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RESEARCH ARTICLE

Automatic dispenser of live Black Soldier Fly larvae to feed poultry

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Abstract

Feeding poultry with live insect larvae stimulates natural behaviour and improves poultry welfare, when poultry has prolonged or frequent access to the larvae. But how to feed live insect larvae to poultry without labour-intensive hand feeding? This paper focusses on the development of a device that overcomes this challenge. A circular device was designed with eight storage compartments, which were filled once a day with live Black Soldier Fly (BSF) larvae (*Hermetia illucens*). A motor controlled the timed rotation of the device multiple times per day, initiating the release of larvae when a compartment was pushed over an outlet. Every 60 minutes, a new compartment was pushed over the outlet, which means that after eight hours all compartments are emptied. To achieve a gradual release of larvae per storage compartment the device was timed to move every 30 minutes half a storage compartment forward. The larval release was recorded every 5 minutes within the 60 minutes. The device was tested at 18 °C, 24 °C and 30 °C, with 3.4 g and 129.8 g BSF larvae per compartment, and with three different outlet types of different size and shape. The larval release rate was influenced by temperature, amount of larvae, outlet type, and interactions between these factors. After placing a new compartment above the outlet, 50% of the larvae were on average released within 6 minutes. After 60 minutes, on average only 0.5% larvae remained in the compartment. Outlets with wider openings are preferred over the outlet with the narrowest outlet because less larvae remained in the compartments. The dispenser fulfilled the low-labour-intensity requirement as filling was only necessary once a day, the release of different amounts of larvae was achieved over several hours. This automatic dispenser provides a valuable tool to investigate the behaviour of poultry fed with live BSF larvae.

Keywords

dispenser of live insect larvae – poultry welfare – behaviour – black soldier fly larvae

1 Introduction

While insects have usually been perceived as pests in agriculture (Van Huis *et al.*, 2013), nowadays several species have been proposed to be multifunctional mini-livestock (IPIFF, 2018; Makkar *et al.*, 2014; Van Huis, 2013). Especially as feed component, insects may play an important role. The poultry production sector is urgently looking for new protein sources which can replace unsustainable soybean and fish meal in livestock diets (OECD/FAO, 2019). An important property of insects, such as fly larvae, is that they can convert low quality substrate into valuable high-quality body mass (Bava *et al.*, 2019; Miranda *et al.*, 2020), which holds

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great potential to contribute to the sustainable development goals (Barragán-Fonseca *et al.*, 2020; Dicke, 2018). Furthermore, because insects are part of poultry diets in nature, they are assumed to have additional functional and bioactive properties. For instance, insects are highly attractive for poultry, and trigger poultry to perform natural behaviour such as foraging (Dörper *et al.*, 2021; Ipema *et al.*, 2020a; Veldkamp and Van Niekerk, 2019). The ability to show this behaviour is an indication of improved welfare (Ipema *et al.*, 2020a; Veldkamp and Van Niekerk, 2019). Poultry is known to experience health and welfare issues in terms of leg problems, poor gait scores, and maladaptive pecking behaviour during rearing (Knowles *et al.*, 2008; Star *et al.*, 2020; Veldkamp and Van Niekerk, 2019). Feeding live insects to poultry might counteract those issues by stimulating activity and foraging behaviour (Ipema *et al.*, 2020a,b). Therefore, insects are highly interesting feed components for poultry, both in terms of nutritional value and behavioural stimulation. Fly larvae such as larvae of the Black Soldier Fly (BSF, *Hermetia illucens*) and Housefly (*Musca domestica*) have been extensively investigated as feed for poultry (Dörper *et al.*, 2021). In the current research, we focused on BSF larvae.

Various methods to feed live larvae to poultry have been described in the literature. Some researchers provided the daily portion of live larvae manually once a day (Bellezza Oddon *et al.*, 2021; Veldkamp and Van Niekerk, 2019), manually at several timepoints per day (Ipema *et al.*, 2020b,a), and continuously over several hours gradually with unmotorized devices (Leushuis and Paul, 2020; Star *et al.*, 2020), while some of those devices required active engagement by the poultry with the device (Ipema *et al.*, 2020a). A comparison between single and multiple feeding moments has shown that frequent or extended access to live insect larvae is a key factor to trigger active poultry behaviour (Ipema *et al.*, 2020a). The behavioural effect seems to be associated to the frequency of feeding (De Jong *et al.*, 2021; Ipema *et al.*, 2020b). For example, when turkeys were fed 10% of their daily feed intake with live larvae, the insects were consumed within 1-2 minutes (Veldkamp and Van Niekerk, 2019). Continuous provision over the day seems required to stimulate longer lasting or more frequent activity.

Pellets are a commonly used feed form in commercial farms. They facilitate a gradual provision of all nutrients over the day, which is key for favourable feed conversion ratios (Xu *et al.*, 2015). Broilers perform selective feeding based on feed characteristics such as flavour (Balog and Millar, 1989), and particle size (Xu *et al.*, 2015). Because live larvae are known to be attractive to poultry and quickly consumed (Ipema *et al.*, 2020b; Star *et al.*, 2020; Veldkamp and Van Niekerk, 2019), the provision of all larvae per day at once might cause a temporary trade-off of pellets for live larvae and an imbalanced nutrient uptake. Therefore, from a nutritional and welfare perspective, it seems best to provide larvae gradually during the day. However, handfeeding at multiple occasions is very labour intensive (Ipema *et al.*, 2020a), which highlights the need to explore new feeding methods.

Furthermore, broiler feed intake changes rapidly over time. After hatching, broilers consume small quantities (~7 g) of feed per day which increases about 19 times before reaching slaughter weight (P. van Boekholt, pers. comm.). Besides, temperature in the broiler house changes over time, starting with 33 °C post-hatching and decreasing to 18 °C toward the end of the production (Hubbard, n.d.). A dispensing system therefore needs to securely release different quantities of insect larvae at a wide temperature range. Here, we aimed to develop a device that can tackle these challenges to improve the provision of live BSF larvae to poultry for the poultry farmer and the birds.

2 Materials and methods

Design

Dispenser

(Numbers in the text refer to the numbers in Figure 1 and Supplementary Table S1)

The dispenser consists of a round storage container (8) with eight separate storage compartments, see schematic overview in Figure 1. The motor (1) turns the storage container (8) so that different compartments will be moved over the outlet (14). The motor can be controlled by an external digital timer (with time setting in seconds). The release of live larvae is initiated as soon as a filled storage compartment of the storage container (8) is pushed over the outlet (14) by the motor. The outlet is always present below one of the eight storage compartments. If all eight compartments are filled at the beginning, live larvae will immediately fall through the outlet (14) because one of the compartments is above the outlet. The outlet is smaller than the bottom of each of the eight storage compartments; therefore, not all larvae within one compartment will get released at once. Initially the larvae that are moved over the outlet are dispensed. The release of the remaining larvae from that compartment depends on the movement of the larvae and the schedule of the digital timer that

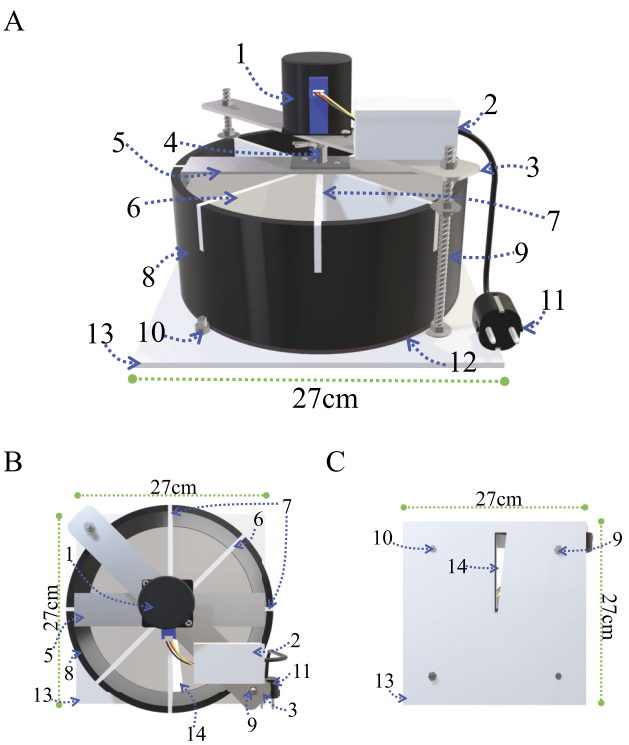

FIGURE 1 Design of a dispenser of live insect larvae. (A) Side view, (B) top view and (C) bottom view. Components: 1 = Synchronous motor; 2 = Distribution box (covering the connections between motor (1) and capacitor (present in the distribution box) and extension cable (11)); 3 = Aluminum plate; 4 = Connection between the motor (1) and aluminum plate (5); 5 = Aluminum plate; 6 = Short divider (held in position by being clamped larvae are stored; the container has eight separate sections; 9 = Lateral frame; 10 = Lateral limitation; 11 = Power cable; 12 = Felt; 13 - Bottom plate; 14 = Outlet. For more details of the parts, see Supplementary Table S1.

determines the rotation of the container (8). In this way, the release of larvae is not predictable for the chickens. The movement of the next storage compartment over the outlet ensures that eventually all remaining larvae will fall through the outlet. The dispenser can be filled with live larvae once or several times per day dependent on the frequency of larvae provision and how many live larvae should get released.

Experimental verification

The following setup was chosen to verify the working principle of the larval dispensing system.

Handling of larvae

Live BSF larvae were obtained once a week (one batch per week) from Protix Biosystems BV (Dongen, the Netherlands) and stored in a climate-controlled room at 10 °C and 70% relative humidity at the Research facility Carus (Wageningen University & Research, Wageningen, the Netherlands). These storage conditions were chosen to reduce the metabolic activity of the larvae, avoiding nutrient use and larval development. During storage, live BSF larvae were mixed with sawdust to keep them dry and clean. Previous handling of the larvae had shown that if larvae are moist, they are able to adhere to the plastic elements used and escape from plastic containers. Approximately one hour before use, the live BSF larvae were taken out of the storage and were transferred to ambient temperature to increasing larval activity and movement. During the reactivation phase the larvae were transported to a climate chamber where the experiments were carried out. To separate the larvae from the sawdust the larvae were passed through a 4 mm sieve and weighed into portions of 3.4 g or 129.8 g. The amount of 3.4 g of live larvae per storage compartment represents the amount of live larvae needed to replace 5% of the dry matter feed intake per 24 one-day-old broilers, and 129.8 g live larvae per storage compartment represent the greatest amount of larvae needed to replace 10% of the dry matter feed intake per 24 seven-week-old broilers before slaughter (P. van Boekholt, pers. comm.). The chosen inclusion levels for live larvae are based on previous research (Ipema *et al.*, 2020a; Star *et al.*, 2020; Veldkamp and Van Niekerk, 2019), while group sizes between 20-28 broilers per pen are commonly used in research setups (Elahi *et al.*, 2020; Ipema *et al.*, 2020a, 2022; Rezaei *et al.*, 2018).

Experimental setup

The release of live BSF larvae by the dispenser was tested at three different temperatures (18 °C, 24 °C and 30 °C) at 65% relative humidity. Slow-growing broilers require a gradual decrease of housing temperatures starting at 33 °C and going down to 18 °C before slaughter (Hubbard, n.d.). Temperatures higher than 30 °C could not be tested due to the upper temperature limit of the climatic chamber. The dispensers were tested two times under the same temperature, one and six days after arrival of the larvae. Six dispensers were built for the experiment. Three different outlet types were created (Figure 2). Two of the six dispensers were equipped with the same outlet type. Of the dispensers with the same outlet type, one was filled with a small (3.4 g) and the other with a large quantity (129.8 g) of live BSF larvae per storage compartment (Figure 2). To measure the release of larvae per storage compartment, only four storage compartments of the dispenser were filled with live BSF larvae, every alternate storage compartment remained empty (Figure 2). This was done because if all storage

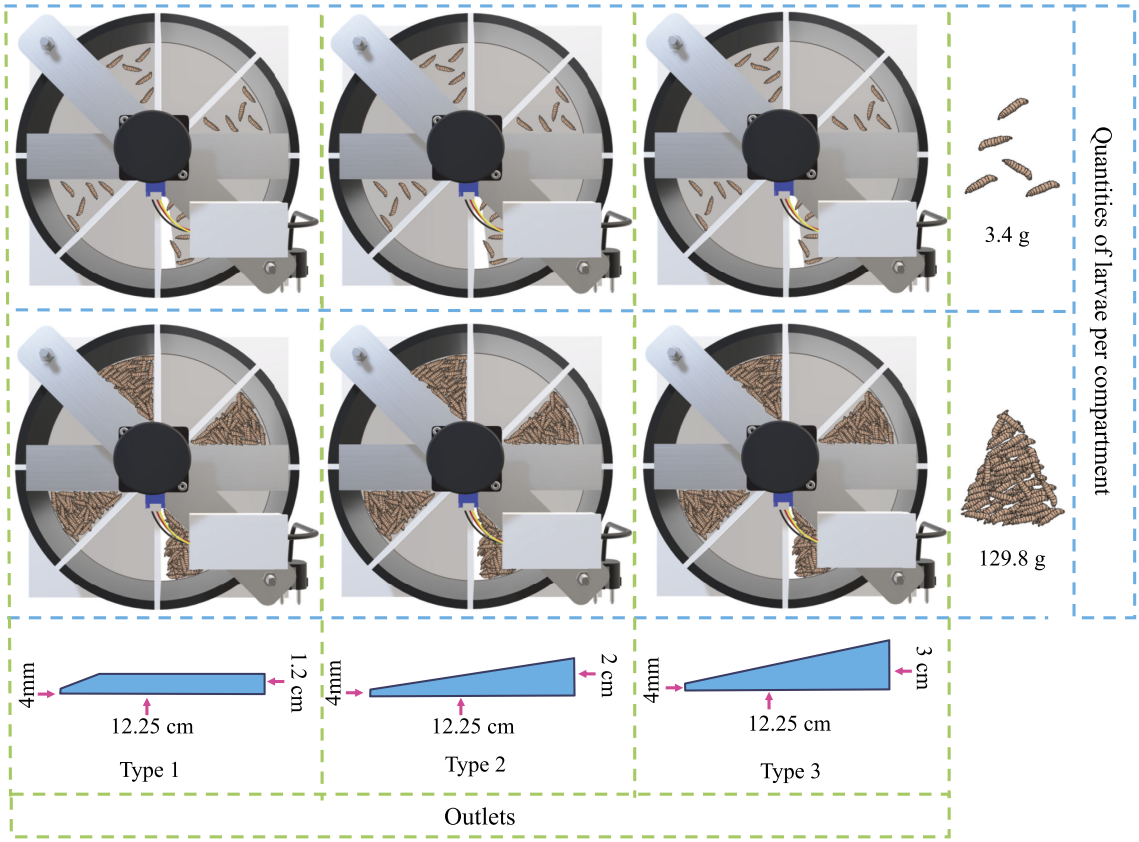

FIGURE 2 Overview of experimental design. Six live larvae dispensers were used in combination with three different outlet types and two quantities of larvae per compartment of the container.

compartments had been filled with larvae, it would not be possible to distinguish the release of larvae between two successive storage compartments. An overview of the setup is given in Figure 2.

Motor timing

The motor (59TYD-7A, Fengtech®, Ningbo, China) turns at a speed of 2 rounds per minute. The timer movement was controlled by a digital timer (DoLike, China) allowing up to 20 different programmed On/Off actions per day. The timer was opened and equipped with a metal oxide varistor (#A2062, Popesq®, Greußen, Germany). The varistor prevented voltage peaks, that occurred when the motors were turned on, from resetting the timer program. The following schedule was used for timing the release of larvae (Table 1). The dispenser has 8 storage compartments (Figure 3). The goal was to release larvae over a time span of 8 hours. This means every hour a new storage compartment needs to be over the outlet. To achieve a gradual release of larvae per storage compartment the motor was timed to move every 30 minutes half a storage compartment forward. Therefore, 16 On/Off actions were scheduled. Since the motor makes a full turn within 30 seconds, 14 of these 16 actions lasted for two seconds and two of these 16 actions were chosen to last for one second instead of two seconds. The device was filled with live larvae at 9:30 am, at that time the dispenser outlet is positioned below the first storage compartment. Larvae in this compartment fall immediately after filling the compartment. After 30 minutes the motor makes a first movement for 2 seconds. The second half of storage compartment number 1 is now over the outlet. With the next motor movement at 10:00 am, the beginning of storage compartment two will be above the outlet. This pattern continues until 17:30 (Table 1).

Measurements

After sieving the larvae, a sample of 20 individual larvae was taken. The larvae were weighed individually to quantify the weight variation among larvae in the different batches and after different days of storage. For the calculation, the mean larval weight per sampling time (six sampling times) was determined. Subsequently, as a measure for variation the absolute deviation from the mean larval weight for every individual larva was calculated. Also, per batch and storage time ten groups of ten larvae were taken to measure the average larval

TABLE 1 Digital timer program used to control the motor movement of the dispenser device. It requires two rotations of the motor to move a new storage compartment of the dispenser over the outlet. The device was filled with live larvae at 9:30 am, at that time the dispenser outlet was already positioned below the first storage compartment. Therefore, for the first storage compartment only one motor movement was required

On/Off	Timepoint on HH:MM:SS	Timepoint off HH:MM:SS	Nr (as in Figure 3) of dispenser storage compartment positioned over the outlet
1	10:00:00	10:00:02	1
2	10:30:00	10:30:02	2
3	11:00:00	11:00:02	2
4	11:30:00	11:30:01	3
5	12:00:00	12:00:02	3
6	12:30:00	12:30:02	4
7	13:00:00	13:00:02	4
8	13:30:00	13:30:02	5
9	14:00:00	14:00:02	5
10	14:30:00	14:30:01	6
11	15:00:00	15:00:02	6
12	15:30:00	15:30:02	7
13	16:00:00	16:00:02	7
14	16:30:00	16:30:02	8
15	17:00:00	17:00:02	8
16	17:30:00	17:30:02	1

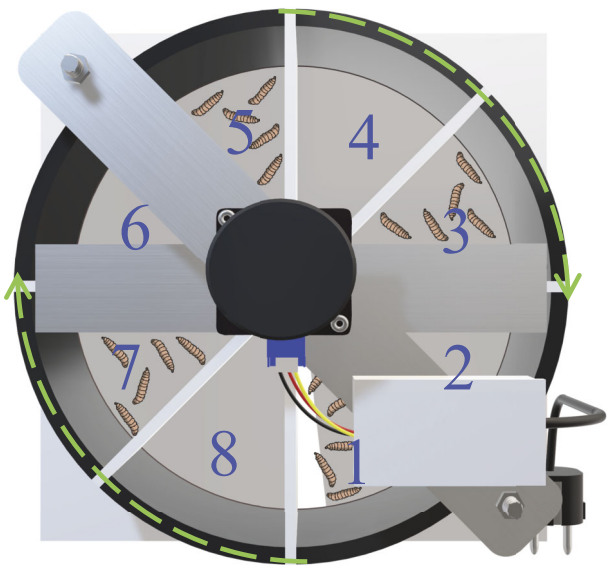

FIGURE 3 Dispenser setup at 9:30 am after filling the live larvae into the dispenser device. Storage compartment one is positioned above the outlet. Larvae in this storage compartment start to fall through the outlet immediately after filling. The green arrows indicate the direction the motor moves the round container with eight storage compartments.

weight. Measurements of the release of larvae from the dispenser were taken between 9:30-10:30, 11:30-12:30, 13:30-14:30, and 15:30-16:30. Within these time periods the weight of the larvae that were released from the dispenser was recorded every 5 minutes. For that purpose, a cup was placed below each dispenser outlet, the cup was exchanged for an empty one every 5 minutes. At the end of a 60-minute-period we recorded if any larvae remained in the storage compartments and recorded the number and weight.

Statistical analysis

All statistical analyses were performed in R 4.1.0. Packages (R Core Team, 2021) used in R were readxl (Wickham and Bryan, 2019), nlme (Pinheiro *et al.*, 2021), lme4 (Bates *et al.*, 2015), lmerTest (Kuznetsova *et al.*, 2017), pbkrtest (Halekoh and Højsgaard, 2014), emmeans (Lenth, 2021), and multcomp (Hothorn *et al.*, 2008).

The variation in larval weight and the average group weights of live larvae were analyzed with linear models including batch (three different batches) and storage time (one or six days) with fixed effects in the model. The model fit was assessed by visual inspection of the residual plots (histogram, standardized residuals vs fitted values). Data of larval weight variation was square-root transformed to achieve normality. Significance was declared at $P < 0.05$ and post-hoc pairwise comparisons were conducted accordingly with P value adjustment using the Tukey or multivariate t-distribution method.

As described above, repeated measurements of released larval weights over time (every 5 minutes during 1 hour, so 13 measurements) per compartment run were obtained. In total there were 144 compartment runs: measurements were taken on 6 days (3 temperature and 2 storage time combinations randomized over 6 days), with 6 dispensers per day (3 outlet types and 2 larval amount combinations randomized over dispensers), and 4 compartments per dispenser. Per compartment run, the 13 released larval weights were transformed into 13 accumulated weight proportions, running from 0 (no larvae released) to 1 (all larvae released). For each of the 144 compartment runs a nonlinear model (either employing an exponential or a logistic curve, allowing for "jumps" during the moments of motor movement) was fitted to estimate the timepoint at which 50% (t50) of the larvae were released from the dispenser system under the different conditions. As the observations on the four compartments per dispenser are considered pseudo replicates, the data set was condensed by averaging observations of the four compartments per dispenser. Afterwards, the data set was analyzed using a linear mixed-effects model with temperature (18 °C, 24 °C and 30 °C), larval storage time, dispenser outlet type (three different types), and amounts of larvae placed into the compartments of the dispenser (3.4 g and 129.8 g) as fixed effects. Additionally, two-way interactions between temperature and amounts, between temperature and dispenser outlet type, and between amounts and dispenser outlet type were added as fixed effects to the model. The model contained test date as a random effect. The variable t50 was log-transformed for analysis. The model fit was assessed by visual inspection of the residual plots (histogram, standardized residuals vs fitted values). Significance was declared at $P < 0.05$ and post-hoc multiple comparisons were conducted accordingly with P value adjustment using the multivariate t distribution method.

3 Results

Larval weight

The average larval weight, as calculated from the weight assessment per group of ten larvae, was significantly affected by batch number ($P < 0.001$) and storage time ($P < 0.001$) (Figure 4). The larvae of the first batch were significantly heavier than the larvae of the following two batches. Larval weight after one day of storage was significantly greater than after six days of storage. There was no significant interaction between batch number and storage time ($P = 0.308$).

Variation in larval weight was not significantly affected by batch number ($P = 0.059$), but it was affected by storage time at 10 °C ($P = 0.046$) (Figure 5). There was a significant interaction between batch number and storage time (batch number * storage time: $P = 0.022$). The variation in larval weight was not different between samples of the same batch stored for either one or six days. Variation in larval weight between different batches was dependent on the storage time. After one day of storage, variation in larval weight was not significantly different between batches, while after six days of storage the first batch had significantly greater larval variation compared to the second batch.

Dispenser test

The time after which 50% of the larvae had been released was affected by temperature ($P = 0.008$), outlet type ($P = 0.001$) and several interactions that will be discussed below. The time after which 50% of the larvae had been released by the dispenser was not significantly influenced by storage time ($P = 0.333$), amounts of larvae dispensed ($P = 0.627$), and the interaction between temperature and outlet type ($P = 0.823$).

The effect of the amount of larvae in the compartment on the time till 50% of the larvae had been released was dependent on the type of dispenser outlet ($P < 0.001$, Figure 6). When using small amounts of larvae, the release of 50% of the larvae was reached the earliest with outlet 1 and the latest with outlet 2. Outlet 3 showed intermediate values. When using large amounts of larvae, the release of 50% of the larvae was reached earliest with outlet 3, followed by outlets 2 and 1. Moreover, dispensers with the same outlet type reached 50% larval release at different timepoints dependent on the amount of larvae added to the dispensers. With outlet 1, 50% larval release was reached earlier when using small amounts of larvae compared to using large quantities. This pattern was the reverse for outlets 2 and 3. For the latter groups, dispensers with small amounts of larvae reached 50% larval release later than dispensers with large amounts.

Temperature affected the dispense rate. The release of 50% of the larvae was reached significantly later at 18 °C compared to 24 °C and 30 °C for both amounts of larvae used ($P < 0.001$, Figure 7). The interaction between temperature and amount of larvae was significant ($P < 0.001$, Figure 7). At 18 °C when using large amounts of larvae, 50% larval release was reached earlier than when using small amounts. In contrast, at 30°C this

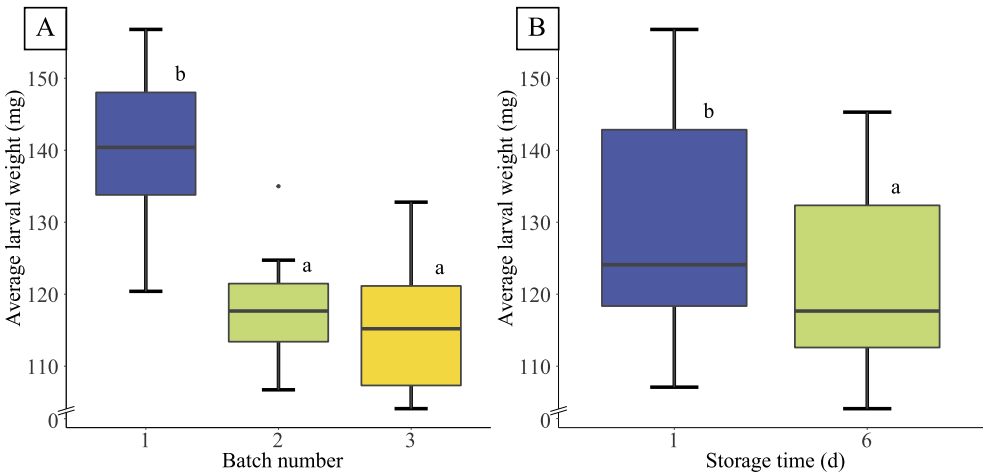

FIGURE 4 Boxplots of the average weight of Black Soldier Fly larvae as assessed for 10 samples of 10 larvae by batch number (A) and by storage time (B). Different letters between groups within one panel indicate significance ($P < 0.05$). The lower whisker of the boxplots represents the smallest observation ≥25% quantile – 1.5 × interquartile range (IQR). The upper whisker represents the largest observation ≤75% quantile + 1.5 × IQR. Points outside the whiskers represent outliers.

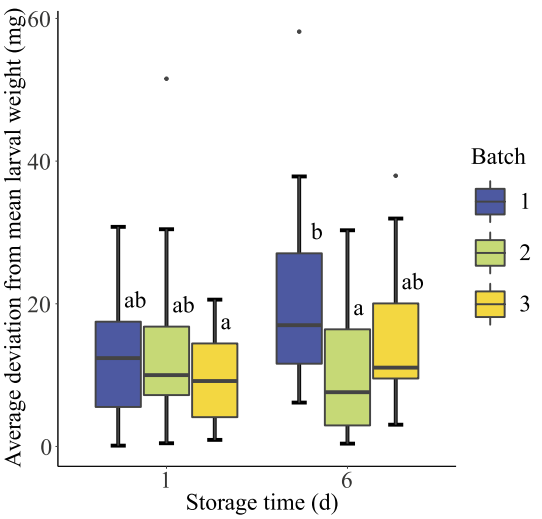

FIGURE 5 Boxplots of absolute deviation from mean larval Black Soldier Fly weight at different batches and storage times. Different letters between boxplots indicate significance ($P < 0.05$). The lower whisker of the boxplots represents the smallest observation ≥25% quantile – 1.5 × interquartile range (IQR). The upper whisker represents the largest observation ≤75% quantile + 1.5 × IQR. Points outside the whiskers represent outliers.

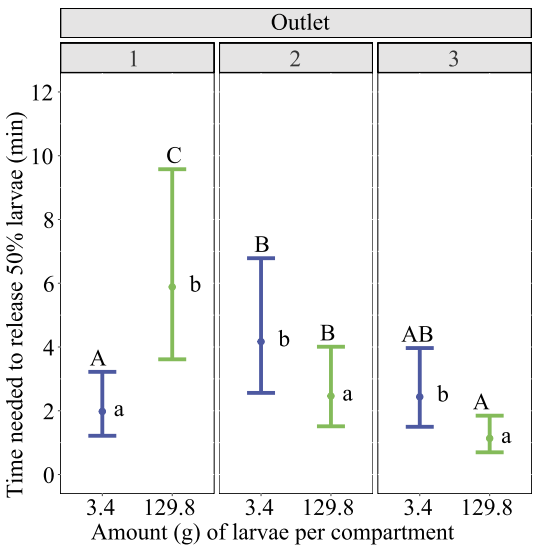

FIGURE 6 Time needed for 50% of Black Soldier Fly larvae to be released from the dispensers. Data is presented as back transformed model-based estimated means with lower and upper confidence interval (95%). Different lower-case letters indicate significance ($P < 0.05$) between different amounts tested within the same outlet type. Different capital letters indicate significance ($P < 0.05$) between different outlet types tested within the same amount of larvae.

effect was the reverse, 50% larval release was reached faster with small amounts than with large amounts. At 24 °C, 50% larval released was reached at the same time independent of the amounts of larvae placed into the dispensers.

After 60 minutes, when one storage compartment was completely pushed over the dispenser outlet, we assessed how many larvae were left in the storage compartment. On average 0.5% (± 0.24% se) of the larvae remained in the storage compartments. The range per outlet type was as follows: outlet 1 (0-31.9%), outlet 2 (0-8.1%), outlet 3 (0-0.8%). Occasionally, motors did not turn the dispensers at the scheduled timepoints or turned counterclockwise. In these cases, the dispensers were turned manually.

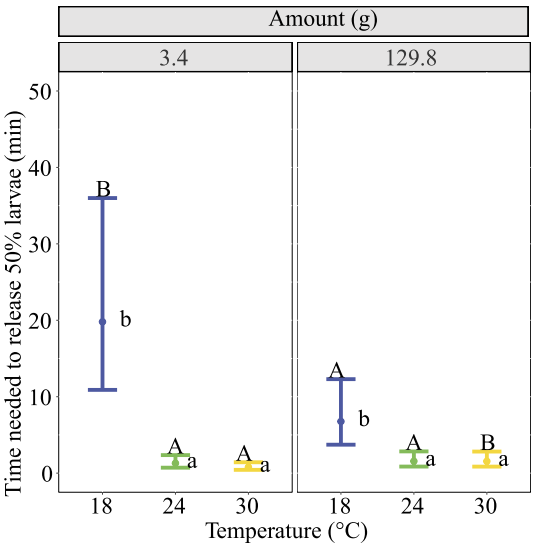

FIGURE 7 Effects of temperature and amounts of Black Soldier Fly larvae added to the dispensers, expressed as timepoint at which 50% of the larvae had been released from the dispensers. Results are presented as back transformed model-based estimated means with lower and upper confidence interval (95%). Different lower-case letters indicate significance ($P < 0.05$) between different temperatures tested with the same amount. Different capital letters indicate significance ($P < 0.05$) between different amounts tested under the same temperature.

4 Discussion

The focus of the current research was to develop a device which automatically and reliably dispenses BSF larvae over an extended period of time, for different amounts of larvae and at different temperatures. Such a dispenser that can be a valuable tool in research on precise replacement of soybean or fishmeal in diets of broilers during their growth from newly hatched chicks to slaughter-ready animals. Because the nutritional and environmental requirements of broilers change during their development (Hubbard, n.d.), the dispenser needs to function accurately under those conditions. With the developed device, larvae were successfully dispensed using two very different amounts of larvae, at low and high temperatures and with different outlet types. For a better visual understanding of how the dispenser functions, see the time-lapse video in supplementary video S1. The video shows larvae being released through a dispenser with a 3 cm outlet. The video was recorded during a preliminary experiment at 25 °C.

The larvae that were received for the research came from a large-scale production facility. For the batches that were received the average larval weight was 124.8 mg, although this value varied between batches. The data represent the variation that must be considered when supplying broilers with live BSF larvae from a commercial source. Moreover, the storage of the larvae during six days at 10 °C and 70% relative humidity affected larval weight. During this storage period, the live larvae lost weight. Weight loss due to cold storage likely results from the loss of water and the proportion of larval dry matter increased over time (Koštál *et al.*, 2016; Yocum *et al.*, 2012). In the present trial, no BSF larval movement was observed during the storage at 10 °C and 70% relative humidity. It is assumed that the BSF larvae were in a reversible chill-coma, which is described as a state in which metabolic turnover is slowed down and the insect becomes immobile (MacMillan and Sinclair, 2011; Mellandby, 1939). In this state, expenditure of energy and loss of dry mass is expected to be low. In previous research, weight loss during storage mainly occurred due to moisture loss (Koštál *et al.*, 2016). Moisture loss and depletion of energy reservoirs might impact the nutritional composition, survival rate, and behaviour of the larvae. This might affect the quality of the larvae as a feed ingredient, but also larval mobility. As a result, this could affect the functioning of the chosen larval dispensing system if that is fully or partially dependent on larval movement. This deserves further investigation. Although larval fresh mass was affected by storage at 10 °C and 70% relative humidity, BSF larval release rate from the dispenser was not affected using the current dispenser system as storage duration did not alter the time until 50% of the larvae were released.

The current study shows that 50% larval release was reached later when the environmental temperature was set to 18 °C, compared to 24 °C and 30 °C. This may result from the design of the dispenser and the behaviour of the larvae. The dispenser outlets do not cover the entire bottom of one storage compartment. Larval release is therefore partially dependent on the motor moving the storage compartments over the outlet, but also on larval movement into the outlet. BSF larvae are ectotherm, i.e. the environmental temperature predominantly defines the body temperature and therefore insect activity (Mellandby, 1939; Willmer, 1991). Optimal temperature for production of BSF larvae is 27 °C, while 16 to 19 °C is reported as the lower threshold for development (Holmes *et al.*, 2016). It is assumed that larval activity in the storage compartments at 18 °C was much lower compared to 24 °C or 30 °C, causing a slower release of BSF larvae. At 18 °C, the release rate of larvae from smaller amounts was slower than for larvae provided in large amounts. This may be the result of

larval aggregation behaviour. BSF larvae aggregate when placed into containers (Shishkov and Hu, 2020). Within larval aggregations the temperature increases due to the production of heat. Thus, group size is a determining factor for the amount of heat produced. The larger the group the more heat is generated (McEachern, 2018). In the current experiment based on the average larval weight (124.8 mg) within the experiment 3.4 g larvae equals about 27 individual larvae, while 129.8 g larvae equals about 1040 individual larvae. It is likely that at higher larval amounts, the heat generated in the aggregations enabled the BSF larvae to be more active than at low larval amounts, leading to a faster release.

The low activity of the larvae might also have consequences for their attractiveness to broilers. Research has shown that broilers fed with either mobile live larvae or immobile dried larvae had different effects on broiler behaviour. Broilers fed with live larvae scattered in the pen showed more foraging behaviour and less resting behaviour compared to broilers fed dried larvae scattered in the pen. In addition, based on personal observations the researchers reported that dried larvae were not consumed by the broilers during the first two weeks, indicating low attractiveness for broilers (Ipema *et al.*, 2022). This may also apply to larvae that are less active due to low environmental temperature. However, it should be noted that also other characteristics may have led to the different behavioural responses of the fast-growing broilers, such as differences in moisture, texture, odour, and palatability between the live larvae and dried larvae (Ipema *et al.*, 2022). It should be noted that the larvae dispenser that is presented in this study extends the dispensing of the larvae over a period of 8 h and with time of being exposed at the temperature of the broiler pen the larvae regain their activity quickly (see Supplementary Video S1).

There was a significant interaction between outlet type and amount of larvae added per compartment of the dispenser. For outlet 1, the release of BSF larvae when using small amounts was faster compared to outlet 2, while outlet 3 had intermediate values. This may be related to the size of the opening at the corners. Outlet 1 has a 1.2 cm wide opening on one side and an opening at the other side that increases in width from 4 to 1.2 mm. The width of outlets 2 and 3 ranges from 4 mm on one side to 2 cm and 3 cm, for outlets 2 and 3, respectively. When BSF larvae are reared in rectangular containers they aggregate in the corners of the container and form piles (Shishkov and Hu, 2020). The BSF larvae are not able to pile up in two out of three corners when using outlet 1. This is different for outlets 2 and 3. At the narrowest corner of the storage compartments (4 mm), larvae might still be able to form small piles, since the 4 mm narrow tip of the outlets is smaller than the average larvae length (1.31 cm) of a 126 mg BSF larva (Addeo *et al.*, 2021). The width of BSF larvae is between 2.02 to 5.99 mm (Ojumoola *et al.*, 2022; Park *et al.*, 2018).

When large amounts of BSF larvae were placed into the dispensers, release rates differed between outlet types. For outlet 1 the release rate of larvae was slower compared to outlets 2 and 3. This outcome might be based on the maximum width per outlet. The larger the maximum width of the outlet, the faster 50% larval release was reached. However, the amount of larvae remaining in a storage compartment after 60 minutes was extensively fluctuating for outlet 1. Up to 31.9% of the larvae were still remaining in the storage compartment, whereas for outlets 2 and 3 this was maximally 8.1% and 0.8%, respectively. As larvae aggregate in corners, the movement within the pile changes over time. At the start of pile formation, most movement takes place at the bottom. Larvae entering from the bottom of the pile push larvae that are already present at the corner up to the top of the pile. This movement continues until the aggregation reaches a stable state at which the weight of the larvae at the top makes it impossible for the larvae below to move (Shishkov and Hu, 2020). Possibly with outlet 1 the larval release rate was slowed down because the larvae occasionally clogged the outlet due to the small outlet width combined with the increasing immobility of the larvae at the bottom. For large quantities of larvae, the larger outlets resulted in faster dispensing of the larvae, and fewer larvae remaining in the storage compartments after 60 minutes.

In order to use live BSF larvae in poultry farms large scale dispensing systems are required if labour-intensive hand feeding is not possible or undesirable. Information about BSF larval behaviour in the literature is scarce (Kortsmit *et al.*, 2022). Our results highlight the need for more research focusing on the behaviour of BSF larvae to improve such systems. Research should focus on pile formation of larvae in different container shapes and sizes, and on the effect of temperature and group size on larvae mobility, heat generation, and pile formation. Moreover, it is interesting to investigate if the nutritional composition of the larvae changes during storage and if larval mobility is influenced after reactivation of the larvae at room temperature.

During broiler development, environmental conditions need to be adjusted according to the needs of the growing broiler (Hubbard, n.d.). This applies to exper-

imental settings as well as commercial broiler production. For instance, one-day-old slow-growing broilers require an environmental temperature of 34 °C, which is stepwise reduced to 18 °C at 40 days of age (de Jong *et al.*, 2021). Also, nutritional requirements change from small amounts to large amounts of feed per broiler (P. van Boekholt, pers. comm.). The current study shows how BSF larvae are released at different temperatures and amounts, with 18 °C and 30 °C as well as 3.4 g and 129.8 g representing extreme values needed in a broiler trial when replacing 5% and 10% of the dry matter feed intake of broilers with live larvae. The environmental settings and inclusion levels are relevant for research as well as practical applications.

The cleanliness of the dispenser is also important for practical applications. Cleaning of the compartments should be considered depending on the cleanliness of the larvae and their extent of defecation. The dispensing system is designed to quickly disassemble it into 3 parts in two steps: First, the R clip at the connection to the engine (Figure 1: 4) needs to be removed. Then the upper nut at the two lateral frames (Figure 1: 9) needs to be unscrewed. This allows for the removal of the first part of the dispenser (Figure 1:1, 2, 3, 4, and 11). Subsequently the second part (Figure 1: 5, 6, 7, 8, and 12) can be taken off, leaving the third part (Figure 1: 9, 10, 13, and 14). The disassembly process can be completed in less than 2 minutes. Afterwards the different parts can be cleaned considering the different materials.

The main goal to develop a larval dispensing system was to automate the release of larvae to avoid labour-intensive and moment-dependent hand feeding. These functions were successfully met by the dispenser presented in this study. Filling the larval dispenser once per day ensured the release of larvae over 8 hours during the day due to the presence of the larvae in separate storage compartments. To ensure that the dispenser makes a full turn, the time schedule of the timer can be extended by adding further on/off switches. Once a storage compartment was pushed over the outlet, the rate of larval release was influenced by temperature, amount of larvae, outlet and interactions between these factors. Nonetheless, larvae were released under all factor combinations, albeit at different rates. On average 50% of the larvae were released from the compartments at 6 minutes. After 60 minutes, on average 0.5% of the larvae remained in the compartments. In this regard, outlets 2 and 3 are preferred over outlet 1 since with the latter outlet more larvae remained in the compartments. It is recommended that in order to make all larvae accessible as feed after a full turn after 8 hours, dispensers should be visually inspected, and any remaining larvae should be released manually. Previous work has shown that effects on broiler behaviour of feeding live larvae to broilers is limited to a short feeding moment (De Jong *et al.*, 2021; Ipema *et al.*, 2020b). Feeding larvae at multiple timepoints per day promotes broilers to display active behaviour (Ipema *et al.*, 2020b). Prolonged access per timepoint might additionally stimulate natural behaviour. Moreover, variation in the rate of larval release might keep the broilers interested in searching for larvae. Whether the provision of live larvae at several timepoints and prolonged access leads to improved broiler welfare requires further studies. It is now of great interest to investigate the behaviour of poultry fed with live larvae using the developed dispenser system. The device can also be used for large practical applications, if necessary, by increasing the device dimensions.

Supplementary material

Supplementary material is available online at: https://doi.org/10.6084/m9.figshare.24573916

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