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House fly larvae harvest yield using three different rations of wheat brand and pig manure as larval development media

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ABSTRACT

The aim of the research was to compare the yields of house fly larvae using different proportions of wheat bran and swine feces as larval development medium, in a randomized block experimental design with five replications: A- 100 % wheat bran; B- 50% wheat bran and 50% swine feces; C- 100% swine feces. Measurements of the temperature of the substrates, as well as the temperature and relative humidity of the place where the flies were developing were made every 24 hours. The larval yield per m2 and kg of substrate, (including the water used to moisten the larval media) were also registered. The wheat bran substrate presented the highest temperature values (36.78 ^{o}C), with values above the ambient temperature. The relative humidity varied between 44 and 68%. The amount of water used per square meter was 28.75; 27.72 and 28.87 L for A, B and C treatments, respectively. The highest yield for all substrates was obtained during the first harvest after six days with the highest values for treatment B with 2869.11 g m^{2-1} and 181.16 g kg^{-1}, respectively. The transformation of the crude protein of the substrates by the fly larvae was between 28.0 and 41.0%, with the highest value for treatment B with 2869,11 g m^{2-1} and 181,16 g kg^{2-1} . No presence of pathogenic agents was observed in the harvested larvae.

Keywords: Water, Insects, Alternative protein, Organic residues, Substrates.

Rendimentos de larvas de moscas em diferentes colheitas com três proporções de farelo de trigo e esterco suíno

RESUMO

O objetivo da pesquisa foi comparar a produção de larvas de moscas domésticas em diferentes proporções de farelo de trigo e esterco suíno como meio de desenvolvimento larval, em delineamento experimental de blocos casualizados com cinco repetições: A – 100% farelo de trigo; B – 50% farelo de trigo e 50% esterco suíno; C- 100% esterco suíno. Medições da temperatura dos substratos, bem como da temperatura e umidade relativa do local onde as moscas estavam se desenvolvendo foram feitas a cada 24 horas. A produção larval por m2 e kg de substrato, incluindo a água utilizada para umedecer o meio larval, também foram registrados. O substrato farelo de trigo apresentou os maiores valores de temperatura (36,78 ^{o}C), com valores acima da temperatura ambiente. A umidade relativa variou entre 44 e 68%. A quantidade de água utilizada por metro quadrado foi de 28,75; 27,72 e 28,87 L para os tratamentos A, B e C, respectivamente. A maior produtividade para todos os substratos foi obtida na primeira colheita após seis dias, com os maiores valores para o tratamento B com 2.869,11 g m^{2-1} e 181,16 g kg-1, respectivamente. A transformação da proteína bruta dos substratos pelas larvas de moscas ficou entre 28,0 e 41,0%, com maior valor para o tratamento B com 2869,11 g m^{2-1} e 181,16 g kg^{2-1} . Não foi observada a presença de agentes patogênicos nas larvas colhidas.

Palavras-chave: Água, Insetos, Proteína alternativa, Resíduos orgánicos, Sustratos.

1. Introduction

Global food demand is undergoing changes never seen before. The trends in these changes involve diets with high consumption of meat and fish, which leads to a huge increase in the demand for raw materials needed to be used as animal feed (Rubio, 2015). Society needs innovation in food for healthy and sustainable products. Pino (2018) points out as a solution for the use of agri-food by-products as insect feed, whose biomass can be used as a source of protein in animal feed and can provide both nutritional and environmental benefits.

Indeed, insects are considered alternative sources of protein to produce animal feed, safe, cheap and sustainable (Lähteenmäki-Uutela et al., 2021). Massive rearing of insects has reduced ecological impact and high efficiency in feed conversion of organic wastes and by-products of low quality as manure or fruit wastes among others (Wang et al., 2013). Insects efficiently converting nitrogenous compounds into valuable proteins and require less use of natural resources, such as land and water resource, per unit of protein produced than protein crops e.g. oilseeds, cereals used as feed of farm animals (van Huis, 2015).

Although there are research using insects for the recycling of waste, with emphasis on pig and chicken manures (Ossey et al., 2012), it is necessary to study the importance of factors such as: temperature, humidity, composition of the same, primarily at laboratory scale and semi-industrial scale (Pastor et al., 2015). The main key species associated to industrial production are *Hermetia illucens* L. (Black soldier fly; Diptera: Stratiomyidae), and *Tenebrio molitor* L. (Mealworm, Coleoptera: Tenebrionidae) that bio-convert agri-food wastes, but the requirements of their life cycle is more complicated and long than that the case of the house fly *Musca domestica* L. (Diptera: Muscidae).

In natural oviposition systems for *M. domestica*, substrates combined to manure have rarely been compared and the available results are non-homogenous (Koné et al., 2017). Comparison of larval bioconversion of three types of manures with the house fly shown the best results with poultry manure (Miranda et al., 2020). However, other authors (Gandal et al., 2019) demonstrated few differences between swine and poultry manure substrates. A relatively low-priced substrate and by-product of wheat industry milling, known as wheat bran, has provided acceptable yields of house fly larvae (Hussein et al., 2017;, Koné et al., 2017; Sanou et al., 2019; Casanovas et al., 2020).

However, previous results showed only the production of fly larvae for a single and/or first harvest, then it is needed to know the subsequent efficiency after a first harvest of the larvae. Also, it is important to evaluate the effect of various proportions of swine feces with wheat bran, in order to guide small producers of insects in the use of these type of wastes. Therefore, the main aim of this study was to compare the successive harvests of house fly larvae using larval media with different proportions of wheat bran and swine feces.

2. Material and Methods

The research was conducted from October to November 2021, in a zinc roofed building (3.80 m x 2.72 m x 2.05 m high), located in the suburban area of the city of Cienfuegos, Cuba. The facility was surrounded by a mesh with 1 cm holes that allowed free access of flies from the outside, favouring natural oviposition. The propylene containers were placed on a table at a height of 85 cm, occupying an area of 81.6 cm^2 and a height of 9 cm. Inside each container, the substrate for larval development was placed at a depth of 3 cm.

Two substrates were used in the experiments: pig manure and wheat bran. The swine feces were taken directly from the pens of pigs in the fattening phase, from clinically healthy animals fed with a compound feed consisting of corn and soybeans. The pig manure was previously exposed to the sun to reduce the humidity content, until a dry matter content of 85%. A tray protected with an anti-aphid mesh was used to avoid contamination by insects. The wheat bran was obtained from a swine breeder, with a dry matter content of 85 %.

Each substrate was moistened with potable water until a homogeneous semi-solid mixture was formed. The addition of water was done every day in the morning. In addition, all substrates were stirred daily after wetting and the amount of water added was measured with a syringe graduated in millilitres, for each substrate, always aiming to reach a semi-solid visual structure. A randomized block experimental design with five replicates was applied, where each container was considered an experimental unit. The following treatments were stablished: A- 100% wheat bran; B- 50% wheat bran and 50% swine feces; and C- 100% swine feces.

The following measurements were taken daily for each replicate of each treatment in the morning (08:00 to 09:00 H): temperature of the substrates; ambient temperature (^oC) and relative air humidity. The maximum and minimum values of each environmental parameter were recorded every 24 hours. The mass of each substrate was weighed (g) on a digital balance with a margin of error of five grams before setting up the experiment. The water used was also measured at the beginning of the experiment. On days that the substrates were stirred with a fork and moistened, the amount of water in ml was measured with a graduated syringe.

To harvest the larvae, it was considered that they were in their third stage, with a size greater than 8 mm,

measured with a ruler. The harvests were distributed as follows: 1st on the 6th day, 2nd on the 9th day, 3rd on the 12th day and 4th on the 14th day after the experiment began. It was defined not to continue the experiment when there was no increase of larvae visually in the containers. In each harvest, 20 larvae were taken at random, replicated three times to determine the weight of a larva, using an Acculab Sartoni Group analytical balance. The larvae were transported to the laboratory in an individual pot with a minimal amount of substrate in order avoid dehydration of the larvae.

The yield of each substrate and the amount of water used for each crop and its total were estimated according to the following formulas: Larvae yield (average) g m^{-2} - [(Total weight of larvae per container * Number of larvae per container) * (10000)]/ (container area); Larvae yield (average) g kg^{-1} - [(Total weight of larvae per container * Number of larvae per container) *(1000)] / (Weight of substrate used); Water consumption (average) ml m^{-2} - (amount of water)/ (10000/container area). From each replicate, before and after the experiment, a 500 g sample was taken to be sent to the Provincial Laboratory of Veterinary Medicine of Cienfuegos, where the proximal analysis was performed, according to AOAC (2005): DM (% dry matter) and CP (% crude protein). The values obtained were used to estimate the crude protein content of the substrates before and after transformation by the fly larvae, in grams per kilogram of dry matter, and to measure the differences between them.

A 50 g sample was taken from each replicate at the beginning of the experiment and a sample of the substrate biotransformed by the fly larvae for bacteriological studies looking for the presence of *Salmonella* spp. and fecal coliforms. The parasitological study was also carried out, for which a pig manure sample was taken to diagnose the presence of coccidia. The samples were processed at the Provincial Laboratory of Veterinary Medicine of Cienfuegos and the following methods were used: Detection of *Salmonella* spp (Microbiology of Food and Animal Feeding Stuffs-Horizontal - Reference Method (ISO 6579:2002, IDT, 2008); Fecal coliforms (Microbiology of Food and Animal Feeding Stuffs-Horizontal - Horizontal - Colony Count technique (ISO 4832:2006, IDT). 2010); Coccidia. Cuban Agricultural Norm for bacteriological sowing - Test methods. (NCAG, 1982).

The statistical package IBM.SPSS v23 (2016) was used to perform an analysis of variance. The assumptions of normality were previously corroborated by the Shapiro-Wilk test and homogeneity of variances by the Levene's test. *Post hoc* tests to identify differences between treatments were performed using Tukey's test. Comparison between crude protein contents (g) of the substrates, before and after transformation by fly larvae, was performed by the related samples test. The P values established were 0.05 and 0.01.

3. Results and Discussion

The temperatures in each substrate showed differences between them, and reached values from 23.34 to 36.78 ^{o}C. The ambient temperature inside the fly house behaved in a range from 24.2 to 28.4 ^{o}C (Figure 1). The wheat bran substrate presented the highest temperature values with respect to the remaining treatments ($P<0.05$) on days 1, 2, 4, 5, 7, 8, 10, 11, 12 and 14, in a range from 36.78 to 24.02^{o}C. The temperature in all substrates was above the ambient temperature inside the fly house, with the highest values in the first five days, when fermentative processes are more intense.

According to INSMET/ Institute of Meteorology. Cienfuegos (INSMET, 2021), the average temperature values of the municipality during the experimental phase ranged between 24.3 and 27.9 °C and the values measured in the fly house were higher. This result is attributed to the location of the fly house and its construction materials. According to Florez et al. (2019), although temperature and diet have a complex impact on larval development, temperature induces larval development. At the same times, at high temperatures, dipteran development is rapid, although their size is reduced. At low temperatures, development is slow, although they have good nutrition, and may take 90% more time than larvae that are subjected to poor nutrition at high temperatures. These authors conclude that the development of larvae in temperature ranges between 25 and 35 °C is considered optimal, but lower temperatures decrease metabolism.

Several authors mention different optimum temperatures for the development of house fly larvae as e.g. Cruz et al. (2002), indicate the best temperature for fly larvae development is between 20 and 26 ^{o}C. However, Casanovas et al. (2021) report temperatures up to 43.0 °C in combined substrates of corn germ and swine feces, with a good performance. Therefore, the temperature of the substrates is within the optimal range for larval development with values between 23.02 and 36.78 ^{o}C. Relative humidity inside the fly house behaved variably during the period evaluated, with mean values between 44 and 68%, although maximum values were obtained during the night period, reaching 76% (Figure 2). The variation of these values is associated with a watercourse that influenced mainly days 6 to 10 of the evaluated period, with values of 34.3 mm to 25.1 mm of rainfall in 24 hours (INSMET, 2021).

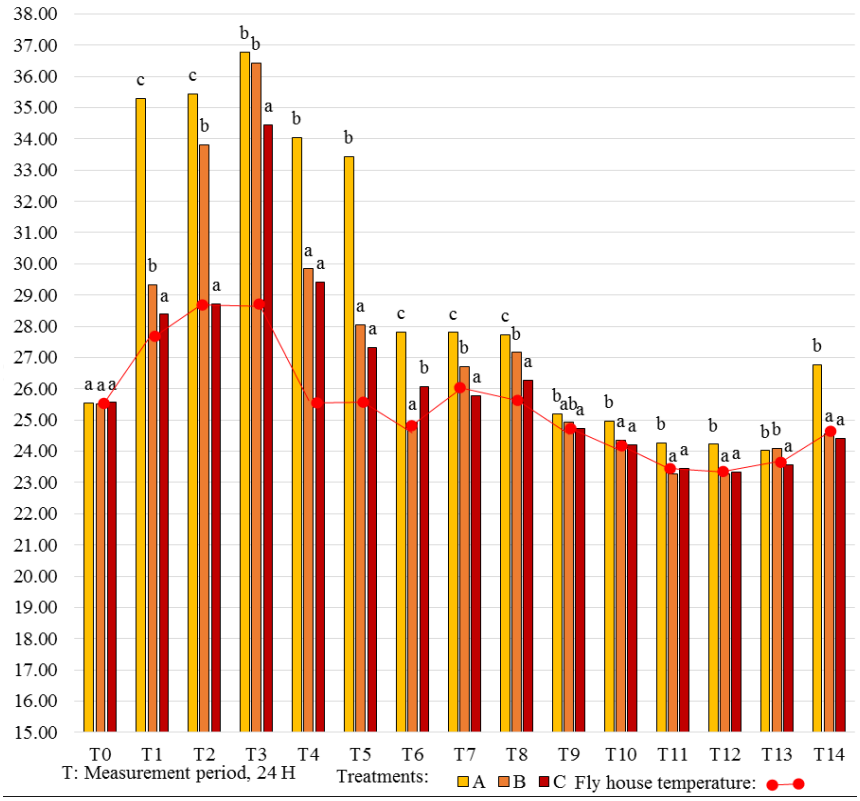

Figure 1. Comparison of temperatures in each substrate and the ambient temperature inside the fly house. Columns with different superscripts differ for P< 0,05 (Tukey)

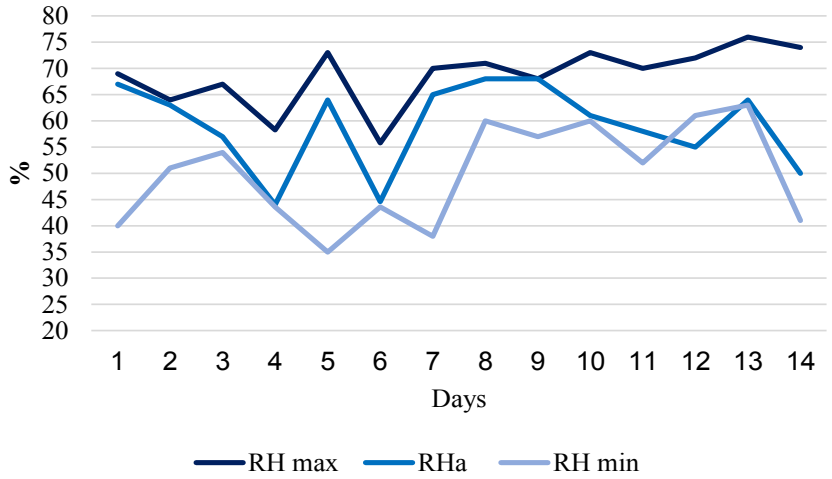

Figure 2. Relative humidity behaviour inside the fly house. Legend: RHmax - Maximum relative humidity; RHa - Average relative humidity; RHmin - Minimum relative humidity.

A determining factor for the development of fly larvae is humidity, since they are very susceptible to dehydration if there is not enough humidity, although excessive values of humidity lead to drowning of the larvae (Feldmeyer et al., 2008). Therefore, the rainy season favors larval production over dry seasons and the knowledge of these limiting climatological factors must be considered for a sustainable and applicable method by producers (Gafar et al., 2019). The optimum relative humidity reported according to Makkar et al. (2014) is between 65 and 70 % with temperatures between 25 and 30° C. Other authors reported optimum relative humidity values between 70-100%. (Sequeira et al. 2001)

Although the relative humidity taken inside the fly house varied, it was similar to that found by the aforementioned authors, and should not have had a negative influence because the substrates were artificially humidified when necessary. The amount of water used to wet the substrates initially was 192 mL for 100% wheat bran, 175 mL for 50% wheat bran with 50% swine feces and 175 mL for 100% swine feces, resulting in a ratio of 1:0.92 water for the first treatment, 1:0.93 water for the second and 1:0.86 water for the third (Table 1).

These values are lower than those reported by Miranda et al. (2020) and Casanovas et al. (2021), who developed the rearing of house fly larvae in wheat bran, using a 1:1 ratio of water to wheat bran. During days 7, 8 and 11 no water was added to the substrates because on those days the city of Cienfuegos was under the influence of a watercourse, which meant that the substrates were moist for larval development. The treatment that required the greatest amount of water for wetting was the pig manure (100%), with the highest values on days 3, 9, and 10.

Although swine feces was not the substrate that required the greatest amount of water for wetting at the beginning of the experiment (175 mL), it was the one that required the greatest amount of water during the 14 days period, explaining the greater amount of water used (162.74 mL) during this period. The amount of water per square meter with three cm thickness of substrates, including the initial water used for wetting, was 32.91, 32,35 and 35.46 L in the total harvests for treatments A, B and C, respectively. For the first harvest it was 87.34, 85.8 and 81.42 % with respect to the total, respectively. This aspect of water use should be considered for planning the production of fly larvae with these substrates, given the importance of this vital resource.

According to Gafar et al. (2019), substrate moisture influences fresh biomass or dry larvae and larval development. Excess water has a negative impact on productivity and as a disadvantage delays larval extraction time. In fly larval production, substrate moisture and larval moisture content are directly proportional. The water resource is vital, its current situation and imperative scarcity entails taking measures to find ways to use it in a responsible and sustainable way. Insect rearing, compared to that of other species, needs low water consumption, requiring eight thousand times less water than cattle farming (Beskin et al., 2018).

For the first harvest the highest number of larvae per treatment was obtained in the treatment based on 50% bran with 50% swine feces, with values of 2910.00 average larvae, and a total of 3381.40 for all harvests, which were higher with respect to the remaining treatments ($P<0.05$). In turn, the lowest larvae count values were obtained in the 100% pig manure treatment with 1721.00 larvae (Table 2). The percentages representing the first harvest for each treatment were: 90.3% for wheat bran, 90.53% for 50% bran with 50% swine feces and 82.5% for swine feces, suggesting that, with an initial harvest, the highest amount of fly larvae can be obtained.

In the comparison of the average weights of the larvae, there are no differences between the weights among treatments, the larvae presented an average weight of 0,007, 0,009 and 0,010 g in the substrates of wheat bran, 50% bran with 50% swine feces and swine feces, respectively. No differences were observed between the first and fourth harvests, which is attributed to the fact that the larvae harvested were in the third larval stage (Table 3).

Table 1. Comparison of the amount of water used per treatment (ml) and initial substrate weight (g).

Days	Treatments			SD ±
	A	B	C	
1	17.00 ^{a}	28.00 ^{b}	30.00 ^{b}	10.90 *
2	12.00 ^{ab}	14.00 ^{b}	10.20 ^{a}	2.22 *
3	14.20 ^{a}	13.60 ^{a}	28.40 ^{b}	7.81 *
4	9.20 ^{b}	4.80 ^{a}	6.00 ^{a}	1.96 NS
5	15.60 ^{a}	17.00 ^{ab}	19.40 ^{b}	3.68 *
6	13.80 ^{b}	12.00 ^{b}	6.00 ^{a}	4.41*
7	0.00	0.00	0.00	0.00
8	0.00	0.00	0.00	0.00
9	4.00 ^{a}	6.60 ^{a}	10.80 ^{b}	3.38 *
10	4.40 ^{b}	6.60 ^{a}	11.40 ^{b}	3.79 *
11	0.00	0.00	0.00	0.00
12	1.28 ^{a}	1.36 ^{a}	1.54 ^{a}	0.59 *
13	19.00 ^{a}	20.20 ^{a}	29.00 ^{b}	6.07 *
14	11.00 ^{a}	9.00 ^{a}	10.00 ^{a}	2.47 NS
Subtotal, 14 days	121.48 ^{a}	133.16 ^{a}	162.74 ^{b}	24.18 *
Initial Day	192.00 ^{b}	175.00 ^{a}	175.00 ^{a}	8.78 *
Total period	313.48 ^{a}	308.16 ^{a}	337.74 ^{b}	24.47 *
Substrate weight, g	206^{a}	194^{a}	192^{a}	12.77 NS

Rows with different subscripts differ for * $P<0.05$. NS- Not Significant (Tukey)

In other research (Miranda et al., 2020), comparing the use of different animal manures, the best results were obtained with hen manure, and they stated that the higher the feeding rate, the better results were obtained in the weight of larvae (4-16%), pupa (16-25%) and adult (8-25%). According to Koné et al. (2017) comparing various types of manure (e.g. chicken, pig and dairy cow feces) the best values were obtained with dairy cow feces which ranged from 0.0174 to 0.0191 g per house fly larvae. Therefore, it is suggested that the composition of the substrates in this research did not influence the weight of the larvae.

Research on other dipteran species such as the black soldier fly (*Hermetia illucens* L.), has shown that the protein and carbohydrate concentration of the diet significantly affects both the fresh and dry weight of the larvae obtained, with the protein value being the most important determinant related to higher weights (Beniers and Graham, 2019). The highest mean yield values (g m^{-2}) for the first harvest and total harvest were found for treatment B (Table 4). The yield factor is determined by the weight and number of larvae, so results in this experiment must be attributed to the greatest greatest number of larvae, since no differences were found in the weight of larvae in any of the treatments.

Yields of the first harvest were always higher with respect to the rest of the harvests, with values of 90.27, 90.55 and 82.51% in grams per m^2 for treatments A, B and C, respectively. Lower results were reported by Casanovas et al. (2020) where fly larvae yield on a fresh weight in the wheat bran substrate, with values of 830.27 g m^{2-1} and 82.37 g kg^{-1}, where it coincided that this treatment presented the highest amount of larvae. If the amount of substrate is increased, it does not necessarily mean an increase in yield, an adequate amount of substrate must be found depending on the dimensions of the opening of the container used (Gafar et al., 2019).

Table 2. Comparison of the number of larvae per treatment.

Harvests	Treatments			SD ±
	A	B	C	
1	1934.80 ^{b}	2910.00 ^{c}	1271.60 ^{a}	793.17 *
2	73.00 ^{b}	136.40 ^{b}	123.20 ^{b}	36.55 *
3	69.60 ^{a}	124.40 ^{b}	116.00 ^{b}	33.60 *
4	65.80 ^{a}	123.60 ^{b}	117.80 ^{b}	35.14 *
Subtotal (2^{a} to 4^{a} ,	208.40 ^{a}	384.40 ^{b}	357.00 ^{b}	90.40 *
Total	2143.20 ^{b}	3214.20 ^{c}	1541.00 ^{a}	562.54

Rows with different subscripts differ for * P<0.05. NS- Not Significant (Tukey)

Table 3. Comparison of average larval weights, grams.

Treatments	1st Harvest	4th Harvest
A	0.007012	0.006262
B	0.009396	0.008186
C	0.010448	0.010200
SD±	0.005195 NS	0.005144 NS

Columns with different subscripts do not differ for * P>0.05, NS- Not Significant (Tukey)

Table 4. Comparison of average yields by treatments.

Treatments	Average yields g m^{-2}	Average yields g m^{-2}	Average yields, g kg^{-1}	Average yields, g kg^{-1}
A	1424.52 ^{a}	1577.92 ^{a}	65.86 ^{a}	72.95 ^{a}
B	2869.11 ^{c}	3169.04 ^{c}	140.85 ^{c}	155.57 ^{c}
C	1395.26 ^{b}	1690.86 ^{b}	69.21 ^{b}	83.87 ^{b}
SD±	945.28 *	941.45 *	32.98 *	34.67 *

Columns with different subscripts differ for* P<0.05, NS- Not significant (Tukey).

According to Barnard and Geden (1993), the influence of temperature and density, is classified as follows: without overcrowding = 1 larva / g manure, moderate overcrowding = 2.5 larvae / g manure, overcrowding = 5 larvae / g manure. The most rapid larval development was observed at 32 ^{o}C with the greatest variation in larval size and with the best survival rates without overcrowding. For this case, the number of larvae per gram of substrate presented a high density, with values from 9.38 to 15.0 larvae for the substrates wheat bran and 50% wheat bran and 50% swine feces, respectively, in the first harvest.

Other research carried out by Hussein et al. (2017); Koné et al. (2017) and Sanou et al. (2019) also highlighted wheat bran as the substrate that produced higher larval biomass compared to other substrates such as cow dung and millet bran. Their results were attributed to wheat bran providing a loose, less consistent, and more aerated structure than the other substrates, which were more compact, with high moisture loss. Larvae were shown to vary in performance depending on the characteristics of the substrate used, including odour, texture, decomposition rate, moisture holding capacity and chemical composition.

The highest larval biomass would be obtained if environmental conditions are favorable. However, the substrate would be rapidly depleted favouring the alkalinization of the medium, creating competition among larvae, which would end up reducing the larval mass (Pieterse and Pretorius, 2013). Therefore, the presence of bacteria or their metabolic products are essential as nutrients for the rearing medium in the development of house fly larvae (Schmidtmann et al., 1992). It is evident that the combination of 50 % wheat bran and 50 % swine manure produced the highest amount of fly larvae with respect to the other treatments, which may offer an opportunity for the utilization of these wastes, as long as the price of wheat bran is low. It would be necessary to carry out a study on the economic feasibility of these results.

In all substrates upon transformation by fly larvae, a decrease in crude protein content per kilogram of dry matter was noted, with values of 54.86 g in wheat bran, 62.89 g in wheat bran 50% with swine feces 50%, and 92.36 g in swine feces ($P<0.05$). This translates into a larval protein conversion ratio of 37.0 %, 35.4 % and 41.0 % for treatments A, B and C, respectively. It is therefore proposed that these values must have been incorporated into the formation of fly larvae (Table 5).

Houseflies have been found to reduce nitrogen in manure, by the metabolic processes carried out by bacteria, which are then the main source of nutrients for the larvae (van Huis 2015), which obtained a reduction 7.5 to 2.6% in poultry manure and in cattle manure, up to 25% on dry basis matter (Hussein et al., 2017). For their part Wang et al. (2013) obtained in swine manure up to 78%. The results obtained could be attributed to the chemical characteristics of the feces, because, according to Mariscal (2007) pig manure contains large amounts of nitrogen in the form of nitrates. Therefore, the management of this waste must be considered, since it can be a pollutant source in ecosystems.

Increasing larval density, can facilitate medium bioconversion, although when food is scarce, house fly larvae may use foods of low nutritional value, such as vegetable protein and crude fibre (Cicková et al., 2015). Therefore, breeding density contributes directly to the rate of substrate conversion, but when breeding density increases, the average rate of substrate reduction decreases (Cheng et al., 2021). This coincides with the results obtained by Casanovas et al. (2020) showing a decrease per kilogram of dry matter in crude protein contents was noted, with values of 23.60 with a conversion ratio in larval protein of 83.95%.

On the other hand, bioconversion of substrates by house fly larvae constitutes a digested residue that can be used as a biofertilizer to improve soil fertility (Leyo et al., 2021). It is concluded that the conversion of nitrogen, represented by the crude protein of the substrates corresponding to the fly larvae, was acceptable with respect to that found in the scientific literature, with values between 28.0 and 41.0%, with the highest value for the treatment of wheat bran 50% with swine feces 50%. It is a well-known concern that the house fly can participate in transmission of some diseases, so regulations are created in many countries for its control in livestock farms (Martínez et al., 2015). Although EFSA (European Food Safety Authority) states that the use of insects and substrates for food production is possible (PROteINSECT, 2016).

In the laboratory results of the bacteriological control for the evaluation of the swine feces, lactose-reducing colonies were identified in the enrichment culture medium Agar Brilliant Green Agar. Therefore, they were confronted to the Salmonella Polyvalent, resulting negative to pathogens. Corroborating this with the biochemistry of the colonies, positive citrate and negative glucose, the presence of *Salmonella* and *E. coli* was ruled out, indicating that the biotransformed substrates, the pig manure and the harvested larvae, did not show the presence of pathogens. Therefore, no pathogens agents were detected in any of the substrates combinations at the beginning and at the end of the experiment. In addition, for the swine feces, the results did not show the presence of coccidia, which is not common in pigs, but can be found.

Table 5. Comparison of crude protein contributions of unprocessed and processed substrates

Treatments	Unprocessed substrates, Day 0	Transformed substrates,	P
A	148.25 ± 1.85	93.39 ± 1.01	0.03 *
B	177.78 ± 2.56	114.89 ± 2.15	0.03 *

Mean values (g) in the same rows differ for * P < 0.05; ** P < 0.01.

Similar results were observed in bacteriological studies on poultry manure and corn germ and swine feces substrates, which was attributed to the temperatures reached in the fermentation process of these substrates (Casanovas et al., 2021). According to Schmidtmann et al. (1992) house fly larvae are also capable of reducing the pathogen load of *E. coli*, *Salmonella enteritidis* and *Campylobacter jejuni* in poultry manure.

Although the biology of house flies has been well studied, mainly focused on the effort to control them, they are considered a vector due to their behavior of roosting in contaminated places and their migratory capacity (Comisión Nacional de Sanidad Avícola/CONASA, 2018). In these results there was no cross-contamination in any of the treatments, nor in the harvested larvae.

4. Conclusions

The temperature in the substrates behaved between 23.34^{o} C and 36.78^{o} C, above the ambient temperature in the fly house, with a relative humidity between 44 and 68 %. The highest fly larvae yield for all substrates was obtained in the first harvest at six days with the highest values for the combination of 50% wheat bran with 50% swine feces, with 2869.11 g m^{2-1} and 181.16 g kg^{-1}. The crude protein transformation of the substrates by fly larvae was between 28.0 and 41.0%, with the highest value for the 50% wheat bran with 50% swine feces treatment. No pathogens were observed in the treatments, nor in the harvested larvae

Authors' Contribution

Enrique Casanovas, head of the research, contributed in the execution of the experiment, data collection, statistical analysis and interpretation of results, writing of the manuscript and final correction of the manuscript. Reina Reyes and Raúl Padilla participated in the data collection and analysis of the results. Alexis Suárez del Villar and Ana Álvarez contributed to the search for updated information on the subject and the correction of the results and their discussion.

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