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Research Article

Effect of Gradually Increasing Levels of Full-Fat Black Soldier Fly (*Hermetia illucens*) Larvae Meal on Layer Performance

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Abstract

Background and Objective: Black Soldier Fly Larvae Meal (BSFLM) has emerged as a promising alternative protein source in poultry nutrition. However, limited research has assessed the impact of gradually increasing full-fat BSFLM on laying hen performance and egg quality. This study aimed to evaluate the effects of graded levels of full-fat BSFLM on the productive performance and egg quality characteristics of ISA brown laying hens. **Materials and Methods:** A total of 200 ISA brown laying hens were randomly assigned to 25 groups, with 8 hens per group. Five diets were formulated containing 0, 3, 6, 9 and 12% full-fat BSFLM (designated as BSFLM0, BSFLM3, BSFLM6, BSFLM9 and BSFLM12). Each diet was replicated five times. Birds were reared for two months under identical conditions. Parameters measured included feed intake, laying rate, feed conversion ratio, egg weight, albumen height, Haugh unit and egg yolk color. Data were analyzed using R software (v4.3.3) with ANOVA at a 5% significance level. **Results:** Increasing levels of BSFLM had a significant linear and quadratic effect ($p < 0.05$) on feed intake and feed conversion ratio. Feed intake declined as BSFLM increased, while feed conversion ratio improved. No significant differences ($p > 0.05$) were observed for laying rate, egg weight, albumen height, Haugh unit, or yolk color across treatments. **Conclusion:** Gradual inclusion of up to 12% full-fat BSFLM in layer diets improved feed efficiency without compromising laying rate or egg quality. However, higher BSFLM levels slightly reduced feed intake. Further research is warranted to evaluate higher inclusion levels and long-term effects on productivity and egg characteristics.

Key words: Egg quality, full-fat BSF larvae, gradual increasing, laying hen performance, feed intake

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

According to the FAO¹, to anticipate the human population growth, the actual animal protein production will need to increase by 60% or more by 2050 and poultry production that grows fast and has favourable feed conversion efficiency is one of the cheapest and easiest means of meeting this anticipated demand in animal protein. Over the past 20 years, egg consumption has risen by around 40%², because eggs are safer for human health³, contain essential vitamins and minerals⁴ and it is particularly a low-cost, high-quality protein food. Enormous resources will be needed to support this increase and the feed being the most challenging because of the limited availability of natural resources, climate change pressure and food-feed-fuel competition¹. Alternative protein sources more sustainable and do not compete with human food production is recommended by current research in response to this situation, particularly the potential of insects^{5,6}. Black soldier fly larvae are an excellent candidate for human and animal protein sources⁷. The BSFL can contain upwards of 40% crude protein, depending on stage of their development and are rich in essential amino acids including lysine, methionine, cysteine, arginine and tryptophan⁶. The BSFL is also concentrated in lipids (28%) and minerals such as calcium and phosphorus⁸.

In broiler diet, the BSFL meal has been successfully employed and improves their performance, feed efficacy, carcass traits, meat features and intestinal macrobiotic⁹⁻¹⁴.

In the layer diet, the results are not similar to broilers. An increase in egg weight and egg shell thickness in whole BSFL larvae compared to the control was observed¹⁵, but a decrease in feed intake, body weight, egg production, egg weight and mass at 17% inclusion of replacing soybean meal was reported¹⁶. Also, Bejaei and Cheng¹⁷ observed an inferior feed conversion ratio and egg production compared to a control treatment that did not receive larvae. Result showed a reduction in egg weight, shell weight, shell thickness and yolk color compared to control¹⁸. These results support that there is an incorporation rate of Black Soldier Fly Larvae Meal (BSFLM) in layer diet, from which laying performance and egg quality are negatively influenced. The objective of this study is to investigate the effect of a gradual increasing rate of full-fat of BSFLM to achieve the best laying hens' performance and egg quality.

MATERIALS AND METHODS

Study area and duration: This study was conducted at the experimental poultry farm of the Regional Agricultural Research Center of the National Institute of Agricultural Research of Maradi (Niger) with natural lighting and

ventilation and lasted for 2 months from August, 2024 to September, 2024.

Experimental house and layers: The experiment was carried out in a 15×7 m henhouse. It has been subdivided into 25 boxes. Each box has a dimension of 2.75 m² (2.75×1 m). The henhouse was cleaned, disinfected and quarantined for two weeks before experimentation started. Two hundred Isa brown laying hens aged 23 weeks and with an average weight of 1483±121 g were used for two months of laying. The experiment was conducted with natural ventilation and lighting conditions with an average internal temperature of 31.85°C and a hygrometry of 39%.

Sanitary and feed management: The chickens were vaccinated against Newcastle and Gumboro diseases. Stress medications, vitamins and calcium supplements were given as needed to prevent post-vaccination reactions, calcium deficit and stress during the experiment.

Millet, wheat bran, peanut meal, fish meal, full-fat BSFLM, lysine, methionine, bone meal, salt, peanut oil and premix were the raw materials used for feed formulation with the essay and error method. All raw materials were crushed separately and weighed according to the formula (Table 1) to make the feed mixture. Full-Fat Black Soldier Fly Larvae Meal (BSFLM) was incorporated into the laying hens' feed at a rate of 0, 3, 6, 9 and 12% to have BSFLM0, BSFLM3, BSFLM6, BSFLM9 and BSFLM12, respectively. The BSFLM0 is the control feed. In all feeds, regardless of the incorporation rate of full-fat BSFLM, the theoretical values of 2900 kcal of metabolizable energy and 18% of crude protein levels recommended by the NRC¹⁹ in layer diet were maintained. The samples of fish meal, peanut meal and full-fat black soldier fly larvae meal were subjected to a chemical analysis by the NIRS method to determine their metabolizable energy concentration, dry matter, crude protein, crude fiber and amino acids content.

Experimental design and data collected: The 200 laying hens were raised on the groundnut as bedding for two months of laying. The hens were distributed randomly among the 25 groups with 8 hens per group. The 5 feeds were distributed randomly among the 25 batches with 5 repetitions per feed.

The layer feed ingested was evaluated by the difference between the quantities distributed and the quantities refused. In each batch, the average feed intake per hen is obtained by dividing the total quantity consumed by the number of hens. The laying hens' performance was expressed in laying rate, which is the relationship between the eggs produced and the number of hens in each batch. It is expressed as a percentage (%).

Table 1: Ingredient composition (%) and nutritional value of experimental feeds used

Ingredients	Full-fat black soldier fly larvae meal				
	0%	3%	6%	9%	12%
Millet	65	67	67	67	66.5
Wheat bran	9	7.25	6	6	5
Peanut meal	10	8.5	9.5	7	9
Fish meal	8.5	7	4.5	4.5	1
Full-fat BSFLM	0	3	6	9	12
Lysine	0.1	0.1	0.1	0.1	0.1
Methionine	0.1	0.1	0.1	0.1	0.1
Bone meal	6	6	5.5	5	5
Salt	0.3	0.3	0.3	0.3	0.1
Peanut oil	0.75	0.5	0.75	0.75	0.95
Premix	0.25	0.25	0.25	0.25	0.25
Total	100	100	100	100	100
Theoretical nutritive value					
Metabolizable energy (kcal/kg DM)	2909.013	2903.593	2913.343	2912.643	2901.163
Crude protein (%)	18.457	18.025	18.099	18.031	18.014
Crude fiber (%)	3.146	3.082	3.127	3.200	3.285
Ether extract (%)	4.503	4.641	4.737	4.868	4.941
Lysine (%)	1.005	1.044	1.070	1.162	1.157
Methionine (%)	0.954	1.161	1.345	1.555	1.724
Calcium (%)	2.231	2.265	2.110	2.061	2.013
Non phytic phosphorus (%)	1.103	1.134	1.086	1.090	1.082

The feed conversion ratio (FCR)¹⁹ is calculated by making the ratio between the average quantity of feed intake and the number of eggs produced. The FCR in g of feed per egg translates the quantity of feed in g needed to produce 1 egg.

Every two weeks, all eggs of the day were collected in batches and weighed at 6 pm using a 0.1 g precision electronic balance. Two eggs of the day are randomly taken per replication for egg quality assessment. Albumen height was measured with a tripod micrometer. The egg weight (W) in grams and the albumen height (H) in millimeters were used to calculate the Haugh unit (HU) using the HAUGH formula:

$$HU = 100 \log_{10} (H - 1.7W^{0.37} + 0.757)$$

The egg color is determined using the Roche fan made of different levels varying from light yellow to dark orange yellow in ascending order of numbers (1 to 15). The level of egg yolk color is defined by taking the average of three observations made by three different persons.

Statistical analyses: The R software (R 4.3.3 version) was used for statistical analysis. The ANOVA at the significance level of 5% was used to analyze the collected data of feed intake, laying hens, feed conversion ratio, egg weight, albumen height, Haugh unit and egg yolk color. Orthogonal polynomial contrasts were used to test the linear or quadratic

response of increasing levels of full-fat black soldier fly larvae meal. The differences between the arithmetic means were compared using the Student-Newman-Keuls (SNK) test to detect the effects of treatments at the 5%, probability level.

Ethical consideration: This experiment was in compliance with the current standards for conducting experiments with animals of the National Institute for Agriculture Research of Niger (INRAN). Ethical issues such as plagiarism, consent to publish, misconduct, data fabrication and/or falsification, double publication and/or submission and redundancy have been checked by all the authors.

RESULTS AND DISCUSSION

Chemical composition of the main protein sources used:

Peanut meal had a higher concentration of metabolizable energy than fish meal and black soldier fly larvae meal (Table 2). The analysis showed that fish meal has a higher crude protein content compared to full-fat larvae meal and peanut meal. As for crude fiber, it is more concentrated in the full-fat larvae meal than in other protein sources used in feed formulation. The lysine and methionine contents of the fish meal were similar to those found in the full-fat larvae meal, but higher than those contained in the peanut meal (Table 2).

Table 2: Chemical composition of the main protein sources used in food formulation

Protein source	Fish meal	BSF larvae meal	Groundnut meal
Metabolizable energy (kcal/kg DM)	1912	1673	2151
Dry matter (%)	94.76	96.20	94.61
Crude protein (%)	55.89	42.28	51.40
Crude fiber (%)	10.62	17.07	5.90
Lysine (%)	2.60	2.28	1.89
Methionine (%)	1.06	1.02	0.46

Table 3: Effect of gradual incorporation of full-fat BSFLM on laying hens' performance

Parameter	Black soldier fly larvae meal (%)					*Contrast		
	0	3	6	9	12	SEM	Linear	Quadratic
FI (g/day)	107.55 ^b	111.22 ^a	101.29 ^c	98.74 ^c	98.25 ^c	16.48	***	***
CLH (%)	48.53	51.30	50.83	48.47	49.75	20.86	NS	NS
FCR (g/egg)	283.77 ^a	271.89 ^{ab}	240.66 ^b	259.13 ^{ab}	248.32 ^{ab}	175.36	*	*

FI (g/day): Feed intake in gram per day, CLH (%): Cumulative laying hens in percentage, FCR (g/egg): Feed conversion ratio in gram per egg, SEM: Standard Error Mean,

*Linear or quadratic response estimated using orthogonal polygonal contrast (NS = Nonsignificant, *** $p < 0.001$ and * $p < 0.05$) and ^{a,b,c}Means in the same row not sharing a same subscription is significantly different at $p < 0.05$

Depending on the authors, BSFLM chemical composition has varied. Breeding substrate could be the major origin of this difference⁶, also, drying methods to obtain non-defatted BSFL significantly influenced chemical composition such as moisture, ash, crude protein, crude fiber, ether extract, nitrogen-free extract, phosphorus and gross energy content²⁰. The full-fat black soldier fly larvae chemical composition obtained in this analysis confirms the observations of certain authors that protein content as well as amino acid profile in BSF larvae is comparable to many protein-rich feedstuffs such as fish meal, soybean meal²¹ and groundnut meals.

Feed intake: The daily laying hens' feed intake was negatively affected by the gradual increase of the full-fat Black Soldier Fly Larvae Meal (BSFLM). Feeds containing less full-fat BSFLM were consumed more. Linearly and quadratically, feed intake decreased significantly ($p < 0.05$) from 6% of full-fat BSFLM level over the entire period of the experiment (Table 3).

This could be linked to the fat content of feeds, which increased gradually with the increase of the full-fat black soldier fly larvae meal rate in the feed. Patterson *et al.*²² found the same trend with full-fat BSFLM, but Mwaniki *et al.*²³ found an increase in feed intake when the defatted level of BSFLM increased. Indeed, fat reduces the laying hens' feed intake²⁴. In general, a high level of lipid in feeds is associated with low digestibility²⁵.

Laying hens: The full-fat black soldier fly larvae meal gradually increasing did not statistically influence ($p > 0.05$) the laying rate (Table 3). Feeds containing 3 and 6% of full-fat BSFLM allowed more laying rates. It started to decrease non-linearly from 9% of full-fat BSFLM in the hen's diet. The protein content in black soldier fly larvae meal can constitute a good source of protein in laying hens' feed. The major factors which can significantly influence the laying rate are the

metabolizable energy concentration, the proteins, lysine contents²⁶, methionine²⁷ and the crude fiber rate²⁸ in layer feeds. Variations of 90 kcal/kg of metabolizable energy in an *ad libitum* diet²⁹, 1% of crude protein²⁶, 0.5% of methionine²⁷ and a variation of 3.4 to 5.5% of crude fiber²⁸ in feed do not significantly affect laying rate. The feeds formulated in this experiment showed similar compositions of these elements. Al-Qazzaz *et al.*³⁰ found a significant variation in laying hens by incorporating black soldier fly larvae meal into the diet of layers because there is a variation in energy and crude protein.

Feed conversion ratio: Feed efficiency increased significantly ($p < 0.05$) with increasing full-fat black soldier fly larvae meal in layer feed. The best feed conversion ratio was obtained with the incorporation rate of 6% (Table 3). But also, 9 and 12% full-fat BSFLM presented better FCR than the control diet and that containing 3% BSFLM, but without significant difference.

The FCR improved with the increase in the fat content of feeds. This could be related to the increased oil content and digestibility of BSFLM6, BSFLM9 and BSFLM12 feeds. Grobas *et al.*³¹ also observed that the incorporation of 10% oil significantly improved the FCR of laying hens compared to 5% incorporation because these diets were more concentrated and had more energy than the control diet. Crude protein and ether extract digestibility were increased quadratically with dietary full-fatted BSFM inclusion levels increased³². Because the fat derived from BSFM is rich in medium chain fatty acids that can have a positive effect on increasing of the energy availability in the intestine^{33,34}, thus promoting growth performance³². However, a significant deterioration in FCR with increasing of defatted black soldier fly larvae meal in diet is noted²³. In fact, the fat content decreased with the increase of defatted BSFLM.

Table 4: Effect of gradual incorporation of full-fat BSFLM on egg quality

Parameter	Black soldier fly larvae meal (%)					*Contrast		
	0	3	6	9	12	SEM	Linear	Quadratic
EW (g)	53.09	52.53	52.78	52.77	53.04	2.83	NS	NS
AH (mm)	9.84	9.28	9.64	9.39	9.66	1.35	NS	NS
HU	99.41	96.89	98.75	97.70	98.64	6.00	NS	NS
EYC (RCU)	3.61	3.99	3.65	3.95	3.91	0.86	NS	NS

EW (g): Egg weight in gram, AH (mm): Albumen height in millimeter, HU: Haugh unit, EYC (RCU): Egg yolk color in rock color unit, SEM: Standard Error Mean and

*Linear or quadratic response estimated using orthogonal polygonal contrast (NS = Nonsignificant and $p > 0.05$)

Egg quality

Egg weight: The gradual increase of full-fat BSFLM did not statistically influence the egg weight either linearly or quadratically ($p > 0.05$) (Table 4). If the control feed (BSFLM0) and BSFLM12 have more weight, the difference with the others does not reach 1 g.

Several authors have also observed that the gradual increase of BSFLM has no linear or quadratic influence on egg weight³⁵⁻³⁷. The egg weight depends mainly on factors linked to the hen (genetic origin and especially age) and secondary of its diet during the laying period³⁸. In early-lay period, egg weight does not vary considerably³⁹. The egg weight increases on average of 0.96 g with the increase in a 10 kcal energy intake³⁸. Other nutritional factors influencing egg weight are the total amino acids, minerals contents⁴⁰ and the fatty acid content⁴¹. The amount of lipids that laying hens obtain from feed is approximately 3 g/day and 5-6 g of lipids is needed for the formation of each egg²⁴. The feeds used for this experiment provided more than 4% of fat.

Albumen height: The full-fat BSFLM incorporation up to 12% in laying hen feed did not statistically influence ($p > 0.05$) the egg albumen height (Table 4). The height decreased with increasing level of full-fat BSFLM, but the difference between the largest height (BSFLM0) and the smallest height (BSFLM3) was 0.56 mm.

At 26 days of laying hen there is a significant difference between the albumen heights depending on the incorporation level of BSFLM, but this difference disappears at 56 days of laying⁴². There are reports that albumen quality decreases with increasing dietary protein and amino acid content⁴³. There was a tendency for less albumen height because feeds containing full-fat of BSFLM in this experiment have more methionine and lysine content than control feed (BSFLM0). But also, albumen percentage and albumen height were all linearly increased by increasing dietary calcium⁴⁴. These results agree with Wu *et al.*⁴⁵, who reported that improving calcium significantly increased albumen weight. The calcium content decreased in feeds containing full-fat BSFLM because the calcium content of the black soldier fly larvae was very low compared to other protein sources³⁷.

Haugh unit: The control feed allowed eggs with a high Haugh unit and the feed containing 3% of full-fat BSFLM allowed the lowest Haugh unit (Table 4). However, the difference between Haugh units induced by the different feeds was not statistically significant ($p > 0.05$).

With defatted-BSFL diets³⁰ or full-fat BSFL diet¹⁷ any significant difference did not detect in the Haugh units. The factors that affect Haugh units are largely related to the egg storage temperature, the hen age, the strain and to a small extent to the crude protein content⁴³. Novak *et al.*²⁶ found that reducing protein intake linearly reduced Haugh units, but dietary effects on Haugh units in early laying hen's period were nonsignificant. The decrease in crude protein content with the increasing of full-fat BSFLM incorporation rate up to 12% was not significantly influence the Haugh unit.

Egg yolk color: Feeds formulated with full-fat black soldier fly larvae meal had more egg yolk color compared to the control feed (Table 4). The difference between egg yolks induced by the different feeds was not statistically significant ($p > 0.05$).

High level of fatty acids such as myristic and palmitic acid in BSFL meals, has been reported that could be transferred to the yolk⁴⁰ and color it. However, yolk color was increased during the overall period of laying hens when feeding reduced crude protein diet⁴⁶. In this experiment, increasing in yolk color may have been due to increasing in millet percentage in the diet contained BSFLM (65% in control diet and 66.5 to 67% of millet in BSFLM diet), which would in turn increase the xanthophyll concentration and egg change yolk color.

CONCLUSION

This study evaluated the effects of gradually increasing levels of full-fat Black Soldier Fly Larvae Meal (BSFLM) on the performance and egg quality of ISA brown laying hens. Results showed that feed intake decreased with higher BSFLM inclusion, while laying rate improved at 3 and 6% levels. Feed conversion ratio was positively influenced at 6, 9 and 12% inclusion rates. Egg weight and albumen height were highest in the control and 12% BSFLM groups. Haugh units were

better with control and 6% BSFLM and egg yolk color improved progressively with BSFLM inclusion. Given these mixed responses, further research is recommended to refine optimal inclusion levels and establish clear feeding guidelines.

SIGNIFICANCE STATEMENT

This study identified the effects of gradually increasing full-fat Black Soldier Fly Larvae Meal (BSFLM) on feed intake, feed efficiency and egg quality in laying hens, which could be beneficial for developing sustainable and alternative protein sources in poultry nutrition. This study will assist researchers in uncovering critical areas of insect-based feed integration that have remained unexplored by many. Consequently, a new theory on the optimal inclusion rate and nutritional impacts of BSFLM in layer diets may be developed.

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