | 7.00 | 6.00 | 7.00 | 6.00 | 5.00 | 6.00 | 5.00 | 4.00 |
| Soybean oil | 4.50 | 3.00 | 1.00 | 4.50 | 3.50 | 1.50 | 4.50 | 3.50 | 1.50 |
| Dicalcium phosphate | 1.95 | 1.80 | 1.60 | 1.80 | 1.70 | 1.60 | 1.60 | 1.56 | 1.54 |
| Limestone | 1.50 | 0.70 | - | 1.40 | 0.70 | - | 1.40 | 0.70 | - |
| L-lysine | 0.45 | 0.41 | 0.39 | 0.36 | 0.32 | 0.30 | 0.30 | 0.28 | 0.26 |
| DL-methionine | 0.16 | 0.13 | 0.11 | 0.16 | 0.14 | 0.13 | 0.13 | 0.11 | 0.09 |
| Salt | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 |
| Vitamin-mineral premix (1) | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 |
| Calculated composition | | | | | | | | | |
| ME, kcal/kg | 3119 | 3119 | 3119 | 3149 | 3149 | 3149 | 3177 | 3177 | 3177 |
| Lysine, % | 1.47 | 1.47 | 1.47 | 1.33 | 1.33 | 1.33 | 1.21 | 1.21 | 1.21 |
| Methionine, % | 0.55 | 0.55 | 0.55 | 0.53 | 0.53 | 0.53 | 0.48 | 0.48 | 0.48 |
| Calcium, % | 0.97 | 0.97 | 0.97 | 0.91 | 0.91 | 0.91 | 0.85 | 0.85 | 0.85 |
| Total phosphorus, % | 0.79 | 0.79 | 0.79 | 0.75 | 0.75 | 0.75 | 0.70 | 0.70 | 0.70 |
| Chitin, % | - | 0.43 | 0.86 | - | 0.40 | 0.80 | - | 0.37 | 0.74 |
| Analyzed composition, % | | | | | | | | | |
| Crude protein | 23.23 | 23.67 | 23.60 | 21.96 | 21.76 | 22.08 | 19.53 | 19.97 | 19.80 |
| NDF | 8.67 | 10.03 | 11.08 | 7.90 | 8.76 | 9.99 | 7.35 | 7.47 | 7.90 |
| ADF | 3.36 | 4.03 | 4.38 | 3.73 | 3.67 | 4.02 | 3.10 | 3.59 | 3.74 |
| ADF-linked protein | 0.42 | 0.91 | 1.02 | 0.68 | 0.82 | 1.00 | 0.48 | 0.79 | 1.09 |
| Ash | 8.34 | 7.50 | 8.20 | 6.90 | 7.46 | 7.18 | 7.46 | 7.43 | 7.57 |

CON, control diet; 25 and 50 BSFLM, BSFLM groups in which the soybean meal was replaced with 25 and 50% BSFLM (black soldier fly larvae meal), respectively; ME, metabolizable energy; NDF, neutral detergent fiber; ADF, acid detergent fiber; (1) Supplied per kilogram of diet: vitamin A 1,600,000 IU; vitamin $D_3$ 300,000 IU; vitamin E 800 IU; vitamin $K_3$ 132 mg; vitamin B1 97 mg; vitamin B2 500 mg; vitamin B6 200 mg; vitamin B12 1.2 mg; nicotinic acid 2000 mg; pantothenic acid 800 mg; folic acid 60 mg; choline chloride 35,000 mg; Mn 12,000 mg; Zn 9000 mg; Fe 4000 mg; Cu 500 mg; I 250 mg; Co 100 mg; Se 50 mg.

*Growth performance*

Health status and mortality were recorded daily during the whole experimental period. The initial body weight (IBW) and the final body weight (FBW) were recorded on days 1 and 35. Daily feed intake (DFI), average daily gain (ADG), and feed conversion ratio (FCR) were determined for the overall experimental period (1 to 35 days)

*Slaughtering procedure*

At the end of the study (35 days), 12 animals (2 birds per pen) from each experimental group were selected based on the average final live weight in each pen and slaughtered by cutting the carotid artery. The head, feet, and abdominal fat were removed from the plucked and eviscerated carcasses, and the carcass weight was recorded. The breast and leg meats were separated into the right and left sides and individually vacuum-sealed. The right breast meats were refrigerated at 4 °C. The abdominal fat, left breasts, and leg meats were frozen at –20 °C until further analyses.

*Chemical composition and meat quality of breast meat*

The proximate composition of freeze-dried left breast meat samples was analyzed using AOAC methods [18]. Refrigerated right breast meats were used to measure meat quality. The breast meat color was measured at 2 different sites (CR-20; Konica Minolta, Ramsey, NJ, USA) to determine the lightness (L*), redness (a*), and yellowness (b*). The pH was measured at the same sites of the pectoralis major muscle using a pH meter (AM-7; Nihonseiki Kaisha Ltd., Tokyo, Japan). The meat samples were then vacuum-sealed in a plastic bag and cooked in a water bath at 80 °C until the core temperature reached 75 °C, as described by Kim et al. [27]. The samples were cooled and dried before weighing to calculate the water-holding capacity (WHC, cooking loss). Using cooked meat core samples (diameter 0.5 inches), shear force was assessed with a Warner-Bratzler shear machine (Model 4465, Instron Corp., MA, USA).

*Fatty acid profiles of insect meal, soybean meal, and leg meat*

Lipid was extracted from the samples using a solvent mixture of chloroform: methanol (2:1) as described by Folch et al.[116]. FA methyl esters were separated using a gas chromatography apparatus (Star 3600; Varian Technologies, Palo Alto, CA, USA) equipped with an Omegawax 205 fused-silica bond capillary column (30 m × 0.32 mm × 0.25 μm film thickness) from the transmethylated samples using a methanolic solution of $H_2SO_4$ (4%). The oven temperature was held at 50 °C for 1 min and then raised to 200 °C at a rate of 25 °C/min. Aliquot of 2 μL of the samples were injected into the injection ports, and the temperatures of the injector and detector were 250 and 260 °C, respectively. Nitrogen was used as the carrier gas at a flow rate of 1 mL/min. The FA composition of the samples was expressed as the percentage (%) of the total detected FA methyl esters.

*Heavy metal analysis*

The BSFLM and leg meat samples (0.5 g) were mixed with nitric acid and hydrogen peroxide for microwave digestion. The resulting solutions were filtered with filter paper and transferred to acid-cleaned tubes. These solutions were diluted with deionized water. The concentrations of heavy metals in the samples were determined by inductively coupled plasma-mass spectrometry (Agilent 7700x; Agilent Technologies, Santa Clara, CA, USA).

*Statistical analysis*

Statistical analysis of the data was performed using the GLM procedure in SAS version 9.4 (SAS, Inc., Cary, NC, USA). Differences among treatments were determined by Tukey's multiple comparison test. An individual bird was considered as an experimental unit for statistical analysis (n = 12 per treatment; two birds per pen). The results are presented as the mean ± standard error of the means. Significance and tendency were defined as $p < 0.05$ and $0.05 < p < 0.10$, respectively.

## 3. Results and discussions

*Growth performance*

The DFI and ADG of the 50% and 100% treatment groups were significantly lower than those of the control group, and they tended to decrease linearly in proportion to the benefit ratio of BSFLM (Table 14). Based on the chitin content in the experimental feed, the decrease in ADG may be associated with the high chitin content in BSFLM. Moula and Detilleux [117] suggested that ADG decreased in proportion to the feed level of insect meal through meta-analysis, which might be a nutrient imbalance, chitin content and morphological changes in intestinal villi. However, they reported that BSFLM was regarded as suitable for poultry without nutrient imbalance. They also said that the cause of the decrease in ADG was due to the morphological change of the intestinal villi. Moniello et al. [118] reported the length of the intestinal villi was reduced in the high-level (7.3~14.6% as fed) BSFLM supplementation group. Moula et al. [119] observed that there was no reduction of intestinal villi in broilers fed low level (2% as fed) of BSFLM.

**Table 14.** Effect of dietary black soldier fly larvae meal (BSFLM) inclusion level on the growth performance of broiler chickens

| Items | Dietary treatment | | | SEM | *P*-value |
|---|---|---|---|---|---|
| | CON | 25BSFLM | 50BSFLM | | |
| IBW (g, d 1) | 42.7 | 43.2 | 42.8 | 0.26 | 0.401 |
| FBW (g, d 35) | 1700.3$^{a}$ | 1619.5$^{a}$ | 1368.8$^{b}$ | 36.01 | <0.001 |
| DFI (g/d) | 70.3$^{a}$ | 70.7$^{a}$ | 62.1$^{b}$ | 0.89 | <0.001 |
| ADG (g/d) | 47.4$^{a}$ | 45.0$^{a}$ | 37.9$^{b}$ | 1.03 | <0.001 |
| FCR (g/g) | 1.49 | 1.57 | 1.65 | 0.05 | 0.062 |

CON, control diet; 25BSFLM and 50BSFLM, BSFLM groups in which soybean meal was replaced with 25% and 50% of BSFLM, respectively; SEM, standard error of the mean; IBW, initial body weight; FBW, final body weight; DFI, average daily feed intake; ADG, average daily gain; FCR, feed conversion ratio.

$^{a,b}$ Values with different superscripts in the same row are significantly different ($P < 0.01$).

*Chemical composition and fatty acid profile of insect meal and soybean meal*

The chemical compositions of BSFLM and SBM are summarized in Table 15. BSFLM contained more crude protein compared to SBM. The FA profiles differed between groups, as shown in Table 16; BSFLM contains the SFA more than 50%, whereas the FA profile of SBM was composed of the PUFA more than half. However, the monounsaturated fatty acid (MUFA) content was similar between the BSFLM and SBM groups.

The FA profile in abdominal fat changed in line with the pattern of that in BSFLM, which contained higher levels of SFAs and lower levels of PUFAs than SBM. However, dietary BSFLM showed weaker effects on the FA profile of leg meat. Similarly, Schiavone et al [81] and Cullere et al. [84] showed that the FA profile of breast meat from broiler chicken and quail was affected by the high SFA content in BSFLM. In the present study, the unchanged FA composition in leg meat was attributed to the relatively lower SFA content in microwave-dried BSFLM when compared to the levels in other studies. Moreover, the FA composition of fat tissues (e.g., abdominal and subcutaneous) is more representative of the dietary FA composition than that in muscle tissue such as breast and leg muscles of broiler chickens [120]. Particularly, it was reported that broilers fed PUFA had lower abdominal fat levels, with more fat deposited in the muscle because of the different roles of SFA and PUFA in the body tissues [112]. This explains the significant difference in the FA composition of abdominal fat but not in leg meat. Loponte et al. [121] was reported that the FA profile of abdominal fat was not changed in broilers fed Tenebrio molitor larvae meal. The different FA concentrations of the Tenebrio molitor and BSF can be attributed to the conflicting result; UFA concentrations are higher in mealworms than BSFL [93].

**Table 15.** The chemical composition (%) of black soldier fly larvae meal (BSFLM) and soybean meal.

| Item | BSFLM | Soybean meal |
|---|---|---|
| Dry matter | 98.53 | 87.95 |
| Crude protein | 61.24 | 45.76 |
| Ether extract | 6.16 | 2.23 |
| NDF | 20.34 | 10.14 |
| ADF | 11.24 | 9.50 |
| ADF-linked protein | 5.54 | 3.46 |
| Ash | 14.98 | 5.52 |
| Chitin [(1)] | 5.70 | - |

NDF, neutral detergent fiber; ADF, acid detergent fiber; [(1)] Calculated data.

**Table 16.** Fatty acid profile (% of total fatty acid methyl esters) of black soldier fly larvae meal (BSFLM) and soybean meal.

| Item | BSFLM | Soybean meal |
|---|---|---|
| Fatty acids | | |
| C10:0 | 1.62 | 0.02 |
| C12:0 (Lauric) | 29.69 | 0.15 |
| C14:0 (Myristic) | 4.39 | 0.46 |
| C16:0 (Palmitic) | 15.85 | 15.87 |
| C17:0 | 0.23 | 0.18 |
| C18:0 (Stearic) | 3.37 | 6.21 |
| Total SFA | 55.15 | 22.89 |
| C14:1 | 0.10 | 0.06 |
| C16:1 *n*-7 (Palmitoleic) | 2.67 | 0.69 |
| C17:1 | 0.23 | 0.09 |
| C18:1 *n*-9 (Oleic) | 19.67 | 22.39 |
| C20:1 *n*-9 (Eiocosenoic) | 0.20 | 0.21 |
| Total MUFA | 22.87 | 23.44 |
| C18:2 *n*-6 (Linoleic) | 15.74 | 46.19 |
| C18:3 *n*-3 (α-Linolenic) | 2.58 | 5.35 |
| C20:2 *n*-6 | 0.20 | 0.19 |
| C20:3 *n*-3 | 0.03 | 0.03 |
| C20:4 *n*-6 (Arachidonic) | 0.28 | 0.14 |
| Total PUFA | 18.80 | 51.87 |
| UFA/SFA | 0.76 | 3.29 |
| *n*-6 | 16.22 | 46.52 |
| *n*-3 | 2.61 | 5.38 |
| *n*-6/*n*-3 | 6.21 | 8.65 |

SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; UFA, unsaturated fatty acid.

*Carcass characteristics, quality parameters, and chemical composition of breast meat*

As presented in Table 17, the live weight was significantly decreased ($p < 0.001$) in the 50 BSFLM group compared to the CON and 25 BSFLM groups. However, no significant difference was observed in the live weight between the CON and 25 BSFLM groups. The carcass weight and percentage of the carcass (% live weight) in the 50 BSFLM group were significantly lower ($p < 0.001$) than in the other groups.

The decreased live and carcass weights of broilers in the 50 BSFLM group could be attributed to the chitin in the exoskeleton of BSFL [113]. It was reported that chitin can negatively affect protein digestibility [115]. Acid detergent fiber (ADF) fractions were analyzed to estimate the chitin contents in the BSFLM and the diets. The amount of the chitin in the BSFLM used as feed in the present study was 5.70%, calculated as follows: chitin (%) = ash free ADF (%) − ADF-linked protein (%), according to Marono et al. [115]. In the present trial, average daily feed intakes in the CON, 25 BSFLM, and 50 BSFLM groups were 70.30, 70.69, and 62.07 g/d, respectively. Therefore, the 25 and 50 BSFLM groups ingested approximately 0.28 and 0.50 g/d of chitin, respectively, considering the inclusion levels of BSFLM in the diets. According to Bovera et al. [122], laying hens (sixteen weeks old) that ingested approximately 0.47 g/d of chitin had lower protein digestibility than those fed on SBM-based diets, and it linearly decreased with an increase in inclusion level of insect meal in the diet. Chitin, which cannot be digested, and the microwave drying process of BSFLM may explain these lowered carcass traits [113,122]. However, the 25 BSFLM group showed satisfactory results for the carcass weight and rate compared to the 50 BSFLM group.

This result agrees with that of Schiavone et al. [81]. study showing that the carcass weight was linearly decreased with the inclusion levels (5, 10, and 15%) of BSFLM and drastically decreased by high inclusion level (15%) of BSFLM. Therefore, including a low level of BSFLM in the broiler diet may maintain the carcass traits.

**Table 17.** Effect of dietary black soldier fly larvae meal (BSFLM) inclusion level on the carcass and breast meat traits of broiler chickens.

| Item | Dietary Treatment | | | SEM | *p*-Value |
|---|---|---|---|---|---|
| | CON | 25 BSFLM | 50 BSFLM | | |
| Live weight (LW), kg | $1.80^{a}$ | $1.76^{a}$ | $1.49^{b}$ | 0.04 | <0.001 |
| Carcass weight, kg | $1.27^{a}$ | $1.25^{a}$ | $1.03^{b}$ | 0.03 | 0.0003 |
| Carcass weight, % LW | $70.67^{a}$ | $71.16^{a}$ | $68.91^{b}$ | 0.53 | 0.0003 |
| pH | $5.75^{b}$ | $5.77^{ab}$ | $5.87^{a}$ | 0.03 | 0.0409 |
| Color | | | | | |
| Lightness, L* | 54.79 | 55.52 | 55.21 | 1.03 | 0.8815 |
| Redness, a* | $3.52^{b}$ | $5.16^{a}$ | $5.13^{a}$ | 0.34 | 0.0023 |
| Yellowness, b* | 8.11 | 8.87 | 8.72 | 0.78 | 0.7661 |
| Cooking loss, % | 23.71 | 23.15 | 22.60 | 0.74 | 0.6207 |
| WHC, % | $60.39^{ab}$ | $60.05^{b}$ | $62.24^{a}$ | 0.57 | 0.0422 |
| Shear force, kg/0.5 inch$^2$ | 2.48 | 2.42 | 2.34 | 0.19 | 0.9017 |
| Moisture, % | 75.75 | 75.85 | 76.27 | 0.25 | 0.3796 |
| Protein, % | 22.43 | 22.27 | 22.21 | 0.24 | 0.8225 |
| Lipid, % | 0.95 | 1.16 | 0.87 | 0.11 | 0.2024 |
| Ash, % | 1.09 | 1.08 | 1.02 | 0.02 | 0.1335 |

CON, control diet; 25 and 50 BSFLM, BSFLM groups in which the soybean meal was replaced with 25 and 50% BSFLM, respectively; SEM, standard error of the means; WHC, water-holding capacity; $^{a,b}$ Values with different superscripts in the same row are significantly different ($p < 0.05$).

No significant differences were found in meat quality parameters, except for the pH, redness (a*) value, and WHC (Table 17). Dietary BSFLM supplementation increased ($p < 0.05$) the pH, redness, and WHC but did not significantly influence the chemical composition of the breast meat. In general, the pH of meat is correlated with the WHC, and changes in the pH of breast meat in broilers fed BSFLM were related to the carcass weight. Therefore, the increased WHC was influenced by the meat pH [123]. Additionally, the pH of meat was negatively correlated with carcass weight, and the decreased carcass weight of broilers fed BSFLM affected the pH of breast meat [124,125]. Although the pH of breast meat was increased by dietary BSFLM, the pH and L* values of breast meat were in normal ranges [126,127]. Bovera et al. [122] also reported that the use of Tenebrio molitor larvae meal in a broiler diet increased the breast meat pH, whereas inclusion of BSFLM did not affect the pH regardless of the carcass weight loss [81]. Additionally, the substitution of BSFLM in a broiler quail diet decreased the pH in breast meat without negatively affecting the carcass weight and dressing percentage [84]. They suggested that the difference in pH occurred because of differences in the muscle glycogen content. These conflicting results for the pH of breast meat in broilers fed insect meal should be interpreted with caution.

According to the meat quality parameters, BSFLM inclusion had a satisfactory effect on the chicken breast meat. The increase in the pH and WHC did not affect cooking loss or shear force. This result indicates that chicken breast meat from broilers fed BSFLM can contribute to consumer acceptance of the meat [125]. However, when BSFLM was included in the broiler diets, a noticeable increase in redness was observed, which agrees with a previous study [81].

They also showed some conflicting results; yellowness was decreased by dietary BSFLM. In contrast, redness was decreased and yellowness was not affected in the breast meat of broiler quails [84]. The reason for the higher level of redness in breast meat in the 25 and 50 BSFLM groups compared with those in the CON group are unclear, although different species and breeds may affect the meat quality [128]. Additionally, a pigment derived from BSFLM and BSFLO may have affected the meat color and egg yolk color [81,90].

*Fatty acid profile of abdominal fat and leg meat*

The effects of the experimental diets on the FA profile of abdominal fat are presented in Table 18. The proportion of SFAs, such as myristic and palmitic acid, was significantly increased, whereas PUFAs, such as linoleic, α-linolenic, and arachidonic acid, were decreased with increasing BSFLM levels ($p < 0.01$). The total MUFA content was also increased ($p < 0.0001$) by dietary BSFLM supplementation. A similar pattern was observed in the FA profile of leg meat (Table 19). However, dietary BSFLM supplementation did not alter the FA profile of the leg meat, contrasting the results for abdominal fat.

**Table 18.** Effect of dietary black soldier fly larvae meal (BSFLM) inclusion level on the fatty acid profile in abdominal fat of broiler chickens.

| Item | Dietary Treatment | | | SEM | *p*-Value |
| --- | --- | --- | --- | --- | --- |
| | CON | 25 BSFLM | 50 BSFLM | | |
| Fatty acids | | | | | |
| C14:0 (Myristic) | 0.43 [c] | 0.68 [b] | 0.85 [a] | 0.03 | <0.0001 |
| C16:0 (Palmitic) | 20.84 [c] | 23.25 [b] | 25.44 [a] | 0.53 | <0.0001 |
| C18:0 (Stearic) | 7.31 | 6.79 | 6.53 | 0.25 | 0.1133 |
| Total SFA | 28.57 [c] | 30.72 [b] | 32.83 [a] | 0.54 | 0.0002 |
| C16:1 *n*-7 (Palmitoleic) | 4.12 [c] | 5.46 [b] | 6.88 [a] | 0.35 | 0.0002 |
| C18:1 *n*-9 (Oleic) | 38.57 [b] | 39.37 [b] | 42.44 [a] | 0.52 | 0.0002 |
| C20:1 *n*-9 (Eicosenoic) | 0.38 | 0.36 | 0.38 | 0.01 | 0.2764 |
| Total MUFA | 43.06 [b] | 45.19 [b] | 49.70 [a] | 0.76 | <0.0001 |
| C18:2 *n*-6 (Linoleic) | 26.28 [a] | 22.28 [b] | 16.27 [c] | 0.98 | <0.0001 |
| C18:3 *n*-6 (γ-Linolenic) | 0.21 | 0.20 | 0.17 | 0.01 | 0.1820 |
| C18:3 *n*-3 (α-Linolenic) | 1.61 [a] | 1.43 [a] | 0.89 [b] | 0.07 | <0.0001 |
| C20:4 *n*-6 (Arachidonic) | 0.26 [a] | 0.18 [ab] | 0.14 [b] | 0.02 | 0.0047 |
| Total PUFA | 28.36 [a] | 24.09 [b] | 17.47 [c] | 1.07 | <0.0001 |
| UFA/SFA | 2.50 [a] | 2.26 [b] | 2.05 [c] | 0.06 | 0.0001 |
| *n*-6 | 26.75 [a] | 22.66 [b] | 16.58 [c] | 1.00 | <0.0001 |
| *n*-3 | 1.61 [a] | 1.43 [a] | 0.89 [b] | 0.07 | <0.0001 |
| *n*-6/*n*-3 | 16.63 [b] | 16.00 [b] | 18.55 [a] | 0.26 | <0.0001 |

CON, control diet; 25 and 50 BSFLM, BSFLM groups in which the soybean meal was replaced with 25 and 50% BSFLM, respectively; SEM, standard error of the means; SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; UFA, unsaturated fatty acid; [a,b,c] Values with different superscripts in the same row are significantly different ($p < 0.01$).

**Table 19.** Effect of dietary black soldier fly larvae meal (BSFLM) inclusion level on the fatty acid profile in leg meat of broiler chickens.

| Item | Dietary Treatment | | | SEM | *p*-Value |
|---|---|---|---|---|---|
| | CON | 25 BSFLM | 50 BSFLM | | |
| Fatty acids | | | | | |
| C12:0 (Lauric) | 0.63 | 0.89 | 0.88 | 0.16 | 0.470 |
| C14:0 (Myristic) | 0.67 | 0.77 | 0.77 | 0.06 | 0.463 |
| C16:0 (Palmitic) | 22.09 | 23.56 | 23.48 | 0.65 | 0.229 |
| C18:0 (Stearic) | 7.19 | 6.98 | 7.41 | 0.24 | 0.496 |
| Total SFA | 30.59 | 32.20 | 32.54 | 0.72 | 0.164 |
| C16:1 *n*-7 (Palmitoleic) | 4.75 | 5.30 | 5.33 | 0.41 | 0.549 |
| C18:1 *n*-9 (Oleic) | 36.23 | 36.35 | 36.68 | 0.67 | 0.904 |
| C20:1 *n*-9 (Eicosenoic) | 1.69 | 1.44 | 1.33 | 0.10 | 0.076 |
| Total MUFA | 42.67 | 43.09 | 43.35 | 0.97 | 0.895 |
| C18:2 *n*-6 (Linoleic) | 23.23 | 21.50 | 20.60 | 1.22 | 0.355 |
| C18:3 *n*-6 (γ-Linolenic) | 0.19 | 0.20 | 0.19 | 0.01 | 0.729 |
| C18:3 *n*-3 (α-Linolenic) | 0.27 | 0.26 | 0.31 | 0.01 | 0.081 |
| C20:4 *n*-6 (Arachidonic) | 0.73 | 0.70 | 0.75 | 0.12 | 0.951 |
| C22:6 *n*-3 (Docosahexaenoic) | 0.10 | 0.10 | 0.09 | 0.02 | 0.883 |
| Total PUFA | 24.52 | 22.73 | 21.94 | 1.32 | 0.413 |
| UFA/SFA | 2.22 | 2.05 | 2.03 | 0.07 | 0.121 |
| *n*-6 | 24.15 | 22.40 | 21.54 | 1.30 | 0.404 |
| *n*-3 | 0.37 | 0.36 | 0.39 | 0.28 | 0.726 |
| *n*-6/*n*-3 | 64.86 | 63.49 | 60.40 | 4.07 | 0.772 |

CON, control diet; 25 and 50 BSFLM, BSFLM groups in which the soybean meal was replaced with 25 and 50% BSFLM, respectively; SEM, standard error of the means; SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; UFA, unsaturated fatty acid.

*Essential elements and undesirable substances in insect meal and leg meat*

The concentrations of essential elements and undesirable substances in the BSFLM and leg meat are summarized in Tables 20 and 21. The concentrations of undesirable substances, such as fluorine, arsenic, lead, mercury, and cadmium, in the BSFLM, were below the maximum permissible levels for animal feeds according to the standards reported by the European Commission [129]. The concentrations of lead and cadmium were also under the maximum permissible levels for chicken meats [130]. Dietary BSFLM increased the concentrations of selenium and zinc in the leg meat, for which maximum permissible levels did not apply.

The safety of chicken leg meat from broilers fed BSFLM must be verified to determine its heavy metal concentrations, ensuring that healthy chickens were provided to consumers and to improve consumer perception. Heavy metals accumulated in all development stages (e.g., larvae, prepupae, and adults); heavy metal concentrations in the larvae were increased with metal concentrations in the food [131]. The concentrations of hazardous heavy metals such as fluorine, arsenic, lead, mercury, and cadmium in BSFLM were below the European Union limits [129]. Moniello et al. [118] also reported concentrations of the toxic elements in insect meal and experimental diets in which SBM was replaced with insect meal (inclusion level in the diets: 7.3 and 14.6%, respectively). The concentrations of the toxic elements in the microwave-dried BSFLM were comparable or lower than those in a previous study [132], demonstrating that the concentrations of the toxic elements were negligible [132]. My results also suggest that the microwave-dried BSFLM is appropriate as an animal feed ingredient with regard to safety. Moreover, the concentrations of lead and cadmium in the leg meat did not exceed allowable levels [130]. In this study, the contents of zinc and sulfur in leg meat were increased by dietary BSFLM but the level of the zinc was in a normal range and not defined by the European Union [132]; the allowable level of sulfur is also not legislated [130]. Sulfur is generally provided by sulfur-containing amino acids (e.g., methionine and cysteine) in humans, and most people may not ingest sufficient amounts of sulfur [133]. These results suggest that BSFL fed food waste was not contaminated, with no negative effects on chicken meat from broilers fed BSFLM.

**Table 20.** Essential elements and undesirable substances (mg/kg) in black soldier fly larvae meal (BSFLM)

| Item | BSFLM | Maximum permissible level [129] |
|---|---|---|
| Essential elements | | |
| Mg | 5600 | NA |
| S | 562.24 | NA |
| Essential trace elements | | |
| Fe | 560.90 | NA |
| Cu | 456.34 | NA |
| Zn | 142.15 | NA |
| I | ND | NA |
| Cr | 59.06 | NA |
| Co | 4.51 | NA |
| Se | 0.48 | NA |
| Mn | 101.08 | NA |
| Undesirable substances | | |
| Al | 3.25 | NA |
| F | 0.01 | 150 |
| As | 0.01 | 2 |
| Pb | 0.01 | 10 |
| Hg | <0.01 | 0.1 |
| Cd | ND | 2 |

EC, European Commission; NA, not applicable; ND, not detected.

**Table 21.** Essential elements and undesirable substances (mg/kg) in leg meat of broiler chickens fed black soldier fly larvae meal (BSFLM)

| Item | Dietary treatment | | | SEM | $p$-value | Maximum permissible level [129] |
|---|---|---|---|---|---|---|
| | CON | 25 BSFLM | 50 BSFLM | | | |
| Essential elements | | | | | | |
| Mg | 233.60 | 232.74 | 222.45 | 4.24 | 0.191 | NA |
| S | 20.73 [b] | 25.19 [b] | 36.53 [a] | 1.80 | <0.0001 | NA |
| Essential trace elements | | | | | | |
| Fe | 7.72 | 8.27 | 9.29 | 0.43 | 0.070 | NA |
| Cu | 0.49 | 0.32 | 0.10 | 0.13 | 0.160 | NA |
| Zn | 11.65 [b] | 12.00 [ab] | 13.43 [a] | 0.44 | 0.035 | NA |
| I | 0.06 | 0.05 | 0.07 | 0.01 | 0.131 | NA |
| Cr | <0.10 | <0.10 | <0.10 | - | - | NA |
| Co | <0.10 | <0.10 | <0.10 | - | - | NA |
| Se | <0.10 | <0.10 | <0.10 | - | - | NA |
| Mn | <0.10 | <0.10 | <0.10 | - | - | NA |
| Undesirable substances | | | | | | |
| Al | 6.48 | 4.45 | 2.62 | 1.65 | 0.319 | NA |
| F | 0.04 | 0.05 | 0.05 | 0.01 | 0.568 | NA |
| As | <0.01 | <0.01 | <0.01 | - | - | NA |
| Pb | <0.01 | <0.01 | <0.01 | - | - | 0.10 |
| Hg | <0.01 | <0.01 | <0.01 | - | - | NA |
| Cd | <0.01 | <0.01 | <0.01 | - | - | 0.05 |

CON, control diet; 25 and 50 BSFLM, BSFLM groups in which the soybean meal was replaced with 25 and 50% BSFLM, respectively; SEM, standard error of the means; EC, European Commission; NA, not applicable: [a,b] Values with different superscripts in the same row are significantly different ($p < 0.05$).

This study demonstrated the effects of microwave-dried BSFLM as a potential feed ingredient in broiler diets. The difference in the FA profiles of abdominal fat between dietary treatments was ascribed to the different FA profiles of BSFLM and SBM. The FA profile showed lower differences in chicken meat than in abdominal fat, and the breast meat showed good quality. Dietary BSFLM did not negatively affect the heavy metal contents in the chicken leg meat. This findings suggest that SBM can be substituted at less than 25% with BSFLM in the broiler diets in terms of carcass weight. Further studies are needed to examine the utilization efficiency and improve the manufacturing process of BSFLM.

Drying processes have extremely important effects on the nutritional value of food products. The microwave drying method is commonly used for food preservation and is suitable for insect processing in terms of microbial stabilization by effectively removing moisture [134]. However, the microwave drying method adversely affects the chemical composition of food by polymerizing protein particles to make them more compact with larger diameters [73]. Such chemical structural change can decrease digestibility by impairing dissolution by digestive enzymes when compared to the conventional drying methods [73,135]. Few studies have evaluated the effects of microwave-dried BSFLM on meat quality traits and safety. In the present study, we used a press-defatted BSFLM derived from larvae reared on food waste. The nutrient composition of BSFL depends on the substrates; however, the protein content of microwave-dried BSFLM was superior to that of SBM [45]. BSFLM and SBM showed opposite FA profiles; BSFLM was rich in SFA, whereas SBM was rich in PUFA. Thus, we predicted that dietary microwave-dried BSFLM would affect carcass traits and the FA composition of the abdominal fat and meat.

# IV. Overall conclusion

This study aimed to assess the potential of black soldier fly larvae-derived ingredients for broiler diets. Firstly, BSFLO could effectively supply broiler with energy instead of SBO and did not cause any detrimental effects on the growth performance of broilers. In addition, the lauric acid-rich BSFLO induced no significant shift in microbial diversity and composition in the cecum of broilers despite the antimicrobial activity of LA.

However, BSFLO could alter the fatty acid composition of breast meat toward lower ratio of UFA/SFA. This lowered ratio could be explained by the raised provision of MCFA (mainly LA) from BSFLO based on the other research results [62,64,65] although this study gave no data of LA ratio of total FAME in breast meat. In addition to the antimicrobial activity of LA, LA-reinforced meat may be advantageous for cardiovascular health of consumers by increasing serum HDL-cholesterol. The accumulation of LA in broiler meat has been also turned out by the earlier studies [62,64,65,136], , but its transfer into eggs was difficult because most of ingested LA were metabolized or elongated by laying hens [137]. Therefore, BSFLO as a reliable source of LA can be utilized to produce functional chicken meat, but not eggs. Besides, Borrelli et al. [138] suggested that BSFLM could significantly lower cholesterol in egg yolks.

Secondly, microwave-dried and press-defatted BSFLM was studied to replace SBM without any deleterious effects on broiler production. Unlike BSFLO, the nutritional bioavailability of BSFLM in broilers might be determined by a nutrient imbalance, antinutritional factors (chitin and phenoloxidase), and the induction of morphological changes in intestinal villi [139] and other various factors. The second experiment showed that the growth performance of broilers could be more reduced in inverse proportion to the inclusion level (7.5% and 15.0%) of BSFLM. Therefore, the lower inclusion level of BSFLM should be recommended depending on the quality of BSFLM. Moula et al. [119] recommended the inclusion level of 2% (as fed) in total amount of feed.

Although a major product in BSFL production is BSFLM rather than BSFLO, there are many challenges to overcome for more efficient use of BSFLM as a feed ingredient (Table 22). The ash content of BSFL was 5 times larger than the other insect species, such as *Tenebrio molitor*, *Alphitobius diaperinus* and so forth [140]. Such high ash contents might be due to the hypodermis of BSF secreting a deposit of $CaCO_3$ [141]. Tomberlin et al. [142] suggested that calcium is stored in forms of $CaCO_3$ in BSFL. The level of Ca is ten times higher than the amount of most other insects [143]. The Ca content variation of BSFL can be mainly ascribed to the nutritional composition of diet for BSFL [144]. The higher levels of ash and Ca in BSFL act as limiting factors in feed formulation.

**Table 22.** Factors affecting bioavailability of BSFL meal and its derivatives.

| Item | Major problems |
|---|---|
| Intrinsic properties | ✓ Deterioration during processing and storage according to chitin content in larvae and phenolic oxidase activity<br>✓ Ash content in larvae: BSFL has an exceptionally high ash content compared to other insects.<br>✓ Chitin contents, 6.7~9.6% of body weight on the dry matter basis |
| Nutritional changes of BSF over the growth stages | ✓ The difference in nutrient content and processing type of food waste: leftover food (wet/dry)<br>✓ Larvae or prepupae |
| Processing methods | ✓ Increased economic cost by increasing electricity consumption by applying the uniform microwave drying (MwD) method<br>✓ MwD can reduce the bioavailability of nutrients due to the increase in particle size by the polymerization of proteins.<br>✓ Endogenous phenoloxidase activity can induce melanization of larvae meal to reducing amino acid quality.<br>✓ Decrease of fat extraction efficiency by denaturation of protein after heat treatment |

The high chitin content of BSFL can lead to overestimating protein content and poor nutrient bioavailability through binding to other nutrients. Finke [47] reported that it was possible to use 6.25 to estimate crude protein content of insects with low chitin content as a nitrogen-to-protein conversion factor, the protein content in insects for edible feed tended to be overestimated [145]. Janssen et al. [140] proposed 4.76 for whole BSFL and 5.60 for protein extract from BSFL as a nitrogen-to-protein conversion factor. [115].

Most of Korean BSFLM producers adopted the dry milling methods for processing BSFL, especially a microwave-drying. But Huang et al. [73] stated that a microwave-drying process diminished *in vitro* amino acid digestibility values of BSFLP compared with a conventional air-drying method. This lowered digestibility was considered because the microwave drying method induces polymerization of protein particles, resulting in reduced surface area due to the increased particle size.

In addition, it has been recently reported that tyrosine in BSFL can be easily converted to melanin by endogenous phenoloxidase activity, thereby reducing the quality of amino acids [74]. Endogenous phenoloxidase can promote the melaninization proceeds during the growth, processing, and even cryopreservation of BSFL. This reaction can be inhibited by heat treatment like blanching at killing [146] or acid treatment during milling process [74].

Improving the rearing and processing methods of BSFL can reduce various anti-nutritional factors as presented above, thus enhance the bioavailability of BSFLM. Nutritional changes occur in the body of BSF during its life cycle. BSF larvae has an excellent adaptive capacity to their diets. This adaptability could help its survival but induce the inconsistent quality as feed ingredients. The primary substrate for BSFL is food waste in Korea, of which chemical composition is largely variable. The nutrient requirement of BSFL is not yet set [147]. The fluctuation of nutrient composition over all life stages of BSF has been reported [9]. The dry matter content of BSF reached the highest level at the early stage of prepupae (day 12), and its crude protein content decreased, and crude fat increased with the growth stage progressed. It is certain that more information about the relation between its growth and diets are needed to establish the nutrient requirements for each growth stage of BSF.

The killing and processing process is necessary to improve the nutrient availability of BSFL-derived ingredients as well as its shelf-life, and safety through sterilization. Blanching and freezing are widely adopted method for the inactivation of BSFL. Blanching could inhibit the melanization of proteins by phenoloxidase and the self-digestion by endogenous enzymes. Freezing could be a humane method to inactivate BSF and could not stop the enzymatic digestion of proteins and the modification of amino acids during the storage [146].

After the inactivation of BSFL/P, the critical process is the drying and defatting in insect meal production. Most, if not all, BSF producers in Korea adopted the microwave drying method. Huang et al. study [73] compared the nutritional values of the microwave-dried and conventional air-dried BSFL suggesting that microwave drying could worsen nutrient digestibility by reducing the contact area of BSFL particles with digestive enzymes with the polymerization of its particles.

Most research on BSFL processing is biased toward improving efficient defatting, not considering the physical properties and nutritional value of BSFL itself. Further studies are necessary to investigate the optimization of BSFL processing methods for improving the nutritional and functional values of BSFL-derived ingredients.

Our society became confronted and thus is under the pressure on how to feed the world, in the realization of further surge in human population, decreased land area for food production, a lack of water resources, and climate change. Although the livestock industry has played a beneficial role in re-cycling wasted food resources as feed ingredients, it has been criticized for its environmental pollution and greenhouse gas emission. One-third of food produced in the world-wide has been wasted before any consumption. FAO (2015) reported that this 'food loss' or 'food waste' was more remarkable in developed than undeveloped countries. The disposal of leftover food has been emerging as one of the most severe social issues in Korea as well.

Livestock can convert inedible feed materials into edible food ingredients such as meat and eggs. Globally, 86% of global livestock feed was estimated to be inedible materials on a dry matter basis [148]. Therefore, animal species are vital players in a circular economy. Insects belong to the kingdom *Animalia*, of which BSF has a critical role as an ecological part of decomposer and primary consumer at the same time. BSFL has a versatile ability to use diverse organic substances as nutrients under unhygienic conditions.

The nutrient composition in BSFL/P was adjustable depending on its ingested substrates and rearing conditions [10,149]. For instance, BSF could adjust its body composition (protein vs. fat) using various organic substrates based on the metabolically adapting function of its fat body [10]. Though these metabolic characteristics act as an advantage in disposing massive organic substrates, their inconsistent quality may conversely provide a disadvantage with the nutritional quality of BSF-derived feed ingredients in protein and lipid content [10,149]. Therefore, numerous factors affect the quality of BSF-derived feed materials for their expanded usage for large feed producers, and economic animal farmers.

BSF is an eco-friendly and economic insect species that can produce excellent quality nutrients by using organic waste. BSF can accumulate nutrients effectively for survival in a harsh environment until it becomes an adult. For this reason, BSFL/P include a high level of protein and fat in its body. These can provide simple nutrient for supplying energy or proteins, but also supply functional substances (e.g., AMPs, lauric acid, and chitins) having antimicrobial activities to animals.

But these properties can be easily changed depending on the feed management, and environmental conditions, and processing methods. Therefore, a holistic approach is needed to maximize multi-functional effects of BSFL/P-derived ingredients from the aspect of breeding, feeding, processing, and evaluations as a feed ingredient.

# V. Summary in Korean

세계 인구의 폭발적 증가와 함께 동물성 유래 식량수요가 증가함에 따라 안정적인 사료자원의 확보는 식량안보 차원에서 중요한 이슈가 되고 있다. 그러나 선진국에서는 오히려 식량의 생산 과정뿐만 아니라 유통과 소비 과정에서 다량의 식량자원이 낭비되고 있는 실정이다. 동애등에 유충은 버려지는 유기자원을 먹이원으로 이용하여 양질의 단백질과 지방을 체내에 축적할 수 있기 때문에 지속가능한 유기자원의 사료화 수단으로서 순환경제의 중요한 역할을 담당하는 생물자원으로 주목받고 있다. 동애등에 유충 유래 원료는 단백질과 지방 같은 다량 영양소를 공급할 수 있을 뿐만 아니라 생리활성을 갖는 물질을 제공할 수 있다. 그러나 동애등에 유충 유래 단백질과 지방을 원료사료로 효율적으로 이용하기 위해서는 대상 동물종에서 급여 효과에 대한 과학적 근거의 확보가 필요하다. 본 연구는 동애등에 유충 오일(BSFLO)과 탈지박(BSFLM)이 육계의 생산성과 육질, 그리고 장건강에 미치는 영향을 구명하기 위해 수행하였다.

첫 번째 급여시험에서는 대두유(SBO)를 BSFLO 로 부분 또는 전량 대체 했을 때 1~5 주령의 육계의 생산성, 계육의 지방산 조성 및 육질에 미치는 영향을 평가하였다. 1 일령 Ross 308 병아리 210 수를 공시하여 대조구, 대두유 50%

대체구 및 100% 대체구, 총 3 개의 처리구(10 반복, 반복당 7 수)에 무작위로 할당하였다. 사료 내 BSFLO 첨가는 육계 생산성, 다리육의 물리화학적 형질, 그리고 가슴육의 관능평가 결과에 영향을 주지 않았다. 그러나 근위의 상대적 중량(g/kg)은 BSFLO 의 SBO 대체수준에 비례하여 감소했다(대조구, 50BSFLO 및 100BSFLO 각각 14.9, 12.5, 및 13.0, $p < 0.05$). 총 지방산 함량 중 포화지방산 비율 (% of 총 FAME, fatty acid methyl ester)은 BSFLO 첨가수준에 비례하여 증가하였으며(대조구, 50BSFLO 및 100BSFLO 각각 27.2%, 27.6%, 및 28.7%; $p < 0.05$), 총 지방 중 단일포화지방산도 대조구, 50BSFLO 및 100BSFLO 각각 43.4%, 44.6% 및 48.6%로 대체수준에 비례하여 유의적으로 증가하였다 ($p < 0.001$). 대조적으로 다가불포화지방산 비율은 대체수준(0%, 50%, 100%)에 비례하여 각각 29.5%, 27.8%, 및 22.7%으로 감소하였다 ($p < 0.001$). 본 연구는 사료 내 BSFLO 를 이용한 SBO 대체는 육계의 생산성에 부정적 영향을 주지 않았으며, 이는 BSFLO 가 육계사료의 지방공급원으로 이용 가능함을 확인하였다.

두 번째 실험은 앞선 연구에서의 동일한 시험축에서 사료 중 BSFLO 가 맹장 내 미생물 조성에 미치는 영향을 조사하기 위하여 수행하였다. 시험기간 최종일 (35 일)에 시험구별 6 수(총 18 수)를 무작위로 선정하여 도계 하였고, 맹장 내 소화물을 채취하여 맹장 미생물 조성 분석에 이용하였다. 총 235,978 개의 유전자 서열을 확인하였으며 3 개 시험구에서 총 4,398 개의 operational taxonomic units 을

확인하였다. 문(phylum) 수준에서는 *Firmicutes* 가 세 개 처리구 모두에서 우점하고 있었으며, 속(genus) 수준에서는 *Faecalibacterium* 이 모든 처리구에서 우점하였다. BSFLO 군과 대조군 사이에 모든 속(genera)의 상대풍부도 간 유의적 차이는 관찰할 수 없었다. 그러나 대조구 대비 50 BSFLO 에서 *Erypsipelatoclostridium* 이 유의적으로 더 풍부하였다 ($p < 0.05$). 육계사료 내 BSFLO 에 의한 SBO 대체는 육계의 맹장 미생물에 부정적인 영향을 주지 않았다.

마지막 실험에서는 마이크로웨이브로 건조 후 탈지한 BSFLM 이 도체특성, 육질 및 복부 지방, 그리고 계육의 지방산 조성, 그리고 중금속 잔류량을 조사하기 위해 수행하였다. 총 126 수(처리구별 6 반복 및 펜당 7 수)의 수컷 병아리를 대조구와 대두박의 25% 대체구 및 50% 대체구에 각각 무작위로 할당하였고, 35 일째 되는 날 도계하고 도체중을 측정하였다. 계육 분석을 위하여 펜당 2 수씩 처리구별 12 수로부터 가슴육과 다리육을 채취하였다.

사료 내 BSFLM 수준이 증가할수록 복부지방의 포화지방산 함량이 증가하였고, 다가불포화지방산 함량은 유의적으로 감소하였다 ($p < 0.001$); 시험구 간 다리육의 지방산조성은 차이를 보이지 않았다. BSFLM 과 다리육 내 중금속 농도는 최대잔류허용수준 이하였다. 그러나 50BSFLM 군의 도체중은 유의적으로 감소하였다 ($p < 0.001$). 마이크로웨이브 건조 BSFLM 은 최대 25% 첨가수준까지

포함했을 때 도체특성, 육질 및 계육 내 지방산 조성과 중금속 잔류량에 부정적인 영향을 주지 않았다.

BSFLO는 대두유와 같은 기존 에너지 사료원을 육계의 생산성, 육질 및 건강에 부정적인 영향을 주지 않고 대체할 수 있었다. 반면 마이크로웨이브 건조 후 압착 탈지한 BSFLM 은 대두박을 완전히 대체할 수 없었으며, 따라서 원료사료로 BSFLM 을 사용하기 위해서는 이에 대한 사료가치가 선행되어야 할 것으로 보인다. 이상의 결과를 종합한 결과, 본 연구의 결과는 BSFLO 와 마이크로웨이브 건조 후 압착 탈지한 BSFLM 이 육계사료 내에서 대두유와 대두박과 같은 기존 원료사료를 대체할 수 있음을 증명하였다.

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