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Research article

Effects of earthworm hydrolysate on health status, laying performance, egg quality, and economic benefit of Cherry Valley laying ducks at the late phase of reproduction

Nguyen Vu Thuy Hong Loan[1,*] and Ngo Hoang Thao Nhung[1]

[1]HUTECH University, Faculty of Veterinary and Animal Science, Ward 25, Binh Thanh District, Ho Chi Minh City 72308, Viet Nam

Abstract

The study aimed to evaluate the effect of the earthworm hydrolysate, including in a complete feed-based diet on laying performance, quality of commercial eggs, and economic return of Cherry Valley (CV) laying ducks at the late phase of reproduction. A total of 100 laying ducks at 48 weeks of age were randomly allocated to 2 dietary treatments, namely CTL (control, in which animals were fed a complete feed and EXP (experiment, in which ducks were fed complete feed and 100 mL of the earworm hydrolysate) and 5 replicates. Animals were kept in the net cage with an earth floor and 10 animals/cage. The results showed that laying rate, number of eggs per week, and egg weight were higher in the EXP than in the CTL, and lower feed consumed per 10 eggs in the EXP than in the CTL ($P \leq 0.05$); egg shape index, york percentage, Haugh unit, and shell thickness were higher in the EXP than in the CTL ($P \leq 0.05$). On the other hand, the profit was higher in EXP than in CTL. In conclusion, the inclusion of earthworm hydrolysate in a complete feed-based diet improved laying performance, egg quality, and economic return of CV laying ducks at the late phase of reproduction. The ducks fed earthworm hydrolysate diet had higher 4.7% laying rate, higher 6.1% selected egg rate for hatchery and 17% more profit than those fed control diet.

Keywords: Cherry Valley laying ducks, Earthworm hydrolysate, Egg quality, Laying performance.

[*]**Corresponding author**: Nguyen Vu Thuy Hong Loan, HUTECH University, Faculty of Veterinary and Animal Science, Ward 25, Binh Thanh District, Ho Chi Minh City 72308, Viet Nam. Email: nvth.loan@hutech.edu.vn. ORCID: 0000-0001-8632-1662.

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INTRODUCTION

Many scientists have reported the possibility of using earthworm meals for the poultry industry (Vu et al., 2009; Prayogi, 2011; Rezaeipour et al., 2014), and they are acceptable sources of proteins, energy, vitamins, fat, and minerals (Sogbesan and Ugwumba, 2008; Doriana et al., 2020). Bahadori et al. (2017) and Parolini et al. (2020) showed that adding earthworms to poultry diets improved nutrient digestibility, increased egg production, improved eggshell quality, and strengthened the immune system. Earthworm protein has a high content of lysine and methionine, and the arginine, lysine, and leucine contents in worms are higher than in fishmeal. Additionally, the content of tryptophan in worms is approximately four times higher than in blood powder (Xiang et al., 2006).

Additionally, hydrolysis of earthworm proteins could be applied in the feed industry. These hydrolyzed proteins have low molecular weights and are rich in peptides and free amino acids that enhance functional properties (Kristinsson and Rasco, 2000). They contained more than ten kinds of proteolytic enzymes (Akazawa et al., 2018). The earthworm hydrolysate can be absorbed directly into the body of other animals and play its role in promoting growth and resisting disease (Sun et al., 2020). Liu et al. (2023) studied the effect of earthworm hydrolysate on production performance, serum biochemical parameters, antioxidant capacity, and intestinal function of Muscovy ducks. The authors reported that the inclusion of 1.5% earthworm hydrolysate increased the average daily gain (ADG) of Muscovy ducks of 16–70 days old, villi length, intestine thickness, and surface area of villi and reduced feed conversion ratio (FCR). Meanwhile, 2.5% improved ADG, abdominal fat yield, breast muscle yield, heart index, spleen index, villi length, crypt depth, intestine thickness, surface area of villi, the percentage of intraepithelial lymphocytes and decreased FCR (42–70 days old and 16–70 days old). These results provide the foundation for preliminary research on the potential principles of earthworm hydrolysate in promoting production performance and adjusting antioxidant function and intestinal functions in the Muscovy duck industry.

Recently, in Viet Nam, earthworm hydrolysate, known as a product of *Perionyx excavates* earthworm hydrolysis using protease hydrolytic enzymes. Earthworm hydrolysate made by *Perionyx excavates* (*giun que* in Vietnamese) contains 100% natural amino acids with all 10 essential amino acids and some peptides. They have been applying for vegetables (Linh et al., 2017), *Macrobrachium rosenbergii* shrimp (Yen and Trung, 2014). Yen and Trung (2014) reported that the application of 3 ml/kg feed per day improved the survival rate of *Macrobrachium rosenbergii* larvae and increased efficiency in hatchery. Additionally, spraying the NACEN-EARTHWORMS earthworm hydrolysate with doses of 10, 20, and 30 mL/8 L on sweet mustard increased yields by 14 – 39% compared to the control (Linh et al., 2017). One of hydrolyzed earthworm products was produced by Vemedim Ltd for poultry (Vemedim, 2020). However, the effects of earthworm hydrolysate on poultry, particularly laying ducks, have not been published yet.

CV ducks are a domesticated 'hybrid' (commercial) bird created by crossing a Pekin with a Rouen and was developed by CV Farms (UK) Ltd. They are world leaders in Pekin breeding stock and created the CV brand which is the commercial name for this duck. They are hardy and friendly ducks making them easy to care for and a popular choice for backyard keepers as well as commercial producers (Weymouth, 2022). The CV ducks were imported to Viet Nam since 1980 from Hungary and other countries. In Ben Tre province, CV ducks have been distributed to private farmer's farms by Haid Feed Company since 2020. The laying ducks are raised on farms and the eggs are collected by the company and are sold to the market. The aim of this study, therefore, to evaluate the effect of earthworm hydrolysate on laying performance and egg quality of CV ducks kept in farmer's households.

MATERIALS AND METHODS

Location and Ethical approval

The experiment was carried out at Minh Phuong family farm in Ben Tre Province, Viet Nam from April to July 2023. The procedures for raising the animals strictly followed the guidance of the Animal Husbandry Law (No. 32/2018/QH14).

Animal and Experimental design

CV laying ducks at 47 weeks of age were raised for 1-week adaptation before starting the experiment. Ducks were raised in cages surrounded by nets, with feeding troughs, watering troughs, and bathing ponds separated so that ducks would not mix between treatments and replicates.

The experiment was arranged in a completely randomized design on 100 CV ducks at 48 weeks of age allocated into 2 treatments and 5 replicates with 10 ducks/pen. In treatment 1 (CTL – control), ducks were fed De Heus complete feed with dry matter content of 87%, crude protein 20%, crude fiber 8%, lysine 1%, methionine + cystine 0.83%, Ca 2.5 – 4.5%, P 0.4 – 0.8% and metabolizable energy 2,800 kcal/kg; while the ducks in the second treatment (EXP – experiment) were fed as CTL and supplemented with 100 mL of earthworm hydrolysate daily. The nutritive values of earthworm hydrolysate as shown in Table 1. Feed for trial and experimental material were produced in the same batch and kept the same quality during experimental time.

To make sure that animals can receive equally, the earthworm hydrolysate was diluted with 2 parts of water and divided by 2 times (8.00h and 14.00h). Due to limitation of bird's cages, the chosen dose (100 mL/day/bird) was followed the recommendation of the earthworm hydrolysate producer. The time for experiment to recording data was 49 days.

Table 1 Amino acid concentration of earthworm hydrolysate

Amino acid	mg/L
Leucine (min)	4,500
Lysine (min)	4,000
Alanine (min)	3,500
Aspartic acid (min)	3,500
Valine (min)	3,000
Glycine (min)	2,500
Phenylalanine (min)	2,000
Glutamic acid (min)	1,500
Serine (min)	1,000
Methionine (min)	1,000
Protease (min)	1,500,00 UI
Water	1 L

Measurements

Health status: Daily observed and recorded the change of body shape, feather condition, and activity level of the duck.

Laying performance: Laying rate, egg yield, egg weight, and feed conversion/10 eggs.

Egg quality: The egg shape index, percentage of yolk and white of the egg, Haugh Unit (HU), and eggshell thickness.

Calculations are as follows: Laying rate. The eggs were manually collected, and their number and the number of laying ducks was recorded daily in each cage, and then calculating the laying rate:

$$\text{Laying rate (\%)} = \frac{\text{Total egg number (egg)}}{\text{Total laying duck number (head)}} \times 100$$

Egg yield was calculated weekly as:

$$\text{Egg yield } (\frac{\text{egg}}{\text{duck}}) = \frac{\text{Total egg number in the week (egg)}}{\text{Total laying duck number (head)}}$$

In each replicate, 10 eggs were randomly collected and weighed every week. Egg weight was an average of 10 eggs:

$$\text{Egg weight (g)} = \frac{\text{Total weight (g)}}{\text{Total egg number (egg)}}$$

Feed conversion to 10 eggs. In fact, the egg was sold by unit therefore, feed conversion per 10 eggs was calculated (FC/10 Eggs):

$$\text{FC/10 eggs} = \frac{\text{Total feed consumed (kg)}}{\text{Total egg number (egg)}} \times 10$$

Shape index was determined simultaneously with mass determination by measuring egg length and width using an electric digital calliper with an accuracy of 0.1 mm. The shape index was calculated as follows:

$$\text{Egg shape index} = \frac{\text{Egg length (mm)}}{\text{Egg width (mm)}}$$

Percentages of york, albumen. The ratio of weight proportion of each part of the egg to total egg weight is as follows:

$$\text{York percentage (\%)} = \frac{\text{Weight of york (g)}}{\text{Weight of egg (g)}} \times 100$$

Shell thickness without shell membrane was measured using a micro-meter (Mitutoyo, Japan), at three locations on the eggs: air cell, equator, and sharp end.

HU represents the interaction between albumen height and egg weight. Eggs were measured for solid albumen height using an electrical digital calliper with an accuracy of 0.1 mm, measuring at the highest position of the solid albumen. Then, combine the data between solid albumen height and egg weight to calculate the HU according to the following formula:

$$\text{HU} = 100 \log(\text{H} + 7.57 - 1.7 \times \text{W}^{0.37})$$

In which, H: solid albumen height (mm), W: egg weight (g)

Economic analysis. Profit was calculated as the difference between total revenue and total expenditure costs. Total revenue from selling eggs and expenses included mainly feed and earworm hydrolysate costs.

Data analysis

Data were presented in the form of the mean (M), and standard error of the mean (SEM). The data were statistically processed by analysis of variance (ANOVA) by the General Linear Model in Minitab v. 16.2 (2010). The difference between the

mean values was determined by the Tukey method at a confidence level of 95%. Statistical model:

$$Y_{ij} = \mu + T_i + e_{ij}$$

Where: μ is the average value; T_i is the effect of the diet; e_{ij} is the experimental error.

RESULTS

Health status

Daily checks were made of the health of CV ducks in the experiment, and it found that ducks in the EXP were healthier in terms of live weight change, good feather condition, and more activity. During the 7 weeks, all ducks were healthy. It was observed that the body weight of CV ducks in the EXP was heavier than that of the CTL. However, ducks can't be weighed when they are laying eggs. The feather condition of ducks in the EXP also improved significantly. Ducks have shiny plumage, less shedding, and brighter colors than in the CTL.

Laying performance

Results on the laying performance of CV ducks in the late phase of reproduction are present in Table 2. Birds fed earthworm hydrolysate showed a significant difference in most of the laying performance parameters than birds fed complete feed ($P \leq 0.05$). The actual laying rate of experimental diet was 2.96% higher than that of control diet. It seems that the earthworm hydrolysate stimulate duck consumed more feed than that of control although the difference was not significantly. Despite of that the FCR of duck fed earthworm hydrolysate was lower significantly than that of control duck (2.87 vs 2.94). Egg yield increased 4.7% in experimental diet versus control diet. It is very interesting that the selected egg rate for hatchery in experimental diet much higher than that of control diet (87.27% vs 81.17% that was 6.10% in experimental diet). The hypothesis was that earthworm hydrolysate stimulate the immune system resulting to better health status so egg having better quality than normal. The reason is of higher laying rate in experimental diet. It means that supplementing 10 mL earthworm hydrolysate in a complete feed diet improves the reproductive performance of CV ducks.

Table 2 Laying rate of CV ducks at the late phase of reproduction

Parameters	Treatments		SEM	*P*-value
	CTL	EXP		
Number of replicate	5	5		
Number of duck per replicate per pen (duck)	10	10		
Total number of duck per treatment (duck)	50	50		
Total experiment day (days)	49	49		
Laying rate (%)	63.83[b]	66.79[a]	0.163	0.005
Difference in laying rate (%)		2.96		
Egg yield (egg/week)	4.47[b]	4.68[a]	0.031	0.002
Total egg during 7 week	1,564	1,636		0.008
Selected egg rate for hatchery (%)	81.17	87.27		
Egg weight (g)	68.73[b]	72.35[a]	0.672	0.003
Total Feed Intake (kg)	460	470		0.671
Feed conversion to 10 eggs (kg)	2.94[a]	2.87[b]	0.023	0.032

[a,b]: *Means in the same row without common letters are different at P≤0.05*

Egg quality

The effect of earthworm hydrolysate inclusion in the diet on egg quality of CV ducks at 48 – 54 weeks of age presents in Table 3. The egg shape index, york percentage, eggshell thickness, and HU were higher in the EXP than in the CTL.

Table 3 Egg quality of CV ducks fed earthworm hydrolysate

Parameters	Treatments		SEM	*P*-value
	CTL	EXP		
Shape index	1.37[b]	1.41[a]	0.031	0.034
Egg york percentage (%)	31.02[b]	33.51[a]	0.383	0.007
Albumen percentage (%)	51.64	50.09[a]	0.465	0.062
Shell thickness (mm)	0.37[b]	0.41[a]	0.011	0.037
Haugh Unit	82.63[b]	84.01[a]	0.602	0.001

[a,b]: *Means in the same row without common letters are different at P≤0.05*

Economic profit

In this study, economic efficiency is calculated on 50 CV laying ducks per treatment raised for 7 weeks at the late phase of reproduction (48 – 54 weeks of age). Ducks at this age have a decrease of laying rate but relatively stable. This CV layers were used to produce duckling so selected duck egg was sold with much higher price (2.5 times) than unselected eggs. Because higher selected egg rate for hatchery so total income from experimental diet was much higher than that of the control diet and the relative figure was 17% higher in profit.

Table 4 Effect of earthworm hydrolysate inclusion in laying duck diets on economic return

Parameters	Treatment		*P*-value
	CTL	EXP	
Expenditure			
Feed Intake (kg)	460	470	0.671
Feed cost (1,000 VND)#	5,796	5,922	0.721
Earworm cost (1,000 VND)	-	245	
Total expenditure (1,000 VND)	5,796[b]	6,167[a]	0.005
Income			
Total number of laying eggs	1,564	1,636	0.008
Selected egg for hatchery (egg)	1,285	1,428	
Total income (1,000 VND)##	6,982[b]	7,556[a]	0.001
Profit (1,000 VND)	1,187[b]	1,390[a]	0.001
Comparison (% as CTL)	100	117	

#: Feed price 12,600 VND; ## Egg breeding price 5,000 VND/piece non-selected egg price: 2,000 VND/piece

DISCUSSION

Health status

In this study, the birds fed earthworm hydrolysate are better feather condition and more active. This proves that earthworm hydrolysate provides the necessary nutrients for the growth and maintenance of good hair condition. Additionally, ducks in the EXP were more active than in the CTL and showed less fatigue and

illness than in the CTL. This could be explained by the effects of nutrients and bioactive compounds contained in earthworm hydrolysate, which help strengthen ducks' immune systems and disease resistance of ducks (Bahadori et al., 2017; Parolini et al., 2020; Sun et al., 2020). Morever, Nguyen et al. (2010) reported that isolated *Bacillus* sp. bacteria from earthworm hydrolysate have strong inhibitory ability on the growth of 3 pathogenic *Vibrio* strains in shrimp. It may apply to poultry as well.

Laying performance

CV ducks have been imported to Viet Nam from Hungary since 1980. Recently, CV ducks have been distributed by Haid Feed Company in Mekong River Delta provinces and raised in farmer farms. Under farmer conditions, they reached egg production of 120 – 130 eggs/year.

In this study, the laying rate, egg yield, and egg weight were higher in birds fed earthworm hydrolysate. These findings were in agreement with some previous studies. Taye et al. (2024) indicated that feeding Bovan Brown laying hens with fresh earthworm at 1.98% in the diet increased egg number/hen/month, hen day egg production, and egg weight. Hesami et al. (2020) also reported that the inclusion of 1.0% and 1.5% earthworm powder improved significantly egg weight, egg production, and egg mass weight of Japanese quails. Similarly, Kim et al. (2014) reported that the inclusion of 1 and 2% of mealworm (*Tenebrio molitor*) meals in diets increased 2 – 3% egg production of chicken layers. However, Nazeri et al. (2021) indicated that supplementing earthworm plus vermin-humus in the diet for Japanese laying quail positively affected growth performance but not egg productivity.

In addition, the egg weight of the CV duck in this study ranges from 68.73 – 72.35 g, which is lower than the previous results of Cao et al. (2022), who reported the egg weight of the CV duck ranged from 79.8 – 86.9 g. Ipek and Sozcu (2017) classified duck eggs into three weight categories: light (<75 g), medium (76 – 82 g) and heavy (>83 g). The egg weight of CV ducks in this study belongs to the group of light duck eggs.

In breeding ducks, feed consumption per 10 eggs is an indicator that is both technically and economically meaningful. It is an indicator to evaluate the economic efficiency of duck production. It was observed that feed intake by CV ducks in the two groups was not different as total feed consumed was consistent in both treatments (Table 4). Meanwhile, Bahadori et al. (2017) reported that earthworm meals can reduce palatability and feed intake in chicken broilers. However, Liu et al. (2023) indicated the better palatability of earthworm hydrolysate; therefore, the results of growth performance show a remarkable increase when Muscovy ducks were fed on an earthworm hydrolysate. The reduced FCR may be one of the reasons for the promoted growth in groups fed on earthworm hydrolysate. In this study, feed conversion to the egg was lower in the EXP due to higher egg yield in this experimental treatment than in the control.

Egg quality

The egg shape index in this study of 1.37 – 1.41 is in agreement with Cao et al. (2022), who indicated that the egg shape index of CV ducks ranged from 1.35 – 1.45. However, the effect of earthworm meal or hydrolysate on the shape index of poultry eggs is not well known yet.

The yolk percentage or weight is also an indicator of special interest to consumers. The york percentages of CV duck eggs in this study of 31.02% – 33.51% are similar to report by Galic et al. (2020) and Cao et al. (2022). Galic et al. (2020) and Cao et al. (2022) indicated that the york percentage of CV ducks ranged from 26.7% – 34.2%. In addition, Nazeri et al. (2021) found that the egg york percentage of Japanese quail was not affected by the inclusion of earthworm meal plus vermin-hums in the diets. This recent study showed that including earthworm

hydrolysate in complete feed-based diet increased the york percentage of CV ducks at the late phase of reproduction.

The eggshell thickness represents durability, strength, and porosity. In this study, the eggshell thickness ranges from 0.37 mm to 0.40 mm and is higher in the EXP than in the CTL. The eggshell thickness in this study is like the results of Galic et al. (2020) and Cao et al. (2022), who reported the CV duck eggshell thickness as 0.36 – 0.41 mm. A recent study shows that supplementing earthworm hydrolysate increases the eggshell thickness. However, Nazeri et al. (2021) reported that the inclusion of 2.5% earthworm meal reduced the eggshell weight and thickness of laying quail. Similarly, Taye et al. (2024) indicated that the inclusion of earthworm meal didn't affect the egg shape index and shell thickness of Bovan Brown layer chickens.

The HU is an indicator of egg white quality, expressing the relationship between egg volume and solid albumen height. The higher the HU, the better the egg quality. In this study, the HU of CV duck ranges from 82.63 – 84.01. The value of HU in recent previous studies ranged from 71.15 (Galic et al., 2019) to 87.2 (Cao et al., 2022). In this study, the HU is higher in the EXP than in the CTL. However, the reports by Nazeri et al. (2021) and Taye et al. (2024) showed no effects of inclusion of earthworm meal in diets for laying quail and laying hens on the HU. Taye et al. (2024) reported that the HU ranged from 84.60 – 88.05 in eggs of laying hen fed commercial feed and commercial feed with 0.66% – 1.98% earthworm meal.

In general, the inclusion of earthworm hydrolysate in a complete feed-based diet improved laying performance and egg quality of CV laying ducks at the late phase of reproduction. Recently, there are many different brands of earthworm hydrolysate, for example Promin, Hiryo, Sfarm, Vemedim... on the market However, earthworm hydrolysate products contain 100% hydrolyzed protein from fresh earthworms mainly *Perionyx excavates.* These products provide nutrients, stimulate appetite, hair growth, shiny coast and bright red wattles, and enhance immunity and disease resistance in poultry (Vemedim, 2020). In addition, earthworm hydrolyses contained more than ten kinds of proteolytic enzymes (Akazawa et al., 2018) and can be absorbed directly into the body of animals and play its role in promoting growth and resisting disease (Sun et al., 2020).

Economic profit

Data in Table 4 show that total collected eggs and profit in CV ducks fed earthworm hydrolysate were higher than in ducks fed complete feed. However, the time to evaluate economic efficiency needs further monitoring to have a more objective assessment between the use of commercial feeds and supplementing hydrolyzed earthworm products in laying duck production.

CONCLUSIONS

Adding 100 mL of earthworm (*Perionyx excavatus*) hydrolysate to the complete feed-based diet of CV ducks at the late phase of reproduction increased reproductivity, egg quality, and economic efficiency while improving animal health. In comparison, the ducks fed earthworm hydrolysate diet had higher 4.7% laying rate, higher 6.1% selected egg rate for hatchery and 17% more profit than those fed control diet. This study provided a scientific basis for the widespread application of earthworm hydrolysate in laying poultry farming.

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AUTHOR CONTRIBUTIONS

Nguyen Vu Thuy Hong Loan and Ngo Hoang Thao Nhung conceived, designed the experiment; Ngo Hoang Thao Nhung performed the experiment; Nguyen Vu Thuy Hong Loan and Ngo Hoang Thao Nhung analysed the data; Nguyen Vu Thuy Hong Loan wrote the paper; all authors reviewed and approved the final manuscript.

CONFLICT OF INTEREST

We have no conflict of interest.

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