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Provisioning of live black soldier fly larvae *(Hermetia illucens)* benefits broiler activity and leg health in a frequency- and dose-dependent manner

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ABSTRACT

Fast-growing broilers spend most their time inactive and are therefore prone to experience leg problems. Environmental enrichment that facilitates intrinsically motivated behaviours can potentially promote activity and reduce leg problems, thereby improving broiler welfare. A promising environmental enrichment method is the scattering of desired feed items, such as insects, which are highly attractive to broilers. We studied the effect of providing live black soldier fly larvae (BSFL) scattered on the litter on broiler behaviour, leg health and performance. One-day-old male broilers were assigned to one of five treatments (eight pens/treatment, nine broilers/pen): a control without BSFL and four treatments with BSFL provided from day 1 onwards in different amounts (5 % or 10 % of estimated dietary dry matter intake; A5 and A10 respectively) and frequencies (two or four times a day; F2 and F4 respectively). All broilers were fed diets formulated to ensure a similar energy and nutrient intake. Broiler weight and leg health were determined on day 42. The behavioural time budget was determined weekly by observations for 7 h per day using 12-min scan sampling, and activity around larval provisioning was determined by 3-min scan sampling from 9 min before, until 27 min after larval provisioning on day 15/16, 29/30 and 40/41. Broilers in all larval provisioning treatments had a different behavioural time budget than controls, with significantly higher levels of foraging behaviour, walking, standing idle and general activity during at least three of the five observation days ($p < 0.05$ compared to controls). The increase in activity was numerically highest and most long-term in A10F4 broilers. Time spent active and in standing posture declined from week 4 onwards in A10F4, whereas for all other treatments this decline occurred already in week 2. Activity during 30 min after larval provisioning was higher for A10 than A5 treatments ($p < 0.05$ for all days) but overall not affected by frequency of larval provisioning. Hock burn occurred less in A10 birds than in controls ($p < 0.05$), and lameness occurred less in A10 and A5F4 birds than in controls ($p < 0.01$). Only A10F2 birds had a lower final weight than controls ($p < 0.05$). In conclusion, the largest amount combined with the highest frequency of larval provisioning applied in this study resulted in the most prominent increase in activity and better leg health, without significantly affecting broiler performance. Further investigation into BSFL provisioning methods is required to determine the optimal method for achieving improved broiler welfare.

1. Introduction

Industrial experts estimate that in 2017 approximately 90–95 % of the European fast-growing broilers obtained a weight of 2–2.5 kg within 6 weeks (Van Horne, 2018). This rapid growth rate attributes to the development of broiler lameness, directly by impairing broiler leg bone development (Kestin et al., 1992; Olkowski et al., 2011) and indirectly through limiting broiler activity (Reiter and Bessei, 2009; Sherlock et al., 2010). Previous reports indicate that the majority of fast-growing broilers exhibit some degree of lameness (Muri et al., 2019), and that between 30 % and 50 % of broilers clearly show a reduced ability to walk (indicated by a gait score of 3 or higher) (de Jong et al., 2019; Vasdal et al., 2018). Lameness can be painful and limit the expression of active behaviours (Danbury et al., 2000). Furthermore, moisture and ammonia aggregate in the litter over time, and broilers that spend a lot of time resting in this litter are more prone to develop contact dermatitis (de Jong et al., 2014). The negative effects of a rapid growth rate on leg health are exacerbated under commercial

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conditions where broilers have limited space and environmental stimulation. The commercial environment can impair broiler welfare by limiting the possibility to perform intrinsically motivated behaviours, diminishing activity levels and thereby increasing the occurrence of associated leg problems (Vasdal et al., 2018). The effects of fast growth and leg problems on activity and behaviour are prominent, as fast-growing broilers spend between 60–80 % of their time sitting (see for example Baxter et al., 2018a; de Jong and Gunnink, 2018).

Several studies have indicated that promoting activity from a young age onwards can advance leg bone development, and even increase activity levels later in life (Reiter and Bessei, 2009; Ruiz-Feria et al., 2014; Vasdal et al., 2018). An additional benefit of increased activity can be that the litter tends to dry easier when periodically mixed by, for example, scratching. This mixing can thus improve the litter quality and reduce the risk of contact dermatitis (de Jong and van Harn, 2012). Therefore, (early) facilitation of activity in broilers may benefit broiler welfare. Various measures, for example increasing the distance between the food and water supply, have been tested with variable levels of success (for review see Riber et al., 2018). Facilitating foraging behaviour is one promising way to promote broiler activity. While providing whole wheat on the litter did not promote activity (Jordan et al., 2011), after scattering mealworms the activity of broilers increased substantially, at least on the short term (Pichova et al., 2016). This could be explained by the observation that broilers are highly motivated to gain access to and consume larvae (Bokkers and Koene, 2002; Clara et al., 2009). In addition, live insect larvae are potentially more attractive to broilers than dead insects, as chickens prefer to interact with moving rather than stationary objects (Jones et al., 1998).

Black soldier fly larvae (BSFL) could potentially be used as effective broiler enrichment. Broilers actively consume these larvae when provided processed (Leiber et al., 2017) or live (Oonincx, 2020, personal communication). In addition, although (Leiber et al., 2017) found no effect of including BSFL meal on broiler performance, other studies have found that BSFL inclusion in the diet can increase broiler growth and feed intake (Dabbou et al., 2018), and increase the broiler's T-helper cell frequency and serum lysozyme activity, improving their nonspecific immune responses (Lee et al., 2018). With the aim of improving the effectiveness of providing insects as enrichment, Pichova et al. (2016) suggested that prolonged access to insects could further increase broiler activity. However, the effect of providing broilers with long-term access to BSFL on the broiler's behavioural time budget has currently not been studied. Also, the effect of BSFL provisioning on broiler leg health and performance remains to be investigated.

The objective of the current study was to investigate the effect of scattering BSFL in the litter on broiler behaviour, contact dermatitis, lameness and performance. Specifically, the consequences of providing larvae in combinations of two amounts (5 or 10 % of estimated dietary dry matter intake) and two frequencies (provided two or four times a day) were studied. We hypothesized that scattering BSFL will promote activity and reduce health problems in broilers, where the largest amount and highest frequency are expected to have the strongest effect as they allow for prolonged interaction with the larvae. An additional aim is to keep broiler performance similar between treatments, as to avoid potential confounding effects of body weight on broiler welfare.

2. Materials and methods

This experiment was conducted between January and March 2019 at the animal experiment facilities of Wageningen University & Research, and the protocol was approved by the Animal Care and Use committee of Wageningen University & Research (Wageningen, The Netherlands).

2.1. Animals and management

Three hundred and sixty one-day old male Ross 308 broiler chicks were obtained from a commercial hatchery. At arrival at the experimental facilities chicks were tagged with a leg ring and randomly distributed over forty pens, resulting in nine chicks per pen. Each pen of 1 × 2 m constituted of one feeder, one drinking line containing five nipples with cups, one 1 m long perch (rectangular bar of 2 × 2 cm, 10 cm high) and a 5 cm deep layer of wood shavings. Visual contact between pens was obstructed by solid panels. Feed and water were provided ad libitum. The IB vaccination (spray) was given at hatch and the NCD vaccination (spray) at eight days of age. Temperature at the start of the experiment was 33 °C and this was gradually decreased to 22 °C at 27 days of age, after which it was kept constant. Humidity was adjusted based on recommendations of the Aviagen Ross broiler handbook. The lighting schedule (20 lx at chick level throughout the rearing period, artificial light) was 23 L:1 D from day 1–3, and from day 4 onwards a schedule of 18 L:6 D was maintained, with the dark period lasting from 00:00 − 06:00.

2.2. Experimental design

Five treatments were included in this study. Broilers in the control treatment did not receive BSFL throughout the experiment. In the other treatments, the amount and frequency of larval provisioning were varied. Broilers received either 5 % or 10 % of the estimated daily dietary DM intake as BSFL (hereafter referred to as A5 and A10, respectively), provided either two or four times a day (F2 and F4, respectively) resulting in, apart from the control treatment, treatments A5F2, A5F4, A10F2 and A10F4. Each treatment was applied to eight randomly selected pens which were distributed (balanced for treatment) over three experimental rooms. A commercial BSFL producer supplied fresh, 14-day-old BSFL weekly. Larvae were stored at 12 °C near the experimental rooms for up to one week. One day before the larvae were provided to the broilers, the larvae were stored at room temperature, to increase their activity at provisioning. Broilers received BSFL on set moments each day (08:00 and 14:00 for the F2 treatments and 08:00, 11:00, 14:00 and 17:00 for the F4 treatments). Provisioning occurred by scattering larvae across the litter throughout the pen.

2.3. Diet composition

All diets were designed to meet or exceed the broiler's nutrient requirements (CVB, 2016). All chicks received a starter feed from day 1–7 (12.46 ME/kg DM metabolizable energy, 22 % crude protein). This starter feed was the same for all treatments; it was not adjusted for larval amount as digestible nutrient intake from larvae was expected to be small during day 1–7. The grower feed provided to the A5 and A10 broilers from day 8–42 was adjusted to account for the estimated nutrient intake from these larvae and ensure a similar nutrient and energy intake for all broilers. Briefly, a mix was designed mimicking the nutritional composition of BSFL, based on analysed values of three samples of BSFL for dry matter, crude protein, crude fat, calcium and phosphorus content. In this mix the fat source was BSFL oil and the protein source was potato protein. This protein source was chosen as protein from dead BSFL is currently not allowed to be used in commercial broiler feed, and not in the feed in this experiment. The total dietary DM intake of all broilers consisted for 90 % of a core feed, and for the control broilers the remaining 10 % consisted of the above-mentioned mix. The A5 broiler's diet contained 5 % of the mix and 5 % live BSFL, and the diet of A10 broilers contained 10 % live BSFL and no mix. Detailed ingredient and nutrient composition of all diets is provided in Appendix A. The results of the analyses on nutrient composition of BFSL were also used to determine the exact amount (g) of fresh larvae to be provided to each pen daily.

Table 1
Ethogram for the behavioural time budget observations.

Item	Description
Behaviour	
Eating	Standing/sitting with head above or in the feeder and/or pecking at feed in the feeder or on the floor.
Drinking	Drinking from nipple or cup beneath nipple.
Defecation	Excreting faeces.
Walking	Locomoting in an upright position with a normal speed or quick steps without performing any other activity.
Shuffling	Half standing/half sitting (hocks might touch the ground or be slightly above the ground) and is moving a few steps after which it sits down.
Standing idle	Standing on the ground without performing any other activity.
Perching	Perching without any other activity.
Resting	Sitting with hocks resting on ground without any other activity, possibly with head on the ground or under wing.
Scratching	Scraping of the litter with the claws.
Ground pecking	Performing pecking movements directed at the ground.
Food running	Running with food in beak while pen mates follow and attempt to grab the food item.
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.
Preening	Grooming of own feathers with the beak.
Wing flapping	Bilateral up-and-down wing flapping.
Pecking pen mate head	Pecking movements directed at the head of a pen mate.
Pecking pen mate other	Pecking movements directed at the body or beak of a pen mate.
Interaction other	Jumping at pen mate, chasing pen mate, threatening pen mate.
Other	Any behaviour not mentioned above.
Posture	
Standing	On floor: hocks not in contact with the litter. On perch: knees not bend.
Sitting	On floor: hocks in contact with the litter. On perch: knees bend.

2.4. Measurements

2.4.1. Behavioural time budget over the day

Prior to observations all broilers were individually marked with one coloured dot, to allow for individual identification. Behaviour and posture (standing/sitting) were observed at 12-min intervals by instantaneous scan sampling on one day at the end of week 1–5 (day 7 14, 21, 28 and 36). This involved seven one-hour periods starting at 08:00, 09:15, 10:45, 12:00, 13:45, 15:00 and 16:30, resulting in 35 scans/broiler/day. Three observers simultaneously observed all pens in the three experimental rooms, and every hour observers switched between rooms. Observers had previously been trained and inter-observer reliability was determined to be sufficient (Fleiss kappa > 0.8) before observations commenced. The ethogram is given in Table 1. At BSFL provisioning times observations started immediately after larvae provisioning.

2.4.2. Behaviour at larval provisioning

At three ages broiler behaviour around all four larval provisioning times was observed in more detail in six pens per treatment for all treatments, including the control group, also if no larvae were provided. For each age, observations occurred during two consecutive days (day 15–16, day 29–30 and day 40–41), and each day three pens per treatment were observed and foraging- and activity-related behaviour was noted according to the ethogram (Table 2). Three-min instantaneous scan sampling started 9 min before and ended 27 min after each larval provisioning time (08:00, 11:00, 14:00 and 17:00), when generally all larvae had been consumed.

2.4.3. Visual health scores

All broilers were visually scored on day 42 on various health parameters. Foot pad dermatitis was scored for both feet on a 5-point scale, with 0 = no lesions and 4 = marked swelling and enlargement of the entire foot pad, necrotic cells covering more than one-half of the total foot pad area (full descriptions by Sami Yamak et al., 2016). Hock burn, breast blisters, cleanliness and gait score were determined according to the Welfare Quality® assessment protocol for poultry (Butterworth, 2009). Hock burn was scored for both hocks on a 5-point scale, with 0 = no lesions and 4 = severe lesions. Breast blisters were scored as being present or absent. Cleanliness was scored on a 3-point scale, with 0 = clean plumage, 1 = slightly dirty plumage, and 2 = large patches of dirty plumage on breast or breast completely covered with dirty plumage. The walking ability (indicative of lameness) of broilers was assessed by encouraging broilers to walk approximately 1 m in the pen and assigning a gait score between 0 (normal, dextrous and agile walk) and 5 (incapable of walking) (Butterworth, 2009).

2.4.4. Performance

All broilers were individually weighed at placement and on day 6, 13, 20, 27, 34 and 42. Feed consumption on pen level was determined weekly by weighing feed remains in the feeder on day 8, 15, 22, 29, 35 and 42. Morbidity and mortality were recorded daily.

2.5. Statistical analysis

2.5.1. Data processing

Based on the intake of feed and larvae, the percentage of the total

Table 2
Ethogram for the behavioural observations at larval provisioning.

Item	Description
Behaviour	
Foraging behaviour	Ground pecking and/or scratching.
Food running	Running with food in beak while pen mates follow and attempt to grab the food item.
Walking	Locomoting in an upright position with a normal speed or quick steps without performing any other activity
Standing idle	Standing without any other activity, on perch or on ground.
Resting	Sitting with hocks resting on ground without any other activity, possibly with head on the ground or under wing.
Agonistic behaviour	Pecking pen mate, jumping, chasing, threatening etc.
Other	Any behaviour not mentioned above.

DM intake consisting of BSFL was determined per pen. All larvae were assumed to be consumed as determined by regular observations and further supported by absence of adult flies in the rooms. The average daily gain (ADG) and average daily DM intake (with and without including larvae) in g/day/chick were determined and based on this the average daily metabolizable energy (ME) intake and the feed conversion ratio (FCR), based on dry matter, were calculated. Behaviours observed during the behavioural time budget observations were combined per chick per day and expressed as the percentage of time spent performing that behaviour. Besides being analysed independently, ground pecking, scratching and food running were combined into "foraging behaviour", preening and dust bathing were pooled into "comfort behaviour", and all behaviours except resting and perching while sitting were pooled into "activity", and these pooled behaviours were analysed in addition to the separate behaviours. Behaviours that occurred in fewer than 0.5 % of the observations (interaction, other, defecation, food-running, dustbathing, pecking pen mate head/other and wing flapping) were not analysed independently. Time spent in standing posture was analysed separately by pooling all behaviours displayed whilst standing. Concerning the detailed observations at larval provisioning times, preliminary analysis showed similar patterns in several foraging-related behaviours. Therefore, these behaviours could be well exemplified by patterns in "active behaviour" (all behaviours except resting), and only "active behaviour" around larval feeding was analysed. For this analysis observations were grouped per pen and expressed as percentage of active chicks. A curve representing activity over time was plotted, and the area under the curve (AUC) before (3 scans) and after (10 scans) larval provisioning were analysed separately. Breast blisters and cleanliness scores above 0 were never observed. Hock burn and foot pad dermatitis scores were combined into absence (score = 0) or presence (score > 0) of the affliction as no scores above 1 were observed, and per chick only the leg with the highest score was included in the analysis. Gait scores were pooled into "absence of lameness" (score = 0), "slight walking abnormality" (score = 1) and "obvious walking abnormality" (score > 1) as scores higher than 2 were uncommon (< 2 % of all broilers).

2.5.2. Data analysis

Data were analysed with SAS 9.4 (SAS Institute Inc., Cary, NC, USA). Performance parameters (ADG, final weight, average daily DM intake, average daily ME intake and FCR based on DM) were analysed using a linear mixed model (MIXED in SAS). The ADG was analysed per week. The average daily DM and energy intake, and the FCR were analysed for the total grower period (day 8–42) only as partial floor feeding applied during the starter period prevented accurate measurements of feed intake. Models included treatment and experimental room as fixed effects, and the random effects of pen nested within room and treatment and (for ADG and final weight) chick nested within pen, room and treatment. To analyse the relationship between activity (percentage of time spent active on the observation day closest to weighing) and ADG a similar model was used with activity as covariate. Behaviours observed in the behavioural time budget observations were analysed with a generalised linear mixed model (GLIMMIX in SAS) using a binomial distribution, logit link function and additional multiplicative over-dispersion parameter. The model included a fixed effect of room, treatment, week (1–5) and the treatment by week interaction, a random effect of pen nested in room and treatment, and a random effect of week with chick as subject nested within pen, room and treatment, with a heterogeneous first-order autoregressive covariance structure. The AUC of activity before and after larval provisioning was analysed for each of the three ages in a mixed model with room, treatment, larval provisioning time (08:00, 11:00, 14:00 and 17:00) and the treatment by provisioning time interaction as fixed effects, and larval provisioning time as repeated effect with pen nested in room and treatment as subject, with a homogenous first-order autoregressive covariance structure. The model also included two contrast statements to test overall effects of amount (5% vs 10 %) and frequency (2x vs 4x) of larval provisioning. Concerning visual health parameters, the distribution of foot pad dermatitis, hock burn and gait scores were analysed in a GLIMMIX with a binary distribution and logit link function for foot pad dermatitis and hock burn scores, and a multinomial distribution and cumulative logit link for gait score. The models included treatment and room as fixed effects and as random effects pen nested within room and treatment and chick nested within pen, room and treatment. Significant fixed effects were analysed further using differences in least square means with a Tukey's HSD correction for pairwise comparisons, except for gait score for which pairwise differences were analysed with a Kruskal-Wallis test. Data are presented as means ± SEM based on pen averages, unless indicated otherwise. P-values below 0.05 were considered statistically significant and P-values between 0.05 and 0.1 indicate a trend. Pairwise differences with $p < 0.05$ are presented in the results.

3. Results

3.1. Behavioural time budget over the day

Treatment had no effect on shuffling (0.5 ± 0.3 %), perching (2.6 ± 0.1 %), stretching (1.4 ± 0.1 %) and comfort behaviour (4.5 ± 0.1 %, $p > 0.1$ for all). For all other analysed behaviours, effects of treatment, week and/or their interaction were found (see below).

3.1.1. Walking

Walking was influenced by treatment, week and their interaction ($p < 0.0001$, Fig. 1A). During the first four weeks, broilers in all larval provisioning treatments spent more time walking than controls without larvae, except for A5F2 in week 2. In week 3, A10F4 broilers also spent more time walking than A5F2 broilers. Compared to week 1, a decline in time spent walking from week 2 onwards was observed in the control, F2 and A5F4 broilers, whereas for the A10F4 broilers this decline started in week 4.

3.1.2. Standing idle

Standing idle was affected by treatment, week and their interaction ($p < 0.0001$, Fig. 1B). Controls spent less time standing idle than broilers in the other treatments in several weeks (A5F2: week 1, 3 & 4, A5F4: week 1–4, A10F2: week 1–5, A10F4: week 1–4). In week 3, A10F4 broilers also spent more time standing idle than A5F2 broilers. A decline in time spent standing idle occurred from week 2 onwards for the controls and A5F2 broilers, whereas for the other treatments time spent standing idle remained relatively constant during the first three weeks, after which a decline occurred.

3.1.3. Eating feed and drinking

The time spent eating feed was influenced by treatment, week and their interaction ($p < 0.0001$, Fig. 1C), and time spent drinking was influenced by treatment and the treatment by week interaction ($p < 0.01$, Fig. 1D). In week 1, controls spent more time eating feed than broilers in all other treatments and A5F2 broilers spent more time eating feed than A10F2 broilers. In week 3, controls spent more time eating feed than F4 broilers and this difference remained in week 4 for A5F4 broilers. Controls also spent more time drinking for one or more weeks than broilers in all other treatments (A5F2: week 1–2, A5F4: week 1, A10F2: week 1–3 & 5, A10F4: week 1, 3 & 5).

3.1.4. Foraging behaviour

The time spent scratching was affected by treatment ($p = 0.001$) and was overall higher in A10F2 broilers (1.7 ± 0.2 %) than in controls (0.8 ± 0.1 %) and A5F4 broilers (0.7 ± 0.1 %). Ground pecking and total foraging behaviour were influenced by treatment, week and their interaction ($p < 0.0001$, Fig. 1E and F), and these behaviours were performed more in all larval provisioning treatments compared to

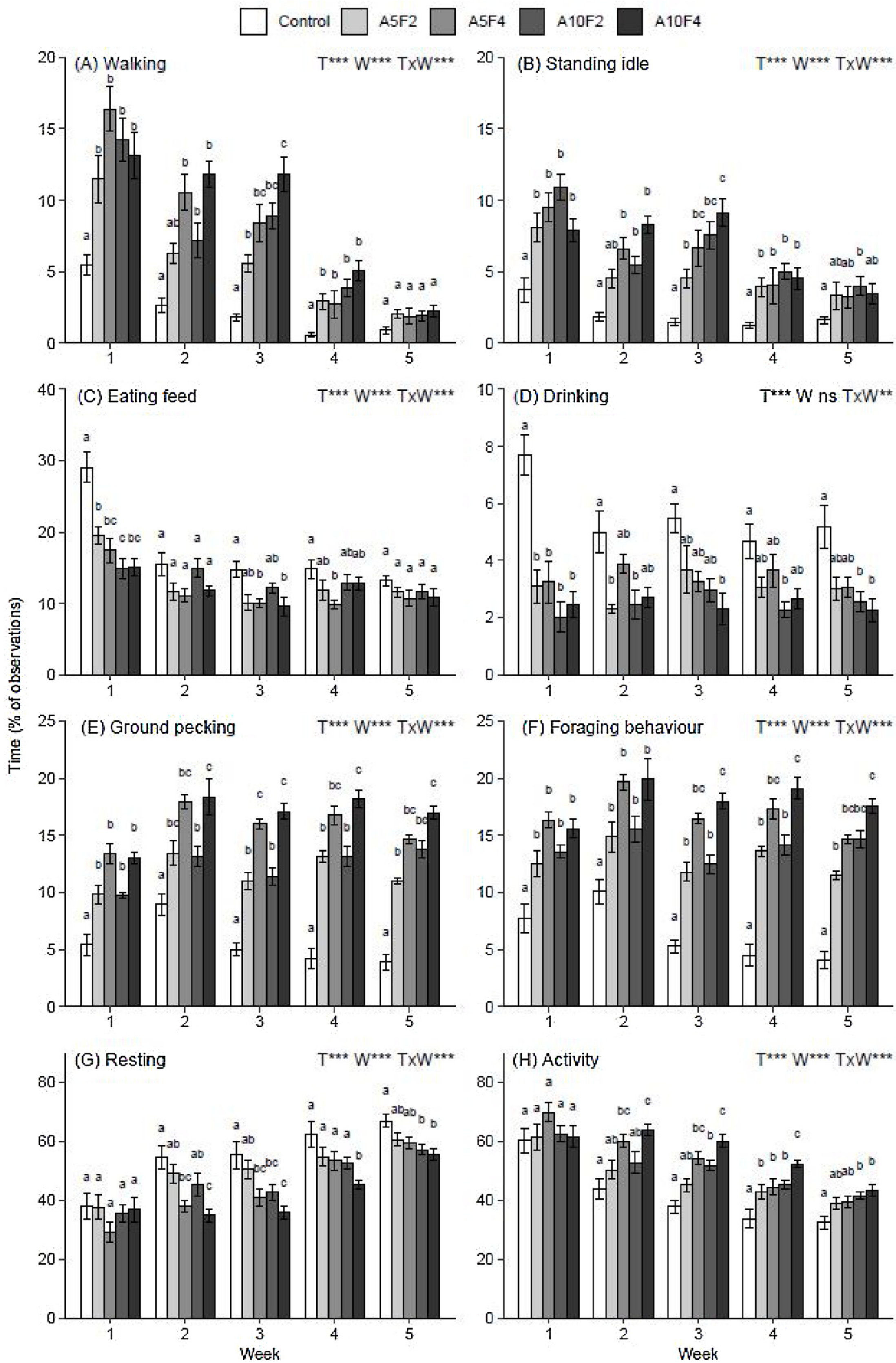

Fig. 1. Behavioural activities (% of observations) of broilers receiving no larvae (Control), or provided with larvae in different amounts (5 % or 10 % of the total dietary DM replaced with larvae, A5 and A10 respectively) and frequencies (two or four times a day, F2 and F4 respectively) scored at the end of week 1-5. Activity encompasses all behaviours except resting and perching while sitting. Foraging behaviour encompasses ground pecking, scratching and food running. Data are presented as means ± SEM. Effects of Treatment (T), Week (W) and their interaction (TxW) are indicated as ns (not significant), ** ($p < 0.01$) or *** ($p < 0.001$). Different letters within one week indicate significant ($p < 0.05$) differences between treatments.

controls in all weeks. In addition, A10F4 broilers spent more time on these behaviours during several weeks than A5F2 broilers (week 3–5) and A10F2 broilers (week 3–4). Time spent on ground pecking (but not total foraging behaviour) increased between week 1 and 2 for all broilers. From week 2 onwards, controls showed a decline in ground pecking and total foraging behaviour, while the occurrence of these behaviours remained relatively constant in all BSFL treatments.

3.1.5. Resting

Resting behaviour was influenced by treatment, week and their interaction ($p < 0.0001$, Fig. 1G). Controls spent more time resting in several weeks than A5F4 (week 2–3), A10F2 (week 3 & 5) and A10F4 broilers (week 2–5). In one or more weeks A10F4 broilers also spent less time resting than A5F2 broilers (week 2–4), A5F4 (week 4) and A10F2 (week 2 & 4). Time spent resting increased from week 2 onwards for the control, F2 and A5F4 broilers, and from week 4 onwards in A10F4 broilers.

3.1.6. Overall activity

Overall activity, i.e. any behaviour except resting and perching while sitting, was affected by treatment, week and their interaction ($p < 0.0001$, Fig. 1H), and was lower in control broilers in several weeks compared to A5F2 (week 4), A5F4 (week 2–4), A10F2 (week 3–5) and A10F4 broilers (week 2–5). Additionally, A10F4 broilers were more active than F2 broilers in week 2–4 and A10F2 broilers in week 4. Time spent active decreased from week 2 onwards for the control, F2 and A5F4 treatments, and from week 4 onwards in A10F4.

3.1.7. Posture

Posture was affected by treatment, week and their interaction ($p < 0.001$ for all, Fig. 2). Time spent in standing posture was lower in controls than in A10F2 and F4 broilers during week 2 − 5. In several weeks A5F2 broilers also showed less time standing than A5F4 (week 2–3) and A10F4 broilers (week 2–4), and in week 2 and 4 A10F2 broilers spent less time standing than A10F4 broilers. No other within-week differences between treatments were observed. Time spent in standing posture decreased from week 2 onwards in controls and F2 broilers, from week 3 onwards in A5F4 broilers and from week 4 onwards in A10F4 broilers.

3.2. Behaviour at larval provisioning

The percentage of active chicks per treatment around each larval

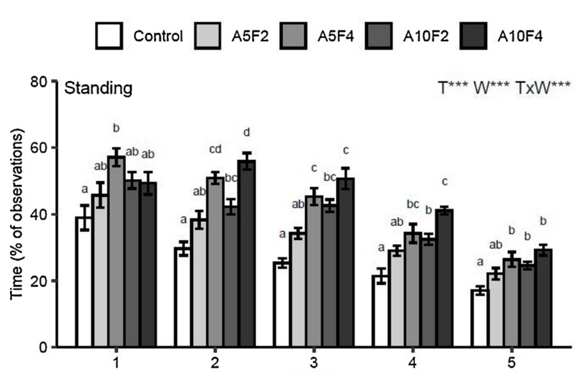

Fig. 2. Posture (% of observations spent standing) of broilers receiving no larvae (Control), or provided with larvae in different amounts (5 % or 10 % of the total dietary DM replaced with larvae, A5 and A10 respectively) and frequencies (two or four times a day, F2 and F4 respectively) scored at the end of week 1–5. Data are presented as means ± SEM. Effects of Treatment (T), Week (W) and their interaction (TxW) are indicated as ns (not significant), ** ($p < 0.01$) or *** ($p < 0.001$). Different letters within one week indicate significant ($p < 0.05$) differences between treatments.

provisioning time at three different ages (15/16, 29/30 and 40/41 days of age) is illustrated in Fig. 3. Broilers in F4 treatments received larvae at all four larval delivery moments (08:00, 11:00, 14:00 and 17:00), while F2 broilers received larvae only at 08:00 and 14:00. Significant differences between the area under the curve of the activity plots before (3 scans) and after (10 scans) larval provisioning are presented below.

3.2.1. Activity before larval provisioning

On day 15/16, activity during the 9 min before larval provisioning, as calculated by the area under the curve, was influenced by treatment ($p < 0.001$) and the treatment by provisioning time interaction ($p = 0.038$). During several provisioning times some BSFL treatments were more active than controls (A5F4: 08:00 & 11:00; A10F2: 08:00; A10F4: 08:00, 11:00, 14:00 & 17:00). On day 29/30 activity before larval provisioning was influenced by treatment ($p = 0.014$), where only A10F4 broilers were more active than controls. On day 40/41 activity before larval provisioning was similar for all treatments (Fig. 3).

3.2.2. Activity after larval provisioning

On day 15/16, activity during the 30 min following larval provisioning, as calculated by the area under the curve, was influenced by treatment ($p < 0.001$), provisioning time ($p < 0.001$) and their interaction ($p = 0.038$). During several provisioning times broilers in some BSFL treatments were more active than controls (A5F2: 17:00; A10F2: 11:00; A10F4: 11:00 & 17:00). The A5F4 broilers never differed in activity from controls, and no differences between BSFL treatments occurred. On day 29/30 and 40/41 activity following larval provisioning was affected by treatment, provisioning time and treatment by provisioning time interaction ($p < 0.001$ for all). Day 29/30 showed a similar trend as day 15/16, with larval provisioning treatments at some time points being more active than controls (A5F2: 08:00; A10F2: 08:00 and 14:00; A10F4: 11:00 & 17:00). On day 40/41 all larval provisioning treatments except A5F4 showed higher activity compared to controls after receiving larvae (F2: 08:00 & 14:00; A10F4: 08:00, 11:00, 14:00 & 17:00). In line with this, controls (which never received larvae) and F4 broilers (which received larvae at all four time points) exhibited consistent activity levels across the four provisioning times, whereas F2 treatments showed higher activity following larvae provisioning at 08:00 and 14:00 than following 11:00 and 17:00 (when no larvae were provided to them). Also, A10F2 broilers were more active after receiving larvae than A5F4 broilers (08:00 & 14:00), and this was reversed when no larvae were provided to A10F2 broilers at 17:00. Similarly, during moments when no larvae were provided to the F2 broilers (11:00 & 17:00) they were less active than A10F4 broilers. When testing for an effect of amount and frequency by using contrast statements, it was determined that treatments receiving a higher amount of larvae were overall more active after larval delivery on all days whereas frequency of provisioning did not significantly affect activity after larval provisioning (Fig. 3).

3.3. Visual health scores

Percentages of chicks with distinct visual health scores are depicted in Fig. 4. The occurrence of foot pad dermatitis was low (< 4 %) and not affected by treatment ($p = 0.99$). Treatment did influence the incidence of hock burn ($p = 0.007$), and post-hoc analysis indicated that the percentage of chicks with hock burn was higher in controls than in A10F2 ($p = 0.033$) and A10F4 ($p = 0.017$). An effect of treatment ($p = 0.005$) was also found on gait score (score of 0, 1 and > 1). Elevated gait scores occurred more in controls compared to A5F4 ($p = 0.003$), A10F2 ($p < 0.001$) and A10F4 ($p = 0.001$) broilers, and they occurred more in A5F2 broilers compared to A10F2 ($p = 0.001$) and A10F4 ($p = 0.014$) broilers.

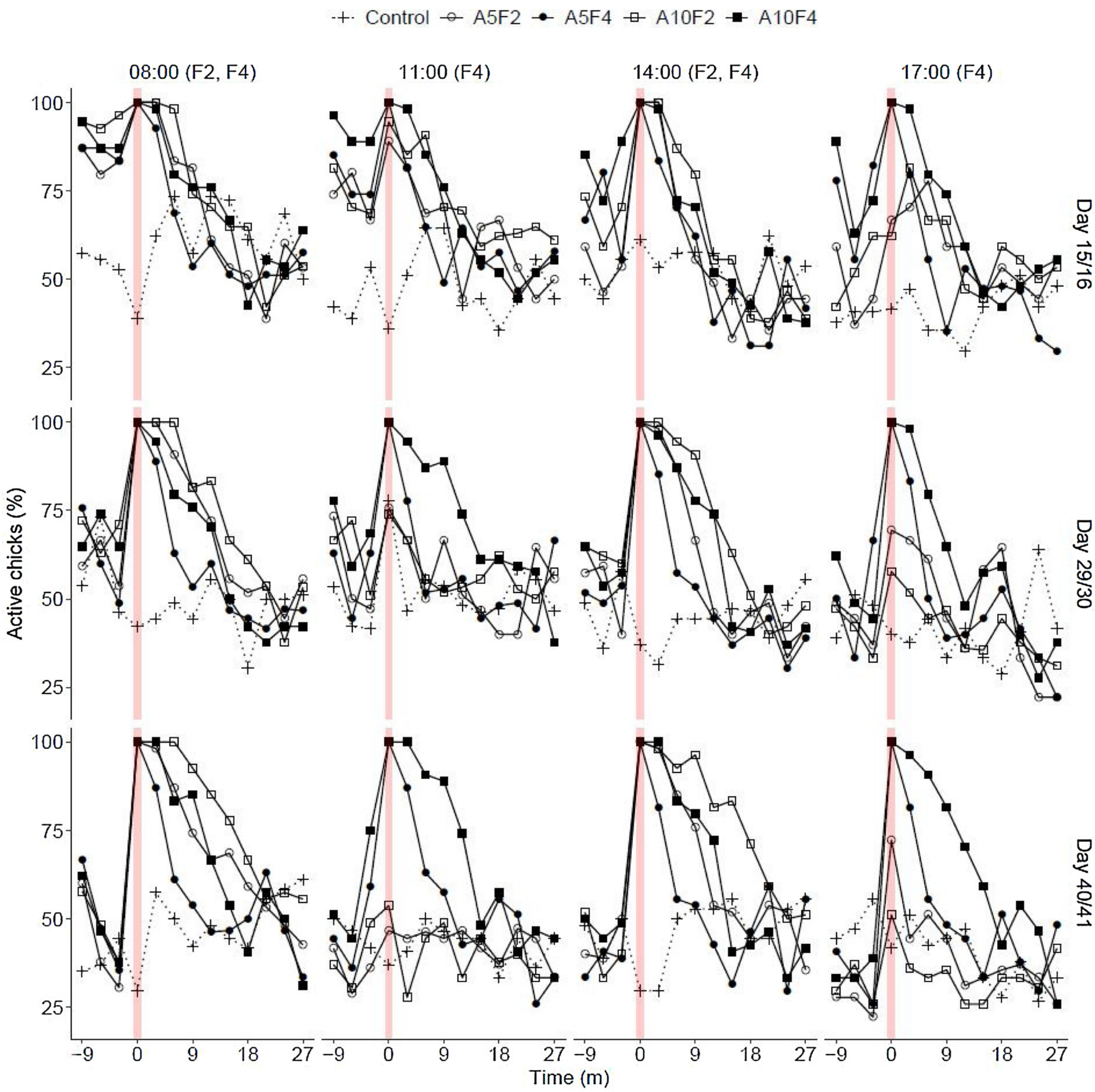

Fig. 3. Percentage of broilers active around larval provisioning moments. Broilers received no larvae (Control), or were provided with larvae in different amounts (5 % or 10 % of the total dietary DM replaced with larvae, A5 and A10 respectively) and frequencies (two or four times a day, F2 (received larvae at 08:00 and 14:00) and F4 (received larvae at 08:00, 11:00, 14:00 and 17:00) respectively). Each row represents one observation period (day 15/16, 29/30 and 40/41), each column represents one moment of larval provisioning (08:00, 11:00, 14:00 and 17:00) with the treatments receiving larvae at that moment between brackets. Instantaneous 3-min scan sampling was done from 9 min before until 27 min after larvae delivery. Larval delivery (at $t = 0$) is indicated by the vertical line.

3.4. Performance

The percentage of the total DM intake consisting of BSFL was 6.3 ± 0.1 % for A5F2, 6.4 ± 0.1 % for A5F4, 12.7 ± 0.1 % for A10F2 and 12.3 ± 0.3 % for A10F4 broilers. Performance parameters are shown in Table 3. Controls had a higher ADG than A10 broilers during days 13–20 and 21–27 ($p < 0.02$ for all) and a higher final weight than A10F2 broilers ($p = 0.049$). Activity added as a covariate in the model for ADG influenced ADG ($p < 0.001$ for all weeks), in addition to removing the significant treatment effect during day 13 − 27. The average daily DM intake excluding BSFL was higher in control broilers compared to all others, in A5F2 compared to A10 broilers and in A5F4 compared to A10F2 broilers. The average daily DM intake including BSFL and the average daily ME intake did not differ between treatments. The FCR based on DM was higher in the F4 treatments than in the F2 treatments, with the FCR of the control treatment being in between. No morbidity (besides the visually scored health problems) or mortality was observed.

4. Discussion

The present study investigated the effect of providing live black soldier fly larvae (BSFL) in different amounts and frequencies on broiler behaviour, leg health and performance. Broilers receiving larvae showed a profound increase in active behaviours compared to controls. In line with our hypothesis, the largest amount of larvae provided at the highest frequency, i.e. 10 % of the dietary DM as larvae spread over four provisioning times a day, seemed most effective in promoting activity and lowering the occurrence of hock burn and lameness, while the final weight of these broilers was not significantly reduced compared to controls.

All broilers receiving BSFL increased their time spent walking,

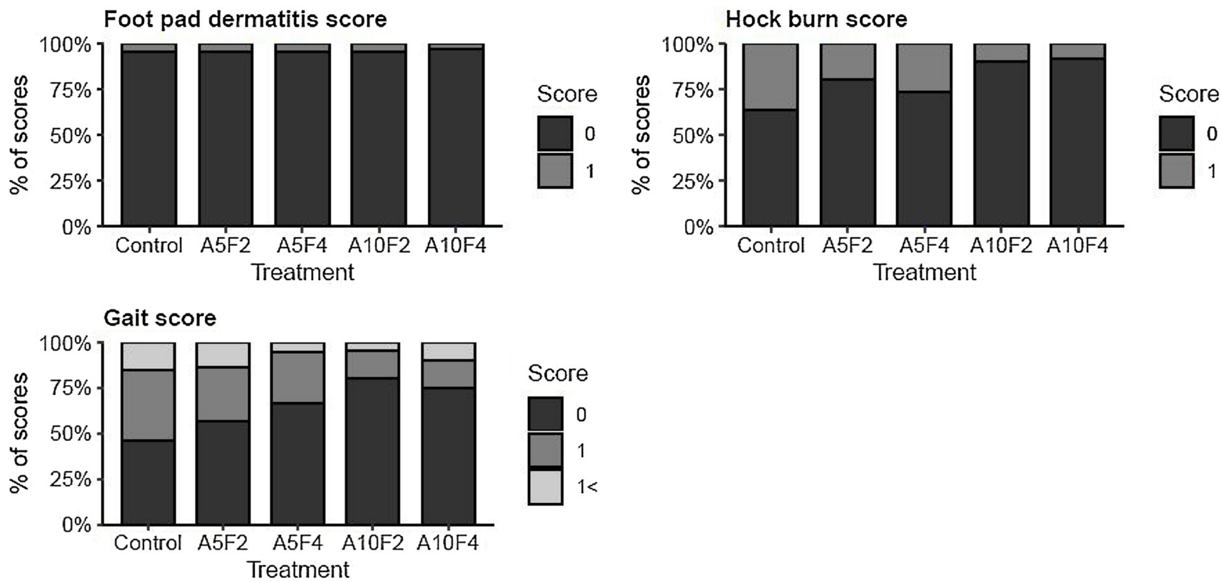

Fig. 4. Percentage of broilers with distinct foot pad dermatitis scores, hock burn socres and gait scores, per treatemnt. Treatments include broilers receiving no larvae (Control), or provided with larvae in different amounts (5 % or 10 % of the total dietary DM replaced with larvae, A5 and A10 respectively) and frequencies (two or four times a day, F2 and F4 respectively).

standing idle, ground pecking and foraging, whilst their time spent resting was decreased compared to controls. Behavioural observations of the daily time budget and around larval provisioning showed that the activity of broilers receiving 5 % of their dietary DM as BSFL provided two times a day was only occasionally elevated. Providing this amount four times a day resulted in a strong but short-term peak in activity around larval provisioning, explaining why the area under the curve of activity around larval provisioning was not affected. However, in most weeks the activity in the daily time budget observations was increased in these A5F4 broilers compared to controls. A comparable increase in activity was observed in broilers receiving 10 % of their dietary DM as BSFL twice a day. Finally, the highest activity level was achieved by broilers receiving 10 % of their dietary DM as BSFL four times a day. In several weeks this group showed more active behaviours than other groups, apart from the controls, particularly the A5F2 group. Furthermore, for all broilers the time spent walking and being active decreased over time and the time spent resting increased over time, which is in line with other studies (Bailie et al., 2013; Baxter et al., 2018b). However, broilers receiving 10 % of their dietary DM as BSFL provided four times a day started showing this decline in activity later in the production period than all other treatments. Increasing and maintaining activity levels from an early age onwards was found to promote good leg bone development in broilers (Reiter and Bessei, 2009), and the observed prolonged elevated activity levels could therefore indicate improved leg health in these broilers.

Our results suggest that providing BSFL shifts the behavioural repertoire of broilers towards more active, natural behaviours such as foraging, and that this shift is strongest when a large amount of larvae is provided in a high frequency. This is in line with previous studies showing modest increases in activity around insect provisioning in small amounts (Oonincx, 2020, personal communication; Pichova et al., 2016), where authors suggested that prolonged access to larvae would further increase broiler activity. Other previously tested enrichment materials such as straw bales or strings caused a temporary elevation of broiler activity only (Bailie et al., 2013; Bailie and O'Connell, 2015), and providing wood shavings, perches and metal chains even reduced

Table 3
Performance parameters of broilers receiving no larvae (Control) or provided with larvae in different amounts (either 5 % or 10 % of the total dietary dry matter replaced with larvae, A5 and A10 respectively) and frequencies (two or four times a day, F2 and F4 respectively).

	Control	A5F2	A5F4	A10F2	A10F4	P - value
ADG (g)						
d1-d6	9.2 ± 0.3	9.3 ± 0.2	8.5 ± 0.3	9.2 ± 0.3	9.7 ± 0.2	0.52
d7-d13	29.5 ± 0.6	29.7 ± 0.6	27.9 ± 0.7	27.6 ± 0.7	29.5 ± 0.6	0.27
d14-d20	55.8 ± 1.0[a]	53.2 ± 1.0[ab]	52.2 ± 1.0[ab]	50.4 ± 1.1[b]	49.9 ± 1.0[b]	**0.01**
d21-d27	82.1 ± 1.6[a]	79.2 ± 1.5[ab]	79.6 ± 1.6[ab]	75.8 ± 1.3[b]	75.4 ± 1.6[b]	**0.01**
d28-d34	102.2 ± 2.1	105.8 ± 2.2	108.2 ± 1.8	103.4 ± 1.6	107.8 ± 2.2	0.26
d35-d42	111.5 ± 2.7	114.6 ± 2.2	112.2 ± 1.9	103.4 ± 2.2	109.2 ± 2.6	0.18
FW (g)	2890 ± 37.9[a]	2902 ± 39.4[a]	2866 ± 38.5[ab]	2726 ± 36.9[b]	2808 ± 42.6[ab]	**0.02**
ADMI[1] (g)						
-BSFL[2]	97.03 ± 1.84[a]	90.67 ± 1.13[b]	89.83 ± 1.12[bc]	82.83 ± 0.61[d]	85.28 ± 2.32[cd]	< **0.001**
+BSFL[3]	97.03 ± 1.84	96.54 ± 1.13	95.7 ± 1.12	95.06 ± 0.61	97.52 ± 2.33	0.86
ADMEI[1,3] (MJ)	1.20 ± 0.02	1.20 ± 0.01	1.19 ± 0.01	1.19 ± 0.01	1.22 ± 0.03	0.85
DMCR[1,3] (g/g)	1.20 ± 0.02[ab]	1.20 ± 0.01[a]	1.20 ± 0.01[a]	1.26 ± 0.01[b]	1.25 ± 0.01[b]	**0.001**

ADG: average daily gain. FW: final weight. ADMI: average daily dry matter intake. ADMEI: average daily metabolizable energy intake. DMCR: dry matter conversion ratio. Data are reported as means ± SEM. Within each row different letters indicate significant ($p < 0.05$) differences between treatments.

[1] Based on the grower period, day 8–42.

[2] Based on intake of feed (meal).

[3] Based on intake of feed (meal) and BSFL.

activity compared to controls (de Jong and Gunnink, 2018). The main difference between these materials and BSFL is likely the strong appetitive value for broilers and the fact that BSFL are alive and moving, which could make them highly interesting for broilers (Bokkers and Koene, 2002; Jones et al., 1998) and therefore more effective in increasing foraging behaviour and thus activity. This is supported by the observation that the increase in foraging-related behaviours was more prominent and long term than that of other behaviours in all BSFL treatments. As being active becomes more energy-expensive with increased broiler weight (Tickle et al., 2018), it is likely that the presence of consumable larvae is required to increase activity levels later in the production period, and that this activity primarily consists of foraging behaviour. This premise is further supported by the observation that the delivery of desirable mealworms also caused a stark increase in foraging behaviours in a previous study (Pichova et al., 2016). Our results suggest that BSFL in contrast to many other enrichment materials strongly motivate broilers to show active behaviour, particularly foraging, until the end of the rearing period, despite the energetic costs.

Apart from affecting broiler activity, the consumption of BSFL influenced broiler performance, even though by mimicking the nutritional value of BSFL in the feed a similar ME intake was achieved for all treatment groups. The relative consumption of BSFL was slightly higher than anticipated (approximately 6 % and 12 % instead of the expected 5 % and 10 % of dietary DM), which could have caused a slight imbalance in amino acid uptake, affecting broiler growth. Broilers receiving 10 % of their dietary DM as larvae showed a reduced growth during day 13–27, and A10 broilers receiving those larvae two times a day had a lower final weight compared to controls. Previous studies found that BSFL consumption in low levels (up to 5 % of the dietary DM) had a neutral (Lee et al., 2018) or positive (Dabbou et al., 2018) effect on broiler growth, whereas diets containing 10–15 % live BSFL resulted in diminished broiler growth and final weight (Oonincx, 2020, personal communication). Chitin could play a role in the observed reduction in broiler growth, as suggested by Dabbou et al., 2018. Chitin can be only partially digested by broilers (Hossain and Blair, 2007; Khempaka et al., 2006), and it can hinder digestibility of crude protein in the broiler digestive tract (Khempaka et al., 2011). In our study, providing 10 % of the dietary DM as BSFL twice a day resulted in relatively large portions of larvae, therefore the digestion-inhibiting effect of chitin could have been strongest for these broilers, resulting in the observed diminished performance. This is also in line with the absence of an effect on final weight of broilers receiving this amount provided four times a day, and thus in smaller portions.

In our experiment broilers were kept at a relatively low stocking density, with good litter quality, which could explain the low occurrence of leg problems compared to commercial conditions (Bessei, 2006; de Jong et al., 2019). Even so, the occurrence and severity of hock burn and lameness was significantly lower in broilers receiving 10 % of their dietary DM as larvae compared to controls, and the severity of lameness was lower in broilers receiving 5 % of the dietary DM as larvae four times a day compared to controls. Leg problems can be painful and inhibit natural behaviour (Danbury et al., 2000), suggesting these broilers experienced improved welfare compared to others. We expect that the beneficial effect of larvae provisioning on leg health will be more prominent under commercial conditions where broilers can benefit more from enrichment, however the applicability of providing BSFL in commercial settings remains to be investigated. For instance, research on the effect of lower light intensities and higher stocking density on BSFL detection and potential negative effects of anticipation of BSFL delivery on bird-directed pecking behaviour deserve attention. In addition, the costs, supply, and distribution methods of the larvae must be considered.

Providing BSFL affected broiler activity, weight and leg health, and these variables may be interconnected. A positive association between leg health and activity was found in our study and in previous studies (Bassler et al., 2013; de Jong et al., 2014). Causality in this relationship is unclear, as activity could promote better leg development and increase leg strength (Reiter and Bessei, 2009), but painful leg problems could also inhibit broiler activity (Danbury et al., 2000). Besides, a link between activity and leg health may also arise from the bi-directional relationship between broiler activity and growth. On the one hand, increased activity levels could result in less energy being available for broiler growth (Tickle et al., 2018). On the other hand, an inherently lower growth rate facilitates higher activity levels as observed in slow-growing broilers (Bokkers and Koene, 2003). In the current study providing 10 % of the dietary DM as larvae provided two times a day improved broiler leg health, however as this group displayed a lower ADG and final weight, the effect might be confounded with body weight. Overall, the causality of the relationship between growth, activity and leg health in the current experiment is unclear and requires further examination. However, the improved leg health and activity levels without significantly reduced growth observed in broilers receiving 5 % or 10 % of their dietary DM as BSFL provided four times a day indicates that improved activity and welfare by larval provisioning can be obtained without impairing performance.

Not only leg health but also specific broiler behaviours are linked to broiler welfare. In the current study, comfort behaviour was not affected by larval provisioning. Similarly, providing mealworms did not promote nor diminish comfort behaviour in broilers (Pichova et al., 2016). In laying hens, comfort behaviour was exhibited in anticipation of a reward, namely access to mealworms (Zimmerman et al., 2011), and it has been suggested that comfort behaviour is an indicator of good welfare (Nicol et al., 2009). However, Moe et al. (2014) found that anticipatory comfort behaviour was not affected by a dopamine blockade in layers, indicating a potential disengagement between comfort behaviour and the reward system in chickens. Other natural behaviours such as foraging behaviour were increased in the current study. Performing such intrinsically motivated behaviours can be rewarding (Moe et al., 2012, 2014), and therefore improve broiler welfare (Bracke and Hopster, 2006), although this was not directly investigated in the current experiment. Further research including other welfare indicators, preferably also assessing affective state, is needed to determine the effect of larval provisioning on broiler welfare, as well as studies on the impact of BFSL provisioning on welfare of broilers kept under commercial conditions.

5. Conclusion

In conclusion, this study showed long-term elevated levels of foraging behaviour and general activity in broilers receiving BSFL, and this effect was largest and most consistent for broilers receiving BFSL in the highest amount and frequency tested, i.e. 10 % of their dietary DM as larvae provided four times a day. Broilers receiving 5 % or 10 % of their dietary DM as BSFL four times a day also experienced improved leg health. These broilers had a similar final weight as controls, despite a temporary period of reduced growth for broilers receiving 10 % of their dietary DM as BSFL four times a day. Thus, by facilitating natural behaviour and activity, and by reducing leg health problems, larval provisioning can benefit broiler welfare. Further studies will focus on strategies facilitating prolonged access of BSFL for broilers.

Declaration of Competing Interest

None.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.applanim.2020.105082.

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