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Organic medium-growing chickens fed live black soldier fly larvae: A welfare improvement study

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Abstract

The overall beneficial effect of live black soldier fly larvae (BSFL) on the welfare of broiler chickens, turkeys, and laying hens has already been discussed in the literature. However, scant information is available regarding the benefits of feeding live BSFL to medium-growing chicken hybrids reared under organic/free-range conditions, and whose welfare is frequently cited as being inadequate. The aim of this research was to advance our knowledge of this topic. To this end, 240 label naked neck birds (Hubbard JA57 hybrid) were assigned, at 21 days of age, to four experimental groups (6 replicates/treatment, 10 chickens/replicate), created according to sex (M/F) and the provision of a 10% live BSFL dietary supplementation (control males, control females, larvae males, and larvae females), and raised until 82 days of age. We performed behavioural observations, a tonic immobility test, and an avoidance distance (AD) test. We assessed feather damage and cleanliness, hock burn, footpad dermatitis, and skin lesion scores, and determined the concentration of excreta corticosterone metabolites (ECM) and the heterophile to lymphocyte heterophile/lymphocyte (H/L) ratio. The behavioural observations demonstrated increased physical and foraging activity ($p < 0.05$) in the live BSFL administered groups compared with C ones, providing valuable data on the explorative and recreational behaviour of this chicken genotype. The results also evidenced the usefulness of live BSFL as a fear reducer in females, as those receiving the BSFL supplement moved closer to the operator during the AD test ($p < 0.01$). No physical injuries or damage were observed on the birds, regardless of whether they received the BSFL supplementation or not. The ECM were unaffected by BSFL supplementation, while the H/L ratio was higher in the larvae groups than in the control ones ($p = 0.050$). In conclusion, live BSFL provision could constitute a powerful tool for improving life quality in medium-growing chickens. Further research is required to clarify the stress modulation role of live BSFL on poultry production.

KEYWORDS
alternative rearing systems, environmental enrichment, free-range chickens, Hermetia illucens larvae, organic farming, poultry behaviour

1 | INTRODUCTION

High productivity farming practices, such as the stocking density, and environmental conditions, such as litter quality, are undeniably the predominant factors generating welfare issues in poultry (EFSA, 2023). Although such matters have mainly been ascribed to fast-growing genotypes, birds reared in organic and free-range rearing systems are not exempt from these problems, especially when the breeding and management practices are not adequate (van de Weerd et al., 2009). Environmental enrichment represents a consolidated way of improving chicken welfare (Riber et al., 2018). Various approaches to enrichment have already been tested and shown to favour the expression of the behavioural repertoire of birds and reduce instances of aggressiveness (Star et al., 2020) and fear (Baxter et al., 2019). Inert materials such as sand, rice hulls, wood and moss-peat shavings can enrich the environment, albeit with some limitations since they are devoid of living organisms and the key feature that attracts the attention of foraging birds: motion (Riber et al., 2018). Environmental enrichment through the provision of live insect can fully satisfy the behavioural requirements of poultry under farming conditions (Carr, 2016; Gariglio et al., 2023; Schiavone & Castillo, 2023). Live insect motility elicits curiosity in chickens. Indeed, chickens naturally spend a remarkable amount of time foraging and pecking the ground, and they eat insects willingly, which form part of the chicken's natural diet (Star et al., 2020). For the present study, BSF (*Hermetia illucens*) was chosen from the few insect species currently allowed for farm animal feeding practice in the European Union due to a number of favourable features, including the environmental sustainability of its production (Gasco et al., 2020; Purkayastha & Sarkar, 2021) and desired animal welfare principles (Schiavone & Castillo, 2023).Various studies have tested the effects of the provision of live larvae on poultry welfare. However, the poultry category encompasses several bird species characterised by broad behavioural patterns and needs, which may elicit different responses. Veldkamp and van Niekerk (2019), for instance, conducted the first study encompassing live larvae as environmental enrichment in turkey poults. They observed a reduction in feather pecking on the back and tail of turkeys supplemented with 10% of black soldier fly larvae (BSFL) compared with controls at 5 weeks of age. Similarly, Star et al. (2020) reported a better plumage condition of laying hens administered 10% live BSFL in comparison with the control groups. Tahamtani et al. (2021) investigated laying hens and found no changes in the behaviour of birds fed 10%, 20%, and ad libitum live BSFL. Moving to broiler chickens, Ipema et al. (2020b) observed that birds fed 5% or 10% live BSFL exhibited more intense foraging behaviour and were generally more active than the controls. Biasato et al. (2022) demonstrated that a 5% dietary inclusion of live BSFL and yellow mealworm (*Tenebrio molitor*) larvae exerted beneficial effects on broiler chickens as it reduced the birds' fear and increased their foraging and activity behaviours, whereas no effects were observed on the excreta corticosterone metabolites (ECM) or on the heterophile to lymphocyte (H/L) ratio during the experiment (Bellezza Oddon et al., 2021; Biasato et al., 2022). No data on the potential to improve bird welfare through insect provision are available on medium-growing genotypes reared in organic farming systems. In such contexts, medium- and slow-growing genotypes exhibit higher activity levels in terms of ambulation, foraging, and overall exploration compared with fast-growing genotypes, better resembling the behavioural patterns of their ancestors. By consequence, research into the behavioural and welfare requirements of these breeds raised under organic/free-range conditions has been widely neglected due to the wide and incorrect interpretation of the organic farming practice as an automatic animal welfare warranty (Riber et al., 2018; van de Weerd et al., 2009). Furthermore, the present study is the first in which the evaluation of live larvae supplementation has been evaluated in both males (M) and females (F) simultaneously, with the aim to investigate if sex may influence the behavioural pattern in response to dietary larvae provision. This is an important consideration for research into poultry welfare, because although being part of the meat production chain, female birds have not been proportionally represented in the welfare and behavioural studies of broiler chickens, especially in relation to alternative rearing systems, for which males have been principally assessed. Broiler chickens might display a different behavioural profile or reactions to surrounding stimuli, in reflection of the distinct attitudes long characterising the two sexes, e.g. boldness in males and prudence in females (Collias & Collias, 1967).

The present research investigated the behaviour and welfare implications of administering diets supplemented with live BSFL to organic medium-growing chicken hybrids.

2 | MATERIALS AND METHODS

2.1 | Ethics

The animal study was reviewed and approved by the Bioethical Committee of the University of Turin, Via Verdi 8, 10124, Turin (Italy) (Prot. No. 814715).

2.2 | Animals and experimental design

The study was conducted at the poultry experimental facility of the University of Turin (north-west Italy, 44.88572, 7.68381). A detailed description of the adopted experimental design and chicken management procedures is provided in Bongiorno et al. (2022), in

which the same birds were considered as for the present trial. Briefly, a total of 240 one-day-old Label naked neck (LNN) birds (Hubbard JA57 hybrid) were divided according to their sex (M and F) and experimental dietary treatment (control [C] vs. live BSFL supplementation [L]), which they received from 21 to 82 days of age. This division produced four experimental groups: control males (CM), control females (CF), larvae males (LM), and larvae females (LF) (6 replicates/treatment, 10 birds/replicate), which were balanced according to the live weight of the birds. The birds belonging to the LM and LF groups received a 10% dietary supplementation of live BSFL, calculated on the basis of the daily estimated feed intake of the birds (Hubbard, 2021). Since the larvae were provided in addition to the feed, they were not considered in the diet formulation. A "first-period" diet was provided from 1–28 days of age (22.92/100 g of crude protein [CP], 15.36 MJ/kg of nitrogen-corrected apparent metabolisable energy [AMEn] as fed), followed by a "second-period" diet from 28–82 days of age (20.55/100 g CP, 14.19 MJ/kg of AMEn as fed) (Verzuolo Biomangimi S.r.l.—Verzuolo, CN, Italy). Water and feed were provided ad libitum and overall growth performance were recorded. Each pen measured 2.2 × 3.5 m, and rice husks were used as the bedding material. A black cloth covered the pens' side walls for the duration of trial to avoid visual contact between control and experimental groups of birds housed in adjacent pens. The birds in each pen had access to an outdoor enclosure (2.2 × 4.5 m) from 49 days of age until the end of the trial to ensure that the birds could spend one third of their lives in an outdoor area in accordance with the European regulations on organic production (European Commission, 2008). The insect larvae were administered to the birds at 11.00 AM daily (excluding Sundays) from 28 to 81 days of age. The larvae were distributed on two plates (18.8 linear cm/bird, 141.3 cm^2/chick, and 30 cm diameter). Two empty plates were introduced into the C pens to simulate the same interaction between the birds and the operator, thus maintaining the live BSFL provision as the sole difference between C and L group treatments (Bongiorno et al., 2022). Sampling days were clustered into the following trial periods: time 0 (T0) = 25–28 days of age; time 1 (T1) = 39–41 days of age; time 2 (T2) = 49 days of age, time 3 (T3) = 61–63 days of age; time 4 (T4) = 76–77 days of age.

2.3 | Behavioural observations

Electronic tablet devices were used to perform the video recordings at 25 (T0), 61 (T3), and 75 (T4) days of age. Four recording replicates/treatment were made, and three time slots (5 min/each) were selected to monitor the birds' behaviours over the course of the same day: in the morning (9.00 AM); during live BSFL provision (11.00 AM); in the afternoon (4.00 PM). The different pens were always assessed in the same order. The time samplings were selected according to the ethogram identified as relevant for the present study and the technical resources available. Bird activity levels are reportedly greater during the morning and late afternoon time than the rest of the day in both domesticated chicken ancestors (Collias & Collias, 1967) and in commercial broiler strains (Foshee et al., 1970; Weaver & Siegel, 1968), although further research is required to understand the birds' behavioural exploitation patterns through the day according to the rearing environment and the resources provided (Bashir et al., 2023). In our case, we selected further times (compatible with the natural photoperiod to which the birds were subjected) both prior to and after the live larvae provision. These two observation time points aimed to detect indirect behavioural differences between the control and live BSFL supplemented groups, to validate the potential of live BSFL in stimulating natural behaviours even when the larvae provision was not actually present (e.g., prosecution of foraging activity to look for other larvae when all the provided ones had been ingested). Furthermore, video recordings were also performed during the administration of live larvae to permit the assessment of bird activity at this time. The video recordings were analysed using BORIS (Behavioural Observation Research Interactive Software v 7.9.7) (Friard & Gamba, 2016). The different kinds of behaviour were ordered into four macro groups: foraging-related behaviour, comfort behaviour, activity behaviour, and social behaviour (Table 1). The occurrence of the specific types of behaviour was registered within each 30 s time slot (considering every continuous behavioural manifestation as one occurrence, regardless of its duration) and corrected according to the number of birds visible in the barn. The recorded ethogram was elaborated considering the previous studies conducted by Biasato et al. (2022), Ipema et al. (2020b), and Veldkamp and van Niekerk (2019).

2.4 | Avoidance distance (AD) test

The AD test was performed to measure chickens' fear, on the basis of the birds' response to the approach of humans (Meuser et al., 2021). The same operator entered the birds' pen, squatted on the litter for 10 s and counted the number of chickens within a distance of 1 m (Welfare Quality®, 2009), between 1 and 2 m, and over a distance of 2 m from him/herself. The test was executed at 27 (T0), 41 (T1), 62 (T3), and 76 (T4) days of age between 3.00 and 4.00 PM.

2.5 | Tonic immobility (TI) test

The TI test was performed according to the methods described in Dabbou et al. (2022) to evaluate the level of fear in chickens. The test was always performed by the same operator in a separate area within the building, out of sight of the other birds. A total of three chickens/pen were randomly selected and labelled with a second wing mark at 26 days of age. These birds were then subjected to the TI test at 26 (T0), 39 (T1), 60 (T3), and 74 (T4) days of age. The test was performed according to the methodology adopted in a previous study (Campo et al., 2007). During the test, a bird was placed on its back on a U-shaped cradle. A slight pressure was applied to the breast of the bird and the time necessary for the bird to stop struggling and become immobile for at least for 10 s was recorded. If the bird righted itself in

TABLE 1 Ethogram of the specific behaviours and activities of the chickens studied.

Categories	Sub-categories	Description	References
Foraging-related behaviour	Ground pecking	Pecking the ground	Ipema et al. (2020b)
	Pecking object	Pecking objects	Veldkamp & van Niekerk (2019)
	Scratching	Moving litter backwards using their claws	Biasato et al. (2022)
	Eating larvae	Pecking larvae from the plates	–
Comfort behaviour	Preening	Self-grooming of feathers using the beak	McCowan et al. (2006)
Activity behaviour	Walking	Walking/running	Biasato et al. (2022)
	Standing	Standing stationary	Veldkamp & van Niekerk (2019)
	Resting	Sitting/lying stationary	Veldkamp & van Niekerk (2019)
	Outside	Entering the outdoor enclosure	–
Social behaviour	Sparring/fighting	Play fighting/fighting	Veldkamp & van Niekerk (2019)
	Chasing	Running after a conspecific	Biasato et al. (2022)
	Pecking conspecifics	Pecking movements directed at a pen mate	McCowan et al. (2006)
	Allopreening	Social preening	Kenny et al. (2017)

less than 10 s, the test was repeated for a maximum of three times. If TI was not induced within three attempts, the assigned score was 0 s. The maximum considered duration of TI was 10 min (600 s). Finally, the TI induction frequency was calculated on the basis of the number of inductions required to induce TI (from 1 to 3 attempts) and expressed as a percentage of the total number of attempts executed.

2.6 | Feather damage and cleanliness

Plumage condition was always assessed by the same operator and considered feather damage and breast feather cleanliness. The assessment was conducted at 28 (T0), 49 (T2), 63 (T3), and 77 (T4) days of age. Feather damage was scored from 0 to 5 after evaluating the wings, tail, thighs and back covering conditions (Aviagen, 2014), where: 0 = fully feathered; 1 = rough; 2 = some broken feathers; 3 = heavily broken feathers; 4 = almost bald; 5 = bald. Breast feather cleanliness was scored from 0 to 4 (Welfare Quality®, 2009), where: 0 = clean; 1 = slight change in feather colouration; 2 = marked change in feather colouration; 3 = spotted litter and excreta stuck to the feathers; 4 = marked litter and excreta stuck to the feathers.

2.7 | Leg health: footpad dermatitis (FPD) and hock burn (HB) scores

The leg health evaluation included both the FPD and the HB scores. The sampling times were the same as those adopted for the feather condition evaluation and skin lesions score (see below), and every observation was carried out by the same operator. The FPD was scored on a scale from 0 to 4 (Welfare Quality®, 2009), where: 0 = no lesions; 1 = minor and superficial lesions of the skin with hyperkeratosis; 2 = moderate and superficial lesions of the skin with hyperkeratosis (less than one quarter of the footpad affected); 3 = severe and deep lesions with hyperkeratosis (one half of the footpad altered); 4 = severe and deep lesions with hyperkeratosis (more than one half of the footpad altered). The HB was evaluated as follows (Welfare Quality®, 2009): 0 = no lesions or mild skin rash; 1 = pronounced skin rash; 2 = moderate skin lesions and blood scabs; 3 = severe but confined skin lesions and necrotic areas (less than one half of the area altered); 4 = severe and extended skin lesions and necrosis (at least half of the area affected).

2.8 | Skin neck and breast lesion scores

The skin condition was assessed at the same time as feather condition. Two areas were considered for the assessment of skin lesions: the neck and the breast. The scoring system adopted for skin lesions on the neck was as follows (Welfare Quality®, 2009): 0 = no lesions or fewer than three pecks (punctiform damage of less than 0.5 cm diameter) or scratches; 1 = at least one lesion of less than 2 cm diameter or three or more pecks or scratches; 2 = at least one lesion of at least 2 cm. The breast-skin lesion protocol considered the presence or absence of erythema and was scored as follows: 0 = normal skin colouration; 1 = intense but contained breast-skin redness (less than half of the breast); 2 = intense and extended breast-skin redness (at least half the breast).

2.9 | ECM analysis

Fresh excreta samples were collected at 26 (T0), 39 (T1), and 74 (T4) days of age from the two birds randomly selected for the TI test. Each bird was

kept in a wire-mesh cage (100 cm width × 50 cm length) until at least 2 g of fresh excreta had been produced and collected in a plastic box under the cage. The samples were stored at −20°C until corticosterone analysis. The ECM analysis was executed according to Costa et al. (2016) and Palme et al. (2013). Briefly, 3 mL of 80% methanol (Sigma Aldrich) was added to 0.25 g of lyophilised excreta in an extraction tube and maintained at −20°C for 2 h to allow the solid phase to settle at the bottom of the tube. After 2 h, the supernatant was transferred to another vial and evaporated under a hood for 14 h. The concentration of ECM was determined using a multispecies enzyme immunoassay kit (K014 - Arbor Assay®) validated for serum, plasma, saliva, urine, dried faecal extracts, and tissue culture media. The inter- and intra-assay coefficients of variation did not exceed 10%, and the sensitivity of the assay was 11.2 ng/g of excreta. Multiple dilutions were conducted to perform the sample analyses (1:4, 1:8, 1:16, and 1:32) and all the regression slopes were parallel to the standard curve (R^2 = 0.989). The mean recovery rate of corticosterone added to dried excreta was 96.5%. According to the manufacturer, the corticosterone kit presents the following cross reactivity: 100% with corticosterone, 12.3% with desoxycorticosterone, 0.62% with aldosterone, 0.38% with cortisol and 0.24% with progesterone. All the analyses were performed in duplicate, and the concentration of ECM was expressed as ng/g excreta dry matter.

2.10 | H/L ratio

During bleeding, at the time of slaughtering, blood samples were drawn from each of the 48 birds selected for slaughter at 82 days of age. An aliquot of 2.5 mL of blood was stored in a tube containing EDTA. Subsequently, a drop of blood was placed onto a glass slide to obtain a smear. May-Grünwald and Giemsa stains (Campbell, 1995) were used to stain the smears, and the samples treated with 1:200 Natt-Herrick solution (Natt & Herrick, 1952). The erythrocyte and leukocyte counts were defined using an improved Neubauer haemocytometer (Salamano et al., 2010). One hundred leukocytes, both granular (heterophil, eosinophil and basophil) and non-granular (lymphocyte and monocyte), were counted on the glass slide and the H/L ratio calculated.

2.11 | Statistical analysis

The data analysis was performed using IBM SPSS Statistics (IBM Corp, 2011). Outlier evaluation was performed for each parameter considered, but no anomalous data were revealed and by consequence removed from the dataset. Subsequently, the data were tested for the homogeneity of variances by means of Levene's test, and both the residuals and the data were tested for normality using Shapiro–Wilk's test. A total of four experimental groups were considered, established according to sex and live BSFL dietary supplementation.

The experimental unit was the pen for the following parameters: feather condition, leg health, skin lesion score, AD test, TI test, ECM (n = 6 per treatment), and behavioural observations (n = 4 per treatment). A single observer performed all the behavioural analyses, while another rater provided feedback on video sequences during the video analysis process to assure observation consistency. The observer was first taught about the importance of scientific detachment and was provided a description of the behaviours to look for. Subsequently, the observer watched and assessed video recordings of replicates not involved in the statistical analyses as training. The sample video recordings were watched and assessed twice, and no discrepancies were observed in the results obtained (intra-rater reliability ICC: ≥0.90) (Bateson & Martin, 2021). Finally, the accurateness of the ethogram provided, and the simplicity of the activities monitored importantly contained the error margin may present within observations and raters (Anderson et al., 2014). The bird was considered the experimental unit for the H/L ratio analyses and for the correlation between TI duration and ECM content (n = 12). Moreover, a general linear mixed model was fitted to analyse feather condition, leg health, and the skin lesion scores (negative binomial response probability distribution with a nonlinear link function-log), TI duration, TI induction frequency and ECM (gamma probability distribution and log-link function), the AD test and video recordings (Poisson loglinear distribution). D, S, and T, and D × S, S × T, and D × T interactions were considered as fixed factors (assessed by means of pairwise comparisons), and the replicate was included in the model as an indicator of the repeated measurements on the same pen. The frequency of the exploited behaviour is intended here as the number of behavioural observations performed during the recordings. A general linear model was used to analyse the H/L ratio, considering S, D, and their interaction (D × S) as fixed factors. Finally, a Spearman correlation test was performed for the ECM concentration and the TI duration, as these data were not normally distributed. All the data were reported as least square means plus the standard error of the mean (SEM). Significant differences were declared for p values ≤0.05. Any behaviour or animal-based welfare measurement observed at a frequency lower than an average of 0.5 times per observation period was not subjected to statistical analysis and thus not reported in the results.

3 | RESULTS

Bongiorno et al. (2022) reported how the final live weight was influenced by both the live BSFL supplementation (+32 g) and sex (M: +772 g). Moreover, the feed conversion ratio was not influenced by the diet but the sex (Figure 1). Due to the characteristics of the two plates upon which the live BSFL were provided, all birds in the two experimental groups, LM and LF, had equal access to larvae during their administration. Therefore, we consider all birds to have eaten the same quantity of BSFL.

3.1 | Behavioural observations

The behavioural observations made in the morning (9 AM), during live BSFL provision (11 AM), and in the afternoon (4 PM) are presented in Tables 2 and 3 and in Figures 2 (F2), 3 (F3), and 4 (F4).

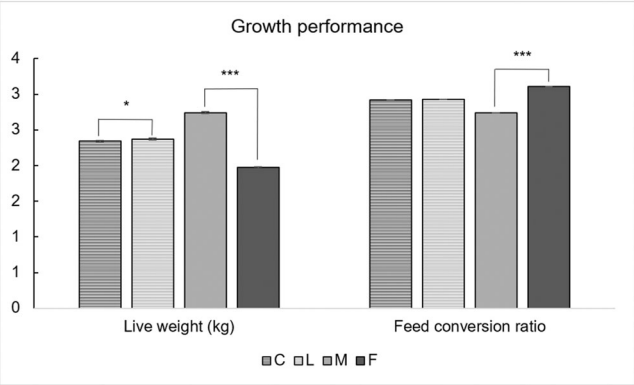

FIGURE 1 The effects of the provision of live black soldier fly larvae on live weight and feed conversion ratio observed in male (M) and female (F) medium-growing hybrid label naked neck chickens ($n = 6$). C, control groups; L, groups supplemented with live black soldier fly larvae; M, males; F, females. *Indicates significant differences within the times at $p \leq 0.05$. ***Indicates differences within the times at $p < 0.001$.

3.1.1 | Foraging-related behaviour

The morning foraging-related behaviour was influenced by the D × S and D × T interactions. CM showed more ground pecking behaviour than LM (6.04 vs. 4.20, $p < 0.001$) (F2Am). Furthermore, fewer L birds performed ground pecking than C birds at T0 (5.25 vs. 10.81, $p < 0.001$), whereas no differences were observed between the L and C birds at T3 and T4 (F2Bm). By contrast, the administration of live BSFL significantly decreased scratching behaviour in both males and females ($p < 0.001$) (Table 2).

An increase in afternoon ground pecking was recorded in sampling periods T3 and T4 than T0 in the supplemented birds only ($p < 0.05$), (T3 vs. T0: 11.25 vs. 4.48, $p = 0.001$; T4 vs. T0: 10.31 vs. 7.47, $p < 0.001$) (F2Al).

At T0, the frequency at which birds in the L groups pecked at objects ("pecking object" frequency) was actually lower than that observed for C birds ($p < 0.001$) (F2Bl); and it was greater in LM than in LF ($p < 0.05$) (F2Cl). Similarly, the scratching frequency was also affected by the interaction D × S, being higher in LF than in LM ($p < 0.05$) (F2Dl), and it was again greater in the C groups than in the L groups at T0 (2.73 vs. 0.79, $p < 0.05$). However, by T3 the trend had reversed, being significantly greater in the supplemented birds (0.27 vs. 1.46, $p < 0.05$) (F2El).

Overall, the foraging-related behaviours in the afternoon were influenced by the diet and by the D × S, and D × T interactions. The CF birds showed a higher ground pecking frequency than the CM birds (6.19 vs. 4.38, $p = 0.001$), whereas no differences were noted between males and females in the experimental group receiving live BSFL supplementation (F2Aa). Scratching behaviour was higher in the L groups with respect to the C groups, at T3 only ($p < 0.01$) (F2Ba). Finally, the larva consumption frequency decreased between T3 and T4 ($p = 0.05$) (Table 2).

3.1.2 | Comfort behaviour

The frequencies of comfort behaviours were less than 0.5 at all three daily observation times; therefore, this behaviour was not analysed.

3.1.3 | Activity behaviour

The diet affected the morning walking behaviour of the female birds, with more LF birds showing walking behaviour than the CF ones ($p = 0.010$) (F3Am). However, the walking and the standing frequencies were both significantly lower at T3 with respect to T0 independently of the diet ($p < 0.001$) (Table 2), whereas the L birds overall displayed a greater standing behaviour than the C ones ($p < 0.05$) (Table 2). The resting frequency was significantly lower in the females than in the males ($p < 0.01$) at all sampling periods (both in the morning and during larvae provision) (Table 2). More CM than CF birds were observed in the outdoor enclosure (1.62 vs. 0.31, $p < 0.001$), with no recorded differences between LM and LF (F3Bm). On the other hand, a greater number of LF than CF birds were recorded to enter the outdoor space during the morning observation (0.99 vs. 0.31, $p < 0.01$) (F3Bm).

During live BSFL provision, the standing frequency was higher in the LM groups than in the LF ones (22.78 vs. 11.32, $p < 0.05$) (F3Al). Furthermore, the standing frequency was overall higher at T0 and at T3 than at T4 ($p < 0.01$) (Table 2), whereas the frequency of resting behaviour increased as the birds became older ($p < 0.001$) and was greater in male than in female birds ($p < 0.001$) (Table 2). Finally, during larvae administration, more C birds were recorded to explore the outdoor enclosure than L birds, albeit only at T4 ($p < 0.01$) (F3Bl).

The frequency of afternoon walking activity decreased in all the groups as the birds became older ($p < 0.001$) (Table 2). Moreover, afternoon walking was significantly less frequent in the CM chickens than in the LM (13.66 vs. 22.20, $p < 0.05$) (F3Ba). Similarly, the frequency of standing activity decreased with age in both the C (at all sampling times) and L groups (between T3 and T4 only) ($p < 0.05$), and standing was more frequent in L birds than in C birds at T4, while the opposite was true at T0 ($p < 0.001$) (F3Aa). As far as use of the outdoor enclosure is concerned, in the C groups more female birds were recorded to use the area than male birds (CF vs. CM: 5.52 vs. 0.62, $p < 0.01$), whereas the opposite trend was true for the experimental groups (LF vs. LM: 0.58 vs. 2.38, $p < 0.05$) (F3Ca).

3.1.4 | Social behaviour

The frequencies of social behaviours were all less than 0.5 at all three daily observation times; therefore, these behaviours were not subjected to analysis.

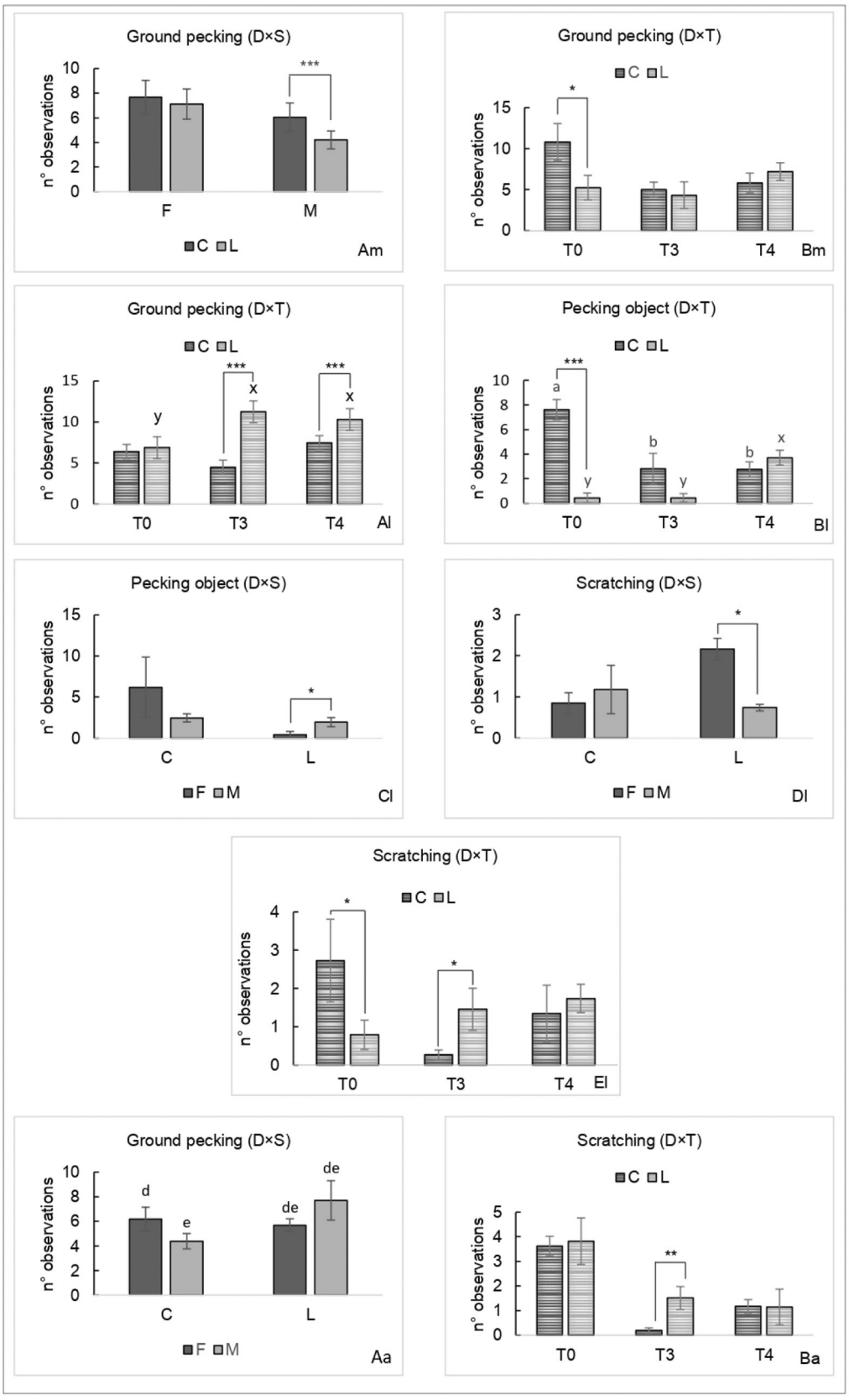

FIGURE 2 (See caption on next page).

3.2 | Ethological tests and animal-based welfare measurements

3.2.1 | Feather condition, leg health, and skin lesion scores

Damage to the birds' feathers, legs, feet, and skin occurred at an average frequency of less than 0.5 times; therefore, these aspects were not considered for statistical analysis.

3.2.2 | AD test

The results of the AD test are reported in Table 3 and Figure 4. Since no chickens were observed within a distance of 1 m from the operator, these data were not subjected to statistical analysis. Moreover, the data for a distance greater than 2 m are not shown since they are complementary to (i.e., the opposite of) the data pertaining 1–2 m.

A greater number of males than females were always recorded within 1–2 m from the operator regardless the diet (4.83 vs. 2.48, $p < 0.001$), except at T3 when no differences between males and females were found (F4A). Moreover, the live BSFL supplementation increased the number of females remaining within 1–2 m from the operator (CF vs. LF: 1.74 vs. 3.52) ($p < 0.01$) (F4B).

3.2.3 | TI test

At T0, a lower percentage of L birds remained immobilised at the first TI attempt compared with C birds (100 vs. 94.27) ($p < 0.05$) (F4C). The TI duration increased between T1 and T3 in both sexes and groups ($p < 0.001$) (Table 3). Only at T0 did the L birds show a longer TI duration than C birds (1.58 vs. 1.16 min) ($p < 0.01$) (F4D).

3.2.4 | Excreta corticosterone metabolites

Both sex and time, but not the diet, had significant effects on the ECM content, which was lower in the females than in the males (36.6 vs. 40.1 ng/g) and decreased over time ($p < 0.001$) (Table 3). No significant correlations were found between the TI duration and the ECM content (data not shown).

3.2.5 | Heterophile/lymphocyte ratio

The H/L ratio was lower in the C groups than in the L groups (0.91 vs. 1.27) ($p = 0.050$), whereas no significant differences were detected for the other fixed and interaction factors (Table 3).

4 | DISCUSSION

Bongiorno et al. (2022) reported how live BSFL supplementation did not impair the growth and slaughtering performance of the birds, with benefits on hepatic function and the immune system. The present research, carried out on the same birds as in the above-cited study, investigated the effects of live insect administration on the behaviour and welfare of this medium-growing chicken genotype.

4.1 | Behavioural observations

4.1.1 | Foraging behaviour

Live BSFL provision was associated with a decrease in morning ground pecking in male groups and a decrease in morning ground scratching frequency in all birds. These findings contrast with those reported by Biasato et al. (2022), Ipema et al. (2020a), and Ipema et al. (2022) for broiler chickens, and by Veldkamp and van Niekerk (2019) for turkeys. The reduced foraging activity observed in these birds in the morning may reflect their possible anticipation of the live BSFL provision at 11 AM, which was able to fully satisfy their behavioural needs.

Interestingly, ground pecking frequency was enhanced during the live BSFL administration, thus suggesting an increase in exploratory behaviour over a short-term period. This finding points towards the potential for further enhancements in terms of behaviour and well-being should larva provision be increased to several times a day (Ipema et al., 2020b). The higher scratching frequency of LF than LM groups may be reconducted to sex differences and how live BSFL administration can interact with them. The in-field observations executed by Collias and Collias (1967) on Red Jungle Fowl, underlined namely the inspection of the surrounding food-sources by a dominant male, which would be a trigger for more intense exploration activity by the hens in the cockerel's wake during their search for food. Veldkamp and van Niekerk (2019) found no significant changes in exploratory activity

FIGURE 2 The effects of the provision of live black soldier fly larvae on foraging-related behaviours performed by male (M) and female (F) medium-growing hybrid label naked neck chickens in relation to the interactions between diet (D), sex (S), time (T), in the morning (m), during BSFL provision (l), and in the afternoon (a) ($n = 4$). diet × time (D × T), diet × sex (D × S), and sex × time (S × T); T0, time 0; T1, time 1; T2, time 2; C, control groups; L, groups supplemented with live black soldier fly larvae. The letters A, B, C, D and E are reported to uniquely identify each graph. *Indicates significant differences within the times at $p \leq 0.05$. **Indicates significant differences within the times at $p < 0.01$; ***Indicates differences within the times at $p < 0.001$. The superscript letters a, b and c indicate significant differences between the female groups or control groups at different times; the letters x, y and z indicate significant differences between the male groups or groups supplemented with live black soldier fly larvae at different times; the letters d, e and f indicate differences among the CF, LF, CM and LM groups at $p \leq 0.05$.

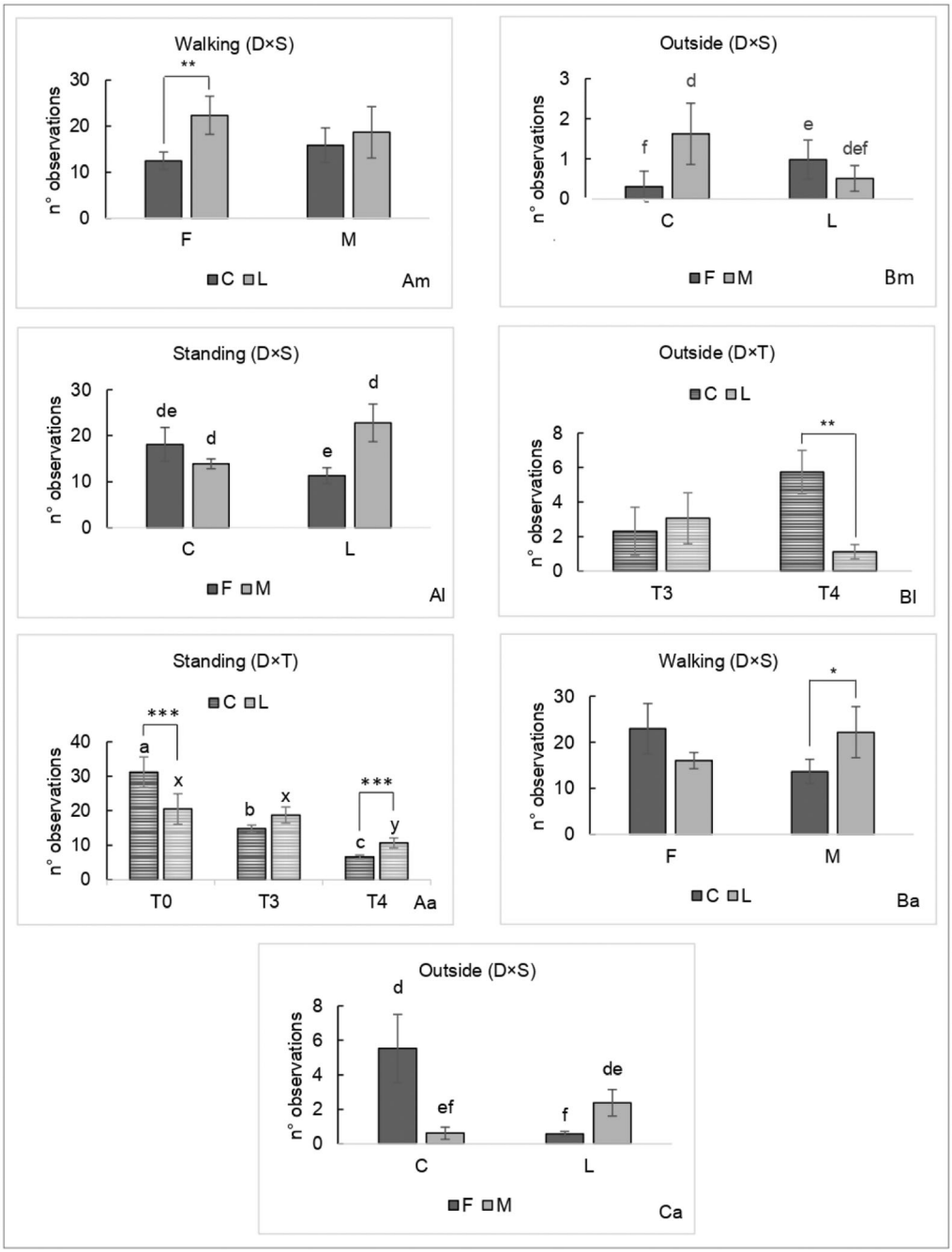

FIGURE 3 The effects of the provision of live black soldier fly larvae on the activity behaviours performed by male (M) and female (F) medium-growing hybrid label naked neck chickens in relation to the interactions between diet (D), sex (S), time (T), in the morning (m), during BSFL provision (l), and in the afternoon (a) ($n = 4$). diet × time (D × T), diet × sex (D × S), and sex × time (S × T); T0, time 0; T1, time 1; T2, time 2; C, control groups; L, groups supplemented with live black soldier fly larvae. The letters A, B and C are reported to uniquely identify each graph. *Indicates a significant difference within the times at $p \leq 0.05$. **Indicates a significant difference within the times at $p < 0.01$; ***Indicates a difference within the times at $p < 0.001$. The superscript letters a, b and c indicate significant differences between the female groups or control groups at different times; the letters x, y and z indicate significant differences between the male groups or groups supplemented with live black soldier fly larvae at different times; the letters d, e and f indicate differences between the CF, LF, CM and LM groups at $p \leq 0.05$.

following the provision of live larvae, whereas other studies (Biasato et al., 2022; Ipema et al., 2020a, 2020b) found provision to stimulate foraging behaviour. The data from our study support the latter effect. A greater number of L birds were observed to engage in scratching at T2, although the opposite was true at T0, when the birds had not yet been exposed to the larvae. Similarly, a greater scratching frequency was observed in the L group than in the C groups at T3 (afternoon). These findings underline how enrichment with live BSFL can foster the expression of natural bird behaviours, albeit starting from opposite trends or absence of

TABLE 2 The effects of the provision of live black soldier fly larvae on the behaviours of male (M) and female (F) medium-growing hybrid label naked neck chickens in relation to diet (D), sex (S), time (T), and their interactions, in the morning (m), during BSFL provision (l), and in the afternoon (a) ($n = 4$).

Class	Behaviour (no. birds)	Diet (D)		Sex (S)		Time (T)			SEM			*p*-value					
		C	L	F	M	T0	T3	T4	D	S	T	D	S	T	D × S	D × T	S × T
During the morning																	
FRB	Ground p.	6.80	5.47	7.39	5.04	7.53	4.65	6.48	0.79	1.09	1.32	<0.001	0.124	0.363	0.004	0.005	<0.001
	Object p.	1.73	1.91	1.83	1.80	2.43	1.30	1.89	0.71	0.71	0.71	0.816	0.923	0.075	0.010	0.462	0.234
	Scratching	2.57	1.24	3.48	0.91	1.53	1.30	2.87	0.61	0.71	0.88	0.013	<0.001	0.100	0.148	0.123	<0.001
CB	Preening	4.35	4.43	4.90	3.94	3.38	4.86	5.16	0.64	0.58	0.90	0.926	0.096	0.114	0.227	0.104	0.651
AB	Walking	14.1	20.4	16.7	17.2	29.3^{a}	14.9^{b}	11.9^{b}	2.91	3.50	4.12	<0.001	0.919	<0.001	0.020	0.587	0.674
	Standing	11.6	19.4	14.3	15.8	28.9^{a}	11.4^{b}	10.3^{b}	2.07	2.79	3.01	0.012	0.754	<0.001	0.076	0.597	0.947
	Resting	7.97	9.03	7.19	10.0	8.72	9.03	7.76	2.17	2.09	2.32	0.494	0.004	0.753	0.482	<0.001	0.096
	Outside	0.71	0.71	0.55	0.91	–	1.71^{a}	0.30^{b}	0.41	0.37	0.64	1.00	0.568	0.003	<0.001	0.546	0.972
During live larvae provision																	
FRB	Ground p.	5.98	9.27	7.01	7.92	6.63	7.10	8.77	0.70	0.73	1.15	0.009	0.478	0.320	0.054	0.049	<0.001
	Object p.	3.89	0.91	1.61	2.19	1.84^{ab}	1.12^{b}	3.19^{a}	0.61	0.69	0.55	0.016	0.663	<0.001	0.042	<0.001	0.029
	Scratching	1.00	1.26	1.35	0.93	1.47^{ab}	0.63^{b}	1.53^{a}	0.22	0.13	0.41	0.463	<0.001	<0.001	0.010	<0.001	0.006
	Eating larvae	–	13.5*	14.4	12.6	–	14.5^{a}	12.4^{b}	–	1.21	0.96	–	0.401	0.050	–	–	0.304
CB	Preening	2.09	2.02	2.39	1.77	1.19^{b}	3.56^{a}	2.05^{b}	0.28	0.27	0.48	0.886	0.179	0.002	0.562	0.100	0.351
AB	Walking	22.7	27.7	24.1	26.1	26.7	25.8	23.0	1.88	3.04	4.13	0.113	0.729	0.456	0.211	0.852	0.006
	Standing	15.9	16.1	14.3	17.8	23.8^{a}	17.1^{a}	10.0^{b}	1.31	1.70	2.87	0.926	0.223	0.007	0.007	0.710	0.889
	Resting	6.90	7.95	5.28	10.4	3.49^{c}	12.8^{a}	9.11^{b}	1.17	0.40	1.02	0.650	<0.001	<0.001	0.971	<0.001	0.324
	Outside	3.64	1.82	4.16	1.62	–	2.66	2.54	0.73	1.03	0.82	0.129	0.139	0.940	0.904	0.001	0.102
During the afternoon																	
FRB	Ground p.	5.21	6.62	5.93	5.81	7.63	4.98	5.33	0.76	0.43	0.97	0.341	0.871	0.083	0.001	0.215	<0.001
	Object p.	1.17	1.14	1.28	1.04	3.44^{a}	0.65^{b}	0.69^{b}	0.22	0.21	0.40	0.935	0.543	<0.001	0.926	<0.001	0.427
	Scratching	0.93	1.87	2.01	0.87	3.72^{a}	0.54^{b}	1.16^{b}	0.33	0.32	0.35	<0.001	<0.001	<0.001	0.285	<0.001	<0.001
CB	Preening	3.41	3.35	4.07	2.81	2.81	3.76	3.66	0.37	0.47	0.63	0.866	0.047	0.446	0.149	0.571	0.899
AB	Walking	17.7	18.86	19.2	17.4	34.3^{a}	20.0^{b}	8.89^{c}	1.92	2.75	3.11	0.748	0.736	<0.001	0.013	0.326	0.100
	Standing	14.5	16.01	14.9	15.6	25.3^{a}	16.6^{b}	8.40^{b}	1.22	2.21	1.78	0.059	0.850	<0.001	0.121	<0.001	0.537
	Resting	6.37	8.19	7.39	7.06	2.46^{c}	14.6^{a}	10.5^{b}	1.12	1.03	0.98	0.128	0.695	0.003	0.312	0.316	0.022
	Outside	1.85	1.17	1.79	1.21	–	2.12^{a}	1.03^{b}	0.52	0.43	0.46	0.334	0.195	0.004	<0.001	<0.001	0.347

Note: diet × time (D × T), diet × sex (D × S), and sex × time (S × T); C, control groups; L, groups supplemented with live black soldier fly larvae; T0, time 0; T3, time 3; T4, time 4; p, pecking. *Arithmetic mean of both sex and times. The superscript letters a, b and c indicate significant differences at $p \leq 0.05$.
Abbreviations: AB, activity behaviour; CB, comfort behaviour; FRB, foraging-related behaviour; SEM, standard error of the mean.

differences before the live BSFL provision onset. Furthermore, greater ground pecking was noted in the CF groups than in the CM groups during the same period of the day, while no differences were detected between the sexes in the supplemented groups. The lack of a difference between sexes in the L groups is probably caused by the stimulation of exploration activity generated by the presence of live BSFL, although the high SEM in the LM groups could be responsible for the insignificant differences observed between the LM and LF groups. Finally, the reduction in larva consumption frequency between T3 and T4 could be attributed to an overall reduction in bird activity over time, as well as to changes in the weather since the trial was conducted in the autumn and winter and larva motility decreases at lower temperatures, thus making them less attractive. In fact, during the trial, the average environmental temperature was 12.8°C (min: 5°C; max: 22°C) in October 2021 versus 7.6°C (min: −1°C; max: 16°C) in November 2021; and the

TABLE 3 The effects of the provision of live black soldier fly larvae on the ethological tests and animal-based welfare measurements performed on male (M) and female (F) medium-growing hybrid label naked neck chickens in relation to diet (D), sex (S), time (T), and their interactions ($n = 6$).

	D		S		T					SEM			*p*-value					
Items	C	L	F	M	T0	T1	T2	T3	T4	D	S	T	D	S	T	D × S	D × T	S × T
AD test, birds in 1–2 m	2.90	4.13	2.48	4.83	1.74^{c}	5.63^{a}	–	5.34^{a}	2.75^{b}	0.25	0.16	0.48	0.003	<0.001	<0.001	<0.001	0.982	<0.001
TII at first attempt, %	95.6	96.4	98.6	93.4	92.7^{b}	97.1^{ab}	–	97.2^{a}	97.1^{b}	1.78	1.89	2.14	0.649	0.076	0.025	0.141	0.022	0.961
TID, min	2.11	2.41	2.18	2.33	0.96^{c}	1.36^{b}	–	4.38^{a}	4.55^{a}	0.17	0.15	0.25	0.336	0.571	<0.001	0.215	<0.001	0.025
ECM, ng/g	38.6	38.1	36.6	40.1	46.0^{a}	38.9^{b}	–	–	31.5^{c}	1.26	1.21	1.78	0.662	<0.001	<0.001	0.219	0.098	0.413
H/L ratio	0.91	1.27	1.06	1.10	–	–	–	–	–	0.13	0.13	–	0.050	0.816	–	0.722	–	–

Note: C, control groups; L, groups supplemented with live black soldier fly larvae; T0, time 0; T1, time 1; T2, time 2; T3, time 3; T4, time 4; diet × time (D × T), diet × sex (D × S), and sex × time (S × T). The superscript letters a, b and c indicate significant differences at $p \leq 0.05$.

Abbreviations: AD, avoidance distance test; ECM, excreta corticosterone metabolites; H/L, heterophile/lymphocyte ratio; SEM, standard error of the mean; TI; tonic immobility; TID, tonic immobility duration.

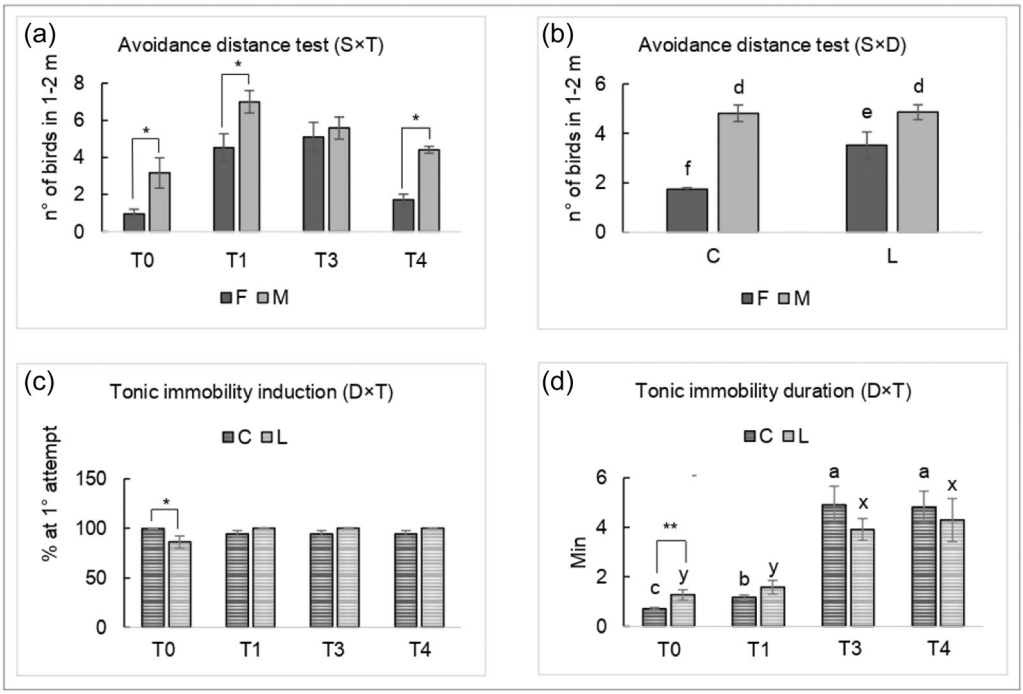

FIGURE 4 The effects of the provision of live black soldier fly larvae on the ethological tests performed by male (M) and female (F) medium-growing hybrid label naked neck chickens in relation to the interactions between diet (D), sex (S), and time (T) ($n = 6$). diet × time (D × T), diet × sex (D × S), and sex × time (S × T); T0, time 0; T1, time 1; T2, time 2; T3, time 3; C, control groups; L, groups supplemented with live black soldier fly larvae. The letters A, B, C and D are reported to uniquely identify each graph. *Indicates significant differences within the groups at $p \leq 0.05$; **Indicates significant differences within the groups at $p \leq 0.01$. The superscript letters a, b and c indicate significant differences between female groups or control groups at different times; the letters x, y and z indicate significant differences between male groups or groups supplemented with live black soldier fly larvae at different times; the letters d, e and f indicate differences among the CF, LF, CM and LM groups at $p < 0.05$ and F and M groups over time.

average relative humidity was 73.5% (min: 52%; max: 95%) in October versus 70.5% (min: 41%; max: 100%) in November 2021, Moreover, a repeated and portioned larvae distribution through the day would have increased the larva consumption frequency and should be considered in future studies.

4.1.2 | Activity behaviour

A higher walking frequency was noted in the LF versus CF groups (morning) and in the LM versus CM groups (afternoon) as a consequence of the increase in bird activity following the start of

live BSFL provision, sustaining the findings of other authors (Biasato et al., 2022; Ipema et al., 2020a, 2022). Furthermore, a greater number of L birds displayed longer standing behaviour than C birds (morning), and during the larva administration more LM birds were observed in a standing position than LF birds. Since male birds reach heavier weights than females and tend to behave as regular broilers as their weight increases, thus reducing their activity levels, the administration of live BSFL could be a promising tool for counterposing the reduction in activity in these birds. Despite this enhancement in standing activity, the overall walking frequency decreased as the chickens became older (morning, afternoon) as did the standing frequency (morning, during live BSFL provision, afternoon), probably due to the weight the birds had gained (Bokkers & Koene, 2003; Jacobs et al., 2021). Moreover, the resting frequency during larva administration increased in both males and females as the birds got older. This might seem surprising as insect provision would be expected to stimulate greater activity in the birds. However, one must bear in mind that the frequency indicates the number of times in which a certain type of behaviour is expressed; therefore, the increased resting frequency, which followed a previous state of active behaviour, is probably linked to the consumption of live BSFL. Thus, resting frequency might even provide an indicator of prior bird activity. We considered behavioural frequencies in the present study but did not collect information on the time budget (due to technical limitations related to the low camera resolution), which would have permitted an analysis of the actual amount of time spent performing each activity. Curiously, more LF birds were observed to enter the outdoor enclosure than CF birds (both morning and afternoon), thus suggesting an enhancement in the explorative activity of the females related to the live BSFL supplementation. At T4, more C birds were recorded to go outside at the time of live BSFL provision than L birds. This might represent an anticipatory behaviour in L birds related to larva administration.

To conclude the interpretation of the bird activity levels, a limitation of this work must be addressed. Since we considered the frequencies of the activities performed by the birds, we could not directly compare the data to those on the behavioural budget of chickens available in literature. However, meaningful considerations can nonetheless be drawn from our data. The number of observations recorded varied by approximately 30% when a statistically significant difference was found, and in some cases a two- or even threefold difference was revealed between groups. The magnitude of these differences sustains the biological relevance of our findings, held in consideration for the genotype reared, the number of birds available for analysis, their already good basal welfare conditions, the duration of the observations, and total number of observations, which encompassed at least ten events per recording period.

4.1.3 | Social behaviour

The diet did not affect the social behaviour of the birds. Indeed, the absence of any negative effects upon the chickens' social patterns should be recognised here as a positive result. In our experimental set-up, the provision of live BSFL did not increase the incidence of aggressive or competitive behaviour in the birds, which might have occurred to ensure their access to the larvae. However, due to the high standard deviations, some of the results obtained may not be significant. This situation may derive from the behavioural variations identified between and within the groups, which could be ascribable to the different personalities in the chickens present in the groups (Rufener et al., 2018; Sibanda et al., 2020; Taylor et al., 2017).

4.2 | Feather condition, leg health, and skin lesion scores

Since all birds had a good basic welfare status, no welfare issues related to feather condition, leg health, or skin lesions occurred, and all the scores were close to zero. Indeed, the low rearing density used here might be considered a limitation of the study as it removed the possibility to observe a potential beneficial effect of live BSFL supplementation on these welfare-related variables. It is broadly recognised that optimal basal welfare conditions diminish the effect of stocking density on lameness (Dawkins et al., 2004) and improves leg health (Hall, 2001) and feather condition (van Hierden, 2003).

4.3 | AD test

In this research, we needed to adapt the Welfare Quality® protocol to our structure and the hybrid used, which is more reactive than fast-growing broilers. Due to the larger pen area provided to each bird compared with those adopted in commercial intensive farming systems, we expected that very few would remain within 1 m of the operator during the avoidance test, which was duly confirmed. However, we noticed a clear distinction between individual bird behaviours, since some of them were more prone to explore the "novelty" element of the pen—namely, the operator squatting on the floor. This created the need to insert the 1–2 m and over 2 m distances classes. Remaining within the 1–2 m location is not necessarily a positive or a negative sign in itself, but it assumes a positive meaning in this context since the birds encountered a human presence on a daily basis, both for their general management and during live BSFL administration. Thus, this relative vicinity of the birds to the squatting operator may reflect their habituation to the presence of humans. Several levels of fear can be observed in poultry, depending on their breed, environmental conditions, and health status, although a basic degree of avoidance and phobia have been recognised as positive welfare indicators inherited from Jungle fowl as a defence against predator attacks (Linares & Martin, 2010). Therefore, the absence of chickens within 1 m from an operator should be considered healthy bird behaviour (Muir et al., 2008).

We observed some interesting results in the 1–2 m AD test. First, compared with the female birds, males expressed higher levels of confidence (T0, T1, T4). This is probably linked to greater ancestral

audacity in males to explore a surrounding environment. This notion derives from the in-field study conducted by Collias and Collias (1967), which found that the exploring-scheme of a flock is determined by the dominant cockerel, who walks in a leader's position through the jungle with his hens behind him. Moreover, hens, being responsible for brooding and offspring protection, might be expected to exhibit more prudent behaviour. This might explain why we noted less confidence in the females towards the operator than in the males, despite all birds being subject to the same bird management practices. Nevertheless, the LF birds were found to move closer to the operator than the CF birds, demonstrating the dietary supplementation to have a positive effect on the human-animal relationship, helping their innate fear to be overridden and permit explorative behaviour, a fundamental objective of welfare-respectful rearing systems (Meuser et al., 2021). The present finding has positive implications for "on-farm" contexts, where operators interact with animals daily, and which might be experienced as a stressor by the birds. However, the positive association of humans with a reward, whether supplied by the operator directly or via an automatic system combined with the visual presence of the operator, could be beneficial for the regular daily management of the birds, with positive outcomes not only on bird welfare but even on production. Our hypothesis is that once the females recognise humans as a source of feed, they might be more motivated to approach them due to their instinct to identify and select feed sources for their future offspring during the parental feeding (Stokes & Williams, 1971).

4.4 | TI test

The TI test is widely used as an indicator of fear in birds (Ipema et al., 2020a). The administration of live BSFL had no effect on the duration of TI, whereas an increase in TI duration was observed at the consecutive experimental time points in both sexes. Similar observations were made for broiler chicken breeders (Brake et al., 1994) and White Leghorns (Campo & Carnicer, 1993). Brake et al. (1994) assumed that the higher weight and the reduced activity level of adult birds were responsible for the prolonged TI over time, a hypothesis which is also consistent with our findings.

4.5 | Excreta corticosterone metabolites

ECM analysis constitutes a reliable method for evaluating stress in birds (Touma & Palme, 2005) since it does not require the birds to be subjected to any invasive procedure (Weimer et al., 2018). In our study, live BSFL supplementation did not cause any variation in the ECM value, although it was lower in the females than in the males, which might be related to a greater level of vigilance in the males than in females. Similarly, Hirschenhauser et al. (2012) and Touma and Palme (2005) indicated sex to be a relevant factor that affects the corticosterone metabolites in droppings, with differences in the excretion peaks and compositions. Moreover, the ECM value subsided with increasing age, probably due to the birds' progressive habituation towards human contact, although further research on this topic is needed.

4.6 | H/L ratio

The H/L ratio is a consolidated indicator of stress in poultry (Gross & Siegel, 1983; Mahboub et al., 2004). Surprisingly, the H/L ratio of the birds in the current work was lower in the C groups than in the L groups ($p = 0.050$). However, considering the absence of exposure of the chickens to intense and prolonged stress conditions, the results of our research might not be related directed to a negative bird experience. On the other hand, a negative effect related to the competition for access to the larvae cannot be ruled out despite no aggressive or competing interactions being observed during the video recordings. Another explanation could lie in the anticipatory behaviour of birds, with stress being positively associated with live larvae provision. Overall, this result sheds light on some uncharted aspects about live BSFL provision in poultry production. Indeed, this appealing form of environmental enrichment and its utilisation should be contextualised on the basis of the structural and managerial peculiarities which characterise each commercial setting, and which have the potential to affect bird welfare and health both positively and negatively. Whether dietary supplementation with larvae causes competition and anticipatory behaviour or nor needs to be investigated. In the case of a positive relationship, then the larva inclusion rate and the influence of the number of administrations per day must also be considered. Useful findings might also be derived from a comparison of distribution methods (e.g., fixed larvae dispenser vs scattered larvae), with a special focus on the more active chicken genotypes. Finally, a clarification of the effect of BSFL supplementation on bird stress might be obtained by combining this parameter with other methods for measuring stress indicators, such as the use of thermal imaging cameras (Jacobs et al., 2023; Nicol, 2020).

5 | CONCLUSIONS

The number of birds reared in the study was determined by the production capacity of the pilot larvae plant. However, practical implications and interesting assumptions on a commercial scale can also be addressed. For example, the low stocking density of the birds might have been responsible for the absence of any significant results on plumage integrity and leg health due to the overall high basal welfare conditions of the chickens. On the other hand, the presence of significant results for other parameters (related to exploration and activity levels) provides firm evidence of the beneficial impact of live larvae provision on poultry production. Furthermore, these effects stand to be amplified in rearing conditions where the maintenance of high welfare standards is more challenging. This concept can be

similarly applied to the behavioural observations and ethological tests. For example, in the AD test, testing the birds in a pen where they have enough space to decide if and how close to approach the operator provides valid information about the fear and exploratory readiness of the birds. Indeed, the data collected could be useful for determining the effectiveness of larva provision on farms, where the test might be biased by the high stocking densities (i.e., the impossibility of birds to roam in the barn as they would like to). In conclusion, this research presents novel information regarding the welfare and behaviour of medium-growing chickens fed live BSFL and farmed in a free-range organic system. It must be stated that the hybrid type and rearing system combination is commonly considered as a direct positive indicator of welfare status. However, this perspective does not provide any certainty in terms of animal welfare and expression of the birds' behavioural repertoire. Therefore, measures should be taken to improve the life conditions of medium-growing chicken genotypes. To this regard, we identified positive effects of live BSFL provision on fear regulation in the birds by means of the AD test, as well as an enhancement in the chickens' exploratory (primarily in female birds) and locomotion activities. The results obtained have implications for commercial-scale contexts, and confirm the potential for live BSFL to stimulate and satisfy the behavioural needs of birds, important for maintaining a healthy flock. Moreover, the distribution of live BSFL by farm operators could have a positive effect on human-animal interactions, helping to avoid stress in these animals and thus any negative effects on production.

AUTHOR CONTRIBUTIONS

Francesco Gai, Achille Schiavone, Valentina Bongiorno, Marta Gariglio, and Ilaria Biasato designed the experiment. Valentina Bongiorno, Marta Gariglio, Eleonora Erika Cappone, and Valeria Zambotto oversaw animal rearing and performed the tests and video recordings. Elisabetta Macchi and Isabella Manenti carried out the corticosterone analyses. SB performed the blood analyses. Valentina Bongiorno and Ilaria Biasato performed the statistical analysis. Valentina Bongiorno and Ilaria Biasato wrote the first draft of the manuscript. Achille Schiavone, Manuela Renna, Ilaria Biasato, and Laura Gasco reviewed the manuscript. Achille Schiavone supervised the study. All the authors contributed to the creation of the manuscript and approved the submitted version.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data are provided by the first author (V. Bongiorno) upon reasonable request. The original contributions presented in this research are included in both the article and Supporting Information Materials. Further inquiries can be directed to the corresponding author.

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