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# **ORIGINAL PAPER**

# Effect of dietary inclusion of infertile egg powder on carcass characteristics and meat quality in broiler chickens

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ABSTRACT. This study evaluated the effects of partially substituting soybean meal (SBM) and maize grain with infertile egg powder (IEP) in broiler diets on carcass characteristics, meat quality, fatty acid, and amino acid (AA) profiles. A total of 196 one-day-old male broilers was randomly assigned to four dietary treatments (7 replicates/treatment, 7 birds/replicate) and reared for 35 days under tropical conditions. The treatments included a 100% basal diet as control (CON), and diets with 4% (IEP4), 6% (IEP6), or 8% (IEP8) IEP replacing part of SBM and maize grain in CON. Results showed that IEP8 increased final body weight, carcass weight, breast meat yield, and abdominal fat mass though the relative percentages of these components remained unchanged. IEP inclusion did not alter carcass quality parameters such as chemical compositions, water holding capacity, cooking loss, or tenderness. However, IEP-fed birds showed elevated breast meat stearic and α-linolenic contents, but reduced oleic acid level compared to CON birds. AA analysis revealed higher threonine, arginine, glycine, alanine, and glutamic acid levels in IEP groups, while phenylalanine, serine, methionine, and isoleucine were lower. In conclusion, substituting up to 8% of maize grain and SBM with IEP can improve broiler growth performance, as well as fatty acid and amino acid profiles in breast meat without compromising its quality. Incorporating infertile egg powder into broiler diets appears to be a promising sustainable alternative protein source in poultry diets.

#### Introduction

The rapid expansion of the poultry industry, particularly broiler chicken production, has successfully met the growing global demand for affordable, high-quality protein. Recently, feed costs have become a primary concern for broiler chicken farmers worldwide, prompting efforts to reduce expenses. A key strategy involves using alternative feed ingredients with competitive prices and valuable nutrient content sourced from industrial by-products (Irawan et al., 2022; Dewi et al., 2024). Infertile eggs, a hatchery by-product of poultry breeding companies, are recognized as a highly nutritious protein alternative for broiler feeds. Infertile eggs without shells contain 31.47% crude protein

(CP), 30.10% ether extract (EE), and 5.455 kcal/kg metabolizable energy (ME) (Ratriyanto et al., 2021). These values exceed those of common broiler feed ingredients such as maize grain and gluten in both energy and protein contents. The high CP content makes infertile eggs a promising partial replacement for soya bean meal (SBM), potentially lowering feed costs given the high price of SBM.

It is estimated that approx. 4–5% of hatching eggs represent a largely underutilised source of high-quality protein. In Indonesia alone, with an annual parent stock population exceeding 300 mln birds (Ministry of Agriculture, 2021), hatcheries produce over 10 000 metric tonnes of infertile eggs per year (Ministry of Agriculture of the Republic

of Indonesia, 2021). This volume represents a significant protein source that could partially replace costly SBM in feed formulations (Won Jun Choi et al., 2021; Zhang et al., 2022). The high protein bioavailability derived from animal products offers important nutritional benefits for animal growth and performance (Choi et al., 2021).

Several studies have demonstrated that infertile eggs can improve the performance of broiler chickens and laying hens (Abiola and Onun Kwor, 2004; Esmailzadeh et al., 2016; Ratriyanto et al., 2020). On the other hand, one study reported that the inclusion of infertile eggs with shells up to 5% did not affect growth performance but improved meat quality (Choi et al., 2021). In contrast, when using shell-free infertile egg powder (IEP) at 8% inclusion, broilers had increased organ weight and body weight gain (Ratriyanto et al., 2021, 2020). The high digestibility of infertile egg protein is particularly beneficial for newly hatched chicks, whose immature digestive systems have limited capacity for enzyme production. The easily assimilated proteins and energy from infertile eggs can therefore support early growth, ultimately leading to higher final body weights in broilers. This is especially crucial during the post-hatch period when chicks require premium-quality nutrients to compensate for their underdeveloped gastrointestinal function (Noy and Sklan, 2002).

In addition, better protein quality in poultry diets enhances protein metabolism and deposition, potentially improving both carcass traits and meat quality. Thus, we hypothesised that dietary inclusion of IEP would positively influence broiler meat quality. The objective of the present study was to evaluate the use of IEP as a feed ingredient on carcass characteristics and meat quality in broiler chickens.

#### Material and methods

This study was conducted in accordance with the principles outlined in the Declaration of Helsin-ki. Formal approval was granted by the Animal Ethics Committee of Universitas Sebelas Maret (No. 780/UN27.20.1/PT.02/2023).

### Infertile egg powder preparation

Infertile eggs were provided by Perseroan Terbatas (PT) Super Unggas Jaya, a commercial breeding farm located in Wonosegoro, Boyolali (Central Java, Indonesia). The IEP was prepared by cracking the eggs, and mixing the albumen and yolk homogenously with 10% wheat flour (w/w) and 10% palm oil (w/w). Eggshells were discarded, and only

the egg contents were used to produce the IEP. The mixture was dried in an oven at 70 °C for 60 min, followed by sun drying. This dried mixture was then ground and the particles were passed through a 1-mm sieve (40 mesh). Nutrient contents of the resulting IEP were determined by proximate analysis according to the Association of Official Analytical Chemists (AOAC International, 2001) and are presented in Table 1.

# Experimental design and diet formulation

A total of 196 one-day-old male Lohman MB 202 broilers (initial body weight:  $38.42 \pm 2.66$  g) were randomly allocated to four dietary treatment groups: control diet = 100% basal diet (CON), 4% IEP inclusion partially replacing SBM and maize grain in the basal diet (IEP4), 6% IEP replacement of SBM and maize (IEP6), and 8% IEP replacement of SBM and maize (IEP8). Each group comprised 7 replicates (pens) with 7 birds per pen (49 birds/treatment). The nutrient composition of the IEP and the experimental diets are listed in Tables 1 and 2, respectively.

Table 1. Nutrient content of infertile egg powder (IEP) (dry matter basis), %

Nutrients	Contents	
Metabolizable energy, kcal/kg*	5454.9	
Crude protein	31.47	
Crude fat	30.10	
Crude fibre	0.59	
Crude ash	1.99	
Calcium	0.05	
Phosphorus	0.18	

'calculated according to Sibbald et al. (1980); metabolizable energy = 3951 + (54.4 × crude fat) – (88.7 × crude fibre) – (40.8 × crude ash)

# Feeding trial

The birds were acclimatised in a brooder from Day 1 to Day 10, and were offered a commercial diet. Subsequently, they were randomly allocated to the experimental units according to the aforementioned design. From Day 11 onwards (average body weight:  $270.53 \pm 5.83$  g), the birds were fed the experimental diets. Standard broiler management practices was applied with ad libitum access to water and feed. The feeding trial lasted until Day 35. At the end of the treatment, two birds of average body weight were selected per replicate (56 total) for carcass and meat quality determination. The birds were slaughtered following a 12-h fasting period, and carcass traits were measured according to the procedure described by Nasr et al. (2021). Organ weights were recorded and expressed as a percentage of body weight (BW).

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Table 2. Composition of starter and finisher basal diet

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Ingredients	Starter, %	Finisher, %
Yellow maize	52.65	51.50
Soybean meal	40.00	35.20
Rice bran	0.00	6.29
Coconut oil	5.00	5.00
DL-methionine	0.15	0.16
Mineral B <sub>12</sub> <sup>1</sup>	1.80	1.45
Premix <sup>2</sup>	0.20	0.20
NaCl	0.20	0.20
Nutrient contents		
metabolizable energy, kcal/kg	3112.5	3152.1
crude protein, %	21.47	20.01
crude fat, %	8.21	8.42
crude fibre, %	5.53	5.89
crude ash, %	5.84	6.10
calcium, %	1.09	0.90
phosphorus, %	0.43	0.38

 $^{\rm 1}$  contained per kg: g: calcium 480, phosphorus 15; mg: iron 4 000, manganese 2 750, iodine 50, copper 200, zinc 2 500, vitamin B $_{\rm 12}$  0.45; IU: vitamin D $_{\rm 3}$  50 000;  $^{\rm 2}$  contained per kg: IU: vitamin D $_{\rm 3}$  200 000; mg: vitamin E 800, vitamin K 200, vitamin B $_{\rm 1}$  200, vitamin B $_{\rm 5}$  50, vitamin B $_{\rm 12}$  1 200, vitamin C 2 500, Ca-D-pantothenate 600, niacin 4 000, choline chloride 1 000, methionine 3 000, lysine 3 000, manganese 12 000, iron 2 000, iodine 20, zinc 10 000, cobalt 20, copper 400, antioxidant 1 000

# Physical properties and chemical composition determination

The breast meat was subjected to chemical composition analysis (moisture, fat, and crude protein) using a near-infrared (NIR) spectroscopy (Model 78,810; Foss, Hillerød, Denmark) according to AOAC official methods (AOAC International, 2001). The samples were ground, accurately weighed and loaded into the instrument following an established and pre-validated protocol provided by the commercial laboratory. Cooking loss was determined using approx. 2 g of breast meat samples by the plastic bag method (Honikel, 1998). Briefly, the samples were sealed in plastic bags, immersed in a water bath for 1 h at 80 °C, cooled down, blotted dry, and reweighed. Cooking loss was calculated as the percentage difference between initial and final weights.

Water holding capacity (WHC) was evaluated following the method described by Warner (2014). A 0.3 g breast meat sample were pressed on a 125 mm-diameter filter paper at 3 000 × g for 3 min. WHC was calculated using the following formula:

WHC (%) = 
$$[1 - (Wa-Wb) / (Wa \times M)] \times 100$$
;  
ere:  $Wa$  – meat weight before pressing.  $Wb$  – me

where: Wa – meat weight before pressing, Wb – meat weight after pressing, and M – moisture content (g). Meattenderness was tested using a Warner Bratzler shear force device (C-LM3B, Beijing, China) following AMSA (1995) guidelines.

# Fatty acid analysis

The fatty acid (FA) profile of breast meat samples was analysed following the AOAC procedure (AOAC International, 2001). Firstly, lipid hydrolysis was carried out using a mixture of diethyl ether and petroleum ether (1:1 v/v). The crude lipid extract was then evaporated under a nitrogen gas stream in a 50 °C water bath. Subsequently, lipids were transmethylated into fatty acid methyl esters (FAME) using 2 ml of methylation reagent (0.66 N KOH in methanol and 14% methanolic boron trifluoride [BF<sub>2</sub>]) and heated for 50 min at 100 °C with periodic shaking. After cooling to room temperature, 1 ml of hexane and 1 g of anhydrous sodium sulphate (Na<sub>2</sub>SO<sub>4</sub>) were added. The upper hexane layer containing FAME was collected and analysed by a gas chromatography (Agilent Technologies, Santa Clara, California, USA) with an injection volume of 1 µl. The separation was performed on an HP 88 column with the oven temperature initially held at 35 °C for 2 min, then increased to 190 °C at a rate of 12 °C/min for 39 min, held for 10 min, and then increased again at 4 °C/min to 240 °C. Injector and detector temperature were set at 260 °C. FA were identified by comparing retention times with FAME standards (37-component mix, Sigma-Aldrich) and quantified using C13:0 as internal standard, with results expressed as percentage of total FA.

# Amino acid composition

The amino acid profile of breast meat was evaluated following the procedure of Schwarz et al. (2005) using HPLC (LA8080, Hitachi high-technologies, Tokyo, Japan). Briefly, fresh breast meat samples were hydrolysed using 6 N hydrochloric acid for 34 h at 110 °C in sealed bottles to prevent oxidation. After drying, the hydrolysates were dissolved in citrate buffer (pH 2.2) and filtrated prior the analysis. Amino acid separation was performed using a C18 Gemini reverse-phase column (5  $\mu$ m, 110 Å, 150  $\times$  4.6 mm) equipped with a security guard column. The mobile phase consisted of two solutions: (A) 40 mmol Na<sub>2</sub>HPO<sub>4</sub> (pH 7.8) and (B) acetonitrile/methanol/water (45:45:10). A gradient elution program was applied (0:100 at 0 min, 79:21 at 8 min, 45:56 at 20 min, 0:100 at 24 min, and 100:0 at 26 min) with a flow rate of 1 ml/min. The column temperature was set at 40 °C and detection was performed fluorimetrically at 340 nm. A reference mixture of amino acids (Sigma Aldrich, Merck KGaA, Darmstadt, Germany) was used for calibration and quantification.

#### Data analysis

The data were analysed for normality using the Shapiro-Wilk test before being subjected to one-way ANOVA. Dietary treatments were included as fixed effects, while replicates (pen) were considered random effects in the model. Post-hoc comparisons were performed using Duncan's multiple range test, with statistical significance declared at P < 0.05 and tendencies discussed at P < 0.10. All analyses were performed using the *lmer4* package implemented in R software, version 3.5.3 (R Core Team, 2019).

## Results

#### Carcass characteristics

All birds fed IEP-supplemented diets showed significantly higher final body weight, carcass weight, and breast weight compared to those fed the basal diet (P < 0.001; Figure 1). Abdominal fat weight was higher (P = 0.003) in the IEP4 and IEP6 groups compared to CON; however, when expressed as a percentage of body weight (% BW), abdominal fat did not differ (P = 0.370) between dietary treatments. Similarly, IEP inclusion in the diets did not influence the carcass yield percentage (P = 0.831) but tended to affect breast percentage (P = 0.053).

#### Meat quality

Dietary inclusion of IEP at levels ranging from 4 to 8% exerted no effect on the physical quality of broiler chicken meat, including WHC, cooking loss, and tenderness (P > 0.10; Figure 2). Similarly, dietary IEP supplementation up to 8% did not alter moisture or protein content of the meat (P > 0.05). However, a tendency for reduced fat content was observed in IEP-fed birds (P = 0.060; Figure 2). All other meat quality characteristics, including WHC,

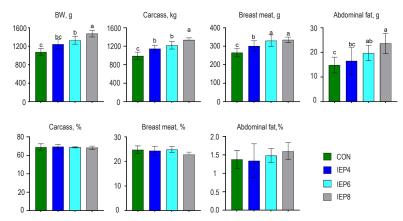


Figure 1. Final body weight, carcass and organ composition of broiler chickens fed diets supplemented with infertile egg powder (IEP) IEP – infertile egg powder; SBM – soybean meal; CON – 100% basal diet; IEP4 – 4% IEP inclusion replacing SBM and maize; IEP6 – 6% IEP inclusion replacing SBM and maize; IEP8 – 8% IEP inclusion replacing SBM and maize. Data are presented as mean value  $\pm$  SEM; SEM – standard error of the mean; ab means within a row with different superscripts are significantly different at P < 0.05

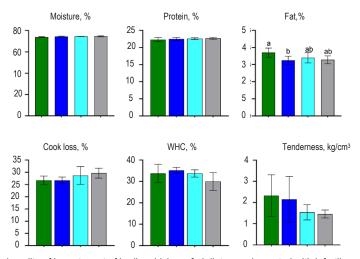


Figure 2. Chemical and physical quality of breast meat of broiler chickens fed diets supplemented with infertile egg powder (IEP)

IEP – infertile egg powder, SBM – soybean meal; CON – 100% basal diet; IEP4 – 4% IEP inclusion replacing SBM and maize; IEP6 – 6% IEP inclusion replacing SBM and maize; IEP8 – 8% IEP inclusion replacing SBM and maize. Data are presented as mean value ± SEM; SEM – standard error of the mean; ab means within a row with different superscripts are significantly different at P < 0.05

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cooking loss, and tenderness were not affected by the dietary treatments (P > 0.10; Figure 2).

# Fatty acid and amino acid profiles of breast meat

The FA composition of broiler breast meat showed significant changes with dietary IEP inclusion (Table 3). IEP supplementation markedly increased stearic acid content (P < 0.001), rising from 0.45% in the CON group to approximately 10% of total fatty acids in IEP-fed groups (4–8% inclusion).

This increase occurred concurrently with a significant reduction in oleic acid content (P < 0.001), decreasing from 38 to 27% of total FA. Notably,  $\alpha$ -linolenic acid levels doubled in breast meat from birds fed IEP4–IEP8 diets compared to controls (P < 0.001).

All birds fed IEP-supplemented diets had higher threonine and arginine concentrations in breast meat compared to those fed the control diet (P < 0.05; Table 4). The IEP4 and IEP6 groups also showed higher glycine and alanine levels in the meat than the

Table 3. Fatty acid composition of broiler breast meat following dietary supplementation with infertile egg powder (IEP)

Variable	Treatment (mean ± standard error of the mean)				Decelor
	CON	IEP4	IEP6	IEP8	— <i>P</i> -value
Caproic acid (C10:0)	0.28 ± 0.11	0.25 ± 0.15	$0.23 \pm 0.08$	0.13 ± 0.12	0.261
Lauric acid (C12:0)	11.14 ± 2.23	$7.53 \pm 3.40$	$6.76 \pm 1.40$	$8.33 \pm 4.64$	0.184
Myristic acid (C14:0)	8.25 ± 2.91	$6.00 \pm 2.45$	6.41 ± 1.95	4.25 ± 1.41	0.084
Palmitic acid (C16:0)	$24.76 \pm 3.38$	$22.02 \pm 6.26$	$24.04 \pm 4.47$	18.98 ± 4.07	0.244
Stearic acid (C18:0)	0.45 ± 0.24 <sup>b</sup>	$9.6 \pm 4.02^{a}$	9.89 ± 2.01a	10.08 ± 1.92 <sup>a</sup>	< 0.001
Oleic acid (C18:1 cis-  ,	37.95 ± 4.02°	27.72 ± 4.84 <sup>b</sup>	27.18 ± 1.17 <sup>b</sup>	26.82 ± 2.84 <sup>b</sup>	< 0.001
Linoleic acid (C18:2 C <sub>9,12</sub> )	$7.70 \pm 1.44$	$6.85 \pm 1.60$	8.33 ± 1.14	7.41 ± 1.26	0.413
α-Linolenic acid (C18:3 C <sub>9.12.15</sub> )	$7.44 \pm 0.55^{\circ}$	14.33 ± 5.73°	14.45 ± 3.69 <sup>a</sup>	13.85 ± 1.70 <sup>a</sup>	< 0.001
MCFA	19.67 ± 2.03 <sup>a</sup>	13.78 ± 1.78 <sup>b</sup>	13.40 ± 1.61 <sup>b</sup>	12.71 ± 1.44 <sup>b</sup>	0.002
LCFA	$78.3 \pm 2.85$	$80.52 \pm 2.07$	$83.89 \pm 2.95$	77.14 ± 3.43	0.213
PUFA	15.14 ± 1.27 <sup>b</sup>	21.18 ± 2.18 <sup>a</sup>	22.78 ± 2.41a	21.26 ± 2.19 <sup>a</sup>	0.027

MCFA – mediumchain fatty acids, LCFA – longchain fatty acids, PUFA – polyunsaturated fatty acids, SBM – soybean meal; CON – 100% basal diet, IEP4 – 4% IEP inclusion replacing SBM and maize, IEP6 – 6% IEP inclusion replacing SBM and maize, IEP8 – 8% IEP inclusion replacing SBM and maize. Data are presented as mean value ± SEM; SEM – standard error of the mean; <sup>ab</sup> means within a row with different superscripts are significantly different at *P* < 0.05

Table 4. Amino acid composition of broiler breast meat following dietary supplementation with infertile egg powder (IEP)

	•	•	•		,	
Variable		Treatment (mean ± standard error of the mean)			- <i>P</i> -value	
variable	CON	IEP4	IEP6	IEP8	r-value	
Aspartic acid	$1.20\pm0.09$	$1.25\pm0.04$	$1.25\pm0.05$	$1.15\pm0.12$	0.213	
Glutamic acid	$2.08\pm0.08^{\text{b}}$	$2.08\pm0.09^{\text{b}}$	$2.26\pm0.04^{\text{a}}$	$2.12\pm0.09^{\text{b}}$	0.006	
Serine	$0.65 \pm 0.13$	$0.60 \pm 0.06$	$0.60\pm0.08$	$0.63\pm0.09$	0.372	
Glycine	$0.59\pm0.19^{\text{b}}$	$0.61\pm0.05^{\text{b}}$	$0.78\pm0.07^{\text{a}}$	$0.79\pm0.04^{\text{a}}$	0.009	
Alanine	$0.81\pm0.19^{b}$	$0.83 \pm 0.04^{\text{b}}$	$1.01\pm0.10^{a}$	$0.99 \pm 0.06^{a}$	0.022	
Proline	$0.66 \pm 0.24$	$0.76 \pm 0.04$	$0.84 \pm 0.05$	$0.80 \pm 0.05$	0.188	
Tyrosine	$0.61\pm0.09$	$0.61 \pm 0.05$	$0.69 \pm 0.06$	$0.62 \pm 0.04$	0.194	
Cysteine	$0.48\pm0.22^{\text{ab}}$	$0.35\pm0.03^{\text{b}}$	$0.52\pm0.08^{\text{a}}$	$0.60\pm0.05^{\text{a}}$	0.031	
NEAA	$7.08 \pm 0.96$	$7.09 \pm 0.99$	$7.95\pm1.12$	$7.70 \pm 0.87$	0.051	
Arginine	$0.91 \pm 0.30$	$0.95 \pm 0.03$	$1.11\pm0.13$	$1.09 \pm 0.07$	0.192	
Threonine	$0.58\pm0.10^{\text{c}}$	$0.69 \pm 0.03^{\text{b}}$	$0.79\pm0.04^{\text{a}}$	$0.71\pm0.03^{\text{ab}}$	<0.001	
Valine	$0.82 \pm 0.09^{\text{c}}$	$0.96\pm0.04^{\text{b}}$	$1.19\pm0.06^{a}$	$1.04\pm0.07^{\text{b}}$	<0.001	
Histidine	$0.54 \pm 0.30$	$0.50\pm0.06$	$0.64 \pm 0.04$	$0.70 \pm 0.04$	0.182	
Methionine	$0.44\pm0.05^{\text{b}}$	$0.55\pm0.12^{a}$	$0.60\pm0.07^{\text{a}}$	$0.60\pm0.03^{\text{a}}$	0.011	
Isoleucine	$0.78\pm0.07^{a}$	$0.67\pm0.05^{\text{b}}$	$0.71\pm0.03^{\text{ab}}$	$0.73\pm0.04^{\text{ab}}$	0.022	
Leucine	$1.00\pm0.06$	$0.88 \pm 0.04$	$1.00 \pm 0.13$	$0.97\pm0.11$	0.166	
Phenylalanine	$0.53\pm0.03^{\text{b}}$	$0.58\pm0.06^{\text{b}}$	$0.58\pm0.05^{\text{b}}$	$0.69\pm0.07^{\text{a}}$	0.002	
Lysine	$0.97 \pm 0.17$	$1.21 \pm 0.40$	$1.10\pm0.11$	$1.08 \pm 0.13$	0.470	
EAA	$6.57\pm0.94^{\text{b}}$	$6.99 \pm 1.05^{\text{ab}}$	$7.72\pm1.03^{\text{a}}$	$7.61\pm1.12^{\text{a}}$	0.042	
TAA	$13.65\pm1.45^{\text{b}}$	$14.08\pm2.0^{\text{b}}$	$15.67\pm1.72^{a}$	$15.31\pm1.64^{\text{a}}$	0.044	

NEAA – non-essential amino acids, EAA – essential amino acids, TAA – total amino acids, IEP – infertile egg powder; SBM – soybean meal; CON – 100% basal diet; IEP4 – 4% IEP inclusion replacing SBM and maize; IEP6 – 6% IEP inclusion replacing SBM and maize; IEP8 – 8% IEP inclusion replacing SBM and maize. Data are presented as mean value  $\pm$  SEM; SEM – standard error of the mean; ab means within a row with different superscripts are significantly different at P < 0.05

CON group (P < 0.05). Among birds receiving IEP-containing diets, only the IEP4 group had a higher glutamic acid concentration compared to the CON group (P < 0.05). Conversely, all IEP-supplemented groups showed reduced phenylalanine levels versus CON (P < 0.05; Table 4). The IEP4 and IEP6 treatments decreased serine content (P < 0.05), with IEP4 additionally lowering methionine and isoleucine concentrations in breast meat (P < 0.05).

# **Discussion**

The present study demonstrated that IEP supplementation could improve carcass yield and quality. The incorporation of 4-8% IEP may have contributed to a higher content of soluble, readily digestible proteins, consistent with the known characteristics of egg protein (Lei and Kim, 2013; Choi et al., 2021). The findings showed that the highest level of IEP (8%) supplementation resulted in the greatest body weight, carcass weight, and abdominal fat compared with both the lower IEP inclusion groups and the control. The improved performance observed in birds fed the IEP8 diet may be attributed to the increased digestible protein content of that formulation. Although few studies have explored the use of hatchery by-product such as IEP, the beneficial effects of highly digestible protein sources in broiler diets have been reported. For instance, inclusion of soybean protein concentrate – a highly digestible protein – has been shown to improve broiler performance (Kiarie et al., 2021). The highly digestible protein content in IEP may have enhanced amino acid availability and absorption in the present study. This hypothesis is supported by established literature demonstrating that digestible proteins improve intestinal morphology and organic acid production (Zhang et al., 2022), potentially explaining the enhanced growth performance. Future studies should directly measure amino acid digestibility to confirm this mechanism. The current findings position IEP as a promising alternative protein source that can optimise broiler production outcomes.

The increase in the level of dietary protein contributed to improved growth performance and final body weight in broiler chickens. Ghahri et al. (2010) reported that varying crude protein levels from 18 to 22% significantly affected body weight gain, although no differences were observed in carcass or breast weights. However, increases in dietary lysine were associated with improvements in carcass and breast weight, suggesting that the amino acid composition of the diet plays a key role in deter-

mining growth and carcass traits. In the current study, increased body weight was accompanied by higher carcass and breast weights, likely due to the amino acid profile of the diets, particularly essential amino acids such as lysine and methionine (Beski et al., 2015). The increased abdominal fat deposition observed in IEP-fed birds aligns with findings by Fouad and El-Senousey (2014), who noted that fat accumulation in birds depends on the availability of lipid substrates in the plasma, derived either from dietary source or *de novo* lipogenesis in the liver. These observations underscore the influence of both the source and level of dietary lipids on overall body fat deposition in poultry.

The increase in the abdominal fat observed in chickens fed diets containing IEP may be attributed to the higher total fat and increased levels of saturated fats resulting from IEP inclusion at levels up to 8%. Sanz et al. (2000) reported that the abdominal fat content was significantly lower in chickens fed diets containing sunflower oil (unsaturated fats) compared to those fed tallow or lard (saturated fats). Similarly, a recent study conducted by Shahryari et al. (2021) showed that increasing the proportion of saturated fatty acids in the diet, even when using sunflower oil, led to greater abdominal fat deposition in broilers. Another possible explanation is the higher ME content of the IEP-containing diets (Table 3), consistent with previous reports showing similar results (Rosa et al., 2007; Shahryari et al., 2021; Tabeidian et al., 2010).

In terms of meat quality, the fat content of meat appeared to increase, as also reflected in the rise in abdominal fat following dietary IEP inclusion. This could be due to the higher ME and fat content of the IEP-supplemented diets. Dietary energy exceeding requirements for growth and maintenance is primarily stored as adipose tissue, IEP supplementation appears to shift nutrient partitioning toward fat deposition. This aligns with established findings that broiler body composition closely reflects dietary fatty acid profiles (Pietras et al., 2021; Shahryari et al., 2021). In this study, cooking loss was not affected by the addition of IEP into the diets, which aligned with findings of Watanabe et al. (2018), who reported no significant correlation between the content of intramuscular fat and meat cooking loss. These results contradict some reports of fat content negatively correlating with cooking loss (Cannata et al., 2010), suggesting that IEP unique composition may differentially influence meat quality characteristics. The present study demonstrated that feeding diets containing up to 8%

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IEP also did not alter meat WHC, which averaged approx. 33%, indicating no clear relationship between fat content and WHC, as reported by Watanabe et al. (2018). The collective findings demonstrate that IEP can be incorporated at 4–8% inclusion rates without compromising standard meat quality parameters.

The inclusion of IEP in broiler feed significantly altered the FA composition of breast meat, producing a 21.3-22.4 fold increase in stearic acid content compared to the control diet. This was the reason for the decrease in monounsaturated fatty acid (MUFA) accumulation. Importantly, IEP supplementation also resulted in a two-fold increase in  $\alpha$ -linolenic acid levels, an essential omega-3 polyunsaturated fatty acid known for its health benefits, including improved metabolic function and cardiovascular protection (Fedor and Kelley, 2009). As a precursor for longer-chain omega-3 fatty acids (Irawan et al., 2022), the elevated  $\alpha$ -linolenic acid levels suggest that IEP supplementation may support the production of nutritionally improved poultry meat.

The results presented in this study indicate a major change in the FA profile of broiler meat induced by the IEP-enriched feed, characterised increased saturated and polyunsaturated fatty acids (SFA and PUFA) and a concurrent reduction in MUFA. This clearly demonstrates a direct relationship between dietary FA composition and subsequent fatty acid deposition in chicken meat, supporting the well-documented principle that modifying dietary fat profiles can effectively manipulate meat FA synthesis and deposition (Fébel et al., 2008; Zelenka et al., 2008; Kartikasari et al., 2012). While these results align with established knowledge about dietary fat manipulation in poultry nutrition, the present study represents the first investigation specifically examining the effects of IEP on broiler meat fatty acid profiles. Therefore, further research should be conducted to elucidate the mechanisms by which infertile egg powder influences meat quality.

The study found that IEP supplementation significantly increased certain amino acids in broiler meat compared to the control group. Threonine content rose by 19.0–36.2% and arginine by 63.8–91.4% in IEP-fed birds. The IEP8 group showed particularly elevated glycine (36.2% higher) and alanine (74.1% higher) levels. In contrast, isoleucine content decreased by 39.7% in the IEP4 group. These changes in free amino acids may influence meat flavour development through Maillard reactions, as previously reported by Rabie et al. (2009). However, the overall impact on meat protein concentration remained nonsignificant, indicating that while IEP modifies spe-

cific amino acid levels, it does not substantially alter the fundamental protein composition of the meat.

#### Conclusions

In conclusion, this study demonstrates that infertile egg powder (IEP) is a suitable alternative protein source for broiler chickens, effectively improving final body weight and carcass yield. The essential and functional amino acids, as well as fatty acids present in infertile eggs contribute to increased α-linolenic acid levels – a valuable omega-3 precursor. While IEP supplementation elevated certain amino acids, it did not substantially alter the overall amino acid profile of the meat. These findings support the practical application of IEP in broiler diets, confirming its potential as a sustainable feed ingredient that maintains meat quality standards. The results indicate that IEP can be successfully incorporated into poultry feeding programs without negatively impacting product quality.

## **Conflict of interest**

The Authors declare that there is no conflict of interest.

#### References

- Abiola S.S., Onun Kwor E.K., 2004. Replacement value of hatchery waste meal for fish meal in layer diets. Bioresour. Technol. 95, 103–106, https://doi.org/10.1016/j.biortech.2004.02.001
- AOAC International, 2001. Official Methods of Analysis, 18th ed. Association of Official Analytical Chemistry. Arlington (USA).
- Beski S.S.M., Swick R.A., Iji P.A., 2015. Specialized protein products in broiler chicken nutrition: A review. Anim. Nutr. 1(2), 47–53, https://doi.org/10.1016/j.aninu.2015.05.005
- Cannata S., Engle T.E., Moeller S.J., Zerby H.N., Radunz A.E., Green M.D., Bass P.D., Belk K.E., 2010. Effect of visual marbling on sensory properties and quality traits of pork loin. Meat Sci. 85, 428–434, https://doi.org/10.1016/j.meatsci.2010.02.011
- Choi W.J., Kim J.H., Han G.P., Kwon C.H., Kil D.Y., 2021. Effects of dietary hatchery by-products on growth performance, relative organ weight, plasma measurements, immune organ index, meat quality, and tibia characteristics of broiler chickens. Anim. Biosci. 34, 1181–1192, https://doi.org/10.5713/ab.20.0755
- Dewi Y.L., Sofyan A., Herdian H., Sakti A.A., Irawan A., Jasmadi J., Anggraeni A.S., Mardawati E., Adriyanto A., et al., 2024. Processing technology to improve seaweed nutritional quality as a feed for poultry: a review and its implementation. Worlds Poult. Sci. J. 80, 207–235, https://doi.org/10.1080/00439339.2023.2270952
- Esmailzadeh L., Shivazad M., Sadeghi A.A., Karimitorshizi M., 2016.

  Performance, intestinal morphology and microbiology of broiler chickens fed egg powder in the starter diet. Rev. Bras. Cienc. Avic. 18, 705–710, https://doi.org/10.1590/1806-9061-2016-0330

- Fébel H., Mézes M., Pálfy, T., Hermán A., Gundel J., Lugasi A., Balogh K., Kocsis I., Blázovics A., 2008. Effect of dietary fatty acid pattern on growth, body fat composition and antioxidant parameters in broilers. J. Anim. Physiol. Anim. Nutr. 92, 369–376, https://doi.org/10.1111/j.1439-0396.2008.00803.x
- Fedor D., Kelley D.S., 2009. Prevention of insulin resistance by n-3 polyunsaturated fatty acids. Curr. Opin. Clin. Nutr. Metab. Care. 12, 138–146, https://doi.org/10.1097/ MCO.0b013e3283218299
- Fouad A.M., El-Senousey H.K., 2014. Nutritional factors affecting abdominal fat deposition in poultry: A review. Asian-Australas. J. Anim. Sci. 27, 1057–1068, https://doi.org/10.5713/ajas.2013.13702
- Ghahri H., Gaykani R., Toloie T., 2010. Effect of dietary crude protein level on performance and lysine requirements of male broiler chickens. Afr. J. Agric. Res. 5, 1228–1234, https://doi.org/10.5897/AJAR10.058
- Honikel K.O., 1998. Reference methods for the assessment of physical characteristics of meat. Meat. Sci. 49, 447–457, https://doi.org/10.1016/S0309-1740(98)00034-5
- Irawan A., Ningsih N., Hafizuddin, Rusli R.K., Suprayogi W.P.S., Akhirini N., Hadi R.F., Setyono W., Jayanegara A., 2022. Supplementary n-3 fatty acids sources on performance and formation of omega-3 in egg of laying hens: A meta-analysis. Poult. Sci. 101, 101566, https://doi.org/10.1016/j.psj.2021.101566
- Irawan A., Ratriyanto A., Respati A.N., Ningsih N., Fitriastuti R., Suprayogi W.P.S., Hadi R.F., Setyono W., Akhirini N., Jayanegara A., 2022. Effect of feeding fermented soybean meal on broiler chickens' performance: A meta-analysis. Anim. Biosci. 35, 1881–1891, https://doi.org/10.5713/ab.21.0546
- Kartikasari L.R., Hughes R.J., Geier M.S., Makrides M., Gibson R.A., 2012. Dietary alpha-linolenic acid enhances omega-3 long chain polyunsaturated fatty acid levels in chicken tissues. Prostaglandins Leukot. Essent. Fatty Acids 87, 103–109, https://doi.org/10.1016/j.plefa.2012.07.005
- Kiarie E.G., Mohammadigheisar M., Kakhki R.A.M., Madsen M.H., 2021. Impact of feeding modified soy protein concentrate in the starter phase on growth performance and gastrointestinal responses in broiler chickens through to day 42 of age. Poult. Sci. 100, 101147, https://doi.org/10.1016/j. psj.2021.101147
- Lei Y., Kim I.H., 2013. Effect of whole egg powder on growth performance, blood cell counts, nutrient digestibility, relative organ weights, and meat quality in broiler chickens. Livest. Sci. 158, 124–128, https://doi.org/10.1016/j.livsci.2013.10.018
- Nasr M.A.F., Alkhedaide A.Q., Ramadan A.A.I., Hafez A.-E.S.E., Hussein M.A., 2021. Potential impact of stocking density on growth, carcass traits, indicators of biochemical and oxidative stress and meat quality of different broiler breeds. Poult. Sci. 100, 101442, https://doi.org/10.1016/j.psj.2021.101442
- Noy Y., Sklan D., 2002. Nutrient Use in Chicks During the First Week Posthatch. Poult. Sci. 81, 391–399, https://doi.org/10.1093/ ps/81.3.391
- Pietras M., Orczewska-Dudek S., Szczurek W., Pieszka M., 2021. Effect of dietary lupine seeds (Lupinus luteus L.) and different insect larvae meals as protein sources in broiler chicken diet on growth performance, carcass, and meat quality. Livest. Sci. 250, 104537, https://doi.org/10.1016/j.livsci.2021.104537

- R Core Team, 2019. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna (Austria)
- Rabie M., Simon-Sarkadi L., Siliha H., El-seedy S., El Badawy A.A., 2009. Changes in free amino acids and biogenic amines of Egyptian salted-fermented fish (Feseekh) during ripening and storage. Food Chem. 115, 635–638, https://doi.org/10.1016/j. foodchem.2008.12.077
- Ratriyanto A., Pratitis S.S.W., Atikah R., 2020. Infertile egg powder as a potential feedstuff for starter broilers. IOP Conf. Ser. Earth Environ. Sci. 518, 012005, https://doi.org/10.1088/1755-1315/518/1/012005
- Ratriyanto A., Suprayogi W.S.P., Lestari A.P., Riandari S.F., Akbar A., 2021. Utilization of infertile egg powder in ration improves the digestive tract development of broiler chickens. IOP Conf. Ser. Earth Environ. Sci. 637, 012069, https://doi.org/10.1088/1755-1315/637/1/012069
- Rosa P.S., Faria F.D.E., Dahlke F., Vieira B.S., Macari M., Furlan R.L., 2007. Performance and carcass characteristics of broiler chickens with different growth potential and submitted to heat stress. Rev. Bras. Cienc. Avic. 9, 181–186, https://doi. org/10.1590/S1516-635X2007000300007
- Sanz M., Flores A., Lopez-Bote C.J., 2000. The metabolic use of energy from dietary fat in broilers is affected by fatty acid saturation. Br. Poult. Sci. 41, 61–68, https://doi. org/10.1080/00071660086411
- Schwarz E.L., Roberts W.L., Pasquali M., 2005. Analysis of plasma amino acids by HPLC with photodiode array and fluorescence detection. Clinica Chimica Acta 354, 83–90, https://doi.org/10.1016/j.cccn.2004.11.016
- Shahryari M., Tabeidian S.A., Foroozandeh S.A.D., Tabatabaei S.N., Toghyani M., Forouzmand M., Habibian M., 2021. Using soybean acid oil or its calcium salt as the energy source for broiler chickens: Effects on growth performance, carcass traits, intestinal morphology, nutrient digestibility, and immune responses. Anim. Feed Sci. Technol. 276, 114919, https://doi.org/10.1016/j.anifeedsci.2021.114919
- Tabeidian S.A., Ghafoori M., Bahrami Y., Chekani-Azar S., Toghyani M., 2010. Effect of different levels of dietary fat on broiler performance and production cost with emphasis on calcium and phosphorus absorption. Glob Vet 5, 54–60
- Warner R., 2014. Measurements of Water-holding Capacity and Color: Objective and Subjective. Encycl. Meat Sci. 2, 164–171, https://doi.org/10.1016/B978-0-12-384731-7.00210-5
- Watanabe G., Motoyama M., Nakajima I., Sasaki K., 2018. Relationship between water-holding capacity and intramuscular fat content in Japanese commercial pork loin. Asian-Australas. J. Anim. Sci. 31, 914–918, https://doi.org/10.5713/ajas.17.0640
- Zelenka J., Schneiderova D., Mrkvicova E., Dolezal, P., 2008. The effect of dietary linseed oils with different fatty acid pattern on the content of fatty acids in chicken meat. Vet. Med. 53, 77–85, https://doi.org/10.17221/1985-VETMED
- Zhang Q., Zhang S., Wu, S., Madsen M.H., Shi S., 2022. Supplementing the early diet of broilers with soy protein concentrate can improve intestinal development and enhance short-chain fatty acid-producing microbes and short-chain fatty acids, especially butyric acid. J. Anim. Sci. Biotechnol. 13, 97, https://doi.org/10.1186/s40104-022-00749-5