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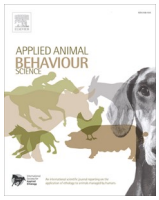

Live black soldier fly larvae (*Hermetia illucens*) provisioning is a promising environmental enrichment for pigs as indicated by feed- and enrichment-preference tests

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A B S T R A C T

Live black soldier fly larvae (BSFL) could function as environmental enrichment for pigs as they are edible, odorous, manipulable, and destructible, though the interest of pigs in live BSFL is unestablished. In this study, three trials were performed to assess this interest in order to verify the suitability of live BSFL as enrichment. Eight (Trial 1 & 2) and 16 (Trial 3) pairs of weaned pigs were included. In Trial 1, the relative motivation of pigs to consume live BSFL compared to corn, raisins and feed pellets was evaluated in no-choice and subsequent two-choice preference tests including all (combinations of) feed items. In Trial 2, the feed items in the no-choice and two-choice tests were provided in tubes that had to be rooted or pushed to access the feed items. In this trial BSFL, corn, feed pellets, and empty tubes were compared. In Trial 1 & 2 each day had two 15 min tests occurring six hours apart. In Trial 3 the long-term interest in live BSFL provided in the tubes for a 5-day period was compared to feed pellets provided in tubes, to jute sacks, and to rubber balls attached to chains. Tubes with live BSFL or pellets were refilled every morning. The relative interest was determined through intake of feed items and/or time spent interacting with the items, as measured by 1.5- or 1 min scan sampling. In Trial 3, the time spent on oral manipulation of pen mates was also scored through scan sampling. In all trials the interaction with and consumption of live BSFL was significantly higher compared to the other items, indicating a clear preference for live BSFL, and pigs remained engaged with BSFL as long as they were accessible. In Trial 3, interest in live BSFL decreased on day 5 compared to day 1, interest in jute sacks and feed pellets decreased on day 3 compared to day 1, and interest in rubber balls attached to chains was continuously low. Pigs also spent less time on oral manipulation of pen mates when having access to live BSFL, pellets, or jute sacks, compared to rubber balls attached to chains. Overall, pigs showed high and extended interest in live BSFL, confirming their suitability as edible environmental enrichment. Pigs were willing to work for access to live BSFL by rooting and pushing tubes, which can be employed when providing live BSFL as enrichment commercially.

1. Introduction

The consequences of incorporating insects into the diet of pigs have been of recent interest, yet their potential effectiveness as environmental enrichment has received little attention. Insects generally have a low feed conversion ratio and can turn biological waste into a high-quality feedstuff that can act as a substitute for increasingly limited feedstuffs such as soybean meal and fishmeal (Makkar et al., 2014; Veldkamp and Bosch, 2015). Black soldier fly larvae (*Hermetia illucens*, BSFL) are suitable to be included in pig diets due to their richness in fat, and in protein with an appropriate amino acid profile for pigs (Barragan-Fonseca et al., 2017; Biasato et al., 2019; Müller et al., 2017). BSFL meal (full-fat or partially or fully defatted) has been included in pig diets without interfering with normal growth, feed intake, nutrient digestibility or intestinal morphology (Biasato et al., 2019; Chia et al., 2019; Crosbie et al., 2020; Spranghers et al., 2018; Yu et al., 2020), or in some cases with beneficial effects on growth and feed/gain ratio (Yu et al., 2020, 2019) and on caecal microbiota composition (Biasato et al., 2020).

BSFL meal has a predominantly nutritional function. Meanwhile, providing BSFL as whole, live larvae could serve an additional function as environmental enrichment and, as such, benefit pig welfare, as was found in broilers (Ipema et al., 2020a, 2020b). Whole BSFL could be applied as environmental enrichment as pigs can not only consume, but also smell, manipulate, and destroy the larvae, aspects that have all been attributed to effective enrichment (Studnitz et al., 2007; Tarou and Bashaw, 2007; van de Weerd and Day, 2009). In barren environments without enrichment, pigs have limited possibilities to perform exploratory behaviour, which often causes them to redirect these behaviours towards pen fixtures and pen mates (Oostindjer et al., 2011; Van de

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Weerd et al., 2005), resulting in stress and injuries (Beattie et al., 2000a; Zonderland et al., 2008). Providing environmental enrichment allows pigs to exhibit a broader range of species-specific behaviour in their home-pen (van de Weerd and Day, 2009). For example, enrichment materials such as straw and peat facilitate exploratory behaviours, while they in turn reduce the occurrence of maladaptive behaviours such as oral manipulation of pen mates (Beattie et al., 2000b; Oostindjer et al., 2011; Vanheukelom et al., 2011). Previous studies have indicated that pigs are especially interested in edible enrichment objects (Durán et al., 2019; Machado et al., 2017; Nannoni et al., 2019) and that they prefer items with food feedback over items without (Holm et al., 2008). In our preceding study we found that scattering small amounts of whole live BSFL on the pen floor during 8 days after weaning increased the piglets' exploratory behaviour towards the floor from approximately 20% to approximately 30% of the observation time, compared to piglets that did not receive larvae. In this study larvae provisioning also decreased pig- and pen-directed oral manipulative behaviours (Ipema et al., 2021).

These results are promising, but the motivation of pigs to interact with and consume live BSFL has not yet been studied. Determining pigs' interest in live BSFL as compared to other feed and enrichment materials will verify potential advantages of using live BSFL as edible enrichment. As effective enrichment often stimulates extended interaction (Studnitz et al., 2007; Van de Weerd et al., 2003), it is also relevant to determine if pigs are willing to work for access to live BSFL, as a way to prolong the interaction with BSFL. Finally, pigs provided with common commercial enrichment items such as a rubber ball attached to a chain often quickly loose interest in them (van de Weerd and Day, 2009), therefore determining the pigs' long-term interest in live BSFL compared to commercially applied items is relevant. To evaluate these aspects, the current study consisted of three separate trials aimed at investigating the motivation of pigs: to consume live BSFL (Trial 1), to work for access to live BSFL provided in an enrichment device consisting of tubes with holes (Trial 2), and to interact with live BSFL provided in the tubes over a 5-day period (Trial 3). In all trials the voluntary interaction with and consumption of live BSFL, as well as the preference for live BSFL over other items was determined. It was expected that pigs would be highly motivated to access and consume live BSFL, and that they would maintain interest in live BSFL longer than in other (commercially applied) enrichment materials.

2. Methods

Three trials were carried out at the animal experiment facilities of Wageningen University & Research (Wageningen, The Netherlands). The experimental protocols were approved by the Animal Care and Use committee of Wageningen University & Research under project license number AVD1040020187184, and they were in accordance with the Dutch animal experimentation law which complies with European Directive 2010/63/EU.

2.1. Animals, housing and management

Thirty-two female, tail docked pigs (Pietrain x TN70, age at start: 38.7 ± 0.8 days, weight at start: 9.7 ± 0.2 kg) were housed in pairs at the experimental facility. They were housed in pens of 2.85 × 1.20 m equipped with a feed trough (12 × 50 cm, with three feeding places) placed in a corner of the pen, a drinking nipple and rubber flooring covered by 5 cm of wood shavings. One chew object (a plastic cross or cylinder attached to a chain) was always present in the pens, except during the experimental days of Trial 3 (see below). Pigs had ad libitum access to water and to a commercial pelleted feed (Optima 3, AgruniekRijnvallei). In the experimental rooms the lights and a radio were on from 07:00–19:00 h, and between 19:00 and 07:00 h the radio was off, and the lights were dimmed. Room temperature was 23 °C at the start of the experiment, and gradually decreased to 21 °C at day 10, after which it remained constant. During all tests, both pigs were present in the pen, and pigs were marked weekly on their back with stock marker spray for individual identification during the behavioural observations.

2.2. Experimental design

Three trials were conducted to determine the relative interest of pigs in live BSFL provided in a feeder (Trial 1, or T1) or in an enrichment device, i.e. tubes with holes (Trial 2, or T2, and Trial 3, or T3) compared to other feed and enrichment items (Table 1). The enrichment device used in T2 and T3 consisted of two horizontally suspended, transparent tubes (32 cm long, 7.5 cm Ø) with four 1 cm Ø holes at the top, that had to be rotated at least 90° for the feed items to fall in the empty feeder below the tubes (Fig. 1A). Pigs could rotate the tubes by pushing and rooting them. T1 and T2 were performed in parallel, with each trial including 8 pens, followed by T3 which included all 16 pens. The provisioning order of the tested materials was based on a Latin square design (Supplementary Table 1).

In T1, the pigs' interest in live BSFL was compared to interest in their regular pelleted feed, canned corn, and raisins. In T2, the pigs' interest in live BSFL was compared to interest in their regular pelleted feed and canned corn, all provided in tubes, and to empty tubes. The comparison with their pelleted feed was included to determine the overall acceptance of live BSFL, while canned corn and raisins were included as these are known palatable feed items for pigs. Corn is attractive for pigs as demonstrated by its common use in feral pig traps (Karlin and Khan, 2020), and raisins are assumed to be attractive for pigs as they have previously been used as edible rewards in several studies (de Jonge et al., 2008; Reimert et al., 2014). The nutritional composition of the feed items can be found in Supplementary Table 2, and the approximate dry matter levels are 36% for BSFL (based on chemical analysis), 88% for pellets (according to manufacturer), 32% for corn and 76% for raisins (de Jong, 2010). A pilot trial indicated that the tubes had a similar release rate in g of fresh material per rotation for live BSFL, corn and pellets. In T3, the pigs' long-term interest in live BSFL provided in tubes was compared to interest in pellets provided in tubes, two jute sacks hanging from the pen wall, and two rubber balls attached to chains hanging from the pen wall (Fig. 1). Rubber balls attached to chains were included as these are commonly used commercially (Bracke et al., 2013), while jute sacks were included as they are highly interesting for

Table 1
Overview of included feed and enrichment items, types of tests, and measurements taken during the tests of Trial 1, 2 & 3. BSFL = Black soldier fly larvae, Pellets = the pigs' pelleted feed.

	Feed/enrichment items	Tests	Measurements
Trial 1	• BSFL • Corn • Raisins • Pellets	• No-choice tests (15 min/test) • Two-choice tests (15 min/test)	• Intake of feed items (% of feed item consumed) • Interaction with feed items (% of scans)
Trial 2	• Hanging tubes containing BSFL • Hanging tubes containing corn • Hanging tubes containing pellets • Empty hanging tubes	• No-choice tests (15 min/test) • Two-choice tests (15 min/test)	• Intake of feed items (% of feed item consumed) • Interaction with tubes and feed items (% of scans)
Trial 3	• Hanging tubes containing BSFL • Hanging tubes containing pellets • Hanging jute sacks • Hanging rubber balls attached to chains	• Long-term enrichment interaction tests (5 days/test)	• Interaction with enrichment items and feed items (% of scans) • Oral manipulation of pen mates (% of scans)

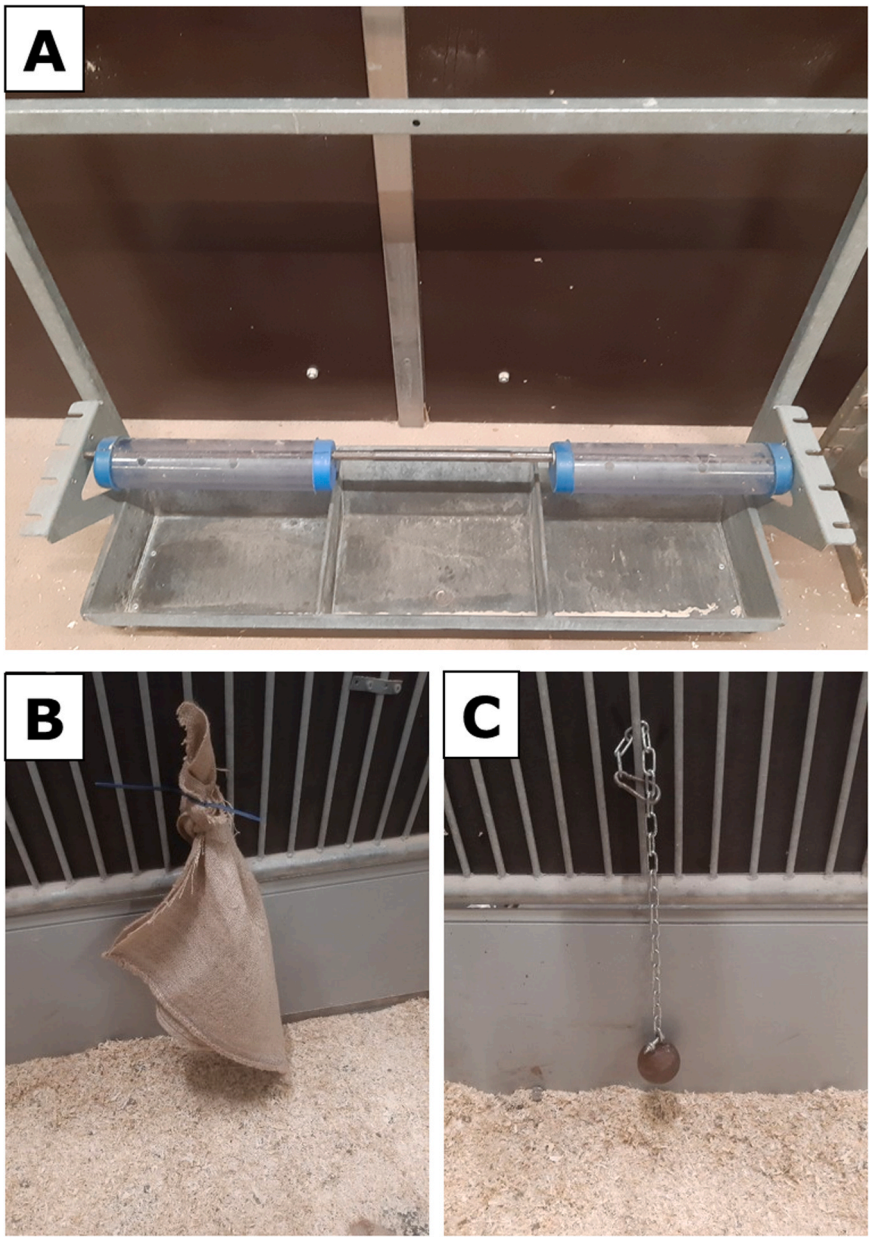

Fig. 1. A) Enrichment device applied in Trial 2 & Trial 3, tubes can be filled with feed items and require turning to get the feed items out; B) jute sack as provided in Trial 3; C) rubber ball attached to a chain as provided in Trial 3.

pigs (Ursinus et al., 2014).

Live, 14-day old larvae were provided weekly (by Bestico B.V., Berkel en Rodenrijs, The Netherlands) and stored at 12 °C near the experimental rooms, together with the other feed items. From approximately 30 min before provisioning, the feed items were stored at room temperature and the canned corn was dried with paper towels. Behavioural observations were done by four observers using the program Observer 14.2 (Noldus Information Technology B.V., Wageningen, The Netherlands). Prior to the experiment the observers were trained, and inter-observer reliability was regarded to be "almost perfect" (Fleiss kappa>0.8)(Landis and Koch, 1977). After each test, the left-over feed items, including any feed that was spilled, were weighed to determine the feed intake.

2.2.1. Trial 1 (T1)

T1 consisted of a habituation period (week 1 & 2, 4 days/week), no-choice voluntary feed intake tests (week 3, 4 days) and two-choice feed preference tests (week 4 & 5, 6 days/week), in which the relative interest in live BSFL compared to feed pellets, corn, and raisins was determined (Table 1). The feed items were presented in a feeder (12 × 50 cm with three feeding places) placed near the pen wall during the test. During habituation, pigs had access to one feed item each day. In the first week of habituation, each day a feeder with 300 g of the feed item was placed in the pen between 09:30–15:30 h, and in the second week of habituation the feeder was placed in the pen for 15 min twice a day, at 10:00 and 16:00 h. Weighing back of left-over feed confirmed that all feed items were sampled in all pens during habituation. During all test days, pigs had access to one (no-choice test) or two (two-choice preference test) feed items for 15 min twice a day, starting at 10:00 and 16:00 h. In each test 300 g of each feed item was provided. In the two-choice preference tests two adjacent feeders each contained one feed item, and each combination of feed items was tested on one day in both weeks, with the location of the feed items balanced between weeks. During the tests, the behaviour of all piglets was scored by instantaneous

scan sampling every 1.5 min, resulting in 10 scans/15 min test. For each scan, the pig was scored as either "Interacting", which included eating or manipulating the feed item and/or exploring the experimental feeder by sniffing, nosing, rooting, or chewing, or "Not interacting".

2.2.2. Trial 2 (T2)

T2 had a similar set-up as T1, except the feed items were provided in the enrichment device with tubes, requiring pigs to work for access to the feed items (Fig. 1A). Instead of raisins, empty tubes were included in T2 (Table 1). For habituation and for the no-choice tests, two tubes, each containing 150 g of the feed item, hanging above a feeder were placed near the pen wall. For the two-choice preference tests, two such devices were placed adjacent to each other, and each device contained two tubes with the same feed item, or with no feed item in case of comparison to the empty tubes. During the first week of habituation, a handful of the feed item was always placed in the feeder beneath the tubes. All pens had sampled all feed items during habituation. Testing started daily at 09:00 and 15:00 h and lasted 15 min. Behaviour was observed as in T1, except that 1 min instantaneous scan sampling was applied in the two-choice preference test, resulting in 15 scans/15 min test. The behaviours scored were "Interacting" which included eating or manipulating the feed, exploring the tubes or the feeder and turning the tubes, and "Not Interacting".

2.2.3. Trial 3 (T3)

In T3, pens were provided with the different enrichment items for five days a week during four consecutive weeks (Table 1). For the live BSFL and the feed pellets, every morning at 08:30 h 300 g was added to each tube, and any feed still in the tubes was taken out and weighed back to determine the daily intake. On day 1, 3 and 5, individual pig behaviours were scored through 1 min instantaneous scan sampling for 5 h a day, starting at 08:30, 10:00, 11:30, 13:00 and 14:30 h. Two observers each observed eight pens in one room, switching rooms every hour. The scored behaviours were "Interacting with enrichment" which included exploring the enrichment, playing with the enrichment, and eating edible enrichment, "Manipulating pen mate" which included (belly) nosing and biting of the other pig, and "Other".

2.3. Statistical analysis

Intake of feed items and behavioural data were averaged per pen prior to analysis. For each feed item in each test the proportion of the provided feed that was consumed was used in the analysis. Behavioural data were grouped per 15 min test (T1 & T2) or per day (T3) and expressed as the proportion of scans in which the behaviour was registered. Data from the two-choice tests were analysed separately for each unique combination of feed or enrichment items. All data were analysed with the statistical software SAS 9.4 (SAS Institute Inc., Cary, NC, USA) with generalized linear mixed models (GLIMMIX in SAS) with a binomial distribution, logit link, and additional multiplicative over-dispersion parameter. Models included 15 min test (T1 & T2) or day (T3) as repeated effect with pen as subject using a compound symmetry covariance structure. Models for T1 and T2 included feed type and test order as fixed effects, though as the effect of test order was never significant this was left out of the final models. Models for T3 included enrichment item, day, and their interaction as fixed effects. P-values below 0.05 were considered statistically significant, and in case of the two-choice tests, this indicated a preference for one item over the other. Significant fixed effects identified by the models on data from the no-choice tests and the data from T3 were further analysed with post-hoc tests on pairwise differences in least square means with a Tukey's HSD correction. Data are presented as pen means ± SEM.

3. Results

3.1. Trial 1

Intake of feed items in the no-choice tests was influenced by feed type ($F(3,21) = 29.3$, $p < 0.001$, Fig. 2). Post-hoc tests indicated differences between the intake of all feed items, in the order of live BSFL > corn > raisins > pellets. The behavioural interaction with the feed items was also influenced by feed type ($F(3,21) = 23.5$, $p < 0.0001$), where pigs interacted more with live BSFL than with all other feed items, and no other differences occurred.

The intake of feed items during the two-choice tests differed for all feed type comparisons (BSFL vs. corn: $F(1,7) = 37.4$, $p = 0.001$, BSFL vs. raisins: $F(1,7) = 80.2$, $p < 0.001$, BSFL vs. pellets: $F(1,7) = 59.4$, $p < 0.001$, corn vs. raisins: $F(1,7) = 7.7$, $p = 0.028$, corn vs. pellets: $F(1,7) = 12.1$, $p = 0.010$, raisins vs. pellets: $F(1,7) = 5.7$, $p = 0.048$), with the order of preference being BSFL > corn > raisins > pellets (Fig. 2). Considering behaviour, the interaction with live BSFL was higher than the interaction with all other items (BSFL vs. corn: $F(1,7) = 103.3$, $p < 0.001$, BSFL vs. raisins: $F(1,7) = 147.5$, $p < 0.001$, BSFL vs. pellets: $F(1,7) = 190.6$, $p < 0.001$). Pigs also interacted more with raisins than with pellets when both were present ($F(1,7) = 8.6$, $p = 0.022$), and there was a trend for pigs interacting more with corn than pellets when both were present ($F(1,7) = 5.4$, $p = 0.053$). There was no difference in interaction when corn and raisins were present simultaneously ($F(1,7) = 0.7$, $p = 0.446$).

3.2. Trial 2

In the no-choice tests, the feed item in the tubes influenced the intake of feed items ($F(2,14) = 21.9$, $p < 0.001$), in the order of live BSFL > corn > pellets as indicated by post-hoc tests (Fig. 3). The behavioural interaction with the tubes also depended on feed type ($F(3,21) = 31.4$, $p < 0.001$). Post-hoc tests identified a difference between all items except between pellets and empty tubes, resulting in the preference order of live BSFL > corn > pellets and empty tubes.

For the two-choice preference tests, a feed item intake preference occurred for all combinations (BSFL vs. corn: $F(1,7) = 89.4$, $p < 0.001$, BSFL vs. pellets: $F(1,7) = 42.7$, $p < 0.001$, corn vs. pellets: $F(1,7) = 27.3$, $p = 0.001$), with the preference order BSFL > corn > pellets (Fig. 3). Pigs interacted more with tubes containing live BSFL than with tubes containing corn ($F(1,7) = 95.3$, $p < 0.001$), pellets ($F(1,7) = 139.4$, $p < 0.001$), or nothing ($F(1,7) = 82.8$, $p < 0.001$). Furthermore, pigs interacted more with tubes containing corn than with tubes containing pellets ($F(1,7) = 50.9$, $p < 0.001$) or nothing ($F(1,7) = 84.0$, $p < 0.001$). The time spent on interacting did not differ when tubes with pellets and empty tubes were present simultaneously ($F(1,7) = 0.9$, $p = 0.372$).

3.3. Trial 3

In T3, pigs consumed more live BSFL than feed pellets provided in tubes (BSFL: 97.3 ± 1.3%, pellets: 22.5 ± 3.4%, $F(1,15) = 68.4$, $p < 0.001$), and consumption was not influenced by day ($F(4,55) = 0.6$, $p = 0.643$) or the feed type by day interaction ($F(4,54) = 1.9$, $p = 0.119$). A main effect of enrichment type ($F(3,45) = 146.0$, $p < 0.001$), day ($F(2,30) = 59.1$, $p < 0.001$), and the enrichment type by day interaction ($F(6,90) = 10.9$, $p < 0.001$) was found on the time spent interacting with the enrichment. According to post-hoc tests on the feed type by day interaction, the daily preference based on the level of interaction was as follows: on day 1 BSFL & jute sacks > pellets > balls, on day 3 BSFL > jute sacks & pellets > balls, and on day 5 BSFL> jute sacks > pellets & balls (Fig. 4). These post-hoc tests also indicated that the time spent interacting with rubber balls attached to chains did not differ throughout the week, while the time spent interacting with jute sacks and tubes containing pellets dropped on day 3 compared to day 1,

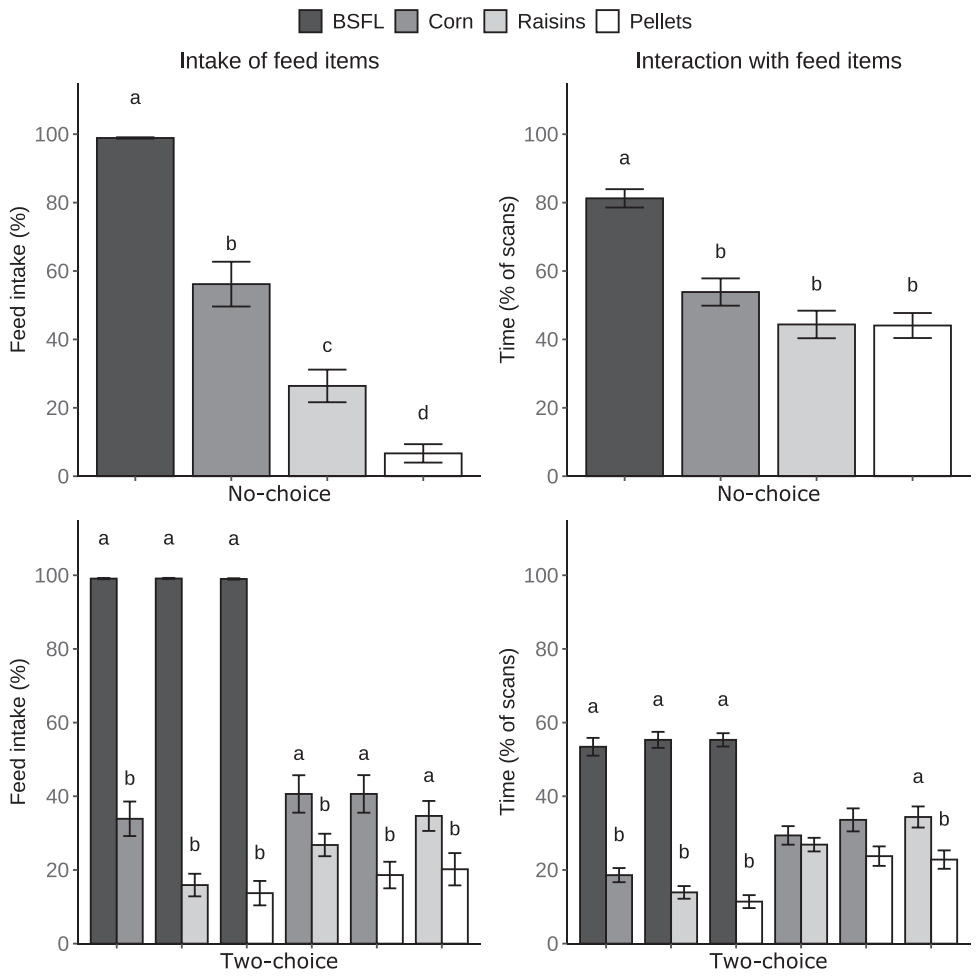

Fig. 2. The percentual intake of each feed item and the time spent on interacting with each feed item provided in no-choice and two-choice tests in Trial 1. BSFL = Black soldier fly larvae, Pellets = the pigs' pelleted feed. For the no-choice tests all bars with different letters differ significantly ($p < 0.05$), for the two-choice tests bars within one feed combination with different letters differ significantly ($p < 0.05$). Data are presented as pen means ± SEM.

after which it did not change. The time spent interacting with tubes containing live BSFL was lower on day 5 compared to day 1.

The time spent on oral manipulation of pen mates was influenced by enrichment type ($F(3,45) = 13.5$, $p < 0.001$) and by day ($F(2,30) = 12.7$, $p < 0.001$), but not by their interaction ($F(6,90) = 1.48$, $p = 0.193$, Fig. 4). Post-hoc tests indicated that oral manipulation of pen mates occurred more when the balls attached to chains were present (2.6 ± 0.2%) compared to when any of the other items were present (BSFL: 1.3 ± 0.1%, jute sacks: 1.6 ± 0.2%, pellets: 1.8 ± 0.2%). On day 1 the time spent on manipulating pen mates (1.3 ± 0.1%) was lower than on day 3 (1.8 ± 0.2%) and day 5 (2.3 ± 0.2%).

4. Discussion

All three trials demonstrate that pigs are highly motivated to interact with and consume live BSFL. Pigs showed relatively more interest in live BSFL compared to their regular feed pellets, canned corn, and raisins, either provided in a feeder (T1), or provided in an enrichment device that required pigs to push and root tubes to access the feed items (T2, only pellets and corn). In T3, pigs also interacted more with live BSFL provided in tubes compared to pellets provided in tubes, jute sacks, and balls attached to chains. The interaction of pigs with live BSFL only went down on day 5, as opposed to day 3 for jute sacks and pellets, while the interest in balls attached to chains was continuously low. Pigs spent more time on manipulating pen mates when they had access to balls attached to chains compared to all other enrichment materials.

In T1 and T2 the results of the no-choice and subsequent two-choice preference tests were in high agreement, all indicating a clear preference for live BSFL over all other feed items. In T1, the intake of live BSFL was always close to 100%, while the time spent interacting with BSFL was around 50–80%. This may be explained by our observation that the last larvae in the feeder were near impossible to reach, which could result in the pigs losing interest over time. Machado et al. (2017) similarly observed that enrichment that required high effort to obtain rewards stimulated exploration less than enrichment that required moderate effort to obtain rewards. The results from T2 also support this notion. Moderate effort was required to obtain the larvae provided in tubes, and here up to 40% of the provided BSFL were still available at the end of the 15 min tests, while pigs spent close to 100% of the scans interacting with the larvae. Both trials therefore confirm that pigs will keep interacting with live BSFL if the larvae can be obtained with moderate effort.

The reason that live BSFL are preferred over corn, raisins, and pellets is likely multi-factorial. It is possible that long-term nutritional consequences for the pigs may have played a role, although with the current set-up this is unlikely. Kyriazakis et al. (1991) claimed that pigs adapted their feed intake to the nutritional composition of the diet only when they had sufficient time to experience the nutritional consequences. Pigs may have experienced the nutritional consequences of consuming the feed items during the first week of habituation in T1 and T2, when each item was available throughout one day. However, during the tests the pigs only had access to individual feed items one day a week for two 15 min periods, separated by six hours without access, limiting the

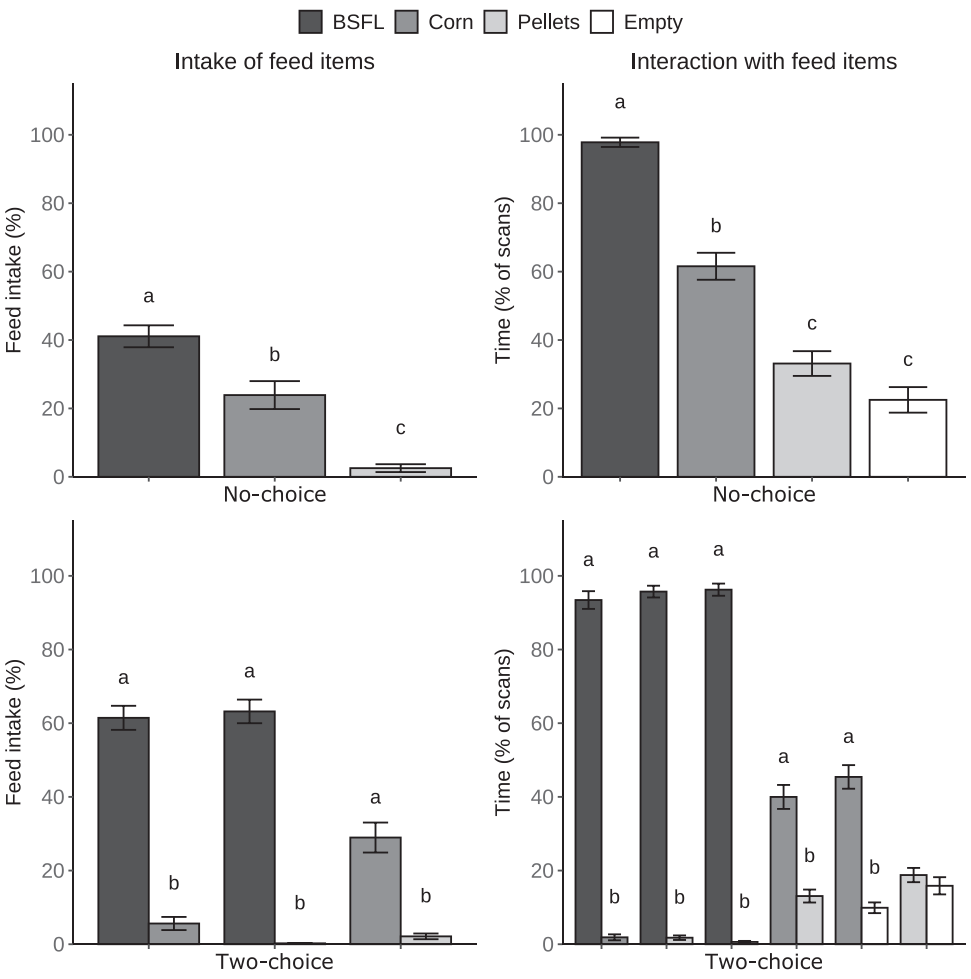

Fig. 3. The percentual intake of each feed item and time spent on interacting with each feed item provided in the enrichment device in no-choice and two-choice tests in Trial 2. BSFL = Black soldier fly larvae, Pellets = the pigs' pelleted feed, Empty = empty tubes in the enrichment device. For the no-choice tests all bars with different letters differ significantly ($p < 0.05$), for the two-choice tests bars within one feed combination with different letters differ significantly ($p < 0.05$). Data are presented as pen means ± SEM.

opportunity to adjust intake based on nutritional consequences.

Alternatively, immediate signals such as taste, odour, and other sensory characteristics influence pigs' intake and interest in feed (Aubé et al., 2019; Solà-Oriol et al., 2009; Van de Weerd et al., 2003). In T1, pigs rarely differed in the time they spent interacting with corn, raisins, and pellets, yet their consumption, and consequently also rate of consumption, differed in the order corn > raisins > pellets. In previous studies, pigs did not prefer hard and fragile feed items (Solà-Oriol et al., 2007), and did prefer items with a higher moisture content that are easier to consume (Aubé et al., 2019; Olsen et al., 2000). These studies support a faster consumption rate of moisture-laden corn as opposed to raisins and pellets, which are dryer, harder, and therefore more difficult to chew. However, pigs preferred consuming and interacting with live BSFL over corn while they have a similar moisture content, indicating that moisture content cannot solely explain this preference. Besides the high moisture content, live BSFL contain at least 3x more fat than the other feed items, and fat is known to improve palatability (Mizushige et al., 2007). BSFL are also richer in protein than corn and raisins, and in line with our results sows were previously found to prefer forages with a high protein level in short-term preference tests (Aubé et al., 2019). While feed pellets contained marginally more protein than BSFL, these are plant-based proteins that can be less preferred by pigs than animal proteins (Solà-Oriol et al., 2011). Like animal protein, BSFL protein is high in glutamic acid (Makkar et al., 2014) which can produce umami flavours that pigs show a preference for (Figueroa et al., 2019).

Finally, items that are novel and/or only periodically available facilitate exploration more than familiar items (Docking et al., 2008; Van de Weerd et al., 2003), explaining the overall preference for live BSFL, corn and raisins over the pellets that were available ad libitum. This is consistent with the observations that pigs in T2 were equally interested in tubes containing pellets and in empty tubes. Overall, while the preference for live BSFL over other feed items is obvious, further research is needed to determine the exact properties that prelude this preference.

In T1 and T2 pigs actively consumed live BSFL. Likewise, in T3 where enrichment materials were provided over a 5-day period pigs consumed almost all live BSFL daily, while the consumption of pellets provided in the enrichment device was much lower. The time spent on interacting with live BSFL throughout the day started off at the same level as for jute sacks, however the interaction with jute sacks decreased already on day 3 while the interest in live BSFL only decreased on day 5, and on this day, it was still higher than the interest in all other items. The prolonged high interest in live BSFL coincides with our previous study where newly weaned piglets remained highly interested in exploring live BSFL for the observed 8 days (Ipema et al., 2021). The reduction in interaction on day 5 may be due to increased familiarity with the larvae, as was previously also observed for other enrichment items over a 5-day period (Docking et al., 2008; Van de Weerd et al., 2003). An alternative explanation is that pigs became more efficient over time in retrieving the larvae from the enrichment device. This is supported by the observation that larvae intake did not differ over days, despite the lower time spent interacting with the tubes containing larvae on day 5. In support of this, in T2 the interaction with live BSFL provided in the enrichment device was similar for the no-choice and the subsequent two-choice preference tests, yet the intake of live BSFL in the latter test was substantially higher (60% vs. 40%), likely because pigs learned how to better retrieve the larvae out of the tubes. Increasing the difficulty of obtaining larvae and/or changing the manner of larvae provisioning over time may counteract this

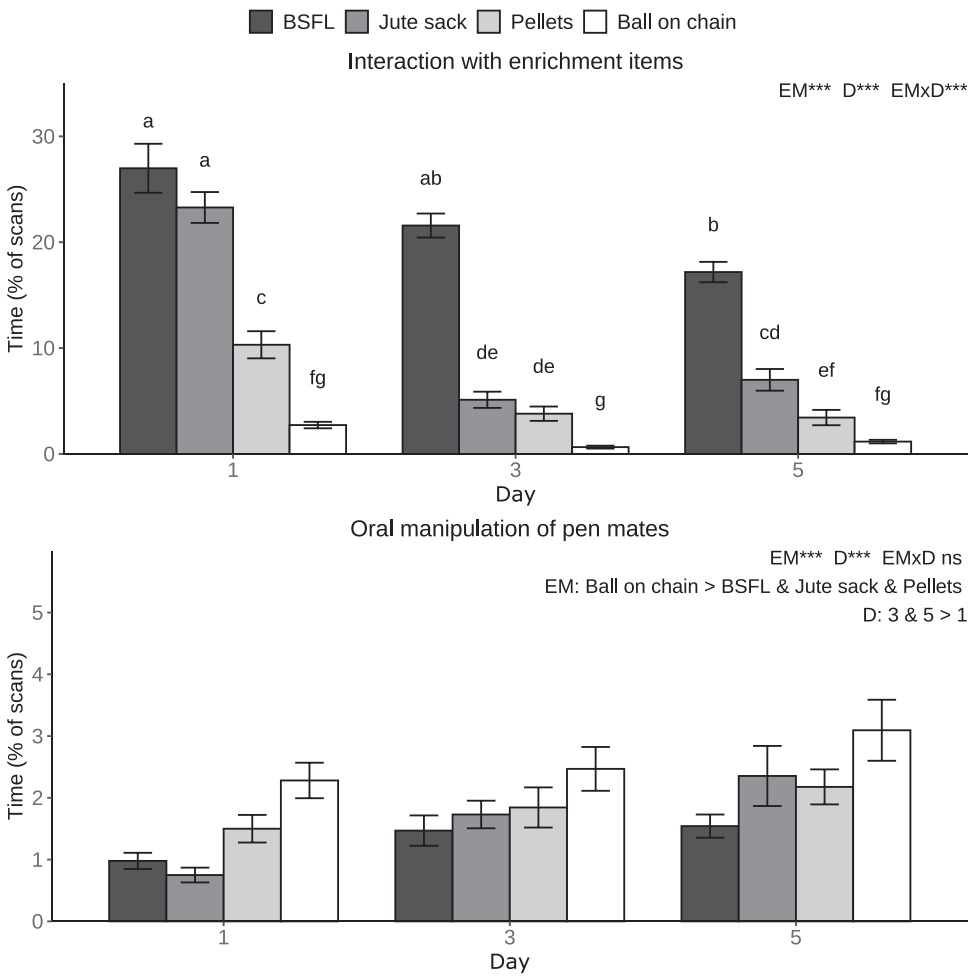

Fig. 4. The time spent on interacting with the enrichment items and on oral manipulation of pen mates in the home pen in Trial 3. BSFL = Black soldier fly larvae, Pellets = the pigs' pelleted feed. Effects of enrichment material (EM), day (D) and their interaction (EMxD) are indicated as ns (not significant), or *** ($p < 0.001$). If EMxD is significant, different letters above bars indicate significant ($p < 0.05$) differences; if EMxD is not significant, the independent differences between enrichment materials and days are indicated. Data are presented as pen means ± SEM.

increased efficiency of pigs in procuring the larvae, and thereby prolong the time they spend interacting with the device that delivers larvae.

In T3, the occurrence of oral manipulation of pen mates was higher in pigs that had access to balls attached to chains than pigs that had access to any other enrichment material. Previous studies found that enrichment material that facilitates rooting, such as straw or peat, is of high interest and reduces manipulation behaviour directed towards other pigs (Oostindjer et al., 2011; Telkänranta and Valros, 2020; Vanheukelom et al., 2011). Balls on chains provide relatively little opportunity for rooting behaviour (Day et al., 2002), which may explain the higher level of oral manipulation of pen mates when pigs only had access to this enrichment. Besides, from all studied items, the balls on chains were most similar to the chew items provided in the pigs' home pen during T1 and T2, and familiarity with such items likely decreased interest in them, reducing their effectiveness as enrichment (Van de Weerd et al., 2003). In the current study the level of pig-directed oral manipulation was likely already low because pigs were housed in pairs with much space and bedding (Beattie et al., 2000b; Chaloupková et al., 2007; Fu et al., 2016), and because regular testing may have functioned as a form of environmental enrichment. Pigs housed under commercial conditions often exhibit more damaging behaviours (Oostindjer et al., 2011; Van de Weerd et al., 2005), therefore live BSFL provisioning could have more pronounced effects under such conditions.

Overall, the observed reduction in maladaptive behaviour compared to balls attached to chains, combined with the greater interest in live BSFL compared to other feed and enrichment items observed in all trials highlights the potential advantage of using live BSFL as environmental enrichment. Accordingly, research into BSFL provisioning under commercial conditions is required, with focus on the consequences for pig behaviour, performance, and health. Commercially, providing other varieties of BSFL such as dried or popped larvae may be more practical and should thus be studied. Also, commercial BSFL provisioning methods should allow all pigs to access the larvae simultaneously, as pigs show synchronized behaviour (Docking et al., 2008) and limited access could result in aggression (Zwicker et al., 2013). Finally, regulated access is likely required to ensure prolonged interest and thereby increased beneficial effects of live BSFL provisioning (Van de Weerd et al., 2003).

5. Conclusion

In conclusion, this study demonstrated that pigs are highly motivated to consume and interact with live BSFL, and they prefer live BSFL over a range of other feed and enrichment items. Over a 5-day period, the occurrence of pig-directed oral manipulation behaviour was reduced in pigs having access to live BSFL in an enrichment device compared to rubber balls attached to chains. These results emphasize the suitability of live BSFL to be used as edible environmental enrichment for pigs. Further research on live BSFL provisioning under commercial conditions is required to establish the total consequences for pigs.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.applanim.2021.105481.

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