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Effect of feeding larvae meal in the diets on growth performance, nutrient digestibility and meat quality in broiler chicken

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ABSTRACT

Larvae meal has been reported as a suitable alternative protein source to fish meal and soybean meal. It contains necessary essential amino acids required in poultry feeding. A feeding trial was conducted to assess the effects of feeding different levels of larvae meal on broiler performance, nutrient digestibility, carcass and meat quality, and bacterial count. Day-old male broiler chicks (216) were raised for 42 days. Birds were weighed and randomly allotted into 6 dietary treatments consisting of 6 replicates (pens) per treatment and 6 birds in each replicate. The dietary treatments were supplemented with earth larvae meal by 0% (control group), 2%, 4%, 6%, 8% and 10%. The feed and drinking water were provided *ad lib.* The findings showed that body weight, average daily gain and total weight gain for group of birds fed 0% larvae meal significantly decreased compared to those chickens fed 8% and 10% larvae meal. A higher crude protein digestibility was found in birds fed with larvae meal. Control group had the highest *Enterobacteriaceae* count compared to the other treatments. Larvae meal supplementation improved the growth performance, nutrient digestibility and meat quality of broiler chicken and decreased excreta *Enterobacteriaceae* counts.

Key words: Broiler, Larvae meal, Meat quality, Nutrients digestibility

Soybean meal and fish meal are conventional feedstuffs used as the main source of protein for poultry diets. This is due to their high nutritious content (Awachat *et al.* 2012, Brah *et al.* 2017). These two feed ingredients are also high in demand by other animals resulting in the higher cost of fish meal and soybean meal. There are attempts to substitute yellow corn and soybean meal with local feedstuffs to decrease the cost of feed, and to achieve the food security in the developing countries (Alshelmani *et al.* 2017b). Hence, it is important to determine alternative sources of protein to replace aforementioned meals. Earth larvae meal is reported as an alternative feedstuff to fish by several researchers (Sogbesan and Ugwumba 2008, Prayogi 2011). Larvae meal contains 64–76% protein content, which is greater than fish (45%) and meat (51%) meal (Prayogi 2011). These all could distinguish larvae meal from plant resources by possessing essential amino acids, fatty acids and reasonable amount of omega-3 in larvae meal (Fadaee 2012). Julendra *et al.* (2012) reported that earth larvae meal (*Lumbricus rubellus*) contains nutrient and antibacterial compounds; and it may be capable to replace in-feed antibiotic growth promoters. There are studies, which have tested earth larvae meal on the growth performance of animals. However, there is still limited information about effect of feeding larvae meal on broiler chicken. Therefore, this experiment was conducted to investigate the effects of different levels of larvae meal on the growth performance, nutrients digestibility, breast yield, meat quality and excreta microflora counts in broiler chickens.

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MATERIALS AND METHODS

Animal and experimental design: Day-old male broiler (Cobb 500) chicks (216) were purchased from a local hatchery and housed for 42 days in battery cages. The birds were weighed and randomly allotted into 6 dietary treatments consisting 6 replicates (pens) per treatment and 6 birds in each. All chickens were wing-tagged and water and feed were provided *ad lib.* Starter and finisher diets (Tables 1 and 2) were offered to the chickens from 0 to 21 days and 22 to 42 days of age,respectively. The larvae meal (black soldier fly; *Hermetia illucens*) was obtained from commercial supplier. The crude protein and crude fat content in larvae meal is about 46.7% and 42.2%, respectively (Ruhnke *et al.* 2018). The larvae meal was

Table 1. Feed compositions of starter diets for broiler chickens fed with different levels of larvae meal

Ingredients (g/kg as fed basis)	Larvae meal (%) 0	2	4	6	8	10
Corn	506.60	506.10	506.10	508.10	508.10	508.10
Soy-bean meal	293.80	295.30	292.10	285.10	285.10	285.10
Larva meal (EHI meal)	0.00	20.00	40.00	60.00	80.00	100.00
Wheat pollard	62.00	54.20	46.50	39.00	29.40	20.20
Crude palm oil	36.00	35.50	35.30	34.20	33.70	33.60
Fish meal 55%	76.00	63.50	54.70	48.50	37.80	26.20
L-Lysine	2.50	2.50	2.50	2.50	2.50	2.50
DL-Methionine	2.00	2.00	2.20	2.50	2.70	2.90
Dicalcium-phosphate 21	9.00	9.00	9.90	11.20	13.00	15.00
Calcium carbonate	7.30	7.10	5.90	4.10	2.90	1.60
Choline chloride	0.60	0.60	0.60	0.60	0.60	0.60
Salt	2.50	2.50	2.50	2.50	2.50	2.50
Mineral premix[1]	1.00	1.00	1.00	1.00	1.00	1.00
Vitamin premix[2]	0.60	0.60	0.60	0.60	0.60	0.60
Antioxidant[3]	0.10	0.10	0.10	0.10	0.10	0.10
Total	1000	1000	1000	1000	1000	1000
Calculated analyses (% as fed)						
Metabolizable energy (MJ/kg)	12.75	12.75	12.75	12.75	12.75	12.75
Protein	21.57	21.52	21.58	21.79	21.53	21.58
Fat	6.02	5.68	7.63	7.82	7.03	7.63
Fiber	3.51	3.14	2.54	2.93	3.07	2.54
Calcium	0.99	0.99	1.01	0.99	1.05	1.01
Total P	0.80	0.75	0.82	0.74	0.77	0.82
Avail P	0.47	0.45	0.55	0.45	0.46	0.55

[1]Mineral premix contains Fe 100 mg, Mn 110 mg, Cu 20 mg, Zn 100 mg, I 2 mg, Se 0.2 mg, Co 0.6 mg. [2]Vitamin premix contains retinol 2 mg, cholicalciferol 0.03 mg, α–tocopherol 0.02 mg, menadione 1.33 mg, cobalamin 0.03 mg, thiamine 0.83 mg, riboflavin 2 mg, folic acid 0.33 mg, biotin 0.03 mg, panthothenic acid 3.75 mg, niacin 23.3 mg, pyridoxine 1.33 mg. [3]Antioxidant contains butylatedhydroxyanisole.

offered at different inclusion rates. In order to meet their nutrient requirements for all treatments, diets were formulated isocaloric and isonitrogenous. The dietary treatments were formulated isocaloric and isonitrogenous and supplemented with larvae meal by 0%, 2%, 4%, 6%, 8% and 10%. Diets were formulated to meet the nutrient requirements of broiler chickens based on NRC (1994). The experimental animals received humane care as outlined and approved by the Institutional Animal Care and Use Committee (IACUC), Universiti Putra Malaysia.

Data and samples collection: Individual body weight (BW) was recorded, and feed intake (FI) per cage was measured weekly. Live weight gain (WG), average daily weight gain (ADG), total weight gain (TWG) and feed conversion ratio (FCR) were calculated. A total of 6 and 12 chickens from each treatment were slaughtered for sampling at weeks 3 and 6, respectively. Breast meat, ileal digesta and excreta were taken for further analyses.

Table 2. Feed compositions of finisher diets for broiler chickens fed with different levels of larvae meal

Ingredients (g/kg as fed basis)	Larvae meal (%) 0	2	4	6	8	10
Corn	580.00	583.00	583.00	583.00	583.50	583.50
Soybean meal	269.00	257.00	236.00	209.50	181.30	154.70
Larva meal (EHI meal)	0.00	20.00	40.00	60.00	80.00	100.00
Wheat pollard	63.74	63.24	67.54	76.44	86.44	95.44
Crude palm oil	34.00	32.00	29.10	26.60	24.10	21.60
Fish meal 55%	10.00	0.50	0.00	0.00	0.00	0.00
L-Lysine	2.50	1.50	1.50	1.50	1.50	1.50
DL-Methionine	2.00	2.00	2.00	2.00	2.00	2.00
Dicalcium phosphate 21	16.46	18.16	18.26	18.36	18.56	18.66
Calcium carbonate	17.40	17.40	17.40	17.40	17.40	17.40
Choline chloride	0.60	0.60	0.60	0.60	0.60	0.60
Salt	2.60	2.90	2.90	2.90	2.90	2.90
Mineral premix[1]	1.00	1.00	1.00	1.00	1.00	1.00
Vitamin premix[2]	0.60	0.60	0.60	0.60	0.60	0.60
Antioxidant[3]	0.10	0.10	0.10	0.10	0.10	0.10
Total	1000	1000	1000	1000	1000	1000
Calculated analyses (% as fed)						
Metabolizable energy (MJ/kg)	13.01	13.03	13.03	13.03	13.02	13.03
Protein	17.12	17.17	17.16	17.13	17.15	17.19
Fat	7.96	7.70	6.76	7.29	7.65	8.40
Fiber	3.58	3.01	2.69	2.47	2.88	2.40
Calcium	1.05	1.04	1.04	1.05	1.05	1.05
Total P	0.80	0.74	0.73	0.73	0.75	0.72
Avail P	0.47	0.45	0.45	0.46	0.46	0.46

[1]Mineral premix contains Fe 100 mg, Mn 110 mg, Cu 20 mg, Zn 100 mg, I 2 mg, Se 0.2 mg, Co 0.6 mg. [2]Vitamin premix contains retinol 2 mg, cholicalciferol 0.03 mg, α-tocopherol 0.02 mg, menadione 1.33 mg, cobalamin 0.03 mg, thiamine 0.83 mg, riboflavin 2 mg, folic acid 0.33 mg, biotin 0.03 mg, panthothenic acid 3.75 mg, niacin 23.3 mg, pyridoxine 1.33 mg. [3]Antioxidant contains butylatedhydroxyanisole.

Breast meat yield: The birds were randomly chosen at 42 days of age, weighed and slaughtered to evaluate breast meat yield, abdominal fat and internal organs (gizzard, liver, spleen).

Apparent nutrient digestibility: An indigestible marker titanium dioxide (TiO_2) was added to the starter and finisher diets (Alshelmani *et al.* 2016). Briefly, the indigestible marker was added to the diet before 4 days of sampling. The ileal digesta were collected and kept at –20°C for further analysis. The nutrients were determined in the feed and digest as well as the marker to calculate the nutrient digestibility.

Evaluation of meat quality parameters: All meat quality measurements were assessed using the breast muscle. Immediately after removal, samples were cut into 4 portions to measure pH, shear force, drip loss, cooking loss and colour. The meat quality measurement was conducted as described by Alshelmani *et al.* (2017a).

Excreta lactic acid bacteria, Enterobacteriaceae *count:* The excreta lactic acid bacteria (LAB) and *Enterobacteriaceae* (ENT) population were determined as per Kareem *et al.* (2016).

Statistical analysis: General linear model of SAS (2014) was used to analyze all the present data. Duncan's Multiple Range Test was used to compare the significant differences between treatments. Orthogonal polynomial contrasts were used to test the linear or quadratic nature of the response to incremental concentrations of larvae meal. Test of statistical significance was considered at P<0.05.

RESULTS AND DISCUSSION

The results of growth performance are shown in Table 3. It is obvious that the BW, ADG and TWG were linear and quadratic significantly improved (P<0.05) by including larvae meal compared to the control. However, no significant differences (P>0.05) were found between birds fed 0%, 2%, 4% and 6% larvae meal. Furthermore, 8% larvae meal had a quadratic significant better (P<0.05) FCR as compared to the control but there was no significant difference (P>0.05) observed among chickens fed 2%, 4% and 6% larvae meal. The results of growth performance are in accordance with many previous findings (Loh *et al.* 2009, Julendra *et al.* 2012, Rezaeipour *et al.* 2014). Moreover, another study suggested that improvement of body weight gain in broilers fed diets with earth larvae meal may be due to the antibacterial characteristics of earth larvae meal (Julendra *et al.* 2012).

Table 4 shows the effect of different levels of larvae meal on abdominal fat, visceral organs and breast meat in broilers chickens at 42 days of age. For the abdominal fat, birds fed 0% larvae meal was quadratic significantly higher (P<0.05) than those fed 2%. However, no significant differences (P>0.05) were found among the birds fed 0%, 4%, 6%, 8% and 10% larvae meal. On the other hand, the broiler chickens fed 10% larvae meal had a quadratic significant lower (P<0.05) gizzard weight than those fed 0% and 2% larvae meal. There was no significant difference (P>0.05) among all treatment groups for liver, spleen and breast meat weight. These results showed that the carcass traits were not influenced by supplementing larvae meal in broiler chicken diets except for the abdominal fat and gizzard weight. In contrast, Rezaeipour *et al.* (2014) revealed that the breast muscle weight increased in broilers fed diets supplemented with high inclusion rate of earth larvae meal in the diets. The increasing in abdominal fat on the group of chickens fed higher levels of larvae meal could be attributed to the medium chain fatty acids of larvae oil (Li *et al.* 2016). In addition, it was reported that larvae meal contain up to 42.2% crude fat (Ruhnke *et al.* 2018).

The apparent nutrient digestibility of starter and finisher diets in broiler chickens fed different levels of larvae meal supplementation are shown in Table 5. Apparent digestibility of crude fat and crude protein were not significantly different (P>0.05) among the treatment groups. This observation is in agreement with the findings of Ijaiya and Eko (2009), who reported that there was no significant difference in apparent nutrient digestibility in starter diet in birds fed different levels of larva meal compared to the control.

Table 3. Growth performance of broiler chickens at 6 weeks of age fed with different levels of larvae meal

Parameter	Larvae meal (%)							Contrasts	
	0	2	4	6	8	10	SEM[1]	Linear[2]	Quadratic
BW (kg)	2.08[b]	2.19[ab]	2.18[ab]	2.19[ab]	2.25[a]	2.21[a]	0.02	*	*
ADG (g)	48.44[b]	51.12[ab]	50.77[ab]	51.06[ab]	52.35[a]	51.46[a]	0.38	*	*
FI (kg)	3.68	3.58	3.64	3.64	3.63	3.84	0.04	NS	NS
TWG (kg)	2.04[b]	2.14[ab]	2.15[ab]	2.14[ab]	2.19[a]	2.16[ab]	0.02	*	*
FCR (feed/gain)	1.81[a]	1.67[ab]	1.69[ab]	1.69[ab]	1.66[b]	1.78[a]	0.03	NS	*

[ab]Means±SEM. Means in the same row not sharing a common superscript is significantly different at P<0.05. [1]SEM, standard error of the means (pooled). [2]Linear or quadratic response estimated using orthogonal polynomial contrasts (NS: nonsignificant; *P<0.05). BW, body weight; ADG, average daily gain; FI, feed intake; TWG, total weight gain; FCR, feed conversion ratio.

Table 4. Effects of feeding different levels of larvae meal on abdominal fat, visceral organs and breast meat in broiler chickens

Parameter	Larvae meal (%)							Contrasts	
	0	2	4	6	8	10	SEM[1]	Linear[2]	Quadratic
Abdominal fats (g)	43.83[a]	27.67[b]	33.50[ab]	38.33[ab]	40.83[ab]	37.67[ab]	1.79	NS	*
Gizzard (g)	38.50[a]	38.33[a]	35.00[ab]	31.17[ab]	31.17[ab]	25.50[b]	1.55	NS	*
Liver (g)	51.17	45.17	48.00	50.67	48.83	44.67	1.65	NS	NS
Spleen (g)	3.83	4.00	4.17	3.67	3.17	3.33	0.24	NS	NS
Breast meat (g)	566.00	537.00	570.67	588.67	591.83	556.67	12.77	NS	NS

[ab]Means ±SEM. Means in the same row not sharing a common superscript is significantly different at P<0.05. [1]SEM, standard error of the means (pooled). [2]Linear or quadratic response estimated using orthogonal polynomial contrasts (NS: nonsignificant; *P<0.05).

Table 5. Apparent nutrient digestibility of starter and finisher diets in broiler chickens fed different levels of larvae meal

Parameter	Larvae meal (%)							Contrasts	
	0	2	4	6	8	10	SEM[1]	Linear[2]	Quadratic
				0 – 21 days					
Dry matter (%)	92.41	92.45	92.22	92.36	92.29	92.40	0.03	NS	NS
Ash (%)	7.16	7.29	7.43	11.99	10.02	7.36	0.75	NS	NS
Crude fat (%)	21.89	18.58	20.15	23.16	28.64	30.93	1.60	NS	NS
Crude protein (%)	55.22	51.99	50.86	54.61	53.44	56.05	0.71	NS	NS
				22 – 42 days					
Dry matter (%)	87.18	87.82	87.67	87.38	87.26	87.41	0.06	NS	NS
Ash (%)	7.41	11.06	9.36	8.032	8.74	9.73	0.31	NS	NS
Crude fat (%)	14.45	16.33	21.36	19.90	17.39	19.19	1.06	NS	NS
Crude protein (%)	35.39[b]	45.25[a]	39.97[ab]	45.39[a]	45.08[a]	41.35[ab]	1.13	NS	*

[1]SEM, standard error of the means (pooled). [2]Linear or quadratic response estimated using orthogonal polynomial contrasts (NS: nonsignificant; *P<0.05). [ab]Means ±SEM. Means in the same row not sharing a common superscript are significantly different (P<0.05).

In the finisher phase, there were no significant differences (P>0.05) for the apparent digestibility of crude fat among the treatment groups. A higher quadratic significant (P<0.05) digestibility of crude protein was observed in birds fed with different levels of larvae meal compared to the control group. This improvement in digestibility was in accordance with the findings of (Rezaeipour *et al.* 2014), who noticed a higher (P<0.05) crude protein digestibility in birds fed diets supplemented with earth larvae meal. The increased digestibility of crude protein has been attributed to the increased length of villi height and crypt depth of jejunum in birds fed with earth larvae meal (Julendra *et al.* 2012, Rezaeipour *et al.* 2014).

The data for meat quality of broiler chicken fed with different levels of larvae meal is shown in Table 6. It can be seen that birds fed 0% and 4% larvae meal had a quadratic lower (P<0.05) drip loss compared to those birds fed 2% and 10% larvae meal. However, no significant differences (P>0.05) were found among 0%, 4%, 6% and 8% larvae meal. This observation could be attributed to the high content of crude fat in the larvae meal, which may affect the drip loss for those birds fed high levels of black soldier larvae in their diets. This is contradicted to the findings of Vu *et al.* (2009), who observed in their research that different levels of larva meal supplementation did not affect drip loss values in chicken breast meat. Also, there were no significant differences (P>0.05) in cooking loss of breast meat among the treatment groups after 24 h chilling storage. This observation corroborates the results of Vu *et al.* (2009) and Zhu *et al.* (2010).

This study observed that there were no significant differences (P>0.05) in instrumental tenderness of breast meat from broilers fed with different levels of larvae meal. Similar to that, Zhu *et al.* (2010) found no significant difference (P>0.05) in muscle tenderness of a fish meal, earth larvae meal powder and mixed groups (fish and earth larvae meal powder) of broiler chicken. The increasing levels of larvae meal supplementation had no influence on Volodkevitch shear force. Corzo *et al.* (2005) and Schilling *et al.* (2010) indicated that broiler chickens require about 8% of larvae meals supplementation to give tender and highly acceptable meat by consumer.

Regarding breast meat pH, no significant differences was found (P>0.05) among the all the treatment groups. Similarly, Vu *et al.* (2009) reported that the different levels of red worm supplementation had no significant effect on pH of breast muscle in poultry.

No significant (P>0.05) differences in colour of meat

Table 6. Meat quality of broiler chicken fed with different levels of larvae meal

Parameter	Larvae meal (%)							Contrasts	
	0	2	4	6	8	10	SEM[1]	Linear[2]	quadratic
Drip loss (%)	3.24[b]	4.36[a]	3.14[b]	3.83[ab]	3.51[ab]	4.41[a]	0.16	NS	*
Cooking loss (%)	18.03	16.92	15.53	15.89	15.29	16.55	0.39	NS	NS
Shear force (g/kg)	987	897	852	856	853	849	0.19	NS	NS
pH	5.82	5.84	5.79	5.80	5.83	5.76	0.01	NS	NS
Meat colour									
L*	50.53	50.67	53.88	50. 55	50.67	49.54	0.34	NS	NS
a*	6.93	6.72	7.32	6.97	7.98	6.61	0.14	NS	NS
b*	15.88	16.15	17.34	15.85	18.34	16.87	0.27	NS	NS

[ab]Means±SEM. Means in the same row not sharing a common superscript are significantly different (P<0.05). [1]SEM, standard error of the means (pooled). [2]Linear or quadratic response estimated using orthogonal polynomial contrasts (NS: nonsignificant; *P<0.05). L*, lightness; a*, redness; b*, yellowness.

Table 7. Excreta lactic acid bacteria and *Enterobacteriaceae* counts in broiler chicken fed with different levels of larvae meal

Parameter	Larvae meal (%)							Contrasts	
	0	2	4	6	8	10	SEM[1]	Linear[2]	Quadratic
Lactic acid bacteria (logCFU/g)	6.54	6.53	6.61	6.57	6.29	6.55	0.06	NS	NS
Enterobacteriaceae (logCFU/g)	5.71[a]	5.28[b]	5.05[bc]	4.81[c]	5.03[bc]	5.27[b]	0.06	NS	*

[abc]Means±SEM. Means in the same row not sharing a common superscript are significantly different (P<0.05). [1]SEM, standard error of the means (pooled). [2]Linear or quadratic response estimated using orthogonal polynomial contrasts (NS: nonsignificant; *P<0.05).

were observed among the treatments. The present results were in consistent with the findings of Vu *et al.* (2009), who reported that no difference in the colour of breast meat was observed among the different levels of red worm supplementation.

Table 7 shows excreta lactic acid bacteria and *Enterobacteriaceae* counts. There was no significant difference (P>0.05) found for the lactic acid bacteria count among the treatment groups. However, T4 had the lowest level of *Enterobacteriaceae* count among all the treatments. In contrast, birds fed 0% larvae meal had a quadratic significant (P<0.05) higher *Enterobacteriaceae* as compared to those birds fed 2%, 6% and 10% larvae meal. Salzet *et al.* (2006) also observed that earthworm *L. rubellus* contained 'lumbricin I' which is a peptide group containing 62 amino acids that possess antibacterial properties. Loh *et al.* (2009) confirmed that fish and soybean meal could be substituted partially with larvae meal in broiler diets. It has also been reported that broilers fed with larvae meal supplemented diet had higher lactic acid bacteria counts but without any effect on *Enterobacteriaceae* count. In contrast, *Enterobacteriaceae* count was found lower in the current study for those broilers fed with larvae meal.

It can be concluded that the growth rate and feed conversion ratios were significantly improved by feeding a larvae meal supplemented diet to broiler chickens. This could be explained by their better apparent nutrient digestibility of nutrients such as protein. Additionally, excreta *Enterobacteriaceae* count was lower in birds fed with larvae meal supplemented diets than the control.

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