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Infertile Egg Powder Improves Nutrient Digestibility, Digestive Organ Development, and Performance of Broiler Chickens

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ABSTRACT

This research investigated the effects of infertile egg powder (IEP) supplementation in the diets of broiler chickens on nutrient digestibility, digestive tract development, and performance. A total of 196 one-day-old male broiler chickens were randomized into four treatments differing in IEP levels, each with seven replicates of seven birds. The treatments were: 100% basal diet as a control (CON), 96% CON + 4% IEP (IEP4), 94% CON + 6% IEP (IEP6), and 92% CON + 8% IEP (IEP8). The observed variables included growth performance, nutrient digestibility, and digestive organ development. The results showed that dietary IEP enhanced the digestibility of dry matter, crude protein, ether extract, and crude ash (p<0.05), most noticeably in the IEP6 and IEP8 groups. However, IEP did not affect crude fiber and nitrogen-free extract digestibility. The birds consuming diets with IEP had heavier and more prolonged (p<0.05) duodenum, jejunum, ileum, and whole small intestines compared to the birds fed the CON diet. Dietary IEP also improved liver and gizzard weight (p<0.05). Feeding IEP did not affect feed intake but increased (p<0.05) crude protein and metabolizable energy intake, leading to an increase (p<0.05) of daily weight gain and concomitantly decreased (p<0.05) feed conversion ratio of the IEP groups. To conclude, IEP from hatchery had a positive response on the improved nutrient digestibility, digestive tract development, and performance of broiler chickens, and therefore, it can be used as a feed ingredient for broiler chickens.

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Introduction

Global meat consumption, including poultry, is forecasted to increase by approximately 14% over the next decade, mainly driven by population growth worldwide (FAO, 2021). The continuous production of hatching eggs from poultry breeding farms results in a large quantity of infertile eggs. Approximately 14-17% of infertile eggs are expected from poultry hatchery, given the standard hatchability of most breeder strains is between 83-86% (Cobb Vantress, 2020; Aviagen, 2021). These eggs are rich in protein, fat, and other bioactive nutrients, such as lysozyme, avidin, and phosvitin (El-Deek et al., 2011; Esmailzadeh et al., 2016).

Eggs also possess a natural balance of essential nutrients (Anton et al., 2006; Sparks, 2006). Dal Santo et al. (2020) have determined that the protein digestibility coefficient in egg powder is 86.81%. Therefore, these infertile eggs could serve as an excellent alternative feedstuff, particularly a protein source for chickens—especially considering that the egg reserves supply all

nutritional requirements during chicken embryonic development (Esmailzadeh et al., 2013).

Infertile eggs can be processed into dried infertile egg powder (IEP), which can further be applied as a feed ingredient without any negative impacts on growth performance (EI-Deek et al., 2011; Esmailzadeh et al., 2013). Processing infertile eggs from the hatchery into IEP is economically relevant to reducing feed costs in broiler chickens. The high nutrient density of IEP could potentially support the growth of the digestive tract and other organs (Esmailzadeh et al., 2016), ultimately supporting growth in all parts of the body, including the muscles, skeleton, and immune system (Lei and Kim, 2013; Esmailzadeh et al., 2016).

Positive impacts of dietary IEP on growth performance and immunity have been previously observed (El-Deek et al., 2011; Esmailzadeh et al., 2013). It is expected that the inclusion of IEP in the diet would improve nutrient availability, leading to growth performance improvement. However, only a few studies have considered the effects of IEP on nutrient digestibility, digestive tract development,

and growth performances of broiler chickens, although Esmailzadeh et al. (2016) have found improvement in villus height and small intestine length among broilers. In particular, highly available protein from IEP might be favorable for broiler chickens in the tropics to diminish heat stress and other environmental challenges. We hypothesize that increasing IEP levels in the diets positively affect broiler chickens' nutrient digestibility, organ growth, and performance. Therefore, the present study investigated the effect of dietary IEP on nutrient digestibility, digestive organ development, growth performance, and nutrient efficiency in broiler chickens.

Materials and Methods

This study was performed in line with the principles of the Declaration of Helsinki. Approval was granted by the Animal Ethics Committee of

Universitas Sebelas Maret (No. 780/UN27.20.1/PT.02/2023).

Infertile egg source and preparation

The infertile eggs were donated by PT. Super Unggas Jaya, a commercial poultry breeding farm in Boyolali, Indonesia. The farm operates ten flocks with a 10,000 capacity of broiler breeders each flock, with a commercial hatchery facility. The IEP was prepared from albumen and yolk, and the eggshell was discarded. The albumen and yolk were mixed homogenously with 10% wheat flour (w/w) and 10% palm oil (w/w). The mixture was dried in an oven at 70°C for 60 min and was sundried thereafter. This dried mixture was then ground to obtain the IEP meal. The nutrient contents of the resulting IEP meal were analyzed following the AOAC procedure (Association of Official Analytical Chemists [AOAC], 2001) and are presented in Table 1.

Table 1. Nutrient content of the infertile egg powder

| Nutrients | Contents |
|---------------------------------|----------|
| Metabolizable energy (kcal/kg)* | 5,455 |
| Crude protein (%) | 31.47 |
| Ether extracts (%) | 30.10 |
| Crude fiber (%) | 0.59 |
| Crude ash (%) | 1.99 |
| Calcium (%) | 0.05 |
| Total phosphorus (%) | 0.18 |

*Calculated according to Sibbald et al. (1976):

Metabolizable energy = 3951+(54.4×crude fat)–(88.7×crude fiber)–(40.8×crude ash)

Experimental design and diet formulation

The experiment was performed on an experimental farm at the Department of Animal Science, Universitas Sebelas Maret, Indonesia. In total, 196 one-day-old male Lohman MB 202 broilers (average body weight [BW]: 38.42±2.66 g) were used in this experiment. The experiment was designed as a completely randomized design with four treatments consisting of seven replicates of

seven birds. The four dietary treatments were: 100% basal diet as a control (CON), 96% CON + 4% IEP (IEP4), 94% CON + 6% IEP (IEP6), and 92% CON + 8% IEP (IEP8). The IEP was substituted with soybean meal and corn to make the diet isonitrogenous. The nutrient contents of the basal and experimental diets are presented in Table 2.

Table 2. Composition of starter and finisher basal diets

| Ingredients | Starter (%) | Finisher (%) |
|--------------------------------|-------------|--------------|
| Yellow corn | 52.65 | 51.50 |
| Rice bran | 0.00 | 6.29 |
| Soybean meal | 40.00 | 35.20 |
| Coconut oil | 5.00 | 5.00 |
| DL-methionine | 0.15 | 0.16 |
| Mineral premix ¹ | 1.80 | 1.45 |
| Premix ² | 0.20 | 0.20 |
| NaCl | 0.20 | 0.20 |
| Nutrient contents | | |
| Metabolizable energy (kcal/kg) | 3,113 | 3,152 |
| Crude protein (%) | 21.47 | 20.01 |
| Ether extract (%) | 8.21 | 8.42 |
| Crude fiber (%) | 5.53 | 5.89 |
| Crude ash (%) | 5.84 | 6.10 |
| Calcium (%) | 1.09 | 0.90 |
| Available phosphorus (%) | 0.43 | 0.38 |

¹ Per kg contains calcium 480 g of, phosphorus 15 g of, iron 4,000 mg of, manganese 2,750 mg of, iodine 50 mg, cup rum 200 mg, zinc 2500 mg, vitamin B12 0.45 mg, and vitamin D3 50,000 IU.

Birds and management

The birds were raised in a brooder from day 1 – 10, where they were fed a commercial feed for

the pre-starter period. Then, they were randomly allotted into four treatment groups, where the pen was the experimental unit. Starting at day 11

² Per kg contains vitamin D3 200,000 IU, vitamin E 800 mg, vitamin K 200 mg, vitamin B1 200 mg, vitamin B2 500 mg, vitamin B6 50 mg, vitamin B12 1,200 mg, vitamin C 2,500 mg, Ca-D-pantothenate 600 mg, niacin 4,000 mg, choline chloride 1,000 mg, methionine 3,000 mg, lysine 3,000 mg, manganese 12,000 mg, iron 2,000 mg, iodine 20 mg, zinc 10,000 mg, cobalt 20 mg, cup rum 400 mg, antioxidant 1,000 mg.

(average BW = 270.53±5.83g), the birds were given one of the four experimental diets. Broilers were raised according to the management guide of the breeder company and fed ad libitum with a continuous clean water supply.

Measurements

Performance

Feed intake (FI) was recorded daily, while it was recorded weekly. Those data were then averaged to obtain the feed intake and average daily gain data. The feed conversion ratio (FCR) was calculated from feed intake and BW data and was corrected for mortality.

Nutrient efficiency

The nutrient efficiency was assessed by the protein efficiency ratio (PER) and the energy efficiency ratio (EER). The PER was calculated by dividing the weight gain by protein intake, while the EER was calculated by dividing the weight gain by 100 kcal metabolizable energy (ME) intake (Nasr et al., 2011).

Nutrient digestibility

The in vivo digestibility study was performed using two birds from each pen at the end of the experiment (day 35). The birds were reared in individual cages and fed the experimental diets over a five-day total collection period, following the procedure of El-Husseiny et al. (2007). The collected excreta were pooled, homogenized in each pen, and sundried. The excreta samples were milled through a 0.5 mm mesh screen before analysis. The crude protein (CP) content of the excreta and diet were determined via the Kjeldahl method, while the dry matter (DM), ether extract (EE), crude fiber (CF), and crude ash (CA) were performed according to AOAC (2001). The nutrient digestibility coefficients were calculated as follows (Attia et al., 2012):

Nutrient digestibility (%) = Nutrient intake (g) - nutrient excreted (g) Nutrient intake (g) ×100%.

Digestive organ characteristics

At the end of the feeding experiment (day 35), two birds from each pen with average BW per replicate were selected for digestive tract measurement. After the feed was withheld for 12 h, the birds were slaughtered to empty the digestive tract. Then, the small intestine, gizzard, and liver were removed. The duodenum, jejunum, ileum, gizzard, and liver were weighed using a digital scale (600 × 0.01g), while the length of the duodenum, jejunum, and ileum were measured with a gauge (Ratriyanto and Sunarto, 2020).

Data analysis

The data were checked for outliers and normal distribution and then were analyzed using a generalized linear model with the following model: $y_{ijk} = \mu + \alpha_i + \epsilon_{ijk}, \text{ where } \mu = \text{the general mean;} \\ \alpha_i = \text{the fixed effect of treatments; and } \\ \epsilon_{ijk} = \text{an experimental error associated with the experimental units (random effect). Duncan's multiple range test was applied to test the significance of the dietary treatment. The statistical analysis was performed using the R program, version 3.5.3 (R Core Team, 2019).}$

Results and Discussion

General observations

All birds were healthy throughout the experiment and readily consumed their feed allowances. The average ambient temperatures in the morning, mid-day, and evening during the experiment were 27.4°C, 33.6°C, and 30.8°C on average, while the average relative humidity levels were 82.3%, 57.0%, and 66.4%, respectively.

Performance

Dietary supplementation of IEP did not affect feed intake (p>0.05). However, CP and ME intakes were higher (p<0.01) for IEP8 than CON (Table 3). All birds consuming IEP from 4 – 8% exhibited greater (p<0.05) average daily gain (ADG) and lower (p<0.05) FCR than the birds fed the CON diet. However, the PER and EER were unaffected by IEP inclusion (Table 3). Overall, IEP6 demonstrated the most remarkable performance improvement compared to CON.

Table 3. Performances and nutrient efficiency (Mean ± SD) of broiler chickens fed IEP

| Items | CON | IEP4 | IEP6 | IEP8 | p Value |
|--------------------------|-------------------------|-------------------------|-------------------------|--------------------------|---------|
| Feed intake (g/d) | 72.03±4.39 | 72.82±5.93 | 78.60±4.81 | 74.55±2.67 | 0.059 |
| CP intake (g/d) | 15.34±0.94 ^b | 16.01±1.41 ^b | 17.42±1.18 ^a | 16.69±0.74 ^{ab} | 0.010 |
| ME intake (kcal/d) | 232.42±14.28° | 244.89±21.73bc | 267.42±18.41a | 255.43±11.67ab | 0.004 |
| Average daily gain (g) | 41.61±3.60 ^b | 46.88±5.20 ^a | 51.08±4.75 ^a | 48.54±3.90 ^a | 0.004 |
| Feed conversion ratio | 1.74±0.09 ^a | 1.56±0.10 ^b | 1.55±0.15 ^b | 1.54±0.08 ^b | 0.008 |
| Protein efficiency ratio | 2.71±0.14 | 2.93±0.18 | 2.94±0.27 | 2.92±0.13 | 0.097 |
| Energy efficiency ratio | 17.89±0.91 | 19.13±1.14 | 19.13±1.73 | 18.98±0.8 | 0.186 |

CP = crude protein; ME = metabolizable energy.

a, b, c Mean in the same row with different superscripts are significantly different (p<0.05).

Nutrient digestibility

Dietary inclusion of IEP increased the digestibility of DM, organic matter (OM), CP, EE, and ash (p<0.05; Table 4), while crude fiber and nitrogen-free extract (NFE) digestibility were similar across dietary treatments. Compared to other

groups, IEP6 exhibited the highest improvement in CP digestibility (75.98% vs. 73.61%), EE digestibility (89.44% vs. 83.92%), and CA digestibility (59.23% vs. 54.5%) compared to the CON group.

Table 4. Nutrient digestibility (%, Mean ± SD) in broiler chickens fed IEP (%)

| Items | CON | IEP4 | IEP6 | IEP8 | p Value |
|-----------------------|-------------------------|-------------------------|-------------------------|-------------------------|---------|
| Dry matter | 78.89±0.69b | 79.69±0.30ab | 80.63±1.35 ^a | 80.28±1.15 ^a | 0.003 |
| Organic matter | 82.60±0.93 ^b | 83.77±0.24ab | 84.55±1.75 ^a | 84.52±1.58 ^a | 0.028 |
| Crude protein | 73.61±0.56 ^b | 76.1±1.33 ^a | 75.98±0.87a | 75.91±1.14a | 0.004 |
| Ether extract | 83.92±1.30° | 88.00±0.63 ^b | 89.44±0.61a | 83.29±1.70° | < 0.001 |
| Crude fiber | 48.45±4.20 | 50.55±6.56 | 49.01±4.24 | 47.45±8.19 | 0.809 |
| Ash | 54.5±3.54 ^b | 54.13±0.74 ^b | 59.23±4.19 ^a | 57.43±4.59ab | 0.041 |
| Nitrogen-free extract | 84.69±1.06 | 84.31±0.79 | 85.22±2.77 | 85.98±1.85 | 0.356 |

a, b, c Mean in the same row with different superscripts are significantly different (p<0.05).

Digestive organ weights and lengths

Dietary IEP increased (p<0.05) the weight and length of digestive organs, including the liver, gizzard, parts, and total small intestine (Table 5). Birds fed IEP diets had heavier liver and small intestines compared to birds fed CON (p<0.05). Only the IEP8 group exhibited heavier (p<0.05) gizzard (17.15 g vs. 14.75 g) and ileum (13.75 g vs. 11.96 g) than the CON group. The IEP8 group also showed the highest increase in the duodenum (14.55 g vs. 11.01 g), jejunum (19.14 g vs. 13.63

g), and the whole small intestine (47.44 g vs. 35.22 g) compared to the CON group (p<0.05). However, only the relative weight of the liver and gizzard was affected by dietary IEP inclusion (p<0.05); the IEP8 birds had the lowest relative liver (1.98% vs. 2.47%) and gizzard (1.12% vs. 1.41%) weight than CO. Parallel to the results obtained for digestive organ weights, dietary IEP increased (p<0.05) the length of the duodenum, jejunum, ileum, and the whole small intestine (Table 5).

Table 5. Digestive organs weight and relative weight (Mean ± SD) of digestive organs to body weight of broilers fed IEP

| Digestive o | rgans | CON | IEP4 | IEP6 | IEP8 | p Value |
|-----------------|--------|--------------------------|--------------------------|--------------------------|--------------------------|---------|
| Liver | W (g) | 25.82±4.60b | 30.99±5.14a | 29.64±3.09a | 30.34±3.03a | 0.049 |
| | R (%) | 2.47±0.51a | 2.38±0.34 ^{ab} | 2.24±0.21ab | 1.98±0.26 ^b | 0.014 |
| Gizzard | W (g) | 14.75±2.31 ^b | 15.75±2.29ab | 16.89±2.08ab | 17.15±1.40 ^a | 0.019 |
| | R (%) | 1.41±0.23 ^a | 1.20±0.09 ^b | 1.27±0.11 ^{ab} | 1.12±0.14 ^b | 0.003 |
| Duodenum | W (g) | 11.01±1.73° | 12.51±1.09bc | 12.71±0.93 ^b | 14.55±1.65 ^a | < 0.001 |
| | R (%) | 1.05±0.19 | 0.96±0.10 | 0.96±0.08 | 0.95±0.14 | 0.141 |
| | L (cm) | 22.07±1.10 ^b | 23.5±0.87 ^a | 23.14±1.11 ^{ab} | 24±1.55 ^a | 0.036 |
| Jejunum | W (g) | 13.63±2.71° | 17.49±2.64 ^{ab} | 15.85±1.89bc | 19.14±1.72 ^a | 0.001 |
| | R (%) | 1.31±0.31 | 1.34±0.12 | 1.20±0.14 | 1.25±0.13 | 0.363 |
| | L (cm) | 50.57±5.12 ^b | 63.21±3.88 ^a | 59.93±3.32a | 62.86±5.52a | < 0.001 |
| Ileum | W (g) | 10.59±1.32 ^b | 11.96±1.34ab | 12.44±1.83ab | 13.75±1.91 ^a | 0.012 |
| | R (%) | 1.01±0.13 | 0.92±0.11 | 0.94±0.13 | 0.90±0.14 | 0.121 |
| | L (cm) | 49.36±4.06 ^b | 57.29±3.60 ^a | 58.79±4.44 ^a | 60.64±3.90 ^a | < 0.001 |
| Small intestine | W (g) | 35.22±4.45° | 41.96±4.43 ^b | 41.00±4.11 ^b | 47.44±4.42 ^a | < 0.001 |
| | R (%) | 3.37±0.54 | 3.22±0.26 | 3.10±0.29 | 3.10±0.36 | 0.127 |
| | L (cm) | 122.00±8.44 ^b | 144.00±6.99a | 141.86±7.11a | 147.50±9.52 ^a | < 0.001 |

W = weight (g); R = relative organ weight to body weight (%); L = length (cm)

Nutrient digestibility

Eggs primarily function as embryonic chambers. Thus, they contain highly digestible nutrients essential for life (Anton et al., 2006). Eggs have an excellent nutritive value and reserve of highly digestible proteins, lipids, vitamins, minerals, and numerous bioactive components (Dal Santo et al., 2020; Anton et al., 2006; Asghar and Abbas, 2012). Thus, it is logical that dietary IEP would improve nutrient digestibility, as demonstrated by the increase in DM, OM, CP, and EE digestibility in this study. Esmailzadeh et al. (2013) suggested that poultry could consume IEP efficiently because of its highly digestible nutrient content. Previous studies also suggested that IEP supports digestive tract development, as indicated by a higher villus and a longer intestine, which increases nutrient absorption in the small intestine (Esmailzadeh et al., 2016). The improvement in digestive organ

development in this study, as indicated by the heavier and longer digestive organs (Table 5), was associated with an improvement in the intact gut area for nutrient absorption, leading to greater nutrient digestibility (Ratriyanto and Prastowo, 2019).

The high-fat content of IEP in this study contributed to the higher ME intake. The high-fat content of IEP may ease digestion along the intestine, allowing for optimal nutrient digestion and absorption (Nutrition Research Council, 1994). Previous studies have confirmed that EE digestibility increased when diets were supplemented with increased EE content (Pesti et al., 1986; Indreswari and Ratriyanto, 2018). Similarly, increasing dietary EE content enhances ME (Sathishkumar and Prabakaran, 2008; Indreswari and Ratriyanto, 2018). Observations in pigs have shown that including dietary spray-dried

a, b, c Mean in the same row with different superscripts are significantly different (p<0.05).

egg and albumen powder improves amino acid digestibility (Zhang et al., 2015). However, Lei and Kim (2013) did not observe any effect of dietary IEP on DM or nitrogen digestibility, although they found the digestibility of energy to be greater.

Digestive organ weights and lengths

This study has shown that dietary IEP improves digestive organ development, as demonstrated by the larger liver, gizzard, and small intestine weights, as well as small intestine lengths. Dietary IEP also provides good nutritional content for supporting digestive tract development, as has been observed previously; digestible nutrients are necessary for supporting digestive tract development and growth in broiler chickens (Esmailzadeh et al., 2016).

broiler chickens, digestive development plays a pivotal role in supporting birds' growth since the chickens are raised in a relatively short time. Improved digestive tract development due to dietary IEP may indicate better nutrient digestion and absorption, which leads to more nutrients being provided to support the birds' optimal growth rate. The enhanced nutrient digestibility in this study confirmed this finding. Also, the longer the small intestine, the more nutrients can be absorbed and used by the birds. It is well known that providing adequate dietary nutrients, both during the starter and finisher periods, is one of the best ways to generate optimal bird performance. IEP's high nutrient content and availability assist digestive tract development in broiler chickens (Anton et al., 2006; Esmailzadeh et al., 2016).

Supporting this study, feeding broiler chickens with a diet of 40g of IEP per kg yielded a longer intestine, a higher villus, and a lower crypt depth, indicating improved small intestine development (Esmailzadeh et al., 2016). The present research has shown that the high nutrient levels in IEP positively impact intestinal morphometry. A well-developed digestive tract supports the growth rate of broiler chickens, as observed in a previous study, which found that dietary IEP improves body weight and FCR in broilers. However, in contrast to the present study, Lei and Kim (2013) have determined that IEP does not affect relative liver and gizzard weights in broilers, but this result may be attributed to the lower levels of IEP used in the diets (1-3%) compared to the levels used in the current study.

Performance

Including dietary IEP altered the ME level of the experimental diets but did not increase feed intake. This finding is in line with previous observations in which dietary IEP did not affect the feed intake of broilers (Esmailzadeh et al., 2016; Ratriyanto et al., 2020). However, the increased CP and ME contents from IEP increased CP and ME intake. Previous observations have also revealed that including IEP in broiler starter diets increases CP and ME intake (Ratriyanto et al., 2020). In

support of this finding, Indreswari and Ratriyanto (2018) have suggested that dietary inclusion of hatchery waste meals increases CP and ME content.

Though increasing the CP and ME content via IEP did not alter feed intake in this study, higher CP and ME intake were plausible to explain the improved growth performance. Therefore, dietary IEP provides more nutrients that support the broiler chickens' optimal growth rate. Additionally, the protein available in IEP has a high biological value and digestibility and a good amino acid balance, leading to enhanced growth rates (Khan and Bhatti, 2001). The nutritional profile of IEP is similar to that consumed by the chicken embryo during the incubation and hatching period, which promotes the chicks' continuous growth (Esmailzadeh et al., 2016). Improvement in growth performance can also be attributed to bioactive egg components, such as essential fatty acids, balanced amino acids, antibodies, and bactericidal enzymes (Schaafsma et al., 2000; Anton et al., 2006).

Decreased FCR following IEP inclusion indicated that broiler-fed IEPs were more efficient at improving body weight gain compared to those without IEPs. IEP contains substantial nutrients. such as protein and fat, which increase the diet's nutritional value. As aforementioned, the growth and development of the birds' digestive tracts could also be a strong reason for the improvement of growth performance (Esmailzadeh et al., 2013). Previous research has shown that dietary IEP increases the villus height and villus: crypt ratio in broilers, leading to a higher capacity for nutrient absorption (Esmailzadeh et al., 2016). Previous observations have also revealed that IEP improves weight gain and FCR in both starters (Esmailzadeh et al., 2016; Ratriyanto et al., 2020) and finisher broilers (Lei and Kim, 2013). In growing layer chicks, supplementing dried egg byproducts was also reported to improve BW and FCR (El-Deek and Al-Harthi, 2009; El-Deek et al., 2011). Similarly, increasing the amount of hatchery waste meal in the diet was found to improve the performance of broiler chickens (Agunbidae et al., 2011). Indonesia native chickens (Akhirini et al., 2021), and male ducks (Jaya et al., 2015).

Conclusion

This study revealed that dietary IEP increased nutrients, most notably in the diets with 6% and 8% IEP inclusion. Dietary IEP also improved digestive organ development. As a result, IEP improved the birds' growth performance and FCR, which are associated with increased protein and energy intake. This finding supports the use of infertile eggs from poultry breeding farms as high-quality feed ingredients for broiler chickens. Further study on immune response, gut microbiome modulation, digestive enzymes, and antioxidant parameters may provide crucial information on how infertile eggs can be used as a functional feed ingredient.

Conflict of interest

The authors declare that there is no conflict of interest.

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Ethical Clearance Statement

This study was performed in line with the principles of the Declaration of Helsinki. Approval was granted by the Animal Ethics Committee of Universitas Sebelas Maret (No. 780/UN27.20.1/PT.02/2023).

Data Availability

The datasets of the current study are available from the corresponding author on reasonable request.

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