AN EXPERIMENT ON THE REUSE OF ARTIFICIAL MEDIUM FOR REARING PINK BOLLWORM, PECTINOPHORA GOSSYPIELLA (SAUNDERS)

by

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การทดลองเลี้ยงหนอนเจาะสมอฝ้ายสืบพันธุ์ Pectinophora gossypiella (Saunders) ด้วยอาหารเทียมที่ใช้แล้ว

An Experiment on the Reuse of Artificial Medium for Rearing Pink Bollworm, Pectinophora gossypiella (Saunders)

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บทคัดย่อ

การเปรียบเทียบการเจริญเติบโตของหนอนเจาะสมอฝ้ายสีชมภู, Pectinophora gossypiella (Saunders) ซึ่งเลี้ยงในอาหารที่ใช้แล้วหรืออาหารที่เหลือจากที่หนอนกินแล้วปราศจากการเพิ่มเติมไวตามินกับอาหารที่ใช้แล้วผสมด้วยไวตามิน 100 เปอร์เซนต์ เช่น โคลีน คลอไรด์ และสารต่อต้านจุลินทรีย์ซึ่งไม่ทนต่อความร้อน (ซูมิคิล-บี,ออร์โอไมซิน และ ฟอร์มัลดีไฮด์)

การประเมินผลอาหารที่ใช้แล้วอีกวิธีหนึ่งคือผสมกับอาหารใหม่ในอัตราส่วนอาหารใหม่ 0,25,50 และ 75 เปอร์เซนต์ เปรียบเทียบกับอาหารใหม่ 100 เปอร์เซนต์

การเจริญเติบโตของหนอนเจาะสมอฝ้ายสีชมภู ซึ่งเลี้ยงด้วยอาหารที่ใช้แล้ว กับอาหารที่ใช้แล้วแต่เพิ่มไวตามินและสารต่อต้านจุลินทรีย์ 100 เปอร์เซนต์ ซึ่งไม่ทนต่อความร้อน ปรากฏว่าไม่มีผลแตกต่างกันนอกจากตัวเต็มวัยที่ออกจากดักแด้ ดักแด้บางตัวซึ่งเลี้ยงด้วยอาหารที่ใช้แล้วตายก่อนที่จะออกเป็นตัวเต็มวัย อาหารที่ใช้แล้วผสมด้วยอาหารใหม่ 75 เปอร์เซนต์ เป็นอาหารที่มีอัตราส่วนการผสมที่ดีที่สุดในการนำอาหารที่ใช้แล้วมาใช้อีก ซึ่งจะมีผลเช่นเดียวกับอาหารใหม่ 100 เปอร์เซนต์ ในการผลิตดักแด้, น้ำหนักดักแด้, การออกเป็นตัวเต็มวัย, อายุของตัวเต็มวัยและการผลิตไข่ของหนอนเจาะสมอฝ้ายสีชมภูตัวเมีย การเพิ่มเติมอาหารใหม่ในอัตราส่วน 0,25 และ 50 เปอร์เซนต์ นั้นให้ผลด้อยกว่าอย่างมีนัยสำคัญในทุกกรณี

ABSTRACT

The development of pink bollworm, Pectinophora gossypiella (Saunders) was compared on rearing on used-diet or the medium remaining after larval use, with no supplements added versus used-diet with 100 percent supplement of vitamins, choline chloride and the heat sensitive antimicrobial agents (Fumidil B, Aureomycin and formaldehyde).

In another evaluation of used diet it was mixed at 0,25,50,and 75 percent with new diet and compared to 100percent new diet.

Used diet with no supplement and used diet with 100 percent supplement of vitamins and heat sensitive antimicrobial agents had no significant effect on the development of the pink bollworm except in adult emergence. Some pupae which developed from unsupplemented used-diet died before moth emergence. Used diet mixed with 75 percent new ingredients was the best rate for reclamation of the used diet and produced the same results in pupal production rate pupal weight, adult emergence, adult longevity and egg production of female pink bollworm when compared to those from diet prepared with 100 percent new ingredients. The other rates supplemented with 0,25 and 50 percent new ingredients were significantly poorer by most criteria.

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1. INTRODUCTION

The pink bollworm, Pectinophora gossypiella (Saunders)[1], is one of the most serious cotton pests in the Southwestern part of the United States. For this reason a sterile release program, The Radiation Control Program, has been established in Arizona. This program has been successful and over a million pupae have been produced per day since 1974. After being irradiated by gamma ray of Co^{60}, the adult pink bollworms have been shipped for release in Southern California.

Since the Radiation Control Program was established, most of the budget has been spent in mass-rearing of the insects, and laboratory rearing costs have continued to increase at alarming rates. Some ingredients have been threatened by world shortages or have become difficult to obtain during critical rearing periods. The combination of these factors warrant an investigation to determine the feasibility of reusing all or parts of the diet that is presently being discarded.

Some of the agar, proteins, carbohydrates, lipids, vitamins and antimicrobial agents are left in the used diet. In addition, about 10 percent of the larvae pupate in the medium without cutting out of the rearing containers to pupate, and thus might supply more protein. The used diet might have adequate essential ingredients for reusing but contamination by various microorganisms, including bacteria, viruses, fungi, and molds, add to the problem. Contamination

of the rearing medium by microorganisms lowers the yields and fecundity of the insects reared on it. If poorer procedures and techniques are executed, diseases can be controlled, otherwise production losses will occur.

To reclaim the used-diet, it may be autoclaved and supplemented with vitamins, choline chloride, and the heat sensitive antimicrobial agents (Fumidil-B, Aureomycin and formaldehyde) which may be lost at high temperatures, or it may be mixed with new diet medium in certain proportion.

The objective of this investigation was to reduce the cost of insect diets. Specifically, investigations were conducted as follows:

1.1 To compare insect production rate from used-diet with no ingredient supplement versus used-diet with 100 percent ingredient supplement of vitamins, and heat sensitive antimicrobial agents.

1.2 To evaluate used-diet supplemented at 0, 25, 50 and 75 percent new ingredients compared to diet prepared with 100 percent new ingredients.

2. METHODS AND MATERIALS

Techniques for rearing these laboratory insects were established in the Standard Operating Procedures of Pink Bollworm, Pectinophora gossypiella (Saunders) Laboratory Rearing Processes of Ridgway et al[2]. In the preparation of pink bollworm rearing medium from new bulk ingredients the amounts were calculated to formulate 3100 ml of total mixture per batch since a 1-gallon capacity commercial waring blender was used. The amount per batch are given in Table 2.1 and Table 2.2

2.1 EXPERIMENT I: To compare insect production rate from used-diet with no supplement versus used-diet with 100 percent supplement of vitamins, choline chloride and heat sensitive antimicrobial agents (Fumidil-B, Aureomycin and formaldehyde).

2.1.1 Used-diet material preparation

The used-diet material in these tests was collected from the half-gallon ice cream cartons used for pink bollworm larval rearing by the Methods Development Rearing Laboratory at Phoenix, Arizona. A separate laboratory for the preparation of diets in these tests was provided. Care was exercised to get used-diet from the same original lot for each test lot.

It was assumed that approximately 50 percent of the used-diet had been consumed. Therefore, then this material was used in diet preparation this fact was considered and twice the original material was prepared. The used-diet was handled in lots of 3100 ml since this was the amount that could be accommodated in a blender, but it was liquified in

a large pot which could be used for two blender lots. For this reason 14 cartons of used-diet were heated for 30 min as water was added to make 6200 ml. After the materials was liquified the pot was placed in an autoclave at 250°F for 20 min. at 15 psi. This amount of used-diet was enough for a replication. A lot of 3100 ml of autoclaved material was blended in a 1-gallon blender for 5 min, then poured into 2 half-gallon ice cream cartons and covered with lids. Another 3100 ml of autoclaved used-diet liquid was blended and to it was added 36 ml choline chloride 10 percent, 15 ml formaldehyde 10 percent, 0.62 gm Fumidil-B, 3.2 gm Aureomycin and finally 12 ml of the vitamin mixture. The temperature of the mixture was measured and vitamins were added at 135 ± 5°F(57 ± 3°c). Above 150°F(65°C) loss of vitamins would occur. The medium was then poured into 2 half-gallon ice cream cartons and covered with lids as before. This diet served as the used-diet, 100 percent ingredient supplement of vitamins and heat sensitive antimicrobial agents.

These diets were left to congeal before they were shredded and infested at approximately 6000 eggs/750gm of shredded diet. The other 4 replications of the experiment followed the same procedere. A total of 180,000 eggs were used in this experiment.

2.2 <u>EXPERIMENT II: To evaluate used-diet supplemented at 0,25,50 and 75 percent with new diet compared to the 100 percent new diet</u>.

Each test replication of used-diet medium was selected and prepared from the same batch of used medium. In this test

there were 5 treatments with 5 replications, with 7 subsamples in each lot. There was a total of 175 subsamples in this experiment.

For the test 35 cartons of used medium from the same batch were poured into 2 pots as previously described. To each pot 350 ml of clean water was added to saturate the diet before it was liquified on the stove as in the previous test. Water was added to bring the final quantity of liquid to 7750 ml. This was heated for 30 min and autoclaved at 250°F for 20 min at 15 psi.

2.2.1 0 percent new diet preparation

Two lots of 3100 ml of the used-diet as prepared above, were blended for 5 min until a homogenous mixture was obtained. This was divided by pouring into 4 half-gallon ice cream cartons. These cartons were covered with lids, and left to congeal before the diet was shredded to yield 14 quarts.

2.2.2 100 percent new diet preparation

The composition of the fresh medium used for rearing pink bollworm larvae was presented in Table 2.1 and Table 2.2. This diet was basically the pink bollworm diet devised by Vanderzant and Reiser [3,4] and later modified by Adkisson et al.[5]; Ouye [6] and Ridgway et al.[2]. The ingredients specified in Table 2.1 made approximately 7 quarts of finished shredded medium.

In preparing a batch of the size indicated in Table 2.1, the agar was dissolved in about 1400 ml of boiling water. The casein was blended in 1000 ml of water and 18 ml of KOH in a 1-gallon capacity blender. Sucrose, wheat germ, alphacel,

Wesson's salts, and methyl paracept were then added and blended into homogeneous mixture. The dissolved agar was added and the mixture was blended for 1 min. The choline chloride, formaldehyde, vitamin, acetic acid, corn oil and dye, Aureomycin and Fumidil-B were added successively with continuous blending. The vitamin suspension had to be agitated well before it was measured and poured into the medium mixture. After blending, the hot mixture was poured into 2 half gallon ice cream cartons and these were covered with lids and allowed to congeal. The second batch of medium followed the same procedure.

2.2.3 25 and 75 percent new diet preparation

Two of the 1-gallon capacity blender lots were used in this preparation. The first batch mixture was used-diet that had been prepared as in 2. (0 percent new diet preparation) and the second batch was fresh medium that had been prepared as in 2.2.2 (100 percent new diet preparation). Twenty-five percent used medium from the first batch was poured into a 1000 ml beaker and to this was added the second batch of fresh medium. After 75 percent used medium was added, the mixtures were blended for 2 min to give a homogeneous condition. It was then poured into 2 half-gallon ice cream cartons and covered with lids to be allowed to congeal.

2.2.4 50 percent new diet preparation

In this mixture the first batch was 0 percent new diet (used medium) as in 2.2.1, and the second batch was 100 percent new diet (fresh medium) as in 2.2.2. The mixture was in two lots, half of the first batch used medium was

poured into a 2000 ml beaker and half the fresh medium from the second batch was added. This was transferred to the blender for a 2 min period. The second lot was then processed in a similar way. Both batches were therefore 50 percent new diet. After blending the hot mixture was poured into 4 half-gallon ice cream cartons and covered with lids and allowed to congeal before it was shredded.

2.2.5 Shredding of media

The third day following medium preparation, shredding was accomplished. The shredded media was put into half-gallon ice cream cartons in the amount of approximately 750 gm/carton. These cartons were then transferred to the egg infesting room. There were 5 replications of this experiment. The carton of shredded diet were infested at approximately 6000 eggs/750 gm shredded diet or per carton. A total of 1,050,000 eggs were used in this experiment. Eggs were cleaned and suspended in agar to infest the media as described by Ridgway et al.[2]

2.2.6 Infesting media with eggs

For infesting, 10 ml of eggs were mixed with 210ml of agar solution which was put in a squeeze bottle to apply 7 ml per half gallon carton of medium to get 6000 eggs per carton after Ridgway et al.[2]. The eggs were spread as uniformly as possible over the medium surface. Lids were placed on the cartons which were then placed on roller racks and transferred immediately to the dark room for holding.

2.2.7 Dark room holding of larval rearing containers

Following the terminology of Ridgway et al.[2], the infested containers were referred to as rearing containers after the eggs hatched, as they contained the larvae during

their "feed out" period. The rearing containers were held at 84±2°F(27±1°C) and 50±10% RH with total darkness for a 9-day incubation and larval development period.

After the 9th day the most advanced larvae began cutting holes in the container to escape and seek pupation sites. Roller racks with 9-day-old rearing containers were transferred to the "cut out area" where Hexcel* (pupation substrate) was placed under the rearing containers.

2.2.8 Larval "cut out" from larval rearing containers

The pupation equipment was prepared by using 2-gallon paper cartons in all replications of Experiment I for comparison of the insect production rate of used-diet with no supplement versus used diet with 100 percent supplement of vitamin and heat sensitive antimicrobial agents; and in Experiment II evaluation of used-diet supplemented at 0,25,50 and 75 percent new diet compared to 100 percent diet prepared with new ingredients for replications I,II and III. However,3-gallon paper cartons were used for replications IV and V because the cartons were taller and prevented the larvae from crawling out and escaping to pupate elsewhere, thereby decreasing the rate of pupal production.

Pupation containers were therefore 2-or 3-gallon capacity cartons of the same diameter open on both ends. The bottoms of the cartons were covered with a 9-inch diameter metal sheet which had been covered with brown paper. Hexcel 8.5-inch diameter was placed in the containers as a pupation

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* A honeycomb-like material (hexagonal cells 3 mm wide,5 mm deep) formed from a polyester film.

site. The diet carton which contained larvae was placed on a wire stand to keep some space between the carton and the Hexcel. Larvae pupated in the Hexcel and connected their cremasters.

The containers as they were placed on the roller racks with mature larvae were maintained in the larval "cut out" area for an 8 day period at 84±2°F, 60±10 percent RH and with lights (similar to moonlight condition). The cartons were moved from the pupation containers after 8 days and the pupation containers were placed in stacks in a storage area off the floor in the larval "cut out" room.

2.2.9 Pupal collection

Pupae were collected from the Hexcel about 48 hrs after the pupations containers were removed from the roller racks. It became necessary at times to remove the pupae from the Hexcel one day earlier or later, depending on factors that might affect pupal maturation rate. Pupae were removed from the Hexcel and transferred to a counting and weighing area with the least machanical abuse possible.

2.2.10 Rate of pupal production

All pupae from each sample were counted on the same day as they were collected, and a percentage of the egg number used in each container were calculated.

2.2.11 Weight/pupa

From each test lot, a sample of 100 pupae were randomly selected and weighed (Mettler balance) to get a record of their average weight at the time of pupation. A total of 3000 and 17,500 pupae were used in Experiment

I and II, respectively.

2.2.12 Rate of adult emergence

From each lot 100 pupae were randomly selected and put in a half gallon with the top covered with 18-mesh plastic screen wire secured by the lid which had the top surface removed. These were maintained in a room for a 10-day period at 82±2°F (27±1°C), 85±5 percent RH. in dim-light to simulate moonlight condition. All pupae from which adults did not emerge were counted after the 10th day.

2.2.13 Rate of egg production

Approximately 400 pupae of each lot were placed in half-gallon cartons and an emply half-gallon carton with 5 holes (2 mm in diameter) in the bottom of each carton. The top carton was covered with 18 mesh plastic screen wire as previously mentioned. These cartons were maintained in a room at 82±2°F (27±1°C), 85±5 percent R.H., in dim-light to simulate moonlight conditions. When the adults emerged, they crawled up into the upper carton through the holes in the bottom of the upper carton. The light was able to pass down through these holes and attracted the adults to crawl upward. Two days after the adults emerged the upper carton was moved to the cold room (40-45°F) to sex the adults. This low temperature was cold enough to anesthetize the adults and made it easier to determine sex without injuring the insects.

The method used for sexing the adults consisted of examination of the tip of the ventral aspect of the abdomen. The males have a characteristic tuft of scales, while the females have a clearly visible bifed ovipositor which lacks

the tuft of scales. From each lot 50 females and 50 males were selected and allowed to mate in a half-gallon carton with 18 mesh plastic screen wire covering the top and bottom. They were maintained in a room at 82 ± 2°F(27 ± 1°C), 85 ± 5 percent R.H. These cartons also served as laying cages.

A plastic screen ring was placed on each cage top in a center position. Paper toweling for the egg ring was cut in the same dimension as the screen and placed over it. Steel weights were attached to the paper rings with clear contact paper. The weight forced the paper partially through the screen mesh, thus the females could deposit their eggs on the paper protruding through the screen. A cotton pad 1-inch square moistened with 7.5 percent sucrose was placed in the center of each cage top to serve as a feeding solution. The feeding pads were serviced daily with move sucrose solution. The egg rings were not collected on the first day of egg deposition; however, they were collected daily for the remainder of the 9-day laying period. Eggs from each lot were weighed daily on a Mettler balance.

A total of 500 female and 500 male adults and 1250 female and 1250 male adults were tested in Experiment I and II, respectively.

2.2.14 Adult longevity

After the last day egg ring collection was made, the adults were 10 days old. The number of live adults from both sexes in each lot were counted and recorded.

TABLE 2.1 Composition of regular medium used for rearing pink bollworms.

Ingredient	Weight or Volume	
Water	2400	ml.
Agar	75	gm.
Locust bean gum[1]	3.78-7.57	gm.
Casein	126	gm.
Sucrose	126	gm.
wheat germ	108	gm.
Alphacel	18	gm.
Wesson's Salts	36	gm.
Methyl paracept	5.5	gm.
Aureomycin	3.2	gm.
Fumidil-B	0.62	gm.
Calco red dye&corn oil(corn oil 1.3 ml)	0.465	gm.
Choline chloride 10%	36	ml.
Formaldehyde 10%	15	ml.
KOH 22.4%	18	ml.
Vitamin mise. (Table 2.2)	12	ml.
Acetic acid 25%	36	ml.

1 Locust beangum was added at 3.78-7.57 gm. per 3100 ml. when the gel strength of the medium dropped to 500 gm/cm^2 for 15 sec. at 1.5 percent agar concentration.

TABLE 2.2 Vitamin mixture

Ingredient		Weight (gm.)
Calcium pantothenate		9.4
Nicotine acid amide		4.8
Riboflavin		2.4
Folic acid		2.4
Thiamine hydrochloride		1.2
Pyridoxine hydrochloride		1.2
Biotin		0.096
B-12		0.0048
Water	to	400 ml.

3. RESULTS AND DISCUSSION

3.1 Rate of pupal production from the Used-Diet supplement Test.

The percentage of pupal production data of pink bollworm larvae developing from used-diet with no supplement versus used-diet with 100 percent supplement of vitamins, choline chloride and heat sensitive antimicrobial agents (Fumidil-B, Aureomycin and formaldehyde) are presented in Table 3.1.1. In an F-test of the data, there was no significant difference in the production rate of pupae. There was, however, a low production rate in each treatment when compared to that of a used-diet supplemented at 0 percent new ingredients in another test, as shown in Table 3.5.1. Thus, it is possible to rear pink bollworm with used-diet with no supplement or with 100 percent supplement of vitamins and heat sensitive antimicrobial agents, but the yield may be affected. Many factors cause low production, such as microbial contamination, high concentration of antimicrobial agents, unsuitable physical texture of medium for feeding, cannibalism because of nutrient deficiency, and perhaps heat stable microbial endotoxins. Some subsamples of used-diet with no supplement were contaminated by the bacterium Bacillus thuringiensis (BT), and perhaps others, virus cytoplasmic polyhedrosis virus (CPV), fungi and molds. It was found in some subsamples that there were larvae with small bodies and large heads. This condition agreed with Moddox[7]. Some infested eggs were perhaps contaminated in egg collection and cleanup as described by Ridgway et al[2].

If proper rearing techniques are followed, diseases can usually be controlled.

In this test it was found that some larvae still remained in the Hexcel when the pupae were collected from the used diet with 100 percent supplement. The low pupal production found in this treatment, particularly in some subsamples is reflected in Table 3.1.1. Antimicrobial agents may inhibit larval development and prolong the larval stage, particularly when there was high concentration according to Cuye[6] and Singh and House[8].

Most of the used-diet could be described as muddy or sticky and the gel characteristics were decreased, which might cause the physical texture of the medium to be unsuitable for larval feeding, as shown by House[9]. Although the used-diet in this test had reduced nutritive concentration, it should not have lacked feeding stimulant because some of the wheat germ was still present. When the physical requirements for feeding were not suitable and the diet was deficient in some nutrients, cannibalism was more likely to occur as suggested by Vanderzant[10].

The exact amount of various vitamins needed by different species of insects may differ greatly. This depends in part on the kind of food needed by the insect. B-vitamin is used as a coenzyme in the metabolism of protein, carbohydrates and fat, and the amount of vitamins needed may depend upon the amount of these nutrients in the diet that is metabolized. Used-diet with no supplement may have had enough

vitamins and choline chloride for supporting growth after being autoclaved since these materials do not suffer loss, as found by Vanderzant[12]. Therefore, the used-diet with 100 percent supplement of vitamins, choline chloride, and antimicrobial agents may have had a high vitamin concentration, but the percent pupal production (Table 3.1.1) and pupal weight (Table 3.1.2) were the same as those developing from used-diet with no supplement. Thus excess vitamins and choline chloride had no effect on pupal production, which agreed with Vanderzant and Reiser[4].

3.2 Pupal weight from used-diet supplement test

An F-test of the data from this test (shown in Table 3.1.2) revealed there was no significant difference in pupal weights. Excess vitamins and choline chloride therefore had no effect on the pupal weight of this insect as mentioned in previous discussion. The pupal weight of pink bollworm larvae developing from used-diet with no supplement is very close to that of larvae developing from diet prepared with 100 percent new diet ingredients (Table 3.5.2). This test showed that used-diet with no supplement can support normal growth, whereas pupal weight of pink bollworm larvae developing from used-diet with 100 percent supplement of vitamins, choline chloride and antimicrobial agents was a little less because of the high concentration of antimicrobial agents as shown by Ouye[6].

3.3 Rate of adult emergence

An F-test of the data (Table 3.3.1) revealed adult emergence was significantly lower than the used-diet with no supplement. This may have resulted from the loss of antimicro-

bial agents since some dead pupae from the treatment of used-diet with no supplement were found after 10 days, whereas in another treatment of used-diet with 100 percent supplement of vitamins and antimicrobial agents, pupae were normal (Table 3.3.1). when compared to that developing from diet prepared with 100 percent new ingredients (Table 3.6.1). Therefore, a high concentration of antimicrobial agents can inhibit diseases, but does not inhibit adult emergence.

3.4 Rate of egg production

An F-test of the data in Table 3.4.1 revealed that there was no significant difference in the number of eggs per female pink bollworm from used-diet with or without supplement. The high concentration of vitamins and antimicrobial agents therefore had no significant effect on egg production. The number of eggs was normal when compared to those developing from diet prepared with 100 percent new ingredients (Table 3.7.1)

3.5 Used and new diet mixture test

In the test for percent pupal production of pink bollworm larvae developing from used-diet supplemented at 0,25,50 and 75 percent new ingredients compared to diet prepared with 100 percent new ingredients, the results are shown in Table 3.5.1. An F-test of the data revealed that there was a significant difference in the pupal production rate of pink bollworm when compared at the 5 percent level of confidence.

There was no significant difference between the mean percent pupal production of pink bollworm larvae

developing from used-diet mixed at 75 percent new ingredients and diet prepared with 100 percent new ingredient; however, there was a significant difference in those developing from used-diet supplemented with 50,25, and 0 percent new ingredients, respectively.

The mean percent pupal production of pink bollworm larvae developing from used-diet supplemented at 50 and 25 percent new ingredients were not significantly different from each other, but they were significantly different from that supplemented at 0 percent new ingredients. The mean percent pupal production developing from used-diet supplemented at 25 and 0 percent new ingredients were not significantly different from each other.

It is possible, therefore, to rear pink bollworm larvae with used-diet supplemented at 75 percent new ingredients and expect about the same yield of pupal production when compared to those reared using diet prepared with 100 percent new ingredients (Table 3.5.1). Both had enough nutrients to support growth, whereas that supplemented at 50,25 and 0 percent diet were significantly poorer, as indicated by their lower pupal yields. Many factors may have caused poorer pupal yields (i.e., too little nutrients to allow proper growth, low feeding stimulant, unsuitable physical texture of diet, and perhaps microbial endotoxins, cannibalism, contamination as previously discussed in section 3.1)

Contamination by disease is one of the important factors that should be considered, but fortunately in this

test there was very low contamination. The protein-carbohydrates-fat ratios in the medium may be very important for good growth. With reference to the 10 percent pupae that were left in used-diet, Roberson[13] indicates that this may have resulted in more protein in the used-diet, thus making the ratio of protein-carbohydrate-fat in used-diet different from that of fresh diet. Fresh diet has the optimum ratio of protein-carbohydrate-fat for normal growth, therefore, the pupal production rate of pink bollworm larvae developing from fresh diet is greater than that developing from used-diet (Table 3.5.1). This finding agreed with Vanderzant and Reiser.[4]

The proper balance of indispensable nutrients in the medium is very important for optimum growth. Insects need amino acids, carbohydrates, lipids, salt minerals and vitamins in a suitable ratio. In the medium in this study amino acids were from casein and wheat germ, the carbohydrates from sucrose and wheat germ, the lipids from wheat germ and corn oil, the salt minerals were supplied from casein, Wessen's salt, and wheat germ. The vitamins were from wheat germ and the pure vitamin mixture, and the antimicrobial agents were from methyl parasept formaldehyde, Aureomycin and Fumidil-B, as shown in Table 2.1 and Table 2.2.

The ratio of the necessary ingredients were in a suitable proportion for optimum growth of pink bollworm in the original medium as described by Vanderzant and Reiser[4]. Vanderzant[14,15]; Ouye and Vanderzant[16]; and Ridgway et al.[12]. The diet prepared with 100 percent new ingredients and the used-diet mixed with 75 percent new ingredients

performed nearly equally (Table 3.5.1). The balance of indispensable nutrients in both of them must have been near optimum for larval growth; while the 0,25,and 50 percent new ingredients diets may have been deficient.

Fat plays an important role in the pupation of the pink bollworm larvae. The ratio of fat in the diet prepared with 100 percent new ingredients (Table 2.1) and that in used-diet mixed at 75 percent new ingredients allowed near optimum larval development, while the 0,25, and 50 percent supplemented diets performed significantly poorer as is indicated in pupal production.

In Table 3.5.1 the percent total pupal production in replications I,II and III were lower than in replications IV and V because the pupation containers used in replication IV and V were taller than those in replications I,II and III and thus prevented larvae from crawling out to pupate outside where they were lost from the test.

In the test for pupal weights of pink bollworm developed from used-diet supplemented at 0,25,50 and 75 percent new ingredients compared to diet prepared with 100 percent new ingredients, results are presented in Table 3.5.2. In an F-test of the data, there was a significant difference in the mean pupal weights of the pink bollworm at the 5 percent level. The 0 percent new ingredients differed from all other means and was the lowest weight. The means of the 50,75 and 100percent ingredients did not differ significantly at the higher weights of pupae.

Methods of growth evaluation such as determination

of the larval instar at any particular time and width of the head capsule are not satisfactory for the pink bollworm because the larvae usually feed inside the medium where they cannot be seen.

After feeding, the larvae leave the rearing containers to pupate outside, thus this is a good stage of compare. Another method used for comparison of the media was the determination of weight of 2-or 3-day-old pupae as reported by Vanderzant and Reiser[4].

According to the discussion of the optimum growth rate of pink bollworm from Table 3.5.2, the average of pupal weight developing from used-diet with 75 percent new ingredients and new diet of 100 percent new ingredients were normal when compared to pupal weights reported by Vanderzant[14].

The higher pupal weight shows in Table 3.5.2 for those insects reared on 50 percent new diet could possibly be explained by cannibalism. In fact the variability between replications suggests that this might have been the case. It has been stated earlier that poor physical condition of higher percentages of used-diet may encourage cannibalism and this would possibly be reflected in higher pupal weights of the survivors.

3.6 Rate of adult emergence

In Table 3.6.1 the percent[illegible] adult emergence from the larvae reared on 0 percent new ingredients was significantly lower than those with all new diet ingredients. There was no significant difference in adult emergence at the 50, 75, and 100 percent new diet ingredients. It is therefore

evident that there was an adequate amount of lipids for promoting adult emergence in the used-diet supplemented at 50 and 75 percent new ingredients.

3.7 Rate of egg production

In the F-test of the data in Table 3.7.1 from the experiment concerning the number of eggs produced per female (fecundity), there was no significant difference at the 5 percent level.

The average number of eggs per female in all media was over 100, whereas 72 eggs per female were obtained from larvae reared on purified casein medium by Vanderzant and Reiser[4] and 98 eggs per female from moths reared on squares by Adkisson[17]. It is possible that larger moths were selected in this test in the sexing procedure and this may have resulted in a bias toward larger numbers of eggs as reported here.

3.8 Adult longevity

In an F-test of the data in Table 3.8.1, there was a significant difference in adult longevity of pink bollworm adults at the 5 percent level. With 100 percent new ingredients, survival was more than twice that of the 0 percent new ingredient test . The means of 0,50,75 and 100 percent new ingredients followed a trend that is perhaps possible to explain. The higher percentage of survival at 25 percent new ingredients cannot be explained. Temperature, moisture and food are important factors in adult longevity. In this test these factors were controlled at near optimum and the adults were fed 7.5 percent sucrose solution.

TABLE 3.1.1 Percent pupal production of pink bollworm from larvae developing on used-diet with no supplement versus used-diet with 100% supplement of vitamins, choline chloride and heat sensitive antimicrobial agents (Fumidil-B, Aureomycin, and formaldehyde).

Vitamins and antimicrobial agents supplemented (%)	Pupal production[1](%) Replication I	II	III	IV	V	Mean(%)	F[2]
0	6.84	7.46	4.92	5.69	8.92	6.77	6.73 ns
100	5.62	4.75[3]	3.45	4.89	2.96	4.33	

1 All data were averages from 3 subsamples with 6000 eggs infested on medium in 2 gallon pupation containess.

2 ns = no significant difference at the 5% level.

3 Data were averaged from 2 subsamples.

TABLE 3.1.2 Pupal weight (mg) of pink bollworm from larvae developing on used-diet with no supplement versus used-diet with 100 percent supplement of vitamins, choline chloride and heat sensitive antimicrobial agents (Fumidil-B, Aureomycin and formaldehyde).

Vitamins and antimicrobial agent supplemented (%)	Pupal weight[1] (mg) Replication					Mean (mg)	F^2
	I	II	III	IV	V		
0	17.15	16.92	14.13	15.76	17.25	16.24	7.69 ns
100	14.68	14.34[3]	14.37	14.27	12.68	14.068	

1 Data were averaged from 3 subsamples with 100 pupae in each.

2 NS = No significant difference at the 5 % level.

3 Data were averaged from 2 subsamples.

TABLE 3.3.1 Percent adult emergence of pink bollworm from larvae developing on used-diet with no supplement versus used-diet with 100% supplement of vitamins, choline chloride, and heat sensitive antimicrobial agents (Fumidil-B, Aureomycin and formaldehyde).

Vitamins and antimicrobial agents supplemented (%)	Adult emergence[1] (%) Replication I	II	III	IV	V	Mean[2] (%)
0	84	89	79	87	96	87[b]
100	97	98	99	98	95	97.4[a]

1 All data were averaged from 100 pupae

2 Means not followed by the same letter differ significantly at the 5 percent level of probability as determined by Duncan's New Multiply Range Test.

TABLE 3.4.1 Number of eggs per female pink bollworm where larvae developed on used-diet with no supplement versus used-diet with 100 percent supplement of vitamins, choline chloride and heat sensitive antimicrobial agents (Fumidil-B, Aureomycin and formaldehyde).

Vitamins and antimicrobial agent supplemented (%)	Number of eggs/female[1] Replication					Mean No. eggs/female	
	I	II	III	IV	V		F[2]
0	97	81	121	104	123	105.2	5.22NS
100	119	113	123	115	124	118.8	

1 All data were averaged from 8-day egg collections with 50 females.

2 NS = No significant difference at the 5 percent level.

TABLE 3.5.1 Percent pupal production of pink bollworm from larvae developing on used-diet mixed at 0,25,50 and 75 percent new ingredients compared to a diet prepared with 100 percent new ingredients.

New ingredients (%)	Pupal production[1](%) Replication I	II	III	IV	V	Mean[2](%)
0	5.53	10.65	10.40	12.05	10.82	9.89 c
25	7.79	13.19	11.54	13.65	18.20	12.87bc
50	10.38	14.81	12.77	16.96	19.81	14.95 b
75	11.19	17.59	18.24	30.76	26.05	20.77 a
100	14.67	17.98	19.33	29.92	27.68	21.92 a
Total	49.56	74.22	72.28	103.34	102.56	

1 All data were averaged from 7 subsamples with 6171 eggs infested in media for replications I,II,III and 6000 eggs for replications IV and V, respectively.
The 2 gallon pupation containers were used for replications I,II,III and the 3 gallon pupation containers for replications IV and V, respectively, which accounted for some better yields.

2 Mean not followed by the same letter differ significantly at the 5 percent level of probability as determined by Duncan's New Multiple Range Test.

TABLE 3.5.2 Pupal weights (mg) of pink bollworm from larvae developed on used-diet mixed with 0,25,50 and 75 percent new ingredients compared to a diet prepared with 100 percent new ingredients.

New ingredients (%)	Pupal weight (mg)[1] Replication I	II	III	IV	V	Mean[2] (mg)
0	15.68	15.41	15.44	15.70	15.37	15.52 c
25	16.24	16.25	16.08	16.30	16.30	16.23 b
50	17.10	17.12	16.35	17.08	16.41	16.81 a
75	15.45	16.49	16.81	16.81	16.85	16.48 ab
100	16.50	15.76	16.24	16.68	16.34	16.30 ab

1 All data are means of 7 subsamples with 100 pupae from each.

2 Means not followed by the same letter differ significantly at the 5 percent level of probability as determined by Duncan's New Multiple Range Test.

TABLE 3.6.1 Adult emergence of pink bollworm developing from larvae developed on used-diet supplemented at 0,25,50 and 75 percent new ingredients compared to diet prepared with 100 percent new ingredients.

New ingredients (%)	Adult emergence[1](%) Replication I	II	III	IV	V	Mean[2]
0	89.86	90.57	91.14	89.57	93.14	90.86 c
25	95.14	95.71	91.14	95.29	95.86	94.63 b
50	97.00	95.86	93.14	95.29	95.14	95.29 ab
75	96.00	95.29	93.71	96.00	95.43	95.29 ab
100	96.00	97.00	96.86	96.29	97.29	96.69 a

1 All data were averaged from 7 subsamples with 100 pupae for each.

2 Means not followed by the same letter differ significantly at the 5 percent level of probability as determined by Duncan's New Multiple Range Test.

TABLE 3.7.1 Number of eggs per female pink bollworm developing from larvae on used-diet supplemented at 0, 25,50 and 75 percent new ingredients compared to diet prepared with 100 percent new ingredients.

New ingredient	Number of eggs per female[1] Replication					Mean no. of eggs/female	
(%)	I	II	III	IV	V		F[2]
0	195	171	81	91	139	135.4	2.31NS
25	171	169	146	120	97	140.6	
50	185	178	114	107	81	133.0	
75	131	80.9	107	88	85	98.4	
100	171	108	121	88	118	121.2	

1 All data were averaged from 8 day egg collection with 50 females

2 NS = No significant difference.

TABLE 3.8.1 Adult longevity as indicated by percentage of adults alive 10 days after emergence of pink bollworm adults developed from larvae developed on used-diet mixed at 0,25,50 and 75 percent new ingredients compared to diet prepared with 100 percent new ingredients.

New ingredients (%)	Adult alive[1](%) Replication I	II	III	IV	V	Mean[2] (%)
0	49	30	25	62	35	40.20 b
25	75	70	73	84	78	76.00 a
50	40	45	38	34	48	51.00 b
75	79	70	63	66	80	71.60 a
100	81	83	86	84	84	83.60 a

1 All data received from 100 adults (50 females,50 males) for each.

2 Means not followed by the same letter differ significantly at the 5 percent level of probability as determined by Duncan's New Multiple Range Test.

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