



UNIVERSITI PUTRA MALAYSIA

***OCCURRENCE OF AFLATOXIN M1 IN URINE SAMPLES, MILK AND
DAIRY PRODUCTS AND THEIR ASSOCIATED FACTORS AMONG
RESIDENTS IN TERENGGANU, MALAYSIA***

FARAH NADIRA BINTI AHMAD

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By

FARAH NADIRA BINTI AHMAD

**Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia,
in Fulfillment of the Requirements for the Degree of Master of Science**

May 2016

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Abstract of thesis presented to the Senate of Universiti Putra Malaysia in
Fulfillment of the requirement for the degree of Master of Science

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May 2016

Chairman : Rosita Binti Jamaluddin, PhD
Faculty : Medical and Health Sciences

Nowadays, in conjunction with dramatic growth of population, food safety matter is of concern. The exposure of fungi producing toxin named mycotoxins is one example of food contamination. Aflatoxin M₁ (AFM₁) is a major metabolite of aflatoxin B₁ (AFB₁) which is one of many naturally occurring mycotoxins. This cross-sectional comparative population (n=206) based study was comprised of two parts. The first part was to study the associations between socio-demographic, socio-economic statuses, the consumption of milk and dairy products and the level of awareness, knowledge, attitude and practice on aflatoxin with the occurrence of AFM₁ in urine sample among residents in Terengganu. The second part was to screen the occurrence of AFM₁ in commonly consumed milk and dairy products and to determine the relationship between the ingestion of AFM₁ through milk and dairy products with the level of urinary AFM₁. A set of questionnaire and a urine container were provided to each subject. Data collection was carried out from August 2013 to December 2013. The selection of subject and collection of milk products were based on systematic and purposive sampling respectively. The competitive enzyme-linked immunosorbent assay (ELISA) method was used to determine the level of AFM₁ in the samples of urine and milk products. Data was analyzed by using SPSS Software version 22.0

Bivariate analyses for the first part observed that urban and female subject were more aware, older and female subjects were more knowledgeable, urban and non-employed subjects had more attitude, rural, high educated, non-employed and subjects received monthly income RM1500 and above were practiced more toward aflatoxin matter. Besides, urban, high educated subjects and subjects with high knowledge and attitude tend to consume high amount of milk and dairy products. In the other hand, subjects below 30 years old and married subjects were observed to be more at risk to be exposed with AFM₁. In term of AFM₁ level, subjects below 30 years old, Chinese and non-employed subjects were exposed with higher level of AFM₁. This study also found significant associations between milk and dairy

products intake and the occurrence of urinary AFM₁. For the second part of the study, AFM₁ was detected in 39.6% of milk and dairy products tested. The level of contamination ranged from 0.9 to 119.1 ng/L. It was observable that 4 samples were above the European Commission limit and all of them were still below the Malaysian Food Regulation 1985 limit. All in all, both urban and rural subjects in Terengganu were slightly exposed with AFM₁ whereas for milk and dairy products commonly consumed by the residents in Terengganu, the exposure of AFM₁ was moderate. This study provided a pioneering data on the occurrence of AFM₁ in milk and dairy products in Malaysia.



Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia
sebagai memenuhi keperluan untuk ijazah Master Sains

**KEJADIAN AFLATOKSIN M₁ DALAM SAMPEL AIR KENCING, SUSU
DAN PRODUK TENUSU SERTA FAKTOR-FAKTOR
MEMPENGARUHINYA DALAM KALANGAN PENDUDUK
DI TERENGGANU, MALAYSIA**

Oleh

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Pada masa kini, sejajar dengan pertumbuhan pesat populasi manusia, keselamatan produk makanan semakin membimbangkan. Pertumbuhan kulat yang menghasilkan toksin yang dipanggil mikotoksin adalah salah satu contoh pencemaran makanan. Aflatoksin M₁ (AFM₁) adalah metabolit utama aflatoksin B₁ (AFB₁) yang mana ia adalah salah satu daripada mikotoksin yang terhasil secara semualajadi. Kajian populasi komparatif rentas (n=206) ini terbahagi kepada dua bahagian. Bahagian pertama adalah untuk mengkaji hubungan antara sosio-demografi, sosio-ekonomi, pengambilan susu dan produk tenusu serta tahap kesedaran, pengetahuan, sikap dan amalan terhadap aflatoksin dengan kejadian AFM₁ dalam sampel air kencing dalam kalangan penduduk di Terengganu. Bahagian kedua adalah untuk menyiasat kejadian AFM₁ dalam susu dan produk tenusu yang kebiasaannya diambil oleh subjek serta menentukan hubungan antara pengambilan AFM₁ melalui susu dan produk tenusu dengan tahap AFM₁ dalam sampel air kencing. Satu set soalan dan satu bekas pengumpulan sampel air kencing diedarkan kepada setiap subjek. Pengumpulan data dijalankan sepanjang Ogos 2013 sehingga Disember 2013. Pemilihan subjek dan sampel susu masing-masing adalah berdasarkan persampelan sistematik dan persampelan bertujuan. Kompetitif *enzyme-linked immunosorbent assay* (ELISA) digunakan untuk mengesan tahap AFM₁ dalam sampel air kencing dan produk tenusu. Data dianalisis dengan menggunakan perisian SPSS versi 22.0.

Analisis bivariat bagi bahagian pertama mendapati subjek wanita dan subjek bandar lebih tinggi tahap kesedaran mereka, subjek lebih tua dan wanita lebih berpengetahuan, subjek bandar dan subjek yang tidak bekerja lebih menunjukkan sikap yang baik serta subjek luar bandar, subjek berpendidikan tinggi, tidak bekerja dan menerima pendapatan bulan sebanyak RM1500 dan ke atas adalah lebih tinggi amalan mengenai aflatoksin. Selain itu, subjek bandar, subjek berpendidikan tinggi, subjek yang lebih berpengetahuan dan menunjukkan sikap yang baik terhadap isu aflatoksin adalah lebih cenderung untuk mengambil susu dan produk tenusu dalam kuantiti yang tinggi. Selain daripada itu, subjek yang berumur 30 tahun dan ke

bawah dan subjek yang telah berkahwin didapati lebih berisiko terdedah dengan AFM₁. Bagi tahap pencemaran AFM₁ pula, subjek berumur bawah 30 tahun, berbangsa Cina dan tidak bekerja lebih tinggi tahap pendedahan terhadap AFM₁. Kajian ini turut mendapati bahawa pengambilan susu dan produk tenusu adalah berkait secara signifikan dengan terjadinya AFM₁ dalam sampel air kencing. Untuk bahagian kedua kajian, AFM₁ dikesan di dalam 39.6% sampel susu dan produk tenusu. Tahap pencemaran berjulat daripada 0.9 hingga 119.1 ng/L. Kajian turut mendapati 4 sampel melebihi had yang ditetapkan oleh *European Commission* dan semua sampel masih tidak melebihi had yang ditetapkan oleh Peraturan Makanan Malaysia 1985. Secara keseluruhannya, subjek bandar dan luar bandar di Terengganu terdedah dengan AFM₁ dalam kadar yang rendah manakala susu dan produk tenusu yang kebiasaanya diambil oleh subjek di Terengganu terdedah dalam kadar yang sederhana. Kajian ini menyediakan maklumat perintis bagi kejadian AFM₁ dalam susu dan produk tenusu di Malaysia.

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfillment of the requirement for the degree of Master of Science. The members of the Supervisory Committee were as follow:

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- the research conducted and the writing of this thesis was under our supervision;
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LIST OF ABBREVIATIONS

AFM ₁	Aflatoxin M ₁
AFB ₁	Aflatoxin B ₁
HPLC	High Performance Liquid Chromatography
AFs	Aflatoxins
AKAP	Awareness, Knowledge, Attitude and Practice
EC	European Commission
ELISA	Enzyme Linked Immune sorbent Assay
HCC	Hepatocellular Carcinoma
LOD	Limit of Detection
LOQ	Limit of Quantification
r _s	Spearman rho
FAO	Food and Agriculture Organization of the United Nations
JECFA	Joint Expert Committee on Food Additives
ISIRI	Institute of Standard and Industrial Research of Iran

CHAPTER 1

INTRODUCTION

1.1 Background of Study

Nowadays, globalisation does not only happen in term of technology, but increase of harmful exposure of food contamination is also a serious concern. Sustainable food supply becomes a challenging strategy in order to overcome shortage and contaminated food sources in the future. Moreover, Keesing et al. (2010) stated that since 1940 until today, more than 300 emerging diseases were discovered in humankind population all over the world and this issue significantly correlates with the diets (Newell et al., 2010). This situation becomes worse as the emergence of new disease towards human who are exposed to different chemicals including carcinogenic substances throughout their life increased vigorously (Afshar et al., 2013). Nevertheless, with a proper diet and controlled standard of food source in term of hygiene, contamination and safety, this global issue can be minimised or overcome successfully.

Food contamination can occur in many ways. One of them is through fungi producing toxin called mycotoxins where the ingestion of this nephrotoxic, immunotoxic, teratogenic and mutagenic toxin in grains by animals and human can cause bad impact towards the public health. Ironically, these toxins are capable of causing acute and chronic effects in human and animals in term of disorders of the central nervous, cardiovascular, pulmonary, intestinal tract systems and finally death (Makun et al., 2010). Aflatoxins (AFs) belong to a group of mycotoxins (O’Riordan & Wilkinson, 2008) where the four naturally occurring aflatoxins are aflatoxins B₁, aflatoxin B₂, aflatoxin G₁ and aflatoxin G₂. Among these four, the most commonly occurring and experiencing high carcinogenic effect is aflatoxin B₁ (AFB₁) (Polychronaki et al., 2008). Many cases of aflatoxin contamination had been reported by researchers all over the world including in Malaysia. In 1995, Lye et al. reported the occurrence of aflatoxin aroused since the outbreak of aflatoxicosis in Perak which resulted in acute hepatic encephalopathy among children originated from aflatoxin contamination. Although this phenomenon happened in the past, it does not mean that the same tragedy would not repeat in the future. As a prevention, many researchers around the world especially in Malaysia started to study food highly susceptible toward aflatoxin (Reddy et al., 2011), the exposure of the mycotoxins in human body (Leong et al., 2012a) and the effective ways (eg. diet) in reducing the level of aflatoxin exposure (Nasrabadi et al., 2013). From that continuous efforts, the exposure of aflatoxin in Malaysia so as other countries can be minimized and thus provide the community a safer food supply.

Ardic et al. (2009) explained that aflatoxin M₁ (AFM₁) is a major metabolite of AFB₁ and this metabolite can be found in milk and urine of humans, dairy cattle and other mammals that have consumed aflatoxin-contaminated food or feed. Secretion of AFM₁ in milk of dairy mammals then is continuously transferred into milk and

dairy products and reach human as the end consumer. On the global scale, the occurrence of AFM₁ in milk and dairy products is a serious concern as they are the main sources for introducing aflatoxins in the human diet (Muhammad et al., 2010). Located strategically in the equatorial area, Malaysia experienced tropical climate with humid and high temperature (Redzwan et al., 2013). These conditions are favorable for mycotoxin producing fungi to proliferate and cause contamination towards agricultural products. Later, the ingestion of AFB₁ contaminated feed by dairy livestock would transfer the AFM₁ into the animal's milk and followed by the collection of milk from the mammals for milk and dairy products industry. Since AFM₁ is not destroyed through pasteurization of milk, it will continue to be present in the powdered milk, yoghurt and other milk-based products (Duarte et al., 2013). Contamination of AFM₁ in milk and dairy products does not have to be questionable since there were many discoveries documented by researchers from Europe (Cano Sancho, 2013), Asia (Zheng et al., 2013) and the Middle East countries (Akrami et al., 2013). Realizing this world phenomenon, the European Commission (EC) had highlighted that the maximum permissible level of AFM₁ in milk and dairy products must not be more than 0.5 ng/kg (Tekinsen & Eken, 2008a) in order to ensure over the limit samples are discarded before reaching the consumers.

Redzwan et al. (2013) mentioned the exposure of AFM₁ does not only appear in food source but can also be detected in human biological samples such as serum AFB₁-DNA adduct, AFB₁-lysine adduct, so as the other metabolites in urine and faeces through the isolation of aflatoxin biomarker as reported by Wang et al. (1999), Mykkänen et al. (2005) and Polychronaki et al. (2008) This method then becomes a powerful tool to determine the molecular epidemiology of aflatoxin exposure of each individual in the community (Redzwan et al., 2013). The variation of urinary level of AFM₁ is dependent on many factors such as lifestyle, environmental factors, genetic susceptibility and nutritional status (Sabran et al., 2012). Hence, with these parameters, the progressive steps should be taken to increase the understanding of AFM₁ exposure in the community. Apart from that, the consumption of milk and dairy products are also said to be interrelated with the variation of AFM₁ in the urine samples (Sabran et al., 2012). In fact, there was a good correlation between AFB₁ dietary intake and urinary excretion of AFM₁ in adults (Zhu et al., 1987). Hence it clearly showed that the starting point of AFB₁ ingestion by the dairy livestock would expose AFM₁ metabolites in human urine sample who consumed milk and dairy products.

Modern and advanced detection tool is not enough to minimize the human exposure of AFM₁ unless the population themselves have sufficient knowledge about the aflatoxin and the food that are contaminated by these toxins. Action to control the aflatoxin contamination on groundnut was actually associated with the knowledge which is linked with the socio-demographic and socio-economic status of the individuals (Redzwan et al, 2012a). Transmitting the information like knowledge becomes one of the mechanisms for health communication capable to change the common health habit (Meyerowitz & Chaiken, 1987). Through this effort, the consumers are able to prevent themselves from being contaminated by aflatoxin and thus achieve better health. The three determinants, which are awareness, attitude and practice, are also important to measure the behavioral factors of human toward the

food contamination like AFM₁ in milk and dairy products. Therefore, with the proper exposure on aflatoxin and their effects on human health, the populations are more educated and more prepared to face with the food contamination challenges after this.

1.2 Problem statement

Nowadays, food contamination issues have aroused significant public concern from all over the world. Rodricks and Stoloff (1977) reported since the first outbreak of aflatoxin tragedy in rye by ergot alkaloid, produced by *Claviceps purpurea* in the early 1960s, many scientific researches were carried out in term of etiology of mycotoxicosis and prevention strategies. Until today, research on aflatoxin has been studied extensively by many countries including Malaysia. An acute outbreak of aflatoxicosis in Malaysia has been reported by Lye et al. (1995) where 78.5 % of the raw peanut kernel samples marketed in Malaysia were contaminated and of 78.5%, 10.71% exceeded the maximum tolerable limit of 15 ng/g. Interestingly, a study by Mohd Redzwan et al. (2012a) observed milk and dairy products consumption was positively associated with the level of AFM₁ in urine sample. Besides, respondents who consumed milk and dairy products above median (67.79 g/day) had high level of AFM₁ compared to their counterpart.

Although AFM₁ has been found to be less carcinogenic and mutagenic compared to AFB₁, it has the ability to exhibit a high level of genotoxic activity. This will cause health risk due to the possibilities to accumulate and damage DNA; particularly at guanine residues (Lafont et al., 1989; Wang & Groopman., 1999). Since in Malaysia many types of milk and dairy products are sold abundantly in the market, the consumers have high possibility to be contaminated by this harmful fungus. Considering this phenomenon of aflatoxin exposure in Malaysia had happened before, it is therefore important to study the association of milk and dairy products acceptability and their consumption pattern with the level of urinary AFM₁ and socio-demographic factors. A positive association was found between aflatoxin in nut and nut products intake with the socio-demographic and dietary status (Leong et al., 2011a). Unfortunately, till today no data had been presented in investigating the relationship between milk and dairy products intake in relation to aflatoxin exposure with different socio-demographic and socio-economic characteristics among urban and rural communities.

The Malaysian themselves are not exposed with the knowledge of aflatoxin in milk and dairy products. Redzwan et al. (2012a) reported that most of the non-academic staff in a faculty at Universiti Putra Malaysia knew about the presence of fungal infection in the foodstuffs, and yet they did not know about aflatoxin. From this data, it clearly shows that the people in Malaysia are still far behind in exploring the knowledge on aflatoxin. The knowledge, awareness, attitude and practice become four important tools to measure the understanding of individuals on food contamination and their implication toward global health. High intake of aflatoxin-contaminated foods among human actually resulted from low awareness and knowledge about fungal infection and aflatoxin contamination in food stuffs

(Redzwan et al., 2012a). Thus, the higher ingestion of AFB₁ and AFM₁ will cause higher detection of AFM₁ in urine samples. So with this explanation, determining the subjects' knowledge, awareness, attitude and practice are crucial as one of the steps to reduce aflatoxin exposure in Malaysia.

1.3 Significance of the study

The result obtained from this research could provide clear guidelines for food supplier or importer to ensure the level of AFM₁ in milk and dairy products does not exceed the Malaysia permissible limit which is 500 ng/L (Food Safety Information System of Malaysia [FoSIM], 2012). Otherwise, the samples must be strictly discarded before entering the market. Many reports had been documented about the content of AFB₁ in agricultural crops like spices, cereals, and peanuts (Masoero et al., 2009). Considering these crops as the main food sources for livestock, the initial steps to ensure low or no aflatoxin contamination present in the feed is really important before the transmission of aflatoxin into milk products. Hence, the collected data could prepare a basic parameter for the Ministry of Domestic Trade, Cooperative and Consumerism to set up a new policy regarding the exposure of aflatoxin. The final results and summary drawn from this study will also be beneficial for policy makers in the central and local governments, extension agents to implement the strict regulation on AFB₁ in food and feed to reduce or avoid the contamination of AFM₁ in milk and dairy products (Iqbal et al., 2013).

Apart from that, by determining the factors affecting the higher level of AFM₁ in urine samples, the Ministry of Health, hospital, higher educational institution and private health related company can conduct an appropriate type of education or intervention programs that can increase the level of awareness among Malaysians. Thus, this can reduce the occurrence level of AFM₁ exposure within various populations. Besides, these results also provide new findings about the pattern of milk and dairy products intake between two different living areas. It is an initial step in understanding milk consumption patterns in detail which eventually leads to further analysis on the relationship between these eating patterns with the exposure of AFM₁.

1.4 Study Objectives

1.4.1 General Objective

To study the occurrence of AFM₁ in urine samples and their associated factors among residents in Terengganu.

1.4.2 Specific objectives

Part 1

- 1) To determine the associations between socio-demographic and socio-economic statuses with the total score of awareness, knowledge, attitude and practice on aflatoxin.
- 2) To determine the associations and relationships between socio-demographic, socio-economic statuses, the total score of awareness, knowledge, attitude and practice on aflatoxin with milk and dairy products consumption.
- 3) To determine the associations and relationships between socio-demographic, socio-economic statuses, milk and dairy products consumption, total score of awareness, knowledge, attitude and practice on aflatoxin with the occurrence of AFM1 in urine samples.

Part 2

- 4) To analyse the occurrence and level of AFM1 in commonly consumed types and brand of milk and dairy products
- 5) To determine the relationship between the ingestion of AFM1 through milk and dairy products consumption and the level of urinary AFM1.

1.5 Null Hypothesis

Ho1 = There are no significant associations between socio-demographic and socio-economic statuses with the total score of awareness, knowledge, attitude and practice on aflatoxin.

Ho2 = There are no significant associations and relationships between socio-demographic, socio-economic statuses, the total score of awareness, knowledge, attitude and practice on aflatoxin with milk and dairy products consumption.

Ho3 = There are no significant associations and relationship between socio-demographic, socio-economic statuses, the consumption of milk and dairy products, total score of awareness, knowledge, attitude and practice on aflatoxin with the occurrence of AFM1 in urine sample.

Ho4 = There is no significant correlation between the ingestion of AFM1 through milk and dairy products and the level of urinary AFM1.

1.6 Conceptual Framework

The conceptual framework (Figure 1.1) showed this study was conducted to investigate the associations between all parameters in the socio-demographic statuses, socio-economic statuses, the consumption of milk and dairy products, score of subjects' determinants (awareness, knowledge, attitude and practice) and the occurrence of urinary AFM₁.



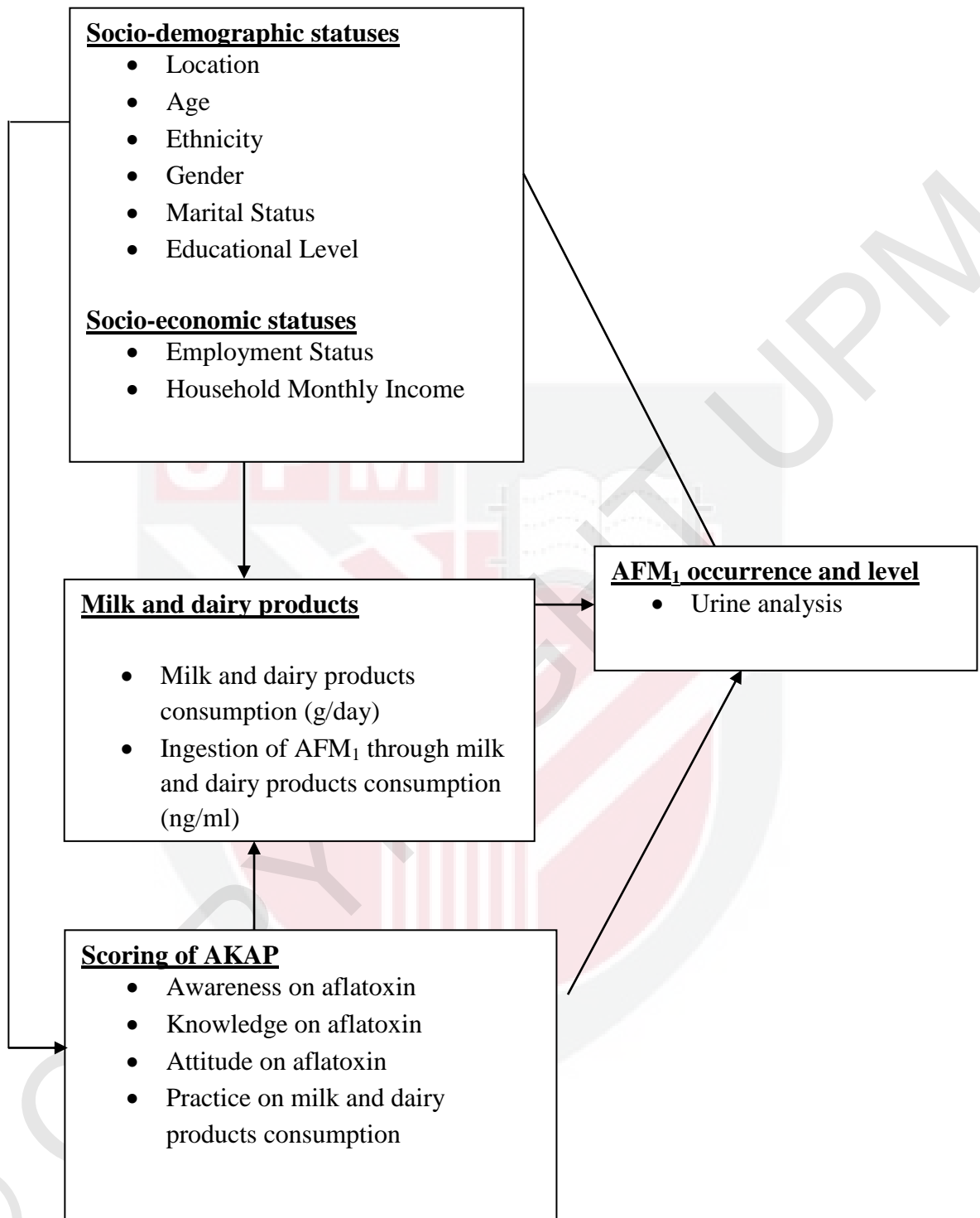


Figure 1.1: Conceptual Framework the Study

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LIST OF PUBLICATIONS

Oral and poster presentations

Farah Nadira A., Rosita J., & Norhaizan M. E. (2015). Screening of aflatoxin M₁ occurrence in selected milk and dairy products in Kuala Terengganu, Terengganu, Malaysia. Nutrition Society of Malaysia 30th Annual Scientific Conference 2 – 3 June 2015 at Renaissance Hotel, Kuala Lumpur, Malaysia (pp. 128).

Farah Nadira A., Rosita J., & Norhaizan M. E. (2016). Screening of aflatoxin M₁ metabolite in urine samples among residents in Terengganu, Malaysia. Nutrition Society of Malaysia 31th Annual Scientific Conference 31 May - 1 June 2016 at Istana Hotel, Kuala Lumpur, Malaysia (pp.62).

Journal

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