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## Influence of earthworm meal supplementation on intestinal physiology and Morphometry in Japanese quails

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**Abstract**

The objective of this study was to evaluate the effect of feed supplement contained Earthworm meal on small intestinal physiology and morphometry of Japanese quail (*Coturnix coturnix Japonica*). An experiment was carried out at Madras Veterinary College in 240 numbers of day old Japanese quails in randomized design with four different dietary supplements viz G1 (0 %), G2 (50 %), G3 (75 %) and G4 (100 %) earthworm meal as replacement for fish meal during 6 weeks of experiment periods. All the animals were randomly divided into four groups with 60 animals in each group (20 animals in each replicate). Proximate analysis of earth worm meal showed that it contains crude protein of 29.49%. Variable measured were intestinal PH, Intestinal enzymes and intestinal morphology. There was no significant change in amylase and lipase activity between groups. At the end of study G4 ( $32.65 \pm 1.56$  IU/ml) showed higher crypt width ( $P < 0.05$ ) compared to other groups G1 ( $28.10 \pm 1.28$  IU/ml), G2 ( $28.96 \pm 1.91$  IU/ml) and G3 ( $30.63 \pm 2.85$  IU/ml). Among the four groups, G4 ( $140.76 \pm 4.90$  IU/ml) and G3 ( $137.25 \pm 3.08$  IU/ml) showed significant increase in trypsin activity in the present study. The results showed that inclusion of earth worm meal at 75% and 100% as a replacement of fish meal significantly increased the intestinal physiology and morphology of Japanese quail compared to control group at 3<sup>rd</sup> and 6<sup>th</sup> weeks respectively. It is concluded that replacement of fishmeal with earth worm meal may be the ideal alternate protein source for better growth performance in Japanese quail.

**Keywords:** earthworm meal, fish meal, intestinal physiology, growth performance

**Introduction**

Japanese quail is a domesticated avian species, has assumed importance worldwide as laboratory bird and is presently commercially exploited for meat and egg production and forms an alternative to poultry farming [5]. Its unique characteristics include small body size, faster growth rate, early sexual maturity, short generation interval, shorter incubation period, high rate of egg production, simple management, ability to withstand wider range of climatic and farm conditions which pose this species as an excellent alternative farming to chicken [1]. Nutrition is one of the most important factors required to obtain normal growth and egg production from quail. Fish meal is a one of the conventional feed stuff which has been used for many years as an important protein source for poultry diet due to high nutrient content [2]. Developing nations like India, the cost of animal protein in feed like fish meal is expensive because it is consumed not only animals but also human. Therefore it is necessary to explore the non-conventional feed stuff which has high protein content so it does not entirely rely on the fish meal [3, 4]. There are many choices of alternate protein sources available to completely or partially replace the fishmeal of the Japanese quail ration, one such is the earthworm meal prepared from the locally available breeds of earthworm (*Eudrilus eugeniae*) [5]. The presence of high protein content in Earthworms meal (60-70%) can be used as a substitute for fish meal. The percentage of protein content in earth worm meal is larger than fish meal 45% and meat 51% [6]. Earthworms possess amino acid profile similar to that of fishmeal and hence can be used as an alternative source of protein in chicken/ Japanese quail ration [7]. This study was aimed to examine the effect of varying inclusion levels of earthworm meal in intestinal physiology and morphometry of Japanese quail.

**Materials and Methods****Preparation of earthworm pits**

Earthworm, *Eudrilus eugeniae*, was obtained from Krishi Vigyan Kendra, Kattupakkam, Kanchipuram District.

The worms were bred during the month of March 2017 and maintained at a pit belonging to Department of Agronomy, Madras Veterinary College, Chennai. The pit was cleared of its previous contents. It was then filled with gravel sand for about 40 cm, followed by layering with soft manure made of cow dung. This was topped further with cow dung and partially decomposed dry leaves alternately. The pit was wetted frequently to maintain suitable dampness for housing earthworms. After preparation of the pit, earthworms (2 kg) were inoculated in the pit and allowed to multiply. The pit was covered with wire mesh at the top to prevent predator intrusions. Adult earthworms with well differentiated clitellum were collected for the preparation of earthworm meal and for the formulation of Japanese quail ration utilized in this study (Fig. 1).



Fig 1: Earth worm pit and Earth worm culture

### Preparation Earth worm meal

Earthworm meal was prepared by powdering incubated earthworms at 60 °C for 24 hours in hot air oven [8], collected by standard protocol for earth worm culture.

### Preparation of Earth worm meal

#### Proximate analysis of earthworm meal

Proximate analysis of earthworm meal was done at Animal Feed Analytical and Quality Assurance Laboratory, Veterinary College and Research Institute, Namakkal (Table 1).

Table 1: Proximate analysis of Earth worm meal in %.

Components	Concentration
Moisture	5.3
Crude protein	29.49
Crude Fibre	1.25
Ether Extract	3.75
Total Ash	45.66
Calcium	2.89
Phosphorus	1.37
Gross Energy	2649 kcal/kg

### Feed Composition and Animals Trails

A total of 240 Japanese quail chicks (*Coturnix coturnix japonica*) from day old to 6 weeks of age were maintained in equicaloric and equinutritious dietary regime at Poultry Research Station, Madhavaram Milk Colony, Chennai – 51. They were fed twice daily at morning and evening with provision of *ad libitum* water. They were divided into four groups, each group consists of three replicate with 20 birds in one replicate and the birds were fed up to 6 weeks. The feed treatment composition was presented in Table 2.

Table 2: Percentage composition of experimental diet in various groups

Groups	Experimental feeding	No. of replicates	No. of Birds / replicate	Total no. of birds / treatment
Group1 (Control group)	Japanese quail basal diet (with 7 % fish meal)	3	20	60
Group 2	Japanese quail basal diet replacing 50 % fish meal with earthworm meal.	3	20	60
Group 3	Japanese quail basal diet replacing 75 % fish meal with earthworm meal	3	20	60
Group 4	Japanese quail basal diet replacing 100 % fish meal with earthworm meal	3	20	60
	Total			240

### pH

The pH of the intestinal contents was recorded by pH strip immediately after sacrifice the bird.

### Amylase activity

Buffered starch substrate (pH 7.0) was prepared by the addition of 13.3g of anhydrous disodium phosphate and 4.3g of benzoic acid to 250 ml of triple distilled water and boiled. A soluble starch (200 mg) was prepared separately in 5ml of cold distilled water and mixed to the boiling mixture. The beaker was rinsed with the additional cold water, boiled, centrifuged for one minute, cooled to room temperature and diluted to 500 ml with triple distilled water. Digesta supernatant was taken and diluted to 1:1000 with 0.9% saline. Estimation of amylase activity of the digesta was assessed as per the method of Coles [9].

One ml of 0.02% buffered starch substrate (pH 7.0) was incubated at 37°C exactly for 8.5min along with 0.1ml of

diluted supernatant solution and one ml of working iodine solution (25g of potassium fluoride with 50ml 0.1N iodine stock solution). The stock solution contained 3.567g of potassium iodate, 45g of iodide, and 9ml of concentrate hydrochloric acid in one liter of triple distilled water. The working iodine solution was made upto 500 ml with triple distilled water. The mixture was thoroughly mixed and the volume was made up to 10ml with triple glass distilled water. The OD was read at 660 nm in a colorimeter, using distilled water as blank.

$$\text{Amylase activity (IU/ml)} = \frac{\text{OD of control} - \text{OD of test}}{\text{OD of control}} \times \text{Volume of supernatant}$$

X Dilution factor

## Trypsin activity

### Principle

A quantitative method for determination of trypsin in intestinal contents uses the substrate benzoyl-arginine-p-nitroanilide. The reaction product, p-nitroaniline can be readily determined by its absorbance at 405nm. The trypsinogen in the intestinal contents is first activated by incubation with a small amount of trypsin.

### Procedure

One ml of sample was mixed with 1 ml of pH 7.6 buffer and incubated for 20 minutes at 37°C. For the assay 100 µl of the incubated sample was added to 3 ml of the substrate previously warmed to 37°C, mixed, and read against a blank of 3 ml of substrate and 1000 µl of pH 7.6 buffers. Reading was taken at 405 nm at 1 minute after sample addition and again at 5 and 10 minutes later, incubating at 37°C between readings<sup>[10]</sup>. A standard curve was prepared by treating 100 µl concentration of samples of the working standards and absorbance at 405 nm was plotted against micrograms per millilitre. Samples were read from the curve.

The results were expressed in IU/ml as per the following formula

$$1\text{U/ml} = \Delta A/\text{minute} * 3.05/0.05 * 1000/9.9 = \Delta A/\text{minute} * 6160/1000$$

Where 3.05 = Total volume

9.9 = Absorbance factor for p-nitroaniline

0.05 = Amount of serum added

since 100 µL contained 50% serum and 50% buffer.

## Lipase activity

### Procedure

The lipase activity of the digesta was determined as per the method of Boutwell<sup>[11]</sup>. The substrate buffer mixture was prepared by stirring five volume of 0.006M phosphate buffer (pH7.0) along with one volume of olive oil emulsion. Twelve millilitres of the substrate buffer mixture was equally transferred into two tubes and warmed in a water bath at 37 °C. To one tube 5ml of the intestinal tissue homogenate was added and mixed thoroughly by gentle inversion. The second tube served as a blank. Both the tubes were incubated at 37°C for 24 hours at the end of incubation. 5ml of tissue homogenate was added to the blank. The mixture was titrated along with 4-6 drops of phenolphthalein against 0.05N sodium hydroxide (NaOH) to get a distinct pink colour. The lipase unit was the quantity of enzyme required to release acid equivalent to 1 ml of 0.05 N NaOH from an olive oil substrate in 24 hours.

$$\text{Lipase activity (IU/ml)} = (T-B)/W \times 5$$

Where,

T - Titre value of test

B - Titre value of blank

W - Weight of intestine content in grams

5 - The dilution factor

## Intestinal Morphology

Quail chicks were sacrificed by humane method at the age of 3<sup>rd</sup> week and 6<sup>th</sup> week. Jejunal tissue samples were carefully cleansed in phosphate buffer saline (0.1ml, pH 7.4) and fixed in 10% buffered formalin for 2 days. all the tissue samples

were sectioned and stained with Haematoxyline and Eosin according to the method described by Bancroft and Marilyn<sup>[12]</sup>. Intact well oriented villus-crypt units were randomly selected at each tissue sample. Villus height was measured from tip of the villus to the villus-crypt junction and crypt depth was measured as the extent of invagination between two villi. Villus width was measured at its base where it was most wider.

## Statistical analysis

The data obtained on various parameters were statistically analysed as per the method of Snedecor and Cochran<sup>[13]</sup> using general linear model in Two-way ANOVA and post hoc analysis were carried out using Duncan's for multiple comparisons using computerized software programme SPSS Ver.20.0.

## Results and Discussion

At 3<sup>rd</sup> week and sixth of age intestinal pH of experimental group, G4 showed significantly lowest ( $P < 0.01$ ) pH compared to other groups G1, G2 and G3. The results were in accordance with Hsu *et al.*<sup>[14]</sup> who suggested that decreased digesta pH recorded due to the accelerated release of H<sup>+</sup> from the carboxyl groups in the hydrolyzed peptide when a higher level of protein source was included in the diet. The results of this study indicates that the maintenance of normal range of pH in all the other three groups showed that the earthworm meal was well accepted by quails after initial proventricular digestion and also there exists an ideal environment for further catalysis by pancreatic enzyme.

Intestinal amylase activity of quails showed no significant changes between the groups both at third week and sixth week and they were within the normal range. This was concurrent with study of Beena<sup>[15]</sup> who reported that the intestinal amylase activity ranged between 103.23±8.08 IU/g and 234.38 ± 38.96 IU/g in Japanese quails and also in accordance with Yang *et al.*<sup>[16]</sup> who reported that there was no significant effect of dietary protein level on intestinal amylase activity in geese. This was contradictory to study of Zhao *et al.*<sup>[17]</sup> reported that there was a significant increase in the amylase activity with higher dietary CP content in the jejunal fluid of peking ducks. However, the levels of pancreatic amylase is reported to be influenced by age, growth and development in Japanese quail<sup>[18]</sup>, but in this study there was no such variation recorded between third and sixth week of age.

Among the four groups, G4 and G3 showed significant ( $P < 0.05$ ) increase in trypsin activity in the present study which was in accordance with Zhao *et al.*<sup>[17]</sup> who reported that there was a significant increase in the trypsin and chymotrypsin activity with higher dietary crude protein content in the jejunal fluid of peking ducks. However earlier studies in avian species regarding dietary changes with regard to level and quality of protein showed that it had little effect and non significant increase of trypsin activity in jejunum but the secretory rate was greater<sup>[19]</sup>. This could have been the reason for the significant increase in G4 and G3 of the present study, as there would be accelerated secretion.

Lipase activity showed no significant difference between groups. Increased intake of fat in the diet expected to increase lipase activity of pancreatic juice. As there was no appreciable changes in lipase activity with respect to the level of inclusion of fat in the present experiment which was in agreement with Ren *et al.*<sup>[20]</sup> who reported that the effects of the dietary protein source on lipase activities in the small intestinal fluid

were not significant and also Zhao *et al.* [21] who reported there was no significant effect in the lipase activity with higher dietary crude protein content in the jejunal fluid of peking ducks.

Structure of intestinal mucosa can reflect the health condition of intestine [22]. In the present study the intestinal villi length, villi width, crypt length and crypt width showed significant increase in G3 and G4 compared to G1 and G2 groups. This result was in agreement with Rezaei *et al.* [23] who reported that the birds fed diets containing high levels of earthworm meal had the biggest jejunum crypt depth. Also

Soltan [24] observed that broilers fed diet containing of glutamine had significantly longer villi in duodenum and jejunum compared to the control group. This was agreed with the findings of Abdulkarim *et al.* [25] Who reported that jejunal villus height: crypt depth ratio increased in birds fed with increased animal protein. Moreover, the higher villi heights certainly contribute to increased surface area for more nutritional absorption. It has been suggested that longer villi would result in an increase surface area and higher absorption of available nutrients as reported by Yasar and Forbes [26].

**Table 3:** Effect of earthworm meal on the intestinal pH and enzyme activity (IU/ml) of Japanese quails (n=24, Mean  $\pm$  S.E.)

Groups	pH		Amylase activity (IU/ml)		Lipase activity (IU/ml)		Trypsin activity (IU/ml)	
	Third week	Sixth week	Third week	Sixth week	Third week	Sixth week	Third week	Sixth week
G1	6.45 $\pm$ 0.13	6.45 $\pm$ 0.08	221.63 $\pm$ 4.60	222.17 $\pm$ 5.40	11.32 $\pm$ 0.53	11.05 $\pm$ 0.43	122.07 <sup>ab</sup> $\pm$ 3.94	122.43 <sup>ab</sup> $\pm$ 3.72
G2	6.52 $\pm$ 0.12	6.63 $\pm$ 0.08	218.42 $\pm$ 3.86	214.06 $\pm$ 5.91	11.35 $\pm$ 0.36	11.33 $\pm$ 0.55	115.20 <sup>a</sup> $\pm$ 3.37	114.25 <sup>a</sup> $\pm$ 3.37
G3	6.21 $\pm$ 0.04	6.48 $\pm$ 0.06	218.95 $\pm$ 3.62	220.31 $\pm$ 3.69	11.21 $\pm$ 0.39	10.70 $\pm$ 0.50	138.27 <sup>bc</sup> $\pm$ 3.38	137.25 <sup>b</sup> $\pm$ 3.08
G4	5.89 $\pm$ 0.17	5.73 $\pm$ 0.16	232.55 $\pm$ 5.92	221.81 $\pm$ 3.94	10.58 $\pm$ 0.47	10.74 $\pm$ 0.39	146.15 <sup>c</sup> $\pm$ 3.96	140.76 <sup>b</sup> $\pm$ 4.90
F value	5.306 <sup>**</sup>	14.675 <sup>**</sup>	2.059 <sup>NS</sup>	0.183 <sup>NS</sup>	0.681 <sup>NS</sup>	0.392 <sup>NS</sup>	0.911 <sup>*</sup>	0.720 <sup>*</sup>

\*\*-. Highly significant ( $P < 0.01$ ), \*-. Significant ( $P < 0.05$ ) and <sup>NS</sup>-. Non significant

Means bearing different superscripts in a column differ significantly between groups.

**Table 4:** Effect of earthworm meal on the intestinal morphology ( $\mu$ m) of Japanese quails (n=24, Mean  $\pm$  S.E.)

Groups	Villi Height		Villi Width		Crypt Length		Crypt width	
	Third week	Sixth week	Third week	Sixth week	Third week	Sixth week	Third week	Sixth week
G1	384.50 <sup>a</sup> $\pm$ 14.50	390.20 <sup>a</sup> $\pm$ 11.02	114.81 <sup>c</sup> $\pm$ 1.20	118.92 <sup>c</sup> $\pm$ 2.20	116.67 <sup>c</sup> $\pm$ 1.02	117.72 <sup>c</sup> $\pm$ 1.46	22.09 <sup>a</sup> $\pm$ 1.09	28.10 <sup>a</sup> $\pm$ 1.28
G2	522.50 <sup>b</sup> $\pm$ 13.82	566.76 <sup>b</sup> $\pm$ 13.42	102.44 <sup>a</sup> $\pm$ 0.92	106.67 <sup>ab</sup> $\pm$ 1.67	99.22 <sup>b</sup> $\pm$ 2.89	100.06 <sup>b</sup> $\pm$ 2.24	23.87 <sup>a</sup> $\pm$ 2.21	28.96 <sup>a</sup> $\pm$ 1.91
G3	576.50 <sup>c</sup> $\pm$ 11.09	630.25 <sup>c</sup> $\pm$ 15.79	108.73 <sup>b</sup> $\pm$ 1.86	104.87 <sup>a</sup> $\pm$ 2.19	157.02 <sup>d</sup> $\pm$ 1.67	160.78 <sup>d</sup> $\pm$ 1.79	25.89 <sup>ab</sup> $\pm$ 1.76	30.63 <sup>ab</sup> $\pm$ 2.85
G4	627.33 <sup>d</sup> $\pm$ 12.78	645.33 <sup>d</sup> $\pm$ 10.76	115.23 <sup>c</sup> $\pm$ 0.78	118.55 <sup>c</sup> $\pm$ 1.89	119.56 <sup>bc</sup> $\pm$ 1.45	125.09 <sup>bc</sup> $\pm$ 2.09	27.78 <sup>b</sup> $\pm$ 2.09	32.65 <sup>b</sup> $\pm$ 1.56
Fvalue	9.098 <sup>**</sup>	8.178 <sup>**</sup>	5.192 <sup>*</sup>	6.098 <sup>*</sup>	6.120 <sup>*</sup>	4.167 <sup>*</sup>	2.897 <sup>*</sup>	2.926 <sup>*</sup>
Fvalue	5.121 <sup>*</sup>		6.202 <sup>*</sup>		3.712 <sup>*</sup>		3.945 <sup>*</sup>	

\*\*-. Highly significant ( $P < 0.01$ ), \*-. Significant ( $P < 0.05$ ) and <sup>NS</sup>-. Non significant

Means bearing different superscripts in a column differ significantly between groups.

## Conclusion

Inclusion of earthworm meal in the Japanese quail diet could improve the digestion efficiency of macronutrient by increasing digestive enzymes. Protein digestibility and performance of intestinal villi has been increased with addition of earth worm meal in the feeding of Japanese quail. Furthermore, production of Japanese quail in earthworm meal was cheaper than fishmeal.

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