ABSTRACT

Aflatoxins are potent carcinogens which have serious biological activities even when present at low concentrations, thus are capable of producing health problems in humans and animals. Their occurrence in food should be regarded as dangerous because of their capability to produce toxicity and carcinogenicity in addition to teratogenic and mutagenic effects.

Contamination of baby food should be of particular concern, especially in a rich country like the United Arab Emirates (UAE), where different varieties of baby food are available and accessible to all infants and children. In addition to that, it has been reported that the young have a greater sensitivity to aflatoxins than the old. Because of this, this study considered some kinds of baby food with an effort to detect the presence of aflatoxins and their possible dietary exposure in infants. For this purpose, a pilot survey (in a form of a questionnaire) was carried out on mothers who frequented the primary health care clinics in Al Ain, UAE, during September/October 1989. The main objectives of the questionnaire were to determine the weight and height of infants and to reveal the commonly practised infant feeding patterns, in order to determine the variety of supplementary foods used. Such supplements were considered for the aflatoxin assessment in this study.

The information obtained from the data generated by the questionnaire showed that 3.5% of the infants depended exclusively on artificial feeding, which consisted mainly of baby milk formulae,
fresh milk and baby food. Moreover, 71.6% of the infants received supplementary food in addition to breast milk and some of the supplements before the end of the first month. However, 90% of the infants received Cerelac either alone or with other supplements. The frequency of the introduction of baby milk formulae and fresh milk was 5 - 6 times/day.

When applying the "Toxoford" index, which is used for assessment of growth through measurement of height and weight, results have shown that all infants included in the study were less than normal. Such a finding revealed the possibility of inadequate infant nutrition.

Initially, the physical characteristics of all possible supplementary food items were examined and analyzed. Also, a microbiological investigation was carried out and it revealed that some of these foods were contaminated with mould. Moreover, isolation and identification of aflatoxin-producing mould confirmed the presence of different strains of *Aspergillus flavus*.

Presence of aflatoxins in baby food indicated that their levels were higher than those which have been established as "tolerated" levels in developing countries. Food samples which were collected from small stores contained a significantly higher quantity of aflatoxins than those obtained from large stores. This could be attributed to less than "proper" storage conditions in the small stores.
Rice is favoured by people of the region and hence, is used by all families of different nationalities not only as a baby food but also for all ages. The questionnaire survey showed that 90% of the UAE citizens preferred to keep rice in storage for a minimum period of 2 months prior to consumption. Aflatoxins have been detected in rice and at high levels. Significant differences were found in the incidence and levels of aflatoxin-contamination between 2 kinds of rice. Long-grain rice had significantly higher levels than those found in short-grain rice.

Aflatoxins were detected in fresh cow and goat milk at levels exceeding those "safe" limits adopted by many countries. Occurrence of aflatoxins was variable in milk samples taken from different farms. Moreover, differences between the 2 animal species were observed. Aflatoxin levels in goat milk were found to be higher than those detected in cow milk. Further work was carried out in order to trace the source of aflatoxins in milk. Animal feeds were tested for the presence of aflatoxin, in which significant quantities were detected. This work confirmed the possibility for the "carry over" of aflatoxins from these contaminated feeds into milk.
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CHAPTER 1

INTRODUCTION

1.1 The United Arab Emirates (UAE)

The UAE is a federated union of 7 Emirates, namely: Abu Dhabi, Dubai, Sharjah, Ajman, Umm Al Quwain, Ras Al Khaimah and Fujairah. All Emirates lie on the Arabian Gulf except the Emirate of Fujairah which lies on the Gulf of Oman (Fig. 1.1). The country lies between latitude 22° and 26° 30 North and longitude 51° and 56° 30 East. The UAE is bounded by the Arabian Gulf and the Gulf of Oman from the North West, to the South by Saudi Arabia and Oman and to the West by Saudi Arabia and Qatar. The climate is mainly divided into 2 seasons; summer (March-September) which is very hot and humid and winter (October-February) which is cool-to-warm. During the period between May to October, the temperature ranges from 38-50°C and in the winter season it ranges from 20-35°C. Rain usually falls in the winter and rarely exceeds five inches annually. The summer is usually humid and it can exceed 90%.

The population of the UAE has exceeded 1.5 million in 1988, with children below 15 years of age forming about 33.6% of the population (Annual Report of Preventive Medicine, 1990). Abu Dhabi is the largest Emirate, with an area of 29,000 square miles and a population of about 500,000 inhabitants, is the wealthiest of
Map showing the location of Al-Ain within the Emirates
the 7 Emirates. The development of the infrastructure in this oil-rich country has helped in establishing high economic standards, which were achieved in a period of not more than 15 years. The presence of several groups of expatriates, who comprise most of the UAE population, has influenced the fast development of the socio-economic picture. With such changes, modifications in the prevalent traditional life style have taken place.

1.2 Primary Care and Health Services in the UAE

The high income per capita attained in the UAE has enhanced the crude birth rate, which was 3.2% in 1989 (Annual Report of Preventive Medicine, 1990). Also, the government encourages citizens to have more births. There are 8 medical districts in the UAE and its inhabitants enjoy a high standard of health care. Health services for children in the UAE are provided through the primary health care clinics. Practically, all women deliver their babies at hospitals and visit primary health care clinics regularly from the beginning of pregnancy. Even though some mothers do not prefer to go to governmental primary health care clinics, they usually go to private clinics on the assumption that they might get better health services. Information which is presently available concerning mothers and child health is limited.

The combination of high socio-economic standards and the improvement of health services has already put the UAE in the category of one of the most developed countries in the world. However, a great deal of scientific work needs to be directed towards
assessing the state of general health of the UAE population, as well as towards identifying prevalent diseases.

1.3 Attitudes and Practices of Infant Feeding in the UAE

The prevalent attitudes and practices of infant feeding among UAE nationals may vary according to the geographical location within the country and the tribe of affiliation. Breast feeding was considered as the best method of infant feeding and was the most dominant method until a few years ago. The presence of expatriates of different nationalities has contributed greatly to such attitudes and practices. In addition to influences stemming from social and/or religious roots, infant feeding patterns would be expected to be affected as more women go to work. Also, members of the health service staff may have a direct impact on the decline of breast feeding, due to such factors as: misleading advice about use of baby formulae, deficiency in health education and communication problems due to language barriers. Other influences may also include the illiteracy of the mother and the effect of the mass-media and of sales representatives through announcement of newly-introduced baby foods. Mothers who work outside the home would be expected to stop breast feeding and introduce bottle feeding considerably earlier than those who are non-working. Also, working mothers would be likely to depend on nurseries or baby sitters for help in child care and rearing, which could be deficient or improper. Although the above factors are implicated, there are no solid data available about commonly used methods of infant feeding in the UAE, or on their suitability.
1.4 Aflatoxins

Aflatoxins are group of mycotoxins which have serious impact on humans and animals by causing health problems that lead to economical losses. Aflatoxins are formed in human food and animal feed as a result of post-harvest invasion of toxigenic strains of Aspergillus flavus and A. parasiticus.

Contamination of baby food or ingredients used in infant feeding by aflatoxins should be of a particular concern, because it has been reported that the young have a greater sensitivity to aflatoxins than the old. Additionally, different varieties of baby foods and ingredients are imported and made available to all infants and children, which necessitates regular checking for compliance with set "tolerated" levels. The conditions under which these foods and ingredients could be far from ideal and would be conducive of mould growth and production of aflatoxins. A survey of aflatoxins was carried out in the city of Abu Dhabi area by the Central Food Laboratory (Saad et al., 1989) and research on animal feed and camel sera was carried out in Al Ain Veterinary Laboratory (Osman and Abdelgadir, 1991). However, research work on baby nutrition, infant foods and their safety from pathogenic microorganisms and poisonous chemicals is deficient in the UAE. Aflatoxins were found in cereals, nuts and camel milk (Saad et al., 1989) as well as in camel tissues and serum (Osman ad Abdelgadir, 1991).
1.5. Dairy Production in the UAE

Dairy production is popular in the UAE; since automation has helped in satisfying the increased demand, encouragement of the government, granting of land and providing of subsidized electricity, animal feed and water by local authorities have made it a profitable business. According to the Abu Dhabi Department of Planning (1988), the total dairy production was estimated to be 6083 tonnes/year. In addition to local production, which covers about 20% of the local demand, the country imports other kinds of dairy products. A dozen or more dairy factories are available in the UAE which produce fresh milk, yogurt, buttermilk and cream. Most of the fodders are imported and few are cultivated under severe water shortage conditions.

1.6. Cancer in the UAE

Like any other developed society, cancer has drawn the attention of local authorities. Cancer is a common cause of death in the UAE among all age groups. This may be due to different factors that followed the drastic social changes in living styles and the eating patterns among locals and expatriates. The government has directed a great deal of effort towards this problem in order to reveal the epidemiological factors involved. Three oncological departments in three specialized hospitals in Abu Dhabi, Al Ain and Dubai, have been established. According to the data obtained from these centers, 200 cases have been reported since 1986. Digestive system, head and neck, urinary organs, haematopoietic cancers are
common among males. Breast cancer followed by reproductive system haematopoietic, digestive and endocrine glands cancers are common among females. Children below 15 years are affected by leukemia and brain cancer. The percentage of deaths as a result of cancer between citizens and expatriates is 58.1 and 41.9% for UAE citizens and non-citizens, respectively (Annual Report of Preventive Medicine, 1990).

1.7 Objectives of the Study

The occurrence of aflatoxins in human and animal feed should be of particular importance to consumers due to their health hazards which cause serious economical problems. The presence of aflatoxins in baby food is of particular concern because of the great sensitivity reported in young animal species. These facts led to the design of this study with an effort to consider the occurrence of aflatoxins in baby food. Information concerning feeding patterns in the infant population were collected to assess their possible exposure to aflatoxins.

A pilot survey was carried out in Al Ain, UAE A questionnaire was distributed through primary health care clinics to which 375 mothers have contributed and the data were generated to evaluate the feeding patterns, kinds of baby food and other weaning food consumed by infants. The identification of supplementary food which mainly composed of milk, cereals or mixture of both and rice, necessitated the survey of aflatoxins in these foods which help in the evaluation of aflatoxin exposure in the infant population in the
region. Nutrient components of tested samples were determined to check whether there are interactions between nutrients and aflatoxins; therefore, tolerated levels may be established in these foods.

Identification of high intake of fresh milk by infants and mothers is of particular concern, as it is known that aflatoxins contaminate milk indirectly when animals ingest aflatoxin-contaminated feeds. With this in mind the survey of aflatoxins in fresh milk is of great importance. Animal products also may be an essential source of indirect human exposure. Aflatoxin occurrence in animal feeds was surveyed in order to establish the source of aflatoxins in milk. Such data may help in animal feed formulation and whether any modifications in their rations will help to reduce animal ingestion of aflatoxins.
CHAPTER 2

REVIEW OF THE LITERATURE

2.1 Baby Nutrition

Generally, nutrition includes all processes involved in supplying the nutritive needs such as food supply and the chain of processes of food utilization and metabolism. The condition or state of nourishment is referred to as the nutritional status which might be either good or poor due to limited food supply, limitation of the essential food components, lack of some constituents needed by the body or contamination with toxins or other inhibitory factors. On the other hand, malnutrition may occur if the food taken in is more than the natural requirement which results in obesity.

Human milk was considered in the past, and up until recently, as the sole method of infant feeding providing the infant with essential nutrients for normal growth and health, especially at critical periods of sickness. In the past, infant feeding consisted mostly of human milk derived from the mother or by a wet nurse. In the old Arabic history, rich families hired nurses to breast-feed their babies. During the 16th Century, breast feeding was the only means and most of the families depended on wet nurses. They believed that a wet nurse of a good origin, who was a healthy and indulged woman, was the best form of infant nourishment. Also, it was believed that the transmission of good characteristics occurred through her milk to the child. Another alternative was the baby
farmers, where unwilling mothers sent their children to. Mother's health and baby suckling were considered as important factors contributing to a standard nutritional status (Committee of Nutrition, 1976).

Human milk has many advantages as a source of nutrients for infants, as it is bacteriologically safe and its colostrum contains immunoglobulins which give the child a substantial immunity to infections (especially enteritis) and usually provide protection from necrotizing enterocolitis (Santulli et al., 1975). Also, breast milk contains low protein which gives better absorption of trace amounts of iron. In addition to the presence of fat, milk provides the calcium and phosphorus required for growth. Breast feeding helps to induce the early physical contact and affinity between mother and infants which is essential for future interactions (AAP Committee on Nutrition, 1972; 1974).

However, some disadvantages make human milk unsuitable and/or inadequate for growth. The Committee of Nutrition (1976) has identified some of them such as: genetic disorders, milk intolerance, concentration of drugs and toxins, inadequate supply and unsuitable nipples. However, this nutrition group did not include aflatoxins, the presence of which in human milk has been reported (Saad et al., 1989).

Research concerned with how long human milk is adequate for the normal physical growth of children has shown that human milk is adequate up to the 9th month in the Baltimore area,
State of Maryland, USA (Chunghae and William, 1980). In some other areas, breast milk was found to be enough for three months only (Neumann and Alpaugh, 1976). The volume and composition of human milk is greatly affected by maternal nutritional status, physical overwork, environmental, psychological and social stress and other factors such as maternal parasitic diseases and genetic disorders (Jelliffe and Jelliffe, 1987). Such factors would affect the quantity and quality of produced mother’s milk and, consequently, its suitability for infant nutrition.

Breast feeding practices are influenced by socio-economic factors such as ethnic, occupation and education. In India, it was found that mothers preferred long periods of breast feeding because it is cheap, results in a healthy child and helps to achieve a reasonable child spacing. An early stoppage of breast feeding occurred when mothers became involved in outside work (Prentice, 1991). In many societies mothers receive care from other family members and this helps and encourages her to breast feed the infant for a longer period.

During the early 18th Century, a number of changes in infant feeding occurred and the wet nurse was required to employ some food other than human milk, so that the use of a solid food in addition to breast milk was apparent. That has marked the first introduction of mixed feeding. Initially, any alternative feed to human milk was limited to bread soaked in milk although cow milk was used as a substitute for the mother’s milk during the 17th and early 18th Centuries (Cone, 1976). Either cow or goat milk with
semolina flour and with addition of egg yolk were introduced by Simon de Vallambet in France in 1565 (Cone, 1976). Also, the use of solid foods such as chewed bread and cooked vegetables had been suggested by an Italian physician in 1577. Weak broth was given with the appearance of the first tooth and with the second tooth minced chicken was fed, after which sugar was added and given with bread boiled in water (Still, 1931). The beginning of the proprietary infant food industry might have been dated back to 1867, when Jestus Van Liebig developed a biochemical classification of organic foodstuffs and the processes of nutrition (Cone, 1976). Van Liebig's mixture was composed of wheat flour, malt flour and cow's milk; cooked with a little bicarbonate of potash that was added to reduce the acidity (Cone, 1976).

Cow milk was considered to be the most appropriate substitute for human milk when unavailability or other factors created problems for breast feeding. During the 19th Century, a lot of deaths among children occurred, 80-90% of which were attributed to the lack of breast feeding (Cone, 1976). In New York (New York Infant Asylum), bottle-fed infants were kept in a room known as the ward of the "dying babies" (McNutt, 1989). Great efforts have been made by pediatricians and nutritionists to produce a milk mixture to suit the infant's nutritional requirements (Simon, 1938).

In the United States, infant food could be divided into three groups; the first group consists of dried cow milk in combination with some cereals and sugars. Examples of this group are: Nestle's
food which contains unchanged starch and Horlick's milk in which the starch was converted into soluble carbohydrates such as maltose and dextrin. The second group contained some form of malted carbohydrates in which starch was completely changed into dextrin and maltose. The third group is composed of pure cereals with fresh cow milk (Cone, 1976). Animal products such as chicken and butter were given to infants when they are nearly 1 year old. By the end of the 19th Century, many foods have been introduced to children under 4 years such as liver, tomatoes, beets, banana, canned, dried and preserved fruits.

Many efforts have been made by researchers to produce an appropriate infant food until the introduction of Rotech's percentage method (Rotech, 1907), in which he suggested that feeding of an individual infant should be equal to his/her digestive capacity. This method has been used from 1890 to 1915, when it was criticized by the American community and was replaced by the "calorimetric" method developed by Rubner and Heubner, in which they suggested that infant feeding should be according to calorimetric requirement (Cone, 1976). In 1909, John Ruhrah suggested some preparations including soybean milk, as a milk substitute, for children with diarrhoea, intestinal disorders and in diabetes mellitus (Ruhrah, 1909). By 1927, evaporated milk was accepted as a food ingredient for infants and in 1930 it became the most widely used in infant formulae (Marriot and Schoenthal, 1929). The idea of using evaporated milk for babies dated back to 1853, when it was introduced by Gail Borden in England and America (Cone, 1976). In infant nutrition, it has become a common practice in the United
Kingdom (UK) and the USA to introduce solid foods from the 3rd month. Examples of the first solids offered included Robinson's baby rice and Milupa rice cereals. From the 6th month and on; milk, egg yolk, nuts and gradually "follow-on" milk should be given. Natural whole grain cereals and bread were given starting from the 8th or 9th month.

Since 1960, the trend in infant nutrition was directed towards "ready-to-use" formulae for hospital use and the pre-diluted formulae for home use (Filler, 1992). In the 1970's, the efforts of physicians and nutritionists were directed towards providing baby formulae: with new sources of protein, medium triglycerides, safe from additives and free of environmental pollutants (Filler, 1992). All concerns, then, were to provide a baby food of good nutritional value; without attention given to the hazards presented by aflatoxins or mycotoxins.

Breast feeding in the USA declined from almost 100% at the turn of the century to an all-time-low in the 1970s to less than 30% at 1 week and to about 5% at 6 months of age (Howard and Weizman, 1992). Due to some factors such as physician practices, hospital routines, educational and governmental programmes; 60% of infants were initially breast-fed in 1984. The prevalence rate of breast feeding in the USA was found to be only 52% (Ryan et al., 1979). Currently, about 50% of infants are initially breast-fed, and less than 20% are still being breast-fed at 6 months. Many factors have contributed to the decline in breast feeding, among which was the introduction of supplementary food especially during the first
week post-partum. Such has resulted in decreased milk production by the mother and the hardness of her nipples which made the baby refuse breast feeding. The decline in breast feeding was found to be influenced by socio-demographic factors; such as the involvement of mothers in social life and for going to work outside the home.

There are many pressures directed towards providing infant formulae products of composition and physiologic function which resemble that of human milk (Filler, 1992). When comparing human milk with artificial milk formulae, many merits could be given to the former. Breast milk contains lactose and fat; which provides 40 and 50% of the calories, respectively. However, it contains higher serum cholesterol when compared to formulae, which are composed of vegetable fat and contain no cholesterol. Breast milk is important for building the body immunity, as about 80% of the protein content of colostrum is immunoglobulin A (IgA). In under developed countries, the risk of infant mortality is five times in artificially-fed infants than that in breast-fed. Breast milk has many advantages such as protection against allergies, inflammatory diseases and from sudden death.

2.2 Fungi (Moulds)

Fungi are found everywhere. Among them is the most essential group in food microbiology, known as "true fungi". Moulds are filamentous fungi belonging to zygomycetes, ascomycetes and deuteromycetes, which are thought to be responsible for food spoilage (Pitt and Hocking, 1985; Samson and Van Reenen-
Mould contamination of stored products is common worldwide. Their light-weight reproductive spores may be carried by air currents to places some distance from their origin. Nevertheless, mould spores are capable of remaining dormant for long periods under conditions which are considered unfavourable for most vegetative cells. Moulds have an economic importance for human life, by influencing the environment chemically and physically through stabilizing the soil, decomposing cellulose and making the chemical elements of lignin and starch available for plants. Man has wisely benefited from abilities of fungi to decompose complex materials in processes such as the ancient process of flax and hemp rotting to free fiber, pectin production as a result of the hydrolytic action brought about by the Mucor species. Aspergillus niger has been employed in ink manufacturing. Diastase is recovered from the action of Aspergillus on caproic acid. Action of A. niger on molasses results in the production of citric acid. Secondary metabolites of some moulds are important in medicine such as penicillin, which is a metabolite of Penicillium chrysogenum.

Mould growth may reduce the nutritional value of stored products, even if mycotoxins are not formed. The moulds thrive in environments where the pH is generally too low, or at least not ideal for many species of bacteria. They consume the most readily available nutrients such as sugars and organic acids as a source of carbon and energy. It is possible for moulds to oxidize these acids to carbon dioxide and water, and to raise the pH to a high enough level for bacterial growth and for food spoilage. The low nutrient
and moisture requirements of some moulds allow them to grow on different habitats such as optical glass surfaces which they are capable of etching. High osmotic pressure is non-inhibitory to the growth of some fungi, as evidenced by mould growth on surfaces of jellies, jams, and preservatives containing a high sugar content. The spoilage of bread and other starchy foods is common during seasons of high temperature and humidity. Fruits are also decayed by fungi, particularly oranges.

2.2.1 Fungal Secondary Metabolites

Primary metabolism involves all processes of building (anabolism) and breaking down (catabolism) of the organic compounds known as carbohydrates, proteins, nucleic acids and lipids. It was defined by Turner (1976) as: “the summation of interrelated series of enzyme-catalyzed chemical reactions to provide the organism with its energy, its synthetic intermediates, and its key macromolecules such as protein and DNA”. Secondary metabolism of moulds and food intoxication was reviewed (Moss, 1992), in which it was explained that moulds use the same rule of primary metabolism common in the majority of living organisms. Moulds use low molecule intermediates during primary metabolism such as malonyl coenzyme A to be used for the synthesis of long chain fatty acids, malonate for the synthesis of sterols and carotenoids and amino acids for the synthesis of proteins.

When conditions are not favourable for active growth, moulds adapt by undergoing morphological and biochemical differentiation
which may result in the production of various compounds of low molecular weight known as "secondary metabolites". Production of a particular secondary metabolite is usually restricted to one or a few species. Fungal secondary metabolites include antibiotics, gibberellins, alkaloids, tremorgens and mycotoxins. Functions of secondary metabolites are varied, some of which play an essential role as inhibitors of environmental competitors as in the case of antibiotics (Katz and Demain, 1977).

2.2.2 Mycotoxins

Mycotoxins are toxic secondary metabolites produced by various moulds under certain environmental conditions that favour their growth and cause health problems to humans and animals. The major mycotoxin producers belong to the genera of *Aspergillus*, *Penicillium* and *Fusarium*. Their mycotoxins result in a wide range of toxicological effects (Watson, 1985). The discovery of mycotoxins as hazardous substances to human and animal health developed in different ways before the final conclusion was drawn, as reviewed by Moss (1992).

Over 300 mycotoxins have been identified, produced by 350 fungal species. Some of these are involved in human diseases including cancer such as ergotism, citreoviridin toxicosis, alimentary toxic aleukia, Balkan endemic nephropathy, stachybotryotoxicoisis and aflatoxicosis (Pohland, 1993).
In the 1930's and 40's, a number of compounds which are now classified as mycotoxins were regarded as "potential" antibiotics. The toxicity of certain mushroom strains has been known for a long time, but other mycotoxin hazards were not recognized. Outbreaks of ergotism have been reported in Europe and the disease known as "St. Antony's fire" was described (Matossian, 1989). In Russia and Eastern Europe, many people suffering from a disease affecting the haematopoietic system resulted in about 60% mortality (Joffe, 1986). The disease was referred to as: alimentary toxic aleukia (ATA), which resulted from ingestion of food contaminated with mould of the Fusarium genus (Pohland, 1993). A mycotoxicosis; which is characterized by severe dermatitis, haemorrhage and rhinitis was observed among farmers handling hay and straw. A toxin was found to be produced by Stachybotrys atra and causes the disease known as stachybotryotoxicosis (Joffe, 1986). A disease known as Balkan endemic nephropathy (BEN) occurred in the rural areas of Bulgaria, Romania and Yugoslavia which resulted in a mortality rate of about 60% because of ingestion of food contaminated with ochratoxin A (Krogh, 1987). Ergotism was reported in India in 1975 (Krishnamachary and Bhat, 1975) and in Africa in 1979 (Derneke et al., 1979). Also, citreoviridin caused acute heart disease, cardiac beriberi among people ingested rice contaminated with P. citreoviride in Japan (Uraguchi and Yamazaki, 1978).
2.2.3 Nomenclature of Mycotoxins

Mycotoxins are produced by groups of fungi and some have been found to be produced by more than one strain or species. The name of each individual mycotoxin is often derived from the fungus which has contaminated the commodity and that was reported to produce the toxin and from where it was isolated. Thus; aflatoxin is an *A. flavus* toxin, which was first isolated from peanut meal and caused death in turkeys (Allcroft and Carnaghan, 1963). The fungus *Penicillium rubrum* produces rubratoxins, which have toxic effects predominantly in the liver and kidney. Ochratoxins are produced by Aspergilli and Penicillia (Ciegler *et al.*, 1972; Hesseltine *et al.*, 1972), but the name was originally derived from *Aspergillus ochraceus* because this was where the toxin was first isolated. *Penicillium cyclopium* was reported to be the predominant mould that produced penicillic acid (Mulinge and Chesters, 1970). The name: ergotamine was derived from the name of the disease ergotism.

2.2.4 Ochratoxin

Ochratoxins are a group of toxins produced by several fungi of the genera *Aspergillus* and *Penicillium*, but the most important producer is *Aspergillus ochraceus* in tropical and subtropical countries and *Penicillium verrucosum* in cooler temperate countries of North Europe (Hesseltine *et al.*, 1972; Ciegler *et al.*, 1972). Ochratoxin A was first isolated from *Aspergillus ochraceus* (Merwe *et al.*, 1965). Ochratoxins were considered as the first major
mycotoxins identified, next to aflatoxins, and two groups: ochratoxin A and B were characterized (Krogh, 1974). Ochratoxins were found to be predominant in colder regions of the world such as Sweden, Denmark, and Canada; where they are produced by *Penicillium verrucosum* (Harwig and Chen, 1974). Their natural substrates include cereals, ground nuts, and beans in South America and Europe. Presence of ochratoxin has not been reported in either hot or semi-hot regions. The optimum conditions for ochratoxin formation in the food was reported to be between 20 and 30°C. Their maximum production occurred at 30°C and with a water activity of 0.935, i.e., 93% equilibrium relative humidity, % dry weight (Bacon et al., 1973). Ochratoxins, which were first recognized in the 1950’s, are nephrotoxins and have been implicated in a chronic disease (BEN), which affected inhabitants of rural areas of Bulgaria, Romania and former Yugoslavia (Krogh and Elling, 1976).

### 2.2.5 Citrinin

Citrinin is a mycotoxin that occurs together with ochratoxin, as a natural contaminant of grains. Both of them are reported as nephrotoxins to experimental animals (Krogh, 1974).

### 2.2.6 Sterigmatocystins

Sterigmatocystin is a mycotoxin, which was isolated from *Aspergillus versicolor* in 1954. The structural formula of sterigmatosystin is a difuroxanthone with a molecular formula of
C\textsubscript{18}H\textsubscript{12}O\textsubscript{6} (Bu'Lock et al., 1962). The first evidence of the natural occurrence of sterigmatocystin was reported in a Canadian wheat sample, which was contaminated with Aspergillus versicolor (Scott et al., 1972). Sterigmatocystin can be produced by other species such as A. flavus, A. parasiticus, A. ruber (Shank, 1976). Some penicillia have been reported to produce sterigmatocystin, such as Penicillium luteum (Dean, 1963). Also, its production was reported to occur in 7 genera of fungi and 22 species of Aspergillus (Bennet and Deutsh, 1986; Frisvad, 1985). Like most other mycotoxins, sterigmatocystin was found to be strain-specific (Hall and Ayres, 1973; Schroeder and Kelton, 1975; Rabie et al., 1977). Hajjar et al. (1989) reported that sterigmatocystin was produced by Aspergillus nidulans on oats and rice. Sterigmatocystin was described as an important intermediate in aflatoxin biosynthesis (Hsieh et al., 1985; Henderberg et al., 1988), with less potency of toxicity, carcinogenicity, mutagenicity and teratogenicity than that of aflatoxins (Van der Watt, 1974; Davis, 1981).

2.2.7 Patulin

Patulin is a mycotoxin which was first described as antibiotic in 1940 following penicillin discovery by Fleming in 1929. Scott and Bullerman (1975) reported its toxicity to a wide range of biological systems including microorganisms, plants and animals as well as its carcinogenicity to mice. Lovett and Thompson (1978) reported the ability of species of Aspergillus and Pencillium to produce patulin at 1.7, 7.2 and 12.8°C.
Patulin was found to be a potent phytotoxin (Polacco and Sands, 1977), which has antibiotic properties (Scott and Bullerman, 1975). Great losses of apples and pears were reported due to patulin production, which usually favours refrigerated temperatures (Sommer et al., 1974). Brian et al. (1956) reported a level of more than 1000 parts per million (ppm) of patulin, as a natural contaminant, in apple sap, juice and cider. Mutagenic and Hepatic effects were produced by patulin when interacting with rubratoxins (Kangsadalampai et al., 1981).

2.2.8 Penicillic Acid

Penicillic acid is a toxin produced by species of Aspergillus, Penicillium and Paecilomyces (Wilson, 1976). It was detected in dried beans at 11.0-18.9 mg/kg (Thorpe and Johnson, 1974). Penicillic acid may be produced in many kinds of foods, provided that the temperature, water activity and the nature of the substrate are favourable for its production (Northolt et al., 1978).

2.2.9 Rubratoxins

Rubratoxins are toxic metabolites produced by Pencillium rubrum and Penicillium purpurogenum that caused serious livestock diseases (Richmond et al., 1980). It was first isolated from mouldy feed that caused serious toxicity in cattle and pigs (Moss et al., 1971). Moss et al. (1969) described the structure of rubratoxin A and B. Both rubratoxins have common features,
rubratoxin A differs from rubratoxin B by addition of two hydrogen atoms at rubratoxin A.

2.2.10 Zearalenone

This toxin is produced by *Fusarium graminearum*, *Fusarium tricinctum*, *Fusarium oxysporum*, *Fusarium sporotrichioides* and *Fusarium moniliforme* at low temperature (12-14°C). However, the common producers were found to be *F. roseum* and *F. graminearum* (Mirocha and Pathre, 1979). Zearalenone was reported to be a sex hormone-like, as it was found to be responsible for hyper-esterogenic syndrome in swine (Christensen et al., 1965). Zearalenone was reported to contaminate cereals in USSR, Taiwan and China (Ueno et al., 1986). Zearalenone residues were detected in animal feeds (Sundlof and Strickland, 1986) and were isolated from corn feed in India (Abbas et al., 1986). Also, it was detected in banana fruits infected with *Fusarium moniliforme* (Chakrabarti and Ghosal, 1986). Sabino et al. (1989) reported the natural occurrence of zearalenone in maize in Brazil.

2.2.11 Trichothecenes

Trichothecenes are a group of more than 20 sesquiterpenoids and are natural contaminants produced by *Fusarium*, *Stachybotrys*, *Myrothecium* and *Trichoderma* (Ciegler, 1978). Fusarium contamination of food and feed commodities was reported to exist throughout the world, especially in geographical regions subjected to a cool and wet climate. The first reported intoxication was the
outbreak of alimentary toxic aleukia (ATA) in Russia during the Second World War. Further evidence of fusariotoxicosis occurred in the USA, which involved feed refusal and vomiting phenomena affecting pigs fed corn infected with *Fusarium graminarium*. Intoxication in humans occurred in Japan among humans who ate contaminated rice or wheat products infected by *Fusarium*. In Russia, intoxication occurred as a result of eating bread baked with scab wheat (Saito and Ohtsubo, 1974).

Fusariotoxicoses are toxic conditions caused by ingesting cereal grains and forages contaminated by different species of *Fusarium*. Effects of the trichothecenes were found to be host-specific and varied according to the different animals species (Gobal et al., 1986). Trichothecenes were found to enhance nitrosamine-induced oesophageal cancer in animals (Craddock et al., 1986). In 1987, an outbreak of trichothecene poisoning occurred in Kashmir Valley, India, and gastrointestinal disorders were apparent as a result of the consumption of bread made from mould-damaged wheat. A toxic strain of *Fusarium* was isolated from scabby wheat in Japan (Ramesh et al., 1989). Trichothecene was found to be produced by *Fusarium sporotrichoides* (Marasas et al., 1984).

### 2.2.12 Aflatoxins

Several hundred thousands of turkey poults died suddenly in England in 1960. This was followed by the death of fourteen thousand ducklings and nine outbreaks in calves. The common factor of these events was the use of Brazilian groundnut in rations
of these animals, which was found to be contaminated with toxic materials (Allcroft and Carnaghan, 1963). Aspergillus flavus was found to be the causative agent and the toxin produced (Aspergillus flavus toxin) was designated the name: aflatoxin (Sargeant et al., 1961).

Aflatoxins are natural contaminants of foods, which are regarded as secondary metabolites produced mainly by Aspergillus flavus and Aspergillus parasiticus, (Sargeant et al, 1961), and more recently A. nomius (Moss, 1971). Aflatoxins are also found in poultry and livestock feed and cause major economical losses in these industries (Jelinek et al., 1989; Pohland and Wood, 1991). Aflatoxin producing organisms are mesophilic and grow on a wide range of substrates when the water activity is greater than 0.85 and with a temperature range of 25-40°C. Aspergillus flavus is considered a saprophytic mesophyte requiring a moisture content of 80-90% relative humidity for growth (Galloway, 1935; Panassenko, 1944). Different amounts of oxygen are required for their vegetative growth and sporulation (Miller and Golding, 1949; Follstad, 1966). Aflatoxins have been reported to be produced by other fungi such as Penicillium puberulum (Hodges et al., 1964), A. ostianus (Scott et al., 1967) and A. ochraceous (Van Walbeek et al., 1968); however, these have not been confirmed. Strains of A. flavus and A. parasiticus vary in the amount of aflatoxins they produce on natural substrates and also in their capability to produce the four aflatoxins. Most of A. flavus strains produced Aflatoxin B₁ while A. parasiticus is capable to produce both B & G groups. some were found to produce only B₁ or G₁ (Hiscocks, 1965).
The physio-chemical properties of aflatoxins and the analytical techniques used for their isolation and identification were described by Van der Zijden et al. (1962). Their chemical structures were illustrated by (Asao et al., 1963), which were confirmed later by X-ray chrystallography (Cheung and Sim, 1964). A comprehensive review of the history of aflatoxins was made by Moss (1972).

Aflatoxins are bis-furano-coumarin metabolites, which are characterized by fluorescent intensity under ultraviolet (UV) light and are relatively unstable to light. They are, however, very stable to elevated temperature; especially in complex food matrices, are soluble in polar solvents but relatively insoluble in water. Since aflatoxin distribution within a given contaminated material is usually uneven, samples and sub-samples are analyzed to reduce errors in the results (Whitaker, 1990).

Aflatoxins includes about 17 compounds but only two major components of aflatoxins were isolated and are referred to as B and G, depending on their fluorescence under UV light at 365 nm (B = blue fluorescence; G = green fluorescence). Of these; B₁, B₂, G₁, G₂ are the main derivatives (Moss, 1972), in addition to M₁ and M₂, which were identified in 1966 as hydroxylated products of B₁ and B₂ excreted in the milk of cows fed contaminated feeds. Aflatoxins B₂a and G₂a were isolated from cultures referred to as A. flavus in 1967. Aflatoxins GM₁ and GM₂ were isolated from sheep and rat urine. Aflatoxins M₂a and GM₂a were isolated from extracts.
of *A. flavus* culture fluids (Heathcote et al., 1973). Other aflatoxins have been isolated such as P₁ and Q₁ (Dalezios et al., 1971). Fig. 2.1 shows some of aflatoxin metabolites.

Aflatoxins might contaminate a wide variety of foods such as cereal and legumes when conditions favour their formation. Lacey (1989) suggested that the largest concentrations of aflatoxins occur under ranges of temperature and water activity that allow growth of mould, and hence toxin formation. Aflatoxin contamination may occur under field conditions before harvest (Moss, 1989). Pitt et al. (1991) suggested that spores of *A. flavus* attach to maize seed head without affecting the plant and remain dormant. Also, invasion of groundnuts with *A. flavus* has been reported at an early stage of growth. Moss (1992) suggested many factors that contributed to aflatoxin formation in maize and groundnut under field conditions such as drought, insect vectors, the presence and nature of inoculum, interaction with other microorganisms and disposition of the plant. Moss (1991) stated that field contamination with aflatoxins is difficult to control due to the effect of climatic conditions. Aflatoxin producers adapt differently to field conditions. For instance, *A. flavus* adapts to the aerial part of the plant and hence is dominant in maize, while *A. parasiticus* is adapted to the soil conditions so is common in peanuts.

General studies were conducted to determine the effect of temperature on aflatoxin formation. Diener et al. (1965) found that the optimum temperature for aflatoxin production by *A. flavus* is
Fig. 2.1: Structures of some Aflatoxins and Metabolites

Aflatoxin B1

Aflatoxin B2a

Aflatoxin Q1

Aflatoxin M1

Aflatoxin Epoxide

Aflatoxicol

Aflatoxin PI

Dihydroxy Aflatoxin
35°C in case of peanuts, while A. parasiticus produced maximum amount at 25-30°C. Sorenson et al. (1967) suggested that the optimum temperature for formation of aflatoxins B₁ and G₁ in rice was 28°C and production of aflatoxin B₁ increased at 32°C while less G₁ was produced.

Effects of relative humidity (RH), water activity (a_w) and moisture content (MC) on aflatoxin production were studied. Relative humidity is defined as the ratio of the actual vapour pressure, as percentage, to saturation vapour pressure at air temperature (Slatyer, 1967). Generally, mould growth is favoured by high relative humidity in combination with high temperature. Diener and Davis (1969) suggested that the lowest relative humidity for aflatoxin production in peanuts was 83% and aflatoxin yield increased as the relative humidity rose to 99%. The water activity a_w (the ratio of water vapour pressure of the substrate to the vapour pressure of pure water at the same temperature and under the same pressure (Scott, 1957). Northolt et al. (1976) reported that growth of A. parasiticus occurs at a_w of 0.83 while no aflatoxin was formed. The limiting water activity for aflatoxin production by A. flavus was 0.78 and 0.83 which suggested that aflatoxins could be produced at conditions of water activity and temperature that were close to the optimum for growth. Moisture content of the substrate was found to influence aflatoxin production by A. flavus and other fungi. Calderwood and Schroeder (1968) reported that rough rice of a moisture content of 24-26% supports the production of large amounts of aflatoxins ranging between 205-750 μg/kg by A. flavus. Van Warmelo et al. (1968) found that aflatoxins were formed in
naturally-infected maize with *A. flavus* at a moisture content of 19.6-25%. Sanders *et al.* (1968) reported that the lower limit for growth and aflatoxin formation in cereal and oil crops was 18.3-18.5% and 9-10%, respectively.

### 2.2.12.1 Aflatoxin Analysis

Many methods have been established for aflatoxin quantification including chromatographic and immunological techniques. Sampling methods are important and are of particular concern for mycotoxicologists, because aflatoxins are usually formed in higher amounts in a small portion of the commodity. In some reports an individual kernel of peanut was found to contain more than 1000000 ng/g, and more than 5000000 ng/g were detected in cotton seeds and more than 400000 ng/g were detected in corn kernels (Whitaker, 1990).

All chemical analysis of aflatoxins include extraction, clean up, concentration, toxin separation, detection and confirmation under UV light. Paper, thin-layer (TLC) as well as liquid and gas-liquid chromatography (LC and GLC) are the main methods used to separate aflatoxins. Recently, immunoassay was used to detect aflatoxins which include enzyme-linked immunoassay (ELISA), affinity columns and radioimmunoassay (RIA). Techniques used to quantify aflatoxin albumin adducts were described by Wild *et al.* (1990), which include enzyme-linked immunosorbent assay (ELISA) performed directly on albumin (direct ELISA), performed on albumin hydrolysate (hydrolysis ELISA) and high performance liquid
chromatography (HPLC), fluorescence detection of AF-lysine adduct after albumin hydrolysis and immunoaffinity purification (Scott, 1989). The detection limits of the three methods were 100, 5.0 and 5.0 Pg AF/mg human albumin and the use of C18 cartridge (Bijl and Van Peteghem, 1985; Wild et al., 1987) or the use of disposable C18 columns (Qian et al., 1984) and immunoaffinity column for clean-up are of particular concern because of their accuracy, sensitivity and simplicity (Mortimer et al., 1987).

2.2.12.2 Aflatoxins in Milk and Milk Products

The most serious group of aflatoxins concerning animals is a metabolite derived from aflatoxin B₁, which is referred to as a milk toxin or aflatoxin M₁ that is nearly toxic as aflatoxin B₁ (Allcroft and Raymond, 1966; Busby and Wogan, 1979). Milk may be susceptible to aflatoxin contamination when lactating animals consumed aflatoxin B₁-contaminated feed. The carry over of aflatoxin B₁ into milk was reported to be 1 to 2% (Price et al., 1985). Recently, ratios of 66:1 and 75:1 were reported for conversion of aflatoxin B₁ in naturally-contaminated feed to aflatoxin M₁ (Frobish et al., 1986).

Acute toxicity of aflatoxin M₁ was found to be similar to that of B₁ (Purchase, 1967); however, its carcinogenicity is less (Van der Linde et al., 1964). Mutagenicity of aflatoxin M₁ was reported to be similar to that of G₁, but it was only 3% of B₁ (Wong et al., 1976). Hepatocarcinogenicity of aflatoxin M₁ was produced by 500 ppb in a male adult Fisher rat with a potency of 2-10% of aflatoxin B₁ (Hsieh et al., 1985). In addition to aflatoxins M₁ and M₂, an isomer
of aflatoxin $M_1$, which is referred to as aflatoxin $M_4$, is thought to be formed as a metabolite of aflatoxin $B_1$ in naturally contaminated milk and dry milk (Lafont et al., 1986). Mutagenicity of aflatoxin $M_4$ is more than that of aflatoxin $M_1$ and $B_1$, as it produced carcinogenic effects in rainbow trout similar to that produced by $B_2$, and was greater than $M_1$ (Lafont and Lafont, 1987).

Presence of aflatoxin $M_1$ in dairy products has been reported in many parts of the world (Purchase et al., 1968; Sabino et al., 1989). Also, its presence has been reported in meat (Bullerman and Aynes, 1968) and in eggs (Stoloff, 1980). Kiermeir (1973) found aflatoxin $M_1$ in German raw milk at levels of less than $1 \mu g/l$. Stubblefield (1979) reported high levels of aflatoxin $M_1$ in milk of cows consuming corn contaminated with aflatoxin $B_1$ in the southeast of the U.S. In the UK, aflatoxins were detected in milk and milk products in 1978 and 1979 (Anon, 1980). In Italy, Finoli et al. (1983) detected aflatoxins in cheese. Riberzani et al. (1983) found that 25% of 233 samples of powdered milk used for babies was contaminated with aflatoxin $M_1$ levels ranging from 2 to 8 ng/l. Boccia et al. (1985) found aflatoxin $M_1$ in 51% samples collected from individual farms at level of 5-146 ng/l and in 67% of samples of commercial milk at the range of 5-30 ng/l. Visconti et al. (1985) detected aflatoxin in 72% of samples of raw, pasteurized and dried milk.

Like other toxins, the presence of aflatoxins in milk dictates the establishment of decontamination or detoxification methods. The objective would be to produce toxin-free product without
affecting the nutritional value of the product (Doyle et al., 1982). Milk which was contaminated with aflatoxins has been treated with various ways to reduce their levels. Naturally-contaminated milk had been treated with bentonite that effectively absorbed the aflatoxins (Doyle et al., 1982). Oxidizing agents such as hydrogen peroxide and riboflavin were used to inactivate aflatoxin M₁ and both agents were effective and capable of the inactivation of 98% of aflatoxins when used together and with pasteurization. Inactivation of aflatoxin M₁ in naturally-contaminated milk was found to be less than in artificially-contaminated (Applebaum and Marth, 1980). Some trials were made on the effect of sulfite, which was effective, but more research is still required to determine its biological safety. Microorganisms were also effective in the removal of aflatoxins from food. In case of aflatoxin M₁, a bacterium known as Flavobacterium aurantiacum NRR B-184 has successfully removed M₁ from naturally-contaminated milk (Lillehoj et al., 1971).

The detection of significant levels of aflatoxin M₁ in milk and milk products has led developed countries to adopt limits of contamination in feeds used for milking animals. A level of 10 μg/kg aflatoxin B₁ was allowed as a “tolerated” level in animal feed by European communities; while Switzerland has adopted levels of aflatoxin M₁ in milk, reconstituted milk and cheese at 50, 10 and 250 ng/l, respectively. These tolerated levels are 500 ng/l in the USA and 100 ng/l in the Netherlands (Piva et al., 1987).
2.2.12.3 Hazards of Aflatoxins to Public Health

The significant role of aflatoxins in livestock diseases and the demonstration of their carcinogenicity have been studied in details and were confirmed (Moss, 1991). Hepatocarcinogenicity of aflatoxin B₁ in experimental animals gave a strong indication about the relationship between exposure to aflatoxin and hepatocarcinoma in humans (Rodricks et al., 1977). The involvement of mycotoxins in human health and their significant hazard have been intensively studied, however, the assessment of human susceptibility to aflatoxin is difficult to make because it is not possible to administer aflatoxin to humans in toxicological experiments. Data from other primates could be applicable to humans. Useful estimates of human susceptibility and involvement have been gained from studies of human food contamination and observation of severe diseases associated with their consumption (Campbell and Stoloff, 1974).

The most important route of contact of aflatoxins to both animals and humans is through ingestion of contaminated food. In addition to the acquired intoxication that might occur, skin contact of toxic compounds, aerosol inhalation of dust containing fungus may occur. Therefore, laboratory workers involved in the isolation of fungi and in toxin extraction should be aware of the hazard of experimental work in their laboratory. Ingestion of contaminated and mouldy food produced liver damage, cholestasis, necrosis, bile duct proliferation and hepatoma (Grant, 1976). Aflatoxin M₁ is considered as a major metabolite in urine which provides a strong
indication of exposure to aflatoxin B₁. Urinary output of aflatoxin M₁ in China was found to be 10% higher during wet seasons, when the consumption of contaminated rice, corn and alcoholic beverages was high (Sun et al., 1985). The average annual mortality rate in Fusui county, China; during 1971-73, from hepatocellular hepatoma due to hepatitis B and aflatoxin contamination was 20 and 112/100,000 persons for females and males, respectively (Yeh et al., 1985).

Another method to detect exposure to aflatoxins is through the measurement of urinary excretion of 8,9-dihydro-8-(7-guanyl 0-9-hydroxy aflatoxin B₁ (aflatoxin gua), which is formed by a reaction with epoxide, considered to be the ultimate carcinogenic form of aflatoxin B₁. This method was used in a pilot study in Kenya, carried out by Autrup et al. (1985) who reported exposure to aflatoxins in 12% of the people studied. Hayes et al. (1984) reported colon carcinoma in two men involved in aflatoxin purification as well as death from cancer among workers in a Dutch oil processing factory, as a result of a respiratory exposure. Also, aflatoxin B₁ was detected in serum samples of Japanese males at a level of 2.562 ng/ml (Silkichi et al., 1984). Aflatoxin hazards to human respiratory system were reported to be severe, as more aflatoxins can be inhaled from the dust of contaminated grains than by ingestion and, especially, that the lung was found capable of activating aflatoxin B₁ (Jonathan et al., 1990). Human exposure to aflatoxins could be detected by the use of immuno-concentration, followed by high performance chromatography. Aflatoxin B₁, G₁, and Q₁ were detected in the sera of 28 patients and workers in
Banepa, Nepal, at high concentrations due to consumption of aflatoxin contaminated food (Denning et al., 1990).

Safety measures have been undertaken in some developed countries to reduce the possibility of aflatoxin hazards to humans and animals. However, in third world countries; contaminated food may be consumed due to some factors such as food shortage, improper farm practices especially during storage, use of fermented food and food spoilage. Moreover, lack of inspection points at the country's entry ports, use of primitive testing methods, lack of sanitation measures and use of some medicinal plants may result in production of certain pathological lesions similar to those produced by mycotoxins (Ovorackova et al., 1977).

The implication of aflatoxin in human health has been considered by the UNICEF Organization of the United Nations. The first meeting to discuss the problem was held in 1962, in relation to the ARLAC infant food programme in Nigeria. Aflatoxins constitute a prime threat to human foods in tropical countries where environmental conditions such as humidity and primitive life enhance their production. Studies have been carried out to detect the relationship between ingestion of aflatoxin-contaminated food and hepatic disease in many countries. Results of testing human food for the presence of aflatoxins and surveying the incidence of human diseases among those involved in food handling and preparation provide specific information on such health hazards. Also, outbreaks or illness of few persons or children after consuming aflatoxin-contaminated food would add information on
such hazards. Additionally, information could be gained from research on the correlation between aflatoxin levels in foods and the incidence of human liver diseases. The danger of aflatoxins have been reported through surveys, and individual cases in different geographical regions throughout the world.

2.2.12.4 Aflatoxins and Liver Cancer

Liver cancer may be caused by many factors such as hepatitis B virus, cell proliferation, nutritional status that produce diseases; in addition to aflatoxins. Epidemiologists were concerned about providing biological information on the role of aflatoxins in liver cancer, since it was reported to cause at least 200,000 deaths per year. A method involving molecular chemistry was developed to provide accurate data about the risk of liver cancer caused by aflatoxins through the detection of the aflatoxin-DNA adduct (AFB-N7-guanine) in urine (Groopman et al., 1993). Other methods were used to measure human exposure to the aflatoxin carcinogen, such as the use of aflatoxin albumin adducts in peripheral blood which was assayed by complementary ELISA and high performance liquid chromatography (HPLC). This method detected a dietary intake of aflatoxins of 1.4 mg/day in a study carried out with residents of Kenebe, West Kiang, and in Gambia (Wild et al., 1992). Also, RIA was used to detect aflatoxin-albumin adducts in serum in Chongming Island, China (Sheabar et al., 1993). Incidence of primary liver carcinoma has been studied in Uganda during 1966-67, where detectable aflatoxin levels were discovered in stored food products. The liver cancer disease, or primary hepatoma, was fairly
uniform over most of the country. Quality of storage, poverty, and food scarcity were found to be the main factors contributing to the ingestion of contaminated food and hence the aflatoxin hazards (Alpert et al., 1971). Significant correlation between the ingested aflatoxin and liver cancer was found in Kenya. High incidence of liver cancer has been found in Taiwan. The factors which contributed to this are mouldy foods (peanuts and sweet potatoes) due to warm and humid climate (Tung and Ling, 1968).

Peers and Linsell (1973) reported that the frequency of liver cancer increases with a decrease in the altitude. The same findings were reported in Switzerland with no seasonal differences in aflatoxin incidence in diets (Keen and Martin, 1971; Peers et al., 1976). Incidence of liver cancer resulting from aflatoxin-contaminated food was reported in many African and South Asian countries (Payet et al., 1966; Shank et al., 1972). In India, aflatoxins were reported as the prime epidemiological and causative agents of infantile liver cirrhosis. Similar findings have been found in Ceylon, Indonesia, West Africa, Costa Rica, Trinidad, Israel, Lebanon, Syria, Egypt, Burma and the Soviet Republic of Tadzhikistan (Robinson, 1967). Aflatoxins were detected in mother's milk of cirrhotic children and in the urine of sick children. In 1974, an outbreak of acute toxic hepatitis with a high fatality rate of adult humans and dogs was reported in India, as a result of ingesting maize contaminated with aflatoxin (Krishnamachari et al., 1975). Gastrointestinal bleeding, bile duct proliferation and predictable fibrosis jaundice, rapidly developed ascites and portal hypertension were the most common clinical signs reported in the
affected persons. A direct relation was reported between liver cancer and human exposure to aflatoxins in the USA where the risk of death from liver cancer was found to each increase by 0.05% per ng/kg of aflatoxin AFB1. The intake of aflatoxins of 253 ng/day may result in an incidence rate of $1 \times 10^{-5}$ for the USA population (Hoseyni, 1992).

In the UAE, aflatoxins were reported in mother's milk (Saad et al., 1989). Aflatoxins B1, M1, P1 and aflatoxin-N7-guanine were isolated from the urine of 30 males and 12 females with ages of 25-64 years in Guangxi autonomous region, China (Groopman et al., 1993).

2.2.12.5 Aflatoxicosis

Aflatoxicosis is a syndrome caused by the ingestion of aflatoxin-contaminated food or feed. Symptoms in some animals were: lack of appetite, reduced growth rate and/or weight loss. Between 70-80% of aflatoxins consumed by animals were found to be excreted through urine, milk and faeces during 24 hours. The remainder showed its effects in kidney, colon, lung as carcinoma and cancerous lesions. Liver was reported to be the primary organ for aflatoxigenic actions which resulted in inhibition of DNA and RNA synthesis (Rogers and Newberne, 1969). Tumors in tissues other than that of liver have been observed in rats. These include: carcinoma of glandular stomach and mucinous adenocarcinoma of the colon (Newberne and Wogan, 1968). The carcinogenic potency of aflatoxins was established in rats, with B1 being the most potent
followed by B₂ and G₂ (Butler et al., 1969; Wogan, 1976). The acute oral single doses for the toxicity of the 4 kinds of aflatoxins in 1-day old ducklings have been found to be 0.36, 0.78, 1.9 and 3.5 mg/kg body weight for B₁, G₁, B₂, and G₂, respectively (Moss, 1972). Acute toxicity for aflatoxins M₁ and M₂ were determined to be 16.0 and 16.4 μg by body weight concentration in ducklings (Purchase, 1967). Animal species have different degrees of susceptibility and carcinogenicity might be affected by some other factors such as nutritional status of the animals (Roger and Newberne, 1969; Newberne and Rogers, 1971). The hormonal status of the animal was found to have a profound effect on aflatoxin carcinogens (Goodal and Butler, 1969).

2.2.12.6 Aflatoxin Hazard to Infants and Children

Aflatoxin hazards to infants and children may be greater than to adults, since their needs for nutrients and energy are greater because of their fast growth. This makes them exposed to a wide range of food and especially dairy products. Aflatoxins were detected in cereals and legumes in the Sudan at levels exceeding the tolerance levels reported in other countries (Abdel-Rahim et al., 1989). Aflatoxins were detected in rice from parboiled and in raw milled rice in Sri Lanka in the range of 185 μg/kg of AFB₁ and 963 AFG₁ (Bandara et al., 1991). Aflatoxins were detected in cereals, rice, corn, crude sugar, peanut products and pistachio nuts (Tabata et al., 1993). Aflatoxins B₁ and G₁ were isolated from mouldy fish extract (Jonsyn and Lahadi, 1992). Also, it was isolated from beef burger at 8 μg/kg, hot dog 5 μg/kg, sausage 7 μg and 3 μg/kg from
luncheon meat in Egypt (Aziz and Yousef, 1991). Aflatoxin contamination was found in dry figs collected from fields, warehouses and processing units in Turkey during 1986 (Boyacioglu and Gonul, 1990). Dry and paste figs imported from Turkey to the UK in 1988-1989 were found to be contaminated with aflatoxins (Sharman and Gilbert, 1991).

Poverty and lack of food may increase the likelihood for child exposure to aflatoxin. Moreover even in rich countries without testing and establishing limits for aflatoxin contamination the hazard may be greater than in those countries with legislation. Children may be exposed to aflatoxins through different routes such as breast milk, follow up milk, supplementary and artificial food and all other daily products which constitute the main staple during infancy and childhood. Human exposure to aflatoxins may start at the prenatal stage and could continue during breast feeding and subsequently during adulthood (Autrup et al., 1991).

Aflatoxins were reported to play a role in kwashiorkor, increase neonatal susceptibility to infection and jaundice, comprise immune response to prophylactic immunization and may play a role in the pathogenesis of disease (Fukal and Brezina, 1991). Aflatoxin M1 was detected in 0.5% of samples of raw milk in a dairy plant producing milk for baby food in Czechoslovakia and the concentration detected was higher than the 0.1 µg/l which is allowed as a tolerated level.
Neonatal and foetal exposure to aflatoxins may increase the incidence of hazard and it was proved in experimental animals that it may affect the immune and hepatic functions and influence growth (Autrup et al., 1991). Aflatoxins were detected in 53% of blood of mothers at delivery in Kenya which was found to have a direct effect on the birth weight of infants (225g) compared to those born to aflatoxin free mothers (De-Vries et al., 1989). Aflatoxin M\textsubscript{1} and G\textsubscript{1} were detected in breast milk of lactating mothers in Zimbabwe (Nyathi et al., 1989), Gambia and West Africa (Zabra et al., 1992). The orally-lethal dose (\textit{LD}_{50}) of aflatoxins in rats was 7 and 7.8 mg/kg of body weight for males and females, respectively (Bourgeois et al., 1971). Children may be affected by lower doses, as in the case of a 15-year old boy, weighing 36 kg, who died after eating cassava-contaminated with 1.7 mg/kg aflatoxins in Uganda (Serck-Hansen, 1970). Also in Senegal, Kenya, Uganda and Gambia; aflatoxin-albumin adduct (AFB\textsubscript{1}-Lysine) up to 350 were detected in the sera of children (Wild et al., 1990; Allen et al., 1992). In 1988, 13 children between 2.5 and 11 years of age died as a result of consumption of contaminated noodles in the State of Perak in Malaysia. Clinical, pathological and histological examinations revealed that death was due to a combination of toxins, amongst which aflatoxins were identified (Cheng, 1992).

Aflatoxins were detected in the serum and urine of 74 children suffering from kwashiorkor in Durban, South Africa (Ramjee et al., 1992). On the other hand, Househam and Hundt (1991) studied the relationship between kwashiorkor and aflatoxins and concluded that aflatoxin had no role in this disease.
Hendrickse (1991) reported that prenatal exposure to aflatoxin and breast-fed infant with aflatoxins in the mother's milk, affect the immune and hepatic functions. Also, the growth and development of these infants were affected and, hence, aflatoxin contamination played an essential role in the etiology of kwashiorkor in the tropics. Aflatoxins B1 and M2 were detected in the serum, urine and stools of children with kwashiorkor and marasmic kwashiorkor (De Vries et al., 1990).

The maximum acceptable levels of aflatoxins in milk and infant food have been established in some countries. This information is shown in Table 2.1.

2.2.12.7 Role of Aflatoxin B1 in Reye Syndrome in Children

Reye et al. (1963) described a group of children with similar clinical and pathological symptoms and concluded that the disease resulted from acute encephalopathy and fatty degeneration of the viscera. The disease started with mild upper respiratory infections; followed by disturbances in consciousness, fever, convulsions, vomiting, altered respiratory rhythm, abnormal muscle tone and abnormal reflexes. Reye disease was recorded in Sydney, Australia, between 1950 and 1966 (Reye et al., 1963) and in Auckland, New Zealand, between 1959 and 1966 (Becroft, 1966). Association between aflatoxin B1 and Reye syndrome has been reported in New Zealand (Becroft and Webster, 1972), Czechoslovakia (Dvorackova et al., 1977), USA and Thailand (Nell et al., 1979).
Table 2.1: Established Maximum Acceptable Levels of Aflatoxins in Milk and Infant Food in some Countries (Van Egmond, 1989)

<table>
<thead>
<tr>
<th>Country</th>
<th>Commodity</th>
<th>Limit µg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Argentina</td>
<td>Infant food based on milk products</td>
<td>0.1</td>
</tr>
<tr>
<td>Austria</td>
<td>Pasteurized fresh milk for infants and children: children foods calculated on reconstituted product</td>
<td>0.01</td>
</tr>
<tr>
<td>Czechoslovakia</td>
<td>Infants food on milk basis calculated as reconstituted product</td>
<td>0.1</td>
</tr>
<tr>
<td></td>
<td>Other infant foods and children foods</td>
<td>1.0</td>
</tr>
<tr>
<td>France</td>
<td>Milk powder for infant foods</td>
<td>0.2</td>
</tr>
<tr>
<td>Federal Republic of Germany</td>
<td>Milk for infant foods</td>
<td>0.01</td>
</tr>
<tr>
<td>Switzerland</td>
<td>Milk for infants or infant foods</td>
<td>0.01 (M₁ + B₁)</td>
</tr>
<tr>
<td>Union of Socialist Soviet Republics</td>
<td>Children food</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Milk and milk products</td>
<td>0.5</td>
</tr>
</tbody>
</table>
2.2.12.8 Aflatoxin in Animal Feed

Natural occurrence of aflatoxins $B_1$, $B_2$, $G_1$ and $G_2$ in various agricultural commodities which are used as animal feed ingredients have been reported in many countries (Jones, 1975). Presence of aflatoxin $M_1$ also was reported in corn, along with aflatoxin $B_1$ (Shotwell et al., 1976). In the USA, aflatoxins have been detected in corn and a maximum level of contamination of 101 mg/kg of aflatoxin $B_1$ was obtained. Moreover, during 1988 corn was affected by a serious drought and higher incidence of aflatoxins was evident in the product of that year (Wood, 1992). In Japan, aflatoxin $B_1$ of up 50 ppb has been detected in peanuts imported from USA (Manabe et al., 1971). In Poland, a survey of occurrence of aflatoxins in 306 samples of animal feed revealed that aflatoxins were present in 12.7% with level of 100 $\mu$g/kg in 4.2% while 2.6% of the samples contained more than 1000 $\mu$g/kg. In the same survey, aflatoxins at a level of 300 $\mu$g/kg has been detected in feed rations used for cattle and sheep (Strzelecki and Gasiorowska, 1974). In Germany, aflatoxin levels ranging between 7 and 300 $\mu$g/kg has been detected in mixed feed (Seibold and Ruch, 1977). In the UK, 95 samples out of 172 samples of animal feeds analyzed for aflatoxins were found to be contaminated with 1-350/ (WHO, 1979). In the USSR, a survey of aflatoxins in domestic and imported commodities showed aflatoxin contamination in 2.2% of corn, 28.3% of cotton seeds and 26.9% in peanuts, where maximum levels of 3650, 600 and 3650 $\mu$g/kg were detected in these crops; respectively (Tutelyan et al., 1989).
CHAPTER 3

MATERIALS AND METHODS

3.1 Survey of Infant Feeding Methods and Growth in Al Ain, UAE

This survey was carried out in the city of Al Ain, about 160 km east of the city of Abu Dhabi. Its population is about 200,000 and consists predominantly of expatriates. However, Al Ain has a high percentage of UAE nationals, when compared to other cities.

A questionnaire (Scheme 1) was distributed to mothers during the period between 1989 and 1990. Mothers were interviewed at 4 primary health clinics in Al Ain. Information pertaining to the baby's sex, age (months), height (cm) and weight (kg) were gathered, with the help of the health-care staff. Data were collected from 375 mothers and 300 children were involved in the assessment of feeding methods and growth. Feeding methods studied included: use of breast milk as a sole method of feeding, supplementation of breast milk with formulae or fresh milk (cow or goat milk) and the supplementation with baby food. For each feeding method, 100 infants (50 males and 50 females) were included in this survey. Weights (Kg) and heights (cm) were measured and the growth was determined by using the Toxford equation for growth which employs weight, height and age for each sex (Abdelgader, 1990), as follows:

\[
\text{Male} = \frac{\text{weight, lb}}{\text{height, inch}} \times \left( \frac{336 - \text{age [month]}}{270} \right)
\]

\[
\text{Female} = \frac{\text{weight, lb}}{\text{height, inch}} \times \left( \frac{336 - \text{age [month]}}{235} \right)
\]
3.1.1 SCHEME 1

The questionnaire used to obtain information about infants, from 375 mothers in Al Ain, UAE
(Prepared by : Nawal Ahmed Osman)

Mothers: Please note the information provided by you is purely for research purposes and will be kept absolutely confidential.

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>(1) Baby sex:</td>
<td>(2) Nationality:</td>
</tr>
<tr>
<td>(3) Mother's age:</td>
<td>(4) Education:</td>
</tr>
<tr>
<td>(5) Occupation:</td>
<td>(6) Baby's age:</td>
</tr>
<tr>
<td>(7) Baby's height</td>
<td>(8) Baby's weight:</td>
</tr>
</tbody>
</table>

Mark the correct answer only. If your answer is other than what is mentioned below, then please write what we have missed in the blank space:

1. Food(s) that is/are used for your child:
   (9) Breast milk only
   (10) Breast milk and other kinds of milk:
   (11) Fresh milk
   (12) Pasteurized cow milk
   (13) Skimmed milk
   (14) Condensed milk
   (15) Baby milk formulas (specify)

Please determine the number of times of breast milk and the number of times of other kinds
(15) Breast milk + some baby (Please specify)
   Other kinds of milk + some baby food
(16) Fresh milk + baby food
(17) Camel milk + baby food
(18) Powdered milk + baby food
(19) Condensed milk + baby food
(20) Baby milk + baby food.

2. Determine the lactation period of your child:
   (21) Not at all
   (22) A few days
   (23) 1-2 months
   (24) 3-6 months
   (25) 7-9 months
   (26) 10-12 months
   (27) 13-18 months
   (28) 18 months-2 years
   (29) more than 2 years

3. Who terminated the breast feeding?
   (30) Medical advice
   (31) The baby
   (32) The mother
   (33) Other factors (specify)

4. Determine the staple food(s) that is used for your child:
   (34) Rice
   (35) Maize
   (36) Wheat
   (37) Sorghum
5. Names of beans and peas that are used for your child:
   - Chickpea
   - Cowpea
   - Broad beans
   - Pigeonpea
   - Lentils
   - Sesame seeds
   - Milon seeds

6. Please specify the main foods from animal sources that are used for your child:
   - Meat
   - Chicken
   - Fish
   - Eggs
   - Cheese
   - Butter
   - Yougurt
   - Butter cream

7. How often you bring your child to the health clinic?
   - At least once a week
   - Once in every 2 weeks
   - Monthly
   - Irregular (please specify)

8. Do you use ready-made baby food? If yes, please specify:
   - Cerelac
   - Milupa
   - Farlay’s rusks
   - Milupa 5 cereals
   - Milupa rice with milk
   - Milupa 3 cereals
   - Fargilia
   - Mixed cereals
9. Please specify the local weaning mixes used for your child:

(70) 2 mixes:

______ + ______
______ + ______
______ + ______

(71) 3 mixes:

______ + ______ + ______
______ + ______ + ______
______ + ______ + ______

(72) 4 mixes:

____ + ______ + ______ + ______
____ + ______ + ______ + ______
____ + ______ + ______ + ______
3.2 Materials

3.2.1 Kinds of Food Used for the Study

3.2.1.1 Supplementary formulae

Milumil baby milk formulae (Milupa)
S26 baby milk formula

3.2.1.2 Follow-up (Raw milk)

Cow milk
Goat milk
Camel Milk

3.2.1.3 Cereal Based Baby formulae

Wheat based formula (Cerelac, Nestle)
Wheat based formula mixed with fruits (Nestle)
Baby food composed of 3 cereals (Milupa)
Baby food composed of 5 cereals (Milupa)
Teething rusks (Milupa)
Teething rusk (Farley's)
Rice mixed with milk (Milupa)

3.2.1.4 Cereals Grains

Short grain rice (imported from Egypt)
Long grain rice (imported from Pakistan)
3.2.1.5 Animal Feed

Maize, barley, oat, soyabean, wheat bran, compound feed and dates.

3.3 Collection of the Samples

Data obtained from the municipality of Al Ain showed that baby food is available in many large and small stores located throughout the city. The city was divided into 4 regions and samples of food were collected from large stores (LS) and small stores (SS). This classification is according to the location of the primary health care clinics from where the questionnaire data was obtained.

3.3.1 Samples of Baby Milk Formulae

During the period of 1989-1990, 16 samples of Milumil and 20 samples of $S_{26}$ were collected. Also, the same number of samples was collected during 1990-1991 from large and small stores.

3.3.2 Samples of Cereal-Based Food

Samples of baby food were collected from each source and kept in sterile bags at $-20^\circ$C until analysis time (Table 3.1).
Table 3.1: Samples of baby food collected for the study

<table>
<thead>
<tr>
<th>Food Type</th>
<th>Sample No.</th>
<th>Sample No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cerelac Wheat</td>
<td>32</td>
<td>32</td>
</tr>
<tr>
<td>Cerelac Wheat + 4 fruits</td>
<td>32</td>
<td>32</td>
</tr>
<tr>
<td>Milupa 5 Cereals</td>
<td>25</td>
<td>18</td>
</tr>
<tr>
<td>Milupa 3 Cereals</td>
<td>28</td>
<td>16</td>
</tr>
<tr>
<td>Milupa Rice with Milk</td>
<td>20</td>
<td>32</td>
</tr>
<tr>
<td>Milupa Teething Rusks</td>
<td>25</td>
<td>30</td>
</tr>
<tr>
<td>Farley's Rusk</td>
<td>28</td>
<td>16</td>
</tr>
<tr>
<td>Baby Milk Formula (Milumil)</td>
<td>16</td>
<td>16</td>
</tr>
<tr>
<td>Baby Milk Formula S_{26}</td>
<td>20</td>
<td>20</td>
</tr>
</tbody>
</table>
3.3.3 Samples of Rice

Hundred samples of rice (50 short grain and 50 long grain) were collected from households of different families of those contributing to the questionnaire of this study. Each sample weighed 150 g and was kept in a sterile plastic bag and was stored at -20°C until the analysis time.

3.3.4 Samples of Raw Milk

Milk samples were collected in sterile 1 litre stoppered bottles, delivered immediately to the laboratory, the first portion was used for nutritional analysis and the second portion was kept at -20°C for aflatoxin analysis.

3.3.4.1 Cow Milk

Cow milk samples were obtained from three different farms designated as A, B and C. Farm C is a large farm that provides Al Ain dairy factory with 50% of its product. A total of 22 samples were collected as follows, 7 samples from farm A, 8 samples from farm B and 7 samples from farm C.

3.3.4.2 Goat milk

Goat milk samples were collected from the same three farms mentioned above; in addition to one more farm, referred to as source D. A total of 34 samples were collected as follows: 6 samples
from farm A, 6 samples from farm B, 13 samples from farm C and 9 samples from farm D.

### 3.3.4.3 Samples of Camel Milk

Twenty-five milk samples collected from camels raised in private farms. Milk samples were collected daily in the morning in sterilized bottles (100 ml). The samples were kept in the refrigerator for analysis.

### 3.3.5 Samples of Animal Feed

Samples of maize, barley, oat, soybean, wheat bran, compound feed and dates were collected in sterile plastic bags from the same sources from where milk samples were obtained and stored at -20°C until analysis time.

### 3.4 Physical Characteristics of Tested Samples

General physical characteristics of each food were recorded such as odor, colour, texture and pH.

#### 3.4.1. Moisture Content

The moisture content was determined by the method of Hart *et al.* (1959). Three sub-samples, each weighing 5 g, from each sample were weighed into moisture tanks which were previously dried at 100°C, and their weights were taken. The moisture tanks
were uncovered and placed with their covers in an oven adjusted to 103±1°C for 72 h. The moisture tanks were then transferred to a desiccator till they reached room temperature and their weights were taken. The moisture content was determined from the reduction in weight, and calculated as a percentage.

3.4.2 Measurement of pH

The method of the official method of (AOAC, 1984) for pH measurement for cereal products was used. Ten grams of each sample of food were weighed in a dry Erlenmeyer flask and 100 ml of boiled water were added at 25°C, shaken until all food particles were evenly mixed. The mixture was processed for 30 minutes shaking and let to stand for 10 min. The supernatant was decanted into an H-ion vessel, and the pH was read after calibrating the potentiometer by buffer solutions of pH 4.0 and 9.0 at 25°C.

3.5 Microbiological Studies of Tested Food Samples

3.5.1 Surface Sterilization

Three samples (each 100 grains of rice) picked randomly and surface sterilized by soaking in 0.01% aqueous mercuric chloride for five minutes, washed with distilled water and dried at 70°C. Three moistened sterile filter papers located in petri dishes where rice grains were plated and incubated at 37°C for one week and examined on daily basis for microbial growth.
3.5.2 Inoculum

In all the experiments, inoculum was prepared by growing moulds; isolated from naturally infected baby food, rice and animal feeds onto plates of a PDA medium, which was incubated at 25-30°C for 7-10 days until good spore production occurred. The fungus *A. flavus* was also inoculated into PDA, MEA and AFPA; then was incubated at both room temperature and 37°C. Daily examination was carried out for visual growth.

3.5.3 Spore Suspension

A spore suspension was prepared according to Karunaratne and Bullerman (1990) by growing *A. flavus* isolated from rice grains on 3 PDA slants for 7 days until good sporulation was obtained. Spores were harvested by washing with a sterile phosphate buffer solution, fortified with 0.05% Tween 80. The spores were loosened by gentle brushing with a swab and filtered 3 times through a sterile cheese cloth. The concentration of the spores was determined by using viable spore count on PDA plates and a concentration of $10^7$ ml$^{-1}$ was prepared as stock.

3.5.4 Fungal Assessment

A count of 100 grains of rice and each animal feed tested were surface-sterilized by soaking in 0.1% mercuric chloride (v/v), washed thoroughly with sterilized distilled water, dried at 70°C for 48 h, then plated on 3 moistened sterile filter papers in a petri dish
and incubated at 30°C for 5 days. Examination of visual fungal growth was made daily. The microbiological flora of baby food and baby milk was determined by shaking 1 g of each sample in 9 ml of sterile distilled water. Ten-fold serial dilutions were prepared and three plates from each medium was used for each dilution. A volume of 0.1 ml of dilution 10⁻¹ was plated on AFPA and DG₁₈% media and 0.1 ml of dilution 10⁻² was used for the NA medium. Plates were incubated at 25°C and observed daily for fungal growth for 14 days.

3.5.5 Aflatoxin Production by Isolate of Aspergillus flavus

A. flavus obtained from naturally-infected seeds of short grain and long grain rice were maintained in Czapek Dox Agar at 40°C. The growth and aflatoxin production ability were tested by cultivating them on Czapek Dox broth (CDB) liquid medium. Twenty four flasks were used for each isolate (3 flasks for each experiment); each flask containing 100 ml of the medium was sterilized by autoclaving at 121°C for 15 minutes, and inoculated with 0.1 ml spore suspension. The flasks were incubated at 37°C on a rotary shaker at a speed of 200 rpm for up to 14 days. Three flasks were removed daily from 4 to 14 days after incubation period autoclaved and were examined for the mycelial weights and aflatoxin content in the remaining culture filtrate. Aflatoxins were extracted from the liquid medium according to the method of Badii and Moss (1988) and analyzed according to Moss and Badii (1982).
3.6 Aflatoxin Determination

3.6.1 Extraction

3.6.1.1 Extraction of Baby Milk Formulae

The official method for AFM in dairy products AOAC (1984) was used for extraction of powdered milk. Ten grams of powdered milk mixed with 100 ml distilled water. Ten grams of diatomaceous earth and 300 ml of acetone were added. The mixture was stirred by a wrist action shaker for 30 min, then was filtered through folded 32 cm Whatman No. 2 filter paper into a 500 ml graduated cylinder. A volume of 275 ml of the filtrate was transferred to a 600 ml beaker containing 20 ml lead acetate solution (200 g of lead acetate trihydrate dissolved in warm water +3 ml acetic acid and made up to 1 litre), stirred and was left to stand for 5 min to precipitate; then 10 ml of a saturated sodium sulphate solution were added and the mixture was filtered. The other 335 ml of the filtrate were transferred to a separator funnel and were extracted with hexane (2x100 ml). The Hexane layer was discarded and the aqueous phase was then extracted with chloroform (3x50 ml). The chloroform was washed with 5% sodium chloride solution and drained through an anhydrous sodium sulphate pad. The chloroform was evaporated on a steam bath, under a gentle stream of nitrogen, to near dryness and the remainder was transferred to a vial and evaporated to dryness and saved for aflatoxin determination.
3.6.1.2 Extraction of Food Samples

The best food (BF) method of AOAC (1975) was used for extraction of baby food. A 5-g sample was blended with 250 ml methanol water mixture (150:100) in a blender and the mixture was filtered through a Whatman No. 4 filter paper, then the filtrate was shaken with hexane (2x100 ml). The aqueous layer was extracted with chloroform (3x25 ml) and the chloroform layers were drained through a bed of anhydrous sodium sulphate. The chloroform was evaporated to near dryness on a steam bath in an extracting fume hood. The residue was dissolved in 10 ml chloroform and was transferred quantitatively to a vial for a determination by TLC.

3.6.1.3 Extraction of Fresh milk

3.6.1.3.1 AOAC Method

The AOAC method for detection of aflatoxin in fresh milk was employed in this study. According to Van Egmond (1981), 75 ml of fresh milk were mixed with 300 ml of methanol, shaken for 3 min and then shaken for 30 min after addition of 25 g of diatomaceous earth. The sample was filtered through a 1 cm pad of diatomaceous earth (S&S filter papers). The residue was washed with 75 ml of methanol. The filtrate was transferred to a separator funnel, then 225 ml of 4% sodium chloride solution were added and the mixture was extracted twice with 100 ml hexane. The hexane layer was discarded and the aqueous phase was extracted with chloroform (3x25 ml). The combined chloroform was washed with 300 ml of 4%
sodium chloride and drained through a pad of anhydrous sodium sulphate. The chloroform extract was evaporated to near dryness and transferred to a vial for evaporation to dryness under a stream of nitrogen.

3.6.1.3.2 Aflatoxin M₁ Easi-Extract Column (TD 120)

A volume of 100 ml of fresh milk and powder milk reconstituted by stirring 10 grams of the milk powder in 100 ml warm water, were centrifuged at 3400 rpm for 15 min, to remove fat. The column was prepared by clamping the empty barrel of the disposable syringe in a vertical position in a stand above a waste collector. The TD120 column was removed from its sachet and both lower and upper caps were removed and the longer end was pushed into the syringe barrel. About 10 ml of PBS were pushed at a slow rate in about 15 sec through the syringe into the column. Aflatoxins were extracted by applying 10 ml of the defatted milk onto the column at a rate of 10 ml/min and the eluent was discarded. The column was washed with 10 ml of PBS and the washings were also discarded. Aflatoxin M₁ was eluted with 2 ml methanol (HPLC-grade) and the eluent was collected in a clean stoppered flask. The volume of the eluent was reduced to 200-300 μl in a water bath adjusted to 20°C with the help of a stream of nitrogen. The reduced volume was measured accurately by using a micro-syringe and the volume was made up to 500 μl with a methanol: water mixture (1:1).
3.6.1.3.3 Extraction of Aflatoxins on a C₁₈ Cartridge

The C₁₈ cartridge was attached to a syringe which was assembled with a vacuum flask at a vacuum of 5 mm Hg. A volume of 5 ml of methanol was added to the cartridge, which was followed by 5 ml of water. The cartridge was removed from the assembly, dried and replaced. The sample of milk was diluted by adding 20 ml of milk to the same amount of warm water and was applied in the cartridge with a gentle flow rate of 30 ml/min, then was washed with 10 ml water-acetonitrile solution. The cartridge was removed, dried with a tissue paper, placed on a silica gel clean-up column, defatted with ether and eluted with dichloromethane-ethanol into a collecting tube, evaporated to near dryness under nitrogen on steam bath and was stored for LC analysis.

3.6.1.4 Extraction of Animal Feed

Aflatoxin extraction was carried out according to the method described by Tutelyan et al. (1989). A 25 g sample of each finely-ground feed was extracted with 125 ml of an acetone:10% aqueous sodium chloride (4:1) for 30 min on a wrist action shaker. The extract was filtered through S&S filter papers and 50 ml of the filtrate were mixed with 50 ml of 10% aqueous lead acetate, which was allowed to stand for 10 min and then was filtered again. A volume of 80 ml of the filtrate was transferred to a separatory funnel, defatted twice with 50 ml hexane and the hexane layers were discarded. Aflatoxins were extracted from the aqueous layer with chloroform (3x30 ml). Chloroform layers were retained through
a pad of anhydrous sodium sulfate and evaporated to near dryness in a steam bath and to dryness with a stream of nitrogen.

3.6.2 The Clean Up Process

3.6.2.1 Clean Up Using AOAC

Aflatoxin in the extract residue was cleaned-up according to the method of AOAC (1990). A small piece of glass wool was fitted on the disc of a chromatographic column and covered with 2 cm of coarse anhydrous sodium sulphate. About 2 g of silica gel were slurried in 10 ml ether:hexane mixture (v/v) in a 15 ml beaker and poured into the column. When the silica gel settled, another 2-cm length of sodium sulphate were applied carefully to the top of the silica gel in the column and the solvent wash was drained to the top of the sodium sulphate layer. The dry extract of the sample was dissolved in about 2-3 ml dichloromethane, applied gently onto the column and the beaker was washed twice and added to the column. The dichloromethane was drained up to the sodium sulphate layer and the column was washed with 25 ml of toluene-acetic acid, then with 50 ml of the ether:hexane mixture and the wash was discarded. Aflatoxins were eluted with 60 ml of a dichloromethane:acetone mixture (v/v) into a 250 ml beaker, evaporated to near dryness on a steam bath and were transferred into 2-4 dram vial, evaporated to dryness under a stream of nitrogen and were kept for aflatoxin analysis.
3.6.2.2 Clean Up Using an Affinity Column

The affinity column was prepared by removing the cap from the top of the affinity column and by cutting off the sealed end. The bottom plug was loosened and the column firmly attached to the 10 ml syringe and placed on the clamp stand. The bottom plug was removed and the hand pump was placed, fully extended on the syringe barrel using a rubber connector to ensure a good seal. A volume of 100 ml of the extract was pushed in at a slow rate, the hand pump was removed and 10 ml water was added to the syringe barrel to wash the column. The washing process was repeated to ensure a thorough washing. The column was eluted by addition of 1 ml HPLC-grade methanol at a flow rate of 1 drop/sec. The eluent was kept in a glass tube, to which 1 ml of distilled water and 1 ml of chloroform were added. This mixture was shaken gently, to allow for settling, and the chloroform layer was drawn by a pipette and was placed into 2-4 dram vial and evaporated to dryness under a stream of nitrogen and kept for aflatoxin analysis.

3.6.3 Methods of Aflatoxin Analysis

3.6.3.1 Thin-layer Chromatography (TLC)

The TLC method described by Van Egmond (1981) was used. Pre-coated keiselgel -60 plates (20x20 cm and 20x10 cm) were used, with a 0.25 mm layer of absorbent. The plates were activated for 2 h at 80°C in an oven, stored in a desiccator over a silica gel
desiccant. The dry extracts of the sample were dissolved in 100 μl chloroform.

3.6.3.1.1 Preliminary TLC (Unidirectional)

Aliquots of 2, 5 and 10 μl from each sample extract were spotted on a TLC plate, 1 cm apart and on an imaginary line 4 cm from the plate bottom. On the sample plate 1, 3 and 5 μl of aflatoxins standards B1, G1, G2 and B2 were spotted. A line 2-4 cm from the top and a line about 0.5 cm from each edge were drawn. A 50 ml of the development solvent [chloroform:acetone, 9:1 (V:V)] were placed into the unlined chromatotank. The plate was withdrawn from the developing tank and was air-dried in the dark for 15 min before viewing under a UV lamp at a wavelength of 365 nm.

3.6.3.1.2 The Two-Dimensional TLC

In this procedure, a line is drawn 1 cm from the bottom of the plate. The plate is turned 90° and another line is marked. A volume of 5 μl of the sample is spotted on the junction of the two lines 2, 4, 6 μl of the standards were spotted at the end of the first direction 4 and 6 μl of the second direction. A spiked plate is prepared, similarly to that discussed above except that 5 μl of standard is applied on top of the sample as an internal standard. The developing solvents were chloroform:acetone (9:1) for the first direction and chloroform: acetone: isopropanol (85:4:10) for the second direction.
3.6.4 Confirmation of the Presence of Aflatoxins

For Aflatoxin B and G confirmation, the method of Smith and McKerman (1962) was used. The presumed aflatoxin spot from the sample and the aflatoxin standard were sprayed with 50% diluted 2M sulfuric acid, which changes the fluorescence to yellow in the presence of aflatoxin. The AOAC (1984) official method was used to confirm the presence of aflatoxin M₁ and derivatization was carried out by the application of 2 μl of trifluoroacetic acid on the sample and standard and the undeveloped plate was placed in an oven at 105°C for about 1 min and developed normally.

3.6.4.1 High Performance Liquid Chromatography (HPLC)

The HPLC used in this study was that of Horwitz (1987). The column was ODS (Spherisorb S5 ODS₂, 25 x 4.6 mm) located in an oven adjusted to 30°C. The dry extract was diluted with methanol: water mixture (1:1) and 200 μl were injected into the column. The mobile phase was water: methanol: acetonitrile (130:70:40) at a flow rate of 0.75 ml/min for food, rice and animal feed. Aflatoxin M₁ used as standard for HPLC was prepared from dry powder obtained from Sigma (Sigma Chemical Company, Fancy Road, Poole, Dorset, BH 17, England). A stock solution of 0.5 ug/ml in dimethylsulfoxide was prepared and stored at -18°C. Working standards were prepared fresh on daily basis by diluting the stock solution 100 fold in methanol. The eluate of the affinity columns and C₁₈ cartridge was concentrated to about 100 μl and injected into HPLC microsorb C₁₈ eluted with methanol: water (1:1) mobile phase at flow rate of 0.75
ml/minute, using LKB pump. Detection was carried by absorbency at 365 nm using LKB UV detector. Peaks were identified by an integrator with reference to the peaks of the standards. Concentration of Aflatoxins (µg/kg) was calculated as follows:

\[
\{H^* \times C \times V_1 \times V \div \{H \times V_1^\# \times W\},
\]

where:

- \(H^*\) = Peak heights of standard and sample, respectively
- \(C\) = Concentration of standard ng/µl
- \(V_1\) and \(V_1^\#\) = volumes of injected standard and sample, respectively
- \(W\) = Weight or volume of sample represented by final extract.

3.7 Analysis of Major Nutritional Components of Milk

Milk samples were analyzed for fat, protein, lactose and total solids by a milk scanner (Multispec M). This instrument functions as an infrared analyzer. Certain of molecular vibrations of the major nutrients pick infrared radiations and are absorbed at specific wavelengths. By measuring the level of absorption at these wavelengths, quantitative determination of these components can be obtained.

3.7.1 Determination of Fat in Milk

A 10ml of homogeneous sample was extracted according to the method of Rose-Cottlieb (1963). The milk sample was treated with 1 ml of 0.88 ammonia and 10 ml ethyl alcohol with mixing.
ml of diethyl ether was added to the tube with vigorous shaking for 1 minute. 25 ml of petroleum ether were added to the tube which was shaken vigorously for half a minute and was left to stand for 30 min until the layers had separated. The ether layer was transferred to a dried flat bottom flask which was connected to rotary evaporator. After solvent evaporation took place, the flask with the contents were dried in an oven adjusted at 60°C. The flask was weighed and the fat % was calculated as follows:

\[
\text{Percentage of fat} = \frac{\text{weight of fat}}{\text{weight of milk}} \times 100
\]

3.7.2 Mineral Analysis

3.7.2.1 Minerals in Milk

10 grams of milk were weighed into an ashing crucible to which 1 drop of glacial acetic acid was added. The milk is heated in a hot plate at 137°C until the residue turned brown and then transferred to a water bath to evaporate the water. The ashing dishes were transferred to a furnace (500±1°C) and were left overnight. The dishes were removed and placed in a desiccator until they reached room temperature. The ash was washed with 10 ml distilled water and transferred quantitatively to a 250-ml beaker, 12 ml of hydrochloric acid and 3 ml of nitric acid were added and the dish was heated gently for 10 min. A volume of 100 ml of hot water was added and the mixture was heated to a boil, then filtered into a 250-ml volumetric flask and was filled up to the mark. The resultant solution was taken for mineral assay by employing Pye

3.7.2.2 Determination of Minerals in Food

5 grams of a mixed sample were weighed into an ashing dish already ignited, cooled in a desiccator and weighed after reaching room temperature. Ignition was made in furnace at 550°C overnight. The ash was washed by 10 ml distilled water and transferred to 250 ml beaker and 12 ml of concentrated hydrochloric acid and 3 ml of concentrated nitric acid were added. The beaker was sealed and heated gently for 10 minutes. 100 ml of boiling water were added and the mixture was heated for another 10 minutes, cooled, washed, and filtered through Whatman No. 2 filter paper in 250 ml volumetric flask and the volume was completed to the 250 ml mark and was kept for mineral analysis using an atomic absorption.
CHAPTER 4

RESULTS

4.1 Survey of Infant Feeding Methods and Growth

The characteristics of the mothers involved in this study are shown in Table 4.1. Several nationalities were included, with the majority being UAE nationals. Mothers age ranged from 15 to 45 years and 69.4% of them were educated while those of the remainder were non-educated. Most mothers, 78.7%, were not committed to outside jobs. The infants included in this study were healthy and were brought to the vaccination units at regular times where the data of this study was collected. The mean age of infants was 9.1±6.3 months, with a minimum age of one month and a maximum of 36 months. The weight of infants was 8.1±2.4 kg and the height was 67.6±11.1 cm.

4.1.1 Exclusive Breast Feeding

Results of the survey showed that exclusive breast feeding is used by 28% of the mothers included in the study for different durations, depending on nationality. The UAE and Omani mothers used to give exclusive breast feeding for a period of 12 months, Jordanians and Palestinians for 9 months, Sudanese and Syrians for 6 months and Egyptians for 3 months. Statistical analysis showed no significance of education, occupation and mother's age when exclusive breast feeding was practiced.
Table 4.1: Characteristics of Mothers involved in the study

<table>
<thead>
<tr>
<th>Character</th>
<th>Number</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nationality:</td>
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<td></td>
</tr>
<tr>
<td>Egyptian</td>
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<td>15.7</td>
</tr>
<tr>
<td>UAE</td>
<td>88</td>
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</tr>
<tr>
<td>Indian</td>
<td>37</td>
<td>9.8</td>
</tr>
<tr>
<td>Jordanian</td>
<td>42</td>
<td>11.2</td>
</tr>
<tr>
<td>Omani</td>
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<td>5.8</td>
</tr>
<tr>
<td>Pakistani</td>
<td>20</td>
<td>5.3</td>
</tr>
<tr>
<td>Palestinian</td>
<td>32</td>
<td>8.3</td>
</tr>
<tr>
<td>Sudanese</td>
<td>32</td>
<td>8.2</td>
</tr>
<tr>
<td>Others</td>
<td>44</td>
<td>11.7</td>
</tr>
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<table>
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<tr>
<th>Mother's age (yr.):</th>
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<th>Percentage</th>
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<tr>
<td>16-20</td>
<td>22</td>
<td>5.8</td>
</tr>
<tr>
<td>21-25</td>
<td>58</td>
<td>15.4</td>
</tr>
<tr>
<td>26-30</td>
<td>166</td>
<td>44.2</td>
</tr>
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<td>31-35</td>
<td>78</td>
<td>20.8</td>
</tr>
<tr>
<td>36-40</td>
<td>36</td>
<td>9.6</td>
</tr>
<tr>
<td>41-45</td>
<td>15</td>
<td>4.0</td>
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</table>

<table>
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<th>Percentage</th>
</tr>
</thead>
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<tr>
<td>Occupied</td>
<td>80</td>
<td>21.3</td>
</tr>
<tr>
<td>Not occupied</td>
<td>295</td>
<td>78.7</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Education:</th>
<th>Number</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Educated</td>
<td>260</td>
<td>69.4</td>
</tr>
<tr>
<td>Not educated</td>
<td>115</td>
<td>30.6</td>
</tr>
</tbody>
</table>
4.1.2 Artificial Feeding

It can be seen from Fig. 4.1, that 3.5% of the infants did not receive breast milk from the beginning and were introduced to artificial food from the first day. Most of the mothers of this group were Egyptians with only 2 mothers from Jordan and one from Oman. The age of the mothers in this group ranged between 26-45 years and none of them were involved in outside commitment, except one woman who was occupied on half-day work. Statistical analysis revealed a highly significant role of the mother's nationality and education in her decision of not practicing breast-feeding (P<0.01).

4.1.3 Mixed Feeding

Only 1.9% of the infants were breast-fed for a few days but less than one month. Significant differences between different age groups, educated and non-educated occupied and unoccupied (P<0.05) were present. Significant differences among nationalities in practicing breast-feeding for a few days and most of them were of the Egyptian nationality (P<0.01). Nearly 6.7% of infants were breast-fed for 2 months only and results showed that about 52% of mothers in this group were uneducated or had minimum education, 95% were housewives who had no other occupational involvement. Their ages ranged between 16-45 years and they were from different nationalities. Statistical analysis showed a non-significant effect of mother's education, occupation and age on practicing breast feeding for this period (P<0.05).
Fig. (4.1) : Trend of Breast Feeding According to Infants Age
Generally, the results showed that 45.6% of infants were breast-fed for a period of 4-6 months and statistical analysis showed no significant differences between mothers of different nationalities, different age groups, different occupations and different educational levels. A significant difference (P<0.01) was present among educated and non-educated mothers and it was observed that the majority of mothers (89%) who practiced breast-feeding for 9 months were non-educated. A highly significant difference (P<0.001) was present among mothers of different nationalities who practiced breast-feeding for 12 months, with the majority being from the UAE.

4.1.4 Supplementation of Breast Milk with Milk Formulae and Other Milk

A wide variety of milk was found to be used by mothers for supplementation of infants with nutrients in addition to breast feeding. Results showed that about 71.6% of the infants received supplementary milk before the end of their first month. The results showed that 29.7% of the infants received baby milk formulae, 26.7% depended on fresh milk (cow or goat milk), 14.2% with skimmed milk and 5.3% with condensed milk. The frequency of breast milk supplementation was found to be 5-6 times per day. Considering the age at which the mother started to supplement the infant with additional milk, the results showed that some mothers started to supplement from the first day of birth. Results also showed that 26% of infants were supplemented with fresh milk at the age of 1-3 months compared to 20.6% who used baby milk formulae and 2.9% who used powdered milk. Pasteurized and condensed milk were not used at this age. Among all mothers, 211
(75%) with no additional occupation were not likely to supplement with fresh milk. No significant difference due to mother's nationality or educational level was observed among mothers of this group. About 80 (25%) of the same group used fresh milk and a highly significant difference was observed among mothers of different nationalities with high educational levels. Mothers from the UAE, Egypt, Oman and the Sudan constituted the higher percentage of mothers that used fresh milk. Educated mothers (67.5%) preferred fresh milk more than non-educated (32.5%). No significant difference was observed among mothers of different nationalities in using fresh milk compared to the significant difference (P<0.05) observed among mothers of different nationalities that did not use fresh milk.

A percentage of 4.8% of the infants were given pasteurized cow milk, in addition to breast milk. The results showed that mothers used this kind of milk and introduced it at the age of 4-6 months. All mothers were not working, from different nationalities and included educated and uneducated. Statistical analysis showed no significant difference between the mothers who used or did not use pasteurized cow milk.

4.1.5 Termination of Breast Feeding

The results have shown a variation in the lactation period among mothers who used breast feeding as the sole form of feeding. The minimum age for stopping breast-feeding, reported for both sexes of babies was 2 months. Generally, milk supplementation
was introduced from the early days of birth. The results indicated that 24.1% of the infants stopped breast milk due to their mother's desire without interfering factors, compared to the same percentage of the mothers (24%) who terminated breast-feeding due to external reasons. Of all mothers, 13.1% stopped breast-feeding because they wanted to. Medical advice had been taken by 2.7% of the mothers to stop breast feeding for reasons such as a medical operation or unsuitability of milk. Statistical analysis showed a non-significant difference due to the mother's occupation, education and age when breast feeding was terminated due to baby or desire or as a result of medical advice. A highly significant difference (P<0.001) was observed among mothers of different nationalities when they decided to terminate breast feeding and this trend was found to be higher within Egyptian, UAE and Omani mothers; compared to other nationalities. A significant difference (P<0.05) was found between educated and uneducated mothers when the breast feeding was terminated by external factors and the results indicated that a higher percentage of educated mothers stopped breast feeding their infants due to this.

4.1.6 Effect of Feeding Methods on Infant Growth

As shown in Table 4.2, the weight of exclusively breast-fed infants was nearly similar in both sexes and age development, while the weights of supplemented infants were slightly better. The weight of infants supplemented with fresh milk was better than that of those supplemented with baby milk formulae during the first
Table 4.2: Influence of feeding methods on infant weight (kg)

<table>
<thead>
<tr>
<th>Feeding method</th>
<th>Age (months)</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th>Age (months)</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1-3</td>
<td>4-6</td>
<td>7-9</td>
<td>10-12</td>
<td>13-15</td>
<td>16-18</td>
<td>1-3</td>
<td>4-6</td>
<td>7-9</td>
<td>10-12</td>
<td>13-15</td>
</tr>
<tr>
<td>Breast milk only</td>
<td>4.5</td>
<td>6.4</td>
<td>7.6</td>
<td>8.8</td>
<td>10.0</td>
<td>10.3</td>
<td>4.5</td>
<td>6.4</td>
<td>7.8</td>
<td>9.4</td>
<td>10.6</td>
</tr>
<tr>
<td>Breast milk + other milk</td>
<td>6.8</td>
<td>7.4</td>
<td>8.5</td>
<td>9.3</td>
<td>10.7</td>
<td>11.3</td>
<td>4.5</td>
<td>7.4</td>
<td>8.3</td>
<td>9.0</td>
<td>9.2</td>
</tr>
<tr>
<td>Breast milk + baby food</td>
<td>6.5</td>
<td>7.1</td>
<td>8.1</td>
<td>9.6</td>
<td>10.7</td>
<td>11.8</td>
<td>5.6</td>
<td>7.0</td>
<td>8.2</td>
<td>9.3</td>
<td>10.3</td>
</tr>
</tbody>
</table>
6 months of age and the weight remained the same up to the 9th month. The weight of fresh milk supplemented infants remained the same and that of formulae improved with the age.

Results in Table 4.3 followed the heights of babies with different feeding methods. It was evident that the height was not initially affected by the nature of the supplementation and remained the same up to the 4th month, while the height of supplemented infants exceeded that of those exclusively breast-fed. Both types of supplement remained the same up to the 9th month after which the heights of infants supplemented with baby milk formulae exceeded that of fresh milk. Heights of female infants were somewhat higher than male heights from the 1st to the 3rd month, while an increase of male height over that of female was evident. Statistical analysis showed no significant difference in the heights of females receiving different feedings.

It can be seen from Fig. 4.2 that the percentage of babies who were exclusive breast-fed started to decline for both sexes at 2 months of age. The percentage of male infants who received exclusive breast feeding during the first 6 months was higher compared to female infants at the same age, however, statistical analysis revealed no significant difference between both sexes. The percentage of infants who received exclusive breast feeding was reduced to 11.2% in both sexes at the end of the first year.
Table 4.3: Influence of feeding methods on infant height (cm)

<table>
<thead>
<tr>
<th>Feeding method</th>
<th>Age (months) Females</th>
<th>Age (months) Males</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1-3</td>
<td>4-6</td>
</tr>
<tr>
<td>Breast milk only</td>
<td>54.0</td>
<td>63.0</td>
</tr>
<tr>
<td>Breast milk + other milk</td>
<td>60.0</td>
<td>70.0</td>
</tr>
<tr>
<td>Breast milk + baby food</td>
<td>60.0</td>
<td>70.0</td>
</tr>
</tbody>
</table>
Fig. (4.2) : Changes of Breast Feeding with Age Groups

Age (Months)

Percentages

- Male
- Female
Fig. 4.3 shows that a higher percentage of female babies were introduced to supplementary fresh milk from the first month of age and up to 18 months. At the age of 4 months 45% of females fed on baby milk formulae compared to 30% male infants. Fresh milk was introduced to 22% of the female infants and 14% of the males. At the age of one year the percentage of male infants were supplemented with baby milk formulae was found to exceed that of females and the results show that still more females were introduced to fresh milk at this age.

The growth of male and female of exclusively breast-fed and of supplemented infants are presented in Tables 4.4 and 4.5. The growth of the breast-fed female infants was less than the normal growth, up to the 5th month. A slight increase occurred during the 6th month, after which the index dropped to the same level. The Toxford index for female infants was the same as that of male in the first month but increased with age from the second to the 5th month where it stayed the same to the 9th month. Statistical analysis showed no significant difference between breast fed and supplemented females while a significant difference (P<0.001) was evident between breast-fed and supplemented male infants. Also, the results indicated in Fig. 4.4, that breast milk supplementation had better effect on growth of males compared to female infants up
Table 4.4: Growth of exclusively breast-fed female and male infants as determined by toxford index

<table>
<thead>
<tr>
<th>Age (months)</th>
<th>Males</th>
<th></th>
<th></th>
<th>Females</th>
<th></th>
<th></th>
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<tbody>
<tr>
<td></td>
<td>Weight (lb.)</td>
<td>Height (in)</td>
<td>Toxford Index</td>
<td>Weight (lb.)</td>
<td>Height (in)</td>
<td>Toxford Index</td>
</tr>
<tr>
<td>1</td>
<td>8.3</td>
<td>20.4</td>
<td>0.5</td>
<td>7.7</td>
<td>21.5</td>
<td>0.46</td>
</tr>
<tr>
<td>2</td>
<td>10.4</td>
<td>21.5</td>
<td>0.4</td>
<td>10.1</td>
<td>22.7</td>
<td>0.57</td>
</tr>
<tr>
<td>3</td>
<td>12.4</td>
<td>22.7</td>
<td>0.5</td>
<td>12.5</td>
<td>23.6</td>
<td>0.67</td>
</tr>
<tr>
<td>4</td>
<td>11.5</td>
<td>23.1</td>
<td>0.4</td>
<td>12.1</td>
<td>19.4</td>
<td>0.80</td>
</tr>
<tr>
<td>5</td>
<td>14.6</td>
<td>24.8</td>
<td>0.5</td>
<td>16.7</td>
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<td>0.77</td>
</tr>
<tr>
<td>6</td>
<td>17.1</td>
<td>26.6</td>
<td>0.6</td>
<td>14.4</td>
<td>28.4</td>
<td>0.75</td>
</tr>
<tr>
<td>7</td>
<td>20.0</td>
<td>26.5</td>
<td>0.5</td>
<td>16.7</td>
<td>25.4</td>
<td>0.70</td>
</tr>
<tr>
<td>8</td>
<td>23.0</td>
<td>28.9</td>
<td>0.6</td>
<td>17.2</td>
<td>27.5</td>
<td>0.78</td>
</tr>
<tr>
<td>9</td>
<td>21.5</td>
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<td>0.6</td>
<td>19.8</td>
<td>29.4</td>
<td>0.84</td>
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</table>
Table 4.5: Growth of female and male infants supplemented with breast milk as determined by Toxford index

<table>
<thead>
<tr>
<th>Age (months)</th>
<th>Males</th>
<th>Females</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Weight (lb.)</td>
<td>Weight (lb.)</td>
</tr>
<tr>
<td></td>
<td>Height (in)</td>
<td>Height (in)</td>
</tr>
<tr>
<td></td>
<td>Toxford Index</td>
<td>Toxford Index</td>
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<td>15.4</td>
</tr>
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</tr>
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<td>20.7</td>
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</tr>
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<td>7</td>
<td>16.4</td>
<td>15.3</td>
</tr>
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<td></td>
<td>25.9</td>
<td>25.7</td>
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<td>8</td>
<td>18.9</td>
<td>18.1</td>
</tr>
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<td>27.7</td>
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<tr>
<td>9</td>
<td>21.5</td>
<td>21.0</td>
</tr>
<tr>
<td></td>
<td>31.4</td>
<td>29.7</td>
</tr>
</tbody>
</table>
Fig. (4.3): Percentage of Males & Females Infants Supplemented with Fresh Milk and Baby Milk Formulae at Different Ages

Male

Female

Age Groups (Months)

Age (Months)

Percentages

- Fresh Milk
- Baby Formulated Milk
Fig. (4.4) : Growth of Breast-Fed and Supplemented Infants as Compared with Toxford Index

- Breast fed Female
- Breast fed Male
- Supplemented Female
- Supplemented Male
to the 4 month after which a better improvement in females was obtained up to the 12th month. At the age of 1 year, the growth of exclusive breast-fed and supplemented male infants was found to be the same. With age development, breast feeding supplementation with milk alone or with other baby foods improved the infant growth.

4.2 Microbiological Studies of Baby Food Samples

Different moulds were detected in different food samples, the highest infection was evident in rice grains while the lowest was in baby milk formulae. A. flavus, A. parasiticus were isolated from all samples except two samples of Milupa food composed of five cereals were found to contain A. parasiticus only. Studies on A. flavus isolated from different foods showed variable growth in different media, different temperature and when incubated for different durations. At incubation temperature of 37°C A. flavus isolated from rice had the best growth followed by A. flavus isolated from S26 and short grains rice and A. flavus isolated form Cerelac. On the other hand; A. flavus isolated from long grains rice had the best growth at 25°C, followed by A. flavus isolated from S26. The other isolates had the same growth. The highest incidence of A. flavus recorded was in both rice samples, followed by cereal food and Cerelac. The lowest incidence was recorded in S26. Highly significant differences in mould incidence due to food type, temperature difference and incubation period (P<0.001) were observed. Cerelac, Cerelac wheat plus four fruits, Milumil and S26
showed detectable mould on DG18 which includes *Cladosporium herbarum*, *Pencillium aurantiogriseum* (in S26), alternaria and some fusaria were detected in 2 samples of Milumil milk

**4.2.1 Mould Infection in Rice**

Results presented in Fig. 4.5 showed that the lowest mould infection observed in intact grains compared to mouldy and damaged ones, however, long grains showed less contamination incidence compared to short grains. Statistical analysis showed a significant effect (P<0.01) of the grading against mould infection.

**4.2.2 Aflatoxin Production by Isolates of Aspergillus flavus, in vitro**

The fungal growth was indicated by the fungal mycelia dry weight. Significant variations between the growth of two isolates were noticed with an increase in incubation time. As can be seen from Table 4.6, the growth of *A. flavus* isolate of long grain rice increased with time up to 10 days of incubation. Results of the *A. flavus* isolated from short grain rice are shown in Table 4.7. It was observed that the growth was less than that of long grain rice and it was not consistent with incubation period. The increase in fungal growth observed by heavy mycelial weight obtained in this study was not correlated with aflatoxin production. On the other hand, the results indicated a highly significant difference in aflatoxin production between the two isolates (P<0.001).
<table>
<thead>
<tr>
<th>Incubation Time (Days)</th>
<th>Dry wt mg/\text{flask}</th>
<th>Aflatoxin μg/\text{flask}</th>
<th>Aflatoxin μg/\text{mg} dry wt</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>420</td>
<td>450</td>
<td>0.9</td>
</tr>
<tr>
<td>5</td>
<td>510</td>
<td>870</td>
<td>0.6</td>
</tr>
<tr>
<td>6</td>
<td>605</td>
<td>910</td>
<td>0.7</td>
</tr>
<tr>
<td>7</td>
<td>695</td>
<td>1029</td>
<td>0.7</td>
</tr>
<tr>
<td>8</td>
<td>1080</td>
<td>1610</td>
<td>0.7</td>
</tr>
<tr>
<td>9</td>
<td>1095</td>
<td>950</td>
<td>1.1</td>
</tr>
<tr>
<td>10</td>
<td>1004</td>
<td>912</td>
<td>1.1</td>
</tr>
<tr>
<td>14</td>
<td>780</td>
<td>890</td>
<td>0.8</td>
</tr>
</tbody>
</table>
### Table 4.7: Mycelial dry Weight and Aflatoxin production by *A. flavus* from short grain rice

<table>
<thead>
<tr>
<th>Incubation Time (Days)</th>
<th>Dry Wt (mg/flask)</th>
<th>Aflatoxin (μg/flask)</th>
<th>Aflatoxin (μg/mg dry Wt)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>320</td>
<td>380</td>
<td>0.8</td>
</tr>
<tr>
<td>5</td>
<td>290</td>
<td>710</td>
<td>0.4</td>
</tr>
<tr>
<td>6</td>
<td>325</td>
<td>800</td>
<td>0.4</td>
</tr>
<tr>
<td>7</td>
<td>450</td>
<td>850</td>
<td>0.5</td>
</tr>
<tr>
<td>8</td>
<td>519</td>
<td>790</td>
<td>0.7</td>
</tr>
<tr>
<td>9</td>
<td>460</td>
<td>688</td>
<td>0.7</td>
</tr>
<tr>
<td>10</td>
<td>410</td>
<td>701</td>
<td>0.6</td>
</tr>
<tr>
<td>14</td>
<td>300</td>
<td>741</td>
<td>0.4</td>
</tr>
</tbody>
</table>
Fig.(4.5): Distribution of Mould Infection in Rice Grains

Discoloured: 45.8%

Intact: 9.0%

Damaged: 45.2%

Long Grains

Discoloured: 43.5%

Intact: 5.7%

Damaged: 50.9%

Short Grains
4.3 Aflatoxins in Baby Milk Formulae

Different methods were used for analysis of aflatoxin from baby milk formulae. Fig. 4.6 shows a chromatogram of aflatoxin M₁ extracted by oxoid affinity column. Table 4.8 shows levels of aflatoxin M₁ (ng/ml). There existed some variations in incidence and levels of aflatoxin in samples collected from two sources. 12 samples of Milumil from small stores were found contaminated with aflatoxin M₁ levels of 0.45-1.21 ng/ml (mean 0.836) compared to 6 samples collected from large stores found to contain 0.43-1.1 ng AF M₁/ml (mean 0.717). Aflatoxin B₁ at level of 0.92 ng/ml was found in one sample from small stores while was not detected in any from large stores.

4 samples of S₂₆ (Table 4.8) from large stores were found to contain aflatoxin M₁ at levels of 0.21-1.1 ng AF M₁/ml (mean 0.553) while 12 samples from small stores were found to contain aflatoxin levels of 0.4-1.0 ng AFM₁/ml (mean 0.616). Aflatoxin B₁ was detected in 4 samples from small stores and 2 samples from large stores at an average of 1.4±0.98 and 1.4 ng/ml, respectively. Generally, a slight difference was observed in the contamination levels in samples from the two sources but statistical analysis by comparing the two means using Sheffe's test for unequal samples showed a significant difference (P<0.05).
Table 4.8: Levels of Aflatoxin M₁ (ng/ml) in baby milk formulae

<table>
<thead>
<tr>
<th></th>
<th>Milumil</th>
<th></th>
<th>S₂₆</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Large stores</td>
<td>Small stores</td>
<td>Large stores</td>
<td>Small stores</td>
</tr>
<tr>
<td>No. of samples</td>
<td>20</td>
<td>20</td>
<td>16</td>
<td>16</td>
</tr>
<tr>
<td>Positive samples</td>
<td>6</td>
<td>12</td>
<td>12</td>
<td>4</td>
</tr>
<tr>
<td>Mean aflatoxin M₁ (ng/ml)</td>
<td>0.717</td>
<td>0.836</td>
<td>0.616</td>
<td>0.553</td>
</tr>
<tr>
<td>SD</td>
<td>0.229</td>
<td>0.24</td>
<td>0.238</td>
<td>0.385</td>
</tr>
<tr>
<td>SE</td>
<td>0.093</td>
<td>0.069</td>
<td>0.060</td>
<td>0.193</td>
</tr>
</tbody>
</table>
Fig. 4.6: Chromatogram of Aflatoxin M₁ extracted by Oxoid affinity column
4.4 The Major Nutrient of Baby milk formulae and Aflatoxin M₁

Nutritional components and aflatoxin amounts in Milumil and S₂₆ are shown in Appendix 1 and 2 respectively. Comparing levels of aflatoxin M₁ (ng/ml) obtained in contaminated samples and the content of fat, protein, carbohydrates and total solids shows no correlation between aflatoxin and these components.

4.5 Incidence of Aflatoxins in cereal-based baby food

4.5.1 Physical characteristics

General characteristics of baby food obtained from different sources are presented in Table 4.9. No significant difference has been observed in the moisture content (MC) of different foods. Also, no significant differences in pH value were evident.

4.5.2 Presence of Aflatoxins in Baby Food

HPLC chromatogram of aflatoxins standards used for identification of present aflatoxins in this study is shown in Fig. 4.7. The use of HPLC with the clean up by C18 allowed a detection limit of aflatoxins up to 0.001 μg/g which is considered as a good sensitivity for determination of aflatoxin in food. Also, it allowed for a good separation of the four components of aflatoxins. The 2-dimensional TLC was valuable in aflatoxin confirmation and detection, where a limit of 0.002 μg/g could be detected in these foods.
Table 4.9: Characteristics of baby food collected for the study

<table>
<thead>
<tr>
<th>Product Description</th>
<th>Manufacture Date</th>
<th>Expiry Date</th>
<th>Moisture Content (mc%)</th>
<th>pH</th>
<th>Consistency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cerelac Wheat - Large stores</td>
<td>May 1989</td>
<td>May 1990</td>
<td>13.8 - 17.6</td>
<td>6.0 - 6.5</td>
<td>Semi powder</td>
</tr>
<tr>
<td>Cerelac Wheat+4 fruits - Large stores</td>
<td>Sep. 1989</td>
<td>Nov. 1990</td>
<td>11.0 - 14.6</td>
<td>5.0 - 5.6</td>
<td>Semi powder</td>
</tr>
<tr>
<td>Milupa 5 cereals - Large stores</td>
<td>Mar. 1989</td>
<td>Mar. 1990</td>
<td>12.0 - 14.5</td>
<td>5.0 - 5.3</td>
<td>Semi powder</td>
</tr>
<tr>
<td>Milupa 3 cereals - Small stores</td>
<td>Mar. 1989</td>
<td>Mar. 1990</td>
<td>11.3 - 18.5</td>
<td>5.4 - 7.0</td>
<td>Semi powder</td>
</tr>
<tr>
<td>Milupa 28 teething rusk - Small stores</td>
<td>Mar. 1989</td>
<td>May 1990</td>
<td>5.0 - 11.2</td>
<td>6.5 - 6.8</td>
<td>Granular</td>
</tr>
<tr>
<td>Milupa rice with milk - Small stores</td>
<td>May 1989</td>
<td>Mar. 1990</td>
<td>10.0 - 10.7</td>
<td>5.5 - 7.8</td>
<td>Granular</td>
</tr>
<tr>
<td>Farley's Rusk - Large stores</td>
<td>May 1989</td>
<td>Mar. 1990</td>
<td>9.0 - 11.1</td>
<td>5.6 - 7.3</td>
<td>Granular</td>
</tr>
<tr>
<td>Farley's rusk - Small stores</td>
<td>May 1989</td>
<td>Mar. 1990</td>
<td>9.2 - 10.7</td>
<td>5.0 - 7.6</td>
<td>Granular</td>
</tr>
</tbody>
</table>
Fig. 4.7: HPLC Chromatogram of Aflatoxins B₁, B₂, G₁, G₂
4.5.2.1 Survey of Aflatoxin Incidence in Baby Food samples Collected from Large Stores

Aflatoxins in baby foods collected from large stores are shown in Table 4.10. Higher aflatoxin levels were observed in plain Cerelac. Also, in Milupa cereals food the level of aflatoxin B₁ detected in Milupa 5 cereals slightly exceeded the level obtained in Milupa 3 cereals. Farley's rusk, on the other hand, contained the lowest amount of aflatoxins B₁, G₁, and G₂. The lowest amount of aflatoxin B₂ was detected in Milupa teething rusks. Statistical analysis showed no significant difference in the incidence and amount of aflatoxins detected in baby food samples collected from large stores.

4.5.2.2 Aflatoxins in Baby food samples collected from small stores

Table 4.11 shows the aflatoxin contamination in baby food collected from small stores during the summer of 1989-1990. The results showed that 77.8-87.5% of the baby food samples analyzed were found to be contaminated with aflatoxins. A large number of contaminated samples (87.5%) was detected in Cerelac wheat, Cerelac wheat+4 fruits, Milupa 3 cereals and Milupa rice with milk followed by Milupa teething rusk (83.3%), Farley's rusk (80%) and Milupa 5 cereals (77.8%).

Aflatoxins B₁, B₂, G₁ and G₂ were detected in all positive samples. Aflatoxin G₁ but not aflatoxin B₁ was recovered in two samples of Milupa 5 cereals. A. parasiticus was isolated from these two samples.
Table 4.10: Means of aflatoxin in baby food samples collected from large stores

<table>
<thead>
<tr>
<th>Aflatoxins ug/kg</th>
<th>Cerelac Wheat</th>
<th>Cerelac Wheat+4 Fruits</th>
<th>5 Cereals (Milupa)</th>
<th>3 Cereals (Milupa)</th>
<th>Rice with milk (Milupa)</th>
<th>Teething rusk (Milupa)</th>
<th>Farley’s Rusk</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n = 32</td>
<td>n = 32</td>
<td>n = 25</td>
<td>n = 25</td>
<td>n = 25</td>
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<td>n = 32</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive samples</td>
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<td>11</td>
<td>4</td>
<td>5</td>
<td>11</td>
<td>9</td>
<td>5</td>
</tr>
<tr>
<td>Mean</td>
<td>7.68</td>
<td>6.78</td>
<td>6.74</td>
<td>5.70</td>
<td>6.40</td>
<td>5.93</td>
<td>4.81</td>
</tr>
<tr>
<td>SD</td>
<td>3.95</td>
<td>2.51</td>
<td>1.32</td>
<td>2.35</td>
<td>1.65</td>
<td>1.71</td>
<td>1.81</td>
</tr>
<tr>
<td>AF B&lt;sub&gt;2&lt;/sub&gt;</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Positive samples</td>
<td>13</td>
<td>6</td>
<td>3</td>
<td>5</td>
<td>8</td>
<td>5</td>
<td>4</td>
</tr>
<tr>
<td>Mean</td>
<td>6.20</td>
<td>5.92</td>
<td>5.41</td>
<td>3.20</td>
<td>5.10</td>
<td>3.20</td>
<td>4.51</td>
</tr>
<tr>
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<td>1.52</td>
<td>1.38</td>
<td>1.29</td>
<td>0.99</td>
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<td>2.10</td>
<td>1.21</td>
</tr>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive samples</td>
<td>8</td>
<td>6</td>
<td>6</td>
<td>3</td>
<td>7</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>Mean</td>
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<td>4.7</td>
<td>6.20</td>
<td>4.92</td>
<td>3.20</td>
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<td>1.51</td>
<td>1.25</td>
<td>1.92</td>
<td>1.94</td>
<td>1.27</td>
<td>1.28</td>
<td>1.74</td>
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<td></td>
<td></td>
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<tr>
<td>Positive samples</td>
<td>4</td>
<td>7</td>
<td>3</td>
<td>2</td>
<td>2</td>
<td>5</td>
<td>3</td>
</tr>
<tr>
<td>Mean</td>
<td>5.91</td>
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<td>6.01</td>
<td>4.91</td>
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<td>5.00</td>
<td>3.90</td>
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<td>2.71</td>
<td>2.45</td>
<td>2.45</td>
<td>0.98</td>
<td>2.10</td>
<td>1.89</td>
<td>2.01</td>
</tr>
</tbody>
</table>
Table 4.11: Means of aflatoxin in baby food samples collected from small stores

<table>
<thead>
<tr>
<th>Aflatoxins ug/kg</th>
<th>Cerelac Wheat</th>
<th>Cerelac Wheat+4 Fruits</th>
<th>5 Cereals Milupa</th>
<th>3 Cereals Milupa</th>
<th>Rice with milk Milupa</th>
<th>Teething rusk Milupa</th>
<th>Farley's Rusk n = 20</th>
</tr>
</thead>
<tbody>
<tr>
<td>AF B1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive samples</td>
<td>28</td>
<td>28</td>
<td>14</td>
<td>14</td>
<td>14</td>
<td>25</td>
<td>20</td>
</tr>
<tr>
<td>Mean</td>
<td>11.67</td>
<td>8.39</td>
<td>9.34</td>
<td>9.19</td>
<td>8.76</td>
<td>7.85</td>
<td>5.39</td>
</tr>
<tr>
<td>SD</td>
<td>1.41</td>
<td>2.23</td>
<td>3.45</td>
<td>1.7</td>
<td>2.22</td>
<td>2.1</td>
<td>1.89</td>
</tr>
<tr>
<td>AF B2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive samples</td>
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<td>22</td>
<td>12</td>
<td>11</td>
<td>12</td>
<td>14</td>
<td>13</td>
</tr>
<tr>
<td>Mean</td>
<td>10.21</td>
<td>8.12</td>
<td>8.21</td>
<td>8.25</td>
<td>7.24</td>
<td>5.11</td>
<td>4.91</td>
</tr>
<tr>
<td>SD</td>
<td>3.72</td>
<td>2.14</td>
<td>3.11</td>
<td>2.11</td>
<td>1.88</td>
<td>0.29</td>
<td>1.74</td>
</tr>
<tr>
<td>AF G1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive samples</td>
<td>10</td>
<td>15</td>
<td>12</td>
<td>10</td>
<td>8</td>
<td>9</td>
<td>11</td>
</tr>
<tr>
<td>Mean</td>
<td>9.71</td>
<td>7.25</td>
<td>8.78</td>
<td>9.0</td>
<td>8.20</td>
<td>7.82</td>
<td>5.1</td>
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<td>SD</td>
<td>1.52</td>
<td>1.95</td>
<td>3.21</td>
<td>1.45</td>
<td>2.59</td>
<td>2.80</td>
<td>1.54</td>
</tr>
<tr>
<td>AF G2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive samples</td>
<td>8</td>
<td>12</td>
<td>9</td>
<td>8</td>
<td>7</td>
<td>9</td>
<td>10</td>
</tr>
<tr>
<td>Mean</td>
<td>9.70</td>
<td>6.69</td>
<td>6.28</td>
<td>7.94</td>
<td>5.4</td>
<td>5.00</td>
<td>3.88</td>
</tr>
<tr>
<td>SD</td>
<td>1.4</td>
<td>1.47</td>
<td>2.14</td>
<td>2.01</td>
<td>3.10</td>
<td>1.29</td>
<td>2.10</td>
</tr>
</tbody>
</table>
4.5.2.3 Effect of Storage conditions on aflatoxin occurrence in baby food samples

Fig. 4.8 represents the percentage of aflatoxin contaminated samples of baby foods collected from large and small stores. As it can be seen, the higher percentage of contaminated samples in each food type were detected within those collected from small stores. Moreover, although food collected was varied in composition, the majority of baby foods analyzed contained the same percentage of contamination as was evident in both kinds of Cerelac, Milupa cereals food and Milupa rice with milk. As presented in Fig. 4.8, baby food collected from small stores contained a significantly higher aflatoxin content than those collected from larger ones (P<0.001). Moreover, the levels of aflatoxins detected in samples from small stores contained higher quantities of aflatoxins compared to those collected from large stores and even aflatoxins were detected in baby food collected from large stores but still the quantities detected were more or less concentrated at the range of less than 10 μg/kg which showed the contamination levels of Cerelac wheat with aflatoxin B<sub>1</sub>.

It was found that 88% of this food collected from large stores contained aflatoxin ranged between 5 and 10 μg/kg, while none of Cerelac samples collected from small stores contained less than 5 μg/kg. The rest of positive samples of the large stores contained less than 5 μg/kg while the majority (46%) of samples of small contained aflatoxin B<sub>1</sub> quantity ranging between 10 and 15
Fig. (4.8) : Distribution of Percentage of Contaminated Baby Food Samples from Large and Small Stores (Limit of detection = 0.01 µg)
ug/kg compared to 36% of the positive samples contained more than 15 ug/kg. The incidence and levels of aflatoxins detected in baby milk formulae was significantly (P<0.001) different when compared using Scheff's test for multiple comparisons.

4.5.2.3.1 Effect of Moisture Content Percentage (%) on Aflatoxin

Table 4.12 shows the effect of moisture content (%) on quantity of aflatoxins present in baby food. It was clear from this table that a highly significant effect (P<0.001) was obtained when studying the effect of the moisture content of the food on aflatoxin production. The average MC was ranging between 14.9 in cerelac wheat plus four fruits and 9.6 in Milupa teething rusks, while level of aflatoxin was ranging between 11.4 and 6.4 μg/kg in cerelac wheat and Farley's rusks, respectively. Comparing the detected level of aflatoxin with the moisture content of the food, statistical analysis showed a positive correlation (r = 0.9) between the moisture content of the baby food and aflatoxin levels.

4.5.2.2 Nutritional components

The fat content of Cerelac wheat and total solid (Table 4.13) were determined and compared with aflatoxin B_{1} of positive samples. Statistical analysis showed no correlation between those components and also no correlation obtained with Mg, Zn, Na and K.
Table 4.12: Correlation between Aflatoxin quantity and moisture content (MC%) of baby food

<table>
<thead>
<tr>
<th>Food type</th>
<th>Moisture content (MC%)</th>
<th>Aflatoxin B&lt;sub&gt;1&lt;/sub&gt; (µg/kg)</th>
<th>Level of significance</th>
<th>Pearson correlation (R)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cerelac Wheat</td>
<td>14.30 ± 1.12</td>
<td>11.4 ± 1.50</td>
<td>&lt; 0.001</td>
<td>0.897</td>
</tr>
<tr>
<td>Cerelac Wheat+4 Fruits</td>
<td>14.9 ± 3.01</td>
<td>10.5 ± 1.20</td>
<td>&lt; 0.001</td>
<td>0.852</td>
</tr>
<tr>
<td>Cereals (Milupa)</td>
<td>14.06 ± 3.30</td>
<td>9.00 ± 2.30</td>
<td>&lt; 0.001</td>
<td>0.902</td>
</tr>
<tr>
<td>3 Cereals (Milupa)</td>
<td>12.50 ± 3.65</td>
<td>9.00 ± 2.30</td>
<td>&lt; 0.001</td>
<td>0.985</td>
</tr>
<tr>
<td>Rice with Milk (Milupa)</td>
<td>11.01 ± 2.04</td>
<td>9.21 ± 2.21</td>
<td>&lt; 0.001</td>
<td>0.983</td>
</tr>
<tr>
<td>Teething Rusk (Milupa)</td>
<td>9.61 ± 1.70</td>
<td>6.61 ± 1.57</td>
<td>&lt; 0.001</td>
<td>0.972</td>
</tr>
<tr>
<td>Farley's Rusk</td>
<td>11.33 ± 2.43</td>
<td>6.44 ± 1.86</td>
<td>&lt; 0.001</td>
<td>0.982</td>
</tr>
</tbody>
</table>
Table 4.13: Correlation between aflatoxin B$_1$ and fat, total solid % of Cerelac wheat

<table>
<thead>
<tr>
<th></th>
<th>AF B$_1$ µg/kg</th>
<th>Fat (%)</th>
<th>Total Solid</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5.0</td>
<td>2.7</td>
<td>2.1</td>
</tr>
<tr>
<td>2</td>
<td>8.0</td>
<td>2.6</td>
<td>3.6</td>
</tr>
<tr>
<td>3</td>
<td>10.0</td>
<td>3.0</td>
<td>2.7</td>
</tr>
<tr>
<td>4</td>
<td>6.0</td>
<td>2.2</td>
<td>2.3</td>
</tr>
<tr>
<td>5</td>
<td>5.0</td>
<td>1.8</td>
<td>2.1</td>
</tr>
<tr>
<td>6</td>
<td>10.0</td>
<td>2.1</td>
<td>2.8</td>
</tr>
<tr>
<td>7</td>
<td>4.0</td>
<td>3.1</td>
<td>2.4</td>
</tr>
<tr>
<td>8</td>
<td>3.0</td>
<td>1.5</td>
<td>2.7</td>
</tr>
<tr>
<td>9</td>
<td>9.0</td>
<td>2.8</td>
<td>2.0</td>
</tr>
<tr>
<td>10</td>
<td>1.2</td>
<td>3.0</td>
<td>2.4</td>
</tr>
<tr>
<td>11</td>
<td>4.0</td>
<td>3.2</td>
<td>2.3</td>
</tr>
<tr>
<td>12</td>
<td>6.5</td>
<td>2.8</td>
<td>2.3</td>
</tr>
<tr>
<td>13</td>
<td>10.0</td>
<td>1.9</td>
<td>2.3</td>
</tr>
<tr>
<td>14</td>
<td>5.0</td>
<td>2.6</td>
<td>2.3</td>
</tr>
<tr>
<td>15</td>
<td>6.0</td>
<td>3.1</td>
<td>2.3</td>
</tr>
<tr>
<td>Mean</td>
<td>6.18</td>
<td>2.56</td>
<td>2.44</td>
</tr>
<tr>
<td>SD</td>
<td>2.72</td>
<td>0.53</td>
<td>0.39</td>
</tr>
<tr>
<td>SE</td>
<td>0.70</td>
<td>0.13</td>
<td>0.10</td>
</tr>
</tbody>
</table>
4.6 Aflatoxin in Rice

Results show that 64% of long grain rice samples were found to be contaminated with aflatoxin at an average of (µg/kg) 13.9 B₁, 11.8 B₂, 9.4 G₁, and 7.5 G₂. Short grain rice, on the other hand, revealed less contamination incidence where 32% samples were found contaminated at levels of (µg/kg) of 9.6, 8.6, 6.8 and 6.4 B₁, B₂, G₁, and G₂, respectively. Statistical analysis showed a highly significant difference (P<0.001) between the two rice kinds and it confirms that aflatoxin contamination was higher in long grains compared to short ones.

4.7 Aflatoxins in raw milk

4.7.1 Cow Milk

Aflatoxin M₁ were found in 20 samples collected from the three farms at levels of 0.37-1.85 ng/ml. Slight variations were found in incidence and aflatoxin levels in the three sampled farms. Although highest contamination incidence was found in milk samples from sources C and B compared to milk samples obtained from source A, the results showed that the maximum level of aflatoxin found was 1.9 ng AFM₁/ml which was detected in milk samples from source A compared to the maximum detected in samples from B and C which found to be 0.9 ng/ml (Table 4.14).
Table 4.14: Distribution of Aflatoxin M₁ ng/ml in samples of raw cow milk

<table>
<thead>
<tr>
<th>Source</th>
<th>No. of samples tested</th>
<th>No. of positive samples</th>
<th>&lt; 0.6</th>
<th>0.61 - 0.75</th>
<th>0.76 - 0.9</th>
<th>&gt; 0.9</th>
<th>Maximum level of aflatoxin M₁ μg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>7</td>
<td>5</td>
<td>-</td>
<td>-</td>
<td>3</td>
<td>2</td>
<td>1.9</td>
</tr>
<tr>
<td>B</td>
<td>8</td>
<td>6</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>-</td>
<td>0.9</td>
</tr>
<tr>
<td>C</td>
<td>7</td>
<td>6</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>-</td>
<td>0.9</td>
</tr>
</tbody>
</table>
4.7.2 Goat milk

Aflatoxin M₁ was found in 21 samples among the 34 samples collected from the four farms at levels of 0.3-4.2 ng/ml. Highest incidence of contaminated samples was found in farm C and D compared to A and B, while the lowest incidence was found in farm B. The highest level of aflatoxin M₁ was 4.2 ng found in milk samples from farm D while the lowest level (1.2 ng) was detected in milk samples from farm C (Table 4.15).

4.7.3 Aflatoxin M₁ in camel milk

16 samples of camel milk were analysed for aflatoxins. Results showed presence of aflatoxin M₁ in 9 samples at levels of 0.8-0.21 ng/ml (mean = 0.442).

4.7.4 Nutritional components

Aflatoxin (ng/ml) and nutritional components of cow, goat and camel milk are presented in Appendices 3, 4 and 5 respectively. The fat content of cow milk collected from source A was correlated with amount of aflatoxin detected in those samples and this effect was found to be significant (P<0.01) when analysed statistically. No correlation was obtained between aflatoxin content and fat in milk samples collected from other two sources.

The fat content of goat milk was compared with AFM₁. No correlation was observed between aflatoxin M₁ and fat content of
Table 4.15: Distribution of Aflatoxin M₁ in goat's milk numbers of milk samples contaminated with Aflatoxin M₁ at range (ng/ml)

<table>
<thead>
<tr>
<th>Source samples tested</th>
<th>No. of positive samples</th>
<th>&lt; 0.5</th>
<th>0.51-0.71</th>
<th>0.71-0.9</th>
<th>&gt; 0.9</th>
<th>Maximum level of aflatoxin M₁ μg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>6</td>
<td>4</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>1.9</td>
</tr>
<tr>
<td>B</td>
<td>6</td>
<td>3</td>
<td>-</td>
<td>2</td>
<td>1</td>
<td>2.9</td>
</tr>
<tr>
<td>C</td>
<td>13</td>
<td>9</td>
<td>3</td>
<td>3</td>
<td>2</td>
<td>1.2</td>
</tr>
<tr>
<td>D</td>
<td>9</td>
<td>5</td>
<td>1</td>
<td>3</td>
<td>-</td>
<td>4.2</td>
</tr>
</tbody>
</table>
milk samples collected from sources A, C and D. Statistical analysis showed a significant correlation $P<0.01$ between fat content of milk samples collected from source B and aflatoxin M₁.

Comparing fat content of camel milk samples and amount of aflatoxin revealed a direct correlation ($r = 0.6$) and the amount of aflatoxins found to be higher in samples that contained high fat percentage ($P = 0.05$).

The protein content of milk samples from different sources was analysed and compared with aflatoxin M₁. No correlation was observed in cow, goat and camel milk. No correlation was found when comparing aflatoxin content in different kinds of milk and components of lactose, total solids, phosphorus, calcium and magnesium.

### 4.8 Occurrence of Aflatoxins in Animal Feed

Table 4.16 shows the distribution of aflatoxin in animal feed samples. Variable contamination incidence and levels of aflatoxin were obtained in different kinds. Most of the samples were found to be contaminated with aflatoxin levels exceeding 20 µg/kg except dates that also contained significant amounts that exceed 10 µg/kg. The highest amounts of aflatoxins detected were in maize and soyabean. Fig. 4.9 shows mould contamination incidence and presence of *Aspergillus* species in different kinds of animal feeds. It was obvious that maize showed the highest contamination incidence where soyabean showed the lowest.
Table 4.16: Occurrence of Aflatoxins (μg/kg) in animal feed

<table>
<thead>
<tr>
<th>Feed</th>
<th>B₁</th>
<th>B₂</th>
<th>G₁</th>
<th>G₂</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maize</td>
<td>11.1 - 67.5</td>
<td>9.1 - 25.7</td>
<td>8.4 - 20.9</td>
<td>6.9 - 18.4</td>
</tr>
<tr>
<td>n = 30</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Barley</td>
<td>8.4 - 35.3</td>
<td>6.8 - 23.5</td>
<td>6.4 - 13.9</td>
<td>4.6 - 10.9</td>
</tr>
<tr>
<td>n = 25</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wheat Bran</td>
<td>12.6 - 29.1</td>
<td>11.3 - 19.8</td>
<td>8.1 - 16.4</td>
<td>9.4 - 17.2</td>
</tr>
<tr>
<td>n = 30</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oat</td>
<td>4.1 - 24.4</td>
<td>5.1 - 21.1</td>
<td>3.1 - 19.4</td>
<td>2.1 - 10.9</td>
</tr>
<tr>
<td>n = 20</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Soybean</td>
<td>8.6 - 40.3</td>
<td>4.1 - 22.9</td>
<td>3.9 - 31.4</td>
<td>5.9 - 15.9</td>
</tr>
<tr>
<td>n = 20</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Compound Feed</td>
<td>15.4 - 36.2</td>
<td>10.4 - 26.1</td>
<td>10.9 - 26.1</td>
<td>11.4 - 26.1</td>
</tr>
<tr>
<td>n = 32</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oats</td>
<td>9.7 - 15.6</td>
<td>7.9 - 12.7</td>
<td>8.9 - 10.2</td>
<td>6.4 - 10.1</td>
</tr>
<tr>
<td>n = 20</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Fig. (4.9) : Percentage of Mould and Aspergillus Contamination in Animal Feed

<table>
<thead>
<tr>
<th>Animal Feed</th>
<th>Mould Infection</th>
<th>Aspergillus SPP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maize</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>Barley</td>
<td>80</td>
<td>60</td>
</tr>
<tr>
<td>Oat</td>
<td>40</td>
<td>20</td>
</tr>
<tr>
<td>Soyabean</td>
<td>20</td>
<td>0</td>
</tr>
</tbody>
</table>
CHAPTER 5

DISCUSSION

Nutritional requirements vary during the early age according to growth and physical activities. The infant body makes some adjustment to the kind of food and adequate requirements for survival. Infant feeding depended entirely on the use of human milk from mother till recently. This trend declined due to development of other nutritional means such as production of infant formula products, which resemble human milk in composition and physiological functions. It appears that infant feeding practices were influenced more by marketing techniques rather than by scientific advice, and due to this, breast feeding was discouraged and baby foods were introduced at an early age.

This study reports infant feeding patterns and growth in a group of healthy infants in Al Ain, UAE. They belonged to a society where family income is high, baby food is available, and it is quite likely that a mother may change her baby's food under the influence of a friend or a neighbour. The results of the general survey confirmed that socio-demographic factors, such as age, education and involvement of mothers in outside work, do not affect the selection of the feeding methods and although most of the mothers were not involved in outside jobs, only 31.9% of the mothers tended to breast-feed to a maximum period of 4-6 months.
Exclusive breast-feeding was found to decline from the age of 2 months and less than 50% of infants were exclusively breast-fed for 6 months. This decline may be attributed to the media-advertised infant formulae, direct role of mother in directing infant feeding without referring to medical advice and to offering of ready-to-use infant formulae in a disposal bottle in hospitals immediately after delivery.

Results showed that 70% of infants are initially breast-fed, and 11.1% were still breast-fed at 12 months of age. Different nationalities seem to have different attitudes in practicing exclusive breast feeding during early infancy. The results indicated that some Egyptians do not like to breast-feed and this decision used to be taken even before delivery. Even for those who were initially breast-fed, the duration was only 3 months. For other nationalities, the duration varied between 6-12 months and some of the mothers were obliged to stop breast-feeding for some reasons of which travel and pregnancy were the most common.

Assessment of nutritional status of exclusively breast-fed and supplemented infants showed a reduced growth compared to the normal, as determined by the Toxford index. This may be attributed to a smaller volume of breast milk produced by mother or that the dietary intake of major nutritional components by mother is inadequate, which results in production of low quality milk. Such factors may contribute to abnormal growth of breast-fed infants. The nutritional status of supplemented infants was somewhat poor, but still an increment in growth was observed from
the 4th month and this may show that breast milk is not satisfactory and needs to be supplemented.

Some mothers of the UAE nationality preferred to give exclusive breast-feeding for 12 months. The results showed that the growth of exclusive breast-fed infants remains constant from the age of 6 months and this practice may have produced a malnutrition condition.

In developing countries some research has reported a strong association between prolonged breast-feeding and malnutrition (Grummer-Strawn, 1993). Thus, prolonged breast-feeding is not recommended because it could produce anaemia if it is practised for more than 6 months, and in this case, it should be supplemented with iron starting from the 4th month (Calvo et al., 1992).

Human milk is not always satisfactory for the infant’s need and many provide a limited energy intake (Walerlow and Thomson, 1979). In some cases it would be satisfactory for 1 month, as it was shown in Jordan (Kimmance, 1970) and for 4 months in Bangladesh (Khan, 1980). In some cases, mothers prolonged breast-feeding and especially among poor families as in Iraq (Mahmood and Freecham, 1987) or in some communities where mothers usually prefer prolonged breast-feeding to extend the infertility period as an effective method of delaying pregnancy (Prentice, 1991).
The supplementary food of infants of UAE citizens and other nationalities composed mainly of baby milk formulae, powdered milk, pasteurized fresh milk, raw goat and cow milk, dairy products, cereals of which rice is the main diet and several kind of nuts. All these kinds of food are liable to contamination by aflatoxins, especially that the environmental conditions in the UAE is characterized by high temperature and humidity and the majority of these foods are imported from other countries and stored for long periods before consumption.

The microbiological tests conducted in this study of some cereal-based baby food, milk formulae and rice have shown the presence of some mould; however, \textit{A. flavus} isolated from different foods and differences in growth of isolates existed in different media, temperature and incubation periods. This may be attributed to differences in nutritional composition of each food. The results showed the presence of some mould such as \textit{Aspergillus}, \textit{Benicillium}, \textit{Cladosporium} and this may be due to some source of contamination during processing or packing. This is in line with finding of Jordal et al. (1993), who detected \textit{Aspergillus} species in pasteurized milk.

The most striking observation was the presence of \textit{Alternaria} species in 3 samples of Milumil baby milk formulae. Species of \textit{Alternaria} are considered as plant pathogens which produce post-harvest decay in many vegetables and fruits. This observation is of great significance in regard to the health hazards of infants, as
some alternaria can produce mycotoxins known as alternariol, alternariol methyl ether and tenuazonic acid (Pohland, 1993).

The study has shown different responses of two kinds of rice grains towards infection with mould and aflatoxin production. Both long and short grain rice were found to be naturally contaminated and showed a high incidence of fungal infection. However, this contamination may take place during storage because the trend used by UAE citizens is to store rice for some time before consumption and rice samples under study were stored for a minimum period of 2 months in home stores. Such may be causative for deterioration of the quality, especially when storage conditions provide the water activity and temperature which enhance mould growth and aflatoxin production.

Isolates of A. flavus from short and long grain rice varied in their growth and amount of aflatoxin produced in vitro. Studies of Codner et al. (1963), Taber and Shroeder (1967), and Osman (1986) on the quantitative production of aflatoxins by different isolates showed different results. These differences may be attributed to many factors such as adequacy of nutrients in the medium used, growing conditions and other requirements for growth and toxin production.

Differences between isolates observed regarding the amount of aflatoxin produced were attributed to the incubation time. Amount of aflatoxin produced by the isolate from long grain increased with time and then decreased. Such a phenomenon may
be explained by the fact that nutrients were reduced in the substrate and became unsatisfactory for growth of the isolate hence it degraded aflatoxins. A similar observation of absence of relationship between fungal growth and aflatoxin production during prolonged incubation was reported by Schroeder (1966).

Generally, environmental conditions are important for the propagation of fungi and mycotoxin production; which are produced whenever the conditions are favourable for their growth. It is possible to get growth and no mycotoxin production. The effect of temperature on the growth of *A. flavus* isolates from different foods was observed in this study and such difference could be due to some factors related to the physiological responses of the isolates. The moisture content of the food is a limiting factor for aflatoxin production. Most of cereal-based foods were found to contain a moisture content exceeding 15% and the moisture content of rice grain exceeded 18%. Such level may be favourable for aflatoxin formation, as Bullerman et al. (1984) reported that most foods with a moisture content above 13% are susceptible to *A. flavus* growth and aflatoxin production. Moreover, foods that have a high oil content may be more susceptible to mould growth at a moisture content below 13%.

The first supplement introduced to infants with human milk is baby milk formulae. The study showed that two formulae were used by mothers more commonly than others and these were Milumil and S26. About 3.5% of infants did not receive breast milk and were introduced directly to baby formulae, in addition to 29% of
infants who received those formulae as supplements to breast milk up to 6 months of age.

Aflatoxin M₁ (ng/ml) was recovered from Milumil and S₂₆ at levels of 0.45-1.21, 0.43-1.1, and 0.4-1, 0.21-1.1 in samples collected from small and large stores, respectively. The present study demonstrated that the daily average intake of milk formula is 500-600 ml per infant which would suggest a dietary intake of aflatoxin M₁ of 215-550 and 105-550 ng per infant per day from Milumil and S₂₆, respectively.

With the advancement of the age of infants, semi solid foods were introduced which were mainly cereals in powered or semi-powdered forms that are mixed with milk or water before being served to the baby. Mothers who contributed in this study have listed many foods of which five groups were selected for the study of aflatoxins. Some of these foods composed of one cereal with other additives; such as Cerelac wheat, Cerelac wheat plus four fruits and Milupa rice with milk while one composed of three cereals and one composed of 5 cereals. The results showed that 90% of the supplemented infants depended on these foods, which in some cases were introduced at a very early age. Some mothers preferred to supplement with baby food instead of using baby milk formulae by diluting the food to a level so that it can be easily taken by the bottle. In most cases, mothers did not follow the manufacturer's "instructions of use" which are commonly printed on the package. The study of aflatoxins in baby food made from cereals is of great significance because large numbers of infants in this study received them as supplements to their diet.
Aflatoxin B$_1$ detected in Cerelac wheat at an average of 11.6 and 7.2 ug/kg in samples collected from small and large stores, respectively. Levels detected in Cerelac wheat plus 4 fruits were 6.7 and 8.3 ug aflatoxins B$_1$/kg in samples of small and large stores. Indeed, these levels are high considering the daily dietary intake of infants who depended on them for their needs. An average of 6.4 and 8.7 µg aflatoxins B$_1$/kg detected in samples of rice with milk collected from large and small stores. Again, these infants may be exposed to more aflatoxins if fed on contaminated meals. Exposure continued even when the infants started to chew food and solid food was introduced, which included Farley's and Milupa rusks. Such were found to contain aflatoxins exceeding 5 µg/kg.

Storage conditions of high temperature and humidity are the main factors which enhance aflatoxin production and may be responsible for the presence of aflatoxins in the baby foods of this study. This also may explain the higher incidence and aflatoxin levels obtained in samples collected from the small stores, which were found to lack proper storage and some of them get baby food from other sources not directly from the main agents, as is the case of large stores. Bad storage is conducive to the growth of mould and aflatoxin production. Other factors are: the nature and composition of the substrate, mould strain, atmosphere, presence of inhibitors, competing microflora and spore load (Karunaratne and Bullerman, 1990). Shelf life of the studied baby foods (6 months) may be enough for aflatoxin formation under improper storage conditions, especially in the summer. Added to that; the containers of baby food are made of metal, which also increase the possibility of aflatoxin
contamination. Such a suggestion may agree with the findings of those reported that storage fungi grow vigorously and produced aflatoxins under bad storage depending on moisture content and container kind and it was found that prolonged storage for a period of 6 months in a metal container increased aflatoxin formation.

The tolerated levels have been established for infant and child's food ranged among zero in Nigeria, 5 µg, B1 in Protugal, but (B1 + B2 + G1 + G2) in France, 1 µg B1 in Czechoslovakia and 3 µg (B1 + B2 + G1 + G2) in Brazil (Van Egmond, 1989). Comparing these levels with the amounts of aflatoxins detected in baby food analyzed in this study (Chapter 4), it was evident that those levels exceeded the tolerated levels determined in all countries. This finding would validate the significance of the need to establish such levels in the UAE. The Cerelac wheat that is commonly consumed by the majority of infants of all nationalities and at different ages was found to contain an average of 11.6 µg aflatoxins B1 when obtained from small stores, and 7.2 µg B1 when obtained from large ones. As indicated on the usage instruction each meal is 40 g and it is recommended to be served at a frequency of 3-4 times per day which indicate dietary exposure to aflatoxin of 1216 ng or 1856 ng per day when Cerelac is obtained from large and small stores respectively, when the infant is fed on contaminated samples. This level may be enough to produce serious health problems from an early age, especially because this food is recommended to start at the age of 4 months and in some cases it is introduced at an earlier age. Direct exposure of infants and children continues with age, as
other weaning food start to be used and aflatoxins have been detected in cereals rusks at levels more than 5 μg/kg.

Presence of aflatoxins in baby foods even as a trace is hazardous and requires more concern. It has been reported in the literature that young animals are more susceptible to aflatoxin hazards than adults due to their growing state. In Human infants the pattern of feeding depended on various sources of nutrients that increase the possibility of more exposure which may be breast milk, baby milk formulae, baby food, milk and other milk products. Health problems produced by aflatoxins in children have been of a particular concern to scientists, as the association between Reye's syndrome and aflatoxins resulted in morbidity and mortality in some children in Thailand in 1963. Aflatoxins B₁ and G₁ were found in livers of two children who died from Reye's syndrome in New Zealand (Becroft and Webster, 1972). Moreover, the effect of aflatoxins on a child's growth started before birth when mothers consumed aflatoxin-contaminated diets as reported in Kenya where the birth weights of females of mothers who consumed aflatoxin-contaminated diets were found to be less than normal. Growth retardation as a result of exposure to aflatoxins was evident in some animals (Butler and Wigglesworth, 1986).

Although the UAE is regarded as being among the developed countries, concerning facilities and high income (estimated as US$21,000/capita), most of the food products are imported from other countries. Baby foods are mostly imported from western countries, were produced and stored under certain environmental
conditions. If proper storage conditions are not provided, this may lead to the deterioration of quality. If *A. flavus* and *A. parasiticus* spores are present and these moulds grow, it is likely that aflatoxin and other mycotoxins may be synthesized, especially as the climatic conditions in the UAE are favourable and provide excellent atmosphere of high temperature and humidity for storage moulds.

The municipalities in the UAE strive to ensure healthy and safe foods for residents. Such covers many issues regarding food controls; however, very little work concerning aflatoxins has previously been carried out. More effort is needed regarding baby food, as it was reported that in 1986 some batches of Cerelac wheat, Rice Cerelac and Wheat banana were collected from large and small stores. Also, it was shown that the chemical and organoleptic characteristics are different for the same products in addition to non-uniformity in product quality (Directive No. 411611, 1986). At that time, a recommendation was made to follow-up the shelf storage of Cerelac which supposed that storage was at 25°C. This indicated that such conditions would not be possible in small groceries, where information about storage conditions of foodstuffs was lacking and this was confirmed by high levels of aflatoxins detected in samples collected from these stores.

Rice is considered as the main staple food for UAE citizens. Rice is imported from abroad and the local governmental authorities keep rice in normal stores, which are not designed to maintain big consignments for long periods. Since rice is purchased through international tenders, a large quantity enough for one year's supply
is imported. These large quantities, under the present storage conditions, face fast deterioration due to high humidity, high temperature, insect infestation, rodents and mould growth. No proper silos are available. Moreover, UAE citizens usually store rice before consumption, based on the assumption that storing will improve flavour.

The findings of this study revealed aflatoxin levels up to 59 μg/kg in rice samples collected from the home stores of UAE citizens. According to Saad et al. (1992), the average daily intake of aflatoxin B1 from rice was suggested to be about 1000 ng/person/day. Such a level could be higher taking into account the contamination levels detected in rice samples in the present study, which were found to exceed the 50 μg/kg tolerance level established in some countries. Aflatoxin contamination of rice is a serious problem that produces health problems, for both mothers and children. The present data about aflatoxin in rice demonstrated that children in the UAE are directly affected by the presence of aflatoxins in rice, since 90% of the mothers relied on rice as weaning food. Moreover, the presence of moulds revealed in this study showed that rice could be nutritionally unsatisfactory for growth of children. The hazard of aflatoxin-contaminated rice starts before delivery, because rice may be consumed by pregnant mothers. Aflatoxin influences on growth, immune and hepatic functions have been reported (Autrup et al., 1991). In Kenya the birth weights of infants of mothers that consumed aflatoxin contaminated food were found to be less than normal. (De Vries et al., 1989). -Presence of aflatoxins in cereals and cereal products
poses serious health problems, as the case of acute liver necrosis where three children died as a result of consumption of rice contaminated to a level of 200 ug/kg in China and the death of more than 100 persons in India as a result of consumption of maize contaminated with 15 mg/kg aflatoxins (WHO, 1979). Moreover aflatoxin-contaminated rice has affected 25 persons in Taiwan, of whom 3 died (Tung and Ling, 1989). In 1988, consumption of aflatoxin-contaminated noodles resulted in the death of 13 children in Malaysia (Cheng, 1992).

Developing countries, where awareness about the hazard of consumption of aflatoxin contaminated food is good, have established regulations to determine the maximum permissible levels of aflatoxin in food which was found to be 50 μg/kg in China, 30 μg/kg in India, 20 μg/kg in USA, 10 μg/kg in Colombia and 5 μg/kg in Czechoslovakia (Van Egmond, 1989).

Raw milk was found to be used as a supplement to breast milk, both goats and cow milk were favoured by UAE citizens and a few of them introduced camel milk at an early age. Twenty-seven per cent of infants received fresh milk. Presence of aflatoxins in milk has confirmed by the heavy aflatoxin contamination of animal feeds analyzed in this study, which gives strong evidence of the relationship between aflatoxin contamination of these feeds and the amount of aflatoxin detected. Aflatoxin M₁ was detected in 68.1% of cow milk samples and in 61.7% of goat milk. Variations in the amount of aflatoxins detected in milk samples obtained from the same source were evident and these may suggest some differences
in the metabolism of aflatoxin B₁ to aflatoxin M₁. This may be dependent on many factors such as animal age, animal physiological status and feed intake. Moreover, carry over of aflatoxins by dairy animals was found to be variable during the lactation period and it was shown that feed consumption rate is high during early and mid lactation than during late lactation (Veldman et al., 1992)

An explanation of the contamination of milk samples with aflatoxins would be the contamination of animal feed. Considering this possibility, a survey was carried out on feed samples collected from the supply of the farms from which milk samples have been collected. Incidence of aflatoxins was evident in all samples of animal feeds, which indicated that the dairy cows and goats consumed contaminated diets and that aflatoxins were transferred to the milk. Veldman et al. (1992) and others described the relationship between aflatoxin B₁ intake per day and aflatoxin content in milk per kilogram that expressed by: aflatoxin M₁ (µg/kg milk) = 1.19 aflatoxin B₁ intake (µg/kg of animal feed per cow per day + 1.9). Application of this formula to the results of this study would mean that the amount of aflatoxin detected in milk from farm A that was 1.1 µg/kg milk would have resulted from the ingestion of 1262 µg aflatoxin B₁, which would be higher than the amount ingested by cows in farm B and C, where aflatoxin intake was found to be 758 µg per day.

The European Community determined 20 µg/kg as a tolerated level of aflatoxin in animal feed for dairy cows assumed to produce
0.1 \mu g/kg aflatoxin M₁ in milk. This level was reduced to 10 \mu g/kg, so as to result in 0.05 \mu g/kg milk (Van Egmond, 1989). When comparing the levels determined in animal feed, clearly all samples containing aflatoxins higher than 10 \mu g/kg would be expected to result in the production of aflatoxin M₁ in milk at a level exceeding 0.05 \mu g/1.

Exposure of infants to aflatoxins from contaminated milk could occur indirectly when mothers consume contaminated milk or directly when infants are fed on this contaminated milk or some of its products. Generally, the consumption rate of milk was found to increase with age. Mothers of infants of this study introduced milk at a frequency of 2-6 times per day, which may lead to that each infant consuming 500-1500 ml per day. Such would be expected to be an exposure of 750-1800 ng of aflatoxin M₁ from cow milk or 1200-2800 ng of aflatoxin M₁ from contaminated goat milk per day.

Some countries have realized the hazards that might arise due to consumption of aflatoxin-contaminated milk by humans, especially among children and infants who rely on milk and milk products for their nutrition. As reported by Van Egmond (1989), those infant and children foods which are based on milk have the lowest tolerance levels among all food kinds. Tolerance (\mu g/kg) in infant foods based on milk products was determined as 0.2 in France, 0.1 in Argentine and Czechoslovakia, 0.05 in Romania and 0.01 in Austria and Germany. It is obvious that the levels detected here in cow and goat milk exceeded the maximum. Tolerance levels of aflatoxin M₁ in milk and milk products have been established in
many countries, which varied among 0.05 in some western countries and 0.5 μg/kg in some American countries, the Soviet Union and Czechoslovakia (Van Egmond, 1989). Compared with this, the levels of aflatoxin detected in milk samples were considered higher than the latter, with the exception of a few samples from farms A, B, C, and D that contained less than 0.5 μg/kg but still their levels were higher than 0.05 μg/kg determined by some western countries.
SUMMARY

The study handled some supplementary infant food used in different feeding patterns during growth and development, with a view to survey levels of aflatoxin contamination.

The results of the experiments can be summarized as follows:

- Different nationalities are available in the UAE, each one had her own style and feeding habits.

- Traditions play an important role in infant nutrition. In addition, the mothers in the UAE have low educational levels and have the tendency to get more children, and hence frequent pregnancies may be a factor in reducing the time of breast-feeding and encouraging the introduction of baby food supplements at an early age.

- Availability of different kinds of baby food and the lack of information about the proper time of their introduction is a prime concern facing nutritionists in the area.

- Aflatoxins were detected at variable levels in some of supplementary milk formulae and cereal based food. There were variations in aflatoxin levels due to sample source where samples from small stores contain higher amounts.
Significant amount of aflatoxin was present in rice grains and low mould infection was detected in intact seeds compared to mouldy and damaged ones.

Aflatoxin M1 was detected in milk of different animals and variations in the amount of aflatoxins were evident due to variations in sources from which milk samples were obtained.

Moreover, animal feeds ingested by these animals showed highly significant levels of aflatoxin contamination and mould infection.

Based on the above findings, the following recommendations are suggested to improve the status of nutrition.

Great care should be given to the mother's nutrition during lactation and the mother should be educated about the importance of nutrients for her growing baby, in order to have a good health and normal growth.

Maternal and child care units should be provided with weight/age charts for normal growth and the personnel should show the mother how to check her child's growth. The growth of infants included in this study compared with Toxford index was less than normal, taking this fact into account together with higher levels of aflatoxins detected in baby food used by those children, further studies are recommended to evaluate exposure of infants to aflatoxins and the health complication produced by consumption of...
aflatoxin contaminated diet that may be one of the factors contributing to in the abnormal growth.

Hazard from aflatoxins to infants occurred directly and indirectly when mothers consumed aflatoxin contaminated foods. This fact should be of particular concern and the food of pregnant and lactating mothers should contain levels of aflatoxins below the tolerated levels determined by developed countries and this needs some effort in determining and checking of aflatoxins in foods and providing of good storage stores that prevents growth and aflatoxin formation.

Storing food under humid and warm conditions enhanced aflatoxins production as evidenced in samples of small stores that were found to contain higher amounts of aflatoxins which increase the hazard of exposure to aflatoxins. These stores scattered throughout the city and accessible to large numbers of families that increase the risk of exposure to aflatoxins. The studies suggest that storage conditions should improve in these stores and responsible staff should be made aware of the problems produced by bad storage.

Significant amounts of aflatoxins found in animal feed confirmed by higher levels obtained in the milks of cows and goats may expose large populations to aflatoxins hazard when consuming products from animals fed on contaminated feeds. Furthermore, the presence of aflatoxins in animal feeds resulted in serious economical losses to animal breeders. Such problems could be
minimized when these feeds are checked for aflatoxins before consumption. In addition to that, owners of these animals should be informed about the danger of storing animal feed in damp and hot places, conditions that enhance aflatoxins production.

Presence of aflatoxins in rice is of particular concern because a large number of the population depend on rice and it is consuming large quantities, hence this study suggest more research about human exposure through quantitative measurement of oxidative metabolites $P_1$, $M_1$ and major aflatoxins metabolites in urine which help in the assessment of this problem.
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143


Appendix 1: Correlation between Aflatoxin M₁ and major component of Milumil

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Appendix 2: Correlation between AFM₁ (ng/ml) and Major Components of S₂₆

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SD: 0.37, 0.55, 2.0, 0.71, 3.3, 13.6, 1.26, 0.33
SE: 0.09, 0.14, 0.55, 0.18, 0.87, 3.5, 0.32, 0.08
Appendix 4: Aflatoxin M\textsubscript{1} (ng/ml) major nutritional components of goat milk

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Appendix 5: Aflatoxin M₁ and major components of camel milk

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