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FYP FIAT

**Effect of Feeding Black Soldier Fly Larvae (BSFL) and Anchovy By-Product on Egg Weight, Fertility, Hatchability and Embryonal Mortality in Japanese quail**

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Honours**

**FACULTY OF AGRO-BASED INDUSTRY  
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## DECLARATION

I hereby declare that the work embodied in here is the result of my own research except for the excerpt as cited in the references.

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## LIST OF SYMBOLS

### Reference number

%	Percentage
n	Total
g	Gram
°C	Degree Celsius



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## LIST OF ABBREVIATION

### Reference number

<b>BSFL</b>	Black Soldier Fly Larvae
<b>FR</b>	Fertility Rate
<b>HR</b>	Hatchability of Incubated eggs/Hatchability Rate
<b>HFE</b>	Hatchability of Fertile Eggs
<b>EPEM</b>	Early Period Embryonic Mortality
<b>MPEM</b>	Middle Period Embryonic Mortality
<b>LPEM</b>	Late Period Embryonic Mortality
<b>CP</b>	Crude Protein
<b>ME</b>	Metabolizable Energy

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## ABSTRAK

Bahan mentah makanan menjadi lebih mahal, terutamanya sumber protein seperti makanan ikan dan debu kacang soya. Sumber protein dalam makanan memainkan peranan penting dalam pembangunan tisu haiwan. Akibatnya, sumber protein yang baik diperlukan untuk pertumbuhan haiwan dan penjanaaan tisu, yang akan memberi kesan kepada prestasi pertumbuhan dan pengeluaran. Majoriti sumber protein yang digunakan untuk menghasilkan makanan di Malaysia diimport dari negara lain, yang mahal dan menjejaskan kos pengeluaran. Sisa ikan bilis dan “*Black Soldier Fly Larvae*” (BSFL) berpotensi untuk digunakan sebagai pengganti protein dalam makanan ayam kerana kos yang lebih murah dan lebih mudah diperolehi. Kajian ini dilakukan untuk memerhatikan kesan makan BSFL, dan ikan bilis oleh produk menjejaskan berat telur, kesuburan, kebolehtetasan, dan kematian embrio dalam puyuh. Rawatan kumpulan kajian ini dibina sebagai kumpulan kawalan yang terdiri daripada 100% hidangan kacang soya sebagai sumber protein, Rawatan 1 dan Rawatan 2 kumpulan yang terdiri daripada 50% BSFL, 50% sisa ikan bilis dan 25% BSFL, 75% sisa ikan bilis sebagai penggantian sumber protein, masing-masing. Telur-telur itu diperolehi dari puyuh berusia 6 minggu. Keputusan yang ditunjukkan untuk berat telur, kumpulan kawalan mencatatkan yang paling rendah. Perbezaan antara berat telur ( $p < 0.05$ ) untuk rawatan 1 dan rawatan 2 kumpulan adalah penting dan ketara pada kumpulan kawalan Pengeluaran telur tertinggi ditemui dalam Rawatan 1 namun Rawatan 2 ditentukan FR tertinggi ( $p > 0.05$ ), HR ( $p > 0.05$ ) dan HFE ( $p > 0.05$ ), masing-masing 100%, 21.53% dan 21.53%. Pada kesimpulannya, analisis ini menunjukkan kesan positif terhadap berat telur, seperti yang dibuktikan oleh kumpulan rawatan 2, yang terdiri daripada 25% BSFL dan 75% produk sampingan ikan bilis sebagai komposisi sumber protein.

**Kata kunci:** “*Black Soldier Fly Larvae*” (BSFL), produk sampingan Ikan Bilis, Kebolehtetasan, Kesuburan, Kematian Embrio

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## ABSTRACT

Feed raw materials are becoming more expensive, particularly protein sources such as fish meal and soybean meal. Protein sources in feed play an important role in the development of animal tissues. As a result, good protein sources are required for animal growth and tissue generation, which will have an impact on growth performance and production. The majority of protein sources used to produce feed in Malaysia are imported from other countries, which is expensive and affects production costs. Anchovy waste and Black Soldier Fly Larvae (BSFL) have the potential to be used as protein substitutes in poultry feed due to less expensive cost and easier to obtain. This study is performed to observe the effects of feeding BSFL, and anchovy by-product on the egg weight, fertility, hatchability, and embryonal mortality in Japanese quail. The group treatment of this study constructed as control group which composed of 100% of soybean meal as protein sources, Treatment 1 and Treatment 2 group which consists of 50% BSFL, 50% anchovy waste and 25% BSFL, 75% anchovy waste as substitution of protein sources, respectively. The eggs were obtained from 6 weeks old quail. The results shown for the egg weight, the control group recorded the lowest. Differences among eggs weight ( $p < 0.05$ ) for the treatment 1 and treatment 2 groups were significant and not significant at the control groups. The highest production of egg was found in Treatment 1 however Treatment 2 was determined the highest FR ( $p > 0.05$ ), HR ( $p > 0.05$ ) and HFE ( $p > 0.05$ ), 100%, 21.53% and 21.53%, respectively. In conclude, this analysis shows a positive impact on egg weight, as evidenced by the treatment 2 group, which consists 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

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## CHAPTER 1

### INTRODUCTION

#### 1.1 Research background

Japanese quail is a type of bird species in the world that belongs to the order Galiformes, the genus *Coturnix*, and the species *japonica*. *Coturnix japonica* is the scientific name for Japanese quail, which varies from the regular quail (*Coturnix coturnix*) (Vali, 2010). As migrant birds, Japanese quail can be found in many countries (Mizutani, 2003). There are some strains of Japanese quail that have developed as a result of the genetic material borne by them, which they inherited from their ancestors.

In recent years, there has been a rise in popularity and demand among Malaysian customers, and it is anticipated that quail rearing will increase dramatically in order to satisfy the high demand of the local and foreign markets (Mnisi & Mlambo, 2018). It is well known that poultry meat is a significant and popular source of protein in Malaysia. This is shown by Malaysia's per-capita poultry intake of 35.5kg in 2011. Malaysia was

one of the world's top poultry buyers as compared to other nations. According to the East Coast Economic Region secretariat, quail demand has increased by 20 to 25 percent each year since 1995 (Berkhout, 2009).

In poultry rearing, high fertility, hatchability, and low embryonic mortality are the principal objective for profitability and benefit of effective to productivity. There are findings stated that fertility, hatchability, and early-stage mortality can be impacted by egg weight, length of capacity period, age of flock, reproducing type, composition of hen diet and sex proportion in poultry species (Uğurlu et al., 2017).

Uğurlu et al. (2017) point out that one of the factors affecting egg development and egg weight is the protein content of the hen diet. Some studies found that the protein levels in diets increased egg production and egg weight (Gunawardana et al., 2008; King'ori et al., 2010; Mohiti-Asli et al., 2012; Shim et al., 2013). Other researchers indicated that feeding low crude protein had no effect on egg development or egg weight (Cho et al., 2004; Khajali et al., 2008). It is well understood in poultry breeding that energy and protein levels in the diet are essential. Leeson and Caston (1996) found that reducing the dietary protein content from 16.80 percent to 14.40 percent in isoenergetic hen diets lowered egg weight. For maximizing egg production in Lohmann Brown laying hens, Li et al. (2013) recorded average optimum metabolism energy (ME) and crude protein (CP) intake levels of 2591 to 2683 kcal/kg and 15.58 percent to 16.64 percent CP. It has also been stated that diets rich in protein but low in energy have a detrimental impact on egg weight and egg development (Li et al., 2013; Steenhuisen & Gous, 2016).

Despite accounting for just 1.96 percent of total marine landings, anchovies are undeniably important and useful, especially to the population. Anchovies were then fermented into budu, or fermented seafood, which is one of Malaysia's most well-known

popular condiments. However, the production of budu often results in anchovies-by-product, which 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 & 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 percent for the head, 16.47 and 15.50 percent for the frame, and 12.05 and 23.90 percent for the viscera, respectively. There were significant variations in the pH and colour properties of anchovy whole fish, fillet, and by-products. Anchovy by-products have high levels of lysine (6-7 percent of total amino acids), leucine (5-6 percent of total amino acids), and a number of essential amino acids, polyunsaturated fatty acids (32-40 percent of total fatty acids), n-3 fatty acids (27-34 percent of total fatty acids), eicosapentaenoic acid+docosahexaenoic acid, and docosahexaen (about 26-32 percent of total fatty acids) as well as different minerals (P, Fe, Ni, Ca, Mn, Na and Zn). These findings indicate that anchovy by-products can be used to make value-added products such as protein powder, protein hydrolysates, fish oils, and mineral supplements.

According to Barragan-Fonseca et al., (2017), body composition of BSFL ranges not only in terms of protein content (ranging from 37 to 63 percent dry matter; DM), but also in terms of fat content, which varies the most (ranging from 7 to 39 percent DM). Though BSFL have a high protein and fat content on average, the type and quantity of food eaten determines the larvae's body composition. The amino acid profile of BSF larval protein shows that it is heavily enriched in lysine (6-8 percent of protein content) and it correlates favourably with published values for animal feed.

Both dried ground BSFL and anchovy by-products will be used and being combined with other ingredients to create a new formulated feed that is packed with all necessary nutrition for Japanese quail consumption and development. It is in order to preserves protein among as substitute soybean meal and fish meal in the quail feed formulation due to the high cost in getting both soybean meal and fish meal.

## 1.2 Problem statement

These days, BSFL is not generally referred to be utilized as one of the ingredients in making poultry feed. The equivalent can be said for anchovy by-products where it is a typical action to be discarded it in any case. Conversely, local farmers are highly leaning on commercial feeds that uses fish meal and soybean meal as its protein source material. The costs have obviously increased, and small-scale farmers have been forced to close their operations because they are unable to manage the costs of production. The significant expense production ultimately compels them to select leftover food or even let poultry animal roam freely to look through food themselves. Hence, this will make an adverse consequence the wellbeing of the poultry animal as it is conceivable that they did not get adequate amount of nutrition they require.

The production of poultry using commercial feed resulted in a high cost of production, causing the price of poultry meat to rise in order to sustain the profit on their earnings. With that in mind, BSFL and anchovy by-products are emerging as a potentially cheaper source of protein, and while there has been a lack of evidence supporting that poultry

animals fed BSFL and anchovy by-products have high egg production and hatchability rates, it is undeniable that it is important to be researched due to the correlation of crude protein to egg production, fertility, and hatchability.

### 1.3 Aim and objective

- 1) To investigate the effect of feeding black soldier fly larvae (BSFL) and anchovy by-product on egg weight, fertility, hatchability of Japanese quails' eggs.
- 2) To evaluate the effect of feeding black soldier fly larvae (BSFL) and anchovy by-product onto embryonal mortality of Japanese quails' eggs.



## 1.4 Hypothesis

$H^0$  = BSFL and anchovy by-product meal have no significant effect onto egg weight, fertility, hatchability of Japanese quails' eggs.

$H_1$  = BSFL and anchovy by-product meal have significant effect onto egg weight, fertility, hatchability of Japanese quails' eggs.

$H^0$  = BSFL and anchovy by-product meal have no significant effect onto embryonal mortality of Japanese quails' eggs.

$H_1$  = BSFL and anchovy by-product meal have significant effect onto embryonal mortality of Japanese quails' eggs.

## 1.5 Scope of study

This study is concerned with animal nutrition. The feeding trial of this newly formulated feed will reveal the most appropriate percentage of BSFL and anchovies by product meal for Japanese quail consumption based on quail health and egg production, fertility, hatchability, and embryonic mortality.

This experiment, in addition to relying on animal nutrition, strongly relies on genetics and animal breeding. This is due to the fact that animal feeding, animal, and offspring are

all inextricably linked. It can be said that the condition should improve if the correct amount of nutrition meal is given by evaluating the effect of BSFL and anchovy by-product meal inclusion in the new formulated feed on the health of the quail and determining the most appropriate ration of the meal for egg generation, fertility, hatchability, and embryonic mortality. It should be remembered that the necessary feed intake for the quails will be based on an ad libitum estimate in order to ensure that the quails received enough feed every day.

#### 1.6 Significance of study

This new formulated feed containing BSFL and anchovies by product meal will undoubtedly be a lifesaver for producers, lowering production costs as the feed becomes more affordable. Through this new formulated feed, the production of eggs, embryonic mortality, fertility, and hatchability off eggs could be determine positive effect.in the egg production and egg weight. As a result, it is important for other farmers to consider BSFL and anchovy by-products legibility as a possible raw material for protein source in poultry feed.

### 1.7 Limitation of study

Environmental difficulties were one of the study's limitations, as the weather in the area may be very erratic. The quails were stressed as a result of the scorching hot temperature as well as the chilly environment caused by heavy rain and monsoon in Malaysia's east coast area, which resulted in mortality or a reduction in laying egg performance. Next, pest management is also one of the elements that contribute to the study's limitations, since birds and rats aggressively assault the feed and eggs.

## CHAPTER 2

### LITERATURE REVIEW

#### 2.1 Production of quails in Malaysia

A Japanese quail is a migratory bird that originated in Japan, Korea, Eastern China, Mongolia, and Sakhalin (Mizutani, 2003). For the past decade, Japanese quails have been one of Malaysia's domesticated birds. Japanese quails are a form of poultry that provides meat and eggs for human consumption. Quail meat has become one of the cheapest animal proteins available in Malaysian markets. The price of a live quail ranges from RM1.80 to RM2.30/kg, while refined quail costs between RM10 and RM28/kg (DVS, 2009). In recent years, there has been a rise in popularity and demand among Malaysian customers, and it is anticipated that quail rearing will increase dramatically in order to satisfy the high demand of the local and foreign markets (Mnisi & Mlambo, 2018). Japanese quail were classified as Galliformes, with the genus *Coturnix* and the species *japonica*. It can be used in many countries due to its characteristics as a migrant bird. It is well known that poultry meat is a significant and

popular source of protein in Malaysia. This is shown by Malaysia's per-capita poultry intake of 35.5kg in 2011. Malaysia was one of the world's top poultry buyers as compared to other nations. According to the East Coast Economic Region secretariat, quail demand has increased by 20 to 25 percent each year since 1995. (Berkhout, 2009).

## 2.2 Quail production farming

Although the market for quail meat is growing, most breeders only work on a small scale. They still use a conventional farming system in which the child replaces the parent, resulting in declining yield and output. Farming quail does not always necessitate large areas because quail breeding is simple and can be handled easily by family members. Aside from that, a collaboration between the Poultry Breeding Centre in Bukit Tengah, Penang, and the Chicken Development Institute Johor Bharu, led by Malaysia's Head of Veterinary Services, was exploring, and studying quail breeding. They were eventually active in raising quality quail pups and breeds for local breeders, known as IKTA Quail (Iqbal, 2018). To summarize what has been stated, quail farming in Malaysia was limited and small-scale farming only.

### 2.3 Quail farming feed

Feed control is an essential aspect of quail bird breeding. A well-balanced nutritious feed guarantees that the birds' development is on target and stable, resulting in solid body weight gain in a short period of time. Typically, the industrial feed has set out a variety of feed choices for various periods of life as well as for various uses. Any of the feed types available are a starter, grower, finisher, and sheet feed. Japanese quails are fed starter feed from the time they hatch until they are 6-8 weeks old. Starter feed has the highest protein content of any feed type. When the chicks reach the age of six to eight weeks, they will be fed grower feed and then finisher feed for meat quail or layer (developer) diet for egg style quail (Linhoss, Thomas Table, & Wells, 2020).

The minimum protein, calcium, phosphorus, and methionine needs for game bird feeds are shown in the table below. To achieve an optimal result, it is important to feed the birds the proper diet. It is crucial that birds kept for egg development must be served developer diets rather than finisher diets. Only laying diets are fed to mature laying/breeder birds. Otherwise, egg development will be limited, and eggs will be thinner shelled (Feeding Quail, 2021).

**Table 1:** Recommended nutritional requirement for quail.

RECOMMENDED NUTRITIONAL REQUIREMENTS				
Diet	Protein (%)	Calcium (%)	Phosphorus (%)	Methionine (%)
<b><u>Bobwhite Quail - Meat-type</u></b>				
Starter (0 - 6 wk)	23.0	1.0	.50	.50
Finisher (6 wk - mkt)	19.0	.90	.50	.40
<b><u>Flight</u></b>				
Starter (0 - 6 wk)	24.0	1.0	.50	.55
Developer (6 - 16 wk)	20.0	.90	.50	.42
<b><u>Breeders</u></b>				
Starter (0 - 6 wk)	23.0	1.0	.50	.50
Developer (6 - 20 wk)	18.0	1.0	.50	.40
Layer (20 wk +)	19.0	2.75	.65	.50
<b><u>Coturnix (Pharaoh) Quail</u></b>				
Starter (0 - 6 wk)	24.0	.85	.60	.50
Finisher (6 wk - mkt)	18.0	.65	.50	.40
Layer (6 wk +)	18.0	2.75	.65	.45

Source: <https://www.thepoultrysite.com/articles/feeding-quail>

It is unavoidable that the cost of feed would strongly influence the cost of production. As a result, increasing demand for maize for livestock in producing countries such as the United States and China has influenced feed prices in Malaysia. According to USDA-NASS, (2022), the prices for the corn increase at 5.47 dollars per bushel in December 2021, compared to 3.97 dollars per bushel in December 2020.



## 2.4 Protein sources for animal nutrition

Soybean meal and fish meal are two major protein sources in Malaysia that are widely used in aquaculture and non-ruminant feeding. For animal feeding in Malaysia, 120,000 metric tons of fish meal and 800,000 metric tons of soybean meal are needed. Aside from that, 400,000 metric tons of soybean meal and 100,000 metric tons of fish meal are expected to meet Malaysia's animal feeding demand (Wong, 2009).

### 2.4.1 Fish meal

The Asia-Pacific fish meal industry began large-scale processing decades ago and is projected to produce 25,000 tons in the future, which is at least triple the volume produced in 1976. In Malaysia, approximately 65,000 metric tons of fish meal are processed annually. It is now projected that the annual value of fish meal production from by-catch in fisheries reached 216,000 metric tons. Due to the rise in the price of fish meal, farmers opted to use soybean meal and fish oil to maintain and suffice the protein source needed for farm feed (Abdul Manaf, 2017).

Aside from that, fish reduction plans run at half capacity year-round, and MARDI has been proactively researching processes for converting fish trash into fish silage as a way of replacing costly fish meal in traditional livestock feeding. Trash fish was a term



used to describe various problems and the relative importance of fish species captured collectively. The word itself is insufficient and has been used widely in various countries, with some referring to fish used for livestock feed and others including both livestock feed and human food (FAO, 2005).

Fish meal is an essential protein source, and the costs attributed to the products are relatively high and costly. Despite the high cost of fish meal, it indeed meets the protein requirements of animal feed. The price of fish meals is determined by the quality of the fish meals, which is influenced by many factors such as organisms, freshness, packaging, storage environment, and shelf life. Both values are rated based on the protein quality as well as the freshness of the fish meal. Many studies have shown that high-quality fish meals result in improved growth efficiency and feed consumption in fish species (Albrektsen, Mundheim, & Aksnes, 2006). Jacobsen & Silva, (2018) define that fish meal and fish oils are made from three types of fish; fish harvested specifically for the purpose of making fishmeal and oil, captures, and fish cuts, lungs, or trimming from fish food used.

#### 2.4.2 Soybean meal

According to Meal, (2016) Soybean meal is the most popular source of protein for livestock animals. It accounts for two-thirds of overall global protein feedstuff production, including all other big oil meals and fish meal. Its feeding importance is unrivalled by any other plant protein source, and it serves as the benchmark against which

other protein sources are measured (Cromwell, 1999). Though it has been a staple of livestock and poultry diets in the United States since the mid-1930s (Lewis et al., 2001), soybean feed production skyrocketed in the mid-1970s and then increased in the early 1990s due to rising demand from developing countries. The expansion of aquaculture and bans on the use of slaughterhouse by-products in feed has also increase the demand for this high-quality protein supply (Steinfeld et al., 2006).

Soybean meal is a by-product of soybean oil production, and different processes result in various goods. Soybean meal is usually marketed based on its crude protein content. Dehulled seeds yield high-protein varieties that produce 47-49 percent protein and 3 percent crude fibre (as fed basis). Other varieties of soybean meal contain less than 47 percent protein and more than 6 percent crude fibre and contain the hulls or a portion of the hulls. The oil content of solvent-extracted soybean meals is usually less than 2%, whereas it reaches 3% in mechanically extracted meals (Cromwell, 2012).

a. Nutritional content in soybean meal

Soybean meal is a highly palatable feedstuff with a high protein content (43 to 53 percent when fed) and low crude fibre content (less than 3 percent for the dehulled soybean meals). It has a relatively strong amino acid composition and contains a lot of lysine, tryptophan, threonine, and isoleucine, both of which are frequently absent in cereal grains. However, the concentrations of cysteine and methionine in monogastric animals

are suboptimal, and methionine supplementation is needed. The digestibility of amino acids is also very high (more than 90% for lysine in pigs and poultry).

Oligosaccharides in soybean meal, such as raffinose and stachyose, cannot be digested by monogastric animals due to the lack of a specific endogenous alpha-galactosidase. Raffinose and stachyose can induce flatulence and diarrhoea, improving the digestive passage rate while decreasing digestion and nutrient absorption. These oligosaccharides have been shown in poultry to reduce nitrogen-corrected actual metabolizable capacity, fibre digestion, and transit time (Parsons et al., 2000; Coon et al., 1990; Rackis, 1975 and Reddy, 1984 cited by Zuo et al., 1996). Soybean meals with low oligosaccharides are now affordable.

Around 60-70 percent of the phosphorus in soybean meal is bound to phytic acid, which is nutritionally inaccessible to monogastric animals and decreases P and other mineral abundance (Wilcox et al., 2000). Inorganic phosphorus must be supplemented, and the addition of phytase can help to mitigate the issue. Low-phytate soybeans are being created, but their productivity remains low (Waldroup et al., 2008).

Soybean meal is low in B vitamins, and a lack of B vitamin supplementation in soybean meal-based diets may lead to reproductive and efficiency issues in sows, older pigs, and hens (McDonald et al., 2002).

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### 2.4.3 Anchovy by-products

In the past decades, the fishery sector had been one of the major playing roles in supplying animal protein to the Malaysian population. Most of the fish are sold in a fresh and chilled form, while those marketed in the live form are directly sent to restaurants with higher prices than markets. One of the fishes gained from the marine activity is anchovy or also known as *Stolephorus* spp. in their scientific terms. They are harvested by the anchovy purse seine method and usually operated in inshore (FAO, 2009). The crop surpassed 22,288 tons in 1997, according to records. While anchovies contributed just 1.96 percent of total marine landings, they are undeniably significant and useful, particularly to the population. Anchovies are then transformed into budu, or fermented seafood, one of Malaysia's most well-known traditional condiments. However, at the end of the manufacturing of budu, the anchovies-by-product will be the waste. It is consisting of their head and guts being discarded rather than used in another way. As a result, there are ways to make use of the by-product.

#### 2.4.4 Black soldier fly larvae (BSFL)

##### a. The uses of BSFL

Poultry processing is heavily reliant on the use of grain in feeds, often used for human consumption, making grains a valuable commodity. When combined with rising chicken consumption, the price of grains skyrockets. A wide range of insects have been studied in the hunt for alternative protein sources for feed and have been found to provide useful protein sources to livestock. It is not only a sustainable and effective alternative, but it is also very environmentally friendly (Cribb, 2010). Because of their ability to be reared using renewable sources and have a favourable feed conversion, insects are regarded as a credible source of protein. Since they are poikilothermic, no energy needed to keep their body temperature stable, allowing them to retain more energy in their bodies and be excellent feed converter performance (Veldkamp et al., 2012). BSFL are considered species of flies that do not exist in normal flies, instead of relying on fruits and staying away from human populations. Essential amino acids in the insect could reduce the use of costly and high-quality fish meal, lowering feed costs.

b. Life cycle of BSFL

The life cycle of BSFL is divided into four stages which are embryo, larva, pupa, and adult, and it takes just 45 days to complete the cycle. During the final larvae stage, the pre-pupae migrate to a dry and suitable place and transform into pupa. Adult flies can become parasites or disease vectors, while female BSFL oviposits only around the margins of the larval food supply, rather than on it, preventing viruses from being passed on to their eggs later (Liu et al., 2017).

c. The nutritional content of BSFL

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 is 45 percent of CP but then increased to 56 percent in neonatal stages after hatching for one day. However, according to Liu (2017), it eventually decreased to 38 percent and 39 percent of CP in mature larvae. BSFL body composition varies among substrates in terms of protein content (ranging from 37 to 63 percent dry matter; DM) and fat content, which varies the most (ranging from 7 to 39 percent DM). While BSFL has a high protein and fat content on average (St Hilaire et al., 2007a; Zheng et al., 2012), the larvae's body composition is determined by nature and quantity of food consumed. Larvae feeding swine manure, for example, have

a higher protein content than those fed cow manure. Nguyen et al. (2015) discovered that larvae feeding fish and liver had higher protein and fat levels than those fed chicken feed. Throughout larval growth, there will be a lot of variances in body shape. For example, crude protein content decreases with age; the highest percentage was recorded for 5 days-old larvae (61%), whereas it was lower in 15 (44%) and 20 (42%) day old larvae (Barragan-Fonseca et al., 2017).

Through reviewed article by Barragan-Fonseca et al., (2017), it is stated that The amino acid profile of BSF larval protein indicates that it is highly enriched in lysine (6-8 percent of protein content), and correlates favourably with reported values for animal feed. In terms of lysine, leucine, phenylalanine, and threonine, essential amino acid amounts in larvae fed swine manure are comparable to those found in soybean meal. When comparing BSF larvae and soy meal values (based on g/16 g N), larvae have higher alanine, methionine, histidine, and tryptophan contents and lower arginine contents than soybean meal.

## 2.5 Diseases affecting Japanese quail, .

Animals are often susceptible to disease, whether it be infectious or bacterial. The same can be said for quail, which is also true because chicken and quail are both considered to be very vulnerable and susceptible to disease. Newcastle disease is one of the most common viral diseases that affect quail (Islam et al., 2016).

Newcastle disease (ND) was first identified in 1926, with the first occurrence of ND involving poultry animals occurring in 1934. Newcastle disease is caused by the Newcastle disease virus infecting poultry and bird populations, and it is well known to inflict lethal damage as well as farm losses. As a health sign, it triggers lung illness, exhaustion, nervous manifestations, and even diarrhoea. ND has a 100% mortality rate and is relatively easy to infect the whole flock due to the virus's mode of dissemination being by inhalation or ingestion (Afonso et al., 2012). The magnitude of the illness, however, is determined by the virus's virulence and strain. It should be remembered that domestic poultry contaminated with NDV have been seen to have poorer productivity, necessitating additional precautions to minimize the likelihood of infection incidence. However, ND was under surveillance, and numerous biosecurity measures were implemented to effectively avoid the recurrence of ND (Leow B.L; Shajaratul Wardah M.Y.; Ramlan M., 2011).

According to quail research in Bangladesh, colibacillosis is the second most common disease among quail (Islam et al., 2016). It is recognized as one of the most common and economically fatal bacterial diseases affecting poultry animals worldwide. Colibacillosis is caused by the contamination of poultry with a pathogenic avian strain of *Escherichia coli*. While the majority of *E. coli* may not qualify as pathogenic bacteria, certain types of *E. coli* have sadly accumulated virulence factors. These strains are known as APEC pathotypes because they contain plasmid-borne pathogenicity islands (PAIs). Infected poultry animals with the bacteria usually exhibit lethal septicaemia, subacute pericarditis, airsacculitis, and cellulitis. Airsacculitis is the most frequent colibacillosis lesion, although it is uncertain if it occurs from the progression of serositis or primary respiratory exposure. (Nolan, 2019).



## 2.6 The operation in pre-incubation egg care

The preincubation time is critical for effective quail propagation. Eggs can be harvested several times a day and kept at 15°C; a kitchen refrigerator is insufficient because it is too cold. When eggs are kept for no more than one week before setting, the best results are obtained. Since quail eggs are prone to shell destruction, they should be treated with extreme caution. The quail's-coloured eggshell made candling impossible. A contaminated incubator or hatchery field is a significant cause of disease and pollution.

After each application, it is advisable to thoroughly wash and disinfect the hatching device with a quaternary ammonium compound or industrial disinfectant. Clean eggs were only used to be incubated because dirty eggs will spread disease or infection. Soiled eggs should be brushed with fine sandpaper or other abrasives; incubated eggs should not be washed. (Randall & Bolla, 2008)

## 2.7 Incubation and hatching process

### 2.7.1 Variables in incubation process

Fertility and hatchability are important reproductive success parameters that are highly susceptible to environmental and genetic factors (Stromberg, 1975). Fertility is the percentage of fertile eggs that hatch from incubated eggs, while hatchability is the percentage of fertile eggs that hatch from incubated eggs. Fertility and hatchability are important reproductive success parameters that are highly susceptible to environmental and genetic factors (Sapp et al., 2004). (Abdurehman & Urge, 2016) explained that it is critical to monitor the same variables of temperature, humidity, ventilation, and egg turning when incubating any bird egg (Feeding Quail, 2021).

The temperature conditions for incubation vary depending on the animal, but most incubators have a temperature variation of 0.2-0.4°C for successful incubation and, as a result, a high hatchability rate. Temperatures greater than 1°C above or below the recommended temperature have a poor tolerance in embryos, and temperatures below this range result in substantial embryonic mortality. Embryos are much more sensitive to temperature changes during the early and late stages of incubation.

During incubation, maintaining consistent relative humidity is more complex and can only be done by increasing the ventilation rate, using adjustable ventilation apertures, and using surface water and water sprays. The embryo's resistance to various humidity levels is higher than its tolerance to temperature, but there are unfavourable effects of

humidity below 40% and above 90%. Maintaining relative humidity at 50-65 percent before the last three days of incubation, where it should be raised to 70-90 percent, results in good hatchability. (IC et al., 2016)

Embryonic development is optimized at a carbon dioxide concentration of 0.4 percent in the air, while carbon dioxide concentrations above 1 percent reduce embryonic growth and increase mortality. The natural atmosphere contains 21% oxygen and 0.04% carbon dioxide. When compared to the pipped chick and the embryo in the intact womb, the hatched chick is more vulnerable to oxygen deviation, implying that ventilation rate and carbon dioxide concentration are more important in the late phase of incubation(IC et al., 2016)

According to IC et al., (2016);Randall & Bolla, (2008), To prevent the embryo growing on the yolk from adhering to the shell membrane, the egg must be rotated or turned. Adherence to the shell membrane is a typical occurrence during fertile egg storage and early incubation (generally the first week). The turning mechanism causes the embryo to rotate and slip in the inner white, allowing it to gain access to additional nutrients for embryonic development. Egg turning can be done 3-6 times a day, with an unequal number of rotations preferred so that the eggs are not in the same place for an extended period of time. The eggs in most incubators are rotated by 90 degrees.

### 2.7.2 Incubation period

The incubation period of quail ranges between 17 and 18 days, depending on the strain and incubation procedures used.(Randall & Bolla, 2008)

**Table 2:** Incubation period for poultry species

(Source:<https://www.thepoultrysite.com/articles/care-and-incubation-of-hatching-eggs>)

<u>Species</u>	<u>Incub. Period (days)</u>	<u>Temp (F.)<sup>1</sup></u>	<u>Humidity (F.)<sup>2</sup></u>	<u>Do not turn after</u>	<u>Humidity Last 3 days<sup>2</sup></u>	<u>Open vent more</u>
Chicken	21	100	85-87	18th day	90	18th day
Turkey	28	99	84-86	25th day	90	25th day
Duck	28	100	85-86	25th day	90	25th day
Muscovy Duck	35-37	100	85-86	31st day	90	30th day
Goose	28-34	99	86-88	25th day	90	25th day
Guinea Fowl	28	100	85-87	25th day	90	24th day
Pheasant	23-28	100	86-88	21st day	92	20th day
Peafowl	28-30	99	84-86	25th day	90	25th day
Bobwhite Quail	23-24	100	84-87	20th day	90	20th day
Coturnix Quail	17	100	85-86	15th day	90	14th day
Chukar	23-24	100	81-83	20th day	90	20th day
Grouse	25	100	83-87	22nd day	90	21th day
Pigeon	17	100	85-87	15th day	90	14th day

Successful hatching is dependent on a thorough knowledge of incubator controls; carefully read the manufacturer's suggestions and save them for future reference. Fan-ventilated (forced-draft) and still-air machines are the two most common forms of incubators. Although a forced-draft incubator is preferred, a still-air system can be effective if used properly. Any versions are specifically made for quail. Japanese quail eggs may be incubated in any kind of chicken egg incubator, but the egg trays in certain machines will need to be adjusted. Eggs should be put big end up in the setting tray.

### 2.7.3 Operation in various types of incubators for quails' eggs.

#### a. Fan-ventilated (forced-draught) incubator.

Forced-draft incubators should be operated at  $37.5^{\circ} \pm 0.3^{\circ} \text{C}$  with humidity of 60% and a wet bulb temperature of  $30^{\circ} \pm 0.5^{\circ} \text{C}$  before the day of 14 of incubation. To keep embryos from adhering to the cell, eggs should be rotate every 2–4 hours. On the 14th day, light a candle and discard any cracked eggs, infertile, or dead embryos. Stop turning the eggs and place them in hatching trays. A separate hatcher should be held at  $37.2^{\circ} \text{C}$  with a relative humidity of 70% and a wet bulb temperature of  $32.2^{\circ} \text{C}$ .

Since the incubator is both a setter and a hatcher, the temperature should be  $37.5^{\circ} \text{C}$ , but the relative humidity should be raised to 70% wet bulb  $32.2^{\circ} \text{C}$  during

hatching. During the hatching process, the hatcher should not be opened. The chicks may be removed on the 17th or 18th day of incubation if all recommended incubation procedures have been followed (Randall & Bolla, 2008).

b. Still-air incubators.

If using a still-air incubator, the usual incubating temperature is 38.3°C for the first week, 38.8°C for the second week, and no higher than 39.5°C before hatching is full. The temperature of the eggs should be determined at the tip. Humidity should be less than 70% wet bulb at 29.4°–30.5°C before the 14th day of incubation, when it should be increased to 70% wet bulb at 32.2°C before hatching occurs in 17 to 18 days. Keeping sufficient humidity in small still-air incubators can be difficult because of need to avoid opening the incubator more often than necessary to transform the eggs and avoid leaving it open for lengthy periods of time.

The eggs must be manually turned at least three times a day, preferably five times. A pencil mark on each egg's side can aid in proper turning. If the temperature in the incubator is not uniform, it may be preferable to transfer the eggs to different places. In hatching trays, newly hatched chicks sometimes sprawl. To avoid this, either crowd the eggs into a narrow area or fasten cheesecloth to the bottom of the hatching tray until the chick hatch (Randall & Bolla, 2008).

## 2.8 Hatchability rate of quail

A study had been conducted by Wahab et al., (2018) found that the hatchability value is a function of the number of chicks hatched and is influenced by various factors. Fertility and egg consistency were the most important factors influencing hatchability and day-old chick weight (Alkan et al., 2008). According to previous research, the element of quail age can impact quail egg weight, which can affect the consistency and development characteristics of the quail eggs, which have a relationship with the quail hatchability rate (Zita, Ledvinka and Klesalova, 2013). Other considerations that can interfere with the effectiveness of the quality of hatched quail chicks or incubation include the turning mechanism and egg location during artificial incubation (Moraes et al., 2008). According to one analysis, the egg geometry, egg consistency, and hatchability differed between various types of Japanese quail strains (Akram et al., 2014).

## CHAPTER 3

### METHODOLOGY

#### 3.1 Designation of group and housing

A total of 36, 6 weeks-old quails were used and reared in battery cages at the Agro Techno Park, University Malaysia Kelantan, Jeli Campus. The quails were divided into 3 groups, which consisted of one control (C), and two treatments group, Treatment 1 (T1) and Treatment 2 (T2). For each group, it has been made up by 3 replicate and each replicate of the group will have 4 quails each: 1 male and 3 female and being fed with different feed according to each group.

T2R1	T1R3	T2R3	T1R2	CR3
CR1	CR2	T2R2	T1R1	

**Figure 1:** The distribution of quails' group.



### 3.2 Animal feeding

Each group were treated with differ in feed. Treatment 1 group were fed the formulated feed consisted of 50% of BSFL and 50% anchovy by-products whereas treatment 2 were fed 25% of BSFL and 75% anchovy by-products constitution of feed and control preference on the other hand was fed with 100% of soybean meal. The newly formulated feed was formulated for layer quails. The feeding quails were operated twice a day: morning and evening. The waterer and feeder were clean every day to avoid contamination. The quails were fed as it has leftover.

**Table 3:** Composition of ingredients and nutrient of formulated feed

<b>INGREDIENTS</b>			
	<b>CONTROL</b>	<b>TREATMENT 1</b>	<b>TREATMENT 2</b>
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
<b>TOTAL</b>	<b>100</b>	<b>100</b>	<b>100</b>
<b>NUTRIENT COMPOSITION (in %, unless stated otherwise)</b>			
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

### 3.3 Collection and storage of eggs

The eggs were collected once a day, early in the morning and being recorded in the log daily. The collected eggs were selected. The selection of eggs depends on the egg weight, the selected of the egg weight is 9-11g because the average of the quail eggs are 10.8g (Tserveni-Goussi & Fortomaris, 2011), clean egg without any dirt and uncracked eggshells to avoid contamination. The selected eggs were placed on trays at the temperature 15°C as suggested by Randall & Bolla, (2008) least than a week and then being transferred to the incubator gently to avoid the eggshell cracked because cracked egg hatch poorly and will contaminate the whole incubator.

### 3.4 Incubation

The incubation for quail ranges between 17 and 18 days. The incubator used is forced-draft incubators. Forced-draft incubators were operated at  $37.5^{\circ}\pm 0.3^{\circ}\text{C}$  with a relative humidity of 60% and a wet bulb temperature of  $30^{\circ}\pm 0.5^{\circ}\text{C}$  before the 14th day of incubation. To keep embryos from adhering to the cell, the incubator was operated to turn the eggs every 2– 4 hours. The incubator was checked daily. The candling process cannot be done because of coloured eggshells. On the 14th day, any cracked eggs will be discarded. The eggs turner was stopped, and the eggs were in hatching trays.

Since the incubator used is both a setter and a hatcher, the temperature should be 37.5°C, but the relative humidity should be raised to 70% wet bulb 32.2°C during hatching. During the hatching process, the hatcher should not be opened. The chicks were removed on the 17th or 18th day (Randall & Bolla, 2008)

### 3.5 Evaluation of incubation results

At the 18th day of incubation, the hatching eggs were monitored, the unhatched eggs were split and examined with the 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 has developed, and more of the yolk sack external to the body and late period embryonic mortality (LPEM) where 2/3 or whole of yolk sack in the body of embryo.

The fertile eggs, chick number of hatched and total eggs were used to measure fertility rate (FR), hatchability rate (HR) or hatchability of incubated eggs, and hatchability of fertile eggs (HFE), respectively. 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 fertility, hatchability rate and hatchability of fertile eggs were measured according to the type of strains and calculated according to Alkan et al., (2008) calculations (Wahab et al., 2018).

$$(\%) \text{Fertility} = \frac{\text{number of fertilized eggs}}{\text{total number of eggs placed into incubator}} \times 100 \quad (1)$$

$$(\%) \text{Hatchability of incubated eggs} = \frac{\text{number of released chicks}}{\text{total number of eggs placed incubator}} \times 100 \quad (2)$$

$$(\%) \text{Hatchability of fertile eggs} = \frac{\text{number of released chicks}}{\text{number of fertilized eggs placed in incubator}} \times 100 \quad (3)$$

### 3.6 Data analysis.

The SPSS Statistic 26 software were used in the data analysis. One-way ANOVA was used to compare egg weight in groups, FR, HR, HFE, EPEM, MPEM, and LPEM values for each groups fed with BSFL and anchovy by-products, soybean meal, and commercial feed, and the Duncan test was used to determine the importance of discrepancies between the groups. FR, HR, HFE, EPEM, MPEM, and LPEM values for each groups were also analysed using chi-square test.,

## CHAPTER 4

### RESULTS AND DISCUSSION

#### 4.1 Egg weight

The means of egg weight for each group are presented in table 4.1. The egg weight for treatment 1 was the lowest compared to the control and treatment 2 group. There was a significant difference among eggs weight ( $p < 0.05$ ) for quails in treatment 1 compared to treatment 2. However, there were not significant difference ( $P > 0.05$ ) in egg weight between control group and both treatment groups. In this study, mean egg weight was 9.40g for the control preferences group; 9.23g for treatment 1 group; 9.97g for treatment 2 group, respectively. These results show that the treatment 2 group recorded the highest egg weight. This showed that the composition of 25% of BSFL and 75% anchovy by-products in treatment 2 groups were influential on the quails.

**Table 4:** Egg and hatching chick weight by groups

<b>Group</b>	<b>Egg weight (g)</b>	<b>n</b>
Control	9.40 ± 0.25 <sup>ab</sup>	15
Treatment 1	9.23 ± 0.17 <sup>a</sup>	26
Treatment 2	9.97 ± 0.19 <sup>b</sup>	26

<sup>ab</sup> is significant differences at the (p<0.05).

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. As a result, the relationship between egg weight and protein levels in the hen diet is critically related. It was also discovered that there is a link between egg weight. According to the previous study, substituting a fish meal with a BSF maggot meal up to 50% affects egg weight considerably. This implies that replacing fish meal with maggot meal resulted in an increase in the weight of quail eggs. The BSFL can replace 50% of fish meals without affecting egg production, egg weight, or eggshell strength. Throughout the study, 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 significant compared to the control group equivalent to Zotte et al., (2019), In the experiment substituting BSFL meal to soybean meal, the weight of the eggs produced by quails was higher (14.5 g) than values commonly found in the literature. In this study, treatment 2 containing 25% BSFL and 75% anchovy by-products substitution of the protein sources recorded the highest egg

weight. It finally points out that there are significantly related that the protein level positively affected egg weight.

#### 4.2 Fertility and hatchability

The data of total egg production, fertile and infertile egg, and chick number was recorded, and the value for the fertility rate (FR), hatchability rate (HR) or hatchability of incubated eggs, and hatchability of fertile eggs (HFE) was calculated as stated in table 5 below. Egg production was determined as 22, 48 and 38 for the control group, treatment 1 and treatment 2 group. The production of eggs of Treatment 1; composition of 50% BSFL and 50% anchovy waste in the feed formulation was the highest. However, the total number of fertile eggs and chick hatched was analysed at the highest within the Treatment 2 group (26) and (4) respectively. Treatment 2 was determined 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 46.67%, 6.67%, 14.29% for the control group, 73.08%, 7.69%, 10.53% for treatment 1 and 100%, 15.83% and 15.83% for the treatment 2 group, respectively.

**Table 5:** Ratio values of egg production, total egg incubated, fertile and unfertile egg, number of chicks hatched, FR, HR and HFE.

<b>Group</b>	<b>Egg production (n)</b>	<b>Total egg (n)</b>	<b>Fertile egg (n)</b>	<b>Unfertile egg (n)</b>	<b>Chick number (n)</b>	<b>FR (%)</b>	<b>HR (%)</b>	<b>HFE (%)</b>
Control	22	15	7	8	1	46.67 <sup>NS</sup>	6.67 <sup>NS</sup>	14.29 <sup>NS</sup>
Treatment 1	48	26	19	7	2	73.08 <sup>NS</sup>	7.69 <sup>NS</sup>	10.53 <sup>NS</sup>
Treatment 2	38	26	26	0	4	100 <sup>NS</sup>	15.83 <sup>NS</sup>	15.38 <sup>NS</sup>

FR: Fertility, HR: Hatchability Rate, HFE: Hatchability of Fertile Eggs

<sup>NS</sup> is not significant, Chi-square test: (p>0.05)

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. However, another study found that low protein diets had a negative effect on egg production (Uğurlu et al. 2017). This finding shows 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. These results were in line with the reports of Abdurehman & Urge, (2016), who revealed that the most impactful egg parameters influencing hatchability are weight, 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 does not generate significant results, but it was revealed in this experiment that the quail produces eggs daily. The results demonstrate that the nutrients provided to the quail were



sufficient for the quail's egg production needs. In line with that, studies 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 group were determined as 46.67%, 73.08%, 100%, respectively. Molapo & Motselisi, (2020) stated that, some researchers agreed that the fertility and hatchability rate was higher in medium sized eggs within the average weight.

As in study conducted by Science, (n.d.), increasing the amount of BSF larvae meal provided had an effect on increasing chitosan levels in the ration. Chitosan has no negative effects. Chitosan can increase the production of the hormone gonadotropin, which functions as a luteinizing hormone-releasing hormone (LHRH) and is responsible for ovulation and egg production. Increased gonadotropins result in better reproductive outcomes and thus have no toxic effects. Throughout the findings, it is well defined that the protein level affects the production of the eggs. To ensure better production of the eggs, the nutrient and protein level of feed must be sufficient and fulfil the nutritional requirements for the layer quail.

### 4.3 Embryonal mortality

The mean values of embryonal mortality for the control group, treatment 1 and treatment 2 groups are given in table 6. It was determined that the highest embryonic mortality mean rates were 0.57; control group, 0.23; Treatment 2 groups, and 0.32; Treatment 1 group, for EPEM, MPEM, LPEM, respectively.

**Table 6:** Embryonic mortality rates for Control, Treatment 1, and Treatment 2 groups.

Group	EPEM	MPEM	LPEM
Control	0.57	0.14	0.14
Treatment 1	0.37	0.21	0.32
Treatment 2	0.35	0.23	0.27

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

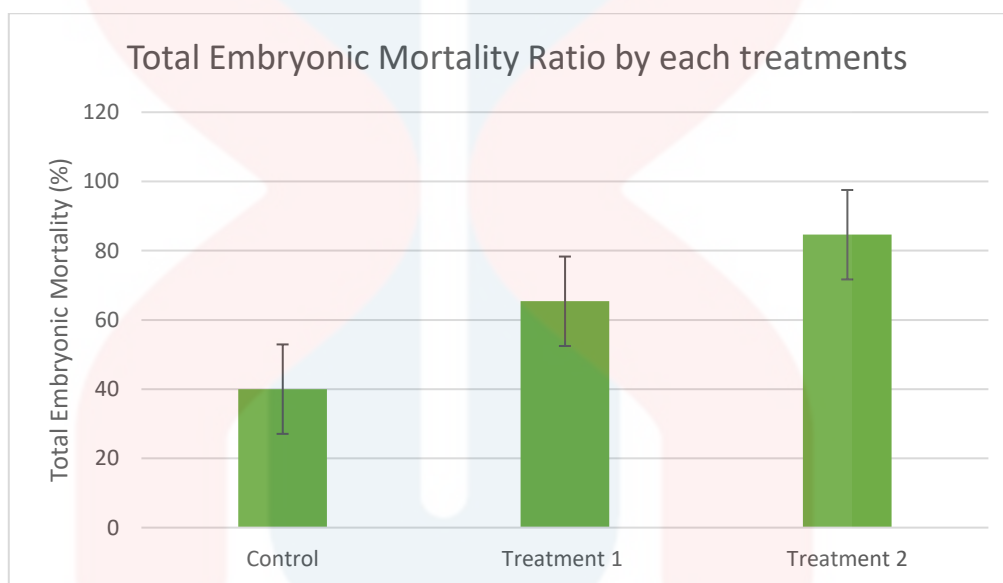
Total percentage of embryonic mortality by each treatment were determined by the total number of embryonic mortality divided by the number of eggs incubated times hundred.

$$\text{Total Embryonic Mortality}(\%) = \frac{\sum EPEM + MPEM + LPEM}{\text{number of eggs incubated}} \times 100 \quad (4)$$

The total embryonal mortality rate for the treatment 2 group (84.62%) was the highest compared to the 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 ( $p>0.05$ ) although there are not statistically significances.

**Figure 2:** The total percentage of Embryonic Mortality by each treatment.



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 tend 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 are not significant ( $p>0.05$ ). In support of these results, Molapo & Motselisi, (2020) underlined those medium eggs often have the lowest embryonic mortality, whereas bigger eggs have the greatest. It was also noted that

medium-sized eggs have the lowest mean score of embryo mortality at the late embryo development stage. According to several studies, bigger eggs have a greater rate of embryo death than medium and tiny eggs at various developmental stages. Since treatment 2 hold the highest egg weight, it is positively linear within the embryonic mortality. The embryonal mortality is positively linked and correlate with the egg weight.

It can be further strengthened with the findings of show that big eggs have higher embryonic mortality than other egg sizes, despite the fact that the differences are not statistically significant ( $p>0.05$ ). The thin eggshells in giant eggs may be a viable explanation for the high mortality rate in large eggs. Eggs with thin shells might lose more moisture during incubation because the thinner eggshell is more porous, resulting in water loss, which is one of the leading causes of embryonic mortality. (Molapo & Motselisi, 2020)

## CHAPTER 5

### CONCLUSION

In conclusion, BSFL and anchovy by products can be offered up to 25% and 75%, respectively in Japanese quail breeder diet without causing any depressive effects on egg and chick production. Thus, combination of these two ingredients is potential to replace up to 40% SBM in quail breeder diet, which can be reflected in lower cost of feed production. The usage of BSFL and anchovy by-products meal as substitution of protein sources, soybean meal in the quail feed were identified to produce the relatively normal and greater eggs of quail especially in size and weight. This was proven by the observation of 12-15g egg that being laid by the hen fed with BSFL and anchovy by-products. Treatment 2 consists of 25% BSFL and 75% anchovy by-products as a substitution for the protein sources recorded the highest egg weight and hatching chick weight.

## RECOMMENDATION

It is strongly advised to be carried out more study in proximate analysis and egg composition analysis in order to better comprehend and fully examine the component in BSFL and anchovy by-product and the impacts influenced on the quails' eggs. In terms of infrastructure, the poultry housing system offered at Agro-techno park, UMK must be expanded to a much higher level to guarantee that the feeding trial can be carried out without interruption, to increase the safety of the quail and researchers, and to ensure more accurate results. Finally, the availability of laboratory equipment, such as the incubator, and the proximate analysis machine should be increased and maintained to allow accurate comparison of gathered data with previous research.

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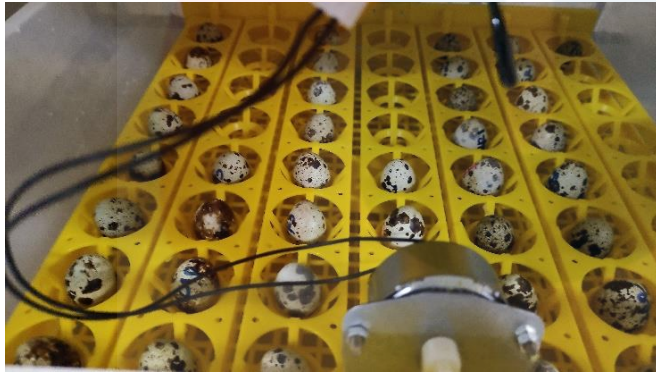
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## APPENDIXES A

Figures below shown the incubation process.





Figures above shown weighing process of hatched chicks.



Figure: The hatched chicks in hatchery

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Figures below illustrate the pest control management



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Figures above demonstrate the condition of eggs caused by the predator.

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## APPENDIXES B

### Descriptives

		N	Mean	Std. Deviation	Std. Error	95% Confidence ... Lower Bound
EggWeight	Treatment 1	26	9.2308	.86291	.16923	8.8822
	Treatment 2	26	9.9615	.95836	.18795	9.5744
	Control	15	9.4000	.98561	.25448	8.8542
	Total	67	9.5522	.97365	.11895	9.3147

		95% Confidence Interval for Mean		
		Upper Bound	Minimum	Maximum
EggWeight	Treatment 1	9.5793	8.00	11.00
	Treatment 2	10.3486	8.00	11.00
	Control	9.9458	8.00	11.00
	Total	9.7897	8.00	11.00

### ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
EggWeight	Between Groups	7.390	2	3.695	4.286	.018
	Within Groups	55.177	64	.862		
	Total	62.567	66			

### Multiple Comparisons

Dependent Variable	(I) Group	(J) Group	Mean Difference (I-J)	Std. Error	Sig.	
EggWeight	Tukey HSD	Treatment 1	Treatment 2	-.73077*	.25752	.017
		Control		-.16923	.30106	.841
	Treatment 2	Treatment 1	.73077*	.25752	.017	
	Control		.56154	.30106	.157	
	Control	Treatment 1	.16923	.30106	.841	
	Treatment 2		-.56154	.30106	.157	



### Multiple Comparisons

Dependent Variable	(I) Group	(J) Group	95% Confidence Interval		
			Lower Bound	Upper Bound	
EggWeight	Tukey HSD	Treatment 1	Treatment 2	-1.3487	-.1129
		Control		-.8916	.5531
	Treatment 2	Treatment 1	.1129	1.3487	
	Control		-.1608	1.2839	
	Control	Treatment 1	-.5531	.8916	
	Treatment 2		-1.2839	.1608	

\*. The mean difference is significant at the 0.05 level.

### Homogeneous Subsets

#### EggWeight

	Group	N	Subset for alpha = 0.05	
			1	2
Tukey HSD <sup>a,b</sup>	Treatment 1	26	9.2308	
	Control	15	9.4000	9.4000
	Treatment 2	26		9.9615
	Sig.		.826	.132
Duncan <sup>a,b</sup>	Treatment 1	26	9.2308	
	Control	15	9.4000	9.4000
	Treatment 2	26		9.9615
	Sig.		.558	.055

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 20.893.

b. The group sizes are unequal. The harmonic mean of the group sizes is used. Type I error levels are not guaranteed.

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## Descriptives

		N	Mean	Std. Deviation	Std. Error	95% Confidence ... Lower Bound
FR	Treatment 1	1	73.0769	.	.	.
	Treatment 2	1	100.0000	.	.	.
	Control	1	46.6667	.	.	.
	Total	3	73.2479	26.66708	15.39624	7.0032
HR	Treatment 1	1	7.6923	.	.	.
	Treatment 2	1	15.3846	.	.	.
	Control	1	6.6667	.	.	.
	Total	3	9.9145	4.76491	2.75102	-1.9222
HFE	Treatment 1	1	10.5263	.	.	.
	Treatment 2	1	15.3846	.	.	.
	Control	1	14.2857	.	.	.
	Total	3	13.3989	2.54767	1.47090	7.0701
EPEM	Treatment 1	1	.36842	.	.	.
	Treatment 2	1	.34615	.	.	.
	Control	1	.57143	.	.	.
	Total	3	.42867	.124135	.071669	.12030
MPEM	Treatment 1	1	.21053	.	.	.
	Treatment 2	1	.23077	.	.	.
	Control	1	.14286	.	.	.
	Total	3	.19472	.046039	.026581	.08035
LPEM	Treatment 1	1	.31579	.	.	.
	Treatment 2	1	.26923	.	.	.
	Control	1	.14286	.	.	.
	Total	3	.24263	.089483	.051663	.02034

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## Descriptives

		95% Confidence Interval for Mean		
		Upper Bound	Minimum	Maximum
FR	Treatment 1	.	73.08	73.08
	Treatment 2	.	100.00	100.00
	Control	.	46.67	46.67
	Total	139.4926	46.67	100.00
HR	Treatment 1	.	7.69	7.69
	Treatment 2	.	15.38	15.38
	Control	.	6.67	6.67
	Total	21.7512	6.67	15.38
HFE	Treatment 1	.	10.53	10.53
	Treatment 2	.	15.38	15.38
	Control	.	14.29	14.29
	Total	19.7276	10.53	15.38
EPEM	Treatment 1	.	.368	.368
	Treatment 2	.	.346	.346
	Control	.	.571	.571
	Total	.73704	.346	.571
MPEM	Treatment 1	.	.211	.211
	Treatment 2	.	.231	.231
	Control	.	.143	.143
	Total	.30908	.143	.231
LPEM	Treatment 1	.	.316	.316
	Treatment 2	.	.269	.269
	Control	.	.143	.143
	Total	.46491	.143	.316

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**ANOVA**

		Sum of Squares	df	Mean Square	F	Sig.
FR	Between Groups	1422.266	2	711.133	.	.
	Within Groups	.000	0	.		
	Total	1422.266	2			
HR	Between Groups	45.409	2	22.704	.	.
	Within Groups	.000	0	.		
	Total	45.409	2			
HFE	Between Groups	12.981	2	6.491	.	.
	Within Groups	.000	0	.		
	Total	12.981	2			
EPEM	Between Groups	.031	2	.015	.	.
	Within Groups	.000	0	.		
	Total	.031	2			
MPEM	Between Groups	.004	2	.002	.	.
	Within Groups	.000	0	.		
	Total	.004	2			
LPEM	Between Groups	.016	2	.008	.	.
	Within Groups	.000	0	.		
	Total	.016	2			

## Group \* FR

### Crosstab

			46.67	FR 73.08	100.00	Total
Group	Treatment 1	Count	0	1	0	1
		Expected Count	.3	.3	.3	1.0
	Treatment 2	Count	0	0	1	1
		Expected Count	.3	.3	.3	1.0
	Control	Count	1	0	0	1
		Expected Count	.3	.3	.3	1.0
Total		Count	1	1	1	3
		Expected Count	1.0	1.0	1.0	3.0

### Chi-Square Tests

	Value	df	Asymptotic Significance (2- sided)
Pearson Chi-Square	6.000 <sup>a</sup>	4	.199
Likelihood Ratio	6.592	4	.159
Linear-by-Linear Association	.490	1	.484
N of Valid Cases	3		

a. 9 cells (100.0%) have expected count less than 5. The minimum expected count is .33.

### Symmetric Measures

		Value	Approximate Significance
Nominal by Nominal	Phi	1.414	.199
	Cramer's V	1.000	.199
N of Valid Cases		3	

## Group \* HR

### Crosstab

			6.67	7.69	15.38	Total
			HR			
Group	Treatment 1	Count	0	1	0	1
		Expected Count	.3	.3	.3	1.0
	Treatment 2	Count	0	0	1	1
		Expected Count	.3	.3	.3	1.0
	Control	Count	1	0	0	1
		Expected Count	.3	.3	.3	1.0
Total	Count	1	1	1	3	
	Expected Count	1.0	1.0	1.0	3.0	

### Chi-Square Tests

	Value	df	Asymptotic Significance (2-sided)
Pearson Chi-Square	6.000 <sup>a</sup>	4	.199
Likelihood Ratio	6.592	4	.159
Linear-by-Linear Association	.023	1	.879
N of Valid Cases	3		

a. 9 cells (100.0%) have expected count less than 5. The minimum expected count is .33.

### Symmetric Measures

		Value	Approximate Significance
Nominal by Nominal	Phi	1.414	.199
	Cramer's V	1.000	.199
N of Valid Cases		3	

## Group \* HFE

**Crosstab**

		HFE			Total	
		10.53	14.29	15.38		
Group	Treatment 1	Count	1	0	0	1
		Expected Count	.3	.3	.3	1.0
	Treatment 2	Count	0	0	1	1
		Expected Count	.3	.3	.3	1.0
	Control	Count	0	1	0	1
		Expected Count	.3	.3	.3	1.0
Total		Count	1	1	1	3
		Expected Count	1.0	1.0	1.0	3.0

### Chi-Square Tests

	Value	df	Asymptotic Significance (2-sided)
Pearson Chi-Square	6.000 <sup>a</sup>	4	.199
Likelihood Ratio	6.592	4	.159
Linear-by-Linear Association	1.089	1	.297
N of Valid Cases	3		

a. 9 cells (100.0%) have expected count less than 5. The minimum expected count is .33.

### Symmetric Measures

		Value	Approximate Significance
Nominal by Nominal	Phi	1.414	.199
	Cramer's V	1.000	.199
N of Valid Cases		3	

## Group \* EPEM

### Crosstab

		EPEM			Total	
		.35	.37	.57		
Group	Treatment 1	Count	0	1	0	1
		Expected Count	.3	.3	.3	1.0
	Treatment 2	Count	1	0	0	1
		Expected Count	.3	.3	.3	1.0
	Control	Count	0	0	1	1
		Expected Count	.3	.3	.3	1.0
Total	Count	1	1	1	3	
	Expected Count	1.0	1.0	1.0	3.0	

### Chi-Square Tests

	Value	df	Asymptotic Significance (2-sided)
Pearson Chi-Square	6.000 <sup>a</sup>	4	.199
Likelihood Ratio	6.592	4	.159
Linear-by-Linear Association	1.337	1	.248
N of Valid Cases	3		

a. 9 cells (100.0%) have expected count less than 5. The minimum expected count is .33.

### Symmetric Measures

		Value	Approximate Significance
Nominal by Nominal	Phi	1.414	.199
	Cramer's V	1.000	.199
N of Valid Cases		3	



## Group \* MPEM

### Crosstab

		MPEM			Total	
		.14	.21	.23		
Group	Treatment 1	Count	0	1	0	1
		Expected Count	.3	.3	.3	1.0
	Treatment 2	Count	0	0	1	1
		Expected Count	.3	.3	.3	1.0
	Control	Count	1	0	0	1
		Expected Count	.3	.3	.3	1.0
Total	Count	1	1	1	3	
	Expected Count	1.0	1.0	1.0	3.0	

### Chi-Square Tests

	Value	df	Asymptotic Significance (2-sided)
Pearson Chi-Square	6.000 <sup>a</sup>	4	.199
Likelihood Ratio	6.592	4	.159
Linear-by-Linear Association	1.080	1	.299
N of Valid Cases	3		

a. 9 cells (100.0%) have expected count less than 5. The minimum expected count is .33.

### Symmetric Measures

		Value	Approximate Significance
Nominal by Nominal	Phi	1.414	.199
	Cramer's V	1.000	.199
N of Valid Cases		3	

## Group \* LPEM

### Crosstab

		LPEM			Total
		.14	.27	.32	
Group	Treatment 1	Count	0	0	1
		Expected Count	.3	.3	.3
	Treatment 2	Count	0	1	0
		Expected Count	.3	.3	.3
	Control	Count	1	0	0
		Expected Count	.3	.3	.3
Total	Count	1	1	1	3
	Expected Count	1.0	1.0	1.0	3.0

### Chi-Square Tests

	Value	df	Asymptotic Significance (2-sided)
Pearson Chi-Square	6.000 <sup>a</sup>	4	.199
Likelihood Ratio	6.592	4	.159
Linear-by-Linear Association	1.867	1	.172
N of Valid Cases	3		

a. 9 cells (100.0%) have expected count less than 5. The minimum expected count is .33.

### Symmetric Measures

		Value	Approximate Significance
Nominal by Nominal	Phi	1.414	.199
	Cramer's V	1.000	.199
N of Valid Cases		3	