# ARTIFICIAL DIETS OF INSECT

Artificial diets were first developed for insects that could easily be maintained in the laboratory on natural food. The insets' food was replaced with substances of known nutrient quality as the nutrients for animals were discovered. Then, diets of known com position, designated as defined diets, were used to determine the nutrient requirements of insects. Practical diets could be mad e by combining unrefined materials with known nutrient supplements. Diets are now widely used for laboratory rearing and many are being used successfully for the mass production of insects. In recent years, many species of plant-feeding insects have been reared, and diets for several parasites and predators also have been used successfully. Some attempts have been made to duplicate the natural food of insects, but most diets have been composed of foods known to have a high nutrient content.

The term artificial diet, when applied to insects is one that has been defined as any diet that is not the natural food of the insect. It includes all the various terms such as synthetic, chemically defined, holidic, meridic and oligidic.

A nutritionally complete diet for most insects in axenic culture must contain all or most of the proteins or amino acids including ten essential ones, carbohydrates, fatty acids, cholesterol, choline, inositol, pantothenic acid, nicotinamide, thiamin, riboflavin, folic acid, pyridoxine, biotin, vitamin B12, beta carotene or vitamin A, alpha tocopherol ascorbic acid, several minerals and water.

When insect is reared on natural food in the laboratory for its mass production, problems like high rate of mortality and requirement of regular fresh food material usually occur in the laboratory. Therefore, the use of artificial diet makes it convenient to rear insect on it to ensure the continous supply of large number of immature stages for various research studies. In the literature, various insect rearing programs have been reported for various purposes like development of insect resistant strains of plants, bioassay of the effectiveness of insecticides, production of food for those entomophagous insects for which there are yet no adequate artificial diets, study of nutritional requirements, mass field releases of predators and parasites of insect pest, growing pathogens such as viruses, evaluation of nutritional quality of cereals inexpensively, source of natural products such as endocrines or pheromones, a continous source of specimens for basic research in morphology, physiology, biochemistry.

**Uses of artificial diets**

- Diets are used for studying insect nutrition
- Used for studying biochemistry, behavior and other biological process
- Testing of compound for physiological effect
- Maintaining colonies
- Mass production of insects for various purpose

**Classification of insect artificial diets**

**1. Holidic diet:**

Diets in which the ingredients can be represented by chemical formulae are known as chemically defined diets or holidic diets.

These diets are used primarily for nutritional studies. Most nutritional studies have been conducted with diets containing one or more of the following ingredients such as agar, protein (casen), vegetable oil, starch and cellulose. With appropriate descriptions, these dets could be designated as defined diets.

**2. Meridic diet:**

Diets which contain one or more undefined substances from plant, animals or micro organisms such as plant tissue, liver extract, and yeast products.

The main characteristics of these diets is that most of the nutrients are provided as pure or refined substances also. The large number of diets for the laboratory rearing of insects is included in this group.

**3. Oligidic diet:**

These diets are made up of crude material. They are designated to imitate the natural food and are assumed to have all the required nutrients with undigestible inert material. These diets are economical and are used of mass rearing of insects.

DEVELOPMENT OF ARTIFICIAL DIETS AND DETERMINATION OF NUTRITIONAL REQUIREMENTS

Artificial diets and the nutrition of insects have been studied over the last 60 years. The introduction of aseptic cultures made it possible to determine the nutrient requirements of insects without the interference of microorganisms. Most of the nutrients for some flies were provided by microorganisms. The fact that insects ate the food that man raised for himself and his animals clearly indicated that insects used some of the same nutrients, undoubtedly the protein, carbohydrate, and fat. The analyses of insects and food confirmed this. In fact, research on insects was based on knowledge of vertebrate physiology and nutrition. Many investigators believed that the major nutrients were the same for insects as for mammals. The differences in physiology between insects and higher animals was so great that the vitamins unique for insects should be sought in their food. Unfortunately, at that time all the vitamins had not been discovered; hence the use of diets composed entirely of known constituents was not possible. But by 1935, Hoskins & Craig stated: "It has been shown in recent investigations that insects resemble mammals in having definite needs for the various kinds of foodstuffs, including minerals and vitamins." Lipid soluble substances also played a prominent role in the search for growth factors. Cholesterol was discovered to be the substance that promoted growth in a blowfly larva on a diet containing ether-extracted peptone and aqueous yeast extract . To this day the requirement of insects for dietary sterols is the only proved unique difference between insect and mammalian nutrient requirements. Insects cannot synthesize the steroid ring; some, however. can modify the side chains. Fats were known to be metabolized and stored for energy, and if not present in the diet, they were synthesized from other foods. But in 1946, Fraenkel & Blewett discovered that certain insects needed polyunsaturated fatty acids. Moths had defects in wing formation and their scales adhered to the pupal cases upon emergence. The defects were comparable to the scaly skin and dermatitis of the rat deprived of polyunsaturated fatty acids. The discovery of sterols and polyunsaturated fatty acids as growth factors was delayed because of the extreme sensitivity of insects to small amounts of lipids. Even traces of fat, sterol, or other lipids in a diet may affect growth and development and invalidate tests with added lipid. Removing lipids completely from natural materials is very difficult. For example, Levinson & Bergmann found it necessary to extract wheat bran at least 20 times with 3 times its weight of chloroform in a Soxhlet apparatus to remove the lipids completely before the bran could be used in diets for the testing of steroids. The work of Fraenkel & Blewett on the nutrition of insects that fed on dry materials formed the basis of much of the nutritional research conducted later. Although the nutrients were not chemically defined and the diets not aseptically prepared, the nutrients were refined sufficiently to permit evaluation of their nutritional contribution, and the diets were dry enough, about 15% moisture, to prevent the growth of all microorganisms except the symbionts present in the insects. The first diets consisted of refined casein, starch or glucose, cholesterol, salts, yeast, and water. The yeast was replaced with B vitamins as they were discovered, and polyunsaturated fatty acids were added. In 1946, a chemically defined diet for Drosophila melanogaster was reported. In this diet, amino acids replaced casein hydrolysate, and biotin, folic acid, and nucleic acid replaced yeast extract. Although growth was better with the protein hydrolysate and yeast extract, the defined diet furnished a starting point for the investigation of unknown factors. Ascorbic acid or vitamin C was another substance that was not recognized as an insect nutrient for many years. In fact, insects of stored products, flies, and roaches grew on diets lacking it; and although widespread in insect tissues, ascorbic acid was believed to be synthesized or to be a fortuitous component from food that was eaten. The first attempts to rear a phytophagous insect, the European corn borer *Ostrinia nubilalis*, on artificial diets were complicated by the necessity of adding a source of an unknown growth factor now known to be ascorbic acid. As nutritional studies on plant-feeding insects increased, ascorbic acid was found to be a feeding stimulant and eventually an essential nutrient for the growth of locusts. Only insects that grew on green growing plants appeared to need i t, but not all such insects did because the tissues of the pink bollworm *Pectinophora gossypiella* contained the vitamin even when the insect was reared on a diet lacking it. The needs for vitamin A or its precursor .a-carotene and vitamin E were not shown until recently. In fact, a very striking effect on the growth of *Plodia interpunctella* was produced by dietary ,a-carotene. Previous reports of growth effects due to fat-soluble vitamins have not been as convincing. The vitamins are effective in such small quantities that very carefully refined lipids sometimes contain them as contaminants. Furthermore, vitamins may be carried from one generation to the next through the egg so that a requirement is not detected unless several generations are reared on the vitamin-free diet. Other growth factors specific for certain groups of insects have been disclosed in dietary studies. Nucleic acids and their components stimulate growth in many Diptera and in several beetles. Carnitine is needed for the growth of tenebrionid beetles. Recently, Neville & Luckey reported that bioflavonoids are growth factors for the cricket *Acheta domesticus.* Rutin was the most active of the compounds tested in a diet containing all the known nu trients for the cricket. However, better growth was obtained with grass which suggested that there was still some o ther factor needed. Plant extracts were required in the diets of the grasshoppers *Melanoplus diiferentialis, M sanguinipes* and *M femurrubrum*. The test diet contained known nutrients except that brewers' yeast was used as a vitamin source. It would be interesting to know if there is any relationship to the fac tors required by the cricket and if the growth factor(s) is similar to the one previously reported for grasshoppers. In testing substances in diets for their growth effects, it is usually assumed that the test die t is nutritional1y complete with respect to the known nutrients for insects. But is it? Perhaps the nutrients are not properly balanced, or a nutrient is present in such a low quantity that the synthesis of another compound is prevented. The unknown growth substance may be that compound. A nutritional1y complete diet for most insects in axenic culture must contain all or most of the following: protein or amino acids including ten essential ones, carbohydra te, fatty acids, cholesterol, choline, inositol, panto thenic acid, nico tinamide, thiamin, riboflavin, folic acid, pyridoxine, biotin, vitamin B'2' /3-carotene or Vitamin A, a-tocopherol, ascorbic acid, several minerals, and water. Sometimes compounds of related structure can substitute for the nutrients listed or they may be needed in addition to those nutrients. An unusual requirement was discovered for the beetle, *Xyleborus ferrugineus*. It could utilize cholesterol or lanosterol for growth and egg production, but it could not pupate unless it also was fed ergosterol or 7-dehydrocholesterol provided by a fungus. Since sterols cannot be synthesized by insects, dietary requirements for sterols often are closely correlated with the sterols present in the natural food material.

## DEVELOPMENT OF PRACTICAL DIETS

After the nutrients for animals were described, diets for many insects could be made simply by combining substances of known nutritional value supplemented with substances such as yeasts and cereal grains found in the natural foods of the insects being studied. However, when the investigation of insects that fed on green plants was started, it became necessary to add host plant material in some cases to satisfy the unknown nutrient or chemical feeding stimulant. In many instances, insects are restricted to a single plant or a family of plants because of the presence of a chemical in the plant which regulates the behavior of the insect. Therefore, in nutritional studies it was necessary to separate the nutrients in the plant from the chemical feeding factors.

The rearing of plant-feeding insects began with the work of Beck and associates and many of the first diets for other insects were based on the diet of the European corn borer. Hence, in addition to defined nutrients, they also included plant extracts of powders usually from the host plant to supply either an unidentified nutrient or a chemical feeding stimulant.

Earle and co-workers believed that the boll weevil *Anthonomus grandis* reared in the laboratory would be more like the native weevil if it were fed a natural protein. Therefore, they prepared an acetone powder from cotton buds to which they added back the salts, vitamins, sterol, and other small molecules that were extracted and incorporated them along with other nutrients into an agar base. Subsequently, they analyzed flower buds for protein amino acids and free amino acids and used the information to prepare a mixture of bud protein, soybean protein, and free amino acids that simulated the content of fresh buds. Larvae reared from this diet were larger than those from other less refined diets and adults laid almost as many eggs in diet as in fresh flower buds. The procedures used in developing the diet were tedious and time consuming. Unfortunately, this kind of approach to devising diets has not been used for many insects. Host plant materials have usually been added without prior treatment to remove anything except water. Thus changes or losses in the nutrient content occur only during blanching, drying, grinding, or cooking and may not be significant if care is taken in the preparation. Moore et al used cotton leaf powder dried either· in the sun or in an oven at 60-70°C in the diet of the boll weevil. The major protein in their diet was egg albumin, however. The leaf powder was mainly used as an oviposition stimulant. A diet in which the major ingredient was cottonseed meal was used to feed both larvae and adults of the boll weevil. A diet containing germinated cottonseed which was blanched to stop enzyme action was used in laboratory rearing and mass rearing of this insect.

In many of the diets in which host plant material was included, the plant material often comprised an important part, but not the major part, of the total amount of nutritive substances. However, there have been several attempts to duplicate natural food by combining fresh ground plant material with water and thickening agents. Most have failed. In recent research with insects that feed within trees, an environment that is difficult to duplicate, diets in which natural food was the major constituent of the diet were moderately effective for rearing.

## MASS PRODUCTION OF INSECTS

The mass production of insects on artificial diets during the last decade has greatly accelerated research on control of pests. Insects have been available for the extraction and identification of pheromones, the release of irradiated insects, the production of viruses, parasites, and predators, and many other studies of their biology and biochemistry. The difficulties of rearing on natural food include physical feeding requirements and environmental conditions and are not always the result of a lack of information on nutritional requirements. Domestic insects such as house flies and cockroaches have been reared on artificial diets for many years and have been the subjects of many nutritional studies. Similarly, insects infesting cereals and other stored products are relatively easy to rear because of their biology and their ability to use dry food. A recent advance was made in the rearing of the Angoumois grain moth *Sitotroga cerealella*, an internal seed feeder which has not been reared because it would not accept powdered mixtures. Chippendale found that larvae would accept ground natural products when they were packed into gelatin capsules, the larvae being introduced into a depression in the diet. Subsequently, the diet was packed into holes in a template to form homogeneous pellets. This procedure would be more suitable for mass rearing. Several diets composed of refined and natural products were tested. The best development of the insect was obtained with a diet composed of corn starch, glycerol, case in, brewers' yeast, and wheat germ. If the amount of wheat germ was increased the brewers' yeast could be omitted. A substitute for grain would make sanitation easier and a uniform diet would produce a more uniform insect.

The screwworm *Cochliomyia hominiyorax*, a parasite of warm-blooded animals, has been reared in vitro on a diet consisting of meat products with suitable extenders. The mass production of this insect for the release of irradiated flies, overwhelming the natural population with sexually sterile males to compete with native females, is already well known. Since meat products are expensive and at times not readily available because of their use in animal foods, a replacement was devised that contained dried whole chicken egg, dried bovine blood, and dried calf suckle (a replacement for milk fed to young calves). After 24 hr the dried egg was omitted, and sucrose added. Later, the amount of sucrose was increased, and dried cheese was added.

Early diets for tephritid fruit flies contained fresh carrots as a major ingredient supplemented with brewers' yeast and inhibitors against mold and bacterial growth. Dry powdered squash, carrots, and other vegetables and fruits were also used, and bran was used in some diets. Dehydrated vegetables and brewers' yeast provided all the nutrients needed for *Ceratitis capitata, Dacus dorsalis*, and *Dacus cucurbitae* . Since carrots and brewers' yeast were expensive, they were replaced successfully with wheat shorts, wheat middlings, and torula yeast, thus reducing the cost by 50%. Schroeder et al used combinations of cottonseed, soybean, and whey protein in place of wheat shorts and middlings supplemented with torula yeast for the three insects. In Costa Rica, Peleg & Rhode substituted wheat germ and bagasse for the carrot powder and used torula yeast instead of brewers' yeast for *C capitata. D. cucurbitae* was successfully reared in India on a thick paste made by boiling powdered rice and water. The olive fruit fly Dacus oleae did not grow on diets used for other fruit flies. Diets for its successful rearing were much more complex and contained brewers' yeast, soy hydrolysate, roasted peanuts, sucrose, olive oil, water, thickening agents, and preservatives. Thirty-five generations were reared on three artificial diets with 80% yields. A simpler diet contained brewers' yeast, chickpea seedlings, and olive oil as the only nutritive substances in the diet, but results depended on the brand of yeast used. Plant-feeding insects, unlike the flies already discussed, do not thrive in the presence of microorganisms and must be provided with clean fresh food to avoid loss of water and ascorbic acid. Environmental control is much more difficult in mass production than in laboratory culture. Of the insects discussed in the book on mass production, only the European corn borer, the pink bollworm, the boll weevil, the cabbage looper, the tobacco horn worm *Manduca sexta*, and *Heliothis* species were reported as being reared with any degree of success on artificial diets. But many more have since been reared on diets that could probably be used for mass production. A recent diet developed for the codling moth which has heretofore been reared on apples included ether-extracted soy meal, wheat germ, apple pomace, wheat starch, sugar, salts, vitamins, preservatives, cellulose, and water. The diet contained no agar and used soy meal instead of the more expensive casein. Other diets also for this insect had a wheat germ base. Navon & Moore used a diet containing autoclaved soy germs, casein, sucrose, torula yeast, ascorbic acid, and water as the principal nutrients. A diet which was quite different from those previously used for *Heliothis zea* was devised by Burton. The principal ingredient was a child food supplement (CSM) composed of cornmeal, toasted defatted soy flour, nonfat dry milk, soy oil, vitamins, and minerals to which was added water, torula yeast, ascorbic acid, preservatives, and agar. Growth of *H zea* was as good on this diet as on previous diets, but some other in sects would not eat it. A wheat soy blend (WSB) consisting of wheat fractions, soy flour, soybean oil, minerals, and vitam ins was substituted for the CSM in the previous diet. The latter diet was accepted readily by *Heliothis* species, and the fall armyworm *Spodoptera frugiperda* developed as well on it as on the bean diet that had been used in the rearing program for several years. Changes were made in the d iet used for mass-r earing the European corn borer. A diet with a wheat germ base was substituted for the diet containing the corn leaf material. Development of the insects reared on the diet with wheat germ for 32 generations was not significantly different from that of insects reared on diet with corn leaf. Raulston modified the diet for the pink bollworm by substituting cottonseed for the casein, omitting the minerals, and cutting in half the amount of vitamins. The cost was 25% less than that of the original pink bollworm diet. Although fluid larval media have worked quite w ell for r earing large numbers of flies which can survive in contact with the diet if a suitable solid support is provided for them, in sects such as aphids that obtain food through membranes have not been reared in large numbers in spite of the success that has been attained in laboratory studies of their nutrition. An insect which sucks fluid from insect eggs and larvae, the predator *Chrysopa carnea* was successfully reared on a diet containing protein hydrolysates. sugar, vitamin s, minerals, cholesterol, vegetable oil, and water. In a later modification, casein and yeast hydrolysate were added, and the mixture was sterilized by autoclaving. The sterile fluid mixture was encapsulated with a waxy plastic coating to prepare artificial insect eggs". These eggs are now being used for the mass production of the insect for field release studies of biological control of crop pests.