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RESEARCH ARTICLE

Evaluation of blow fly, *Chrysomya megacephala* (Calliphoridae: Diptera) as an alternate source of protein in broiler feed

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Abstract

Poultry industry is one of the fastest growing agri-businesses in the world. However, the usage of expensive soybean meal and fishmeal as poultry feed ingredients is making it less profitable and environmentally unsustainable. Insects are becoming more popular as an alternate protein source in poultry feed because they are more sustainable, cost-efficient and eco-friendly than most of the conventional poultry feed sources. In the present study, we replaced 4%, 8% and 12% soybean meal with blow fly, *Chrysomya megacephala* (Calliphoridae: Diptera) larvae and evaluated its impact on growth performances, hematological parameters, intestinal morphometry and meat quality of Ross 308 broiler. For comparison, we also evaluated commercially available full fat *Hermetia illucens* (Stratiomyidae: Diptera) larvae. Three hundred and fifty 1-day-old chicks with initial weight of 40.28 g/chick were randomly divided into seven experimental diets (5 pens per treatment and 10 birds per pen). All the dietary treatments were isocaloric and isonitrogenous, meeting the nutrient requirements of the broilers. The results revealed that the broiler fed on 12% *C. megacephala* or *H. illucens* had significantly higher ($P < 0.001$) live weight, average daily weight gain and better feed conversion ratios. The diets containing 12% *C. megacephala* or *H. illucens* significantly improved the blood haematology and serum bio-chemistry in the broiler. The gut histological indices of jejunum and ileum such as villus height (Vh), crypt depth (Cd), villus width (Vw), and Vh/Cd ratios also improved by the feeding of 12% *C. megacephala* and *H. illucens* diets. The broiler fed on 12% *H. illucens* diet showed higher L^* (lightness) and a^* (redness), while those fed on 12% *C. megacephala* showed higher b^* (yellowness) in breast meat (*Pectoralis major*). Both species performed equally well in growth performance, haematology, serum bio-chemistry, gut histology and meat quality. It was concluded that replacement of soybean meal with 12% *C. megacephala* or *H. illucens* larvae improves the growth performance, blood haematology, gut histology and meat color and lightness traits of broiler and thus can be used as an alternate source of protein in broiler feed.

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Keywords

Chrysomya megacephala – *Hermetia illucens* – soybean meal – growth performance – health status – broiler

1 Introduction

Insects have gained much attention in recent decades as a potential food and feed source to combat the rising issues of human and livestock food insecurity (Heuel *et al.*, 2022). Insects are the important component of the diets of wild birds and free-range poultry chicks (Cullere *et al.*, 2018). Their potential as a valuable alternative food source stems from their higher protein content (30-45%), well-balanced nutritional profile (35% fats, 5-8% calcium and 0.6-1.5% phosphorus) and efficient mass conversion ratios (Van Huis and Oonincx, 2017; Gasco *et al.*, 2020; Lu *et al.*, 2022). The chitin contents in insect exoskeleton fluctuate from 5.9% to 8.7% and contain hypocholesterolemic and antioxidant that have positive impacts on human and animal health (Cutrignelli *et al.*, 2018). Moreover, the prebiotic qualities of chitin improve the immune system in chicken besides preventing the development of Gram-negative bacteria in the large intestine (Ngo and Kim, 2014; Biasato *et al.*, 2018).

The black soldier fly BSF (*Hermetia illucens*) (Diptera: Stratiomyidae) and yellow mealworm (*Tenebrio molitor*) (Coleoptera: Tenebrionidae) are the most frequently used insects in poultry feed (Diener *et al.*, 2011; Parry and Weldon, 2023). Both have exhibited promising results as an alternate of soybean meal (SBM) in poultry feed and significantly enhance the growth and quality of meat (Yu *et al.*, 2019; Vasilopoulos *et al.*, 2023). Moreover, utilization of insects as poultry feed can substantially reduce the environmental burden of bio-waste stream as they can be successfully transformed into high-quality protein sources (Ewusie *et al.*, 2018; Smetana *et al.*, 2019; Van Huis, 2020; Gold *et al.*, 2021). Their utilization in poultry feed is environmentally more sustainable than the conventional soybean meal SBM and fish meal (FM) (Smetana *et al.*, 2016). This shift has also mitigated the sole reliance of poultry growers on SBM and FM (Varelas, 2019; Tran *et al.*, 2024).

Poultry meat is rich in proteins (up to 34.5%) and micronutrients while low in fat, making it a nutrient-dense food (Pereira and Vicente, 2013). Moreover, it plays a crucial role in human nutrition by providing substantial amount of polyunsaturated fatty acids (PUFA) and essential amino acids leading to high digestibility and protein efficacy ratio (PER) (Cartoni Mancinelli *et al.*, 2022). The demand of poultry meat is continuously increasing with the increase in global population, but its price is directly influenced by feed prices. SBM has long been used as a commercial diet for poultry (Dittoe *et al.*, 2019) however, the rising cost of production, as well as the associated greenhouse gas emissions and water pollution, makes it a contentious feed material (Karlen *et al.*, 2012; Ahiwe *et al.*, 2018). Therefore, small and commercial farmers in low-income countries can utilize insects as a potential alternative to SBM for poultry production (Fruci *et al.*, 2023).

Insect farming is a new business venture that can improve food security and income by providing a less expensive protein source for poultry industry, lowering food-feed competition, and generating job opportunities for the small farmers (Veldkamp and Bosch, 2015). However, utilization of a certain insect species in poultry feed depends on local conditions, production scale and economic considerations (Savoldelli *et al.*, 2020; Chaix-Bar *et al.*, 2023). Presently, exotic insect species from tropical regions (like BSF) are being employed in the manufacturing of poultry feed, which necessitates a large budget to establish an environment that is favorable for their mass reproduction (Lourenço *et al.*, 2022). Native insect species perform better under local conditions and may also have high fecundity, quick growth, and good nutritional value. They may also require less capital for mass multiplication and therefore their commercial viability must be explored (Hanboonsong *et al.*, 2013).

Dipterans in general have a quick life cycle with a short larval stage. Their rapid growth and reproduction contribute to efficient mass production, providing a consistent and abundant supply of biomass (Cammack and Tomberlin, 2017). Many Dipterans are detritivores and consume decaying organic matter. This makes them a valuable contributor towards sustainable and circular agricultural system (Hore and Banerjee, 2017). Blow flies can be very important candidate in this regard due to their ability to efficiently convert bio-wastes into useful biomass, their short lifecycle, high fecundity rate and self-harvest due to wandering behavior before pupation (Richards *et al.*, 2009a; Parry *et al.*, 2017). Therefore, their rapid processing of organic waste minimizes the chances of pathogen survival, making the resulting larvae safer for use in poultry feed (Richards *et al.*, 2009b; Joosten *et al.*, 2020). Therefore, exploring the potential

of locally available insect species as a protein source in the poultry sector is thus a pressing need for sustaining poultry birds' productivity. To the best of our knowledge, *Chrysomya megacephala* has already been studied for replacement of FM, but not previous attempts have been made to replace the SBM. Therefore, in the present study, we evaluated the effects of replacing SBM with native *Chrysomya megacephala* on growth performances, blood parameters, gut histology, and meat quality in broiler chickens. For the comparison, we evaluated commercially used *H. illucens*.

2 Materials and methods

Ethics approval

The chicken handling and utilization was in accordance with the regulation of Pakistan accepted by the Ethical Review Committee (NO. DR/495), University of Veterinary and Animal Sciences (UVAS), Lahore, Pakistan.

Birds and housing conditions

The feeding trials were conducted at Avian Research and Training Centre (ARTC), UVAS, Lahore. One day old Ross 308 broiler chicks (n = 350) were purchased from A.W. Breeders, Lahore, Pakistan. These birds belonged to the same parental group and vaccinated against infectious diseases at the hatchery. The chicks were randomly assigned to seven dietary treatments (5 pens/treatment and 10 birds/pen) and raised for 35 days. The seven dietary treatments with increasing replacement levels included 4%, 8% and 12% of SBM with blow fly *C. megacephala* and BSF *H. illucens* (labeled as CM4, CM8 and CM12 for blow fly and HI4, HI8 and HI12 for BSF) and a control treatment of basal diet for the comparison. Rice hull was used as a bedding material in pens, the birds were fed and watered *ad libitum* during feeding weeks. The bedding material was properly raked during the experimental weeks. The shed temperature was maintained at 34 °C for 5 days and gradually reduced by 2 °C/week to 26 °C, which was maintained until the end of the experiment. Artificial lighting was provided at a pattern of 18 h of light alternating with six hours of darkness. The broiler chicks were vaccinated against Newcastle disease and infectious bronchitis by spraying in the hatchery. The health status and mortality were visually observed daily during whole feeding intervals.

Formulation of diets

Blow flies were reared on meat wastes and BSF on fruit wastes at the insect rearing laboratory of the Department of Entomology, The Islamia University of Bahawalpur, Pakistan. Fully grown blow fly and BSF larvae were dried at 70 °C for 24 hours and ground to a meal (powder) for chemical analysis and feed formulation. The experimental ration was mixed in mash form for feed formulation in a feed mill unit at the Department Animal Nutrition, UVAS, Lahore, Pakistan. All these diets were isonitrogenous and isocaloric. The ingredients of experimental diets are presented in Table 1.

Chemical analyses

Before the formulation of diets, insect meals were subjected to preliminary analysis for proximate analysis using the Official Analytical Chemists International methods (AOAC, 2005). The amino acid profiles of *C. megacephala* and *H. illucens* and SBM were analyzed by LC-4500 Compact HPLC System JASCO (Alikwe *et al.*, 2010). After feed formulations, proximate analyses of all the experimental diets were performed to determine the dry matter, crude protein, crude fiber and fat contents (AOAC, 2005). Mineral contents of diets were also determined through atomic absorption spectrometry (STA-4800 Spectrophotometer, Stalwart Analytics). The concentration of metabolizable energy in *C. megacephala* and *H. illucens* meals as well as GM-SBM was calculated from chemical profiles and the regression equation as defined in the Nutrient Requirements of Poultry (INRA, 2004). The nutrient contents, minerals and ambient metabolize energy per kg of the remaining feed components contained in experimental diets was estimated based on tabular data from the Nutrient Requirements of Poultry. The nutritional value of diets was determined from analyses results and tabular data from the Nutrient Requirements of Poultry. All experimental diets fulfilled the nutrient requirements of broiler as given in the Nutrient Requirements of Poultry (INRA, 2004). The chemical composition of the experimental diets is detailed in Table 2.

Growth performances

Effect of experimental diets on the productive performances was assessed as described by Gariglio *et al.* (2019). The birds live weight (LW) of individual bird was recorded at the beginning and at week (wk) 1 to 5th. Average daily weight gain (ADG) and daily feed intake (DFI) of individual bird were estimated at an individual level at the end of the growth period. Feed conversion ratio (FCR) was calculated for each growth interval (wk 1 to 5) as well as for the overall experimental period.

TABLE 1 Ingredients of the experimental diets (% as fed)

Ingredients	Experimental diets						
	Cont.	HI4	HI8	HI12	CM4	CM8	CM12
Corn, grain	47.50	47.50	52.50	58.50	48.70	52.50	58.50
Wheat bran	10.08	9.57	5.79	2.86	9.00	5.48	2.56
Rice polishing	5.00	5.00	5.00	2.00	5.00	5.00	2.00
Oil soybean	2.50	2.00	1.00	0.60	2.50	2.20	2.00
Soybean meal	20.00	17.00	13.50	11.00	16.00	12.50	10.00
Canola meal	8.00	9.00	9.00	9.00	9.00	9.00	9.00
Fish meal	5.00	4.00	3.50	2.50	4.00	3.50	2.50
HI meals	–	4.00	8.00	12.00	–	–	–
CM meals	–	–	–	–	4.00	8.00	12.00
L-Lysine HCl	0.08	0.09	0.09	0.12	–	–	–
DL-methionine	0.07	0.06	0.04	0.03	0.10	0.12	0.14
Threionine	0.07	0.08	0.08	0.09	–	–	–
Common salt	0.40	0.40	0.40	0.40	0.40	0.40	0.40
Limestone	1.20	1.20	1.00	0.80	1.20	1.20	0.80
Micro Min Premix	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Vitamin Premix	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Total	100.00	100.00	100.00	100.00	100.00	100.00	100.00

Minerals-Vitamin Premix supplied per kg of diet (formulated and mixed at feed manufacturing site): copper = 16 mg; iodine = 1.25 mg; iron = 20 mg; manganese = 120 mg; selenium = 0.30 mg; zinc = 110 mg; vitamin A = 12,000 IU; vitamin D3 = 5000 IU; vitamin E = 80 IU; vitamin K = 3.2 mg; thiamine = 3.2 mg; riboflavin = 8.6 mg; niacin = 65 mg; pantothenic acid = 20 mg; pyridoxine = 4.3 mg; biotin = 0.22 mg; folic acid = 2.20 mg; vitamin B12 = 0.017 mg.

TABLE 2 Nutrient composition and energy contents of the experimental diets

Chemical composition	Experimental diets						
	Control	HI4	HI8	HI12	CM4	CM8	CM12
Dry matter (% DM)	89.40	89.30	89.50	89.40	89.30	89.40	89.40
[1]Crude protein (% DM)	22.23	22.22	22.24	22.21	22.18	22.19	22.20
Ether extract (% DM)	5.76	5.98	6.03	6.08	4.74	5.69	5.78
Crude fiber (% DM)	4.92	5.01	4.98	4.96	5.00	4.94	4.88
Crude ash (% DM)	3.62	3.85	3.96	3.80	3.82	3.80	3.69
Nitrogen free extract (% DM)	62.35	61.23	61.60	62.18	61.88	61.92	63.00
Calcium (% DM)	0.75	0.77	0.78	0.74	0.77	0.78	0.74
Available phosphorus (% DM)	0.60	0.61	0.59	0.54	0.61	0.59	0.54
Gross energy (kcal/kg)	4,606	4,593	4,598	4,601	4,595	4,602	4,592
Metabolizable energy (kcal/kg)	2,857	2,843	2,849	2,851	2,843	2,849	2,852
Lysine	1.24	1.25	1.24	1.25	1.25	1.24	1.25
Methionine	0.47	0.47	0.47	0.47	0.47	0.47	0.47
Threonine	0.92	0.92	0.92	0.92	0.92	0.92	0.92
Valine	1.18	1.18	1.19	1.19	1.18	1.19	1.19
Arginine	1.30	1.30	1.30	1.30	1.30	1.30	1.30
Leucine	1.77	1.78	1.77	1.77	1.78	1.77	1.77
Isoleucine	0.90	0.91	0.92	0.92	0.91	0.92	0.92

[1]Nitrogen-to-protein conversion factor for insect diets and other ingredients was 4.76 or 6.25 as described by Bovera *et al.* (2015) and Janssen *et al.* (2017).

Slaughtering procedures

At day 35, 10 birds per treatment were randomly selected on the basis of the average final body weight and slaughtered by following the Halal Islamic method with sharp knife. Carcasses were de-feathered and eviscerated after the removal of head. The body weight of the bird was determined before slaughter, while the carcass weight was determined after bleeding and plucking using an electronic scale of 0.01 g accuracy level. Breast muscles (*Pectoralis major – PM*) were deboned, packed in string polyethylene bags and chilled at 4 °C for 24 h for the assessment of meat quality parameters by following Ab Aziz *et al*. (2020).

Haematology and serum bio-chemistry

Two birds per replicate were used to determine haematology, 2.5 mL blood was collected at slaughter in EDTA and serum-separating tubes. Blood smears were prepared from drops of blood without anticoagulant for complete blood count (CBC) (Campbell, 1995). The red (erythrocytes) and white (leukocytes) cell counts in the blood were determined and the tubes without anticoagulant were permitted to clot in a standing position at room temperature for two hours. The serum was separated by centrifugation at 700 *g* for 15 minutes and the creatinine, glucose, cholesterol, total protein (T. protein), albumin (Alb), globulin (Glob), and uric acid levels were determined using Automatic analyzer (Microlab 300 Semi-Automatic Chemistry Analyzer) (Germana *et al*., 2010). All of these tests were performed at the University Diagnostic Lab (UDL), UVAS, Lahore, Pakistan.

Gut histology

The gut histology of the slain birds was performed at the Department of Anatomy and Histology, UVAS, Lahore, Pakistan. Jejunum and ileum segments of approximately 5 cm were excised and flushed with 0.9% saline to remove feed contents and then preserved for at least 72 h before processing in 10% formalin solution. Then these sections were placed on slides and stained with Lilee Meyer hematoxylin and counter-stained with eosin yellow. After staining, the transverse sections were visualized with the light microscope and the images were analyzed by ImageJ software (Ferreira and Rasband, 2012). Villus height (Vh), villus width (Wd), crypts depth (Cd) and Vh/Cd were measured from 20 well-oriented villi/bird (Laudadio *et al*., 2012; Qaisrani *et al*., 2014).

Meat quality

Meat quality traits of the broiler chicken fed on different experimental diets were assessed at the Department of Meat Science and Technology, UVAS, Lahore, Pakistan. Debone *Pectoralis major* were chilled at 4 °C for 24 h to assess the effect on meat quality traits in terms of meat colour, cooking and drip losses and pH. Meat pH was calculated from three places of a breast portion using a pH meter (WTW, pH 3210 SET 2). One portion of breast was utilized to assess the drip loss and other for cooking loss. In order to determine drip loss, the breast portion was packed in plastic bags after weighing it and then stored at 4 °C in a refrigerator (Kaić *et al*., 2021). Furthermore, breast samples were placed in plastic bags and cooked in a water bath at 82-85 °C until the core temperature reached 72 °C in order to estimate the cooking loss. These samples were then chilled and weighed, and cooking loss was estimated by subtracting the end weight from the initial weight. Meat colour was measured using a Minolta CR-410 colorimeter after 24 hours post-slaughter for several colours such as lightness (L^*), redness (a^*), yellowness (b^*), chroma (C^*) and hue (H^*) (Priolo *et al*., 2002). Cooked breast samples were placed in polystyrene trays and cooled in a chiller at 4 °C. The breast samples were cut parallel to the direction of the muscle fibers using a scalpel handle blade into a rectangular form of approximately 1 h × 1w × 2 L cm. Finally, the Warner-Bratzler shear force (N/cm^2) was measured using a V slot blade with the help of Texture analyzer (TAXT plus texture analyzer, UK).

Statistical analysis

Growth performances were calculated on pen basis while blood haematology, gut histology and meat quality were determined per individual bird basis. Data regarding growth performances, blood haematology and meat quality were subjected to one-way ANOVA followed by Duncan's multiple range test as the post hoc analysis. Polynomial contrasts were also applied to test the linear and quadratic responses by increasing replacement levels of SBM with *C. megacephala* and *H. illucens*. General Linear Model (GLM) was applied to study the effects of diets, intestinal segments and interaction between diets and intestinal segments on the intestinal morphometric indices of the broiler chickens. The fixed factors included diets, intestinal segments and their interaction. Repeated measure ANOVA was performed to calculate the Least Square Mean of the intestinal morphometric indices in relation to dietary treatments and intestinal segments. Statistical analysis was performed according to the "General Linear Mod-

TABLE 3 Effects of *H. illucens* and *C. megacephala* diets on growth performances of the broilers

Items	Control	*Hermetia illucens*			*Chrysomya megacephala*			SEM	*P*-value				
		HI4	HI8	HI12	CM4	CM8	CM12		ANOVA	[1]HI Lin.	[1]HI Quad.	[1]CM Lin.	[1]CM Quad.
Live weight (g)													
DOC	40.28	39.76	40.0	40.8	39.58	41.09	40.79	0.05	1.00	1.00	1.00	1.00	1.00
wk1	141.8^{e}	141.8^{e}	157.8^{c}	169.6^{b}	152.0^{d}	165.6^{b}	179.8^{a}	2.33	<0.001	<0.001	0.002	<0.001	0.012
wk2	361.0^{f}	374.8^{e}	409.0^{c}	445.2^{b}	391.6^{d}	450.4^{b}	475.0^{a}	6.81	<0.001	<0.001	<0.001	<0.001	<0.001
wk3	781.2^{g}	819.0^{f}	871.4^{d}	953.2^{b}	853.0^{e}	922.4^{c}	968.4^{a}	11.15	<0.001	<0.001	<0.001	<0.001	<0.001
wk4	1288.6^{f}	1345.4^{e}	1406.2^{d}	1510.0^{a}	1358.4^{e}	1441.8^{c}	1494.8^{a}	13.03	<0.001	<0.001	<0.001	<0.001	<0.001
wk5	1782.6^{f}	1836.4^{e}	1919.4^{c}	2036.0^{a}	1867.6^{d}	1970.0^{b}	2034.2^{a}	15.7	<0.001	<0.001	<0.001	<0.001	<0.001
Average daily weight gain (g)													
wk1	18.57^{b}	18.86^{b}	18.91^{b}	21.55^{a}	19.6^{b}	19.49^{b}	21.7^{a}	0.3	0.006	0.009	0.1	0.008	0.008
wk2	28.92^{e}	30.03^{de}	32.19^{c}	35.92^{b}	31.18^{cd}	36.98^{b}	39.17^{a}	0.66	<0.001	<0.001	0.111	<0.001	<0.001
wk3	56.45^{d}	60.05^{c}	62.53^{c}	68.94^{a}	61.8^{c}	65.23^{b}	67.35^{ab}	0.75	<0.001	<0.001	0.172	<0.001	<0.001
wk4	67.2^{c}	71.59^{b}	71.92^{b}	76.00^{a}	69.11^{bc}	69.98^{bc}	70.31^{a}	0.56	<0.001	<0.001	0.889	0.025	0.025
wk5	64.83^{e}	67.77^{d}	69.60^{cd}	72.19^{b}	69.55^{cd}	71.61^{bc}	74.86^{a}	0.6	<0.001	<0.001	0.844	<0.001	<0.001
Daily feed intake (g)													
wk1	25.06	24.8	24.2	24.06	23.97	23.71	24.6	0.16	0.245	0.245	0.046	0.245	0.046
wk2	51.6	51.6	50.09	49.14	51.77	51.51	50.69	0.28	0.071	0.071	0.079	0.071	0.079
wk3	96.8	95.0	95.62	94.4	95.37	95.22	95.28	0.27	0.370	0.36	0.099	0.36	0.099
wk4	128.0^{bcd}	129.9^{ab}	127.5^{cd}	126.63^{d}	128.6^{abcd}	129.43^{abc}	130.22^{a}	0.32	0.012	0.012	0.015	0.012	0.015
wk5	179.2	178.2	178.66	180.25	179.98	179.48	179.20	0.57	0.977	0.977	0.653	0.977	0.653
Feed conversion ratio (g/g)													
wk1	1.33	1.30	1.28	1.14	1.24	1.19	1.16	0.02	0.091	0.032	0.384	0.01	0.431
wk2	1.79^{a}	1.73^{ab}	1.56^{c}	1.37^{de}	1.66^{b}	1.39^{d}	1.30^{e}	0.03	<0.001	<0.001	0.073	<0.001	<0.001
wk3	1.72^{a}	1.58^{b}	1.53^{b}	1.37^{d}	1.54^{b}	1.46^{c}	1.42^{cd}	0.02	<0.001	<0.001	0.627	<0.001	0.019
wk4	1.91^{a}	1.82^{bc}	1.78^{c}	1.67^{d}	1.86^{ab}	1.85^{abc}	1.85^{abc}	0.02	<0.001	<0.001	0.771	0.123	0.888
wk5	2.77^{a}	2.63^{b}	2.57^{bc}	2.5^{cd}	2.59^{bc}	2.51^{bcd}	2.40^{d}	0.02	<0.001	0.001	0.524	<0.001	0.112

DOC = day old chick; HI4 = 4% *H. illucens*; HI8 = 8% *H. illucens*; HI12 = 12% *H. illucens*; CM4 = 4% *C. megacephala*; CM8 = 8% *C. megacephala*; CM12 = 12% *C. megacephala*; SEM = Standard error of mean; ANOVA = Analysis of variance; [1]Polynomial contrast; HI lin. = *H. illucens* linear; HI quad. = *H. illucens* quadratic; CM lin. = *C. megacephala* linear; CM quad. = *C. megacephala* quadratic; The difference of superscript in the same column represented the statistically significant difference ($P < 0.05$).

els > Univariate" procedure at alpha 0.05. The results are presented as the mean and standard error of the means (SEM). Data was analyzed using SPSS software package (version 21 for Windows, SPSS Inc., Chicago, IL, USA).

3 Results

Growth performances

The birds did not show any clinical sign and had very low mortality (3.1% ± 0.24) in all the dietary treatments. The effect of insect meals on the growth performances of broilers, i.e. LW, ADG, DFI and FCR, all along the feeding intervals are presented in Table 3. There was a significant difference ($P < 0.001$) in LW and ADG among all the diets. The highest LW and ADG were observed in 12% *C. megacephala* diet, while the lowest were in control diet. The DFI did not show a significant difference ($P > 0.05$) among all the experimental diets except Wk4 ($P < 0.05$) where the maximum DFI was found in 12% *C. megacephala* diet and the minimum in 4% *C. megacephala* diet. FCR differed significantly ($P < 0.001$) among all the dietary treatments across the feeding intervals, except for wk1 ($P > 0.05$). The maximum FCR was recorded for control diet while the minimum for both 12% *C. megacephala* and *H. illucens* diets.

The Polynomial contrasts showed that there were a linear and quadratic differences in LW among all the dietary treatments of *H. illucens* and *C. megacephala* across the observation weeks. The ADG differed linearly among *H. illucens* diets while it differed linearly and

quadratically among *C. megacephala* diet. DFI was linearly and quadratically significant for *H. illucens* and *C. megacephala* diets only during Wk4. There was a linear difference of FCR among the dietary treatments of *H. illucens* across all the observation weeks while it differed linearly and quadratically among *C. megacephala* dietary treatments.

Haematology

Complete blood count (CBC)

The complete blood count of the broiler chicken is summarized in Table 4. There was a highly significant difference ($P < 0.001$) among *H. illucens* and *C. megacephala* diets in terms of complete blood counts except for MCHC, monocytes and eosinophils ($P > 0.05$). The Hb and HCT were the maximum in 12% *C. megacephala* and *H. illucens* diets while the minimum in 8% *H. illucens* diet. The highest RBC was found in 12% *C. megacephala* and *H. illucens* diets and the lowest in 4% *C. megacephala* diet. The maximum MCV, heterocytes, and lymphocytes were recorded in 12% *C. megacephala* diet and the minimum in control diet. MCH was highest in control diet while the lowest in 4 and 8% *H. illucens* diets. The maximum platelet was in 12% *C. megacephala* and the minimum in control diet while TLC was highest in 12% *C. megacephala* and the lowest in 4% *H. illucens* diet.

The polynomial contrast showed that there were a linear and quadratic differences in *H. illucens* and *C. megacephala* diets in terms of Hb, MCV, platelets, heterocyst and lymphocyte. There was only quadratic difference in *H. illucens* diets in terms of RBCs, HCT and MCH. However, HCT differed linearly among the dietary treatments of *C. megacephala*.

Serum biochemistry

The serum biochemistry parameters of the broilers are shown in Table 4. There was a significant difference ($P < 0.001$) in serum chemistry traits among all the experimental diets except albumin ($P > 0.05$). The maximum creatinine, glucose and uric acid levels were recorded in control diet while the minimum in 12% *C. megacephala* and *H. illucens* diets. The highest total protein and cholesterol was found in 12% *C. megacephala* diet and the lowest in control diet. Globulin was maximum in 12% *C. megacephala* diet but the minimum in 4% *C. megacephala* diet.

The polynomial contrasts showed that there were a linear differences of creatinine level among *H. illucens* diets while a quadratic difference in globulin among *H. illucens* diets. Glucose, cholesterol and total protein differed linearly and quadratically among the *H. illucens* diets. There was a linear difference in creatinine, albumin and uric acid while the quadratic difference cholesterol, total protein and globulin among the *C. megacephala* diets. The glucose level differed linearly and quadratically among the dietary treatments of *C. megacephala*.

Intestinal morphometry

The effects of the diets, intestinal segments and interaction between diets and intestinal segments on gut morphometric indices of the broiler chickens are shown in Tables 5 and 6. There was a significant difference ($P < 0.001$) in diets, intestinal segments and their interaction between diets and intestinal segments on the gut morphometric indices (Table 5). There was a highly significant difference ($P < 0.001$) in Vh, Cd, Vw and Vh/Cd among all the treatments. The maximum Vh, Vw and Vh/Cd were recorded in 12% *H. illucens* and *C. megacephala* diets while the minimum was in control diet. The Cd was the highest in control diet and the lowest in 12% *C. megacephala* diet. The jejunum showed the higher Vh and Cd, Vw and Vh/Cd than ileum (Table 6).

Meat quality

The effect of experimental diets on the meat quality traits of the broilers were summarized in Table 7. There was a highly significant difference ($P < 0.001$) in meat quality traits except meat pH and shear force ($P > 0.05$) among all the experimental diets. The cooking loss and drip loss were the maximum in control diet and the minimum in 12% *H. illucens* diet. The L^* and a^* were the highest in 12% *H. illucens* while the lowest in 4% *H. illucens* diet. The maximum b^* and H^* were found in 12% *C. megacephala* diet and the minimum in control diet.

The polynomial contrasts showed that for *H. illucens* diets, the cooking loss was linearly significant; L^* and a^* were quadratically significant; b^* was linearly and quadratically significant. For *C. megacephala* diets, there was a linear and quadratic differences of cooking loss, L^* and b^*; linear difference of drip loss and a^*. The H^* was quadratically significant in *H. illucens* diets while linearly in *C. megacephala* diets.

4 Discussion

Protein demand is increasing all over the world, therefore there is a need to identify alternative protein sources to fulfill human needs. Because of its high nutritional content and low manufacturing costs, insect meal

TABLE 4 Haematology and serum bio-chemistry of the broilers fed on *H. illucens* and *C. megacephala*

[1]Traits	Control	*Hermetia illucens*			*Chrysomya megacephala*			SEM	*P*-value					[2]Reference value
		HI4	HI8	HI12	CM4	CM8	CM12		ANOVA	[1]HI Lin.	[1]HI Quad.	[1]CM Lin.	[1]CM Quad.	
Haematology														
Hb	10.5^{b}	9.7^{c}	8.80^{d}	11.90^{a}	9.40^{c}	9.80^{c}	12.10^{a}	0.21	<0.001	0.003	<0.001	<0.001	<0.001	8.6-21.5
RBC's	2.9^{cd}	2.9^{cd}	3.50^{ab}	3.70^{a}	2.80^{d}	3.20^{bc}	3.80^{a}	0.08	<0.001	0.6	0.002	0.02	<0.001	2.0-7.0
HCT	33.6^{b}	31.0^{bcd}	28.60^{d}	38.80^{a}	31.60^{bc}	30.80^{cd}	38.20^{a}	0.70	<0.001	0.06	0.007	0.424	<0.001	3.6-48.6
MCV	88.0^{c}	88.9^{c}	101.90^{b}	108.12^{b}	102.90^{b}	107.46^{b}	115.90^{a}	1.83	<0.001	0.033	<0.001	0.002	0.044	64-132.4
MCH	36.2^{a}	27.8^{c}	27.50^{c}	34.58^{ab}	32.20^{b}	31.82^{b}	34.74^{ab}	0.71	<0.001	0.394	<0.001	0.088	0.089	20.5-70.43
MCHC	35.4	35.4	35.06	34.54	36.01	33.36	35.96	0.28	0.16	0.259	0.645	0.751	0.828	20.4-64.3
Platelets	15040^{d}	13300^{d}	18000^{c}	20600^{b}	15400^{d}	18100^{bc}	30400^{a}	954.38	<0.001	0.043	0.006	<0.001	<0.001	9300-38300
TLC	12400^{c}	6640^{d}	16560^{b}	11000^{c}	12000^{c}	17500^{ab}	19000^{a}	713.87	<0.001	0.026	0.851	<0.001	<0.001	3.8-19.5
Heter.	30.0^{e}	48.0^{b}	38.00^{cd}	40.00^{cd}	35.00^{d}	50.00^{b}	60.00^{a}	1.70	<0.001	<0.001	<0.001	<0.001	<0.001	25.8-63.2
Lym.	36.0^{e}	48.0^{d}	58.00^{bc}	61.00^{b}	46.00^{d}	57.00^{c}	66.00^{a}	1.70	<0.001	<0.001	<0.001	<0.001	<0.001	44.9-70.3
Mono.	2.00	2.00	2.00	2.00	2.00	2.00	2.00	0.14	1.00	1.00	1.00	1.00	1.00	1.0-7.1
Eo.	2.00	2.00	2.00	1.00	2.00	2.00	2.20	0.14	0.282	0.076	0.176	0.82	0.723	3.8-49
Serum bio-chemistry														
Creatinine	0.60^{a}	0.50^{ab}	0.40^{abc}	0.30^{bc}	0.50^{ab}	0.40^{abc}	0.25^{c}	0.03	0.018	0.025	0.286	0.052	0.221	0.13-0.9
Glucose	214^{a}	201^{ab}	196^{b}	135^{e}	174^{c}	171^{c}	154^{d}	4.73	<0.001	<0.001	<0.001	<0.001	0.001	180-326
Cholesterol	122^{c}	140^{b}	152^{a}	155^{a}	142^{b}	152^{a}	153^{a}	2.11	<0.001	0.029	<0.001	0.062	0.007	33.3-266
T. Protein	2.58^{c}	3.12^{a}	3.20^{a}	3.3^{a}	2.72^{bc}	3.04^{ab}	3.3^{a}	0.06	<0.001	0.01	0.007	0.186	0.004	3.0-4.9
Alb.	1.60	1.44	1.50	1.32	1.36	1.30	1.30	0.03	0.094	0.06	0.908	0.007	0.914	1.1-5.5
Glob.	1.60^{bc}	1.80^{ab}	1.80^{ab}	1.40^{c}	1.40^{c}	1.60^{bc}	1.90^{a}	0.06	<0.001	0.06	<0.001	0.499	0.004	1.0-3.5
Uric Acid	4.10^{a}	4.08^{a}	3.60^{bc}	3.50^{c}	3.5^{c}	3.90^{ab}	3.42^{c}	0.04	<0.001	0.073	0.085	0.039	0.197	1.3-19.3

[1]Hb g/dl = hemoglobin; RBC's × 10^{6} / ul = red blood cells; HCT % = hematocrits; MCV fL = mean corpuscular volume; MCH pg = mean corpuscular hemoglobin; MCHC = mean corpuscular hemoglobin concentration; Platelets/ul; TLC × 10^{3}/ul = total leucocytes; Heter. (%) = heterocyst; Lym. (%) = lymphocytes; Mono. (%) = monocytes; Eo. (%) = Eosinophils; Creatinine mg/dl; Glucose mg/dl; Cholesterol mg/dl; T. Protein g/dl = total protein; Alb. g/dl = albumin; Glob. g/dl = globulin; d Uric Acid g/dl; [2]Reference values were derived from the University Diagnostic Lab, UVAS, Lahore, Pakistan. Polynomial contrast; HI lin. = *H. illucens* linear; HI quad. = *H. illucens* quadratic; CM lin. = *C. megacephala* linear; CM quad. = *C. megacephala* quadratic.

TABLE 5 Effects of diet, intestinal segments (jejunum and ileum) and interaction between diet and intestinal segments on the intestinal morphometric indices of the broiler chickens

Index	Fixed effects	[3]df	F	*P*-value
Vh (μm)	[1]Intestinal segments	1	1666.65	<0.001
	[2]Diets	6	76.41	<0.001
	Intestinal segments × Diets	6	17.00	<0.001
Vw (μm)	Intestinal segments	1	124.55	<0.001
	Diets	6	32.75	<0.001
	Intestinal segments × Diets	6	20.86	<0.001
Cd (μm)	Intestinal segments	1	124.50	<0.001
	Diets	6	6.94	<0.001
	Intestinal segments × Diets	6	5.72	<0.001
Vh/Cd (μm)/(μm)	Intestinal segments	1	336.96	<0.001
	Diets	6	15.05	<0.001
	Intestinal segments × Diets	6	2.98	0.008

Vh = villus height; Cd = crypt depth; Vh/Cd = villus height to crypt depth ratio; [1]Two intestinal segments, jejunum and ileum; [2]Seven dietary treatments; Control; HI4 = 4% *H. illucens*; HI8 = 8% *H. illucens*; HI12 = 12% *H. illucens*; CM4 = 4% *C. megacephala*; CM8 = 8% *C. megacephala*; CM12 = 12% *C. megacephala*; GLM = Generalized linear model, [3]Degrees of freedom.

has the potential to replace soybean meal in poultry feed. Insects have a higher protein content than other animals and plant foods such as beef, chicken, fish, soybeans, and maize (Teffo *et al.*, 2007). In the present study, we evaluated different replacement levels of *C. megacephala* and *H. illucens* with soybean meal in terms of productive performance, blood parameters, gut histology, and meat quality in broiler chickens.

Growth performances

In the present study, the highest live weight of the broiler (15% increase) was observed in 12% *C. megacephala* and *H. illucens* diets as compared to the conventional soybean diet. Insects are rich in high-quality essential amino acids (e.g. methionine 2.2%, lysine 6.1% in BSF) (Zielińska *et al.*, 2015), and vitamins as compared to SBM i.e. methionine 0.65%, lysine 2.95% (Akhtar and Isman, 2018). On the other hand, insects have superior quality fatty acids (e.g. 21% lauric acid, 32% oleic acid and 16% palmitic acid in BSF) than SBM i.e. 12% lauric acid, 18% oleic acid and 13% palmitic acid (Islam *et al.*, 2022). The proteins and fatty acids are the growth premotor that play a key role in growth performance of the broilers (Kouřimská and Adámková, 2016). The nutritional makeup of dipterans (BSF and house fly) are well-balanced that contains important vitamins and minerals that ensure the robust and quick growth in the birds (Adli, 2021). Moreover, Onsongo *et al.* (2018) and Kierończyk *et al.* (2023) found that the body weight of chicken increased 5% to 61.63% when fed on 5% to 20% *H. illucens* as compared to SBM. In another study, chicken gained more body weight by replacing 50% and 100% fish meal with *M. domestica* (Đorđević *et al.*, 2008). Previously, it has been proved that *C. megacephala* larvae increased the 739% weight in Asian stinging catfish (*Heteropneustes fossilis*) (Satter *et al.*, 2022). Our findings that diet positively affects growth performance are consistent with those of other studies that propose insects as nutrient-dense feed ingredients for poultry feed (Khusro *et al.*, 2012; Józefiak and Engberg, 2015; Naderiboroojerdi and Rajabzadeh, 2022; Koutsos *et al.*, 2023; Vasilopoulos *et al.*, 2023).

In case of daily feed intake, no significant difference was observed among the tested and control diets. However, during the 4th week, more intake was observed in 12% *C. megacephala* diet. This is might be due to the palatability of the diet ingredients and age-related digestibility of the birds (Abdollahi *et al.*, 2018; Dabbou *et al.*, 2018). In case of feed conversion ratio, more feed conversion ratio was observed in control as compared 12% *C. megacephala* and *H. illucens*. It is might be due to change in the nutritional composition of the diet. Elahi *et al.* (2020) evaluated the FCR was reduced at 8% inclusion of *M. domestica* diet in the broiler chickens. In another study, feed conversion ratio in broiler decreased when fed on 8% yellow mealworm as compared to silkworm and SBM diets (Khan *et al.*, 2018). A study from Italy, also reported that 15% *H. illucens* decreased the feed conversion ratio in Ross 308 broiler (Dabbou *et al.*, 2018). It is possible to reduce FCR by substituting con-

TABLE 6 Least square means of intestinal morphometric indices in broiler chickens in relation to diets and intestinal segments

Index	Fixed effects	Effects level	Least square mean	*P*-value
Vh (μm)	Diets	0% Control	1139.10^{d}	<0.001
		4% *H. illucens*	1233.63^{bcd}	
		8% *H. illucens*	1271.84^{bcd}	
		12% *H. illucens*	1504.79^{a}	
		4% *C. megacephala*	1182.60^{cd}	
		8% *C. megacephala*	1339.82^{abc}	
		12% *C. megacephala*	1428.77^{ab}	
	Intestinal segments	JE	1558^{a}	<0.001
		IL	1278.13^{b}	
Cd (μm)	Diets	0% Control	160.93^{a}	0.001
		4% *H. illucens*	155.48^{ab}	
		8% *H. illucens*	144.39^{bc}	
		12% *H. illucens*	144.36^{bc}	
		4% *C. megacephala*	153.93^{abc}	
		8% *C. megacephala*	146.05^{bc}	
		12% *C. megacephala*	142.70^{c}	
	Intestinal segments	JE	155.07^{a}	<0.001
		IL	144.23^{b}	
Vw (μm)	Diets	0% Control	55.28^{b}	<0.001
		4% *H. illucens*	55.79^{b}	
		8% *H. illucens*	60.18^{b}	
		12% *H. illucens*	77.92^{a}	
		4% *C. megacephala*	56.60^{b}	
		8% *C. megacephala*	63.16^{b}	
		12% *C. megacephala*	84.95^{a}	
	Intestinal segments	JE	73.56^{a}	<0.001
		IL	56.12^{b}	
Vh/Cd (μm)/(μm)	Diets	0% Control	7.96^{c}	<0.001
		4% *H. illucens*	8.60^{abc}	
		8% *H. illucens*	9.12^{abc}	
		12% *H. illucens*	10.02^{a}	
		4% *C. megacephala*	8.36^{bc}	
		8% *C. megacephala*	8.52^{bc}	
		12% *C. megacephala*	9.53^{ab}	
	Intestinal segments	JE	10.33^{a}	<0.001
		IL	7.41^{b}	

JE = Jejunum; IL = Ileum; The difference of superscript in the same column represented the statistically significant difference ($P < 0.05$).

TABLE 7 Effects of dietary replacement of broilers' diets with *H. illucens* and *C. megacephala* diets on meat quality of the broiler

Traits	Control	*Hermetia illucens*			*Chrysomya megacephala*			SEM	*P*-value				
		HI4	HI8	HI12	CM4	CM8	CM12		ANOVA	[1]HI Lin.	[1]HI Quad.	[1]CM Lin.	[1]CM Quad.
Cooking loss	33.01^{a}	29.25^{b}	26.38^{c}	23.47^{d}	34.50^{a}	26.99^{c}	26.22^{c}	0.67	<0.001	<0.001	0.25	0.027	<0.001
Drip loss	3.29^{a}	2.47^{bc}	2.67^{b}	1.97^{c}	2.45^{bc}	2.16^{bc}	2.07^{c}	0.1	0.002	0.357	0.41	0.771	0.015
Meat pH	6.09	6.10	6.20	6.22	6.07	6.14	6.20	0.02	0.41	0.26	0.965	0.42	0.238
Shear Force	60.96	62.45	60.71	60.53	59.80	61.60	60.19	0.27	0.128	0.376	0.278	0.558	0.64
L*	49.76^{cd}	49.14^{d}	54.35^{ab}	57.53^{a}	53.01^{bc}	53.41^{b}	57.14^{a}	0.65	<0.001	0.375	<0.001	0.013	0.011
a*	15.70^{ab}	12.37^{d}	15.49^{abc}	16.08^{a}	14.10^{c}	14.47^{bc}	15.99^{ab}	0.28	<0.001	0.079	0.001	0.405	0.013
b*	12.58^{d}	18.01^{b}	15.47^{c}	15.75^{c}	16.25^{c}	16.50^{c}	19.53^{a}	0.39	<0.001	0.004	<0.001	<0.001	0.023
C*	22.38	21.58	21.67	21.76^{b}	21.75	21.76	21.27	0.31	0.937	0.526	0.468	0.296	0.757
H*	41.04^{d}	49.73^{bc}	43.15^{d}	50.13^{b}	43.21^{d}	47.54^{c}	54.61^{a}	0.83	<0.001	0.264	<0.001	<0.001	0.971

L* = lightness; a* = redness; b* = yellowness; C* = chroma; H* = Hue; HI4 = 4% *H. illucens*; HI8 = 8% *H. illucens*; HI12 = 12% *H. illucens*; CM4 = 4% *C. megacephala*; CM8 = 8% *C. megacephala*; CM12 = 12% *C. megacephala*; SEM = Standard error of mean; ANOVA = Analysis of variance; [1]Polynomial contrast; HI lin. = *H. illucens* linear; HI quad. = *H. illucens* quadratic; CM lin. = *C. megacephala* linear; CM quad. = *C. megacephala* quadratic; The difference of superscript in the same column represented the statistically significant difference ($P < 0.05$).

ventional protein sources with insect meal due to high nutrient density, balanced amino acid profile, palatability, digestibility, energy efficiency, and lower antinutritional factors (Tschirner and Simon, 2015). This leads to improved growth rates and more efficient conversion of feed into body mass in broilers (Barragan-Fonseca *et al.*, 2018).

Haematology and serum biochemistry

The current study provided crucial information about the effect of dietary treatments on haematology of broiler. The Hb, RBC, HCT, TLC, MCV, platelet counts, heterophil, and lymphocyte counts were the maximum in 12% *C. megacephala* and *H. illucens* diets. Synthesis of blood cells is mainly associated with the dietary composition of feed. Different diet compositions have different impact on the production of RBC (Lessire *et al.*, 2017). Our findings were in consonance with the findings of Schiavone *et al.* (2017) who stated that the blood traits were not affected by replacing 50 to 100 soybean oil with *H. illucens* larvae in broiler chicken (Ross 500). This contradiction might be due to difference in breed and feed composition of the boiler. Lessire *et al.* (2017) determined that the efficient use of insect protein increased blood flow and improved the health of broiler chickens.

In serum biochemistry traits, creatinine, cholesterol, uric acid, and glucose were the lowest in 12% *C. megacephala* and *H. illucens* diets. Overall metabolic functioning of the birds enhances with the decrease of creatinine, cholesterol, uric acid, and glucose levels (Sypniewski *et al.*, 2020). However, in previous studies 4% SBM replacement (Kim *et al.*, 2020) and 100% soybean oil replacement with *H. illucens* have no effect on the serum biochemistry including creatinine, uric acid and glucose level (Schiavone *et al.*, 2018; Kim *et al.*, 2020; Sypniewski *et al.*, 2020). The discrepancy in the current study and prior studies might be due to differences in breed, trial conditions, age and composition of diet (Danieli *et al.*, 2019; Kawasaki *et al.*, 2019).

In our study, total protein and globulin were the maximum in 12% *C. megacephala* diet. The total proteins and globulin are generally influenced by isoprotenic and isoenergetic compositions of dietary treatments (Bovera *et al.*, 2016). Another study elicited that high globulin and low albumin/globulin ratios were enhanced the immune response and disease resistance in birds (Griminger and Scanes, 1986). The nutritional compositions of the diet may strengthen the immune system, increasing the resistance of broiler chickens to illnesses and infections (Schiavone *et al.*, 2017).

Gut histology

In our finding, villus height increased while crypt depth was decreased in 12% *C. megacephala* and *H. illucens* diets. The maximum Vh and Vh/Cd ratio was also recorded in 12% *C. megacephala* and *H. illucens* diets. Chitin contents, antioxidant peptides and antimicrobial peptides – obtained from insect-based diets – are vital in the development of gut in broiler chickens (Henry *et al.*, 2018; Antonopoulou *et al.*, 2019; Elahi *et al.*, 2022). Villi and crypts of small intestine are the absorptive epithelium well known for efficient nutrient digestion and assimilation (Wang and Peng, 2008). Poor nutrition leads to short villus that indicates poor gut development (Cano-Cebrián *et al.*, 2022). Short villus and deeper crypt impose negative impact on animal performance i.e. poor digestion and reduced nutrient absorption and higher rate of cell turnover. Resultantly more energy is spent for digestive tract operation rather than its growth (Qaisrani *et al.*, 2014). Biasato *et al.* (2016, 2017, 2018) found no change in gut morphometric indices in free-range or broiler chickens when fed on diets with 7.5% and 5% inclusions of *T. monitor*, respectively. The variations in the development of Vh, Cd, Vw, and Vh/Cd are due to the anatomical location and structural characteristics of jejunum and ilium (Li *et al.*, 2022). Jejunum is usually more involved in the absorption of nutrients than ileum (Goodman, 2010). These findings suggest that 12% *C. megacephala* and *H. illucens* have the potential to be a valuable dietary replacement of soybean that helps to improve the gut histology and resultantly the bird health.

Meat quality

Meat quality traits influence sensory characteristics e.g. meat colour, pH, tenderness (shear force), and water-holding capacity (Yang *et al.*, 2011; Christiansen, 2013). Our findings revealed that the cooking and drip losses decreased 21% and 37%, respectively, with the increasing levels of soybean replacement i.e. the lowest in 12% *C. megacephala* and *H. illucens* diets. The cooking and drip losses in meat are positively associated with its pH (Altmann *et al.*, 2020) and negatively associated with its juiciness (Toscas *et al.*, 1999). Earlier, it has been demonstrated that replacing 7.8% SBM with *H. illucens* resulted in a 13% decrease of cooking and drip losses in broiler Ross 308 (Leiber *et al.*, 2017).

Meat colour and texture are the two most important quality features of poultry meat that directly influence the perception of a consumer. In the current study, L^* and a^* of the meat was maximum in 12% *C. megacephala* and *H. illucens* diets. The maximum b^* value

was recorded in the 12% *C. megacephala* diet. The meat colour changes with the fatty acid compositions of the diets (Adeyemi and Sazili, 2014; Mir *et al.*, 2017) that is might be due to presence of carotenoids in the formulated diet (Slimen *et al.*, 2023). Carotenoids are important pigments in *H. illucens* that provide an attractive colour to egg yolk, which is a desirable visual characteristic (Karadas *et al.*, 2006; Finke, 2013). The poultry meat colour could also be affected by pre-slaughtering conditions (i.e. diet composition, handling and transportation) and procedures (i.e. human slaughtering method and blood removal) (Fletcher, 2002). In the prior study, 20% *H. illucens* diet improved the meat quality traits in Ross 308 broiler due high amount of lauric acid, myristic acid and eicosapentaenoic fatty acid in diets (De Souza Vilela *et al.*, 2021).

Most of the studies did not show any change in the meat pH, colour, moisture loss, shear force or fatty acid profile at 4% to 15% replacement of SBM with *H. illucens* and *M. domestica* (Dahiru *et al.*, 2016; Dabbou *et al.*, 2020; Elahi *et al.*, 2020; Kim *et al.*, 2021). Previously Schiavone *et al.* (2019) reported more meat a^* in broiler chickens when fed on 15% *H. illucens*. Contrarily, quail breast meat showed less redness at 10% and 15% *H. illucens* (Cullere *et al.*, 2018). Such discrepancies may happen due to the diet compositions and metabolic differences within the species of the commercial broilers (De Souza Vilela *et al.*, 2021).

5 Conclusion

The current study presents novel information on the effect of different replacement levels of SBM with native *C. megacephala* – in poultry feed – on the growth and welfare of broiler. The results were statistically similar with the commercially used BSF. Diet containing 12% *C. megacephala* and *H. illucens* exhibited the best growth performances of broiler chickens, haematology, gut histology and meat quality. The improved feed intake and feed conversion ratio – as important parameters of growth performance – highlight the potential of insect-based diets as a cost-effective and sustainable alternative to chicken growth. The observed variation in meat quality in terms of colour, drip loss, cooking loss, etc., provide important information on dietary manipulation that may influence consumer acceptance. The results are particularly important for feedstuff manufacturers and producers who believe in sustainable production and consumption. Future research warrants to explore optimal inclusion levels of other native inset species and their long-term impacts on sustainability, cost-effectiveness and nutritional quality of the poultry production.

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Author contributions

MS, MB and AS: conceptualization. MS, AS, MB and GAC: methodology. MS, AA and AS: data analysis. MS, AA, AS, IUH, AZG, MSH and MB: writing – original draft preparation. AS, MB, IUH, AZG, MSH and A.A: writing – review and editing. MB, AA, AZG, MSH and AS: visualization. MB and AS: supervision. AA, AZG and MSH: funding acquisition. AS: project administration. All authors have read and agreed to the published version of the manuscript.

Conflict of interest

The authors declare that they have no competing interests.

Data availability statement

The original contributions presented in the study are included in the article, further inquiries can be directed to the corresponding authors.

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