Survey of Consumption Pattern, Exposure, and Risk Assessment of Aflatoxins in Different Animal Livers

Soltani, Mahya
Student Research Committee, Department of Food Science and Technology, School of Nutritional Sciences and Food Technology, Kermanshah University of Medical Sciences, Kermanshah, I.R. IRAN

Sadeghi, Ehsan
Department of Food Science and Technology, School of Nutritional Sciences and Food Technology, Kermanshah University of Medical Sciences, Kermanshah, I.R. IRAN

Mahaki, Behzad
Department of Biostatistics, School of Health, Social Development and Health Promotion Research Center Research Institute for Health, Kermanshah University of Medical Sciences, Kermanshah, I.R. IRAN

Shirvani, Hooman
Departments of Agriculture, Payame Noor University, Tehran, I.R. IRAN

Fallah, Maryam
Student Research Committee, Department of Food Science and Technology, School of Nutritional Sciences and Food Technology, Kermanshah University of Medical Sciences, Kermanshah, I.R. IRAN

Motamedi, Parisa
Department of Food Science and Technology, School of Nutritional Sciences and Food Technology, Kermanshah University of Medical Sciences, Kermanshah, I.R. IRAN

Mohammadi, Reza*
Department of Food Science and Technology, School of Nutritional Sciences and Food Technology, Kermanshah University of Medical Sciences, Kermanshah, I.R. IRAN

ABSTRACT: *Aflatoxins are a group of toxic and carcinogenic metabolites produced by fungal species that are found in a variety of foods. Due to the high consumption of liver in Iran and especially in Kermanshah province, in this study consumption patterns of liver types (Sheep, Cow, and Chicken), the aflatoxin levels of liver types (B_1 and G_1), and hazard indexes including Estimated Daily Intake (EDI) and Margin of Exposure (MOE) were investigated. Results showed that males had the highest liver consumption (52.3%) than females with a marked tendency toward consuming sheep liver (80.7%). The results of HPLC analysis indicated that aflatoxin G_1 was detected in all types of the liver. Also, the mean concentration of aflatoxin in samples taken from autumn to winter in cows, sheep, and poultry liver was 1.823, 0.7605, and 0.446 μg/kg. The results of EDI show that the cow liver was 2.33 ng/kg bw/day and above the threshold and the MOE level for all three liver types showed a high risk of cancer with the chicken liver incurring the highest risk with MOE = 78.2. Therefore, it is required to adopt an effective strategy regarding community education, attention to food safety, and liver consumption in Kermanshah city.*

KEYWORDS: *Consumption pattern; Questionnaire; Aflatoxin; Liver.*

***** *To whom correspondence should be addressed.*
+ E-mail: r.mohammadi@kums.ac.ir
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INTRODUCTION

Nowadays, food consumption pattern has a fundamental role in human life, so it has an undeniable role in the development of non-communicable diseases such as cancer, and cardiovascular disease, maintaining good health, and preventing diseases. Countries have different social cultures and food consumption patterns, so the relationship between food consumption patterns and people's health has recently been considered by experts to provide preventive methods to patients [1, 2]. Offal is the internal organs or cavities of an animal which include the liver, heart, kidneys, lungs, intestines, brain, tongue, tail, and legs [3]. In many cultures, it is used as an important nutritional resource. The liver as a part of offal, is enormously popular in Iran and is recognized as a quality protein source traditionally sold on the market and consumed more than other offal parts [4-6]. It is also a rich source of a variety of vitamins including A, B, D and minerals such as iron and zinc, which, according to studies, can have a higher nutritional value than meat. However, it is the site where toxin components such as heavy metals, insecticides, pesticides, dioxins, and mycotoxins are accumulated [7, 8]. Mycotoxins are toxic compounds that remain in the liver for a long time [9]. The most dangerous mycotoxin is aflatoxin B_1 produced by *Aspergillus flavus* and *Aspergillus parasiticus* [10, 11]. Aflatoxins are produced by inappropriate storage conditions of food products or raw materials that contaminate foods such as grains, nuts, dried foods, milk and other dairy products, meat, eggs, and animal feed [12-16]. Many studies have been conducted on the presence of aflatoxins in Iranian animal feed (barley, wheat bran, wheat dry pulp, and canola meal) and their results indicate that many of them are over-limited to be contaminated with aflatoxin. This can be due to the traditional forage storage system, climate change across seasons, humidity and changes in fresh plant feed in the spring and then winter wheat forage. It then enters the animal body through the food chain and can contaminate various animal products and organs such as meat and liver [17]. The maximum total intake of aflatoxins in general by the EU is set at 2-15µg/kg for food and feed [18]. On the other hand, the amount of aflatoxin permitted by the FAO is 0-50 µg/kg and by the American Food Administration (FDA) it is 20 µg/kg [19]. Aflatoxins B_1 are in the first group of carcinogenic compounds which can cause diseases such as liver cancer, chronic hepatitis, jaundice, cirrhosis, and impaired nutritional synthesis, plus it increases the risk of cancer if the Hepatitis B virus is present [20]. Therefore, given the high consumption of liver in Iran, especially in Kermanshah province, and also due to the contamination of livestock feed with aflatoxins, the purpose of this study is to survey the consumption patterns of the liver in Kermanshah city in 2018-2019 as well as the measurement of aflatoxin levels B_1 and G_1 in all types of liver used in two periods of six months (spring & summer and winter & autumn) to raise awareness about food safety in the community.

EXPERIMENTAL SECTION

Sampling

In separate months between seasons with hot and cold weather (autumn & winter and spring & summer) in 2019, 180 liver samples including cow liver (60), sheep liver (60), and chicken liver (60) were purchased from the retail store and important slaughterhouses of Kermanshah with the weight of 500 g. Then, the samples were transferred to the quality control laboratory of the university in appropriate packaging at 4 ° C and stored at -18 °C until extraction.

Materials

The standard of Aflatoxin (B_1, B_2, G_1, and G_2) with a purity of 99% was used by Sigma-Aldrich, UK. The immunoaffinity column (AflaStar and OchraStar) was prepared from Romers, USA, for purification. HPLC grade solutions including water, acetonitrile, methanol, Sodium chloride, and Tween 20 were purchased from Merck Company (Darmstadt, Germany) for the extraction of samples. Buffer tablet was purchased from Medicago Company Uppsala Sweden for pH stability during analysis.

Preparation of Samples

Extraction of liver samples

The analysis of aflatoxin was performed according to the method by *Fan et al*. (2013) with some changes [15]. Liver samples were placed at ambient temperature for 30 minutes. 25g of Livers mince for 7-10 min with a meat grinder separately, and then fully blended samples were mixed with 5g of NaCl and 100 mL of methanol and water at 80:20 and then mixed with a magnetic stirrer for 20 minutes. The mixture was applied to a Whatman filter paper No.1 (Whatman Inc., Clifton, NJ, USA). A total of

10 mL of the solution was added into 40 mL Phosphate Buffer Saline (PBS) containing 0.1% Tween 20 which passed through the immunoaffinity column containing specific antibodies and the ability to isolate very small amounts of this contaminant. Finally, the column was washed with acetonitrile and the liquid was collected. At the end of extraction, the column was washed in 2 steps: In the first stage: the column was washed with 10 mL of phosphate buffer at a flow rate of 10 mL/min, and in the second step:1 mL of methanol and 1 mL of water were used for column washing, respectively. The solutions were dried in the vicinity of nitrogen gas and diluted20 mL of volume with distilled water. Finally, 150 µL of the sample was injected into the HPLC apparatus equipped with RPC18 column and fluorescence detector at wavelength 365-435 nm.

HPLC conditions

All the procedures at this stage were followed according to the method described earlier with the changes according to the conditions. In this research, Chromatographic separation was carried out by the Knauer Azarov model with Post-column derivatization (UV, Lc Tech) and an RF-20A fluorescence detector (Shimadzu, Kyoto, Japan). Aflatoxins were separated by RPC18- Knauer columns in the wavelength 365-435 nm and the column temperature of 40 °C. Mobile phases included water and acetonitrile (9:1) and the injection was performed at 150 µL.

Analysis Validation

Validation was performed using AOAC methodology [21]. To determine the linearity of the standard curve, calibration, and the validity of the method, initially standard concentrations (0.4, 1.2, 2.0, 2.8, 3.6, 5.6, and 7.2 µg/kg) were injected into HPLC and regression equation y=35609x-10.971 was obtained at R^2=0.997 to calculate the concentration of samples. Furthermore, the Limit of Detection (LOD) and the Limit of Quantification (LOQ) were carefully determined. The precision of the test method was specified by adding toxins to the non-contaminated samples at three concentrations (2.5, 5, and 7.5µg/kg) and four replicates for aflatoxins. Then, the recovery rate was calculated and the final amount of aflatoxin was reported based on µg/kg units. The final amount of aflatoxin was reported based on µg/kg units.

Instrumentation

Information for liver consumption was collected through a questionnaire whose validity and reliability was confirmed in Iran. Initially, the population data of 8 municipalities of Kermanshah were obtained from the city planning and management organization. Then, using a statistical method with an accuracy of 0.5 and a confidence level of 0.95, 496 questionnaire items was designed. The questionnaire was completed randomLy, and face-to-face interviews were conducted with people from 18 to 60 years of age. The questionnaire consisted of two parts: the first part addressed demographic characteristics (Age, Weight, Gender, Education, Marital Status, and Occupation), and the second part patterns of consumption (Nutrition, Consumer, Health, and Safety Questions) of the liver.

Risk Assessment

The development of liver cancer and its association with the intake of aflatoxin has been considered by the Food and Agriculture Organization (FAO) and the World Health Organization (WHO). Therefore, the Margin of Exposure (MOE) was determined to measure the risk of carcinogenesis and genotoxic carcinogenicity according to Eqs (1) and (2) [22]:

(1) Estimated Daily Intake (EDI) =Average concentration of AF×Average daily consumption/Average body weight

(2) MOE=BMDL10/EDI

BMDL10= 170 ng/kg bw/day for Aflatoxin [23]**.**

MEO of less than 10,000 indicates a danger to human health, while the MOE of more than 10,000 low public health concerns

Statistical analysis

The data were expressed in tables and graphs using $sspss_{21}$ software. Quantitative variables were defined as the mean ± standard deviation and qualitative variables were characterized by frequency (number and percentage). Firstly, the Kolmogrov-Smirnov test was run to evaluate the normal distribution of quantitative data. ANOVA procedure and Kruskal-Wallis test were then employed to compare normal variables in the three groups. Also, in order to investigate the toxin level, the factorial analysis was performed in a Completely Randomized Design (CRD) with six replications used for the classification of samples at 1% level.

RESULTS AND DISCUSSION

Characteristics of respondents

Through 496 distributed questionnaires, according to table 1, both central and dispersion indices were determined regarding demographic characteristics for a total number of 8 districts of Kermanshah City. Results showed that the average age of the respondents was 32.22 and the highest frequency was 23 years old (9.6%)**.** The maximum age was 60 and the minimum was 18 years old. Also, married people 52.3% had the largest population. In this research, the males had the highest frequency of liver consumption (52.1 %) and the people's average weight was 70.25 kg and the people with 60 kg had the highest frequency of 5%. *Vicence et al*. studied the consumption pattern of chocolate and cocoa among the students where various indicators such as gender, educational level, age, and weight were expressed using the dispersion test and it was found that the average age of the people was 24.10 and most of the respondents were males. Also, the Average Body Mass Index for the subjects was 0.8 kg/m^2 and chocolate bars were the most consumed among students [24].

ANOVA Analysis

The results of ANOVA analysis showed that there were significant differences in age, education, and marital status among 8 regions ($P \leq 0.01$), and no significant difference was observed in weight, sex, and occupation (Table 1). For consumption pattern questions, a significant difference ($P \leq 0.01$) was detected between the frequency of the consumption of chicken liver and cow liver, the use of clean and hygienic restaurants, and the use of hygienic packages of the liver and the consumption of liver at home**.** There was no significant difference in other questions. *Niyonzima et al.* examined the daily consumption and bacteriological quality of meat in Rwandan families. The results showed that the highest consumption among the types of meat was related to beef with a significant difference from other types [25].

Consuming, nutritional and hygienic tendencies

In the present study, people who were not interested in consuming the liver were sidelined and in total 80.70% of people tended to consume the liver (Table 1). Among various regions, the highest percentage of liver consumption is in area 3 (98.32%), because this area is the main center of liver sales. Additionally, the lowest percentage of liver consumption was in area4 (72.58%), due to its far distance from the distribution and sale of the liver. Also, among the liver types, the tendency to consume sheep liver was the highest at 85.2%. In a study by *Pieniak et al.*, the pattern of fish consumption and labeling of fish in Poland were studied. Dispersion analysis was performed for different indices such as the interest in fish species. Results demonstrated that 52.7% of respondents did not eat wild fish and 41.5% did not like fish at all. In a study of poultry consumption patterns in Belgium and awareness of the risk factors of Campylobacter bacteria [26]. *Sampers et al.* reported that the longest duration of meat refrigeration was one to three months and poultry meat consumption in Belgium was 92.75% according to dispersal analysis [27]. In relation to the types of liver consumption, barbecuing with 80.8% had the highest response and 29.7% of the study population sometimes preferred fat with the liver. This could be due to Iranians' interest in consuming grilled liver. In this study, most respondents are keen to consume liver from clean and healthy restaurants, which can be due to people's awareness of food safety and health. However, in Iran, liver is sold locally. Partial awareness of the disadvantages and benefits of the liver was the most frequent response with 49.5% and most respondents were interested in consuming the liver at home (Table 1).

Correlation

The results of correlation (table 2) between demographic parameters and questions showed that there was a significant and positive correlation between education, weight, and job and most of the questions. Also, there was a positive and significant correlation between the percentage of liver consumption and demographic parameters of the job, education chooses between types of food in the restaurant, use of liver packs with a health license, and Awareness of the benefits and harms of the liver. *Hiamey et al.* addressed the concerns about the consumption of street foods in Ghana. In this study, the correlation between parameters was determined to show that there was a significant negative correlation between food safety and environmental concerns as well as between street food consumption, health, and food safety concerns and environmental concerns [2]. *Cabral et al.* studied food choices in Africa. In this study, a good correlation was reported between nutritional and dietary aspects, food content, and eating habits [28].

Table 1: Frequency of liver consumption and individuals' health and nutrition tendencies in 8 areas of Kermanshah city.

	Parameters	Mean Square	Mean (Std. Deviation)	Median	Mode	Amount	Most Answers
Demographic	Age	394.50**	32.22 (11.798)	28.00	23	9.6%	
	Weight	283.17ns	70.86(13.427)	70.00	60 kg	5%	
	Sex	0.051ns	1.48(.500)	1.00	1	52%	male
	Level of education	2.46**	3.42(.948)	3.00	3	37.4%	Secondary education
	marital status	0.94**	1.52(.500)	2.00	2	52.3%	Married
	Job status	1.84ns	2.49(1.045)	3.00	3	32.6%	Unemployed
Consumption pattern	Liver consumption percentage	538.17**	80.78(80.20)	80.70	3	98.38%	aerie 3
	Tends to consume liver (Q^1)	1.71ns	2.58(1.056)	3.00	2	30.4%	medium
	Liver Type Preference (Q^2)	0.166ns	2.00(.392)	2.00	2	85.4%	liver of sheep
	Consume Chicken liver (Q^3)	3.715**	3.83(.934)	4.00	4	38.1%	Once every three months
	Consume sheep liver (Q^4)	1.48*	3.53(.813)	4.00	4	49.8%	Once every three months
	Consume cow liver (Q^5)	3.19**	4.22(.815)	4.00	4	43.8%	Once every three month
	Type of liver consumption (Q^6)	0.525ns	1.36(.761)	1.00	1	81.1%	Barbecue
	Tends to consume liver with fat (Q^7)	1.434ns	2.31(1.046)	2.00	2	29.9%	Sometimes
	The tendency to be a barbecue type (Q^8)	1.212ns	2.84(1.116)	3.00	4	35.2%	minced meat kebab
	Choose between types of food (Q^9)	2.720*	1.75(1.086)	1.00	1	59.8%	Kebab
	The tendency to consume liver in clean and healthy restaurants (Q^{10})	3.640**	3.17(.984)	3.50	4	50%	Too much
	Use of liver packs with a health license (Q^{11})	1.80**	2.19(.769)	2.00	3	40%	Sometimes
	Awareness of the benefits and harms of liver (Q^{12})	3.077**	2.15(.913)	2.00	3	49.5%	Somewhat

ns: Non Significant. **Significant at the (P≤ 0.05) probability levels.* ***Significant at the (P≤ 0.01) probability levels.*

Table 2: Correlation coefficients between demographic indicators and meaningful questions.

Parameters	Age	Weight	Education	Job status	Q^3	Q^4	Q^5	Q^9	Q^{10}	Q^{11}	Q^{12}
Weight	.439	1									
Education	.156	.808*	1								
Job status	.305	.834**	.776*	1							
Consume Chicken liver (Q^3)	-.072	.702	.827*	.660	1						
Consume sheep liver (Q^4)	.322	.798*	.854**	.674	.846**	1					
Consume cow liver (Q^5)	.333	.795*	.680	.680	.776*	.926**	1				
Choose between types of food (Q^9)	.593	.823*	.854**	.770*	.659	.905**	.787*	1			
The tendency to consume liver in clean and healthy restaurants(Q^{10})	.228	.665	.716*	.694	.821*	.642	.538	.655	1		
Use of liver packs with a health license (Q^{11})	.310	.811*	.818*	.861**	.547	.749*	.724*	.817*	.385	1	
Awareness of the benefits and harms of liver (Q^{12})	.484	.888**	.843**	.826*	.577	.836**	.784*	.918**	.465	.963**	1
Percent	.488	.740*	.786*	.784*	.366	.608	.504	.816*	.390	.915**	.911**

**Significant at the (P≤ 0.05) probability levels.* ***Significant at the (P≤ 0.01) probability levels.*

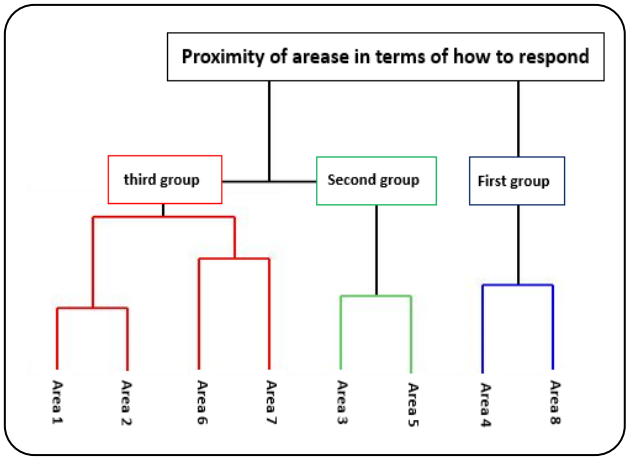

Fig. 1: Dendrogram derived from demographic indices and study questions for eight regions.

Grouping the regions according to the pattern of liver consumption

Fig. 1 shows cluster analysis based on all parameters for 8 regions. The results indicated that these areas can be divided into 3 groups based on all features. Consequently, areas 4 and 8 which are geographically close together were lower in terms of all demographic parameters and questions and the lowest points belonged to these two areas. Areas 3 and 5, which had the highest average consumption of liver, were in the second group because of their proximity to liver centers. Also, there were four areas 1, 2, 6, and 7 in the third group, which were similar in terms of distribution and type of response.de *Carvalho-Ferreira et al.* investigated the tendency to consume fat and overeating by cluster analysis for the three variables of sweetness, fat content, and calorie, where it was shown that participants did not use low-fat and sweet foods [29]. *Trafialek et al.* studied the health practices of food vendors using a questionnaire based on the cluster test. The questions related to safety were clustered and the ones with similar ranks were concentrated side by side so that their significant differences from other clusters were identified [30].

Evaluation of aflatoxin concentration

Table 3 shows the aflatoxin concentrations and risk assessment indicators for liver types. In this research, LOQ levels for aflatoxins B_1 and G_1 were 0.3 and 0.1 ng/mL and LOD levels for aflatoxins B_1 and G_1 were 0.1 and 0.03 ng/mL, respectively which indicates good accuracy of the method. Also, the calibration curve was linear and aflatoxin concentrations in liver types calculated using standard concentrations. Fig. 2 shows a chromatogram of a positive liver sample contaminated with aflatoxins B_1 and G_1. Analysis of the laboratory data showed that out of 180 analyzed samples, cow, sheep, and chicken liver were contaminated by aflatoxin G_1 with a mean total concentration of 1.441 µg/kg, 0.688 µg/kg, and 0.384 µg/kg, respectively which can be due to the type of animal feed consumed in Iran, namely straw and alfalfa dried and stored. The results of this study are in line with the results *Rodriguez-Blanco et al.* [31]. They studied the levels of aflatoxin in feed and the prevention of aflatoxin M_1 in dairy products and found that the highest levels of aflatoxins were in low-humidity animal feed (maize silage, alfalfa silage, barley silage, okara, soya bean husk, straw, and dehydrated alfalfa), which showed a high concentration of aflatoxin G_1. *Omeiza et al.* investigated the levels of aflatoxin B1intypes in animal feed (fresh and stored). Their results showed that animal feed with grain and concentrate origin is closely related to the formation of aflatoxin B_1but, aflatoxin B_1 levels were lower in grassland and dry grassland than in other livestock feeds which can be attributed to the presence of *Aspergillus flavus* in concentrated animal feed and its nutrient richness [32]. Cattle and chicken liver samples taken from autumn-winter were contaminated with aflatoxinB_1 which could be due to the high consumption of animal feed such as seeds (corn, soy, and barley) and concentrate compounds during this time period. *Amirkhizi et al.* studied chicken liver and egg samples in Iran for aflatoxin B_1 levels. In this study, it was found that a high percentage of samples, including 72% of liver and 58% of eggs, were contaminated with aflatoxin and the level of aflatoxin contamination was between 0.30 to 16.36 µg/kg [5]. Also, in another study, they measured the level of ochratoxin and zearalenone in egg and chicken samples at the market level and the results indicated that 35% of chicken samples and 28% of egg samples were contaminated with these toxins [33]. According to the analysis of variance (Table 4), there was a significant difference between the types of liver and sampling season and an interaction effect between sampling season and livers for aflatoxin G_1 level at 1% level that investigates these differences, the means were compared at 1% level. The mean comparison results in Table 3 indicate that cow liver in the autumn-winter period had the highest amount of aflatoxin G_1 with a mean of 1.823 µg/kg and chicken liver had the lowest mean

Table 3: Calculated parameters for aflatoxin G_1 concentration and determination of EDI and MOE in different types of cow, sheep and chicken liver used in Kermanshah market.

Type of liver	Time span	Number of sample	Replication	Mean I	SD I	Rang I	Mean II	SD II	Rang II	EDI	MOE	Average daily consumption	LOD	LOQ	Recovery
Cow	Autumn to Winter	30	6	1.823[a]	0.482	1.29-2.35	1.441[a]	0.522	0.939-2.350	2.33	396.1	100	0.1	0.3	%86.56
	Spring to Summer	30	6	1.059[b]	0.133	0.939-1.25									
Sheep	Autumn to Winter	30	6	0.7605[c]	0.061	0.679-0.857	0.688[b]	0.096	0.510-0.857	0.97	164.9	100			
	Spring to Summer	30	6	0.6155[d]	0.063	0.51-0.676									
Chicken	Autumn to Winter	30	6	0.4463[e]	0.027	0.404-0.482	0.384[c]	0.079	0.234-0.482	0.46	78.2	85			
	Spring to Summer	30	6	0.3233[e]	0.063	0.234-0.398									

Mean I: Mean of Aflatoxin G_1 concentration in measured Seasons. SDI: Standard deviation of Aflatoxin G_1 concentration in measured Seasons. Rang I: variation range of Aflatoxin G_1 concentrations in measured Seasons. Mean II: Mean of Aflatoxin G_1 for each liver type. SD II: Standard deviation of Aflatoxin G_1 for each liver type. Rang II: variation range of Aflatoxin G_1 for each liver type. LOD: limit of detection LOQ: limit of quantification

aflatoxin G_1 in two six-month intervals which were not significantly different from each other. It could be due to the type of constant feed of chicken throughout the year. The reason for the difference in the mean concentration of aflatoxin in cow and sheep liver can be free grazing in spring and less use of dry forage resulting in a lower concentration of toxin. Overall, the mean concentration of aflatoxin was higher in the autumn-winter period than in the spring-summer period. As a result, the amount for all three types of cow, sheep, and chicken liver was 1.823, 0.760, and 0.4463 µg/kg, respectively, which may be due to moisture and appropriate conditions for the growth of aflatoxin-producing fungi in the animal feed storage which entered the food chain. Herzallah studied aflatoxin M_1, M_2, B_1, B_2, G_1, and G_2 levels in milk (raw and pasteurized milk of sheep, cow, and goat), eggs, and beef samples of local markets in Jordan from winter to spring [21]. The results showed that aflatoxin levels were higher in winter (0.15- 6.6 µg/kg in fresh meat) which was related to aflatoxin levels and winter weather conditions compared to spring and green pastures as animal feed. In two six-month intervals, the mean concentration of aflatoxin G_1 in the chicken liver was 0.384 µg/kg which was lower than cow and sheep liver, due mainly to the shorter longevity of chickens and the consequent accumulation of less toxin in chicken liver than in beef and sheep liver. Also, cow liver with a mean of 1.44 µg/kg had the highest level of toxin, which could be due to the greater longevity of cows and consuming more daily feed. *Hussain et al*. studied the effect of different concentrations of aflatoxin-containing diet and the age of broilers on the amount of aflatoxin B_1 remaining in the liver and muscle. In this research, three different concentrations of aflatoxin B_1 were added to the diet of 7, 14, and 28dayold chickens, and the results showed that after 14 days, they showed a higher level of toxicity in the liver and muscle [34].

Cancer risk assessment for types of liver

Due to the high consumption of liver in Iran and Kermanshah province, in particular, risk assessment indices such as MOE and EDI were calculated. EDI index was calculated based on daily consumption of liver, mean toxin concentration, and mean body weight (70.86 kg) for daily intake of aflatoxin by types of the liver. The results showed that the EDI of sheep liver, chicken liver, and cow liver were 0.97 ng/kg bw/day, 0.46 ng/kg bw/day, and 1.33 ng /kg bw/day, respectively which is higher than the recommended level.

Given that aflatoxins are known to be a group of genotoxic and carcinogenic compounds, many scientific committees around the world have reduced their daily intake of aflatoxins to a reasonable minimum. The European Food Safety Authority recognizes daily intake of more than 0.017 ng/ kg bw/day for aflatoxins as a public

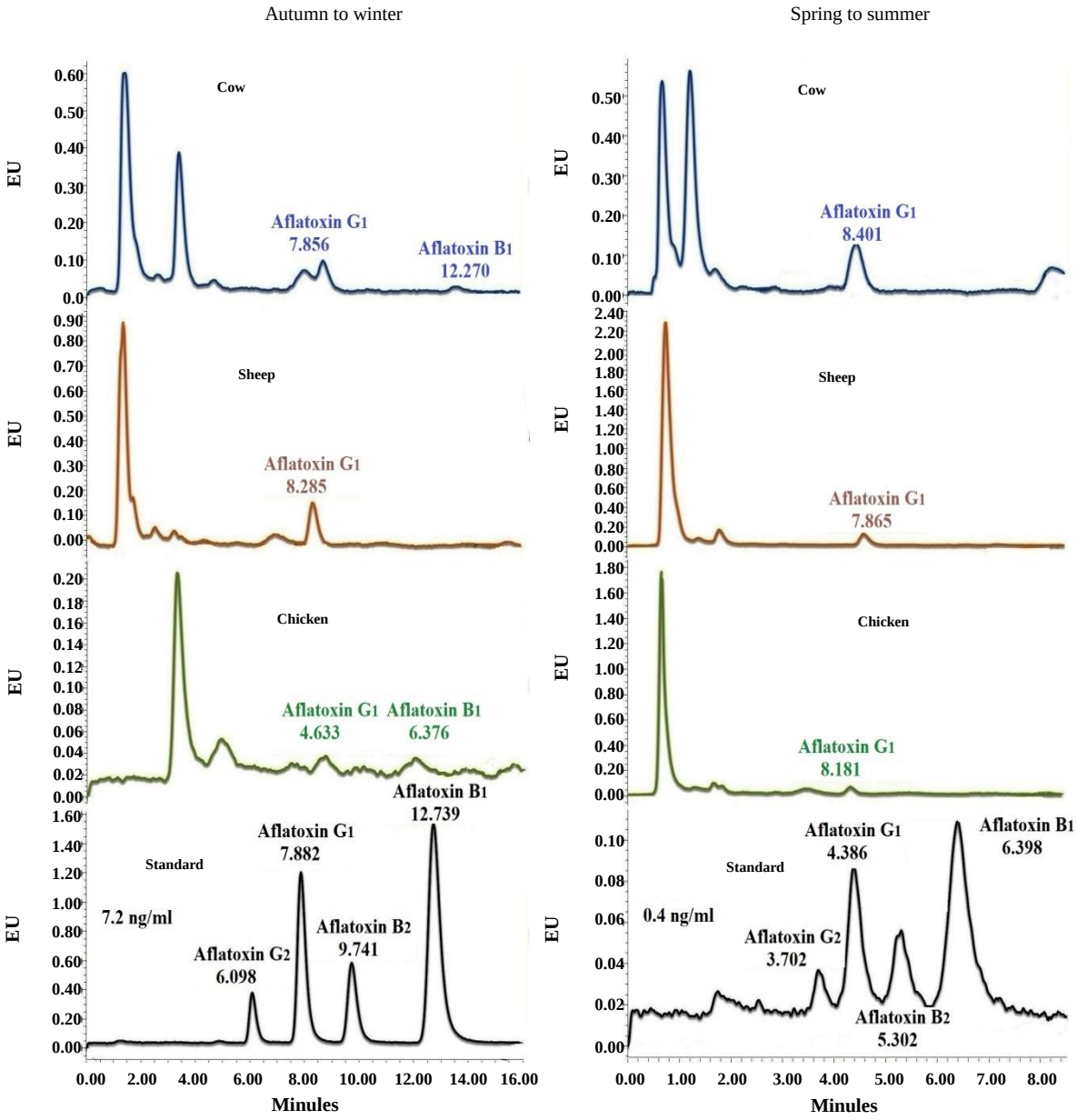

Fig. 2: Chromatogram of liver samples (cows, sheep, and chicken) over the analysis period (spring to summer) and (autumn to winter).

health problem [35]. Even the European Commission has stated that exposure to aflatoxin in 1 ng /kg bw/day or less can lead to liver cancer [22]. One of the other important indicators that are used to calculate the number of toxic compounds in food is the MOE which estimates the margin between the dosage of a substance and human exposure to cancer. Although the mean concentration of aflatoxin G_1 in all three liver types was lower than the standard level, the MOE showed that the values obtained for all three types of liver were lower than 10,000 which could potentially trigger cancer. Chicken liver with MOE = 78.2 has the highest risk and can cause concern about liver consumption. In one study after determining the concentration of aflatoxin and ochratoxin in cheese calculated the levels of EDI, MOE, and cancer risk [36]. The mean concentration of aflatoxin B_1 and the level of MBO were .610 and 2982, respectively, which indicates health risk for aflatoxin M_1 and B_1. *Martinez-Miranda et al.* examined the levels and risk of

Table 4: Analysis of variance for aflatoxin concentrations in different seasons for liver samples.

Sources of Variation	Degrees of freedom	Mean of squares
Liver type	2	0.985*
Season	1	0.253*
Liver Type × Season	2	0.048*
Error	30	0.007
Coefficient of variation	9.70%	

**Significant at the (P≤ 0.01) probability levels, respectively.*

aflatoxins (G_1, G_2, B_1, and B_2) in bread, rice, and arepa in the adult population of Colombia. The results showed that the EDI and MOE values for AFB_1 by rice consumption were 3.93 and 60.8 respectively [21]. Typically, the MOE of aflatoxin G_1 in all types of liver reported in this study is in line with MOE of aflatoxin B_1 obtained from cereals in other parts of the world such as China (between 24.1- 1272); Pakistan (between 10 and 69), Vietnam (2674), Iran (1417-4250), Netherland (8684.2-9916.7) [37-41].

CONCLUSIONS

Based on the completed questionnaire analysis, results showed a high level of interest in the liver as part of offal and especially sheep liver, and men are more likely to consume liver Laboratory results showed that aflatoxin G_1 was observed in types of the liver. Also, aflatoxin G_1 levels in liver samples were higher in the spring-summer than in the autumn-winter periods. Aflatoxin B_1 in winter and autumn samples was observed in both cattle and sheep liver. MOE levels for all three liver types showed a high risk of cancer and it was found that chicken liver had the highest risk. The mean concentration of aflatoxin G_1 in all three liver types was lower than the standard level. However, given the high consumption of liver in Kermanshah city and the results of risk assessment indices (EDI and MOE), it can be of concern to the health of the community. Overall, assuming that some countries, such as Iran, have a high consumption of the liver, the results of this study can raise public awareness of the risks associated with excessive consumption of the liver.

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