The substitution of fish meal with larvae of Hermetia illucens supplemented with Trichoderma sp on quail's nutritional utility and egg production

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The substitution of fish meal with larvae of *Hermetia illucens* supplemented with *Trichoderma* sp on quail's nutritional utility and egg production

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Abstract

The study purposed to evaluate the effect of substitution of fish meal by larvae of *Hermetia illucens* (HI) supplemented with *Trichoderma* sp on quail's nutritional utility and egg production. Two hundred quails aged 9 weeks with the bodyweight of 172.7 ± 12.79 g were randomly placed on 5 dietary treatments, i.e. HI 0 (100% fish meal), HI 25 (75% fish meal + 25% (HI + 2% *Trichoderma* sp), HI 50 (50% fish meal + 50% (HI + 2% *Trichoderma* sp), HI 75 (25% fish meal + 75% (HI + 2% *Trichoderma* sp), HI 100 (100% (HI + 2% *Trichoderma* sp). Data were analyzed using analysis of variance in a completely randomized design.

Nutrient digestibility, metabolic energy, nitrogen retention percentage and feed conversion did not differ among groups (p> 0.05). Feed intake, metabolic energy consumption, nitrogen retention amount, and egg production decreased with 100% substitution of *H. illucens* larvae compared to controls (p<0.05). *H. illucens* larvae with 2% *Trichoderma* can replace fish meal up to 75% in the diet without detrimental effects on egg production.

Keywords: *BSF, digestibility, conversion, egg, TME*

Introduction

Protein is a major limiting factor and the most expensive component of feed-in poultry rations. Poultry feed price in developing countries has consistently increased due to dependence on fish meal imports and soybean meal. For this dependence, it is necessary to find alternative sustainable protein sources (Khan et al 2016). Fish meal is a high-quality source of protein used in poultry diets. However, because the price of fish meals is high, then look for alternative protein sources. Some substitution studies of fishmeal by larvae/prepupae of *H. illucens* (HI) / black soldier fly (BSF) have been carrying. The results cannot reach 100% depending on the larval/prepupa age.

Nutrient content of HI larvae reached of the dry matter 44%, 42% protein, and 35% fat, moreover, their profile of amino acids and fatty acids have in common with the fish meal (Sheppard et al 2002; Newton et al 2005; Newton et al 2007; Fahmi et al 2006; Bonso 2013).

The used of HI larvae at various ages give different results. Gunawan et al (2012) stated that the 2-week old maggots fed on the manure of quails, ducks, broilers and laying hens had a metabolic energy value of 3,121; 3,373; 3,250; and 3,028 (kcal/kg) and nitrogen retention 75.8; 64.0; 74.5; and 70.6%. According to Dengah et al (2016), substitution fish meal by HI larvae aged ten days in broiler diets (15% fish meal content) more than 50% were reduced feed consumption and body weight gain at the finisher period. Widjastuti et al (2014) stated that the substitution of fish meal (10% in diets) with HI meal more than 50% reduced to quail egg production.

One obstacle in using HI is chitin as an exoskeleton component of insects, which poultry cannot digest chitin. Chitin content in a fish meal is 0% and in HI is 9.6% (Kroeckel et al 2012), the chitin content of HI larvae is lower than that of pupae. Pupa of HI has high crude protein and amino acid score, however, low digestibility than HI larvae (CP 56.1 vs 52.1; AAS 93 vs 79.2). The increase in chitin in the pupa caused low digestibility (Bosch et al 2014). The chitin content of HI larvae is estimated to be 5.4% (Finke 2012). High chitin levels in insects harm feed intake and interfere with protein use (Longvah et al 2011), thereby reducing the availability of nutrient utilities that affect growth performance (Kroeckel et al 2012). Marono et al (2015) study that in vitro chitin harmed protein digestibility. Addressing chitin and low palatability is the key to developing HI prepupae as an alternative protein source.

Trichoderma is a fungus that can produce chitinase enzymes which can degrade chitin. *Trichoderma* sp. is known to have chitinase, β-1,3-glucanase, and protease (Elad et al 1982; Sandhya et al 2004). Chitinase enzyme could split the chitin polymer into N-acetyl glucosamine monomer units (Ulhoa and Peberdy 1991; Isahak et al 2014). Research by Mulyono et al (2019) states that the addition of 2% *Trichoderma* sp can improve the digestibility of HI larva

To best our knowledge, there are no data on the substitution of fish meal with HI larvae supplemented using *Trichoderma* sp could be found in the literature. Therefore, this present study of purposed to evaluate the effect of substituting fish meal by different levels of HI larvae meal that was supplemented with 2% *Trichoderma* sp in the diet of laying quail.

Materials and methods

The study was conducted at the Laboratory of Nutrition and Feed Science Faculty of Animal and Agricultural Sciences, Diponegoro University. The *H. illucens* larvae were obtained from the layer farm in Kalisidi village, West Ungaran, Semarang, Central Java, Indonesia. The inoculums were TRICHOR-TM with *T. viridae* (10^8 CFU/g), *T. harzianum* ($7x10^8$ CFU/g), *Trichoderma* sp, ($7x10^8$ CFU/g) that purchased from CV Pradipta Paramita Solo, Central Java, Indonesia.

Animal and Diets

This study used 200 of 9-weeks female quails (*Coturnix coturnix japonica*) with an average body weight of 172.7 ± 12.79 g. They were randomly allocated to 20 pens consisted of five treatments and four replicates each. Each treatment was composed of 10 quails. The experiment using a completely randomized design with five treatments and four replications. The treatments given are: HI 0 (100% fish meal), HI 25 (75% fish meal + 25% (HI + 2% *Trichoderma* sp), HI 50 (50% fish meal + 50% (HI + 2% *Trichoderma* sp), HI 75 (25% fish meal + 75% (HI + 2% *Trichoderma* sp), HI 100 (100% (HI + 2% *Trichoderma* sp). The diets were formulated from yellow corn, soybean meal, fish meal, BSF meal, mineral mix, and *Trichoderma* sp, as shown in Table 1.

Table 1. The Composition and nutrient contents of experimental diets

Feed ingredients	Treatments				
	HI 0	HI 25	HI 50	HI 75	HI 100
Yellow Corn (%)	56.9	56.6	56.6	56.1	55.8
HI + 2% *Trichoderma* (%)	0.00	2.12	4.24	6.36	8.48
Fish meal (%)	8.00	6.00	4.00	2.00	0.00
Soybean meal (%)	29.1	29.3	29.4	29.6	29.7
Premix (%)[1]	1	1	1	1	1
CaCO3 (%)	5	5	5	5	5
Total	100	100	100	100	100
Calculate Nutrient					
Moisture (%)	11.7	11.6	11. 6	11. 6	11. 6
Ash (%)	6.73	6.67	6.63	6.58	6.53
Crude protein (%)	20.8	20.8	20.9	20.9	20.9
Ether extract (%)	3.24	3.32	3.40	3.48	3.55
Crude fiber (%)	5.09	5.11	5.14	5.16	5.19
Metabilizable energy (kkal/kg)[2]	2,921	2,927	2,932	2,937	2,943
Calcium (%)	2.48	2.49	2.50	2.50	2.51
Phosphorus (%)	0.54	0.52	0.51	0.49	0.47
Lysine (%)	1.33	1.25	1.16	1.07	0.99
Methionine (%)	0.42	0.39	0.36	0.333	0.29
ME/CP ration	141	141	141	141	141

[1] 1Supplied per kg of diet: vitamin A 12,500 IU, vitamin D 32,500 IU, vitamin E 7.5 IU, vitamin K 2 mg, vitamin B1 2.5 mg, vitamin B2 4 mg, vitamin B6 1 mg, vitamin B12 0.012 mg; biotin 0.2 mg, folic acid 0.5 mg, vitamin C 50 mg, nicotinate acid 50 mg, Ca-D Pantothenate 4 mg, choline chloride 15 mg, copper 5 mg, iron 25 mg, iodine 0.2 mg, manganese 60 mg, selenium 0.2 mg, zinc 70 mg, cobalt 0.2 mg, zinc bacitracin 21 mg, lysine 160 mg, DL-methionine 50 mg, threonin 4 mg. 2 Values were obtained based on the formula according to Bolton (1967)

Parameter measured

Nutrien utility

Nutrient digestibility, nitrogen retention, and metabolic energy were measured using the total collection, modified from Mulyono et al (2019). Briefly, 100 quails were placed in 20 metabolic cages (60×45×30 cm) and fed for four days in the collection periods.

Experimental feeds were weighed, and 1% ferric oxide was added on the first and on the last day of the collection period to identify the excreta derived from the experimental diet. Therefore, in the first collection, non-marked excreta were discarded and in the last collection. The excreta were collected in the plastic tray at the bottom of the cage. All contaminants, such as feed particles and feathers, were discarded. The excreta were weighed and then sun-dried to obtain the air-dry weight. The content of proximate components such as moisture, ash, crude protein, ether extract and crude fibre of diets and excreta were analyzed by applying the AOAC method (AOAC 1990). The contents of gross energy (GE) were determined using a bomb calorimeter by Parr (2013).

Nutrient digestibility (ND) was calculated according to Onimisi et al (2008) with the following equation:

ND = (nutrient intake-nutrient excreta)/nutrient intake*100%

Nitrogen retention (NR) was the difference between nitrogen consumption and nitrogen excreted through faeces and urine after corrected by endogenous nitrogen excretion, and it was calculated as follows:

NR (%) = [N intake – (N excreta - N endogenous)]/ N intake × 100%

Intake of NR (g/bird), the intake total nitrogen retention was multiplying the percentage of nitrogen retention (%) by average dietary intake (g/bird) over the five weeks of the study.

Metabolizable energy (kcal/kg), calculated based on the equation suggested by Sibbald (1989), namely:

TME= [((Fi×GEf) – (E×GEe)) + (FEm+UEe)] / Fi

Where:

TME: true metabolizable energy (kcal/g)

Fi: feed intake (g).

E: excreta (g).

GEf: gross energy of feed sample (kcal/g)

GEe: gross energy of excreta (kcal/g)

FEm: faecal metabolic energy (kcal/g)

UEe: indigenous urinary energy (kcal/g)

Quails Performance

The feed intake was calculated using the daily feed minus the remaining feed (g/bird/day). The feed conversion ratio was calculated from feed intake (g) divided by egg production (g). Quail-day egg production (QDP) was calculated by dividing the number of eggs by the number of live quail (%).

Statistical analysis

Data were analyzed by the analysis of variance procedure using the SPSS 16.0 statistical software. The differences between the treatments′ means were examined by Duncan's multiple range test (Steel et al 1997). The significance level was $p<0.05$.

Result

Nutrient utility

Fish meal replacement by 100% HI larvae supplemented with 2% *Trichoderma* was not affected to nutrients digestibility, percentage of NR (%), and metabolic energy content. However, its decreased NR quantity and the intake of TME (Table 2; Figure 1; Figure 2).

Table 2. Nutrient digestibility, nitrogen retention and metabolizable energy

Variables	Treatment					SEM	*p*
	HI 0	HI 25	HI 50	HI 75	HI 100		
Organic matter digestibility (%)	86.0	85.5	83.9	85.4	83.8	0.47	0.494
Ether extract digestibility (%)	90.6	90.0	90.17	90.4	89.75	0.20	0.705
Crude fibre digestibility (%)	43.8	48.3	44.5	45.0	46.7	1.19	0.794
Crude protein digestibility (%)	89.0	88.4	87.7	88.1	87.6	0.30	0.652
Nitrogen Retention (%)	88.6	87.8	86.7	87.3	86.7	0.39	0.506
Nitrogen Retention (g)	4.17^{a}	3.99^{ab}	3.98^{ab}	3.88^{bc}	3.72^{c}	0.04	0.005
TME (kkal/kg)	3072	3053	3029	3055	2976	17.3	0.482
TME intake (kal//d)	69.6^{a}	66.6^{a}	66.7^{a}	64.9^{ab}	61.0^{b}	0.84	0.007

TME = true metabolizable energy, $^{a-c}$ Means in a row with different superscripts differ at p<0.05

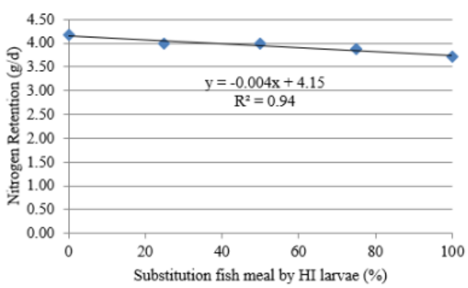

Figure 1. Effect of substitution fish meal by HI larvae on nitrogen retention

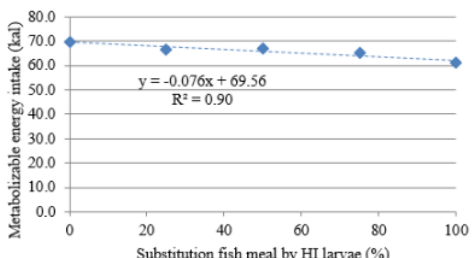

Figure 2. Effect of substitution fish meal by HI larvae on the intake of metabolizable energy

Quails performance

There was a decrease in feed intake, protein intake and egg production ($p<0.05$) due to the increased percentage of larvae HI to replace fish meal in the diet (Table 3; Figures 3 and 4). Despite these negative trends, the response was no difference in feed conversion.

Table 3. Feed intake, QDP, FCR and mortality

Variables	Treatment					SEM	*p*
	HI 0	HI 25	HI 50	HI 75	HI 100		
Feed intake (g/d)	22,7[a]	21,9[ab]	22,0[ab]	21,3[b]	20,5[c]	0.20	0,000
QDP (%)	76,4[a]	75,5[a]	72,8[a]	70,6[ab]	66,7[b]	1,09	0,014
FCR	3,03	3,04	3,1[illegible]	3,03	3,18	0,03	0,516

QDP: quail day production, FCR: feed conversion ratio; SEM: standard error of the mean. a-c Means in a row with different superscripts differ at $p<0.05$

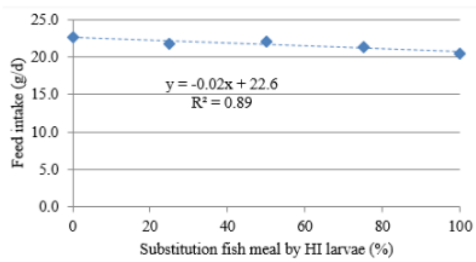

Figure 3. Effect of substitution fish meal by HI larvae on feed intake

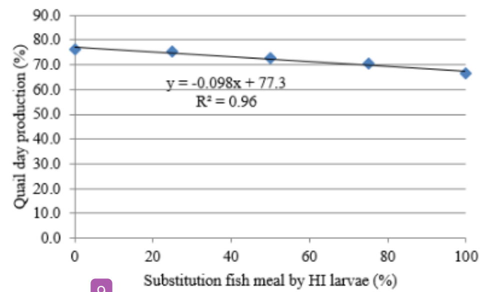

Figure 4. Effect of substitution fish meal by HI larvae on egg production

Discussion

Nutrien Utility

The digestibility of diets in the study was no significant (p>0.05) because the rations consumed were almost the same quality (iso-energy and iso-protein). The organic matter digestibility of the diets includes the constituent feed ingredients' breakdown, including protein, fat, carbohydrates, and crude fibre. Organic matter's digestibility, mainly protein and fat, was measured to verify the quail's nutrients utilization. Protein and fat are the egg's main compounds; its absorption was essential for egg production metabolism. Factors affecting digestibility are composition and proportion of ration materials, rations' chemical composition, ration protein levels, and mineral content (Law et al 2015). The high digestibility indicates that the higher the body absorbs feed substances.

The high value of protein and fat digestibility in each treatment (Table 2) indicated that the quail could effectively digest and absorb the protein and fat. The protein digestibility is relatively the same, because of the protein content in the same ration. Protein digestibility depends on the diet's ingredients and the amount of protein that enters the digestive tract (Cruz et al 2005; Widodo et al 2013).

H. illucens larvae's replacement fish meal up to 100% (HI 100) reduced nitrogen retention amount (p < 0.05). Nitrogen retention influenced by several factors, namely: feed consumption, protein consumption and protein digestibility. Protein consumption and nitrogen retention were directly proportional. Feed and protein intake decreased in HI 100, leading to the lowest nitrogen retention rates.

The metabolic energy intake (cal/bird/day) was calculated by the average total dietary intake (g/day) were multiplied by the metabolic energy content of the diets (kcal/kg). Substitution of fish meal by 100% HI larvae with *Trichoderma* 2% reduced quails' metabolic energy intake (p <0.05).

The metabolizable energy intake at HI 100 was lowest than the HI 0, HI 25, HI 50, and HI 75 treatments (p<0.05). Metabolic energy intake influenced by the diets consumption and the metabolic energy content of the diets. Feed consumption and energy consumption had a positive relationship, the higher the feed consumption, the higher the metabolic energy consumption. The feed intake of HI 100 treatment decreased, leading to lower metabolic energy consumption than HI 0, HI 25, HI 50, and HI 75 treatments. It was consistent with Barzegar et al (2020) that the lower energy consumption resulted in lower egg production.

Quail performance

Feed consumption

Substitution fish meal by 75% larvae HI were reduced feed consumption. Quails feed intake reduced at HI 75 and HI 100 (p <0.05). This study differs with Harlystiarini et al (2020) that the HI larvae meal in the diet did not affect the diet palatability. On the other, it was higher than the study Widjastuti et al (2014) consumption decreased at the level of 50% in the laying quails, 50% in hen layers (Agunbiade et al 2007). Atteh and Adeyoyin (1993) study that the fish meal's replacement rate of more than 10% causes low feed consumption and broiler performance. Dengah et al (2016), stated that substitution above 50% reduces ration consumption in broiler the finisher phase. According to (Awoniyi et al 2003), replacing fish meal (4% in the ration) with maggot meal at a level greater than 50% reduces broiler rations' consumption. Metabolic energy content in all treatments was equivalent. However, feed intake decreased at HI 75 and HI 100 (p<0.05), which was presumably due to increased chitin in the ration. High chitin levels cause low palatability (Bosch et al 2014). Decreased feed intake led to a reduction in energy consumption and other nutrients. A lower amount of ration consumption will result in fewer nutrients used for production (Scott et al 1982; Scott 2005).

Egg production

The quail's egg production among HI 0, HI 25, HI 50, and HI 75 not differed; however, declined in HI 100 ($p<0.05$). This result was higher than Widjastuti et al (2014) that substitution of fish meal (10% in rations) with HI meal to a limit of 50% on egg production.

Nutrient utility between treatments was not significantly different; however, egg production decreased according to lower diet consumption. The egg production was affected by the feed consumed; the low consumption reduced production. On the other hand, high consumption increased egg production. This study inline with Kobayasi et al (2002), the quail egg production influenced by feed consumption. The results of regression analysis with the dependent variable egg production and the independent variable feed consumption, show the equation $Y = - 19.3 + 4.24X$ with a value of R-sq = 0.583, and $p = 0.000$. This equation shows that every 1 gram increased in feed consumption will increase the QDP by 4.24 %. The R-sq value explains that 58.3% of egg production caused by feed consumption. Besides, feed consumption appears to be in line with egg production (Harlystiarini et al 2020).

Feed conversion ratio

Substitution fish meal by 100% HI larvae supplemented with 2% *Trichoderma* was not affected ($p> 0.05$) in feed conversion. The result inline with (Ansyari et al 2012; Gariglio et al 2019), however different from (Dengah et al. 2016) that substitution of fish meal with 100% BSF larvae decreased feed efficiency in broiler finisher.

The FCR was not significantly different due to the nutrient quality of HI 0, HI 25, HI 50, HI 75, and HI 100 had the same quality, similarly in digestibility, percentage of nitrogen retention and density of metabolic energy. In this study, based on feed conversion, it was possible to replace all fishmeal with 100% HI larvae supplemented with 2% *Trichoderma* without affecting FCR levels.

Conclusion

- *Hermetia illucens* larvae supplemented with 2% *Trichoderma* can be replaced fish meal up to 100% in the diet without detriment on nutrient utility and feed conversion.
- Substitution fish meal by *Hermetia illucens* larvae supplemented with 2% *Trichoderma* can be used up to 75% in the diet without detrimental effect on egg production.

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