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Provision of black soldier fly larvae (Hermetia illucens) in different ways benefits broiler welfare and performance, with largest effects of scattering live larvae

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ABSTRACT

Including black soldier fly larvae (BSFL) in broiler diets has the potential to benefit broiler welfare and increase production performance, but the effects of dietary BSFL likely depend on the way BSFL are provided. In this study we aimed to discern the effects of different BSFL forms and provisioning methods by providing male broilers with no BSFL (CON), processed BSFL meal and oil incorporated in the feed pellets (INC-F), dried BSFL in the feeder on top of the feed (D-F), or dried or live BSFL scattered through the pen (D-S and L-S, respectively), and evaluating various indicators of broiler welfare and production performance. In all dietary BSFL treatments 8% of the total dietary dry matter content was replaced with BSFL. Dried and live larvae were provided in four equal daily portions at 08:00, 11:00, 14:00, and 17:00. Compared to a diet without BSFL, scattering dried or live larvae through the pen increased active behaviors, though only live larvae increased the time broilers spent standing. Broilers in the D-F, D-S and L-S treatments had higher average daily body weight gain during some periods, and they had higher final weights, despite L-S broilers having a lower total dry matter intake than CON broilers. Furthermore, the dry matter conversion ratio of INC-F, D-S and L-S broilers was reduced. At the end of the rearing period, pens in all dietary BSFL treatments had better litter quality than CON pens. Furthermore, food pad dermatitis was less severe for INC-F and D-S broilers than for CON broilers, and for L-S broilers than for broilers in all other treatments, and hock burn severity was less for L-S than for CON broilers. Broiler lameness, cleanliness, plasma natural antibody titers, and whole blood serotonin were not influenced by dietary BSFL treatment. Feather corticosterone concentrations were affected by treatment, though without any significant post-hoc differences. Our results indicate that BSFL meal and oil, and dried and live BSFL are all promising feed ingredients for broilers as they all benefit some aspects of broiler welfare and production performance. Scattering BSFL through the pen results in more welfare benefits than providing BSFL in the feeder, with live BSFL having the most beneficial effects on broiler welfare.

1. Introduction

Black soldier fly larvae (BSFL) are considered a suitable feed ingredient for broilers as they contain sufficient quantities of micro- and macronutrients (e.g., protein, fat, minerals, vitamins and fibers) [1,2]. They can be reared on a wide range of biological waste streams [3,4] and compared to fishmeal and soybean meal BSFL rearing is expected to use less land and water and to produce less greenhouse gas emissions, thereby contributing less to global warming [5,6]. Additionally, BSFL contain compounds with prebiotic and/or antibiotic functions, such as chitin and antimicrobial peptides, that could benefit broiler immunity and intestinal functioning [1,7,8].

Before August 2021 only whole live insects and insect fat could be included in livestock feed in the EU, but commission regulation 2021/1372 changed this to also allow processed insect protein to be included in livestock feed. The range of insect forms allowed in livestock feeds is thus expanding, and in the future, it may include other forms such as whole dried larvae. Different insect forms will have varying effects on broiler physiology, behavior, and welfare. For example, dietary full-fat or defatted BSFL meal has been reported to increase broiler body

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weight gain and feed intake, enhance innate immune activity, and stimulate a more diverse caecal microbiota composition [9–12]. Conversely, dietary BSFL oil did not affect broiler body weight gain and feed intake, and it had minimal effects on gastro-intestinal tract development [13–15]. Provision of live BSFL in feeding trays also did not affect body weight gain, but it did cause a more diverse caecal microbiota composition [16,17]. Variability in the observed effects of different BSFL forms will partly be due to different inclusion levels across studies (ranging from 1 to 20%). For example, a meta-analysis on dietary insects for poultry indicated that replacing more than 10% of the diet with insects often reduces poultry body weight gain [18]. Additionally, the different processing methods required for the different BSFL forms play a role. For example, heat treatment reduces moisture content, changes product texture, and can cause lipid oxidation and protein denaturization, which in turn can change the nutrient availability and palatability of BSFL, affecting broiler functioning [19,20]. Processing methods may also alter immunomodulating compounds in BSFL [21], potentially causing differences in immunological response parameters such as natural antibodies. The level of defatting also plays a role, as partially defatted BSFL meal was found to be more digestible than highly defatted BSFL meal for broilers [22].

Not only the BSFL form but also the provisioning method influences how broilers respond to dietary BSFL, especially considering broiler behavior. The fast growth rate of broilers can hinder leg development and reduce their ability to be active [23], and consequently broilers spend up to 70% of their time sitting near the end of the rearing period [24,25]. Many common welfare issues of broilers, such as contact dermatitis and lameness, are partly a result of their low activity levels and are exacerbated by their barren, unstimulating housing environments [26,27]. Incorporating BSFL meal or oil in feed pellets is unlikely to stimulate activity and thereby improve leg health, which is supported by the observation that neither dietary BSFL meal nor BSFL oil influenced the occurrence of foot pad dermatitis [14,28]. Also, providing small amounts of live BSFL in feeding trays once a day stimulated broiler foraging behavior without influencing leg health parameters [29]. In contrast, two recent studies showed that regularly scattering small amount of live BSFL through the pen or providing live BSFL in tubes that had to be manipulated to access the larvae stimulated foraging behavior and activity and, in some cases, reduced the occurrence of activity-related leg problems [30,31]. Moreover, one of these studies demonstrated that broilers with frequent or prolonged access to live BSFL were less fearful [30], suggesting that providing live BSFL also benefits the affective state of broilers and thereby promotes welfare in a broad sense. As such, different BSFL forms and provisioning methods may also differentially influence parameters linked to affective states such as corticosterone and serotonin production [32,33]. Dried larvae are more suitable for commercial use than fresh live larvae, as the latter cannot be stored for long periods of time and their high moisture content may cause feed safety risks related to degradation and microbial spoilage [19]. It is, however, unknown whether offering dried BSFL will have welfare benefits similar to those of live BSFL, as chickens seem particularly attracted to moving prey [34]

Distinct combinations of BSFL forms and provision methods are thus expected to differentially affect broilers. Therefore, the aim of the current study was to determine the effects of different BSFL forms (i.e., BSFL meal and oil incorporated in the feed, dried BSFL, and live BSFL) and different provisioning methods (i.e., in the feeder or scattered across the pen) on various indicators of broiler welfare (e.g., behavior, health, corticosterone and serotonin concentrations) and production performance (e.g., body weight gain, feed intake). We hypothesized that incorporating BSFL meal and oil in the diet and providing dried BSFL in the feeder would be less effective in stimulating broiler activity and therefore less beneficial for welfare than scattering dried or live BSFL through the pen. In addition, we expected that scattering live BSFL would be more attractive to broilers and thereby more proficient in improving broiler welfare than scattering dried BSFL. Furthermore, based on the expected effect on activity, it is possible that the different BSFL supplementations strategies will have differential effects on broiler production performance.

2. Methods

This experiment was carried out at the research facility of For-Farmers (Bathmen, The Netherlands). The experimental protocol was approved by the Animal care and Use committee of Wageningen University & Research, under project license number AVD1040020187184. The protocol was in accordance with the European Directive 2010/63/EU on the protection of animals used for scientific research. The ARRIVE guidelines for reporting animal experiments were accounted for in this study [35].

2.1. Animals, housing, and management

At the start of the experiment 1680 one-day-old male Ross 308 broilers were obtained from a commercial hatchery and randomly distributed across 60 pens at the experimental facility, resulting in 28 broilers per pen. Each pen of 1.45 × 1.45 m contained one feeder (1.42 × 0.2 m), one drinking line containing 6 nipples with cups, and a 1 cm layer of wood shavings. After placement, per pen ten randomly selected broilers without signs of health problems were given a neck tag for individual identification. These broilers were the focal broilers for individual measurements throughout the experiment. Feed and water were available ad libitum throughout the 35-day experiment, and all broilers received routine vaccinations. The lighting schedule was 23L:1D on day 1–2, 20L:4D on day 3–7, 18L:6D on day 8–33 and 20L:4D on day 34–35. The temperature was 34 °C during the first two days, after which it was gradually decreased to 20 °C on day 35.

2.2. Experimental design

The experimental room was divided in 12 blocks of five adjacent pens, and within each block pens were randomly assigned to one of five treatments. Broilers in the control (CON) treatment did not receive any black soldier fly larvae (BSFL). In the four BSFL treatments, 8% of the ingredients from the CON pellets was replaced by BSFL on a dry matter (DM) basis as follows: BSFL meal and oil incorporated in the pellets and thus provided in the feeder (INC-F), dried whole BSFL provided in the feeder (D-F), dried whole BSFL scattered through the pen (D-S), or live BSFL scattered through the pen (L-S). For the INC-F treatment, the ratio between BSFL meal and oil was chosen to have a similar protein to fat ratio as whole BSFL (see Supplementary Tables S1-S3 for the dietary details). The dried and live larvae were provided in equal portions four times a day (08:00, 11:00, 14:00 and 17:00). Protix B.V. (Dongen, The Netherlands) supplied the BSFL meal (ProteinX) and oil (LipidX), and Bestico B.V. (Berkel en Rodenrijs, The Netherlands) supplied the dried and live BSFL. Live larvae were supplied weekly and stored at 10 °C near the pens until provisioning.

All broilers were fed a three-phase diet, with starter feed provided on day 1–9, grower feed provided on day 9–27, and finisher feed provided on day 27–35. The composition of all pellets was adjusted to similar protein, fat, and energy intakes among the dietary treatments based on preliminary analyses of the composition of the applied BSFL forms, assuming DM intake was unaffected by the BSFL supplementation strategy. All dietary treatments were designed to meet or exceed broiler dietary requirements [36].

2.3. Measurements

2.3.1. Home-pen behavior and posture

Eight pens/treatment were included in the home-pen behavioral observations. Before observations, the ten focal broilers per pen were marked with a colored dot (stock marker) for individual identification.

Both behavior and posture of all focal broilers were scored by means of 6-min instantaneous scan sampling on day 12, 23 and 33 (ethogram in Table 1), using a tablet with the program Observer 3.3 (Noldus Information Technology B.V., Wageningen, The Netherlands). Behavior was observed for seven 1-hour periods, starting at 08:00, 09:30, 11:00, 12:15, 14:00, 15:30 and 17:00, resulting in 70 scans/broiler/day. If an observation period included the provisioning of dried or live BSFL, the larvae were provided immediately before the first sampling point of each pen. Four observers simultaneously observed ten pens each, switching pens every observation period. Before observations, all observers had been trained and inter-observer reliability was deemed sufficient (Fleiss kappa > 0.8, [37]).

2.3.2. Litter quality

On day 34 the litter quality was scored according to a protocol adjusted from Van Harn et al. (Table 2) [38]. The level of friability was scored on a scale from 1 (completely friable litter) to 5 (completely clumped litter), and the level of wetness was scored on a scale from 1 (completely dry litter) to 5 (very wet litter).

2.3.3. Visual leg health and cleanliness scores

On day 34 the lameness of four randomly selected focal broilers per pen was assessed by prompting the broilers to walk at least 1 m in the pen and assigning a gait score between 0 (normal, dexterous, and agile walk) and 5 (incapable of walking) [39]. On day 35 all focal broilers were visually scored on several leg health parameters and cleanliness. Foot pad dermatitis on both feet was scored on a scale of 0 (no lesions) to 4 (marked swelling and enlargement of the entire foot pad, necrotic cells covering more than half of the total foot pad area) [39,41]. Hock burn on both hocks was scored on a scale of 0 (no hock burn) to 4 (severe lesions) [39]. Cleanliness of the belly was scored on a scale of 0 (feathers and/or skin completely clean) to 2 (feathers and/or skin have severe discoloration and mattered, clumped feathers of > 10 cm) [40]. Full descriptions of scores are present in Table 2.

Table 1
Ethogram for home-pen behavioral observations. Both behavior and posture were scored at each scan sampling point.

Item	Description
Behavior class	
Eating from feeder	Having head above or in the feeder and/or pecking at feed or larvae in the feeder.
Drinking	Drinking from nipple or cup beneath nipple.
Standing idle and Walking	Standing, walking (locomoting in upright position with a normal speed or quick steps) or shuffling (half standing/half sitting and moving a few steps before sitting down), without performing any other behavior.
Defecating	Excreting feces.
Resting	Sitting with hocks resting on ground without performing any other behavior, possibly with head on the ground or under wing.
Foraging	Performing pecking movements directed at the ground, or scraping the litter with claws, or food running (running with food in beak while pen mates follow and attempt to grab the food item).
Comfort behavior	Grooming of own feathers with beak, or dust-bathing (performed with fluffed feathers while lying, head rubbed on floor, wings opened, scratching at ground, distributing substrate over body).
Stretching	Stretching of wing and/or leg.
Wing flapping	Bilateral up-and-down wing flapping.
Agonistic behavior	Jumping at pen mate, chasing pen mate, threatening pen mate, pecking movements directed at head of pen mate.
Pecking pen mate	Pecking movements directed at the body or beak of pen mate.
Other	Any behavior not mentioned above.
Posture class	
Standing	Hocks not in contact with the litter.
Sitting	Hocks in contact with the litter.

Table 2
Litter quality, visual leg health, and cleanliness scores.

Measure	Score	Description	Reference
Litter quality scores			
Friability	1	Completely friable litter.	[38]
	2	25% of litter is clumped.	
	3	50% of litter is clumped.	
	4	75% of litter is clumped.	
	5	Completely clumped litter.	
Wetness	1	Completely dry litter.	[38]
	2	Mildly moist litter.	
	3	Moist litter.	
	4	Wet litter.	
	5	Very wet litter.	
Visual leg health and cleanliness scores			
Gait	0	Normal, dexterous, and agile.	[39]
	1	Slight abnormality, but difficult to define.	
	2	Definite and identifiable abnormality.	
	3	Obvious abnormality, affects ability to move.	
	4	Severe abnormality, only takes a few steps.	
	5	Incapable of walking.	
Foot pad dermatitis[1]	0	No lesions.	[39]
	1	Raised central pad, reticulate scales are separated, with or without small, black necrotic area(s).	
	2	Marked swelling of the foot pad, black reticulate scales forming scale-shaped necrotic areas, with necrosis evident on less than one-quarter of the total foot pad area.	
	3	Marked swelling and enlargement of the entire foot pad, necrosis extending up to one-half of the total foot pad area.	
	4	Marked swelling and enlargement of the entire foot pad, necrotic cells covering more than one-half of the total foot pad area.	
Hock burn[1]	0	No evidence of hock burn.	[39]
	1	Minimal evidence of hock burn.	
	2	Minimal evidence of hock burn.	
	3	Evidence of hock burn.	
	4	Evidence of hock burn.	
Cleanliness[1]	0	Clean feathers.	[40] modified from [39]
	1	Discolored feathers.	
	2	Severe discoloration and mattered, clumped feathers > 10 cm.	

[1] See reference for detailed illustrations of individual scores.

2.3.4. Immunological and hormonal measures

On day 35 the four focal broilers per pen of which the gait was previously assessed also had blood and feather samples taken for immunological and hormonal measures. Per broiler, 2 ml blood was collected in EDTA-containing tubes. Half of the blood was stored as whole blood at −80 °C until analysis. The other half was centrifuged at 5000 x g for 10 min at room temperature (RT), after which plasma was collected and stored at −20 °C until analysis. Additionally, the second and eight primary feathers of each wing were clipped, and the four feathers from each broiler were collected in a bag and stored in the dark until analysis. All laboratory analyses were performed blind to treatment.

2.3.4.1. Plasma natural antibodies. Plasma was used to determine IgM, and IgG natural antibody (NAb) titers against keyhole limpet hemocyanin (KLH) by ELISA. Natural antibodies are antigen-binding antibodies without known exposure to the antigen, and they play a role in innate immunity [42]. After thawing, plasma was pre-diluted 1/10 for IgM and IgG binding KLH in dilution buffer (PBS containing 0.5% horse serum and 0.05% Tween®20), based on a pilot. Briefly, 96-wells plates were coated with a coating buffer (5.3 g/L Na_2CO_3 + 4.2 g/L $NaHCO_3$, pH

9.6) containing 2 μg/ml KLH. All washing steps were done with tap water containing 0.05% Tween. After washing, plates were incubated for 90 min at RT with a serial 4-step dilution in dilution buffer, resulting in 1:40, 1:160, 1:640 and 1:2560 test dilutions. Duplicate standard positive plasma samples (a pool of male plasmas) were stepwise 1:1 diluted with dilution buffer. After washing again, plates were incubated for 90 min at RT with goat-anti-chicken IgM labelled with horse radish peroxidase (PO) (1:10,000, GASwIgM/PO, Bethyl Laboratories Inc., Montgomery, USA) or goat-anti-chicken IgG labelled with PO (1:10,000, GASwIgGFC/PO, Bethyl Laboratories Inc., Montgomery, USA) in dilution buffer. After washing again, plates were incubated with tetramethylbenzidine for approximately 15 min at RT, after which the reaction was stopped with 1.25 M H_2SO_4. Absorbance was measured with a Multiskan Go (Thermo scientific, Breda, The Netherlands) at 450 nm and expressed relative to that of the standard positive control sample. Antibody titers are $\log_2$ values of dilutions that gave an extinction closest to 50% of E_{max}, with E_{max} representing the highest mean extinction of the standard positive sample present on all plates.

2.3.4.2. Whole blood 5-HT. Whole blood serotonin (5-Hydroxytryptamine, or 5-HT) was measured according to Bolhuis et al. [33]. In short, after thawing 1 ml whole blood was pipetted into 50 ml tubes and 2 ml NaCl solution (9 g/L), 1 ml ascorbic acid solution (3%), 5 ml phosphate buffer (2 M K_2HPO_4, pH 10.0) and 20 ml n-butanol were added. Tubes were shaken for 5 min and centrifuged at 1000 x g for 15 min. Fifteen ml of the butanol layer was pipetted into new tubes after which 2 ml 0.1 M HCl and 25 ml cyclohexane were added. These tubes were shaken for 20 s and centrifuged at 1000 x g for 4 min. The cyclohexane/butanol layer was removed, and 1 ml of the acidic phase was pipetted into a new tube containing 0.3 ml 12 M HCl. Tubes were vortexed shortly and samples were measured at 295/540 nm on the Aminco-Bowman fluorescence spectrofluorophotometer (PerkinElmer Inc., Waltham, USA). Absorbance was compared to a standard curve, and 5-HT levels were expressed as nmol/ml.

2.3.4.3. Feather corticosterone. To extract feather corticosterone (CORT), all feathers were cleaned by placing them in demi-water for 10 s and gently rubbing them with a tissue, after which they were air dried overnight. The calamus, downy bars and tip of the feathers were removed and after this the length of the feathers was determined. Then, the vanes were collected by cutting next to the rachis, and vanes from the four feathers of each broiler were combined and weighed to the nearest 0.1 mg. The vanes were cut in flakes of <3 mm^2 and thoroughly mixed. A sub-sample of approximately 35 mg (weighed to the nearest 0.1 mg) was placed in an Eppendorf tube with 3 metal beads (3.2 mm stainless steel balls, Cat. No. 11079132ss, Biospec Products, Bartlesville, USA). These tubes were dropped in liquid nitrogen for 1–2 min, and immediately thereafter they were placed in a Tissuelyser (Qiagen, Hilden, Germany) at 30 Hz for 5 min. This step was repeated three times. Then, 0.5 ml PBS was added to each sample and the samples were placed in a rotator (IKA Loopster, Staufen, Germany) at 300 rpm for 24 at RT, after which they were frozen at -20 C° until analysis.

After thawing, the PBS extract was centrifuged at 1000 g x for 5 min and the supernatant was pipetted in Eppendorf tubes for analysis. CORT concentrations were determined in duplicate by using a commercial CORT ELISA kit (Enzo Life Sciences, NY, USA) following a standard protocol (see online manual http://www.enzolifesciences.com/ADI-900-097/corticosterone-eia-kit/). The CORT concentrations were expressed as a function of the feather length (pg/mm).

2.3.5. Production performance

At placement and on day 9, 19, 27 and 35 the average weight and feed intake of all broilers were determined on pen level. Additionally, on these days all focal broilers were weighed individually to determine if the focal broilers were representative of the whole pen. Morbidity and mortality were recorded daily.

2.4. Statistical analysis

2.4.1. Data processing

During the experiment, 48 broilers (of which 19 focal broilers) died from health issues (CON $n = 3$, INC-F $n = 8$, D-F $n = 12$, D-S $n = 15$, L-S $n = 10$) and they were excluded from analysis. If a focal broiler died, it was immediately replaced by a randomly selected pen mate, and this was accounted for in the analyses. The behaviors and postures observed in the home-pen were averaged per broiler per day and expresses as a percentage of the total observations for each day. Behaviors that occurred in more than 5% of the observations (comfort behavior, drinking, standing idle and walking, foraging, and resting) were analyzed. Additionally, the behavior "eating from feeder" was analyzed as it is an indicator for pellet intake, and in case of the D-F treatment also for dried larvae intake. Based on the pellet intake measures the average daily dry matter intake in g/broiler/day with and without larvae was calculated, as well as the dry matter conversion into body weight gain. Per broiler only the leg with the most severe foot pad dermatitis or hock burn score was included in the analysis. The score "4″ for both foot pad dermatitis and hock burn was present in less than 1% of the broilers, therefore this score was combined with score "3″ for both parameters. To assure normality of residuals from general linear mixed models, a Grubbs test was applied to all continuous data, and the indicated outliers (mostly deemed a result of sampling errors or health problems) were omitted from analysis of the average daily gain (d1–9 five outliers, d9–19 four outliers, d19–27 five outliers, d27–35 five outliers), the final weight (three outliers), and the feather corticosterone concentration (three outliers) of focal broilers. The focal broilers were deemed a reliable representation of the total pen as treatment effects on the average daily gains and final weights of the focal broilers were similar to that of the total pen weights (Supplementary Table S4).

2.4.2. Data analysis

The statistical software SAS 9.4 (SAS Institute Inc., Cary, NC, USA) was used to analyze the data. All general linear mixed model residuals showed normality, except for the feather CORT concentration, which was ln transformed to achieve normality. All general and generalized linear mixed models included a fixed effect of dietary treatment and a random effect of block, and all models with individual broilers as experimental units additionally included a random effect of pen nested in treatment and block.

The proportion of observations in which the different behaviors and postures were shown were analyzed with generalized linear mixed models (GLIMMIX in SAS) using a binomial distribution, logit link function, and an additional multiplicative over-dispersion parameter. Besides the aforementioned fixed effect of dietary treatment, these models included a fixed effect of day and the treatment by day interaction. Additionally, these models included a repeated effect of day with broiler as subject, using a heterogenous first-order autoregressive covariance structure. The average daily gain and final weight (measured at pen and individual level) and the average daily feed intake, dry matter conversion ratio, plasma antibody titers, whole blood 5-HT concentration, and feather CORT concentration (measured at individual level) were analyzed with general linear mixed models (MIXED in SAS). The model for final weight included d1 wt as covariate. Significant fixed effects were further analyzed using differences in least square means with a Tukey HSD correction

As litter quality scores contained empty subclasses, these scores were analyzed with a Kruskal-Wallis test, and in case of significant treatment effects a Dwass-Steel-Critchlow-Fligner multiple comparisons test was used for pair-wise comparisons. Leg health scores were analyzed with the GLIMMIX procedure using multinomial distribution and cumlogit link, and cleanliness scores were analyzed with the GLIMMIX procedure using a binary distribution and logit link. Significant fixed effects on

health and cleanliness scores were further analyzed using estimate comparisons with Bonferroni correction.

Data are presented as pen means± SEM unless indicated otherwise. Effects were considered significant at $p < 0.05$ and a tendency at $0.05 < p < 0.1$.

3. Results

3.1. Home-pen behavior and posture

The time spent on comfort behavior was only influenced by day ($F_{(2,1150)} = 3.03$, $p = 0.049$, Fig. 1), with no significant post-hoc differences. The time spent on the behaviors eating from feeder, drinking, standing idle and walking, foraging, resting (Fig. 1), and the time spent in standing posture (Fig. 2) were influenced by treatment, day, and the treatment by day interaction, and pairwise significant ($p < 0.05$) differences are described below.

3.1.1. Eating from feeder

The time spent on eating from the feeder was influenced by treatment ($F_{(4,35)} = 28.93$, $p < 0.001$), day ($F_{(2,1150)} = 44.01$, $p < 0.001$, Fig. 1), and the treatment by day interaction ($F_{(8,1150)} = 4.43$, $p < 0.001$). On d12 CON broilers spent more time eating from the feeder than broilers in all other treatments, and broilers in the INC-F treatment spent more time eating from the feeder than broilers in the L-S treatment. On d23 and 33 broilers in the CON, INC-F and D-F treatments spent more time eating from the feeder than broilers in the D-S and L-S treatments. The time CON broilers spent eating from the feeder did not change over time, while it increased from d12 to 23 and d23 to 33 for D-F broilers and it increased from d12 to 33 for INC-F, D-S and L-S broilers.

3.1.2. Drinking

The time spent drinking was influenced by treatment ($F_{(4,35)} = 6.85$, $p < 0.001$), day ($F_{(2,1150)} = 18.23$, $p < 0.001$), and the treatment by day interaction ($F_{(8,1150)} = 3.73$, $p < 0.001$, Fig. 1). On d12 L-S broilers spent less time drinking than all other broilers, and on d23 they spent less time drinking than CON, INC-F and D-S broilers. The time spent drinking did

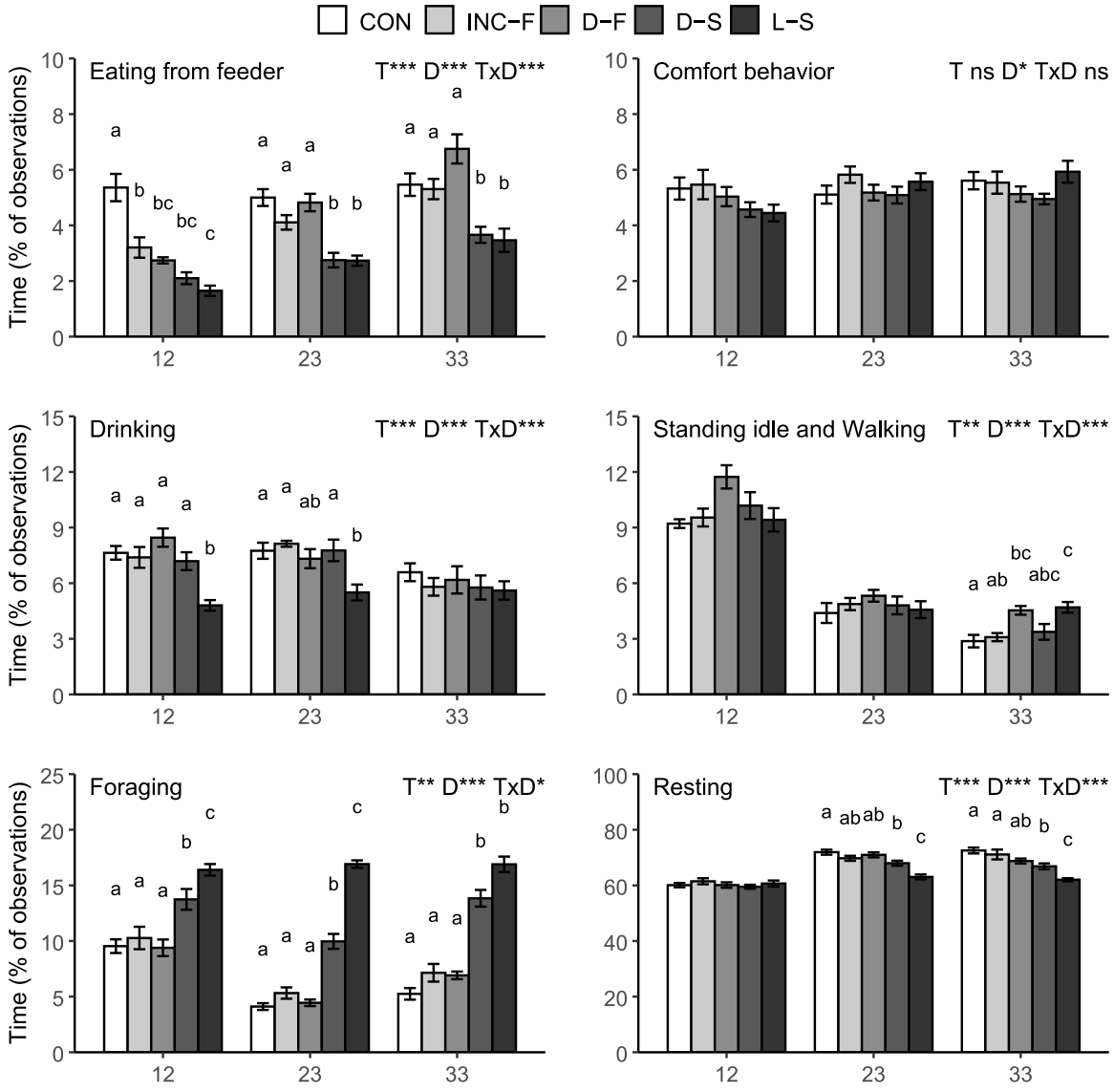

Fig. 1. Home-pen behavior (% of observations) of broilers receiving no larvae (CON), larvae meal and oil incorporated in the feed (INC-F), dried larvae in the feeder (D-F), dried larvae scattered through the pen (D-S), or live larvae scattered through the pen (L-S). Data presented as means± SEM. Effects of Treatment (T), Day (D), and their interaction (TxD) are indicated as ns (not significant), * ($p < 0.05$), ** ($p < 0.01$) or *** ($p < 0.001$). Different letters within one day indicate significant ($p < 0.05$, Tukey's HSD correction) differences between treatments.

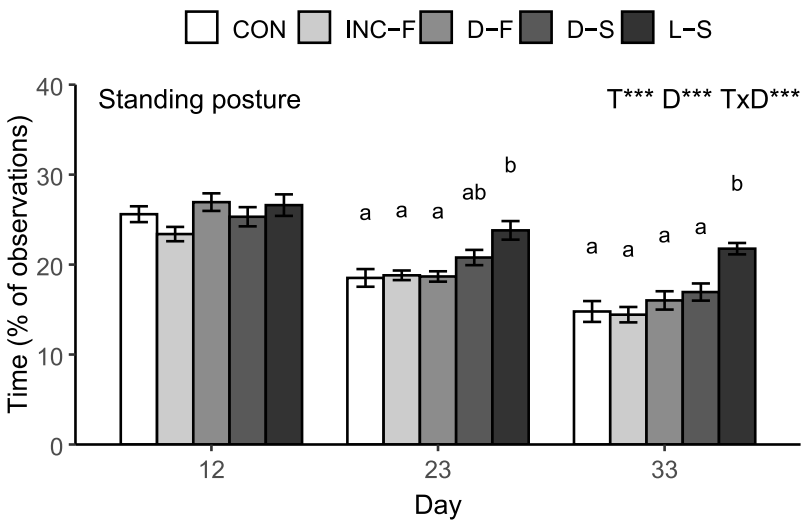

Fig. 2. Time spent in standing posture (% of observations) of broilers receiving no larvae (CON), larvae meal and oil incorporated in the feed (INC-F), dried larvae in the feeder (D-F), dried larvae scattered through the pen (D-S), or live larvae scattered through the pen (L-S). Data presented as means± SEM. Effects of treatment (T), Day (D), and their interaction (TxD) are indicated as *** ($p < 0.001$). Different letters within one day indicate significant ($p < 0.05$, Tukey's HSD correction) differences between treatments.

not differ between treatments on d33. CON and L-S broilers did not change in their time spent drinking over time, while INC-F and D-S broilers spent less time drinking on d33 than on d23, and D-F broilers spent less time drinking on d33 than on d12.

3.1.3. Standing idle and walking

The time spent standing idle and walking was influenced by treatment ($F_{(4,35)} = 5.54$, $p = 0.002$), day ($F_{(2,1150)} = 467.47$, $p < 0.001$), and the treatment by day interaction ($F_{(8,1150)} = 3.36$, $p < 0.001$, Fig. 1). On d12 and 23 the time spent standing idle and walking did not differ between treatments. On d33 CON broilers spent less time standing idle and walking than D-F and L-S broilers, and INC-F broilers spent less time standing idle and walking than L-S broilers. The time spent standing idle and walking decreased from d12 to 23 and d23 to 33 for CON, INC-F and D-S broilers, while it decreased only from d12 to 23 for D-F and L-S broilers after which it stayed constant.

3.1.4. Foraging

The time spent foraging was influenced by treatment ($F_{(4,35)} = 98.81$, $p < 0.001$), day ($F_{(2,1150)} = 129.84$, $p < 0.001$), and the treatment by day interaction ($F_{(8,1150)} = 18.59$, $p < 0.001$, Fig. 1). On all days D-S and L-S broilers foraged more than CON, INC-F and D-F broilers. Additionally, on d12 and 23 L-S broilers foraged more than D-S broilers. L-S broilers did not differ in their time spend foraging on different days. INC-F and D-F broilers spent less time foraging on d23 than d12, and on d33 than d23. CON broilers spent less time foraging on d23 and 33 than d12, and D-S broilers spent less time foraging on d23 than on d12 and 33.

3.1.5. Resting

The time spent resting was influenced by treatment ($F_{(4,35)} = 19.14$, $p < 0.001$), day ($F_{(2,1150)} = 205.04$, $p < 0.001$), and the treatment by day interaction ($F_{(8,1150)} = 10.31$, $p < 0.001$, Fig. 1). On d12 there was no difference in time spent resting between treatments. L-S broilers spent less time resting than broilers in all other treatments on d23 and 33. D-S broilers spent less time resting than CON broilers on d23 and 33 and then INC-F broilers on d33. The time spent resting of L-S broilers did not change over time, whereas in the other treatments it increased from d12 to 23 after which it remained constant.

3.1.6. Standing posture

The time spent in standing posture was influenced by treatment ($F_{(4,35)} = 12.81$, $p < 0.001$), day ($F_{(2,1150)} = 219.61$, $p < 0.001$), and the treatment by day interaction ($F_{(8,1150)} = 6.08$, $p < 0.001$, Fig. 2). Treatment did not influence the time spend in standing posture on d12. On d23 and 33 L-S broilers spent more time standing than CON, INC-F and D-F broilers, while on d33 L-S broilers also spent more time standing than D-S broilers.

3.2. Litter quality

Treatment influenced both litter friability and wetness ($p < 0.001$, Table 3). CON pens had less friable litter than D-F, D-S and L-S pens, and CON pens had wetter litter than pens in all other treatments.

3.3. Visual health and welfare scores

There was a tendency for treatment to affect gait score ($p = 0.098$) and cleanliness ($p = 0.052$, Fig. 3). Foot pad dermatitis was influenced by treatment ($p < 0.001$), with the L-S broilers having less severe foot pad dermatitis scores than broilers in all other treatments, and the INC-F and D-S broilers having less severe foot pad dermatitis scores than CON broilers. Hock burn was also affected by treatment ($p = 0.002$), where L-S broilers had less severe hock burn scores than CON broilers (Fig. 3).

3.4. Immunological and hormonal measures

Feather CORT concentration was affected by treatment ($p = 0.037$), but there were no significant post-hoc differences between treatments. Plasma IgM natural antibody titers against KLH tended to be influenced by treatment ($p = 0.059$). Plasma IgG natural antibody titers against KLH and whole blood 5-HT were not influenced by treatment (Table 4).

3.5. Production performance

The production performance parameters of the entire pens are shown in Table 5. During several days the broilers' average daily gain was influenced by treatment (all $p < 0.01$). During d1–9 D-F and L-S broilers grew faster than CON broilers, with INC-F and D-S broilers in between. During d9–19 D-F, D-S and L-S broilers grew faster than CON and INC-F broilers. During d19–27 the D-F and D-S broilers grew faster than CON broilers, and during d27–35 treatment did not influence broiler average daily gain. The final weight was also influenced by treatment ($p < 0.001$). The final weight of D-F and L-S broilers was higher than that of CON broilers, and the final weight of D-S broilers was higher than that of CON and INC-F broilers.

When calculating the average daily dry matter intake, we assumed that all larvae provided were indeed consumed (Table 5, Supplementary Table S5), though this may not have been the case for all pens (see

Table 3
Frequencies of visual litter quality scores (1 = completely friable or dry litter, 5 = completely clumped or very wet litter) of pens with broilers receiving no larvae (CON), larvae meal and oil incorporated in the feed (INC-F), dried larvae in the feeder (D-F), dried larvae scattered through the pen (D-S), or live larvae scattered through the pen (L-S). Significant treatment effects ($p < 0.05$) are indicated in bold, and within the "sum of scores" rows different superscript letters indicate significant ($p < 0.05$, DSCF correction) differences between treatments.

Measure	Score	CON	INC-F	D-F	D-S	L-S	Test-statistic and df	P-value
Friability score	1	0	0	0	0	0	$H_{(4)} = 23.24$	**<0.001**
	2	1	3	7	3	7		
	3	1	4	5	8	4		
	4	4	4	0	1	1		
	5	6	1	0	0	0		
	Sum of scores[1]	579.5[a]	414[ab]	239.5[b]	341.5[b]	255.5[b]		
Wetness score	1	0	0	0	0	0	$H_{(4)} = 26.54$	**<0.001**
	2	0	4	8	8	2		
	3	3	6	4	3	10		
	4	9	2	0	1	0		
	5	0	0	0	0	0		
	Sum of scores[1]	597[a]	368[b]	234[b]	253[b]	378[b]		

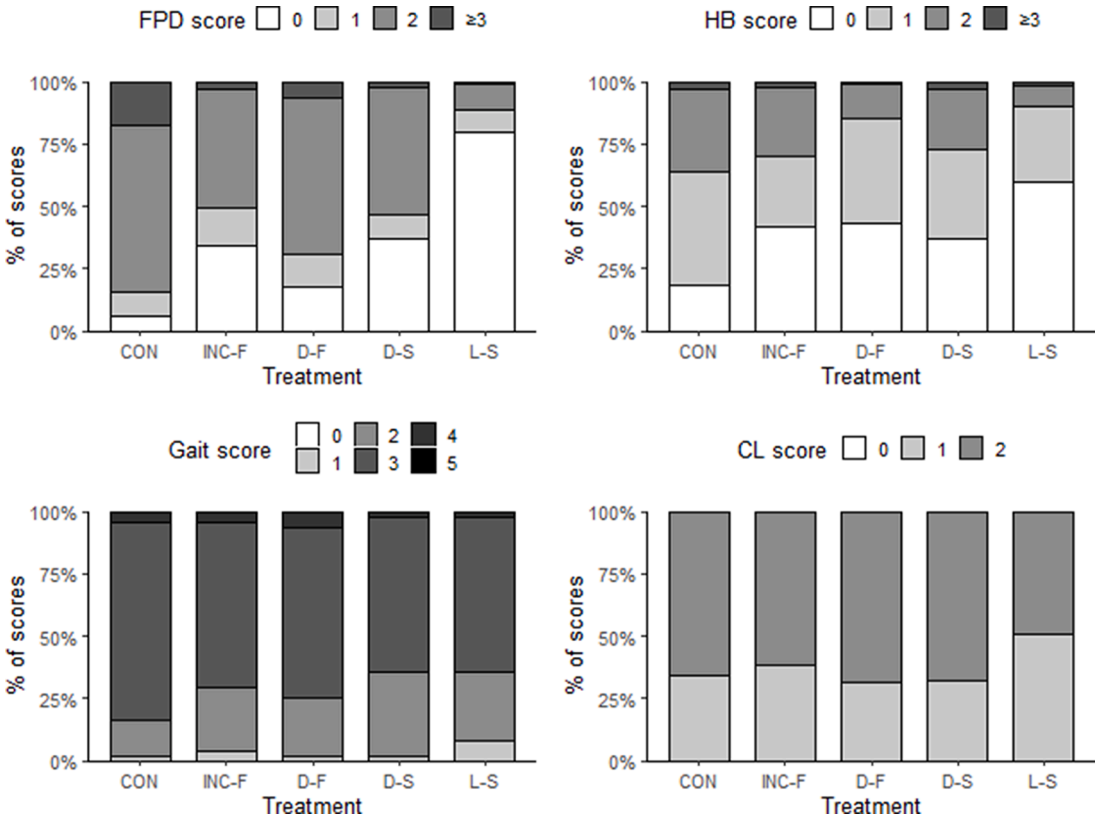

Fig. 3. Percentage of broilers with each foot pad dermatitis (FPD), hock burn (HB), gait, and cleanliness (CL) scores (higher scores equal worse leg health or cleanliness) of broilers receiving no larvae (CON), larvae meal and oil incorporated in the feed (INC-F), dried larvae in the feeder (D-F), dried larvae scattered through the pen (D-S), or live larvae scattered through the pen (L-S). Please note, no birds received a score of 0 for gait and CL, and no birds received a score of 5 for gait.

Section 4.5). Based on this calculation, the BSFL percentage of the total dry matter consumption was estimated to be 8.5 ± 0.1% for D-F broilers, 8.7 ± 0.1% for D-S broilers, and 9.1 ± 0.1% for L-S broilers

Treatment influenced the average daily dry matter intake of pellets ($p < 0.001$). L-S broilers had a lower intake than broilers in all other treatments, and D-F and D-S broilers had a lower pellet intake than CON and INC-F broilers. Treatment also influenced the estimated average daily dry matter intake of pellets and dried or live BSFL combined ($p < 0.001$). Here, L-S broilers had a lower intake than broilers in all other treatments, and D-F broilers had a higher intake than INC-F broilers. Periodic differences in average daily dry matter intake are shown in Supplementary Table S5.

The dry matter conversion ratio (DMCR) was influenced by treatment ($p < 0.001$). The DMCR of L-S broilers were lower than that of broilers in all other treatments, and the DMCR of INC-F and D-S broilers was lower than that of CON broilers.

4. Discussion

In this study we investigated the effects of replacing 8% of the dietary dry matter intake of broilers with black soldier fly larvae (BSFL) as meal and oil incorporated in the pellets (INC-F), as dried larvae provided in the feeder (D-F) or scattered through the pen four times a day (D-S), and as live larvae scattered through the pen four times a day (L-S) on various indicators of broiler welfare and production performance. The four different BSFL inclusion methods all did not affect or improved the

Table 4
Blood plasma KLH-IgG and KLH-IgM antibody titers, whole blood 5-HT concentrations, and feather corticosterone (CORT) concentrations of broilers receiving no larvae (CON), larvae meal and oil incorporated in the feed (INC-F), dried larvae in the feeder (D-F), dried larvae scattered through the pen (D-S), or live larvae scattered through the pen (L-S). Data presented as means ± SEM. Significant treatment effects ($p < 0.05$) are indicated in bold.

Measure	CON	INC-F	D-F	D-S	L-S	Test-statistic and df	P-value
Plasma KLH-IgG titer	2.4 ± 0.1	2.6 ± 0.1	2.6 ± 0.1	2.6 ± 0.1	2.5 ± 0.1	$F_{(4,55)} = 0.67$	0.618
Plasma KLH-IgM titer	3.1 ± 0.1	3.3 ± 0.1	2.9 ± 0.1	3.2 ± 0.1	3.4 ± 0.1	$F_{(4,55)} = 2.42$	0.059
Whole blood 5-HT (nmol/ml)	44.4 ± 1.3	46.0 ± 1.9	44.2 ± 2.4	46.0 ± 1.8	43.2 ± 2.1	$F_{(4,55)} = 0.58$	0.680
Feather CORT (pg/mm)	0.44 ± 0.13	0.24 ± 0.06	0.30 ± 0.11	0.41 ± 0.14	0.38 ± 0.12	$F_{(4,55)} = 2.76$	**0.037**

investigated parameters compared to a diet similar in protein, fat, and energy content but without BSFL (CON treatment), though the specific responses varied between treatments. Generally, broiler welfare benefitted most from scattering live BSFL, followed by scattering dried BSFL and then BSFL meal and oil incorporated in the pellets. Also, broiler production performance was increased most by providing dried or live BSFL.

4.1. Home-pen behavior and posture

Compared to the controls, D-S and L-S broilers performed more foraging behavior throughout the whole rearing period (on average 12.5% and 16.7% of the observed time, respectively, vs. 6.3% of time of controls). Furthermore, they performed more standing idle and walking and less resting near the end of the rearing period. The L-S broilers typically spent more time on active behaviors than D-S broilers, and only the L-S broilers showed more standing postures than controls on d23 and 33. Contrarily, INC-F and D-F broilers did not show more active behaviors than controls. The increased activity as a result of scattering larvae was also observed in previous studies using live BSFL [30,31] or mealworms [43], and our study shows that scattering larvae throughout the pen promotes activity in contrast to providing dried larvae localized in a feeder. Performing natural behaviors such as foraging is considered essential for good welfare as it satisfies intrinsic motivations [44,45] and the benefits can extend to a broiler's affective state (e.g., reduced fearfulness) and health (e.g., reduced leg problems, discussed in Section 4.3) [30,46]. Live larvae may be more interesting to broilers than dried larvae because their movement can be attractive to birds [34], and/or due to consequences of the drying process such as reduced moisture contents and changed odors that may make dried larvae less palatable [47], though unraveling the exact reasons will require more extensive research. Based on our observations, scattering live larvae through the pen is most advantageous for promoting broiler activity.

On d12 and 23, the time spent drinking was lower for L-S broilers compared to broilers in all other treatments. This is likely a results of the high moisture content of live BSFL, and is in line with what we found previously [31]. As expected, the time spent eating from the feeder was lower in D-S and L-S broilers compared to CON broilers on all observation days, because 8% of their diet was provided outside of the feeder. On d12 INC-F and D-F broilers also spent less time eating from the feeder than CON broilers, although their average daily intake from the feeder was not lower during this period. Previous studies have suggested that diets including insect meals or oils are more palatable than diets without [48,49], which could have resulted in faster consumption of the more palatable diets by INC-F and D-F broilers. However, as the observed differences did not persist throughout the rearing period it is difficult to pinpoint the exact cause.

4.2. Litter quality

We observed that pens in all BSFL treatments had dryer litter than CON pens, and additionally pens in D-F, D-S, and L-S treatments had more friable litter than CON pens. The increased activity of D-S and L-S broilers likely regularly tousled the litter, which is known to promote drying and improve litter quality [50,51]. However, as INC-F and D-F pens also showed better litter quality without broilers being more active, it can be assumed that the consumption of larvae also improved litter quality independently from activity. Previous studies indicate that diets including BSFL meal had high fat digestibility [12], which can be beneficial for litter quality as fecal lipid compromises litter absorption abilities [52]. However, in contrast to the current study, former studies on dietary BSFL found either no effects or a decrease of litter quality [28,30], and these discrepancies between studies highlight the need to further explore the mechanisms through which dietary BSFL affects litter quality.

Table 5
Production performance on pen level of broilers receiving no larvae (CON), larvae meal and oil incorporated in the feed (INC-F), dried larvae in the feeder (D-F), dried larvae scattered through the pen (D-S), or live larvae scattered through the pen (L-S). Data are presented as means ± SEM. Significant treatment effects ($p < 0.05$) are indicated in bold, and within each row different superscript letters indicate significant ($p < 0.05$, Tukey's HSD correction) differences between treatments.

Measure	Period	CON	INC-F	D-F	D-S	L-S	Test-statistic and df	P-value
Average daily gain (g/d)	d1–9	22.6 ± 0.3^{a}	230±0.1^{ab}	23.7 ± 0.2^{b}	23.4 ± 0.3^{ab}	23.8 ± 0.3^{b}	$F_{(4,44)} = 4.50$	**0.004**
	d9–19	61.3 ± 0.6^{a}	61.6 ± 0.3^{a}	64.8 ± 0.4^{b}	64.2 ± 0.4^{b}	65.8 ± 0.7^{b}	$F_{(4,44)} = 17.15$	**<0.001**
	d19–27	102.4 ± 1.3^{a}	103.1 ± 0.9^{ab}	107.0 ± 1.3^{b}	107.6 ± 1.3^{b}	104.0 ± 1.4^{ab}	$F_{(4,44)} = 4.16$	**0.004**
	d27–35	122.4 ± 1.2	124.4 ± 1.2	123.6 ± 1.5	125.5 ± 1.5	124.6 ± 2.5	$F_{(4,44)} = 0.57$	0.688
Final weight (g)	d35	2660 ± 19.7^{a}	2694 ± 11.4^{ab}	2758 ± 9.8^{bc}	2772 ± 18.9^{c}	2747 ± 16.1^{bc}	$F_{(4,43)} = 9.88$	**<0.001**
Average daily dry matter intake of pellets (g/d)	d1–35	93.4 ± 0.7^{a}	92.4 ± 0.3^{a}	86.9 ± 0.6^{b}	85.8 ± 0.6^{b}	81.3 ± 0.6^{c}	$F_{(4,44)} = 73.48$	**<0.001**
Estimated average daily dry matter intake of pellets and larvae (g/d)*	d1–35	93.4 ± 0.7^{ab}	92.4 ± 0.3^{b}	95.0 ± 0.6^{a}	94.0 ± 0.6^{ab}	89.4 ± 0.5^{c}	$F_{(4,44)} = 13.73$	**<0.001**
Dry matter conversion ratio (g/g)	d1–35	1.25 ± 0.002^{a}	1.23 ± 0.003^{b}	1.24 ± 0.008^{ab}	1.22 ± 0.004^{b}	1.16 ± 0.004^{c}	$F_{(4,44)} = 49.63$	**<0.001**

Note: *Based on the assumption that all larvae are consumed.

4.3. Visual leg health and cleanliness scores

The percentage of broilers with a score above 0 for foot pad dermatitis (FPD, 63.3%), hock burn (HB, 61,3%), lameness (100%), and cleanliness (100%) are in the range of those found in previous studies [53–55]. The improved litter quality in the BSFL treatments partially coincides with improved leg health, as INC-F and D-S broilers had less severe FPD scores, and L-S broilers had less severe FPD and HB scores than CON broilers. Both FPD and HB have been linked to poor litter quality, as high levels of moisture and ammonia cause contact dermatitis [26,56,57], therefore improved litter quality can benefit broiler leg health. In contrast to our results from the INC-F treatment, previous studies found that providing dietary BSFL meal or oil did not affect the severity of FPD [14,28], and the mechanisms of this effect require further attention.

Only L-S broilers had reduced HB severity, and that may be because they showed the highest activity and time spent standing. Increased activity reduces the time that hocks are in contact with the litter, limiting the development of hock burns [26]. This is in agreement with our previous study that found reduced HB severity after live BSFL provisioning only in treatments with the most active broilers [31]. Also, D-F broilers did not show a reduced severity of FPD and HB despite the improved litter quality, which could be because their activity level was similar to that of the CON broilers. In line with this, providing live larvae in feeding trays also did not influence FPD and HB [29], and it is possible that this provisioning method causes fast consumption of whole larvae which does not facilitate long-term foraging behavior. Furthermore, D-F broilers had a higher average daily gain and final weight than CON broilers (discussed in Section 4.5), and higher weights can increase the risk of contact dermatitis due to increased pressure of the skin against the litter [58,59], possibly outweighing any benefits of improved litter quality. In previous studies several treatments applying live BSFL provisioning did not affect FPD and HB severity, however in these treatments the occurrence of leg health problems was low, presumably due to beneficial rearing conditions (e.g., lower stocking densities or litter supplementation) [30,31].

Lameness severity can also be reduced by regular activity as this stimulates leg development [27,56] and it can be increased by higher daily body weight gains because of the extensive load this places on the legs [60,61]. The D-S and L-S broilers had both increased activity and higher body weight gains than controls, and these effects may have canceled each other out, explaining why gait score was not influenced by dietary BSFL. This is consistent with a previous study in which regular provisioning of live larvae reduced broiler lameness but also reduced their average daily gain [31]. Cleanliness of the broilers was also not influenced by dietary BSFL in the current and a previous study [30]. Some studies indicate a link between cleanliness and litter quality [26] though others find no such connection [62], and cleanliness has also been linked to other parameters such as leg health and performance [26,56]. While there were no significant differences in lameness and cleanliness scores, there was a tendency for dietary BSFL to benefit these parameters, which warrants further investigation.

4.4. Immunological and hormonal measures

Both IgG and IgM titers against KLH were not affected by dietary BSFL. It has been suggested that the prebiotic compounds in BSFL (e.g., chitin, antimicrobial peptides) and their derivatives can modulate humoral and cell-mediated immunological responses of broilers [7,63], though evidence of immuno-enhancing effects of dietary BSFL is scarce and contradictory. For example, blood leukocyte concentrations were increased by including up to 20% of BSFL meal in broiler diets [12], but in another study this concentration was unchanged by up to 15% inclusion of BSFL meal [9]. One study found that replacing 6.5% of the diet of layer hens with BSF pupa for 15 weeks increased serum IgG concentrations [64], and another study found that mealworms fermented with probiotics increased broiler IgG levels while not affecting IgM levels after a *Salmonella enteritidis* challenge compared to controls [65], but the current study could not corroborate these results. It is possible that an immunological challenge is required to observe any immunomodulatory effects of dietary BSFL, which warrants experimental investigation.

Whole blood serotonin (5-HT) concentrations were also not influenced by dietary BSFL. Whole blood 5-HT reflects storage of 5-HT and thus long-term 5-HT system functioning [66]. Relatively higher whole blood 5-HT levels have been linked to reduced fear-related behavior in layer hens [33] and pigs [67], and 5-HT depletion has been associated with pessimistic affective states in pigs [68]. Providing broilers with environmental enrichment such as perches and dust baths [25], or live BSFL in tubes [30], was found to reduce fearfulness, though in these studies, 5-HT levels were not investigated. Our results do not suggest an effect of dietary BSFL on 5-HT concentrations, though the relationship between dietary BSFL, 5-HT system functioning, and broiler affective states remains to be studied.

Feather CORT concentrations are considered a novel indicator of long-term stress in broilers [32,69]. Compared to previous reports, the observed feather CORT concentrations were similar [70] or lower [69] and differences between studies are expected to be a result of different feathers used or alternative processing methods [71]. Despite a main effect of treatment, there were no significant differences between individual treatments in the feather CORT concentration, which is in line with results on excreta CORT previously measured after daily provisioning of small amounts of live BSFL [29]. Numerically, the feather CORT concentration was highest in the control treatment and lowest in the INC-F treatment, which might suggest that consuming BSFL can lower chronic stress in broilers. INC-F broilers had less severe leg problems than controls, and better leg health has previously been linked to reduced stress in broilers [72]. However, as broilers in several other BSFL treatments also showed improved leg health, even to a larger extent, but had similar feather CORT concentrations as controls, it cannot be excluded that other factors affected feather CORT accumulation in our study. A recent paper demonstrated that contamination with feces can increase feather CORT concentrations, even when feathers were washed prior to analysis [73]. As the friability and wetness of the litter as well as time spent standing, which may influence exposure to feces, were all influenced by treatment, it is possible that potential effects of stress on feather CORT concentrations were confounded with effects of contact with fecal matter. Future studies are needed to affirm whether and how different BSFL provisioning methods affect broiler stress.

4.5. Production performance

Broilers in the INC-F treatment did not differ in average daily feed intake from CON broilers. This is in line with preceding studies that observed no difference in feed intake when BSFL meal [74,75] or oil [14,15,22] was incorporated in broiler diets at similar inclusion percentages, and confirms that including 8% processed BSFL in broiler diets does not negatively influence feed intake. As anticipated, D-F and D-S broilers consumed less pellets but had a similar daily total dry matter intake as CON broilers. It must be noted that personal observations indicate that several broilers from the D-F and D-S treatments did not consume the dried larvae during approximately the first two weeks of the trial, and this could have resulted in an over-estimation of the daily total dry matter intake and the dry matter conversion ratio. It is possible that young broilers had difficulty eating the rigid larvae, or that they disliked the sensory properties of the dried larvae. Additional studies that record dried BSFL consumption in more detail are required to further understand the influence of dried BSFL provisioning on broiler performance and welfare throughout different life stages.

In contrast to broilers that received dried larvae, L-S broilers had a lower daily pellet intake than all other treatments, and their daily total

dry matter intake was also lower than that of CON broilers. This contradicts previous studies that did not observe reduced total dry matter intake when up to 10% of the dry matter intake consisted of live BSFL [17,30,31]. However, pigs that received up to 20% of their diet as live larvae did show a reduced total dry matter intake [76]. Differences between studies in the effect of feeding live BSFL on daily dry matter intake could be caused by differences in dietary composition (e.g., different protein and energy levels) or experimental set-up (e.g., stocking density), as these parameters affect feed intake [77,78]. Live BSFL have a higher moisture content and consequently a higher volume at similar dry matter weights than dried larvae, and it is plausible that this increased the stomach fill and thereby satiety in broilers, as broilers are known to eat to their maximum physical ability [79]. This could have resulted in a lower motivation to consume pellets and thus an overall lower dry matter intake.

Despite having a similar or lower daily dry matter intake, the average daily gain of broilers in the D-S and L-S treatments was higher during several days than that of controls, resulting in a higher final weight and lower dry matter conversion ratio (DMCR). These results are not in line with previous studies that found either similar or temporarily lower average daily gains in broilers that received live larvae, which was mainly attributed to their increased activity [17,30,31]. However, in these studies live BSFL provisioning had no or minimal effects on broiler leg health [30,31]. While higher broiler body weight gains can impair leg health (as discussed in Section 4.3), alternatively leg health can influence broiler body weight gain. For example, body weight gain was reduced by inducing FPD through wet litter [26] and by inducing lameness through bacterial chondronecrosis with osteomyelitis [72]. As such, any benefits of BSFL provisioning on activity and leg health may have improved the body weight gain of D-S and L-S broilers.

However, D-F broilers also had increased average daily gains and a higher final weight on d35 without higher activity levels or improved leg health, and INC-F broilers had a lower DMCR than controls, even though their feed intake and average daily gain only differed numerically. These results suggest that not only activity and leg health play a role in broiler production performance. Several studies on dietary BSFL meal did indicate higher average daily gains at similar feed intake levels [12,80], sometimes resulting in a lower feed conversion ratio [80]. In these studies, the beneficial effects of dietary BSFL on broiler production performance were attributed to the nutrition composition of BSFL, including their prebiotic and antibiotic compounds (e.g., chitin and antimicrobial peptides (AMPs)), however the exact cause remains to be studied. Furthermore, in the current study diets were adjusted based on estimated digestibility levels from studies including BSFL meal, as to the authors' knowledge the digestibility of diets containing dried and live larvae has not been investigated. Underestimation of the digestibility of diets including BSFL may have led to higher metabolizable energy and/or protein contents in these diets, which in turn could have increased production performance. Research into the effect of dietary dried and live BSFL on nutrient digestibility is required to unravel their role in broiler performance, and to understand why in-feed BSFL meal and oil, dried BSFL, and live BSFL affect the studied parameters of broiler production performance differently.

5. Conclusion

Replacing 8% of the diet of broilers with BSFL meal and oil incorporated in feed pellets, dried larvae provided in the feeder or scattered through the pen four times a day, or live larvae scattered through the pen four times a day all increased broiler average daily gains and/or dry matter conversion ratio, and improved litter quality. Incorporating BSFL meal and oil in the diet and scattering dried or live larvae through the pen improved broiler leg health, and scattering larvae also increased broiler activity. Plasma natural antibodies and whole blood serotonin concentrations were not influenced by dietary BSFL. Feather CORT concentrations were affected by BSFL provisioning, though post-hoc differences between treatments were absent. Overall, we confirmed that processed and live BSFL can benefit broiler welfare and increase broiler production performance. Scattering BSFL through the pen results in more welfare benefits than providing BSFL in the feeder, with live BSFL having the strongest effects on broiler behavior and leg health, and therefore being most beneficial for broiler welfare.

Declaration of competing interests

The authors declare no competing interests.

Data Availability

Data will be made available on request.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.physbeh.2022.113999.

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