**Journal of Animal & Plant Sciences, 35(1): 2025, Page: 273-284**
**ISSN (print): 1018-7081; ISSN (online): 2309-8694**
https://doi.org/10.36899/JAPS.2025.1.0022

# ERI SILKWORM PUPAE MEAL AS A FISH MEAL REPLACEMENT IN JAPANESE QUAIL STARTER DIET

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## ABSTRACT

A 28-day growth performance trial was conducted to assess the potential of Eri Silkworm (ES) pupae as a partial or complete substitute for fish meal in Japanese quail (*Coturnix japonica*) diets. A comparative analysis of the nutrient, mineral, and essential amino acid composition of ES pupae, mulberry silkworm (MS) pupae, and fish meal was carried out. A total of 490 ten-day-old male Japanese quail, divided into seven treatment groups, each having seven replicates and 10 quails per replicate. The treatments included a control (without ES pupae), 25, 50, 75, and 100% ES pupae substitution, 75% MS pupae, and a commercial quail feed (positive control). The data on weekly body weight (WBW), body weight gain (BWG), feed intake, carcass weight, dressing percentage, and serum biochemical profile were collected. Results showed that 75% ES pupae meal significantly improved weight gain ($p < 0.05$) compared to commercial feed and MS pupae. The ES pupae exhibited superior nutritional profile, rich in essential amino acids (methionine, cystine, threonine, and lysine) and possessed higher fat content (24.36%) than fish meal and MS pupae. These findings suggest that ES pupae meal can replace up to 75% of fish meal in Japanese quail diets without compromising growth performance, offering a promising solution to reduce reliance on costly fish meal and promote sustainable poultry production.

**Keywords:** Eri silkworm pupae meal, Japanese quail, Growth performance, Carcass quality, Serum parameters, Mulberry silkworm pupae meal.



Published first online February 02, 2025 Published final February 18, 2025

## INTRODUCTION

India's persistent struggle with malnutrition, evidenced by its ranking of 111 out of 125 countries in the Global Hunger Index 2023, underscores the critical need for nutritional interventions. A significant contributing factor to this issue is the inadequate protein intake among the Indian population. Despite the recommended dietary protein intake for Indian adults being 0.8–1.0g per kg body weight, the current average intake falls short at 0.6g per kg body weight (ICMR, 2023). This deficiency necessitates the consumption of more protein-rich foods.

Poultry, as the primary source of animal protein in India, plays a vital role in bridging the nutritional gap prevalent in the country. However, the high cost of poultry production poses a substantial challenge to this endeavor. According to Elahi *et al*. (2022), feed expenses account for a staggering 60-80% of the total rearing cost, making it the most significant contributor to the overall expenditure. To bolster nutritional security and affordability, reducing production costs is vital. A viable solution lies in replacing expensive fish meal with insect meals, a cost-effective and nutrient-rich alternative protein source. Silkworms offer a promising solution to reduce feed costs in poultry production. Mulberry Silkworms (MS) and Eri Silkworms (ES), widely cultivated in India's agro-industry, generate underutilized pupae rich in protein (54-62%) and essential nutrients ideal for poultry. This sustainable alternative surpasses traditional protein sources like fish meal in cost-effectiveness (Altomere *et al.* 2020; Zegeye, 2020).

Bhuiyan *et al.* (1989) and Mangisah *et al.* (2004) highlights MS pupae as affordable, protein-rich (54-62%), and fat-rich ingredients. Notably, de-oiled pupae contain approximately 11% nitrogen (Sarmah *et al.* 2012), making them a valuable feed supplement for various livestock, including poultry, fish, and pigs. Studies have demonstrated the nutritional benefits and palatability of MS meal in poultry feed. Khatun *et al.* (2005) and Loselevich *et al.* (2004) found MS meal to be palatable and acceptable to broiler birds. Bovera *et al.* (2015) reported chitin in MS meal acts as a prebiotic, enhancing immune response. MS pupae contain an ecdysteroid that stimulates protein synthesis and tissue formation (Fagoonee, 1983). Research by Koudela *et al.*

(1995) showed MS pupae meal supports growth performance in Japanese quails. Most importantly, ES pupae possess a unique nutritional profile, offering a competitive advantage over MS pupae as a valuable protein source. Specifically, ES pupae contain α-linolenic acid, omega fatty acids, high lipid content (26%) and favorable Na/K ratio.

Japanese quails, characterized by rapid growth and short production cycles, require high-protein diets for optimal performance. To address this necessity, the potential benefits of silkworm pupae in quail diets have been explored by many researchers, however, further research is needed to optimize its inclusion levels. This study builds upon existing knowledge, seeking to optimize pupae inclusion levels. Specifically, it compares growth performance in Japanese quails fed diets supplemented with varying levels of ES pupae, MS pupae, and fish meal.

## MATERIALS AND METHODS

The ES and MS silkworm were reared in the Department of Entomology, Annamalai University, Tamil Nadu, India (11.3908° N; 79.7148° E) for adequate quantity of pupae production. Whereas, the Japanese quails (*Coturnix japonica*) were reared in the Division of Animal Husbandry. The first instar ES larvae were reared using tender castor (*Ricinus communis* L.) leaves, and mature leaves were used as the larvae grew to subsequent instars. The cocoons were harvested on the fifth day and pupae were collected. Similarly, MS larvae were reared using mulberry leaves following the standard protocol (Devaiah and Dayashankar, 1982), and pupae were extracted.

Five-day-old pupae were kept at 60°C for 12 hours for moisture removal. The grains (corn, pearl millet, and finger millet), pupae, and fish meal were ground separately for proper mixing while making experimental diets. Isocaloric and isonitrogenous feeds were prepared by mixing the grain ingredients with the protein components (ES pupae and fish meal) at different proportions, and with other feed ingredients (Table 1). The MS pupae mixed with fish meal in the proportion 75:25 and commercial quail feed (Krishi Quail Feeds, Krishi Nutrition Company Private Limited, Tamil Nadu, India) were used as positive control diet. The treatments were replicated seven times with ten birds per replication. The experimental diets were fed to ten-days-old male quails reared using the commercial feed. The color of the breast feathers determined the sex, with the male displaying brownish-red feathers and the female typically having tan (gray) feathers with black speckles (Homma *et al.* 1965). They quails were housed in cages (2m$^2$ per replication comprised of ten birds), each corresponding to an experimental unit. An average temperature, relative humidity, and photoperiod of 24°C, 65% RH, and 16:8 (Light: dark hours), respectively, were maintained. The pre-weighed quantities of feeds and water *ad libitum* for each replicate were kept, and feed intakes were measured. The feeding was done for 28 days.

**Table 1. The ingredients and nutrient composition of experimental diets**

| Ingredients | Feed without ES pupae meal | Feed with 25% ES pupae meal | Feed with 50% ES pupae meal | Feed with 75% ES pupae meal | Feed with 100% ES pupae meal | Feed with 75% MS pupae meal |
|---|---|---|---|---|---|---|
| **Grain powder** | | | | | | |
| (Corn, Pearl millet, and Finger millet, each 21 grams) | 63 | 63 | 63 | 63 | 63 | 63 |
| Anchovies Fish meal | 30 | 22.5 | 15 | 7.5 | - | 7.5 |
| Eri Silkworm pupae meal | - | 7.5 | 15 | 22.5 | 30 | - |
| Mulberry Silkworm pupae meal | - | - | - | - | - | 22.5 |
| Monodicalcium phosphate | 2 | 2 | 2 | 2 | 2 | 2 |
| Limestone | 2 | 2 | 2 | 2 | 2 | 2 |
| Salt | 1.5 | 1.5 | 1.5 | 1.5 | 1.5 | 1.5 |
| Vitamin and Mineral premix* | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 |
| Choline Chloride | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 |
| **Nutrient Composition (%)** | | | | | | |
| Crude protein | 25.3 | 25.5 | 25.4 | 25.5 | 25.2 | 25.6 |
| Crude fibre | 4.2 | 4.3 | 4.4 | 4.6 | 4.4 | 4.7 |
| Ash | 3.11 | 2.90 | 3.16 | 3.31 | 3.12 | 2.97 |
| Methionine | 0.41 | 0.33 | 0.37 | 0.40 | 0.38 | 0.31 |
| Lysine | 0.44 | 0.49 | 0.43 | 0.48 | 0.44 | 0.47 |
| Metabolizable energy (KJ/g) | 18.10 | 18.03 | 18.95 | 18.44 | 18.30 | 18.25 |

*Grow B-Plex – Vitamin and Mineral Premix, Growel Agrovet Private Limited, Bangalore-560064, India. Composition (100 gm): Vitamin B1: 100 mg; Vitamin B2: 30 mg; Vitamin B6: 40 mg; Vitamin B12: 50 mcg; Vitamin E: 25 mg; Methionine: 2420 mg; Lysine: 500 mg; Niacinamide: 500 mg; Biotin: 1 mg; Calcium Pantothenate: 60 mg; Iron: 5 mg; Copper: 1.5 mg; Cobalt: 100 mcg; Manganese: 4.5 mg; Iodine: 5 mg; Magnesium: 8 mg; Zinc: 1 mg; Selenium: 2 mg.

**Data Collection:** Data on initial body weight, weight at weekly intervals, and final body weight were measured individually for all birds. The BWG and FCR were worked out. At the end of the experiment, pre-slaughter weight of the birds was measured and, 28 birds per treatment (4 birds per replication) were slaughtered through decapitation, followed by three minutes of bleeding time, de-feathered manually, and carcass weights were measured. The dressing percentage of quails was determined by the following formula.

$$\text{Dressing percentage} = \frac{\text{Weight of Carcass}}{\text{Weight of Live bird}} \times 100$$

Blood samples were collected from the slaughtered birds and serum biochemical parameters were analyzed. The nutrient and mineral compositions (AOAC, 1990), and amino acids profile (Longvah *et al.* 2011) of experimental diets, eri silkworm pupae, mulberry silkworm pupae, and fish meal were determined.

**Data Analysis:** The data were analyzed by analysis of variance technique following Completely Randomized Design and using SPSS (version 20). The mean values were compared by following Duncan's Multiple Range Test (Duncan, 1955) and Tukey's test based on the nature of experiment and considering p<0.05 as significant. The graphs were prepared by Graph pad Prism Software (version 10).

## RESULTS AND DISCUSSION

The highest weight gain from 7 to 28 days was noted in birds fed diet containing 75% ES pupae meal (p<0.05). The quails fed 75% ES diet resulted in higher body weight gain than the control (P≤0.01) and those fed with a diet without ES pupae meal (P≤0.0001). These birds reached a maximum cumulative weight gain of 172.7g, that represents a 17.64% increase compared to the control group, thus demonstrating their superior performance (Figure 1 & 2).

Initially, non-significant effects were noted among birds fed diet without ES pupae meal (protein supplemented entirely by fish meal) and commercial feed. However, it recorded as the second-best treatment. During the second week, commercial feed resulted in significantly better performance and it overtakes the feed without and 50% ES pupae meal treatments. However, throughout the experiment the poor performance was noted in birds fed 25 and 100% ES pupae meal (Table 2). Retes *et al.* (2022) found that a crude protein level of 20-22% was optimal for quail growth. This is consistent with the current study in which all diets were formulated to have protein levels as reported earlier for proper weight gain in quails. This also indicated that the differences among treatments are due to difference in ingredients and their levels rather than due to difference in protein contents of the diets. Miah *et al.* (2020) reported similar results in chickens and indicated that higher amounts of MS pupae meal in diet led to lower feed intake and slower weight gain. This was likely due to poor palatability and high chitin contents of pupae meal. Additionally, increasing MS pupae in the diet can decrease the omega-6/omega-3 fatty acid ratio, which can affect overall health. However, in contrast to these, Wang (2010) reported that complete replacement of dietary protein with MS pupae meal resulted in the best performance. The poor performance with ES pupae meal 25 and 50% might be attributed to low methionine contents as indicated in the nutrient composition of the diet. The methionine contents of ES pupae meal 75% diet were similar to control diet thus having better performance. The diet with ES pupae meal 100% resulted in poor performance might be due to high indigestible chitin contents. These findings warrant taking into account for methionine and chitin contents of the diets.


**Figure 1. Impact of Eri Silkworm Pupae Meal Inclusion on Cumulative Weight Gain of Japanese Quail Between 7 and 28 Days of Age**

Values indicated with ns – non-significant; * - P ≤ 0.05; ** - P ≤ 0.01; **** - P ≤ 0.0001 (Tukey's test)

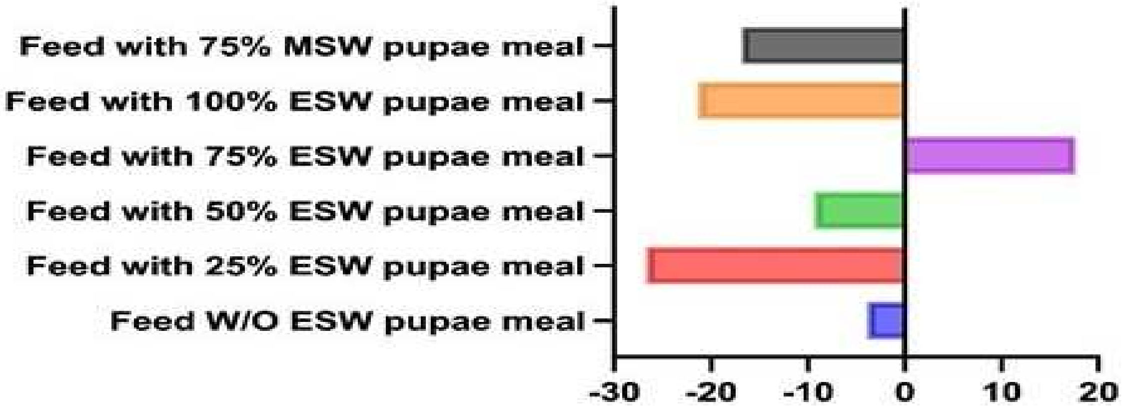


**Figure 2. Percent Increase in Cumulative Weight Gain (up to 28 days) of Japanese Quail Fed Diets Containing Eri Silkworm Pupae Meal Compared to a Positive Control Diet**

**Table 2. Effect of Dietary Eri Silkworm Pupae Meal on the Weekly Body Weight, Feed Intake, and Feed Conversion Ratio of Japanese Quail**

| Treatments* | Mean weight of quail (in grams) | | | | | Feed Conversion Ratio (FCR) | Total Feed Intake (g) |
|---|---|---|---|---|---|---|---|
| | Initial Weight | Days After Feeding | | | | | |
| | | 7 | 14 | 21 | 28 | | |
| **Feed without ES pupae meal** | 40.71 | $72.83^{ab}$ | $110.1^{b}$ | $147.4^{bc}$ | $182.0^{bc}$ | $2.62^{b}$ | 370 |
| **Feed with 25% ES pupae meal** | 38.42 | $61.63^{cd}$ | $91.6^{c}$ | $121.4^{d}$ | $146.2^{d}$ | $3.97^{f}$ | 425 |
| **Feed with 50% ES pupae meal** | 39.57 | $69.34^{b}$ | $105.3^{b}$ | $141.2^{bc}$ | $172.8^{bc}$ | $2.87^{c}$ | 383 |
| **Feed with 75% ES pupae meal** | 39.85 | $79.85^{a}$ | $125.5^{a}$ | $170.6^{a}$ | $212.6^{a}$ | $2.06^{a}$ | 365 |
| **Feed with 100% ES pupae meal** | 40.42 | $58.50^{d}$ | $91.7^{c}$ | $124.7^{d}$ | $152.0^{d}$ | $3.90^{e}$ | 433 |
| **Feed with 75% MS pupae meal** | 41.90 | $67.92^{bc}$ | $101.5^{bc}$ | $136.0^{cd}$ | $164.0^{cd}$ | $3.30^{d}$ | 406 |
| **Commercial quail feed (Positive Control)** | 40.57 | $73.40^{ab}$ | $112.7^{ab}$ | $152.9^{b}$ | $187.4^{b}$ | $2.59^{b}$ | 379 |
| **SE(d)** | NS | 0.237 | 0.293 | 0.340 | 0.376 | 0.058 | - |
| **C.D (0.05)** | NS | 0.475 | 0.587 | 0.681 | 0.753 | 0.117 | - |

a-f Means in the same column with different superscripts differ significantly (P<0.05).
*ES – Eri silkworm; MS – Mulberry silkworm.
C.D – Critical Difference; SE(d) – Standard error of difference.

Similarly, Khatun *et al.* (2003) noted that increasing MS pupae meal in diet led to better growth, meat yield, and profitability. However, Valerie *et al.* (2015) reported that replacing only 50% of the fish meal by silkworm pupae was the best balance. Dutta *et al.* (2012) observed drop in feed intake and weight gain when more than 50% of fish meal was replaced with MS pupae meal. They believed that MS pupae might contain substances that interfere with nutrient digestion. Likewise, harmful effects were also noted when Muga silkworm pupae was used (Mahanta *et al.* 2004). Studies also reported poor nutrient absorption in chickens due to high chitin content of MS pupae (Razdan and Pettersson, 1994; Makkar *et al.* 2014). Previous research has shown that chickens possess intrinsic chitinolytic activity in their gastrointestinal tract, enabling them to digest chitin with an efficiency ranging from 67% to 92% (Han *et al.* 1997). However, the quail can partially digest chitin, the deacetylation process converts chitin into chitosan. Chitosan is known to form complexes with dietary nutrients, primarily proteins and lipids, which can hinder nutrient degradation and reduce overall digestibility (Jayanegara *et al.* 2020). Additionally, the bioactive substance 1-deoxynojirimycin (1-DNJ) is known for its antihyperglycemic and anti-obesity properties. It effectively inhibits α-glucosidase enzymes, which are responsible for breaking down starch into absorbable monosaccharides in the intestine or by competitively inhibiting specific enzymes involved in glycogenolysis, glycoprotein, and saccharides hydrolysis (Gao *et al.* 2016). Zotte *et al.* (2021) found that quails fed diets made from full-fat or defatted MS pupae had poor fattening results due to the presence of chitin and DNJ. Tsuduki *et al.* (2013) reported that even a small amount of 1-DNJ (0.107 mg/100 g) in the diet could inhibit starch digestion in growing quails, reduce fatty acid synthesis and increase fatty acid oxidation that leads to less fat accumulation.

Kongsup *et al.* (2022) investigated the impact of incorporating Eri silkworm pupae (5%, 10%, and 15%) into broiler diets and indicated that a 10% inclusion rate led to increased weight gain, cold carcass weight, and improved feed conversion ratio. Conversely, a higher inclusion rate of 15% resulted in negative outcomes. The authors attributed these performance variations to differences in amino acid profiles and digestibility between soybean meal and ES pupae meal. This might the reason of poor growth performance of quails with low and high level of ES pupae meal. In the present study the fish meal was substituted with ES pupae meal resulted in better performance at a 75% replacement level. This suggested that the source of nutrients, rather than the mere quantity, significantly influences treatment efficacy. Eri silkworm pupae offer a broader spectrum of nutrients compared to conventional fish meal. Consequently, diets relying heavily on fish meal (25% and 50% ES pupae meal) exhibited suboptimal performance. Growth performance in birds is influenced not only by the level of insect meal addition but also by the meal's chemical composition and digestibility (Hong *et al.* 2020; Chodova and Tumova, 2020). Zotte *et al.* (2024) found that adding 4% silkworm pupae meal (SWM) to broiler chicken feed during the grower-finisher phases increased n-3 fatty acids in both breast and leg meat, improving the omega-6/omega-3 ratio. Likewise, Singh *et al.* (2023) demonstrated that adding full-fat silkworm pupae meal (SWM) to laying quail feed, at levels of 8% and 12%, increased egg production but also resulted in higher feed consumption compared to the control group. While increasing SWM inclusion led to a larger edible portion of the egg, it also reduced shell weight. These findings suggest that silkworm pupae meal can be a suitable dietary supplement for laying quails.

The average carcass weight and dressing percentage of male Japanese quail were 126 g and 71%, respectively (Murali, 2019). Quails fed 75% ES pupae meal diet showed significantly ($P \leq 0.01$) higher carcass weights than those fed control diet, feed without ES pupae meal, and 75% MS pupae meal ($P \leq 0.0001$). However, non-significant differences were observed in dressing percentage among the treatments (Figure 3 & 4). Quails fed 75% ES pupae meal-based diet resulted in significantly higher levels of serum total protein, cholesterol, and antioxidants than the control group ($p<0.05$). In contrast, quails fed diet with 25 and 100% ES pupae meal resulted in significantly low levels of biochemical parameters. These findings suggests that 75% ES pupae meal diet provided better nutrition to quails thus better live performance with higher levels of blood biochemical parameters was noted. Anggraeni *et al.* (2016) investigated the effects of different ratios of silkworm pupae powder extract (maceration extraction method with 95% ethanol as a solvent), residues of pupae powder extract and pupae powder on blood profile of Japanese quail and concluded that addition of pupae powder extract at 10% significantly improved the white blood cells and better weight gain. Non-significant difference observed pertaining to the erythrocytes, hemoglobin, and packed cell volume among different treatment groups. This finding indicates that silkworm pupae did not negatively impact the serum characteristics of quails.

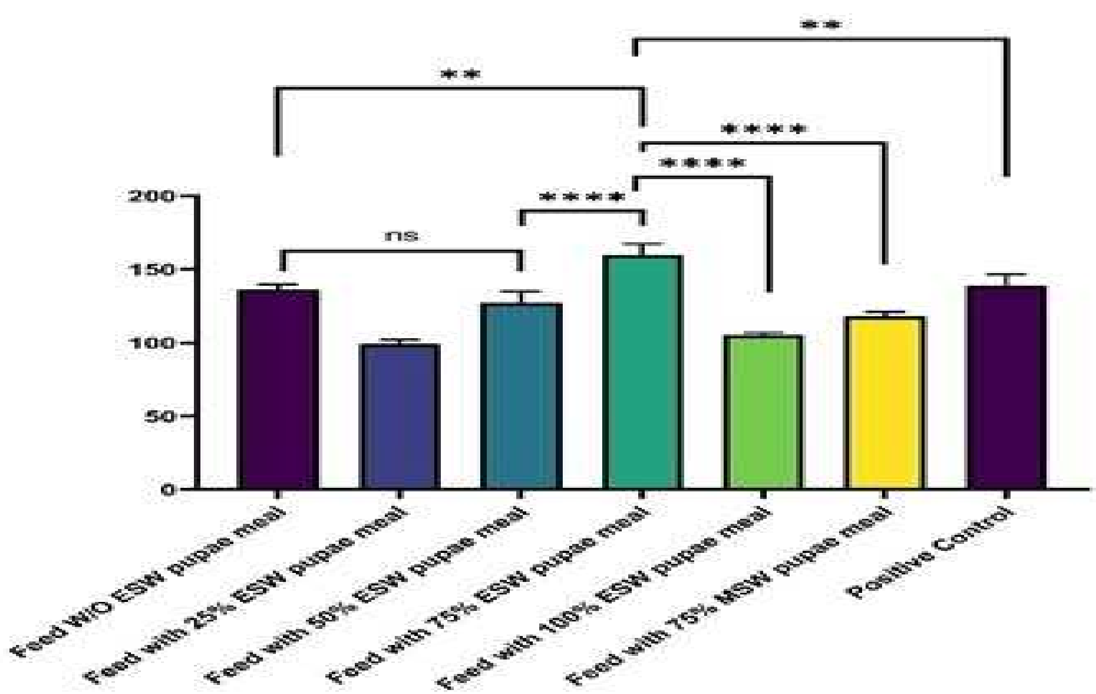


**Figure 3. Carcass Weight of Japanese Quail Fed Diets Containing Eri Silkworm Pupae Meal**

Values indicated with ns – non significant; ** - P ⩽ 0.01; **** - P ⩽ 0.0001 (Tukey's test)

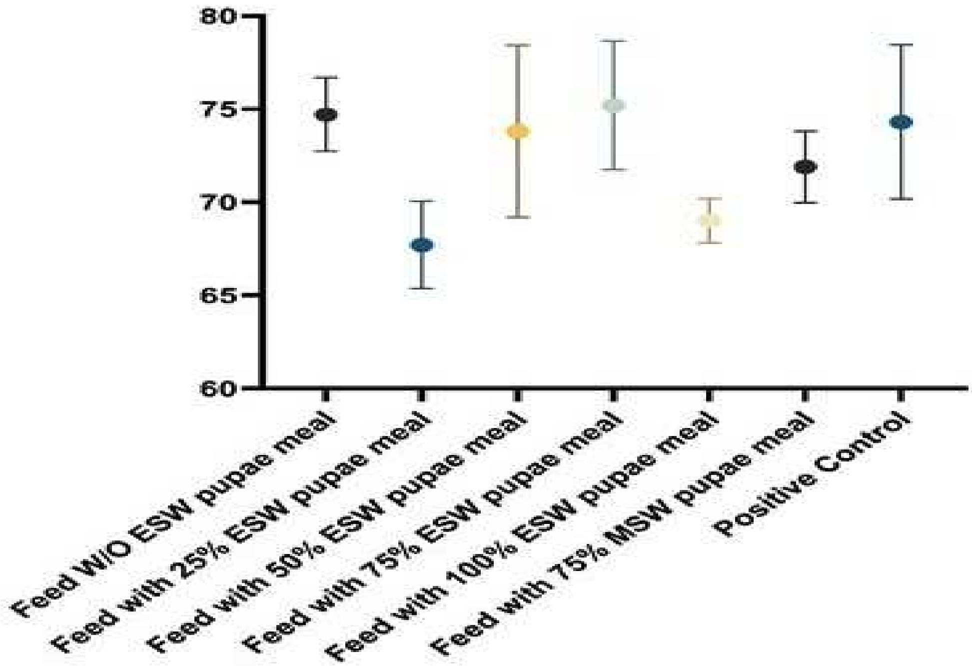


**Figure 4. Dressing Percent of Japanese Quail Fed Diets Containing Eri Silkworm Pupae Meal Non-significant values recorded among treatments (p>0.05) (Tukey's test)**

Actually, utilization of eri silkworm pupae meal in animal feed is the novel strategy, only a one or two studies were done so far. Thereby the use of mulberry silkworm pupae and other insect meals in various poultry feed studies was compared in the discussion chapter. Higher alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities highlight the stressful condition of an organism. Non-significant values pertaining to ALT and AST activities recorded among all treatment groups (Table 3) indicated no harmful effects of dietary treatments on birds. The variations in MS and ES pupae nutrient composition might be the reason for the better performance of 75% ES pupae meal-based diet over corresponding MS pupae meal-based diet. The ES pupae meal contains 1.31 times more fat, 1.16 times more protein, and 1.12 times more zinc than the MS pupae meal. Further, it contains four times fat than the fish meal ($p<0.05$). Importance of proline in whole-body protein synthesis compared with other amino acids (Wu *et al.* 2011) justified better quails' growth in ES pupae meal-based diet than MS pupae meal and fish meal-based diets. Further, ES pupae meal's total amino acid contents were optimal and comparable with MS pupae and fish meal (Table 4 & 5). Both the proximate composition as well as the essential amino acid composition of ES pupae were almost similar as recorded by Longvah *et al.* (2011). The non-significant effect on blood ALT and AST activities indicated the safety of the meal for quail birds.

**Table 3. Serum Biochemical Profile of Japanese Quail Fed Diets with Varying Levels of Eri Silkworm Pupae Meal**

| Treatments* | Total protein (g/dl) | Cholesterol (mg/dl) | ALT (U/ml) | AST (U/ml) | Total antioxidant (mM/L) |
|---|---|---|---|---|---|
| **Feed without ES pupae meal** | 5.6±0.05[c] | 103±0.05[d] | 38.1±0.04 | 30.9±0.03 | 5.3±0.07[c] |
| **Feed with 25% ES pupae meal** | 3.4±0.03[d] | 102±0.03[d] | 38.7±0.08 | 31.1±0.09 | 3.1±0.04[d] |
| **Feed with 50% ES pupae meal** | 5.5±0.15[c] | 114±0.03[b] | 37.9±0.10 | 30.4±0.06 | 5.4±0.18[c] |
| **Feed with 75% ES pupae meal** | 10.4±0.10[a] | 121±0.15[a] | 38.6±0.03 | 30.7±0.05 | 9.96±0.10[a] |
| **Feed with 100% ES pupae meal** | 3.5±0.07[d] | 91.9±0.04[e] | 39.0±0.03 | 29.9±0.10 | 3.4±0.16[d] |
| **Feed with 75% MS pupae meal** | 5.8±0.03[c] | 115±0.07[b] | 38.0±0.05 | 30.7±0.07 | 6.1±0.04[b] |
| **Commercial quail feed (Positive Control)** | 6.8±0.02[b] | 109±0.17[c] | 37.8±0.20 | 30.9±0.01 | 6.50±0.21[b] |

[a-e]Means in the same column with different superscripts differ significantly (P<0.05).
Values are not significantly different.
*ES – Eri silkworm; MS – Mulberry silkworm.

**Table 4. Nutrient Profile, Mineral Contents and Amino Acid Compositions of Eri Silkworm Pupae, Mulberry Silkworm Pupae, and Fish Meal.**

| Nutrients* | Eri silkworm pupae meal | Mulberry silkworm pupae meal | Fish meal |
|---|---|---|---|
| **Protein (%)** | $60.6 \pm 0.22^{b}$ | $51.95 \pm 0.11^{c}$ | $67.0 \pm 0.32^{a}$ |
| **Fat (%)** | $24.36 \pm 0.10^{a}$ | $18.60 \pm 0.23^{b}$ | $6.10 \pm 0.91^{c}$ |
| **Carbohydrates (%)** | $3.55 \pm 0.09^{c}$ | $5.60 \pm 0.89^{a}$ | $4.35 \pm 0.33^{b}$ |
| **Zinc mg/100 g** | $7.34 \pm 0.23^{a}$ | $6.50 \pm 0.67^{b}$ | $4.55 \pm 0.39^{c}$ |
| **Iron mg/100 g** | $23.8 \pm 0.71^{b}$ | $34.50 \pm 0.78^{a}$ | $19.0 \pm 0.78^{c}$ |
| **Calcium mg/100 g** | $67.0 \pm 0.91^{c}$ | $70.1 \pm 0.50^{b}$ | $83.90 \pm 1.20^{a}$ |
| **Phosphorus mg/100 g** | $56.0 \pm 1.90^{a}$ | $45.0 \pm 1.50^{b}$ | $39.6 \pm 0.40^{c}$ |
| **Methionine (g /100 g)** | $2.19 \pm 0.10^{c}$ | $3.50 \pm 0.11^{b}$ | $3.90 \pm 0.20^{a}$ |
| **Cystine (g /100 g)** | $1.11 \pm 0.13^{c}$ | $2.35 \pm 0.52^{a}$ | $1.60 \pm 0.11^{b}$ |
| **Lysine (g /100 g)** | $7.90 \pm 1.10^{b}$ | $8.90 \pm 0.45^{a}$ | $7.10 \pm 0.41^{c}$ |
| **Threonine (g /100 g)** | $3.94 \pm 0.43^{c}$ | $4.70 \pm 0.82^{a}$ | $4.30 \pm 0.55^{b}$ |
| **Phenylalanine (g /100 g)** | $6.10 \pm 0.22^{a}$ | $5.80 \pm 0.19^{b}$ | $4.12 \pm 0.71^{c}$ |
| **Leucine (g /100 g)** | $5.90 \pm 0.63^{c}$ | $7.70 \pm 1.01^{b}$ | $7.90 \pm 0.33^{a}$ |
| **Aspartic Acid (g /100 g)** | $9.51 \pm 0.23^{b}$ | $10.90 \pm 0.34^{a}$ | $9.14 \pm 0.80^{c}$ |
| **Serine (g /100 g)** | $4.98 \pm 0.64^{a}$ | $4.10 \pm 0.83^{b}$ | $4.78 \pm 0.28^{a}$ |
| **Proline (g /100 g)** | $6.12 \pm 0.92^{a}$ | $4.50 \pm 0.32^{b}$ | $4.50 \pm 0.83^{b}$ |
| **Glycine (g /100 g)** | $4.39 \pm 0.92^{c}$ | $5.11 \pm 0.78^{b}$ | $6.24 \pm 0.69^{a}$ |
| **Alanine (g /100 g)** | $5.90 \pm 0.27^{b}$ | $4.80 \pm 0.97^{c}$ | $6.39 \pm 0.22^{a}$ |

[a-c]Means in the same row with different superscripts differ significantly (P<0.05).

**Conclusion:** The present study highlights the potential of eri silkworm pupae meal as a valuable alternative protein source to fish meal in Japanese quail diets. The 75% replacement rate with eri silkworm pupae meal optimizes growth performance and overall health parameters**.** Excessive incorporation of the meal (beyond 75%) negatively affected growth performance, underscoring the importance of a balanced dietary approach.

**Acknowledgment:** The authors are very grateful to the Department of Entomology and Division of Animal Husbandry, Annamalai University, Tamil Nadu, India for providing all the necessary facilities to conduct the experiment.

**Authors Contribution:** TS and SK conceived and design the experiment. RKG executed the study, data collection, and carry out the sample analysis. RKG, TS, and SK all were involved in the preparation of the manuscript. TS and SK revise and finetuned the manuscript.

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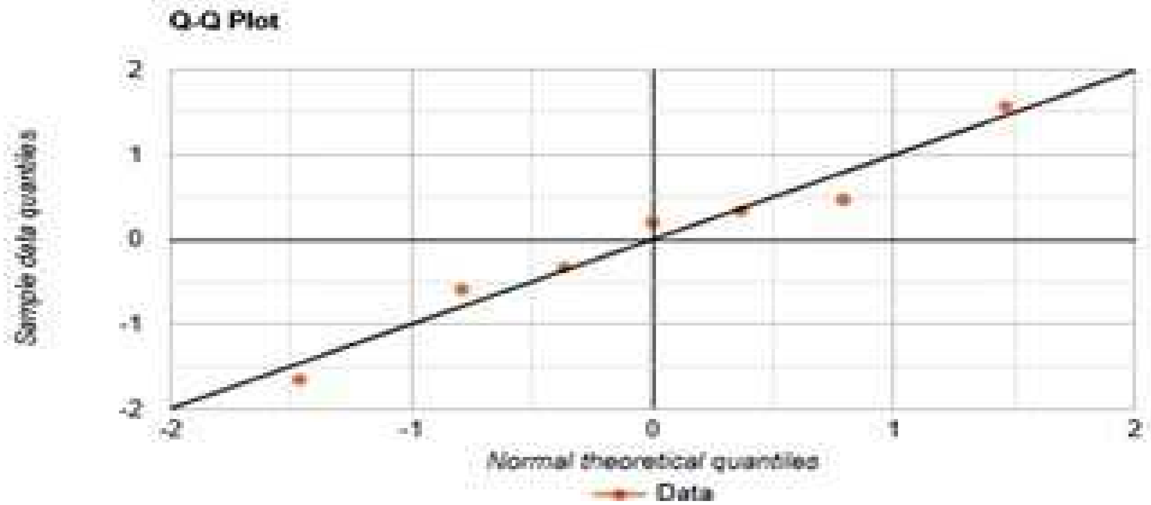
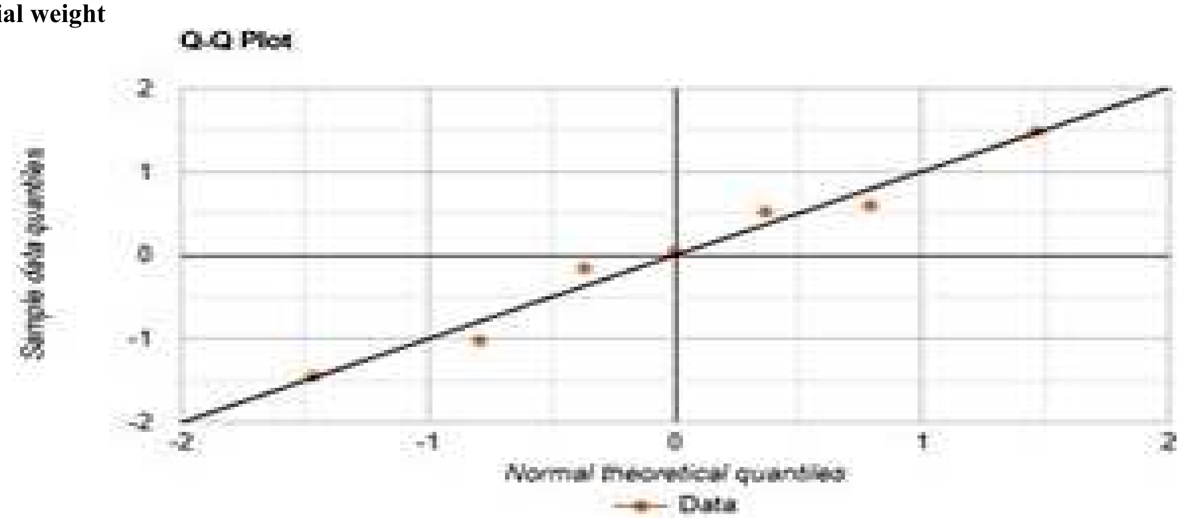
**Supplementary data pertaining to normality test suggested by the reviewer**

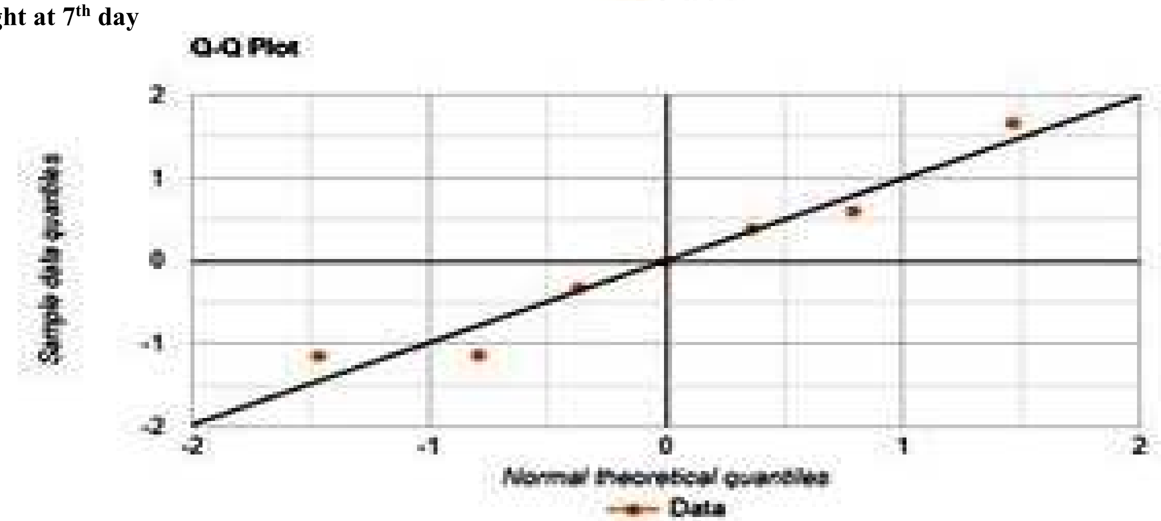
**Shapiro-wilk test** by using Statistical kingdom online software (https://www.statskingdom.com/shapiro-wilk-test-calculator.html)



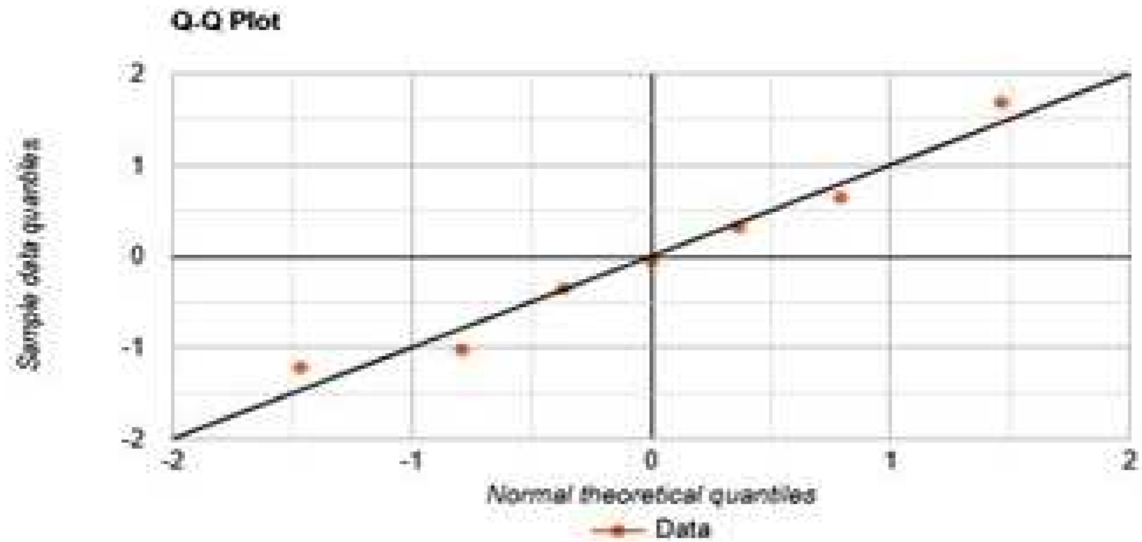

**Initial weight**



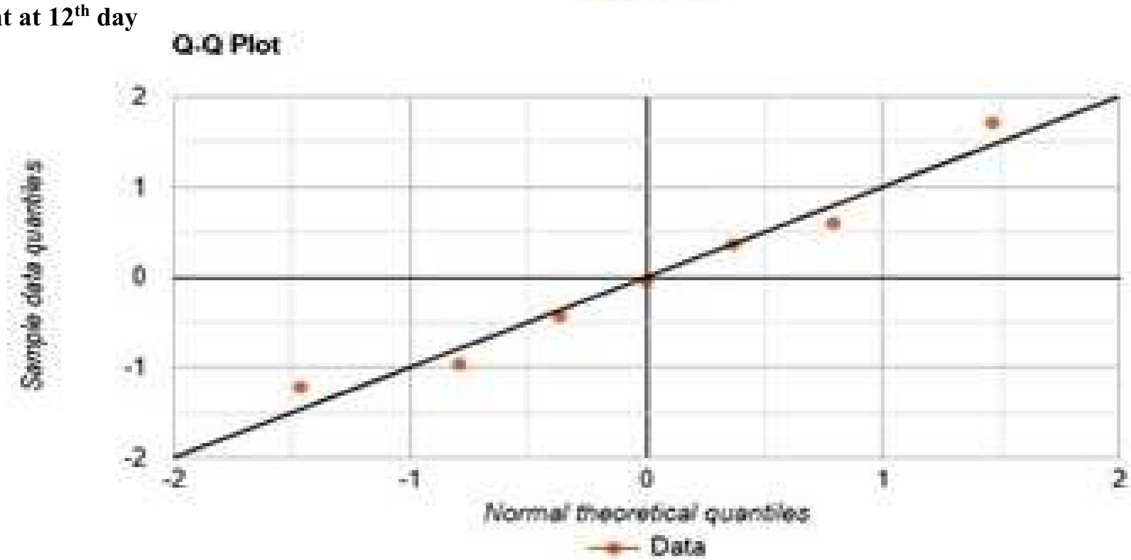

**Weight at 7th day**



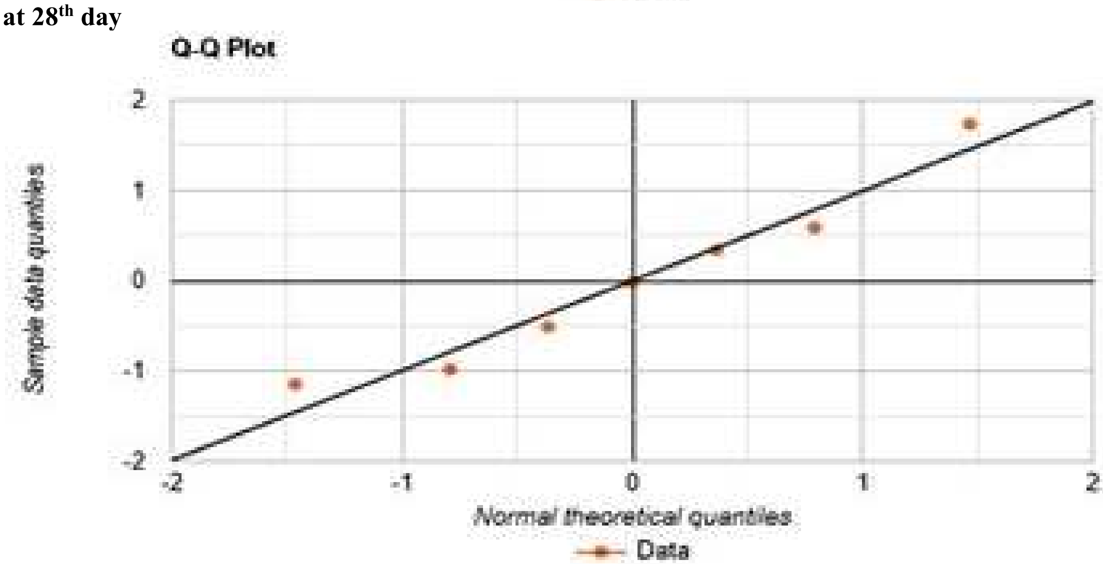

**Weight at 14th day**




**Weight at 12th day**




**Weight at 28th day**




**Final weight gain**

All the data were normally distributed hence, data were subjected to parametric test (one way-ANOVA) in the present research.

**Anova: Two-Factor Without Replication**

| *SUMMARY* | *Count* | *Sum* | *Average* | *Variance* |
|---|---|---|---|---|
| Feed without ES pupae meal | 5 | 553.04 | 110.608 | 3190.909 |
| Feed with 25% ES pupae meal | 5 | 459.25 | 91.85 | 1898.8 |
| Feed with 50% ES pupae meal | 5 | 528.21 | 105.642 | 2864.507 |
| Feed with 75% ES pupae meal | 5 | 628.4 | 125.68 | 4760.026 |
| Feed with 100% ES pupae meal | 5 | 467.32 | 93.464 | 2110.352 |
| Feed with 75% MS pupae meal | 5 | 511.32 | 102.264 | 2443.339 |
| Commercial quail feed (Positive Control) | 5 | 566.97 | 113.394 | 3485.237 |
| Initial Weight | 7 | 281.44 | 40.20571 | 1.170495 |
| 7 | 7 | 483.47 | 69.06714 | 52.92812 |
| 14 | 7 | 738.4 | 105.4857 | 145.4481 |
| 21 | 7 | 994.2 | 142.0286 | 287.7024 |
| 28 | 7 | 1217 | 173.8571 | 515.2762 |

**ANOVA**

| *Source of Variation* | *SS* | *df* | *MS* | *F* | *P-value* | *F crit* |
|---|---|---|---|---|---|---|
| Rows | 4172.754 | 6 | 695.4589 | 9.059396 | 3.19484E-05 | 2.508188823 |
| **Columns** | **81170.29** | **4** | **20292.57** | **264.3412** | **1.81895E-19** | 2.776289289 |
| Error | 1842.398 | 24 | 76.76659 | | | |
| Total | 87185.44 | 34 | | | | |

The column represents the weekly weight measurements, so it is highlighted.