Effect of feed supplement containing earthworm meal
(*Lumbricus rubellus*) on production performance of quail
(*Coturnix coturnix japonica*)

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Abstract. The objective of this study was to evaluate the effect of feed supplement (FS)
containing earthworm meal (EWM) on production performance of laying quails. Twenty
weeks-old of 360 *Coturnix coturnix japonica* quails were used in a Completely
Randomized Design (CRD) with three dietary treatments A= CD (control without FS), B=
CD + 0.250 % of FS, and C= CD + 0.375 % of FS during 6 weeks of experimental period.
Each treatment in 4 equal replicates in which 30 quails were randomly allocated into 12
units of cages. Variable measured were feed intake, feed conversion ratio, feed efficiency,
mortality rate, hen day production, egg weight, and egg uniformity. Data were statistically
analyzed by One Way ANOVA and the differences among mean treatments are analysed
using Duncan’s Multiple Range Test (DMRT). The results showed that administration of
0.375% FS based on earthworm meal, fermented rice bran, and skim milk impaired the feed
conversion ratio and increased the feed efficiency. The experimental treatments did not
effect on feed intake, mortality, hen day production, egg weight, and egg uniformity of
quail. It is concluded that administration of feed supplement improved the growth
performance of quail.

1. Introduction
In recent years, breeding of quail has taken an important place in alternative poultry production.
Many factors such as hatchability and fertility rate, male/female ratio, genetics, age, egg quality,
nutritional status and live weight of breeders affect quail’s performance [1]. Consumers demand on
quail meat and eggs (*Coturnix coturnix japonica*) increase annually hence the quail production
establish significantly in the last decade. One way of increasing protein supply is to diversify. The
Japanese quails have the potential to serve as an excellent and cheap source of animal protein for
consumers [2]. The optimum performance of livestock depends largely on the quality and quantity
of their dietary nutrients. To obtain high growth and reproductive rates of quail, the given feed
should fit the nutritional needs. In the quail starter phase requires a higher protein content of 22-
24% in the feed formula, whereas in the grower phase protein needs to be 20-22% [3].

Feed additives commonly added into feed formulation in order to improve the quail performance. Feed additives are non-nutritive substances used in poultry feed including antibiotics,
enzymes, antioxidants, pellet-binders, antifungal, colored pigments and flavoring agents [4]. For
many years feed additives have been widely used to increase animals’ performance and

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1
recently it is used in poultry industry to improve growth, feed efficiency and layers performance [5]. Many studies have reported that earthworm meal can be used as one alternative source of animal protein in feed [6]. Earthworm meal contained high protein content (63.06%) as nutrient source for animal [7]. Essential amino acid of earthworm meal was dominated by isoleucine (1.98% of dry matter basis) [7]. Earthworm L. rubellus contained ‘lumbricin I’ which had antibacterial activity, included in peptide group which contained 62 amino acids [8]. Amino acid utilized as proteins are primary constituents of structural and protective tissues, including skin, feathers, bone, ligaments, as well as muscles and organs. Amino acids are used as poultry feed supplement for optimum performance and also used in livestock health care [9].

Sofyan et al. [10] reported that supplementation of feed supplement containing earthworm meal in broiler feed increased the final body weight, decreased feed intake, and improved feed conversion. Prayogi [11] stated that the addition of earthworm meals improved weight gain and lower feed conversion of quail. Rezaeipour et al. [12] reported that the addition of 10% earthworm meal Eisenia foetida increased weight gain, severe chest muscles, and protein intake of broiler chickens.

This study aimed to determine the effect of feed supplement contains earthworm meal on production performance of layer quail (Coturnix coturnix japonica).

2. Materials and methods

2.1. Preparation of feed supplement

Preparation of EWM refers to [13]. Earthworms are separated from the media and removed the dirt by washing. Then worms soaked in cold water 4 °C for 24 h. Formic acid 80% is added as much as 3% by weight of the worms. The worm is milled using a blender to become a paste and the paste of the worm is dried in an oven at 50 °C for 12 h and sieved to obtain a homogeneous particle size of ±40 mesh.

Fermented rice bran (FRB) is prepared according to [14]. Rhizopus sp. is isolated from tempeh and cultured in Potato Dextrose Agar (PDA) medium for 3-5 days of incubation. This isolate is propagated in PDB medium and incubated 5-7 days to obtain liquid inoculum. Rice bran is mixed with 5% of chitin-chitosan production waste (w/v) and 35% of warm water (v/v). The inoculums of Rhizopus sp. is added to the mixture (3.5% v/w) after the previous mixture temperature was 30-40 °C. The mixture is incubated for 7 days at room temperature (27 °C and 75% of RH). After incubation, fermented rice bran dried under the sun heat (2 days), prior to dry in oven (temperature 70 °C) for 2 hours until the moisture content was less than 10%. The fermented rice bran is ground and sieved to obtain a homogeneous particle size of ±60 mesh.

Skim milk is used in this study and categorized as food grade. After prepared the EMW with size of ±40 mesh, FRB with particle size of ±60 mesh, and skim milk then materials are mixed homogeneously with ratio 25:45:30 (w/w/w) and called as feed supplement. Nutrient content of EWM, FRB, and skim milk is performed on Table 1.

2.2. Experimental procedure

A total number of 360 quails Coturnix coturnix japonica aged 20 weeks were tested in a completely randomized design (CRD) with 3 treatments A= CD (control without FS), B= CD + 0.250 % of FS, C= CD + 0.375 % of FS. Each treatment in a 4 equal replicates in which 30 quails were randomly allocated into 12 units of multilevel cages.

Quails were kept under similar condition of management at temperature 27-28 °C with adequate water and feeding spaces provided throughout the experimental period lasting for 6 weeks. Light is provided for 24 h to avoid pilling and death. Through quail is known to be resistant to disease, anti-stress and antibiotics were administered through water at various times to check against possible disease outbreak. Good hygiene and biosecurity were ensured throughout the experimental period.
Feed supplement is given daily and mixed into the diet (0, 2.5, and 3.75 g of FS/kg of total diet). This study used commercial diet (CD) as diet contained 20% crude protein (CP). Nutrient content of diet based on laboratory analyses are presented in Table 2.

2.3. Data collection
Laying percentage (HDP) is calculated as number of eggs produced per hen divided by the number of days of the experimental period. Egg mass production per hen per day is calculated as laying percentage multiplied by average egg weight of the hen. Average egg weight is determined per hen. Feed intake is determined per replicate. Feed conversion ratio (FCR) is calculated as grams of feed consumed per gram of egg mass [5].

2.4. Data analysis
The commercial diet (CD) were analyzed for proximate composition according to the Association of Official Analytical Chemists [16]. Statistical analyses were performed with Costat [17]. The average data per replicate (30 hens) were treated as the experimental unit. Performance data were subjected to analysis of variance (ANOVA) and the differences among mean treatments were analyzed for significance using Duncan's Multiple Range Test [18].

3. Result and discussion

3.1. Quail growth performance
Earthworms are known as a protein rich source of cattle feed. The dry matter of earthworms has been found to be around 20 to 25% of the fresh weight. This contains around 60% of protein, 7-10% of fat, 8-10% of ash, 0.55% of Ca and 1% of phosphorus [19]. Nutrient composition of earthworm meal (EWM), fermented rice bran (FRB), skim milk, and feed supplement (FS) are presented in Table 1. Nutrient content in commercial diet (CD) with FS supplementation level are performed in Table 2.

| Nutrient content  | EWM       | FRB       | Skim milk | Feed supplement (FS) |
|-------------------|-----------|-----------|-----------|----------------------|
| Dry matter (%)    | 11.43 ± 0.04 | 92.46 ± 0.07 | 89.41 ± 0.14 | 8.01 ± 0.09          |
| Ash (%)           | 5.15 ± 0.06   | 8.40 ± 0.01   | 8.21 ± 0.10   | 6.21 ± 0.11          |
| Crude protein (%) | 55.87 ± 0.06 | 11.69 ± 0.36 | 22.33 ± 1.40 | 24.13 ± 0.06         |
| Fat (%)           | 16.39 ± 0.18   | 12.51 ± 0.28   | 0.19 ± 0.05   | 8.99 ± 0.22          |
| Crude fiber (%)   | 0.17 ± 0.01    | 13.30 ± 0.89    | NAa          | NAa                  |

NA (not available)

Table 2. Nutrient content (DM basis) of the diet used in the experiment

| Nutrient content  | Treatment A | Treatment B | Treatment C |
|-------------------|-------------|-------------|-------------|
| Moisture (%)      | 12.31 ± 0.23 | 11.82 ± 0.30ab | 11.49 ± 0.60b |
| Crude protein (%) | 20.54 ± 0.42 | 20.60 ± 0.72 | 19.76 ± 0.13 |
| Crude fat (%)     | 3.72 ± 0.20ab | 3.94 ± 0.56a | 3.05 ± 0.19b |
| Ash (%)           | 11.13 ± 0.28ab | 12.58 ± 0.68a | 11.89 ± 0.17ab |

Note: Average in the same row with different superscript shown significantly differ (P<0.05)
A= CD (control without FS)
B= CD + 0.250 % of FS
C= CD + 0.375 % of FS

3
Table 2 showed that protein content in diet (20%) was in accordance with the requirements of feed quality for quail in layer phase around 20% [20]. Quails convert dietary protein content in diet into body protein (lean meat) and egg production, insufficient of protein concentration of feeds may diminish the economic and productive performance of quail. The utilization of dietary crude protein by poultry are affected by many factors such as sex, age, disease, nutritional status, protein balance, presence of anti-nutritional factors, feed waste and poultry genotype [21,22].

The effect of FS containing of EWM in dietary on quail performance are presented in Table 3. Administration of FS did not significantly affect (P>0.05) feed intake (FI). It is estimated that administration of EWM did not affect the feed palatability, hence it did not affect the feed consumption. Feed consumption is affected by environmental temperature, also the quality and quantity of ration [23]. Contrary, Sofyan et al. [10] reported that administration of feed supplement containing EWM decreased feed intake of broilers. Bahadori et al. [24] stated that 1-3% of earthworm (E. fetida) meal in diet decreased feed intake of broiler chicken compared to control diet without EWM. Japanese quail are less efficient to convert feed to body tissue than broilers, but quail can utilize low energy diets more efficiently than fowl [25]. Shoukry et al. [26] stated that Japanese quail consumed higher metabolizable energy per Kg body weight than chicken. This higher energy cost for egg production in Japanese quail is due to high losses of metabolizable energy from different way such as the energy costs of thermoregulation where, the mature Japanese quail expended about 6-10% of its metabolic rate to adjust a new level of metabolism under temperature fluctuation condition [27]. Another way is the calorific values of fat and carbohydrates in quail lays are highly energy egg compared to chicken [26]. Morever, metabolic body size of Japanese quails are larger than the chicken, thence the metabolizable energy is high losing. This condition indicates that Japanese quail are different in the energy efficiency compared to chicken.

| Table 3. Production performance of quail |
|------------------------------------------|
| **Observed parameter**                   | **Treatment**   |
|                                         | A              | B              | C              |
| Feed intake (g/hen/d)                    | 23.26 ± 0.66   | 22.86 ± 0.80   | 22.85 ± 0.91   |
| Feed conversion ratio                    | 2.97 ± 0.13<sup>a</sup> | 2.82 ± 0.08<sup>ab</sup> | 2.77 ± 0.14<sup>b</sup> |
| Feed efficiency (%)                      | 33.77 ± 1.47<sup>a</sup> | 35.52 ± 0.99<sup>ab</sup> | 36.23 ± 1.82<sup>b</sup> |
| Mortality (%)                            | 3.33 ± 2.72    | 1.67 ± 3.33    | 3.33 ± 3.85    |

Note: Average in the same row with different superscript shown significantly differ (P<0.05)
A= CD (control without FS)
B= CD + 0.250 % of FS
C= CD + 0.375 % of FS

The lowest FCR observed in this study, which is defined as the ratio between feed intake and egg production, occurred with 0.375% FS administration in the diet. Feed conversion can be used as surrogate marker of the egg production coefficient, whereby a smaller value indicates a more efficient use of feed to produce an egg. Supplementation of EWM in diets could increase body weight and impaired feed conversion [11]. Istiqomah et al. [28] reported that administration of bio-supplement containing earthworm extract decreased feed conversion (FCR) of broiler. Administration of earthworm E. foetida meal in broiler feed decreased the feed conversion [12,24] and net protein ratio (NPR) [12]. In this study the FCR value was considerably similar with previous study 2.91 [2].

Administration of 0.375% FS significantly (P>0.05) increased the feed efficiency (P>0.05). Rezaeipour et al. [12] reported that protein efficiency percentage increased with increasing level of earthworm meal from 5% to 10% numerically but did not significantly different. Marks [29] suggested that the variation in feed efficiency is primarily the result of differences in metabolic efficiency and is approximately 40% genetically supplement. Murakami et al. [30] that dietary
protein had no significant influence on feed efficiency when laying quails were fed diets with different protein levels.

Administration of feed supplement did not significantly (P>0.05) affect mortality of quail. Mortality rate were 1.67 and 3.33% for 6 weeks of experimental period. Health status of the hens was good and mortality during the experiment was acceptable (30 birds, 1.67 and 3.33%). Mortality is caused by pullorum disease (infection of \textit{Salmonella pullorum}) with clinical signs such as white color of excreta, lower feed intake, shortness of breath, feathers puckered, and wings move weak. The overall mean livability of female layer Japanese quails were 97.12±0.22% (mortality rate 2.88%) [31].

3.2.  Performance of egg production

Performance production of quails are shown in Table 4. There were no difference (P<0.05) on hen day production (HDP) among treatments. In this study, the protein content in diet administered by FS (Table 1) did not significantly different (P>0.05) with control diet, therefore the administration of FS did not affect the formation of egg and egg production during the experimental period. This result was probably due to adequacy of nutrient content between treatments that lead to a healthy quail, so it did not affect the process of egg and egg production is running normally. Karimy et al. [32] also reported that addition of earthworm meal (\textit{L. rubellus}) did not affect egg production of quail.

Tuleun et al. [1] reported that hen day egg production (HDEP) on 20% CP diet was 81.67% for thirteen weeks study period. In this study, HDP ranging from 78.17 to 81.50% was considerably good. According to Mufti [33] eggs production are affected by lighting and the protein content in diet. Grindstaff et al. [34] also reported that the process of egg formation is influenced by the nutrients in the diets consumed by poultry.

| Observed parameter          | Treatment   |
|-----------------------------|-------------|
|                             | A           | B           | C           |
| HDP (%)                     | 78.17 ± 6.63| 79.37 ± 5.94| 81.50 ± 4.99|
| Egg weight (g/egg)          | 10.06 ± 0.19| 10.24 ± 0.20| 10.17 ± 0.31|
| Egg mass (g/hen/d)          | 7.86 ± 0.54 | 8.13 ± 0.27 | 8.29 ± 0.51 |
| Egg uniformity (%)          | 65.45 ± 13.52| 70.00 ± 4.33| 70.45 ± 7.62|

Note: Average in the same row with different superscript shown significantly differ (P<0.05)

A= CD (control without FS)
B= CD + 0.250 % of FS
C= CD + 0.375 % of FS

Based on Table 4, administration of FS did not significantly (P>0.05) affect egg weight of quail. The results obtained were still at the normal range of 6-16 g [35]. Bertechini [36] stated that normal range of quail’s egg weight were 10-12 g. Tuleun et al. [2] reported that quail egg weight on 20% CP diet was 9.75 g and stated that egg weight depended greatly on daily protein intake. Quail egg weight, shape and colour can vary greatly among different females in a population, but are quite specific and consistent for one female [37]. The relationship between Japanese quail egg colour, shell quality and internal features is confirmed in some experiments inter alia carried out by Taha [38].

Administration of FS did not significantly (P>0.05) affect egg uniformity of quail. In this study, quail eggs showed lower uniformity, with high egg weight variation compared to Nepomuceno et al. [39]. According to Murakami et al. [30], the variation of egg quail weight is between 10 to 11 g, considering the deviation from 7 to 14 g.

Hen day production (HDP) among treatments was observed 6 weeks (figure 1).
Figure 1. Weekly hen day production in A treatment (CD without FS), B treatment (CD + 0.250 \% of FS), and C treatment (CD + 0.375 \% of FS).

The result showed that peak production during 6 weeks of experimental period is observed in week 24 of age then decreased in week 25 of age. Quail hens attain peak production between 12-15 weeks and it is expected that as the birds increased in age, the number of small sized eggs would decrease consistently, while the medium and large sized eggs would increase consistently as well [40]. Japanese quails in the age group of 11 to 14 weeks resulted HDP of 76.93 ± 0.46\% [31]. This optimum egg production could be achieved when body metabolism run well. This optimum metabolism are affected by environment factor (temperature, relative humidity, and lightning) and nutrition. At laying period, 22-24 °C is the required temperatures and the relative humidity should not exceed 60\% during these production phase [36]. The differences observed for the peak of lay could be as a result of variations in the environments in which the experiments were conducted.

4. Conclusion
The result showed that administration of 0.375\% of feed supplement (FS) based on earthworm meal, fermented rice bran, and skim milk impaired the feed conversion and increased the feed efficiency. However it did not affect hen day production, egg weight, and egg uniformity of quail. It is concluded that administration of feed supplement containing earthworm meal have potential to improve the growth performance of quail.

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