J. King Saud Univ., Vol. 23, *Agric. Sci.* (1), pp. 1-17, Riyadh (2011/1432H.)

An Innovative System for Production of Protein from House Fly Larvae (*Musca domestica*) Using Poultry Manure for Animal Consumption

A. E. Ghaly and F. N. Alkoaik
Department of Agricultural Engineering, College of Agriculture and Food Sciences,
King Saud University, Riyadh, Kingdom of Saudi Arabia

(Received 9/10/1430H.; accepted for publication 15/2/1431H.)

Keywords: Insects production, Housefly, Poultry manure, Protein, Amino acids, Nutrition, Animal consumption, UV radiation, Sterilization.

Abstract. The feasibility of producing safe protein concentrate from chicken manure through the intensive production of housefly (*Musca domestica*) was investigated. A production system consisting of a manure sterilization unit, an egg production unit and a larvae production unit was constructed and evaluated. A larva harvesting technique and protein processing methodology were developed. The sterilization of poultry manure using ultraviolet radiation was found effective for small flow-rates. The effectiveness of the system was dramatically decreased when the flow-rate increased. Retention time was a very important factor in the sterilization process. Variation in hatching time of the house fly eggs lead to uneven containment of life stages in the desired containers which resulted in the presence of larvae in egg hatching units and pupae in larvae units. The separation of eggs, larvae and flies from the manure was not easy. A new larvae harvesting system should be designed to collect larvae before pupation. The house flies are efficient converters of food into protein. In dry weight basis the larvae contain 64% protein, 16% fat and 16 essential amino acids. The larvae could be harvested dried and used as a protein supplement in animal feeding.

Introduction

Fish meal has traditionally been the primary source of protein for poultry, swine and aquaculture species (William and Barlow, 1996; Hardy and Tacon, 2002) with the poultry industry consuming 60% of the annual supply of total fish meal produced (Hardy, 1996). Studies have shown the possibility to replace up to 80% of fish meal protein with animal by-product meals and still retain the same growth rates in aquaculture species (Lillamen, 2002). However, there have been recent restrictions on the use of rendered animal proteins due to concerns from bovine spongiform encephalitis or BSE (Hardy and Tacon, 2002).

There are also many animal feeds available that are based upon soybean and maize. However, in countries that do not grow soybean or maize, fish meal remains the major source of protein for animal feeds (Hardy and Tacon, 2002). Furthermore, most plant-derived protein sources lack one or more essential amino acids (MacFarlane, 1978) and, therefore, cannot be used as a complete replacement for fish meal. Work has been done in an effort to replace all or part of the fish meal diet in aquacultures with soybean protein as it has a higher protein and amino acid content than other plant based proteins (Kaushik *et al.*, 1994; Carter and Hauler, 2000).

Most of the fish meal produced is from whole fish (with only a small amount from fish processing waste material) meaning that there is less fish available for human consumption. With finite resources and decreasing global fish stocks there is competition for fish for both animal and human consumption (Tacon, 1998). It is also estimated that more than half of the plant mass produced by farmers is used as feed for animals (Flachowsky, 2002). Therefore, large quantities of land are used for the purpose of providing food for animals and, thus, provide more strain on food producers trying to keep up with the rising demand for human food. Therefore, a new method for animal feed production must be

* Corresponding author: Dr. Fahad N. Alkoaik; E-mail: falkoaik@ksu.edu.sa; Tel.: +966-1-467-8753.

developed using appropriate technology suitable for underdeveloped countries.

The technology to be developed must be effective in producing a high quality protein supplement at an affordable price. A suitable feed material must be found which is inexpensive to obtain, can be produced locally in sufficient quantities, has no further use and possess sufficient nutrients that can be converted into animal protein. Materials which are inexpensive and available worldwide in large quantities are animal and poultry wastes. A comparison of the nutrient concentrations in various animal manures (Table 1) shows that poultry manure has considerably higher concentrations of the elements (nitrogen, phosphorus and calcium) necessary for the manufacturing of animal protein (Pontenot and Ross, 1981). The dry matter of poultry manure contains a considerable amount of protein (Table 2) which is lost when the manure decomposes (Pontenot and Ross, 1981). Therefore, the nutrient rich poultry manure can be used as a substrate for the production of insects for animal consumption.

A new source of feed for animals must be utilized from another source than traditional plants and animals. Insects are very efficient converters of feed to protein (more than five times that of beef) and fast growing organisms, contain all the essential amino acids required for animal and human growth and can be reared on low substrate material such as manure (DeFoliart, 1982).

Objectives

The aim of this study was to investigate the feasibility of producing a protein concentrate from chicken manure through the intensive production of houseflies. The specific objectives were: (a) to design, construct and evaluate a production system consisting of a manure sterilization, egg production and larvae production units, (b) to develop a larvae harvesting technique, and (c) to develop and evaluate an insect protein substrate processing methodology.

The Housefly (*Musca domestica*)

The housefly, *Musca domestica*, belongs to the Muscidae family of the genus *Musca.* The insect is characterized by being slender to robust, usually strongly bristled fly, dull in color (black, grey or yellowish). It rarely has brightly colored hairs and it resembles bumble bees. Their wings are usually unmarked, sometimes with cross veins. The adult housefly is 12-14 mm long. The life cycle of the housefly (Fig. 1) is made up of four separate stages: egg, larvae, pupae and adult. The adult female fly begins to lay eggs at four days of age in the nearest deposit of biodegrading materials with preference being given to animal and food wastes. A single adult fly will oviposit hundreds of eggs, but only about 80% will hatch. The eggs are approximately 1 mm long and 0.001 mm in diameter. The eggs are held together by a sticky mucus. The flies continue to lay eggs until they are 9 days old. The eggs will hatch after 2 days and the larvae will appear. The larvae (maggots as they are commonly known) are 5-7 mm in length and white-greyish in color. They are very mobile and can consume large quantities of food. The larval stage continues for 3-4 days. The larvae will begin to search for a place to pupate. In the pupal stage, the larvae form a hard brown shell around them and remain in the shell for 4-5 days. The shells will then become a dark brown to black in color, a sign that they are within hours of emergence as adults (Larrian and Salas, 2008).

Table 1. Nutrient concentrations in various solid animal wastes (Pontenot and Ross, 1981)

Animal Type	Dry Matter (%)	Nutrient (%)				
		N	P	K	Ca	Mg
Poultry	51.80	31.3	0.96	1.06	2.24	0.38
Beef	23.40	20.3	0.13	0.51	0.29	0.12
Dairy	18.30	12.7	0.13	0.44	0.31	0.11
Swine	28.20	23.5	0.33	0.29	0.79	0.22

Table 2. Composition of poultry manure dry matter (Pontenot and Ross, 1981)

Parameter	Content (%)
Energy	52.0 – 55.0
Protein	30.0 – 40.0
True Protein	11.0 – 13.0
Crude Protein	13.0 – 15.0
Ash	20.0 – 30.0
Calcium	7.0 – 9.0
Phosphorus	1.7 – 2.5
Potassium	1.8 – 2.5

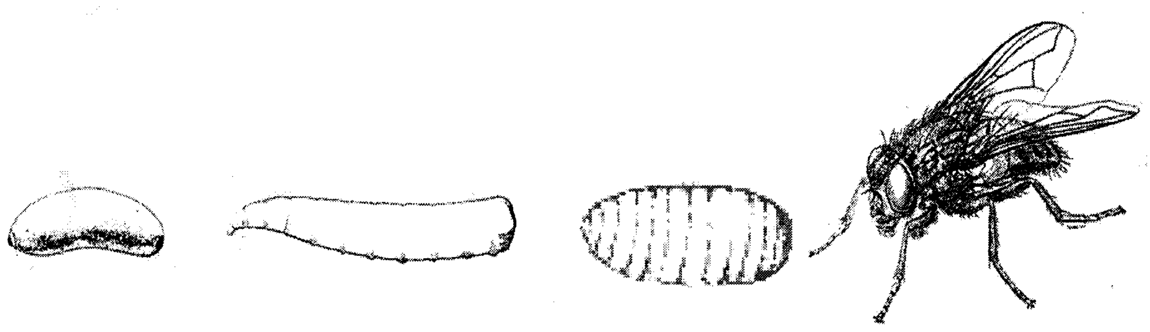


Fig. 1. Life cycle of house fly.

System Development

A protein production system was designed to produce a high quality protein substrate from poultry manure through the intensive production of houseflies. A colony of breeding flies was obtained from Agriculture Canada Research Station in Kentville, Nova Scotia which had been established in a temporary egg production unit in the Biotechnology Laboratory at Dalhousie University, Malifax, Nova Scotia. Figure 2 shows a schematic diagram for the various steps involved in the production process. The production facility (Fig. 3) consisted of: (a) manure feeding and harvesting system, (b) a manure sterilization system, (c) an egg production system, (d) a larval production system, and (e) a harvesting unit.

Manure feeding system

The manure feeding system (Fig. 4) consists of: (a) a manure storage unit, and (b) a manure feeding unit. The manure storage unit was a tank constructed out of 10 gauge stainless steel sheet metal with an overall height of 255 mm. The upper section was a rectangular box (102 mm wide, 210 mm long, and 108 mm deep) which then narrows down to a 38-mm diameter cylinder. Located directly above the circular opening in the bottom of the tank is a ribbon auger which moved the manure out of the storage tank and into the manure handling and mixing unit. The auger was made out of stainless steel rod with a 5-mm diameter. The flight has a diameter of 76 mm at the top, decreasing gradually (for 102 mm) down to a 27-mm and then remaining at a constant diameter. The auger was driven by an electric motor (1/8 horsepower Model No. HSA40, Dynetic System, Elk River, MN) mounted rigidly on the top of the feeding tank. The motor rotates at 5 RPM with sufficient torque to prevent binding.

Manure sterilization system

The manure sterilization system consisted of: (a) an ultraviolet radiation lamps, and (b) outer casing. The outer casing of the UV unit was designed to rigidly hold the manure handling and mixing unit at a distance from the ultraviolet radiation lamp. It was made from 12 gauge stainless steel sheet metal and consisted of the lid, bottom and support stands. The lid and bottom together form a 16-sided cylinder, 930 mm in length and 360 mm in diameter. The lid was formed by seven of the 16 surfaces for the UV lamps, while the bottom section was formed by the remaining nine surfaces. The back of the lid is hinged to the back of the bottom section of the casting by a 930-mm long piano hinge secured by 10 machine screws. The front of the lid has an extra surface attached to it, which overlaps the bottom section by 70 mm to ensure that the ultraviolent radiation does not come in contact with the human eye before the power to the lamps is terminated. Two handles are fixed to the front of the lid to aid in the opening and closing. The two support stands are made of 300 mm x 310 mm stainless steel sheet metal. They are fixed to the ends of the casing with six 12.5 mm x 6.25 mm machine bolts and nuts. The ultraviolent radiation was produced by G-30T8 germicidal lamps. The germicidal lamps operate at a wavelength range between 260 nm and 280 nm, which is the most effective for killing microorganisms (American Ultraviolet Co., Lebanon, IM). The lamps are 914 mm long and 25.4 mm in diameter and were held in 914 mm fluorescent light fixtures. The lamps operated on a pre-heat circuit with a ballast starter switch which was designed such that an attempt to open the casting surrounding the lights will terminate the flow of current to the lamps. Eight separate lamp/fixture combinations were arranged in an octagonal configuration with each lamp being 50 mm from the outer surface of the cylindrical tube. Four 12.5 mm x 6. 25 mm UNF hex head nuts and bolts were used to fasten each fixture to the casting.

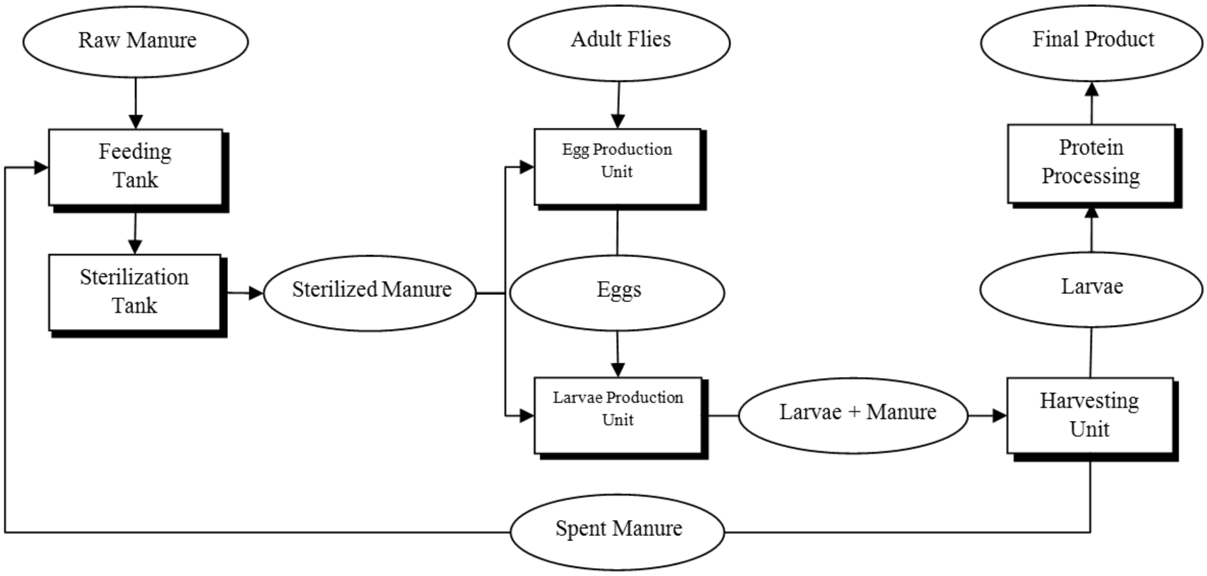


Fig. 2. Schematic diagram showing the various steps of insect protein production.

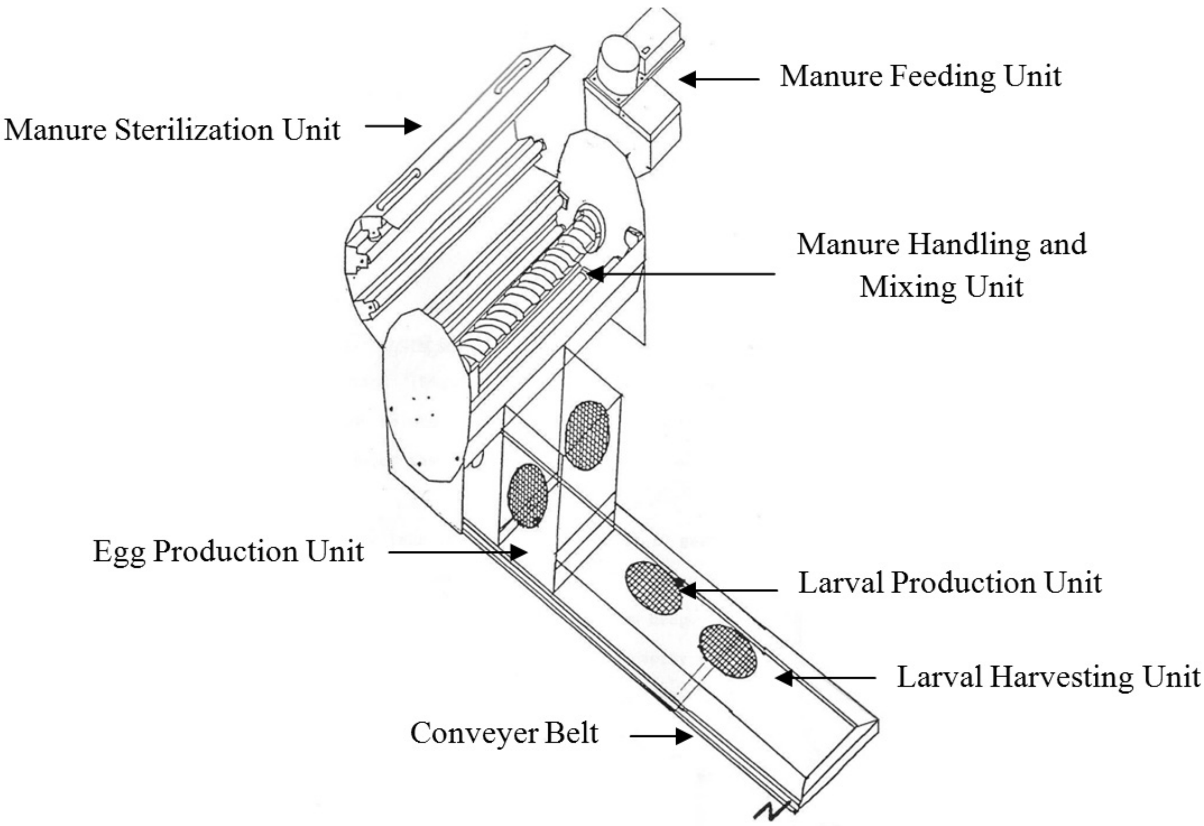
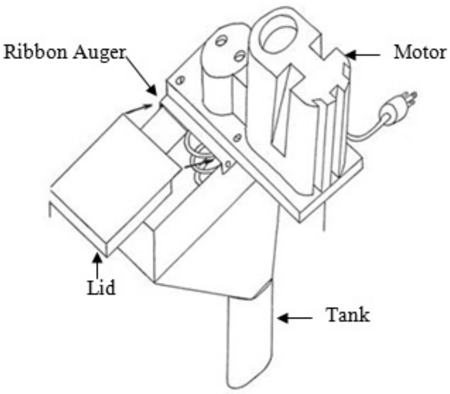
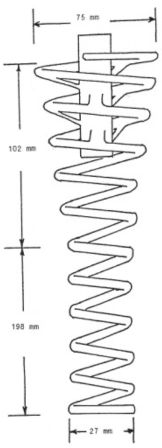


Fig. 3. Protein production facility.



(a) Unit assembly.



(b) Ribbon auger.

Fig. 4. Manure feeding unit.

Manure handling and mixing system

The manure handling and mixing unit (Fig. 5) is an auger running in a cylindrical tube that transfers the manure from the manure feeding unit through the ultraviolet radiation unit and into the egg production unit. The auger was driven by an electric motor.

The cylindrical tube was made from TYPE 214 fused quartz glass (York Glassware Services Ltd., York, UK) which has 90% transmittance of the two wavelengths, 260 nm and 280 nm. The cylindrical tube is 803 mm in length with an outside diameter of 61 mm and an inside diameter of 55 mm. A 32-mm hole was formed with intense heat 120 mm from one end. An outlet tube (with an outside diameter of 42 mm, an inside diameter of 32 mm, and a length of 85 mm) was annealed then fused onto the cylindrical feeding tube around the hole. To reduce the effect of the forces involved in rigidly mounting the fragile quartz tube and provide removable end seals in which the agitation unit can rotate, end caps were designed and constructed out of PVC plastic.

The inlet end cap was formed with two separate sections. The upper section is a 75-mm thick cylinder with a 100-mm diameter. Four 4-mm holes were drilled equidistant apart on a radius of 40 mm. A cylindrical section (5 mm deep with an internal diameter of 62 mm) was machined out of one side. A second cylindrical section (60 mm deep with a diameter of 45 mm) was machined out of the same side. A 60-mm wide plane was surfaced off the outside of the upper section and a 42-mm diameter hole was bore into the center of the plane until it reached the interior of the end cap, creating an elbow joint. A 22-mm hole was drilled in the end of the upper cap for the manure handling and agitation auger and a 15-mm nylon bushing was inserted. The lower section of the end cap was a collar with an outer diameter of 100 mm and an internal diameter of 18 mm. There were four holes drilled such that they lined up with the holes in the inner section. A 4-mm, 45°, chamfer was machined off the upper inside edge. A 62-mm internal diameter, 5 mm thick polyurethane rubber o-ring was placed in the wedge shaped groove which was formed when the upper and lower section were assembled. As the adjusting bolts were tightened the wedge groove reduces in area, forcing the o-ring towards the center of the cap which caused a clamping effect on the tube. This clamping effect did not produce large enough strains to damage the quartz glass tube.

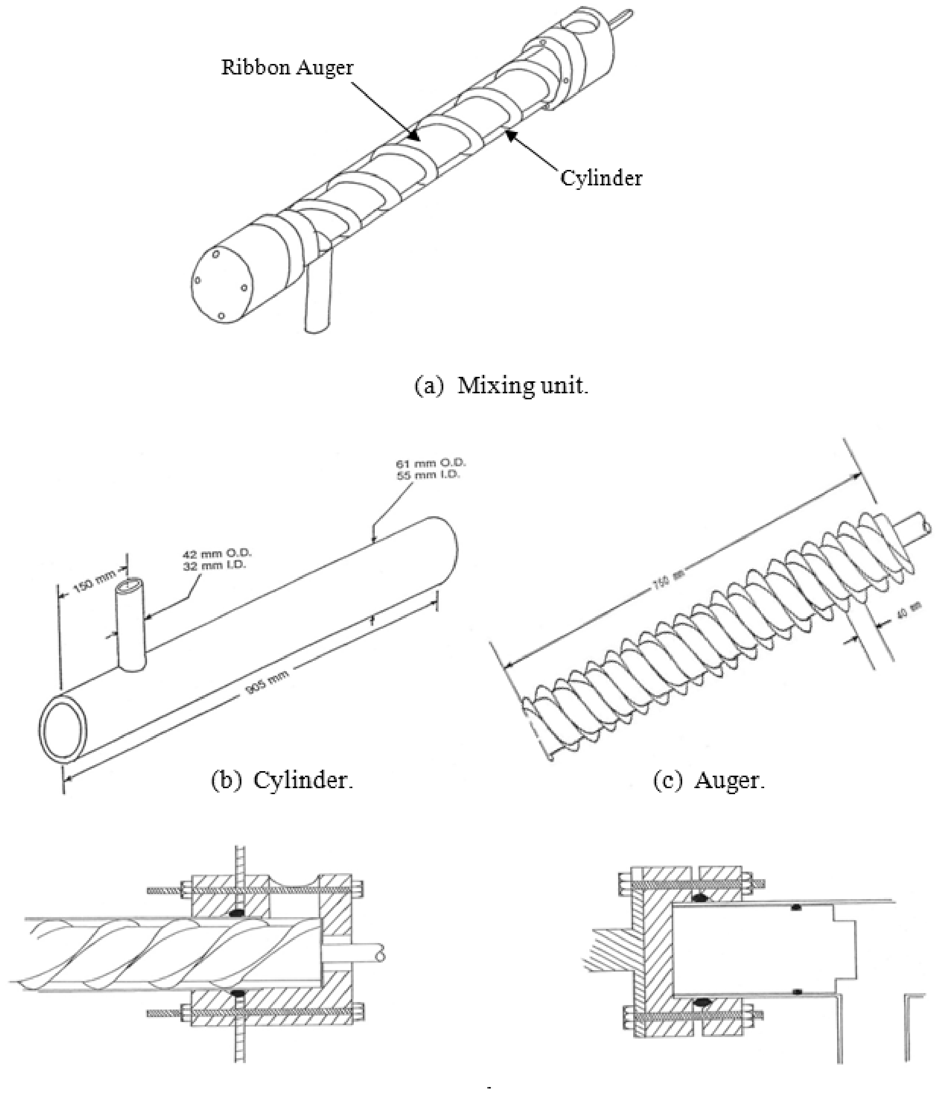


Fig. 5. Manure handling and mixing unit.

The outlet end cap is formed with two separate sections. The upper section is an end plate 20 mm thick and 100 mm in diameter. Four 4-mm holes were drilled equidistance apart on a radius of 40 mm. A cylindrical section with a diameter of 62 mm and a depth of 15 mm was machined out of one side. The lower section of the end cap was a collar with an outer diameter of 100 mm, an internal diameter of 62 mm and a thickness of 18 mm. There were four holes drilled such that they lined up with the holes in the upper section. A 4-mm, 45° chamfer was machined off the upper inside edge. A 62-mm internal diameter, 5 mm thick polyurethane rubber o-ring is placed in the wedge shaped groove which was formed when the upper and lower sections are assembled. As the adjusting bolts were tightened, the wedge groove reduced in area, forcing the o-ring toward the center of the cap which caused a clamping effect on the tube. To provide a bearing to centralize the rotation of the auger and to prevent the sterilized manure from passing the outlet tube, a plug was inserted. The plug was a cylindrical tube of Teflon, 55 mm in diameter and 100 mm long, with a 5-mm step machined out of it.

An auger was designed and constructed out of stainless steel. The auger body was a 770-mm long cylinder with a 45-mm outer diameter and a 35-mm internal diameter. A 5-mm diameter stainless steel rod was arc welded to the tube using 1020 rods. The flighting was helically wound around the tube with a 40-mm pitch. A 2-mm thick face plate was arc welded inside the auger cylinder at the inlet end and a 13-mm diameter shaft was arc welded perpendicular to the plate in the center. The shaft on the inlet end of the auger is 80 mm long, with a 5-mm wide, 2.5 mm deep key way cut into the end. There was a 75-tooth sprocket mounted on the end of the shaft driving a number of 30 chains connected to the motor output shaft.

The auger was driven by an electric motor (1/8 horsepower HST21 GK Heller Corp., Muskegon, MI) operating at 7-9 RPM. The motor was mounted onto the support stand at the inlet end of the outer casing with four 12.5 mm by 20 mm hex head UNF bolts and nuts. The output shaft of the motor had a 65-mm diameter, 20-tooth sprocket mounted on it and secured with a 5 mm x 5 mm key.

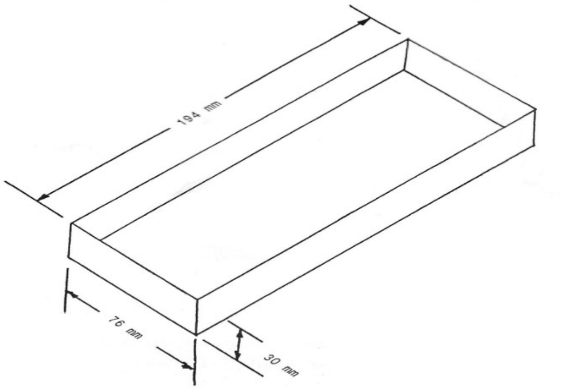
Egg and larvae production system

The natural breeding habits of the housefly were researched and it was found that they prefer to lay their eggs on fresh manure more than any other material (Hogsette, 1996). However, the manure used in this study for egg and larvae production was sterilized in the manure mixing and handling system. The egg and larvae production system (Fig. 6) consists of: (a) sterilized manure trays, (b) an egg production chamber, (c) a larvae production chamber, and (d) a conveyor belt. To transport small quantities of the sterilized manure through the breeding chamber, a reusable container was necessary. A sterilized manure carrying tray was designed and constructed from 10 gauge sheet metal. A number of trays were constructed to fulfill the requirements of the breeding chamber and the larvae production chamber. The trays were 194 mm long, 75 mm wide and 30 mm deep. Each tray held 220 cm^3 of sterilized manure.

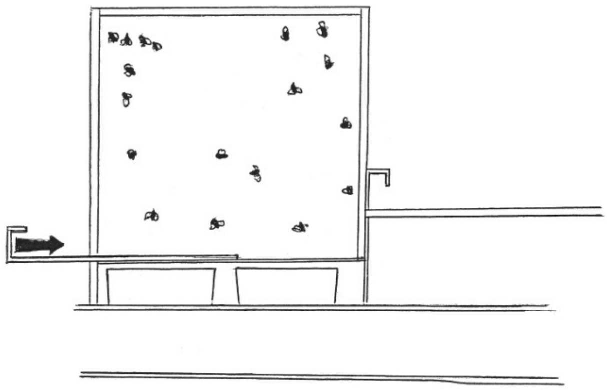
The egg production chamber was a controlled environment in order to optimize the adult housefly's ability to reproduce in great numbers. The chamber was made from a Plexiglas container which had a width of 208 mm, a length of 178 mm, and a height of 280 mm. The chamber had an open top and bottom. Located on the bottom of the inlet side of the container was a 208 mm wide, 55 mm high tray inlet. There was a similar opening on the outlet side. This tray inlet was partially covered by a 196-mm wide, 38 mm high, 6 mm thick door, upwardly hinged on its top edge by two small screws, pinning it from both sides. A 2-mm wide, 196 mm long slot was cut out of the inlet side of the container and the inlet door. At a height equal to that of the bottom edge of the slot, two 5-mm wide railing were fastened to the insides of the chamber. These two slots provided support for the safety divider. The safety divider was designed to prevent the adult flies from escaping, either out of the tray inlet or into the larvae production system, by restricting their movement to the upper section of the chamber. The safety divider was a 200 mm x 196 mm plate made out of stainless steel sheet metal.

To control the humidity and the temperature of the egg production chamber, two 102-mm diameter air intake holes were drilled into the side of the chamber. The holes were covered with a fine plastic fly screen. The screens were securely fastened to the Plexiglas sides with an epoxy-resin combination.

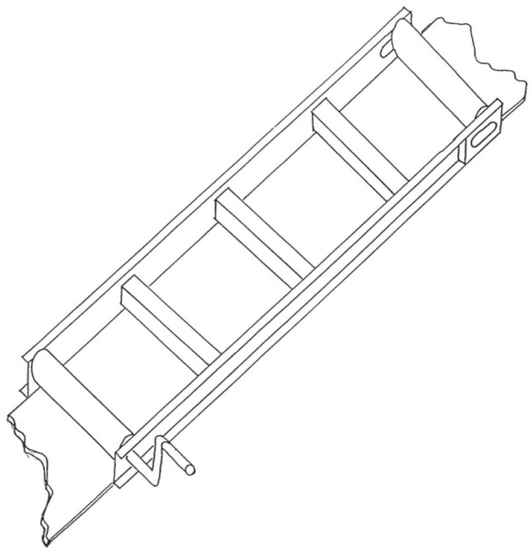
The larvae production chamber was a rectangular box made of 6 mm thick Plexiglas with an open top and bottom. The chamber was 208 mm wide, 427 mm long, and 90 mm high. Located on the bottom of the inlet side of the chamber was a 208 mm x 55 mm tray inlet opening. A similarly dimensioned try outlet opening appears on the opposite side. A 196 mm x 38 mm, 6 mm thick door was hinged to the top of the tray outlet opening. A fine mesh plastic fly screen was stretched over two 100-mm diameter holes cut out of the Plexiglas top. The screens were fastened to the Plexiglas with an epoxy-resin mixture. The larvae production chamber fit around the conveyor unit in the egg production system. The tray inlet side of the larvae production chamber is physically connected to the tray outlet of the egg production unit. There is a 2-mm wide slot cut along the center 196 mm of the connection between the larvae and egg production chambers.



(a) Sterilized manure carrying tray.



(b) Adult flies and eggs separation box.



(c) Conveyor unit.

Fig. 6. Egg and larvae production system.

To prevent any adult flies from escaping into the larvae production chamber, a divider was placed between it and the egg production chamber. The divider was a 196 mm x 110 mm, 1.5 mm stainless steel sheet which could slide up and down between the two chambers. Two 6-mm diameter holes were drilled 60 mm from the top of the sheet and located 60 mm from each side. When the divider was lifted to allow the trays to move from the egg production unit to the larvae production unit, two 6-mm diameter pegs were placed in the holes preventing the outlet/inlet openings from closing.

The conveyor unit was designed to move the sterilized manure trays through the egg production chamber. The egg production chamber was placed 100 mm from one end of the conveyor. The extended sides of the egg production unit fit around the sides of the conveyor belt with the hinged inlet facing opposite the direction of the belt rotation. The conveyor unit was a 194-mm wide, 2 mm thick rubber belt with an overall length of 2,640 mm. The belt was tightly stretched between two 206 mm wide, 50 mm diameter aluminum stock rollers. The belt tension was adjusted by turning the adjustment screws on the frame.

The conveyor unit frame consisted of two 1,420 mm long, 25 mm thick, 50 mm wide aluminum rails. Each rail had a 22-mm hole cut out of each end with four 6-diameter holes drilled equidistance apart around a 27-mm radius. At the opposite end a 20-m wide, 45 mm long slot was cut out. This slot was part of the adjustable tension mechanism. The two rollers ran in bronze bushings. Three square sections spacers were arc welded to rails to keep them sufficiently spaced apart as to allow easy rotation of the belt. To rotate the belt, a simple crank was fixed to the roller on the outlet side of the egg production chamber.

Experimental Procedure

Manure collection and preparation

The manure was obtained from a local poultry house in Truro, Nova Scotia. Four plastic bags were suspended under four different cages until they were full. The manure was then transferred to 60-liter plastic containers and transported to the Biotechnology Laboratory at Dalhousie University in Halifax, Nova Scotia. The manure characteristics are shown in Table 3.

To prepare the manure for use in the sterilization system, it was necessary to remove any large pieces of foreign matter which might become lodged in the manure handling unit. The manure was screened through a No. 4 Taylor Series sieve (W.S. Tyler, St. Catherine's, Ontario). The screened manure was collected in a catch basin. Ten 10-ml samples were randomly removed and diluted with 1,000 ml of water and used for bacterial count. The remaining manure was frozen for three days to kill any invading arthropods.

Various dilutions of the screened manure to water were prepared to find the optimum moisture content for movement through the auger and porosity to support the larvae. Three dilutions of manure and water were prepared: 1 kg of manure with 500 ml of water, 1 kg of manure with 750 ml of water, and 1 kg of manure with 1000 ml of water. The manure feeding unit was then filled and the system operated. The optimum dilution rate was found to be 1 kg of manure with 750 ml water (moisture content of ≈70%).

Time required for sterilization

The ultraviolet dosage required to kill 99.9% of various microbes is shown in Table 4. Calculations of the time requirements and the necessary dosage were performed using a safety factor of 2. The number of lights and distance from the cylindrical feeding tube were varied from 4 to 8 and from 10 to 100 mm, respectively. The lamp intensity was 85 W/cm^2. The required dosage of ultraviolet radiation was calculated as follows:

Table 3. Some characteristics of raw poultry manure

Parameter	Value
Moisture Content (% wb)	47.0
Dry Matter (% wb)	53.0
Total Protein (% db)	38.0
Crude Protein (% db)	16.0
Initial Organic Matter (% db)	73.0
Initial Ash (% db)	27.0
N (% db)	9.2
K (% db)	1.2
Ca (% db)	2.4
Mg (% db)	0.4

Table 4. Ultraviolet dosage required to kill 99.9% of various microbes

Bacteria	Relative Resistance*
Bacillus subtilis	1.7
Bacillus subtilis spores	3.3
Clostridum botulinum	1.6
Dysentary bacilli	0.6
Escherichilia coli	1.0
Salmonella typhimurium	2.3
Salmonella typhosa	1.0
Stretococcus aureus	1.0
Streptococcus viridians	0.6
Bacteriophage (E. coli)	1.0
Adenovirus	0.7

*one relative resistance unit = 7000 W sec/cm^2

$$Required\ Dossage = (Relative\ Rating)(Relative\ Resisteance)(Saftey\ Factor) \quad (1)$$

The relative resistance of 7000 W sec/cm^2 was selected and the required dosage was estimated to be 838 W sec. The exposure time was then calculated as follows:

$$Exposure\,Time = \frac{Required\ Dosage}{Lamp\ Intensity} \quad (2)$$

Effect of auger speed on manure sterilization

To evaluate the effectiveness and efficiency of the manure sterilization system, the prepared manure was placed in the manure feeding unit. The electric motor was turned on and adjusted to 1 RPM. The manure as then allowed to run through the manure sterilization system and three random samples were taken at the outlet tube of the cylinder for microbial analysis. The above process was repeated while the electric motor was operated at 2.5, 5.0, 7.5 and 10.0 RPM.

Egg production

One hundred housefly pupae from a stock provided by the Entomology Division of Agriculture Canada Research Station, Kentville, Nova Scotia were placed in two sterilized manure carrying trays. The trays were placed side by side on the rotating conveyer belt and were transported into the egg production chamber by rotating the crank on the conveyer unit. The pupae turned into a dark brown to black in color approximately one hour before hatching into adult flies. Once the adult flies left the sterilized manure carrying trays, and the egg production chamber divider was moved into place to ensure that flies cannot escape. After four days, the tray inlet door of the egg production unit was opened and two trays of sterilized manure were introduced into the egg production unit by moving them along the conveyor. The larvae production chamber divider and the egg production tray inlet door were then opened, two additional trays of sterilized manure were placed on the conveyor belt and the conveyor was cranked clockwise until the initial tow trays were in the larvae production chamber. The larvae production chamber divider was then re-inserted and the tray inlet door was closed. The egg production chamber divider was then removed and the adult flies were once again allowed to lay eggs.

Larvae production

After the trays carrying sterilized manure and eggs had entered the larvae production unit, the eggs hatched into larvae. The sterilized manure provided both food for the larvae and a medium for the larvae to live in. The larvae were allowed to mature in the larvae production chamber for two cycles of egg production or four days. When the trays of larvae reached the tray outlet door of the larvae production unit (four days), they peaked in size and were ready to be harvested. The larvae were then separated from the manure. The larvae manure-mixture was spread approximately 10 mm thick on No. 14 Taylor Series sieve (W.S. Tyler, St. Catherine's, Ontario). A 100-Watt incandescent bulb was supported approximately 10 mm above the upper surface of the manure and turned on. The larvae were extremely phototactic having an incredible aversion to light. They congregate near the bottom of the screen, the darkest spot, where they will wriggle thought to be caught in the basin below. A random 100 were allowed to pupate to continue the production of eggs. The remainder of the larvae were prepared for protein processing.

Experimental Analyses

Bacterial count

The bacterial count was done for 1 gram of manure in 1000 ml of water giving a dilution factolor of 10^3. The agar plates were cultured with a smear of the solution and incubated at 35°C in a Shel Line incubator (Sheldon Manufacturing, Model No. 2020, Cornelius, Oregon). A plate count was performed after 48 h. The number of colony forming units were calculated and recorded from the following equation:

$$CFU = (Total\ Colonies\ Counted)\ x\ (Dilution\ Factor) \quad (3)$$

Moisture content

The moisture content was determined gravimetrically using 50 live worms. The oven dry method procedure described in APHA (1990) was followed. The 50 live worms were first weighted using a Mettler scientific balance (AE 2005, Mettler Instruments, AG, Greifensee, Zurich, Switzerland). They were dried in a convection oven (Isotemp oven, Model No. 655F, Fisher Scientific, Montreal, Quebec) for 24 hours at 105°C. The dried samples were then removed from the oven, left to cool in a dessicator and weighed. The moisture content was calculated as follows:

$$MC = \frac{M_1 - M_2}{M_1}\ x\ 100 \quad (4)$$

where:

MC is the moisture content (%)
M_1 is the initial weight (g)
M_2 is the weight of the dried sample (g)

Ash content

The ash content was determined gravimetrically on live weight basis according to the procedure described in APHA (1990). The 50 dried samples were placed in a muffle furnace (Isotemp muffle furnace, Model No. 186A, Fisher Scientific, Montreal, Quebec) for 30 minutes at 550°C. They were then removed, left to cool in a dessicator and weighed using a Mettler scientific balance (AEZOOS, Mettler Instruments, AG, Greifensee, Zurich, Switzerland). The ash content was calculated as follows:

$$AC = \frac{M_3}{M_1}\ x\ 100 \quad (5)$$

where:

AC is the ash content (%)
M_3 is the weight of the material remaining after burning the dry sample (g)

Protein content

The protein analysis was carried out on a live weight basis using 30 mass reared larvae. The live weight of the larvae was recorded using a Mettler scientific balance (AE 2005, Mettler Instruments, AG, Greifensee, Zurich, Switzerland). The larvae were subsequently frozen and dried in a freeze dryer (Labconco FreeZone, Cat No. 10-271-16, Fisher Scientific, Montreal, Quebec) for 24 hours. The larvae were ground using a laboratory grinder (Waring Laboratory, Cat No. 14-509-18, Fisher Scientific, Montreal, Quebec). The total protein was determined using the Tecator Kjeltec Auto Analyzer (Model-1026, Fisher Scientific, Montreal, Quebec). The freeze dried larvae were transferred to the macro 250 mL digestion tubes. One “Kjeltab” (containing 3.5 g K_2SO_4 and 0.0035 g Se) and 3.0 mL of distilled water were added to the samples in the digestion tubes. The samples were digested at 420°C for 30 minutes in a digestion block heater (Tecator Digester System, 20 Model-1016, Fisher Scientific, Montreal, Quebec). The digestion tubes were removed and allowed to cool for 10 minutes. Then, 30 mL of distilled water was added to each of the digestion tubes. The test tubes and the digests were transferred to the Auto Analyzer. The constants A and B for the equipment were set at 0.00 and 1.862, respectively. The titrant acid and the predetermined blank sample were set at 0.2127 M and 0.01, respectively. Distillation, titration and calculation were performed automatically. The protein percentage was computed from the following equation:

$$PC = \frac{Displayed\ Result}{W_s} \quad (6)$$

where:

PC is the protein content (%)
W_s is the weight of the sample of live weight larvae (g)

Fat content

The fat content was carried out on a live weight basis using 50 larvae. The live weight was recorded using a Mettler scientific balance (AEZOOS, Mettler Instruments, AG, Greifensee, Zurich, Switzerland). The larvae were then frozen and dried in a freeze dryer (Labconco FreeZone, Cat No. 10-271-16, Fisher Scientific, Montreal, Quebec) for 24 hours. The worms were ground in a laboratory grinder (Waring Laboratory, Cat No. 14-509-18, Fisher Scientific, Montreal Quebec). The fat content was determined using an ether extraction technique according to the procedure described in the Official Method of the Association of Official Analytical Chemists (AOAC, 1975). Hot ether was percolated through a porous receptacle filled with ground material from the freeze dried larvae for 24 hours. The fat was released from the dry matter and collected at the bottom of the apparatus. The receptacle was removed, dried in a vacuum oven (Isotemp oven, Model No. 655F, Fisher Scientific, Montreal, Quebec) for 24 hours at 105°C and then reweighed. The change in weight corresponded to the fat content of the original sample. The fat percentage was computed from the following equation:

$$FC = \frac{W_f}{W_s} x\ 100 \qquad (7)$$

where:

FC is the fat content (%)

W_f is the weight of fat extracted (g)

Amino acid profile

The amino acids (alanine, arginine, cysteine, glutamic, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, serine, threonine, tryptophan, tyrosine, and valine) were determined using the HFB-IBA (Heptafluorobutyric isobutyl esters of amino acids) Amino Acid Derivatization Kit (Cat. No. 18094, Alltech Associates, Inc., Deerfield, Illinois). First, 50 mg of dried worms were weighed using a Mettler scientific balance (AEZOOS, Mettler Instruments, AG, Greifensee, Zurich, Switzerland) and placed in a small reaction vial. An amount of 3 mL of 0.2 M HCl was added to each vial and the solutions were heated to approximately 110°C using a block heater (Model 16500-10, Hach Chemical Co., Loveland, CO) for 30 hours. Then, the vials were removed from the heater and dried under a stream of dry nitrogen. 1.25 mL of acetyl chloride (Cat. No. 18094B, Alltech Associates Inc., Deerfield, Illinois) were slowly added to 50 mL of isobutanol and the mixture was added to each vial (which contained dry sample). The vials were capped and heated at 110°C for 45 minutes. The vials were uncapped and heated at 115°C under a stream of nitrogen to remove excess reagent. Then, the vials were removed from the heater and cooled in an ice bath (Microprocessor Controlled 280 Series Water Bath, Precision, Winchester, Virginia) for approximately 5 minutes. 3 mL of methylene chloride and 2 mL of HFBA (Cat. No. 18094A, Alltech Associates Inc., Deerfield, Illinois) were added to each vial. The vials were then capped and heated at 100°C for 4 hours. The vials were removed from the heater and after cooling to ambient temperature, excess reagent was evaporated under a stream of dry nitrogen. The dried samples were redissolved by adding 2 mL of ethyl acetate and injected into the gas chromatograph (Model-HP5890 Series II, Hewlett, Palo Alto, CA). The amino acids profile was determined from the output of the gas chromatograph.

Results and Discussion

Ultraviolet light intensity

The UV light intensity and exposure time calculated for various numbers of lamps and distances from the feed tube are presented in Table 5 and Fig. 7. The sterilization time increases exponentially as the distance between the UV lamps and feed tube increased. Increasing the number of UV lamps from 4 to 8 reduced the sterilization time by one half.

Bacterial count

The bacterial counts for the untreated manure were 345,000/g manure. The effect of auger speed and number of lamps on the bacterial counts is given in Table 6 and Fig. 8. The sterilization of manure with ultraviolet radiation was found to be effective for the small flow rates and 8 UV lamps. The effectiveness of the system was found to be dramatically decreased with an increase in auger speed and/or the use of 4 lamps. 100% destruction efficiency was obtained when the system operated at 1.0 rpm using 8 UV lamps. When the system operated without 8 UV lamps, 100% destruction efficiency was never achieved. The sterilization effect could be achieved by the modification of various parameters including: (a) increasing the overall length of the tube illuminated by the ultraviolet lights, (b) serrating the auger flighting to aid further in mixing the manure, (c) stopping and starting the auger at small intervals of time, and (d) increasing the diameter of the quartz cylinder. Other parameters can also be changed such as slowing down the auger speed and increasing the auger diameter (reducing the anuallar space), but these would result in a decrease in output volume.

Larvae production

The hatching rate of flies from pupae is given in Table 7. The table also indicates the percentage of successful hatchings. Problems were encountered as a result of the variance in hatching times. Eggs which were laid early in the cycle could hatch into larvae in the egg production chamber and pupate in the larvae production chamber. Through more intensive breeding operations and larger colonies the flies can be better prepared for this system.

Table 5. UV light exposure with various lamp configurations and distances

Number of Lamps	Distance from Lamp to Feed Tube (mm)	Duration (h)
4	100	3.12
	50	0.80
	10	0.31
8	100	1.60
	50	0.40
	10	0.15

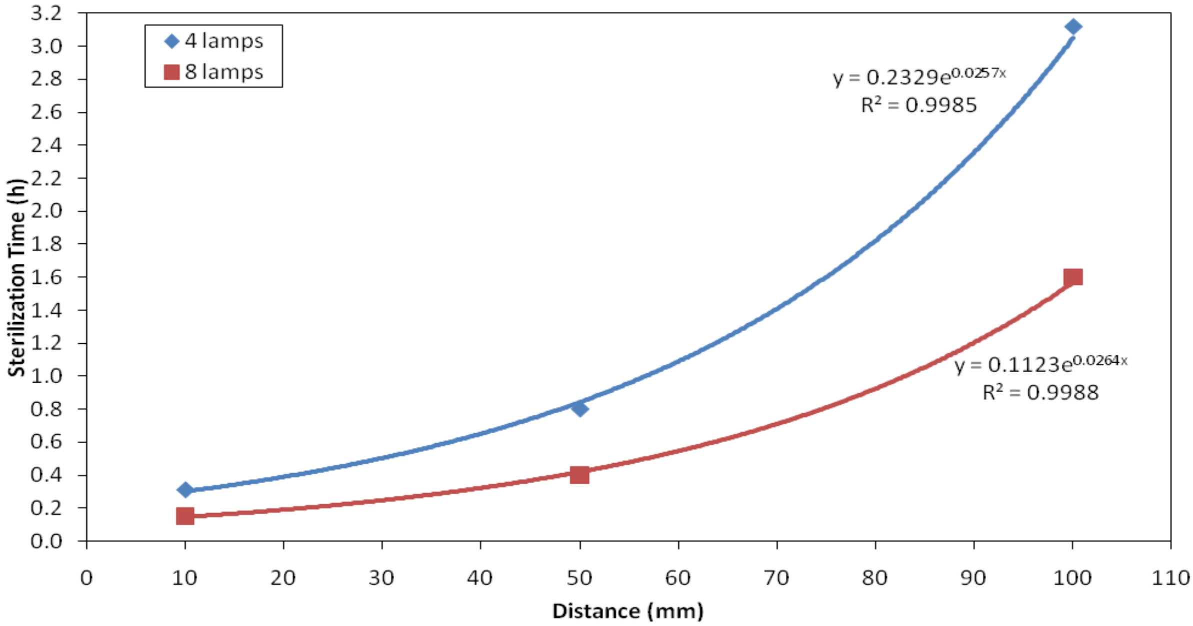


Fig. 7. Sterilization time for two different UV lamp configurations.

Table 6. Bacterial counts at various auger speeds and number of UV lamps

RPM	4 Lamps		8 Lamps	
	Bacterial Counts (x 10^3/g manure)	Reduction (%)	Bacterial Counts (x 10^3/g manure)	Reduction (%)
1.0	77 $\pm$ 4	75	0	100
2.5	164 $\pm$ 14	58	12 $\pm$ 3	97
5.0	217 $\pm$ 12	45	58 $\pm$ 8	83
7.5	246 $\pm$ 7	37	130 $\pm$ 12	67
10.0	268 $\pm$ 13	32	158 $\pm$ 14	60

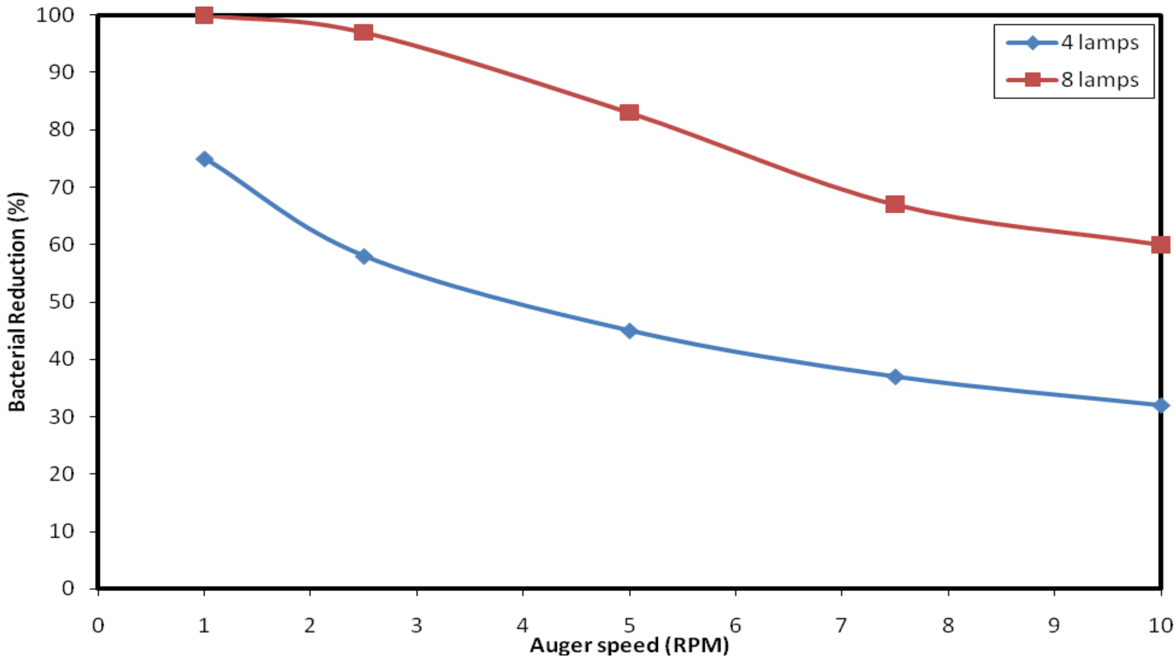


Fig. 8. Bacterial reduction of manure.

Table 7. Hatching rate of flies

Duration(days)	Hatching Rate
0	No Hatching (100 pupae)
1	No Hatching (100 pupae)
2	10% Hatched
3	38% Hatched
4	60% Hatched
5	90% Hatched
6	10% Dead pupae

The separation of the flies from the eggs and the manure from the larvae showed flaws in the design which would have to be rectified in a scale-up operation. The simple sliding door failed to segregate the flies into the upper section of the egg production chamber and as a result flies were consistently being lost into the next chamber. Possible solutions to this problem are: (a) the enlargement of the egg production chamber, to give the flies more possibility of being on the upper side of the divider, and (b) the reduction in the height of manure carrying trays and inlet and outlet doors. This reduction would provide less room on the bottom half of the divider to prevent flies from residing there.

Problems were also encountered with the separation of the larvae and the manure. The system of driving the larvae through the screen was found to be ineffective in this study as the larvae were allowed to mature to their maximum size in the manure, at which time a sizeable portion of the larvae had already started to pupation. This was compounded by the fact that the final drying of the manure to drive the larvae out took time which allowed the remaining larvae to pupate. The variance in the pupation time of the flies can be controlled, as mentioned earlier, by the same methods mentioned in controlling the hatching rates. The solving of the second factor in this problem is not so easy and a new method should be developed which is quicker and just as easy to operate.

Protein production

The chemical analyses of larvae are shown in Tables 8 and 9. The protein and fat content, of the larvae, calculated on a dry basis, were 63.2% and 15.3%, respectively. They energy content was found to be 406.1 kcal/100g of larvae.

Table 8. Nutritional value of dried larvae

Parameter	Value
Moisture Content (%)	13.7
Ash (%)	3.9
Protein (%)	63.2
Fat (%)	15.3
Carbohydrate (%)	3.9
Energy (kcal/100g)	406.1

Table 9. Amino acid profile in larvae

Amino Acid	Housefly Larvae (g/100g)
Alanine	5.9
Arginine	3.9
Cysteine	4.6
Glycine	1.2
Glutamic	6.7
Histidine	1.1
Isoleucine	5.0
Leucine	7.0
Lysine	4.9
Methionine	1.5
Phenylalanine	4.2
Serine	3.7
Threonine	2.9
Tryptophan	0.9
Tyrosine	4.1
Valine	5.3

Conclusions

Housefly larvae (*Musca domestica*) were successfully grown on a feed of poultry manure in the designed protein production system. Houseflies were found to be a suitable food source for animals with high protein, fat and energy contents of 63.2%, 15.3% and 406.1 kcal/100g, respectively. To achieve 100% bacterial reduction in the manure, 8 UV lamps must be used at an auger speed of 1 RPM. A second ultraviolet light chamber can be added in series with the first, essentially doubling the sterilization effectiveness of the total system. This has the added advantage of allowing the total volume flow to be increased. Further research can be performed into the optimum moisture content of the manure. As ultraviolet light has better penetrating power in water, the higher the moisture content of the manure the more effective the sterilizing power of the lights. This is offset by the larvae's inability to breathe under water and therefore an optimum solution can be found from these two constraints.

The simple sliding door system failed to separate the adult flies from the larvae production chamber leading to mixing of the life stages. This leads to less adult flies being present in the egg production chamber. Possible solutions to this problem are (a) the enlargement of the egg production chamber, to give the flies more possibility of being on the upper side of the divider, and (b) the reduction in the height of manure carrying trays and inlet and outlet doors. This reduction would provide less room on the bottom half of the divider to prevent flies from residing there.

Also, the system of separating the larvae from manure by driving the larvae though a screen was found to be ineffective as the larvae were allowed enough time to pupate in manure, thus limiting the yield of the larvae in the harvesting unit.

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نظام مبتكر لإنتاج البروتين من يرقات الذباب المنزلي عن تغذيتها على مخلفات الدواجن للاستهلاك الحيواني

عبدالقادر غالي، وفهد بن ناصر الكعيك

قسم الهندسة الزراعية، كلية علوم الأغذية والزراعة،
جامعة الملك سعود، الرياض، المملكة العربية السعودية

(قدم للنشر في ١٤٣٠/١٠/٩هـ؛ وقبل للنشر في ١٤٣١/٢/١٥هـ)

ملخص البحث. تم دراسة إمكانية إنتاج البروتين المركّز من يرقات الذباب المنزلي (*Musca demostica*) النامية على مخلفات الدواجن من أجل استخدامه كغذاء للحيوانات. ولتحقيق ذلك فقد تم تصميم نظام الإنتاج المكون وتقييمه من وحدة تعقيم مخلفات الدواجن، ووحدة إنتاج البيض، ووحدة إنتاج اليرقات. كما تم تطوير أنظمة حصد اليرقات واستخلاص البروتين. وأثبتت التجارب أن استخدام الأشعة فوق البنفسجية لتعقيم مخلفات الدواجن فعالة لمعدلات التغذية المنخفضة، بينما انخفضت فعالية نظام التعقيم بصورة جذرية عند زيادة معدل تغذية المخلفات في جهاز التعقيم. كما وُجد أن زمن الاستبقاء عامل مهم خلال عملية التعقيم. ولوحظ من التجارب أن الاختلاف في زمن فقس بيض الذباب المنزلي يؤدي إلى تداخل في مراحل الحياة المختلفة لهذه الحشرة، مما أدّى إلى ظهور يرقات في وحدة فقس البيض وعذاري في وحدة إنتاج اليرقات. كما لوحظ أن عزل اليرقات من المخلفات لم يكن سهلاً. ويقترح تصميم نظام جديد لحصد اليرقات لجمعها قبل تطورها لمرحلة الحشرة الكاملة. وأثبتت النتائج أن الذباب المنزلي ذو كفاءة عالية لتحويل المخلفات إلى بروتين، حيث تحتوي اليرقة على ٦٤٪ بروتين و ١٦٪ دهون وأحماض أمينية مهمة، ويمكن حصاد اليرقات وتجفيفها واستخدامها كمصدر للبروتين في العلائق الحيوانية.