



## Effect of Raising Laying Hens in Intensive, Continuous and Rotational Free-Range Systems on Egg Production and Yolk Cholesterol Content

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**ABSTRACT.** This study aimed to evaluate the effects of raising laying hens in intensive cage and various free-range systems on hen-day production (HDP), egg weight, egg mass, yolk cholesterol, and yolk protein content. A total of 80 Isa Brown laying hens, aged 15 weeks and with an average starting weight of 1.20 kg, were assigned to four different rearing treatments: P1 (intensive cage system), P2 (continuous free-range system), P3 (rotational free-range system with two relocations), and P4 (rotational free-range system with four relocations), each with four replicates. Data were analyzed using analysis of variance (ANOVA), and significant differences ( $P < 0.05$ ) among treatments were further evaluated using Duncan's multiple range test. The results showed that the rearing system significantly influenced daily body weight gain (DBWG), daily feed intake (DFI), HDP, egg weight, egg mass, feed conversion ratio (FCR), and yolk cholesterol content ( $P < 0.05$ ), but had no significant effect on yolk protein content ( $P > 0.05$ ). In conclusion, continuous free-range systems led to higher HDP and egg mass, while the intensive cage system resulted in greater egg and body weight, along with higher feed intake. The continuous free-range system was associated with a lower FCR, and the rotational free-range system with four relocations yielded the lowest yolk cholesterol levels.

**Keywords:** egg production, free-range, intensive cage, laying hen, yolk cholesterol

### INTRODUCTION

Most commercial laying hen farms utilize intensive housing systems due to their simplicity and management efficiency (Adi et al., 2019). The intensive rearing system confines chickens to cages, limiting their movement (Andris et al., 2022). This method offers several economic advantages, including space efficiency, ease of monitoring, practicality, and reduced exposure to predators (Setiawati et al., 2016).

Despite these benefits, the intensive system presents several challenges. One major concern is stress, which can negatively impact bird health, reduce productivity, and increase mortality rates (Abbas et al., 2021). Additionally, intensive systems are associated with high labor and feed costs. If nutritional requirements are not adequately met, chickens may become inefficient and prone to stress. The initial investment in cages and equipment also adds to the overall production costs (Andris et al., 2022).

In recent years, increasing attention has been paid to animal welfare in poultry farming. Free-range rearing systems, which allow chickens to express natural behaviors, are considered more humane and can enhance egg quality and food safety (Dikmen et al., 2017). Studies have shown that eggs from free-range systems contain higher levels of vitamin A (Anderson, 2018) and lower cholesterol content (Ghanima et al., 2020)

compared to those from intensive systems. These nutritional differences may result from the availability of additional natural feed sources in free-range environments.

Free-range systems can be categorized into continuous and rotational models. In continuous systems, chickens remain in a single pasture area, whereas in rotational systems, they are periodically moved between different pasture plots (Zhou et al., 2019). According to Khatifah et al. (2022), rotational free-range systems provide chickens with greater access to natural nutrients, which can improve their overall health and productivity.

This study aimed to investigate the effects of raising laying hens in intensive cage systems and various free-range rearing models-continuous and rotational-on hen-day production (HDP), egg weight, egg mass, yolk cholesterol content, and yolk protein content.

### MATERIALS AND METHODS

#### Equipment and Materials

The equipment used in this study included experimental cages, paddocks, feeders, drinkers, light bulbs, feed scales, digital scales, egg trays, centrifuge tubes, water bath, test tubes, spectrophotometer, analytical balance, Kjeldahl flasks, fume hood, volumetric flasks, Erlenmeyer flasks, drop pipettes, and distillation flasks. The materials utilized were 80 Isa Brown laying hens, feed ingredients (concentrate, corn, rice bran, mineral mix, and forage), vitamins, Newcastle Disease (ND) vaccine, dewormer, acetone, alcohol, chloroform, Liebermann–Burchard reagent,

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selenium, sulfuric acid ( $\text{H}_2\text{SO}_4$ ), distilled water, 2% boric acid ( $\text{H}_3\text{BO}_3$ ), indicator solution, and 30% sodium hydroxide ( $\text{NaOH}$ ).

#### Animals

This study used 80 Isa Brown strain laying hens aged 15 weeks, with an average initial body weight of 1.20 kg. The hens were assigned to two rearing systems: 20 hens were placed individually in an intensive cage system, while the remaining 60 hens were distributed across three free-range systems (20 hens per system), corresponding to different rearing patterns.

#### Cage and Paddock Preparations

The cages used in the intensive system were constructed from wood and bamboo, each measuring  $30 \times 30$  cm and roofed with thatched leaves. Each cage housed two chickens and was equipped with a drinking water source and feeding containers made from paralon pipes.

The experimental land was divided into four paddocks, with the following dimensions and designations:

1.  $7 \times 7$  m - Intensive cage system (P1)
2.  $7 \times 7$  m - Continuous free-range system (P2)
3.  $7 \times 12$  m - Rotational free-range system with two relocations (P3)
4.  $7 \times 12$  m - Rotational free-range system with four relocations (P4)

Each paddock was subdivided into four sub-paddocks, resulting in 12 paddocks in total, as described by [Khatifah et al. \(2022\)](#). Five chickens were allocated per paddock, totaling 60 chickens in the free-range systems. Paddocks were considered as replicates. The land in each paddock was naturally covered with overgrown grass. In the continuous free-range system, paddocks were fixed, while in the rotational systems, paddocks were mounted on wheels to facilitate movement between plots.

#### Rearing Management

The rearing period lasted for 35 days. In the intensive and continuous systems, chickens remained in the same cages or paddocks throughout the study. In contrast, in the rotational systems, chickens were relocated either twice (P3) or four times (P4) over the course of the experiment.

#### Feeding Management

All birds were provided a formulated diet consisting of corn, concentrate, rice bran, and mineral mix composition presented in Table 1. The nutrient composition of the feed was designed based on the nutritional requirements for laying hens as recommended by the [NRC \(1994\)](#). Chickens in the free-range systems also had access

to natural feed sources such as grasses, insects, and earthworms.

Feeding was conducted twice daily: 30% of the ration was given at 05:00 WITA, and the remaining 70% at 15:00 WITA. Clean drinking water was provided ad libitum.

#### Experimental Design

The research was conducted using a Completely Randomized Design (CRD) consisting of four treatments with four replicates each. Each replicate represented an experimental unit composed of five chickens. The treatments were defined based on four different rearing models for laying hens, as follows:

P1= intensive cage system

P2= continuous free-range system

P3= rotational free-range system with 2 times removal

P4= rotational free-range system with 4 times removal

#### Observed Parameters

##### Body Weight Gain (BWG)

The body weight gain of chickens was determined by measuring the difference in body weight from the beginning of the rearing period to the end of the 35-day maintenance period, with weekly weighing intervals ([Nugraha et al., 2017](#)).

##### Daily Feed Intake (DFI)

Daily feed intake was measured as the total amount of feed consumed by each replicate group per day. It was calculated by subtracting the leftover feed from the total feed provided each day ([Sulaiman et al., 2019](#)).

##### Hen Day Production (HDP)

Egg production was recorded daily throughout the 35-day study period. Hen-day egg production was calculated using the following formula ([Sulaiman et al., 2019](#)):

$$\text{HDP} = \frac{\text{daily egg count}}{\text{number of hens}} \times 100\%$$

##### Egg Weight (EW)

Egg weight was measured by weighing each egg produced daily throughout the rearing period using a digital scale.

##### Egg Mass (EM)

Egg mass was calculated as the product of hen-day production (HDP) and the average egg weight (EW) recorded daily. The following formula was used ([Nurmeiliasari et al., 2020](#)):

$$\text{EM} = \frac{\text{HDP} \times \text{Egg weight}}{100\%}$$

### Feed Conversion Ratio (FCR)

Feed conversion ratio was calculated to determine the amount of feed required by chickens to produce a unit of egg mass. The following formula was used (Sulaiman et al., 2019):

### Yolk Cholesterol Content (mg/100g)

The cholesterol content in egg yolks was determined using the Liebermann–Burchard method with a spectrophotometer. First, 1 gram of egg yolk was placed into a centrifuge tube containing 10 mL of a solvent mixture composed of acetone and alcohol in a 1:1 ratio. The mixture was stirred until homogenous and then heated in a water bath until boiling. After reaching the boiling point, the tube was removed and allowed to cool to room temperature.

Once cooled, the sample was centrifuged for 15 minutes. The resulting supernatant was transferred into a clean test tube and reheated in a water bath until all the liquid evaporated, leaving a residue. This residue was dissolved in chloroform and homogenized. After proper dilution, 2 mL of the Liebermann–Burchard reagent was added. The mixture was then placed in a dark room for 5 minutes to allow color development. A green coloration indicated the presence of cholesterol.

The intensity of the green color was measured using a spectrophotometer at a wavelength of 680 nm. The resulting absorbance values were used to determine cholesterol concentration by referencing a standard cholesterol regression equation (Ohoilulin et al., 2023).

### Yolk Protein Content (%)

The protein content in egg yolks was determined using visible light spectrophotometry following the Biuret method. Egg yolk samples were first centrifuged for 10 minutes. Then, 1 mL of the resulting supernatant was collected and mixed with 4 mL of Biuret reagent. The mixture was left to stand at room temperature for 30 minutes to allow for color development.

Subsequently, the absorbance was measured using a visible light spectrophotometer. The absorbance values displayed on the screen were recorded and used to calculate protein content by referring to the linear regression equation generated from the calibration curve (Ramadhani et al., 2019).

### Data Analysis

All collected data were analyzed using analysis of variance (ANOVA). If the results indicated a significant difference among treatments

( $P < 0.05$ ), further analysis was conducted using Duncan's Multiple Range Test to compare means.

## RESULTS AND DISCUSSION

### Egg Production

The egg production performance of laying hens reared under intensive and various free-range systems was observed during the rearing period. Parameters evaluated included body weight gain (BWG), daily feed intake (DFI), hen-day production (HDP), egg weight (EW), egg mass (EM), and feed conversion ratio (FCR). The results are presented in Table 2.

### Body Weight Gain (BWG)

Laying hens reared in intensive and free-range systems, across various patterns, had a significant effect ( $P < 0.05$ ) on body weight gain (BWG). The highest BWG was observed in both the intensive (T1) and continuous free-range systems (T2), which were higher than those in the other treatments. In contrast, the lowest BWG was recorded in the rotational free-range system with four times paddock removal (T4). The stress caused by the periodic removal of paddocks, especially the more frequent removals in T3 and T4, resulted in a significant decline in BWG. This finding aligns with the study by Daud et al. (2017), which indicated that stress negatively affects body weight gain in chickens. Factors such as management practices, feed quality, and environmental conditions can contribute to these effects.

A positive correlation between BWG and daily feed intake (DFI) was observed. The highest DFI was recorded in the intensive rearing system (T1), which is consistent with Prastio et al. (2022), who noted that increased feed consumption contributes to greater body weight gain.

### Daily Feed Intake (DFI)

Laying hens reared in intensive and free-range systems, with different patterns, showed a significant effect ( $P < 0.05$ ) on DFI. The DFI values for all treatments were below the ISA Brown standard of 101 g/b/d for 20-week-old hens (ISA Brown Commercial Management Guide, 2014).

The highest DFI was observed in the intensive rearing system (T1), likely due to the restricted activity of hens, which allowed them to consume more feed. Fitra et al. (2021) stated that intensive systems aim to minimize chicken activity, ensuring more efficient feed consumption and maximizing production levels. In contrast, chickens in the free-range systems consumed less

feed, possibly due to the additional forage available. As observed by [Mangalisu \(2017\)](#), free-

range chickens engage in more activity, which may reduce their feed intake.

**Table 2.** The egg production of laying hens reared in cages and free-range systems

| Parameters         | Treatment               |                         |                        |                        |
|--------------------|-------------------------|-------------------------|------------------------|------------------------|
|                    | T1                      | T2                      | T3                     | T4                     |
| BWG (g/b/w)        | 55.0±4.08 <sup>b</sup>  | 50.0±4.08 <sup>b</sup>  | 41.3±2.50 <sup>a</sup> | 38.0±2.88 <sup>a</sup> |
| DFI (g/b/d)        | 93.8±2.59 <sup>b</sup>  | 78.5±5.16 <sup>a</sup>  | 83.2±9.11 <sup>a</sup> | 81.4±6.00 <sup>a</sup> |
| HDP (%)            | 39.3±2.60 <sup>bc</sup> | 46.0±2.44 <sup>c</sup>  | 33.5±3.22 <sup>b</sup> | 22.0±2.16 <sup>a</sup> |
| Egg weight (g/egg) | 50.7±1.00 <sup>b</sup>  | 48.4±0.62 <sup>ab</sup> | 48.0±0.40 <sup>a</sup> | 47.7±1.00 <sup>a</sup> |
| Egg mass (g/egg)   | 19.8±2.44 <sup>bc</sup> | 22.2±2.18 <sup>c</sup>  | 16.1±3.25 <sup>b</sup> | 10.5±2.51 <sup>a</sup> |
| FCR                | 4.7±0.47 <sup>b</sup>   | 3.5±0.19 <sup>a</sup>   | 5.0±0.80 <sup>b</sup>  | 8.0±1.16 <sup>c</sup>  |

Note: <sup>a,b,c</sup> superscripts that differ between treatments indicate significant differences ( $P<0.05$ ). T1= intensive rearing system, T2= continuous free-range rearing system, T3= Rotational free-range rearing system with 2 times removal, T4= rotational free-range rearing system with 4 times removal.

### Hen Day Production (HDP %)

The different rearing systems significantly ( $P<0.05$ ) affected hen day production (HDP). In the intensive cage system (T1), HDP matched the ISA Brown standard of 38% for 20-week-old hens (ISA Brown Commercial Management Guide, 2014). The continuous free-range rearing system (T2) showed higher HDP than the standard, while the rotational free-range system with four times paddock removal (T4) had the lowest HDP, which was below the standard.

The differences in HDP between the continuous and rotational free-range systems were attributed to stress from the periodic removal of paddocks. The highest HDP in the continuous free-range system may be due to the availability of supplementary feed from plants growing on the land. Sufficient quantity and quality of feed contribute to higher egg production, as noted by [Sulaiman et al. \(2022\)](#).

### Egg Weight (EW)

Significant differences ( $P<0.05$ ) in egg weight were found among the rearing systems. Egg weights ranged from 47.7 g to 50.7 g, which were lower than the ISA Brown standard of 51.4 g for 20-week-old hens ([ISA Brown Commercial Management Guide, 2014](#)). The highest egg weight was observed in the intensive rearing system (T1). The body weight of hens positively correlated with egg weight, which is consistent with the findings of [Setiawati et al. \(2016\)](#) and [Utomo \(2017\)](#), who found that as chicken age and body weight increase, so does egg weight. Several factors, including genetics, body weight, and feed, influence egg weight.

### Egg Mass (EM)

Egg mass also varied significantly ( $P<0.05$ ) between the rearing systems. Egg mass values

ranged from 10.5 g/egg to 22.2 g/egg. In the intensive cage system (T1), egg mass aligned with the ISA Brown standard of 19.5 g/egg for 20-week-old hens ([ISA Brown Commercial Management Guide, 2014](#)). The highest egg mass was recorded in the continuous free-range system (T2), which was above the standard. In contrast, the lowest egg mass was observed in the rotational free-range system with four times paddock removal (T4). The lower egg mass in the rotational systems was linked to reduced HDP and egg weight, likely due to the stress experienced by the hens.

The relationship between egg production and weight is well-documented, with higher egg production and egg weight contributing to greater egg mass, as stated by [Mawaddah et al. \(2014\)](#).

### Feed Conversion Ratio (FCR)

The FCR was significantly ( $P<0.05$ ) affected by the rearing systems. The FCR in the rotational free-range system with four times paddock removal (T4) exceeded the ISA Brown standard of 5.1/d egg ([ISA Brown Commercial Management Guide, 2014](#)). On the other hand, the lowest FCR was found in the continuous free-range system (T2), which was below the standard.

A lower FCR indicates more efficient feed consumption, while a higher FCR indicates inefficiency. The low FCR in the continuous free-range system (T2) was likely due to the low feed consumption combined with higher egg production. The availability of additional forage likely provided a variety of nutrients, helping to enhance egg production. This is consistent with the findings of [Prastio et al. \(2022\)](#), who noted that increased feed consumption without a corresponding increase in body weight leads to a higher FCR.

### Yolk Cholesterol Content and Yolk Protein Content

The yolk cholesterol and protein content of eggs from laying hens reared in intensive and free-range systems are presented in Table 3. The different rearing systems significantly ( $P<0.05$ ) affected yolk cholesterol levels. Cholesterol content in eggs from all groups ranged from 344.3 to 413 mg/100g. This range was lower than the values reported by [Anderson \(2018\)](#), which ranged from 156 to 160.4 mg/50g, but higher than those reported by [Ohoilulin et al. \(2023\)](#), which ranged from 539.9 mg/100g to 547.4 mg/100g. These discrepancies may be attributed to variations in the age of the chickens, the nutritional composition of the feed, and the breed of chickens used in different studies.

Rearing laying hens in intensive and free-range systems with varying patterns is expected to reduce yolk cholesterol levels. The lowest cholesterol levels were observed in eggs laid by hens reared in rotational free-range systems with four paddock removals (T4). Increased movement between pastures enhanced the hens' activity, allowing them to forage and select their preferred feed, which was abundantly available in the fields. This finding aligns with [Anderson \(2018\)](#) and [Popova et al. \(2020\)](#), who reported that eggs from free-range hens tend to have lower cholesterol levels compared to those from intensively reared chickens. The free-range system allows chickens to access a wider variety of feeds, including insects and worms, which can be beneficial for reducing cholesterol levels ([Fitra et al., 2021](#)).

**Table 3.** Yolk cholesterol and yolk protein content of eggs laid by laying hens in different rearing systems

| Parameters                         | Treatment                     |                               |                               |                               |
|------------------------------------|-------------------------------|-------------------------------|-------------------------------|-------------------------------|
|                                    | T1                            | T2                            | T3                            | T4                            |
| Yolk cholesterol content (mg/100g) | 413.0 $\pm$ 3.24 <sup>b</sup> | 350.3 $\pm$ 3.88 <sup>a</sup> | 412.5 $\pm$ 4.50 <sup>b</sup> | 344.3 $\pm$ 2.25 <sup>a</sup> |
| Yolk Protein content (%)           | 17.7 $\pm$ 0.48               | 17.1 $\pm$ 0.27               | 17.8 $\pm$ 0.32               | 17.9 $\pm$ 0.25               |

Note: <sup>a,b</sup> superscripts that differ between treatments indicate significant differences ( $P<0.05$ ). T1= intensive rearing system, T2= continuous free-range rearing system, T3= Rotational free-range rearing system with 2 times removal, T4= rotational free-range rearing system with 4 times removal

The protein content of egg yolks from laying hens reared in both intensive and free-range systems ranged from 17.1% to 17.9%. This range was higher than the values reported by [Sahara et al. \(2020\)](#), which ranged from 14.2% to 15.3%. The difference in protein content may stem from variations in livestock breed, age, and the protein content of the feed. According to [Lestari et al. \(2015\)](#), the protein content of the feed, in the form of amino acids absorbed by the liver, is subsequently converted into proteins and directed to the ovaries for the egg formation process.

### CONCLUSION

Laying hens reared in both intensive and free-range systems can lead to higher hen day production (HDP) and egg mass in continuous free-range systems. The intensive system tends to produce eggs with higher body weight gain (BWG), egg weight, and daily feed intake (DFI). In contrast, the continuous free-range system results in a lower feed conversion ratio (FCR). The rotational free-range system with four removals (T4) leads to lower egg yolk cholesterol levels.

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