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REVIEW ARTICLE

Application of black solider fly larvae as alternative source of protein in poultry nutrition. A Review

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Abstract

This review gives an overview on black soldier fly larvae meal (BSFM) as substitute ingredients available to the chicken business, their sustainability, and strategies for promoting them as important protein sources. Traditional sources of protein for chicken feed include soy beans and canola meal. The cost of poultry feeds is largely comprised of energy and protein sources. This is so because in chicken feed, 25–40% of the feed is based on sources of protein, whereas 50–65% of the feed is based on energy. The best strategy to lower the cost of feed is to offer affordable resources or to locate and utilise alternative resources. Insects have attracted a lot of interest as alternative protein sources to replace traditional protein sources used in animal production because of the rising demand for feed protein sources and environmentally friendly production. The nutritional benefits of BSFM, which is distinguished by a balanced amino acid composition and a high concentration of protein, energy, mono- and poly-unsaturated fatty acids, have proven to be the most attractive to the feed industry. Previous studies have demonstrated that the dietary low inclusion of BSFM as an alternative to soybean oil has positive effects on energy availability, attributed to improving growth performance and meat quality in species of poultry. These encouraging results suggested that there would be a promising practical application of the BSFM in the formulation of poultry diets.

Keywords: Insects protein; Feed cost; Nutritive value; Performance; Poultry.

1. Introduction

The biggest issue facing the global farm animal sector is a lack of protein feed resources. For chickens, insects are a well-known alternate protein source. There are numerous insects that can be used in poultry diets. Insect larvae flourish in dung and organic waste. To protect themselves from microbial diseases, they produce antimicrobial peptides, which may also be useful in chicken feed. The growth efficiency, nutritional digestibility, intestinal health, and immunological function of chickens can all be enhanced by feeding them feed containing antimicrobial peptides. Compared to standard feedstuffs, insect meal contains a higher concentration of important amino acids. The most often utilised insect meals in broiler and laying hen diets include black army fly, mealworm, housefly, cricket/grasshopper/locust (Orthoptera), silkworm, and earthworm. In intensive poultry production systems, feed is the most important input and accounts for 60-80 % of total production costs (Paul *et al.*, 2017). Protein is one of the main compartments of poultry feed and is one of the major contributors

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to the finished feed costs. However, the increasing demand of poultry feeds has led to considerable increase in feedstuff prices. It has been predicted that traditional sources of protein for poultry will become scare and more expensive. Furthermore, beside the rising prices of feedstuff, the traditional and conventional protein sources in poultry feed such as soybean (SBM) and canola meal are failing to meet the increasing demand due to both sector and human population growth. It is possible to improve the productivity and sustainability of farming by using unconventional feed ingredients like insects, microalgae, or even food waste in animal production. Insects offer the potential to partially or completely replace current lipid feed sources. Furthermore, because of their suitable nutritional quality, insects have a potential as a feed ingredient (Veldkamp and Bosch, 2015). The ability of black soldier fly larvae meal (BSFM) to minimize the volume of organic waste disposed of in landfills makes them a potential substitute feed ingredient for chicken diets. In particular, its crude protein concentration (53%) and crude fat content (58%) on a DM basis make BSFM an appealing element in monogastric animal feed due to its nutritious profile (Ewald *et al.,* 2020; Ruhnke *et al.*, 2018; Spranghers *et al.,* 2017). Furthermore, the rearing environment of BSFM may be adjusted (e.g., temperature, substrate), which enables production with relatively low nutritional variability, amino acid variability, and fatty acid variability (Ewald *et al.,* 2020; Spranghers *et al.*, 2017). So that many countries which do not produce soybeans get to consider BSFM as alternative protein supplement to partially replace the SBM in poultry feed. To the best of our knowledge, no studies have yet been conducted to ascertain the effects of soybean oil replacement by BSFM larva on growth performances, carcass criteria, and histological features of broilers. Knowledge about the suitability of BSFM as poultry feed ingredient is currently limited. This research provides an overview of BSFM as possible substitute ingredients for the chicken industry, as well as information on their sustainability and strategies for promoting them as significant sources of protein.

2. Entomology and Distribution of Black Solider Fly (BSF)

The BSF is a true fly (Diptera), belonging to the Stratiomyidae family. Its expansion across all continents was aided by climatic change (leek, 2017). Because of this, the Black Solider Fly now occurs naturally across over 80% of the planet between latitudes 46 N and 42 S. (Martinez-Sanchez *et al.,* 2011). They are frequently regarded as one of the potential sources of protein because of their capacity to transform a sizable amount of organic waste (1.3 billion tons annually) into biomass that is rich in proteins (Veldkamp *et al.,* 2012). Live, sliced, dried, or crushed forms of black soldier fly larvae are all used. Only the adult BSF breeds, searches for mates, and lays 500–900 eggs in cracks near decomposing waste (Diener *et al.,* 2011). The adult fly has no mouthparts and does not even eat for the duration of its brief life. They exclusively eat as larvae, do not sting or bite, and are not linked to the spread of illness. Because the insect does not eat while it is an adult, its larvae are quite huge (220 mg), allowing them to store all the resources needed to nourish the adult (Makkar *et al.,* 2014). Depending on the size of the maggot and the type of food being consumed, the larvae can feed swiftly at a rate of 25 to 500 mg of fresh matter/larvae/day (Makkar *et al.,* 2014). The larvae crawl away from the trash during the final stage of development and towards a dark spot to pupate. In rearing facilities, this migratory behavior is used to self-collect (Diener *et al.,* 2011). BSF can tolerate weather extremes, but they thrive best in temperatures of 29 to 31 C and relative humidity levels of 50 to 70%. (Makkar *et al.,* 2014). In its life cycle, the BSF goes through five stages: egg, larvae, prepupae,

pupae, and adult. BSF is thought to have a 40-day life cycle, but this can vary depending on the habitat and the nourishment offered (Alvarez, 2012). It takes 4 days for eggs to hatch into larvae, which have a drab appearance and attempt to avoid light (Newton, 2015). The larvae consume food and organic waste for the majority of their lifespan, quickly converting it to fat, protein, and calcium. Lower temperatures are manageable since the larvae's feeding action and metabolism produce some heat, allowing them to develop in colder climates. At the ideal temperature, larvae will reach full size (20 to 25 mm) in about 4 weeks, but this period may vary depending on the species.

3. Nutritional profile BSFM

Rendering fat from the animal's body that is not intended for human consumption and crude vegetable oils make up the majority of the lipid sources utilised in poultry diets. Due to its high nutritional value, digestibility, and metabolizable energy content compared to other vegetable oils, soybean oil is used as an element in chicken diets (Fascina *et al.,* 2009). However, the current scarcity of soybeans and their high cost have sparked an increased interest in finding new substitute lipid sources for chicken feed. Insects offer the potential to partially or completely replace current lipid feed sources. Comparison of Black solider fly larvae with some insect's meal, soybean meal and fish meal were studied. In addition, insects have the potential to be used as a feed additive due to their suitable nutritional content and higher acceptance by chickens (Veldkamp and Bosch, 2015; Leiber *et al.,* 2017). In fact, wild birds and free-range poultry naturally ingest insects in their adult, larval, and pupal forms (Rumpold *et al.,* 2016). Most of the focus on insects as a feed source in recent years has focused on their protein content (Belforti *et al.,* 2015; Gasco *et al.,* 2016; Renna *et al.,* 2017). The use of fully or partially defatted larvae and the resulting differences in nutrients, as well as comparing treatment groups that were not developed based on the same concept, result in significant variations in the BSFM composition between experiments (Dabbou *et al.,* 2018; Onsongo *et al.,* 2018). The_amino acids composition of Black solider fly larvae meals versus conventional meal was also studied. BSFM has high amino acid profile, lipid content and calcium content (Cullere et al., 2016; Schiavone *et al.,* 2017). The BSFM contains up to 45% of lipids (Li *et al.,* 2016). The medium-chain fatty acids, which make up 21.4% to 49.3% of the total profile and include lauric_acid and its esters, are likewise abundant in the FA profile (Tran *et al.,* 2015; Ushakova *et al.,* 2016). The meal made from insect larvae is rich in crude protein, which ranges from 440 to 690 g/kg, and fat, which ranges from 230 to 470 g/kg (Veldkamp *et al.,* 2012). So, Insect has been demonstrated to be a suitable protein source for broiler chickens.

4. BSFM Economic efficiency and recommendations in poultry diets.

There are currently restrictions on how widely BSFM can be used in the poultry sector. Depending on the nation, the cost of the BSFM could not be comparable with that of traditional protein meals, and the scale needed to create BSFM at an affordable price necessitates significant modifications to the industry's current systems for producing the food. However, as previously mentioned, due to the abundance of natural resources and a weak currency for importing protein meal, BSFM is a desirable feed element for animal production systems in some countries. Before it is taken into consideration for many poultry diets, the indicated optimal inclusion level of 15% to 20% will need to be developed in many countries to reduce cost and increase volume of BSFM production. In the diet of broilers, black soldier fly meal may be used in place of fish meal, soybean meal, or even soybean oil. In the feed of domestic chickens, fish meal was successfully replaced by BSFM up to 15% (Manangkot *et al.,*

2014). Increased dressing percentage and protein deposition in meat were seen in Cobb 500 broiler chickens fed on a diet containing 33% BSFM (4% in diet) in place of fish meal (Mohammed, 2017). While diets containing 100% BSFM with inadequate methionine levels lowered feed intake, protein conversion ratio, and growth performance in Ross 308 broiler chickens, diets containing 50% or 100% BSFM with an optimum amino acid ratio as a replacement for soybean meal increased growth performance (Moula *et al.,* 2018). In place of soybean meal, Ross 308 broiler chickens performed better in terms of growth and CP deposition when fed a diet containing 75%/50% BSFM (75% for starter phase and 50% for grower phase), or 50% or 100% BSFM with extended amino acids supply (Neumann *et al.,* 2018). Furthermore, 50% BSFM and 100% BSFM with extended amino acids supply produced superior protein quality model parameter and net protein utilisation. Cobb 500 broiler chickens successfully ate BSFM at a rate of 15% [53], while Ross 308 male broiler chickens successfully switched from soybean oil to 100% BSF fat (Schiavone *et al.,* 2018). Better growth performance and higher gizzard weight were observed in Cobb 500 broiler chickens fed diets containing 5% BSFM as a replacement for soybean meal and fish meal; 10% BSFM increased meat quality; and 15% BSFM decreased cooked pectoral muscle aroma and taste, total feed cost, and increased gross profit margin (Onsongo *et al.,* 2018).

5. Effects of BSFM on growth performance

According to Sihem *et al.* (2018), replacing soybean meal in broiler diets with BSF (5%, 10%, and 15%, respectively) increased body weight (BW), body weight gain (BWG), and feed intake (FI) during the starting phase but may have a negative impact on the FCR and gut morphology. When 5%, 10%, 15%, and 20% of BSF larvae were substituted for soybean meal in broiler diets, significant gains were made in body weight, body weight gain, and feed intake; however, broilers who received 20% BSFM experienced a 10% decrease in FCR, and birds given 20% BSFM for 42 days may have experienced a reduction in immune response energy expenditure (De Souza Vilela *et al.,* 2021). In diets containing Tenebrio molitor meal at 23.7% for 32 days, improved FCR in broiler chickens from 4.1 to 3.6 (Bovera *et al.,* 2016). Similar to this, many studies have discovered that feeding insect meal to broilers had a good impact on their performance, including increased BW, BWG, or lower FCR in at least one phase of the trial (Dabbou *et al.,* 2018; Gariglio *et al.,* 2019; Khan *et al.,* 2018; Loponte *et al.,* 2017). When broilers were fed BSFM at various inclusion levels up to 15%, Onsongo et al. (2018) did not observe any effects of insect meals on feed intake, FCR, or According to numerous studies, BSFM is the best insect protein for improving the growth performance of broiler chickens. When given a diet containing 2.6% BSFM and an increased amino acid supply, Ross 308 broiler chickens' growth performance were enhanced (Neumann *et al.,* 2017). The feed efficiency of Cobb broiler chickens fed diets containing 5% BSFM was improved by, 7.5% than control and 10% BSFM still resulted in improved growth (Dahiru *et al.,* 2016). Diet containing 20% BSFM fed to Ross 308 male broiler chickens improved the meat quality by increasing concentrations of lauric acid, myristic acid, and eicosapentaenoic fatty acid; however, partly reduced the total PUFA (De Souza Vilela *et al.,* 2021). When Ross 308 broiler chickens were fed diets containing 5% or 10% BSFM, their growth performance improved; however, 15% BSFM in the diet reduced feed efficiency and caused an increase in crypt depth and a decrease in villus height, as well as an increase in the villus height to crypt depth ratio (Dabbou *et al.,* 2018).The amount of growth performance and the feed efficiency rose when Ross 308 broiler chicks were fed diets containing (3% or 6%) BSFM, but the weight

gain was decreased (Choi *et al.,* 2013). Improved feed efficiency, greater net protein consumption, and reduced body crude fat deposition were seen in Ross 308 male broiler chickens fed the diet containing 23% or 21% BSFM (Brede *et al.,* 2018). Better growth performance were observed in Cobb 500 broiler chickens fed diets containing 5% BSFM as a replacement for soybean meal and fish meal (Onsongo *et al.,* 2018). Increased growth performance was seen in Cobb 500 broiler chickens fed on a diet containing 33% BSFM (4% in diet) in place of fish meal (Mohammed, 2017). While diets containing 100% BSFM with inadequate methionine levels lowered feed intake, and growth performance in Ross 308 broiler chickens, diets containing 50% or 100% BSFM with an optimum amino acid ratio as a replacement for soybean meal increased growth performance (Moula *et al.,* 2018). In place of soybean meal, diets containing 75%/50% BSFM (75% for starter phase and 50% for grower phase), or 50% or 100% BSFM with extended amino acids supply, or both, resulted in improved growth and CP deposition in Ross 308 broiler chickens (Neumann *et al.,* 2018). Additionally, the net protein utilisation and higher protein quality model parameter were obtained by the 50% and 100% BSFM with extended amino acids supply. While Ross 308 male broiler chickens successfully converted from soybean oil to 100% BSF fat, Cobb 500 broiler chickens successfully ate BSFM at a rate of 15% (Schiavone *et al.,* 2018).

6. Effects of BSFM on Nutrient digestibility

To increase the sustainability of chicken diets, insects may be used in poultry feed. There are numerous insects that can be used in poultry diets. Compared to standard feedstuffs, insect meal includes more important amino acids (Al-qazzaz and Ismail, 2016). For poultry diets, the insects can be employed in live (fresh), dried, and paste form (Khan, 2018; Elahi *et al.,* 2020). Due to the water content in fresh or live insects, which increases decomposition, antibacterial activity, and the Millard response, a dried insect is thought to be acceptable for chicken diets (Elahi et al., 2020). When high quantities of defatted BSFM (17%) were added to broiler diets, observed a decline in both DM and CP digestibility (Bovera *et al.,* 2016; Cutrignelli *et al.,* 2018). In contrast, when smaller levels (up to 9%) of insect meal of BSFM were introduced to the diets of broilers, showed no effect on nutrient digestibility (Benzertiha *et al.,* 2019; Gariglio *et al.,* 2019). Khempaka *et al.* (2006) showed a decrease in DM digestibility only when 8%, 12%, and 16% of shrimp meal were added to broiler diets.
De Souza Vilela *et al.* (2021) reported that replacement of soybean meal by 5%, 10%, 15%, and 20% of black solider fly larvae meal in broiler diet had no effect on DM, crude protein, crude ash and calcium or phosphorus digestibility, however, there was an increase in crude fat digestibility. Another study estimated that BSF larvae meal for the broiler meals are excellent sources of the total tract apparent digestibility of crude protein (51%), ether extract (99%), gross energy (69%), dry matter (53%) and (66%) organic matter of (De Marco *et al.,* 2015). A strong source of protein and energy, black soldier fly meal (BSFM) is also full of essential and optional amino acids, saturated, monounsaturated, and polyunsaturated fatty acids (PUFA), vitamins, and minerals (Chu *et al.,* 2020; Kawasaki *et al.,* 2019; Nyakeri *et al.,* 2017). In BSFM, the proportion of crude protein (CP) varied from 35% to 61% and crude fat content ranges from 7% and 42% of BSFM's weight (Chu *et al.,* 2020; Mohammed *et al.,* 2017; Liu *et al.,* 2021). Numerous research revealed that BSFM is the best insect protein for enhancing broiler chicken growth performance, carcass composition, and meat quality. The growth performance and nutrient digestibility of Ross 308 broiler chickens were improved in the starting phase by feeding them a diet containing 2.6% BSFM and an extended amino acid supply

(Neumann *et al.,* 2017). Cobb broiler chickens fed diets containing 5% BSFM exhibited greater feed efficiency, 7.5% BSFM led to superior growth (Dahiru *et al.,* 2016). When diets containing (3% or 6%) BSFM were provided to Ross 308 broiler chicks, the amount of feed efficiency, and nutrient digestibility increased (Choi *et al.,* 2013). When fed a diet containing 23%/21% BSFM (starter/grower phase), male Ross 308 broiler chickens showed improved feed efficiency and nutrient digestibility (Brede *et al.,* 2018). Increased nutrient digestibility of Cobb 500 broiler chickens were observed in fed on a diet containing 33% BSFM (4% in diet) in place of fish meal (Mohammed, 2017). While diets containing 100% BSFM with inadequate methionine levels lowered feed intake, protein conversion ratio in Ross 308 broiler chickens, diets containing 50% or 100% BSFM with an optimum amino acid ratio as a replacement for soybean meal increased nutrient digestibility (Moula *et al.,* 2018). When fed a diet comprising 75%/50% BSFM (75% for starter phase and 50% for grower phase) or 50% or 100% BSFM with prolonged amino acids supply in place of soybean meal, Ross 308 broiler chickens fared better in terms of CP digestibility (Neumann *et al.,* 2018). A better protein quality model parameter and net protein utilisation were also achieved by 50% and 100% BSFM with extended amino acids supply. As opposed to Ross 308 male broiler chickens, Cobb 500 broiler chicks successfully converted from soybean oil to 100% BSF fat at a rate of 15% (Schiavone *et al.,* 2018). In comparison to hens fed a 25% MWM diet, Ross 708 broilers fed BSFM had a higher coefficient of total track apparent digestibility for ether extract (De Marco *et al.,* 2015). Increased proportions of saturated fatty acids and decreased proportions of polyunsaturated fatty acids (PUFAs) were observed in breast meat from diets containing 100% BSFM in place of soybean oil, but growth performance, haematological parameters, carcass quality, and meat quality were unaffected (Schiavone *et al.,* 2018). Ross 708 broiler chickens' breast meat had less cholesterol when black soldier fly fat was used to replace 50% of the soybean oil; however, 100% BSF fat increased the overall amount of saturated fatty acids and decreased the MUFA and PUFA in the breast and leg meat (Cullere *et al.,* 2019). In comparison to birds fed 2.5% fully defatted BSFM, Ross 308 male broiler chickens fed the same diet showed higher crude fat digestibility and apparent metabolizable energy (Schiavone *et al.,* 2017), BSFM was a part of Ross 708's diet. The ability to develop suitable insect-based diets for broilers is, however, constrained by the lack of knowledge currently available about insect digestibility in chicken.

7. Effects of BSFM on carcass criteria

Cullere *et al.* (2016) demonstrated that diets containing 10% and 15% BSF larvae meal as a substitute for SBM and soybean oil had no negative effect on carcass weight, the weight of breast muscles or their proportion in total carcass weight in growing broiler quails. In a study by (Bovera *et al.,* 2016), carcass traits were not adversely affected by the complete replacement of SBM with BSF larvae meal in broiler chicken diets (from 30 to 62 d of age). It has been observed that feeding a diet with 8% fresh BSF larvae meal to local chickens from 30 t0 80 days old did not affect the carcass yield, pectoral muscles and thigh weight (Moula *et al.,* 2018). According to numerous studies, BSFM is the best insect protein for improving the growth performance, carcass composition, and meat quality of broiler chickens. When given a diet containing 2.6% BSFM and an increased amino acid supply, Ross 308 broiler chickens' growth performance and nutritional digestibility were enhanced (Neumann *et al.,* 2017). The feed efficiency of Cobb broiler chickens fed diets containing 5% BSFM was higher, 7.5% BSFM increased the weight of the thighs and decreased the pH of the meat, and 10% BSFM still resulted in improved growth (Dahiru *et al.,* 2016).Broiler

chickens fed diets containing 5% BSFM had less abdominal fat, 10% BSFM had more carcass weight and breast percentage, and 15% BSFM had more body weight, abdominal fat, meat redness, meat protein, monounsaturated fatty acids (MUFA), and less polyunsaturated fatty acids (PUFA) in the breast meat (Dahiru *et al.,* 2016).Better higher gizzard weight were observed in Cobb 500 broiler chickens fed diets containing 5% BSFM as a replacement for soybean meal and fish meal; 10% BSFM increased breast weight and overall acceptability of cooked pectoral muscle; and 15% BSFM decreased cooked pectoral muscle aroma and taste, total feed cost, and increased gross profit margin (Onsongo *et al.,* 2018). Breast meat from diets using 100% BSFM in place of soybean oil showed increased proportions of saturated fatty acids and lower proportions of polyunsaturated fatty acids (PUFAs), but growth performance, haematological parameters, carcass quality, and meat quality were unaltered (Schiavone *et al.,* 2018). Black soldier fly fat reduced the cholesterol in the breast meat of Ross 708 broiler chickens when it was substituted for 50% of the soybean oil; however, 100% BSF fat increased the overall quantity of saturated fatty acids in the breast and leg meat (Cullere *et al.,* 2019). Ross 308 male broiler chickens fed the same diet as birds fed 2.5% completely defatted BSFM displayed increased crude fat digestibility and apparent metabolizable energy (Schiavone *et al.,* 2017).

8. Effects of BSF on blood parameters

The blood parameters provide details about the health of the birds. The broiler chickens were kept in physiologically appropriate conditions, confirming that neither BSF meal nor BSF fat had any impact on the health of the poultry. Dabbou *et al.* (2018) reported that replacement of soybean meal by 5%, 10% and 15% BSF larvae meal in broiler diet no significant effects were observed on blood serum parameters, except for phosphorus concentration. Increasing the level of BSF dietary meal increased the level of P, with the highest level at 10% dietary BSF larvae meal in comparison to other groups, although no effects were observed on Ca and other blood and serum parameters. However, these findings are not consistent with several other studies that did not notice any significant changes in serum level of Fe and Mg in broiler diets that were fed BSF larvae meal (Marono *et al.,* 2017; Loponte *et al.,* 2017). Serum AST and ALT activities are typically associated with liver injury, and when they rise, they serve as signs of liver necrosis (Hyder *et al.,* 2013). The lack of an impact on ALT or AST activity indicates that BSF fat may not have a deleterious impact on the health of the hepatopancreas or liver. The current study's findings concur with those of Li *et al.* (2016), who demonstrated that using HI larva fat in place of soybean oil in the diets for broilers and juvenile Jian carp, respectively, had no adverse effects on the animals' blood traits and supported the nutrient sufficiency of these diets. According to numerous studies, BSFM is the best insect protein for improving the growth performance, Health status of broiler chickens. When given a diet containing 2.6% BSFM and an increased amino acid supply, Ross 308 broiler chickens' growth performance and liver function were enhanced (Neumann *et al.,* 2017). While growth performance and haematological indicators were unaltered, breast meat from diets containing 100% BSFM in place of soybean oil showed increased proportions of saturated fatty acids and lower proportions of polyunsaturated fatty acids (PUFAs) (Schiavone *et al.,* 2018). Black soldier fly fat reduced the cholesterol in the breast meat of Ross 708 broiler chickens by 50%, although 100% BSF fat increased the overall quantity of saturated fatty acids in the breast and leg meat (Cullere *et al.,* 2019). The blood parameters offer insight into the health of the birds. The broiler chickens' physiological conditions proved that BSF had impact or not on the animals' state of health. Chitin in the BSF larva meal may have a chelating effect,

explaining this behaviour. So that BSF larvae meal might possibly be utilised in place of soybean meal in broiler diets. To determine the maximal level of BSF larvae that can exist without impairing consumer and poultry health standards, additional research is necessary.

9. Conclusion

Considering the precautions that prevent the development of certain substances that are detrimental to consumers in insect biomass, using insects as sustainable protein sources in the diet of chickens may help achieve food security and help solve its problem. It may be possible to effectively maintain the chicken industry's economic viability by feeding insects fed on organic wastes and employing those insects as poultry feed. BSF is a remarkable selection insect for large-scale production, high growth performance, low mortality, superior disease resistance, and protein quality in order to meet this objective. Therefore, BSF larvae meal has the potential to replace soybean meal in broiler diets. However, more investigation is necessary to determine the maximal concentration of BSF larvae that can exist without impairing consumer and poultry health standards.

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The authors disclosed no conflict of interest starting from the conduct of the study, data analysis, and writing until the publication of this research work.

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