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RESEARCH ARTICLE

Partial Replacement of Soybean Meal with Insect Meal in Layer Hen Diets: Effects on Egg Production and Quality

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ABSTRACT

A total of 192 and 32-week old Hy-Line Brown laying hens were assigned to 12-week feeding trials to four dietary treatments that provided 0%, 4%, 8%, or 12% of their soybean meal protein from BSFLM, with 48 hens per replicate cage and 6 replicate cages per dietary treatment. The egg production, egg weight, feed intake and feed conversion was recorded every week, while the egg quality parameters such as shell thickness, shell breaking strength, Haugh unit, yolk colour and yolk to albumen ratio were measured every two weeks. Hen-day egg production, egg weight and feed conversion were not significantly different from the control at either dietary inclusion rates of 4 and 8% BSFLM, while yolk color scores were found to be significantly higher and progressively increased with increasing levels of BSFLM inclusion, possibly due to the presence of carotenoid in the larvae substrate. In comparison, 12% BSFLM inclusion significantly lowered hen-day egg production in comparison with control, egg weight, shell thickness, shell breaking strength and Haugh unit, suggesting that there is a threshold at which egg production and quality begin to be adversely affected. The results indicate that up to 50% inclusion of dietary soybean meal protein (8%) in layer diets could be replaced with BSFLM without compromising egg production or quality, and improved yolk pigmentation, but higher inclusion rates were detrimental to both egg production and internal and shell quality, possibly because of the higher proportion of dietary chitin and its effect on calcium utilization.

1. Introduction

The world's most common protein source in commercial layer hen diets is soybean meal, which has an excellent amino acid profile and high digestibility [1]. Growth in soybean production has, however, been associated with land-use and deforestation in the key producing areas, and soybean prices have been highly volatile at international markets caused by weather conditions, trade policy, and competing demands from the biofuel and livestock industries [2]. To address both these environmental and economic challenges, there has been a growing focus on the need to find substitute protein sources that can help alleviate the need for relying on soybean meal (SBM) in poultry feed. The high crude protein content, preferential essential amino acid composition, and potential to rear the black soldier fly larvae on organic waste substrates make it an attractive option as a sustainable protein feed and a valuable means of valorization of organic wastes [2,3]. Besides its protein content, BSFLM also contains carotenoid pigments from the larval rearing substrate that may affect the egg yolk pigmentation, which is a very important commodity trait in the egg industry due to consumers demand for deep yellow-orange egg yolk [4].

Although these potential benefits exist, the high chitin content of BSFLM, due to the presence of the larval exoskeleton, is a concern for nutritive digestibility and the possible impact on calcium metabolism, which is especially significant for laying hens with high daily calcium requirements for eggshell production [3,4]. The lower

utilization or availability of calcium may have negative effects on the quality of the eggshell, which is an economically valued characteristic associated with egg breakage and marketability [5]. The optimal inclusion level of BSFLM in layer diets to maximize the protein replacement benefits, while minimizing any potential chitin related issues, is still under investigation as optimal inclusion levels have been reported to vary from study to study depending on the method of processing the larvae, and the type of basal diet [2,4].
This study is aimed to systematically evaluate the effects of the graded BSFLM inclusion (0%, 4%, 8%, and 12%) on egg production performance and egg quality traits in laying hens to find the optimum level of inclusion that maximizes soybean meal replacement in egg production without compromising egg production and quality.

2. Materials and Methods

2.1 Hens, Housing, and Experimental Design

One hundred ninety-two (192) 32-week-old, uniform body weight and egg production rate, commercial Hy-Line Brown laying hens were chosen and randomly assigned to four dietary treatments with six replicate cages per treatment and eight hens per cage (n = 48 hens/treatment) in a completely randomized design. The feeding trial was carried out in a laying house where hens had individual nest access, had ad libitum access to feed and water and a standardized lighting program for 12 weeks after a dietary adaptation phase of 2 weeks.

2.2 Dietary Treatments

Black soldier fly larvae were fed on a standard organic manure and collected at the prepupae stage for larval meal production by drying them in an oven, extracting the fat and milling them. Four isonitrogenous and isocaloric diets were prepared that provided 0% (T0, control), 4%, 8% (T2), or 12% BSFLM, replacing 0%, 25%, 50% and 75% of the dietary soybean meal protein, respectively, on a digestible amino acid basis, and all diets provided an adequate supply of calcium and available phosphorus based on breed nutrient requirements. The summary of diet formulation and BSFLM level has been presented in Table 1.

Table 1. Dietary treatment groups and black soldier fly larvae meal (BSFLM) inclusion levels used in the 12-week layer hen feeding trial.

Treatment	Insect Meal Inclusion (% of diet)	Soybean Meal Protein Replaced (%)	Hens per Treatment (n)
T0 (control)	0%	0	48 (6 cages × 8)
T1	4%	25	48 (6 cages × 8)
T2	8%	50	48 (6 cages × 8)
T3	12%	75	48 (6 cages × 8)

2.3 Egg Production Performance Measurements

Hen-day egg production percentage was determined by collecting and counting eggs on a daily basis within a cage while the average egg weight was determined within a cage by recording total egg weight weekly. Feed intake was measured at the cage level once a week, and was used to determine average daily feed intake and feed conversion ratio in grams of feed per gram of egg.

2.4 Egg Quality Assessment

The egg quality was evaluated every two weeks with 6 eggs randomly collected from each cage (36 eggs/treatment). After removing the membrane, the thickness of the eggshell was measured at three locations (air cell, equator and sharp end) with a digital micrometer, and the shell breaking strength was measured using a texture analyzer equipped with a compression probe. The standard internal egg quality measure Haugh unit was calculated by using albumen height and egg weight. Yolk color was determined by using the Roche yolk color fan (1–15 scale) and yolk and albumen were separated and weighed to determine the yolk-to-albumen ratio.

2.5 Statistical Analysis

One way analysis of variance (ANOVA) with dietary treatment as the main effect and cage as the experimental unit was used for data analysis. Tukey's Honestly Significant Difference (HSD) test was used to separate treatment means at $p \leq 0.05$. The linear and quadratic effects with increasing level of BSFLM were tested using polynomial contrasts. Values are given as a mean ± standard error (SE); means with different superscript letters in the same column are significantly different.

3. Results

3.1 Egg Production Performance

Egg production per hen/hen-day, average egg weight and feed conversion were not significantly different between the 12-week control (T0) and inclusion of either 4% (T1) or 8% (T2) BSFLM ($p > 0.05$). The hen-day egg production (85.2 ± 1.6%) and average egg weight (60.7 ± 0.6 g) were significantly lower in hens fed the 12% BSFLM diet as compared to the control (91.4 ± 1.2% and 62.8 ± 0.5 g, respectively), though feed intake did not differ significantly between the two diets. The FCR of hens raised with the 12% BSFLM diet (2.22 ± 0.05 g feed/g egg) was significantly poorer than that of the control (1.96 ± 0.03 g feed/g egg). The significant linear and quadratic effects of BSFLM inclusion level on hen-day egg production and feed conversion confirmed that the hen-day egg production and feed conversion were maintained at low-to-moderate levels of BSFLM with a decrease at the highest inclusion level. The summary of production performance of eggs is shown in Table 2.

3.2 Egg Quality

There was no significant difference in shell thickness, Haugh unit and shell breaking strength among the control, 4% and 8% BSFLM treatments, but a significant reduction in all three characteristics occurred with the 12% inclusion treatment compared to the control, suggesting a decrease in both internal and external egg quality at the highest inclusion rate. Carotenoid pigments from the BSFLMM substrate were deposited, which resulted in a significantly increasing and progressive yolk color score with increasing inclusion rates from 7.8 ± 0.2 (control) to 10.2 ± 0.3 (12%). There was no significant difference in the ratio of yolk to albumen between the treatments. The results of the quality of eggs are presented in Table 3 and Figure 1.

Table 2. Egg production performance of laying hens fed graded levels of black soldier fly larvae meal (BSFLM) over a 12-week feeding trial.

Treatment	Hen-Day Egg Production (%)	Avg. Egg Weight (g)	Feed Intake (g/hen/day)	Feed Conversion (g feed/g egg)
T0 (control)	91.4 ± 1.2[a]	62.8 ± 0.5[a]	112.3 ± 1.5[a]	1.96 ± 0.03[a]
T1 (4%)	92.0 ± 1.1[a]	63.1 ± 0.6[a]	111.8 ± 1.4[a]	1.93 ± 0.03[a]
T2 (8%)	90.6 ± 1.3[a]	62.5 ± 0.5[a]	113.1 ± 1.6[a]	2.00 ± 0.04[a]
T3 (12%)	85.2 ± 1.6[b]	60.7 ± 0.6[b]	114.6 ± 1.7[a]	2.22 ± 0.05[b]

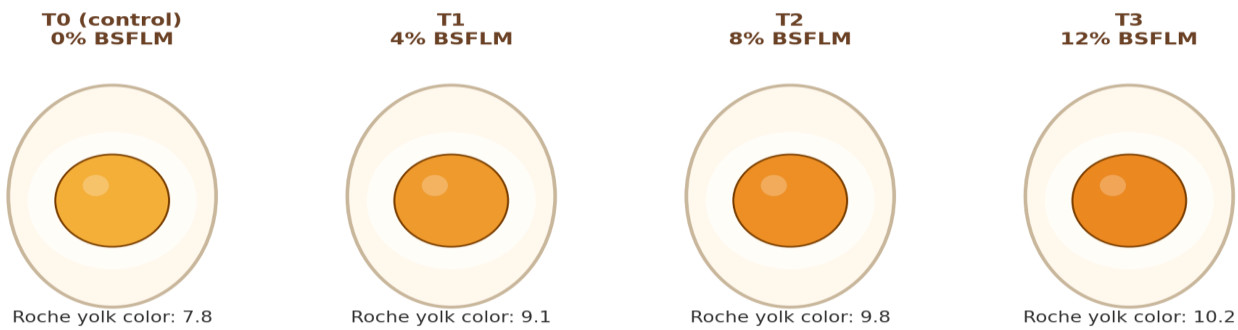
Different superscript letters within a column indicate significant differences (Tukey's HSD, $p \leq 0.05$).

Table 3. Egg quality traits of laying hens fed graded levels of black soldier fly larvae meal (BSFLM), assessed biweekly over the 12-week trial

Treatment	Shell Thickness (mm)	Haugh Unit	Yolk Color (Roche scale)	Yolk:Albumen Ratio	Shell Breaking Strength (N)
T0 (control)	0.352 ± 0.006[a]	84.1 ± 1.0[a]	7.8 ± 0.2[b]	0.36 ± 0.01[a]	38.4 ± 0.9[a]
T1 (4%)	0.358 ± 0.005[a]	85.0 ± 0.9[a]	9.1 ± 0.2[a]	0.36 ± 0.01[a]	39.2 ± 0.8[a]
T2 (8%)	0.361 ± 0.006[a]	84.6 ± 1.1[a]	9.8 ± 0.2[a]	0.37 ± 0.01[a]	39.9 ± 1.0[a]
T3 (12%)	0.339 ± 0.007[b]	80.3 ± 1.2[b]	10.2 ± 0.3[a]	0.35 ± 0.01[a]	36.1 ± 1.1[b]

Different superscript letters within a column indicate significant differences (Tukey's HSD, $p \leq 0.05$)

Figure 1. Egg quality traits of laying hens fed graded levels of black soldier fly larvae meal (BSFLM), assessed biweekly over the 12-week trial



4. Discussion

Based on the results of this study, black soldier fly larvae meal can be used as a partial substitute for up to 50% (8% inclusion level) of dietary soybean meal protein to achieve satisfactory egg production performance without reducing egg production while providing the added advantage of improving yolk pigmentation in all diets evaluated. This result is in line with the potential of BSFLM as a potential sustainable protein source in commercial layer diets and consistent with the existing knowledge on the use of insect meal in laying hen nutrition [5,6].

The increasing trend of yolk color score with the increase of BSFLM inclusion had a dose-dependency, this is consistent with the fact that the black soldier fly larvae contain carotenoid levels that are dependent on the pigment composition of the rearing substrate [6,7]. The pigmentation benefit may be a valuable co-benefit of the inclusion of BSFLM in production systems, not just as a protein source but for the market value of the yolk color, which is a desirable attribute for consumers in many markets and can be reduced or even avoided with the use of synthetic or marigold derived carotenoid pigments [4,6].

The reduction in egg production, egg size and feed conversion reported at 12% inclusion is equivalent to what has already been reported in broiler chickens fed high levels of BSFLM, most likely a result of the cumulative chitin intake with increasing inclusion level, which is poorly digested by the mono-gastric digestive system, and may be responsible for reducing overall nutrient and energy utilization [8]. Reduced shell thickness and shell break strength at the highest inclusion rate are of special concern for commercial egg production because if shell quality is compromised, egg breakage during handling, transport and storage can counterbalance the economic advantage of soybean meal reduction [9].

Mechanisms responsible for the declining shell quality at high BSFLM inclusion may be a result of either reduced digestibility or availability of dietary calcium and phosphorus in the presence of high levels of chitin; in some monogastric species chitin is reported to interact with divalent cations and affect gut pH and mineral solubility, but these mechanisms were not directly explored in the present study and may be important for the current experiment [8,10]. The concurrent decrease in Haugh unit at 12% inclusion, which was associated with decreased albumen quality, may be a general nutrient utilization effect and hen metabolic status effect at this inclusion level, and not a shell-specific effect [11].

The results of this study may not necessarily apply to other hen strains with different production cycles or to other BSFLM processing methods (e.g., enzymatic chitin reduction or fermentation) since they may cause different negative effects if the hen strain and processing method are changed. In addition, this study did not directly measure the digestibility of Ca and P, bone mineralization or gut microbiota composition which would add mechanistic insight into the cause of depressed shell quality at high inclusion rates [6,8,11]. The application of mineral digestibility trials, production testing over a complete laying cycle, and economic evaluation would be useful to incorporate into future studies to further develop practical recommendations for the incorporation of BSFLM in commercial layer hen production [12].

5. Conclusion

This study showed that addition of black soldier fly larvae meal to the diets of laying hens at up to 8% (replacing about 50% of the soybean meal protein) did not adversely affect egg production performance or egg quality, and did result in better yolk color for all meal inclusion levels tested. The egg production, egg weight, feed conversion ratio, eggshell quality and internal egg quality were all significantly reduced with inclusion at 12%, which was probably related to the increased chitin content in the diet and interference with calcium and nutrient utilization. Based on the results, BSFLM is a nutritionally suitable and sustainable alternative to soybean meal in the nutrition of layer hens when it is included in moderate amounts, providing environmental and product quality advantages to egg industry.

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Declaration of conflict of interest

The authors no conflict of interest.

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