International Journal of Agricultural Technology 2022Vol. 18(4):1381-1392
Available online http://www.ijat-aatsea.com
ISSN 2630-0192 (Online)

The differences in hatching chick weight, fertility, hatchability, and embryonic mortality of Japanese quail fed Black Soldier Fly Larvae (BSFL) and anchovy by-product as protein sources

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Abdul Aziz, M. R., Rusli, N. D., Rahman, M. M., Zulhisyam A. K., Harun, H. C., Al-Amsyar, S. M., Mahmud M. and Mat, K. (2022). The differences in hatching chick weight, fertility, hatchability, and embryonic mortality of Japanese quail fed Black Soldier Fly Larvae (BSFL) and anchovy by-product as protein sources. International Journal of Agricultural Technology 18(4):1381-1392.

Abstract The anchovy waste and Black Soldier Fly Larvae (BSFL) have the potential to be used as protein substitutes in poultry feed because they are less expensive and easier to obtain than other sources of animal protein. The results from this study showed for the chick weight, the control group recorded the lowest weight with 6 grams compared to the Treatments 1 and 2 with 7.5 grams respectively. The egg weight was significantly different ($p<0.05$) for the Treatment 1 (50% BSFL + 50% anchovy waste) and 2 (25% BSFL + 75% anchovy waste), while the differences among chick weight ($p>0.05$), on the other hand, it had no significant differed in all groups. The highest egg production was found in Treatment 1. However, Treatment 2 was resulted the highest FR ($p>0.05$), HR ($p>0.05$) and HFE ($p>0.05$) as 100%, 21.53% and 21.53%, respectively. The total embryonic mortality rate for the treatment 2 (84.62%) was the highest ($p>0.05$) as compared to 65.38% and 40% for the treatment 1 and control group, respectively. In conclusion, this analysis showed a positive impact which was evidenced by the treatment 2 group consisting of 25% BSFL and 75% anchovy by-products as a protein source composition.

Keywords: Black soldier fly larvae (BSFL), Anchovy by-products, Hatchability, Fertility, Embryonal Mortality

Introduction

The new global economy, quails have become an attraction in increasing the consumers' demand for them, especially in Malaysia. Among Malaysian, poultry meat is well-known and well-consumed as a source of protein. It is preferable to consume white meat more than red meat. According to DVS (2020), Malaysians consumed about 49.3 kilograms of poultry per

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capita in 2020. This is expected to hit about 51.28 kilograms per capita by 2025. Malaysians consumed more poultry meat in 2019 than the OECD average. The trend of quails' meat consumption links positively with the consumption of poultry meat. The Japanese quail is a member of the order *Galliformes*, genus *Coturnix* and specific epithet *japonica*. (*Coturnix japoni*ca) is the scientific name for Japanese quail, distinct from common quail (*Coturnix coturnix*). As a migrant bird, the Japanese quail can be found in Japan, Korea, Eastern China, Mongolia, and Sakhalin (Mizutani, 2003). The rise in popularity and demand among Malaysian customers is anticipated that quail rearing would increase dramatically to satisfy the high demand of the local and foreign markets (Mnisi and Mlambo, 2018).

High fertility, hatchability, and low embryonic mortality are the primary goals of poultry rearing for profitability and the value of effective productivity. According to Uğurlu *et al*. (2017)., egg weight, length of capability time, flock age, breeding type, hen diet composition, and sex proportion affect fertility, hatchability, and early-stage mortality in poultry species and also pointed out that the protein content of the hen diet is one of the factors affecting egg development and egg weight as it is accompanied by some studies that showed the positive increment between protein level in the diet composition and egg production and egg weight. It is well understood that the composition of energy and protein level in the diet is essential for the breeder. As supported by study that discovered reducing the dietary protein content of isoenergetic hen diets from 16.80% to 14.40% reduced egg weight. Li *et al*. (2013) found that the average optimal metabolism energy (ME) and crude protein (CP) intake levels for optimizing egg production in Lohmann Brown laying hens were 2591 to 2683 kcal/kg and 15.58% to 16.64% CP. It has also been reported that diets high in protein but low in energy have a negative effect on egg weight and growth (Li *et al.*, 2013).

In order to fulfil the nutrition requirement of the layer to produce eggs in optimum condition, the farmers are burdened with the feed cost. However, the cost of raw feed materials is increasing, especially for protein sources such as fish, bone, and soybean (Kari *et al.*, 2022; Kari *et al.*, 2021). Protein sources of feed play a vital role in the development of animal tissues. As a result, good protein sources are needed for animal growth and tissue development, which impact growth efficiency and development (Rahman *et al*., 2021). Most protein supplies used to manufacture feed in Malaysia are imported from other countries, which is expensive and influences production costs. As a result, it is important to find local raw materials that can be used to substitute or complement manufactured protein supplies (Mat *et al.*, 2022). On the other hand, anchovy waste and Black Soldier Fly Larvae (BSFL) have the potential to be used as protein substitutes in poultry feed (Jansen, 2018). Anchovy waste and BSFL are less costly and more accessible to obtain than fishmeal and soybean meal.

Anchovies-by-products consists of the head and guts of anchovies that are discarded rather than used in another way. As a consequence, the by-product can be used in a variety of ways. According to the findings of a report conducted by Gencbay and Turhan (2016), the gross yield of by-products from anchovy was approximately 32% of the entire fish based on wet weight. Anchovy by-products had protein and fat contents of 13.39 and 10.02% for the head, 16.47 and 15.50 % for the frame, and 12.05 and 23.90% for the viscera, respectively.

The black soldier fly (*Hermetia illucens*) is a world traveller. It has extended through all continents and into neotropical regions (Kinasih *et al.*, 2018). About its wasp-like nature, BFS is entirely harmless (Mat *et al*., 2021). It does not transmit any established viruses, and it does not bite or sting. BSFL proliferate and have a high feed turnover rate (Shumo *et al.*, 2019). In ideal conditions, full-size larvae can be harvested in just two weeks. The time required is determined by the temperature and the consistency of the feed (Al-Qazzaz *et al.*, 2016). There are some variations in importance in the nutritional content of BSFL at various periods of the life cycle. The crude protein in the egg was 45% of CP but then increased to 56% in neonatal stages after hatching for one day. There are some variations in importance in the nutritional content of BSFL at various periods of the life cycle (Cockcroft, 2018). However, crude protein content decreases with age; the highest percentage was recorded for five days-old larvae (61%), whereas it was lower to 15 (44%) and 20 (42%) day old larvae (Barragan-Fonseca *et al.*, 2017).

In part to improve quail breeding production, this study aimed to determine the effects of feeding black soldier fly larvae (BSFL) and anchovy by-product on egg and hatching chick weight, fertility, hatchability, and embryonal mortality in Japanese quail.

Materials and methods

Designation of group, housing and feeding

A total of 36 six-week-old quails were used and reared in battery cages at the Agro Techno Park, University Malaysia Kelantan, Jeli Campus. The quails were divided into three groups, which consisted of one Control (C1), and two treatments group, Treatment 1 (T1) and Treatment 2 (T2). Each group was made up of three replicates and each replicate of the group had four quails each, one male and three female which being fed with different feed according to each group. Each group was treated with different in feed as in Table 1. Treatment 1 was fed the formulated feed consisted of 50% of BSFL and 50% anchovy by-products, whereas Treatment 2 was fed 25% of BSFL and 75% anchovy by-products constitution of feed and control, on the other hand, it was fed with 100% of soybean meal. The newly formulated feed was formulated for layer quails (NRC, 1994; Yan *et al.*, 2010). The feeding of quails was operated twice a day, morning and evening. The drinker and feeder are cleaned every day to avoid contamination. The quails were fed ad-lib and the leftovers were measured every morning.

Table 1. Composition of ingredients and nutrient of formulated feed

	INGREDIENTS		
	CONTROL	**T1**	**T2**
Anchovies by product	0	19.45	28.5
BSFL	0	18.55	9.5
Corn meal	33.29	33	32.51
Whole wheat meal	10.79	18.3	21.49
Soybean meal	40.18	0	0
Dicalcium phosphate	4.29	3.45	3
L lysine	2.29	1.45	1
Dl methionine	2.29	1.45	1
Limestone	2.29	1.45	1
Vegetable oil	2.29	1.45	1
Premix	2.29	1.45	1
TOTAL	100	100	100
NUTRIENT COMPOSITION (% as if not stated)			
Energy {Kcal/kg}	3080.756	3030.979	2970.07
Crude protein	19.966	19.72	20
Crude fibre	1.582	0.806	0.884
Crude fat	3.592	6.009	5.412
Ash	5.08	3.761	2.841
Moisture	11.112	12.475	13.769

TI: Treatment 1 (50% BSFL + 50% anchovy waste)
T2: Treatment 2 (25% BSFL + 75% anchovy waste)

Collection and storage of eggs

The eggs were collected once a day, early in the morning, and recorded daily. The selection of eggs depended on the egg weight; the selected egg weight was 9-11g because the average of the quail eggs was 10.8g, and it varied which depending on the age of the flock (Tserveni-Goussi and Fortomaris, 2011), cleaned egg without any dirt and uncracked eggshells to avoid contamination. The selected eggs were placed on trays at the temperature of

15 ℃ for as suggested by Randall and Bolla (2008) at least a week and then being transferred to the incubator gently to avoid the eggshell cracked because cracked egg hatch poorly may contaminate the whole incubator.

Hatching process and evaluation of incubation results

The incubation for quail ranged between 17 and 18 days. The used incubator is forced-draft incubators. Forced-draft incubators were operated at 37.5±0.3 ℃ with a relative humidity of 60% and a wet-bulb temperature of 30±0.5 ℃ before the 14th day of incubation. Eggs were turned every 2– 4 hours to keep embryos from adhering to the cell. The incubator was checked daily. On the 14th day, any cracked eggs were discarded. The eggs turner was stopped, and the eggs were in hatching trays. The used incubator was both setter and hatcher, the temperature was 37.5 ℃, but the relative humidity raised to 70% wet bulb 32.2 ℃ during hatching. During the hatching process, the hatcher was not opened. The chicks were removed on the 17th or 18th day according to method of Randall and Bolla (2008).

On the 18th day of incubation, the hatching eggs were monitored. All groups' hatching chicks' weight was determined by weighing them one by one, on electronic scales sensitive to 5 g when the chicks' bodies thoroughly dried up. In addition, the unhatched eggs were examined with naked eyes. In the macroscopic study, the stage of embryo formation at death was divided into three potential death stages. Early-stage embryonic mortality (EPEM), which embryo formed, filling the eggshell, and development of eyes, middle period embryonic mortality (MPEM) where feathers were developed, and more of the yolk sack external to the body and late period embryonic mortality (LPEM) were 2/3 or whole of yolk sack in the body of the embryo.

The fertile eggs, chick number of hatched and all eggs were used to measure fertility rate (FR), hatchability rate (HR) or hatchability of incubated eggs, and hatchability of fertile eggs (HFE). EPEM, MPEM, and LPEM were also measured as early-stage embryo mortality divided by fertile eggs, middle period embryo mortality per fertile eggs, and late-period embryo mortality divided by fertile eggs, respectively. All the data measurement of the hatchability rate were measured according to the type of strains and calculated according to Wahab *et al.* (2018).

$$(\%) Fertility = \frac{number\ of\ fertilized\ eggs}{total\ number\ of\ eggs\ placed\ into\ incubator}\ x\ 100$$

$$(\%) Hatchability\ of\ incubated\ eggs = \frac{number\ of\ released\ chicks}{total\ number\ of\ eggs\ placed\ incubator}\ x\ 100$$

$$(\%) Hatchability\ of\ fertile\ eggs = \frac{number\ of\ released\ chicks}{number\ of\ fertilized\ eggs\ placed\ in\ incubator}\ x\ 100$$

Statistical analysis

All data were analysed using One-Way Analysis of Variance (ANOVA) by IBM SPSS statistics version 23. The differences among means were identified using Duncan's multiple range tests. The level of significance was determined at $p<0.05$ for all analyses.

Results

Egg weight and hatching chick weight

The means of egg weight and hatching chick weight for each group are presented in Table 2. The egg weight for Treatment 1 was the lowest compared to the Control and Treatment 2 group. Meanwhile, for the chick weight, the control group recorded the lowest. There was a significant difference among eggs weight ($p<0.05$) for quails in Treatment 1 compared to Treatment 2. However, there were no significant difference ($P>0.05$) in egg weight and hatching weight between control group and both treatment groups. In this study, the mean egg weight and hatching chick weight were 9.40 g and 0.00 g for the control group; 9.23 g and 0.58 g for Treatment 1 group; 9.97 g and 1.31g for Treatment 2 group, respectively. These results showed that the Treatment 2 group recorded the highest hatching chick weight and egg weight. It showed that the composition of 25% of BSFL and 75% anchovy by-products in treatment 2 groups were influenced on the quails.

Table 2. Egg and hatching chick weight by groups

Group	n	Egg weight (g)	n	Hatching weight (g)
Control	15	9.40 ± 0.25^{ab}	1	6.00
Treatment 1	26	9.23 ± 0.17^{a}	2	7.50 ± 0.71
Treatment 2	26	9.97 ± 0.19^{b}	4	7.50 ± 0.58

^{ab} is significant differences at the ($p<0.05$).

Fertility and hatchability

The data of total egg production, fertile and infertile eggs, and chick number were recorded, and the value for the fertility rate (FR), hatchability rate (HR) or hatchability of incubated eggs, and hatchability of fertile eggs (HFE) were stated in Table 3. Egg production was determined as 22, 48 and 38 for the Control group, Treatment 1 and Treatment 2 groups. The production of eggs of Treatment 1; composition of 50 % BSFL and 50 % anchovy waste in the feed formulation showed the highest. Treatment 2 was the highest FR (p>0.05), HR (p>0.05) and HFE (p>0.05). The fertility rate (FR), hatchability rate/ hatchability of incubated egg (HR), and hatchability of fertile eggs (HFE) were determined as 71.43%, 3.57%, 8.34% for the control group, 73.81%, 7.74%, 10.56% for Treatment 1 and 100%, 21.53% and 21.53% for the Treatment 2 group, respectively.

Table 3. Values of egg production, total egg incubated, fertile and unfertile eggs, number of chicks hatched, FR, HR and HFE

Group	Egg production (n)	Total egg (n)	Fertile egg (n)	Unfertile egg (n)	Chick number (n)	FR (%)	HR (%)	HFE (%)
Control	22	15	7	8	1	71.43	3.57	8.34
T1	48	26	19	7	2	73.81	7.74	10.56
T2	38	26	26	0	4	100	21.53	21.53

FR: Fertility, HR: Hatchability Rate, HFE: Hatchability of Fertile Eggs
TI: Treatment 1 (50% BSFL + 50% anchovy waste)
T2: Treatment 2 (25% BSFL + 75% anchovy waste)

Embryonal mortality

The mean values of embryonal mortality for the control group, Treatment 1 and Treatment 2 groups within two cycles are given in Table 4. It was determined that the highest embryonic mortality mean rates were 0.75 in control group, 0.20 in treatment 1 and 2 groups and 0.32 in treatment 1 group for EPEM, MPEM, LPEM, respectively.

Table 4. Embryonic mortality means rates for Control, Treatment 1 and Treatment 2 groups

Group	EPEM	MPEM	LPEM
Control	0.75 ±0.25	0.08 ±0.08	0.08 ±0.08
Treatment 1	0.37 ±0.07	0.20 ±0.09	0.32 ±0.02
Treatment 2	0.35 ±0.02	0.20 ±0.08	0.23 ±0.10

EPEM: Early Period Embryonic Mortality, MPEM: Middle Period Embryonic Mortality, LPEM: Late Period Embryonic Mortality.

The total embryonic mortality rate for the Treatment 2 (84.62%) was the highest as compared to 65.38% and 40% for the Treatment 1 and control group, respectively. It was observed that there was a linear increase between protein composition in the feed and EPEM (p>0.05), MPEM (p>0.05), and LPEM (p>0.05).

Discussion

Egg weight and hatching chick weight

According to Uğurlu *et al.* (2017), many researchers reported that different protein levels in hen diets had different effects on egg weight in poultry species. There also reported the constructive outcomes of dietary protein on egg weight (Cho *et al.*, 2004). As a result, the relationship between egg weight and protein levels in the hen diet is critically related (Shim *et al.*, 2013). It was also discovered that there was a link between egg weight and hatching chick weight. According to the previous study, substituting a fish meal with a BSF maggot meal up to 50% affected egg weight. This implies that replacing fish meal with maggot meal resulted in to increase in the weight of quail eggs. The BSFL replaced 50% of fish meals without affecting egg production, egg weight, and eggshell strength. The substituting BSF maggot meal for fish meal produced the exact weight of eggs as the control diet by up to 75%. (Widjastuti *et al*., 2014). This study resulted in an increase in egg weight for both treatment groups and was significantly compared to the control group. In this study, Treatment 2 contained 25% BSFL and 75% anchovy by-products substitution of the protein sources which recorded the highest egg weight and hatching chick weight. It finally pointed out that there are significantly related that the protein level positively affected egg weight and hatching chick egg.

Fertility and hatchability

In poultry breeding, it has been reported that protein diet and energy levels are essential factors in egg production. Some studies found that increasing dietary protein content increased egg production (Petek, Baspinar and Ogan, 2003). However, another study found that low protein diets had a negative effect on egg production (Uğurlu *et al.*, 2017). This finding showed no statistically significant difference between the different sources of protein and protein composition in the feed between fertility and hatchability of eggs. It may be due to the shell thickness of the eggs and the egg weight (Deeming and Wadland, 2002). These results were in line with the reports of Abdurehman and Urge (2016) who revealed that the most impactful egg parameters influencing hatchability are weighted, shell thickness and porosity, and content consistency. Maurer *et al*. (2016) also firmly agreed that the current research would not affect the total production of eggs by quail if the intake of maggot meal increased or decreased. It did not generate significant results, but it was revealed in this experiment that the quail produces eggs daily. The results demonstrated that the nutrients provided to the quail with sufficient for the quail's egg production. There were reported that the substitution of the BSFL as the protein source did not affect the production of eggs (Widjastuti *et al*., 2014). The fertility rate for the Control group, Treatment 1 and Treatment 2 groups were determined as 71.43%, 73.81%, 100%, respectively. Molapo and Motselisi (2020) stated that some researchers agreed that the fertility and hatchability rate was higher in medium sized eggs within the average weight.

Embryonal mortality

One crucial factor for hatching characteristics and embryonic mortality is the protein content of the breeder diet. A study with chukar partridges found that high protein levels tended to increase infertility while decreasing hatchability. However, fertility, hatchability of total and fertile eggs, and embryonic mortality were higher in the high protein hen diet than in the low protein hen diet (Uğurlu *et al*., 2017). This study recorded that the total embryonal mortality rate for the Treatment 2 group (84.62%) was the highest compared to the 65.38% and 40% for Treatment 1 group and Control group, respectively, even though the differences were not significant ($p>0.05$). In support of these results, Molapo and Motselisi (2020) underlined those eggs often had the lowest embryonic mortality, while bigger eggs had the greatest. It was also noted that medium-sized eggs had the lowest mean score of embryo mortality at the late embryo development stage. According to several studies, bigger eggs had a greater rate of embryo death than medium and tiny eggs at various developmental stages. Treatment 2 holds the highest egg weight; it was positively linear within the embryonic mortality. The embryonal mortality was positively linked and correlated with the egg weight.

Acknowledgements

Authors would like to express deep appreciation to the Malaysian Ministry of Higher Education for the Fundamental Research Grant Scheme (FRGS/1/2019/WAB01/UMK/02/7), Public-Private Research Network (PPRN) (R/PPRN/A0700/00130A/004/2021/00941) and Research Management and Innovation Centre of Universiti Malaysia Kelantan for the Rising Star Grant Scheme (R/STA/A0700/00130A/003/2021/00933).

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(Received: 11 August 2021, accepted: 30 May 2022)