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THE EFFECT OF A DIET ON THE OCCURRENCE OF OCHRATOXIN A IN BODY FLUIDS

S u m m a r y

Background. Ochratoxin A (OTA), a secondary metabolite of fungi, is produced by fungi of the *Aspergillus* and *Penicillium* genera. It is supplied to the body with food consumed by people. The purpose of this study was to determine the occurrence of ochratoxin A in human body fluids in relation to diet. A part of the study group consisted of women who were donors from a breast milk bank. The aim of this study was to determine the correlation of ochratoxin A in body fluids (milk, urine, serum) and the diet of the women covered by the study. The study material consisted of 60 urine samples, 60 milk samples and 60 blood samples. Ochratoxin A in milk and urine was determined by HPLC with fluorescence detection. For the determination of ochratoxin A in blood serum, we used a liquid chromatography-mass spectrometer (LC - MS/MS) method.

Results and conclusions. As a result of the analysis, it was found that as the frequency of consumption of dried grape fruit increases, there is a significant increase in the level of OTA in breast milk. Next, it was shown that as the frequency of beer consumption increases, there is a significant increase in the level of OTA in maternal urine. Finally, it was shown that a significant increase in maternal serum OTA levels occurs with an increase in the frequency of consumption of dried fruit. OTA was found in 4 out of 60 milk samples, in 40 out of 60 urine samples and in 60 out of 60 serum samples. The surveyed women's average age was 31 (ranging from 22 to 41). Given all the respondents, 46 lived in the city, 14 in rural areas, 35 were economically active, 22 were not, and 3 were on maternity leave. On average, they had 2 children (from 1 to 4). Of all children being fed, 35 were male and 25 female. On average, delivery occurred on the 37th week of pregnancy (between the 27th and 42nd week).

Keywords: mycotoxins, ochratoxin A, diet, body fluids

Introduction

Ochratoxin A (OTA), a secondary metabolite of fungi, is produced by fungi of the *Aspergillus* and *Penicillium* genera [20, 19, 30, 27, 13] OTA, due to favorable weather

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and microclimatic conditions, is found almost all over the world. It occurs in raw materials and products that are stored in an inadequate manner [33, 17]. It is commonly found in materials such as: wheat, barley, corn, oats [19], rice, millet, rye [22], cereal products and their derivatives – flour, bread, pasta, cereal flakes [22,7], cocoa and cocoa products [7], coffee (roasted and green), beer, grapes, wine (red and white), grape juice, spices [7, 10, 6, 31] milk [27, 33], legumes [31], dried fruit [7].

The European Union Agency, which provides scientific advice on food chain risks, European Food Safety Authority (EFSA), has set a maximum permissible weekly intake of OTA of 120 ng/kg b.w. [9], while the Joint Expert Committee on Food Additives (JECFA) has set the weekly intake of OTA at 100 ng/kg b.w. [16]. OTA is mostly excreted from the body in urine, feces [25, 8], indirectly in bile [21] or milk [23]. In addition, OTA has a long half-life in humans – about 35 days [9]. OTA has nephrotoxic, hepatotoxic effects, is teratogenic, mutagenic and immunosuppressive [19, 32, 3, 1, 26]. Initially, the International Agency for Research on Cancer (IARC) classified OTA as Group 3 (not classified as a human carcinogen) in 1987 [14], but as the years passed and greater knowledge of its toxicity was gained, OTA was classified in 1993 as Group 2B (possibly carcinogenic to humans), making it a carcinogen [15].

Over the past few years, there has been a noticeable increase in interest in the effects of mycotoxins on human organisms. The relationship between the frequency of consumption of certain foods and OTA levels in milk, urine and maternal serum is the main hypothesis included in this work. An analysis of existing studies has shown that many issues concerning mycotoxins and their effects on human organisms have not been described. This became the motivation for conducting the research described in this paper, which had several objectives – to determine the correlation of the occurrence of ochratoxin A in body fluids (milk, urine, serum) and the diet of the women covered by the study.

Research material and methods

On April 24, 2018, the Bioethics Committee at the Nicolaus Copernicus University in Torun, Collegium Medicum in Bydgoszcz, Poland, issued approval no. KB323/2018 for the study "Detection and comparison of ochratoxin A levels in body fluids (milk, urine, blood) of breastfeeding women". The study material consisted of 60 urine samples, 60 milk samples and 60 blood samples. The samples were collected between December 2018 and November 2019. A part of the women examined were the donors from a breast milk bank in Toruń. Each of the women undergoing the study filled out a questionnaire about their general health and diet. The questionnaire included foods that could potentially contain OTA and be adapted to the diets of breastfeeding women.

The surveyed women's average age was 31 (ranging from 22 to 41). Given all the respondents, 46 lived in the city, 14 in rural areas, 35 were economically active, 22 were not, whereas 3 were on maternity leave. On average, they had 2 children (from 1 to 4). Out of all children being fed, 35 were male, 25 female. On average, delivery occurred on the 37th week of pregnancy (between the 27th and 42nd week). Spearman correlation was used for statistical analysis.

Methods for determining ochratoxin A in milk

Milk samples were thawed immediately before extraction. First, they were centrifuged in a centrifuge for 15 minutes at a centrifugation speed of 7,500 rpm. After centrifugation, 20 cm^3 of milk was passed through an OchraPrep immunoaffinity column (R-Biopharm Rhône LTD). After the samples passed through the column, they were washed with 20 cm^3 of deionized water and air dried. The next step was to elute the OTA through 1.5 cm^3 of ACN:MeOH mixture (3:2). In the final step, the samples were evaporated to dryness in a stream of nitrogen at 40 °C. Immediately before the samples were analyzed by liquid chromatography with fluorescence detection (HPLC - FLD), they were dissolved in 1 cm^3 of mobile phase.

Methods for the determination of ochratoxin A in urine

A 10 cm^3 portion of urine was diluted with 10 cm^3 of 5 % $NaHCO_3$ solution. The samples were then mixed on a vortex. The next step involved the filtering of the solution through a smooth filter. A 10 cm^3 filtered solution was put into an OchraPrep immunoaffinity column (R-Biopharm Rhône LTD). After the samples passed through the column, they were washed with a 10 cm^3 portion of distilled water and air dried. In the next step, OTA was eluted by 2 cm^3 of MeOH. The final step was evaporation of the mixture in a stream of nitrogen at 40 ^oC. Immediately before the samples were analyzed by liquid chromatography with fluorescence detection (HPLC - FLD), they were dissolved in 1 cm^3 of mobile phase.

Methods for the determination of ochratoxin A in serum

A 20 µl portion of internal standard (^{13}C-OTA) and 20 µl of β-glucoronidase were added to 1 cm^3 of serum. The samples were then incubated for 18 hours at 37 ^oC. After the incubation, 1 cm^3 of MeOH and 1.96 cm^3 of ACN were added to the mixture. Subsequently, the solution was vortexed for 2 minutes and centrifuged in a centrifuge for 10 minutes at 7,000 rpm. A 3 ml portion of the supernatant was transferred to a 50 cm^3 tube and evaporated in a stream of nitrogen. After evaporation, the samples were dissolved in 1 cm^3 of MeOH, first for 3 minutes in an ultrasonic cleaner and subsequently for 5 minutes in a shaker. In the next step, 25 cm^3 of PBS was added to the solution. The solution was quantitatively transferred to an OchraPrep immunoaffinity column (R-Biopharm Rhône LTD). After the sample passed through the column, it was washed

with 20 cm^3 of distilled water and air dried. OTA was eluted by 1.5 cm^3 of MeOH:CH_3COOH (98:2) into a 2 ml tube. Subsequently, it was evaporated in a stream of nitrogen at 40 ^oC. Immediately before LC - MS/MS analysis, the samples were dissolved in 150 µl of H_2O:MeOH (7:3) mixture.

Chromatographic methods for the analysis of test samples

Ochratoxin A in milk and urine was determined by HPLC with fluorescence detection. The HPLC system (Merck Hitachi) consisted of an L-2130 pump, L-2300 column oven, L-2200 autosampler, L-2480 fluorescence detector and LiChrospher® 100 RP-18 column (250 × 4 mm, 5 µm) with precolumn (4 × 4 mm, 5 µm), mobile phase: ACN:2 % CH_3COOH (70:30), flow rate: 1 cm^3/min, injection volume: 50 µl.

For the determination of ochratoxin A in blood serum, we used a liquid chromatography-mass spectrometer (LC - MS/MS) method. The chromatograph used in the study was a Shimadzu Nexera: pump (LC30AD), autosampler (SIL30AC), oven (CTO20AC), and detector - 5500 Qtrap mass spectrometer (Sciex). Chromatography column: Kinetex C18 100 × 2.1 mm 2.6 um, mobile phases: A:5m $MAcONH_4$ + 0.1 % AcOH in H_2O; B: 5 M $AcONH_4$ + 0.1 % AcOH in MeOH; flow rate: 0.3 cm^3/min, injection volume 10 µl, oven temperature 40 °C.

Results

In order to verify the hypothesis assuming a relationship between the frequency of consumption of certain food types and the level of OTA in the mother's milk, urine and serum, a series of Spearman rank correlation analyses were conducted. The characteristics of the distributions of the independent variables involved in those analyses are included in Figures 1 – 6. OTA was found in 4 out of 60 milk samples, in 40 out of 60 in urine samples and in 60 out of 60 serum samples.

As a result of the analysis, it was found that as the frequency of consumption of dried grape fruit increases, there is a significant increase in the level of OTA in breast milk (rho = 0.28; $p < 0.05$). The strength of this correlation is weak. Next, it was shown that as the frequency of beer consumption increases, there is a significant increase in the level of OTA in maternal urine (rho = 0.36; $p < 0.01$). The strength of this correlation is moderate. It was also found that a significant decrease in maternal serum OTA levels occurs with an increase in the frequency of consumption of cow's milk (rho = -0.26; $p < 0.05$) and spices (rho = -0.40; $p < 0.01$), with the strength of the former correlation being weak and the latter being moderate. Finally, it was shown that a significant increase in maternal serum OTA levels occurs with an increase in the frequency of consumption of dried fruit (rho = 0.26; $p < 0.05$) and legumes (rho = 0.31; $p < 0.05$), with the strength of the first correlation being weak, while the second correlation is moderate. A summary of the results obtained is shown in Table 1.

The women whose milk was detected with OTA were 29, 35, 35 and 37 years old. Three of them were city residents, while one was a rural resident. Three boys and one girl were born in this group. The average birth weight of the children was about 2567.5 grams. The birth occurred on average on the 35th week of gestation. Three of the women in the study breast-fed, while one with pumped milk. One woman was tandem feeding. For two of the subjects, this was the fourth child, for one the second, while one woman had a firstborn. Three of the women ate white bread daily, while one ate dark bread. Two of the women did not eat dried fruit, while they ate cereal and porridge every day. Other two women drank ground coffee every day and ate chocolate. One of the women in whose milk OTA was detected consumed nuts, cow's milk and cream cheese every day.

The women in whose urine OTA was detected were on average 31 years old. Out of these women, 28 were urban residents, while 12 were rural residents. In this group, 23 boys and 17 girls were born. The average birth weight of the children was about 2937.38 grams, and they were delivered on the 36th week of gestation. Out of these women, 28 were breastfeeding, six with pumped food, six with mixed food (breast and pumped), and Four women were tandem feeding. For two women it was the fourth child, for four it was the third one, for 16 it was the second one, while for 18 it was a first-born. The woman with the highest urinary OTA was 35 years old, a rural resident, and gave birth to her first child on the 31st week of gestation - a girl weighing 1570 g. The baby was fed with pumped milk. The woman consumed white bread, wheat gluten and chocolate every day, and drank ground coffee. Once a week, the woman ate dark bread, offal, nuts, cow's milk, eggs and dried vine fruit.

OTA was detected in the blood of all women tested. The women's average age was 31. Out of these women, 46 were city residents and 14 were rural residents. In the study group, 25 girls and 35 boys were born. The average birth weight of the children was 2965.6 g, and delivery occurred on average on the 37th week of gestation. There were 39 women who breast-fed, ten feeding with pumped milk, and 11 feeding in a mixed manner, whereas five women were tandem feeding. For two women it was the fourth child, for six it was the third one, for 24it was the second one, and 28 had first-borns.

The women whose serum OTA levels were the highest consumed white bread, cereal, cow's milk and cream cheese every day, and dark bread, cornflakes and chicken eggs once a week.

Table 1. Spearman's rho correlation analysis between the frequency of consumption of individual food types and the content of OTA in the mother's milk, urine and serum

Tabela 1. Analiza korelacji rho Spearmana pomiędzy częstością spożycia poszczególnych pokarmów a zawartością OTA w mleku, moczu i surowicy matki

Variable / Zmienna	OTA in mother's milk [ng/cm^3] / OTA w mleku matki [ng/cm^3]	OTA in mother's urine [ng/cm^3] / OTA w moczu matki [ng/cm^3]	OTA in mother's serum [ng/cm^3] / OTA w surowicy matki [ng/cm^3]
White bread / Pieczywo białe	0.05	-0.12	-0.18
Dark bread / Pieczywo ciemne	-0.07	0.02	0.24
Cereal flakes / Płatki zbożowe	0.07	-0.03	0.12
Corn flakes / Płatki kukurydziane	-0.20	0.04	-0.05
Giblets / Podroby	-0.12	-0.09	-0.11
Ground coffee / Kawa mielona	0.05	-0.01	0.10
Instant coffee / Kawa rozpuszczalna	-0.22	-0.08	-0.12
Green coffee / Kawa zielona	-0.06	0.08	0.09
Wine / Wino	-0.07	0.13	0.01
Beer / Piwo	-0.09	0.36**	0.15
Grape juice / Sok z winogron	-0.11	-0.05	-0.16
Wheat gluten / Gluten pszenny	0.00	0.13	0.10
Chocolate / Czekolada	0.09	0.18	0.17
Cocoa / Kakao	-0.10	0.04	-0.18
Groats / Kasze	0.15	-0.05	-0.05
Rice / Ryż	0.06	0.05	-0.20
Nuts / Orzechy	0.05	-0.02	0.12
Cow's milk / Mleko krowie	-0.15	-0.10	-0.26*
Cheese / Ser żółty	-0.22	-0.14	-0.02
Blue cheese / Ser pleśniowy	-0.08	0.00	-0.06
Hen eggs /Jaja kurze	-0.05	-0.07	-0.07
Licorice / Lukrecja	-0.03	0.00	0.16
Spices / Przyprawy	-0.17	-0.04	-0.40**
Raisins / Rodzynki	0.28*	0.03	0.23
Dried fruit / Suszone owoce	-0.06	-0.11	0.26*
Legumes / Rośliny strączkowe	0.07	0.09	0.31*

Explanatory notes / Objaśnienia: *$p < 0.05$ **$p < 0.01$ ***$p < 0.001$

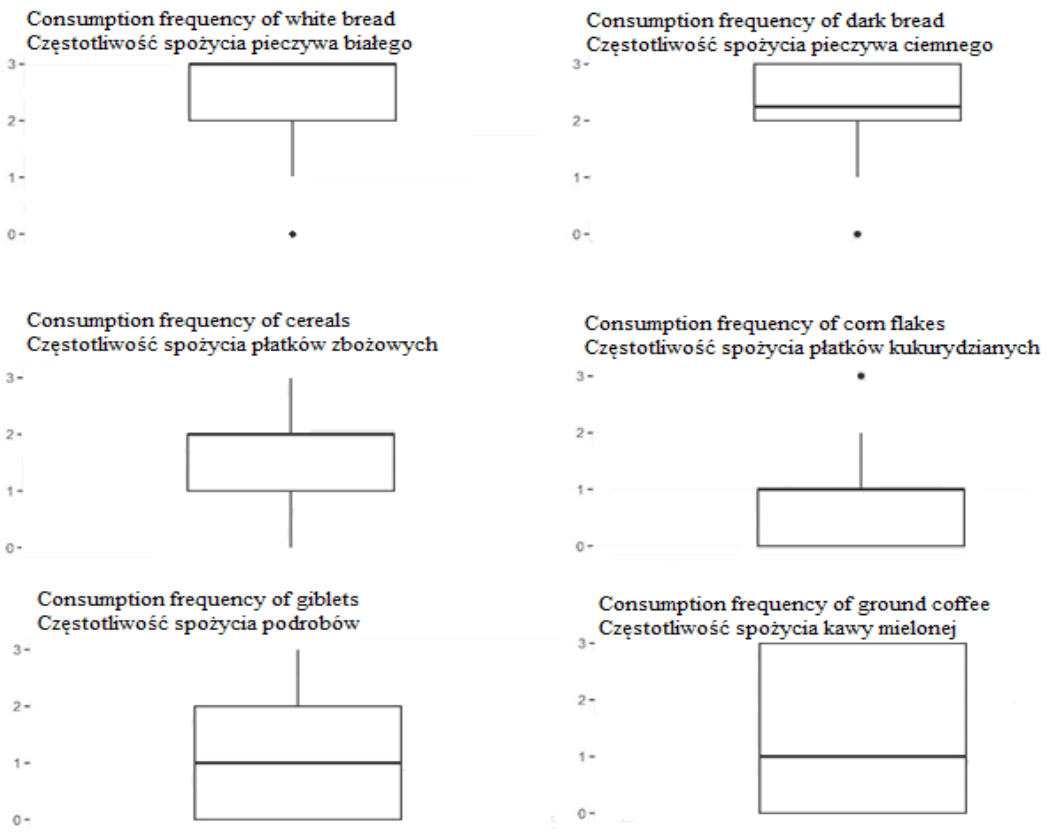

Figure 1. Distribution of values of the variable "frequency of consumption": white bread, dark bread, cereals, corn flakes, giblets, ground coffee

Rycina 1. Rozkład wartości zmiennej "częstotliwość spożycia": pieczywo białe, pieczywo ciemne, płatki zbożowe, płatki kukurydziane, podroby, kawa mielona

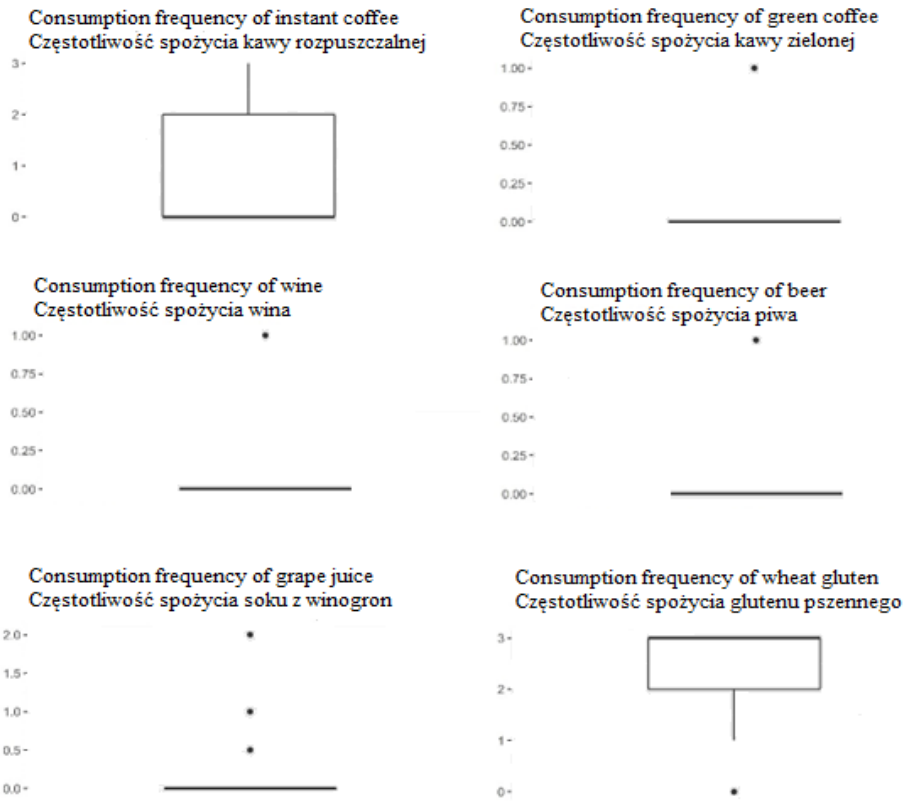

Figure 2. Distribution of values of variable "frequency of consumption: instant coffee, green coffee, wine, beer, grape juice, wheat gluten"

Rycina 2. Rozkład wartości zmiennej "częstotliwość spożycia: kawa rozpuszczalna, kawa zielona, wino, piwo, sok winogronowy, gluten pszenny"

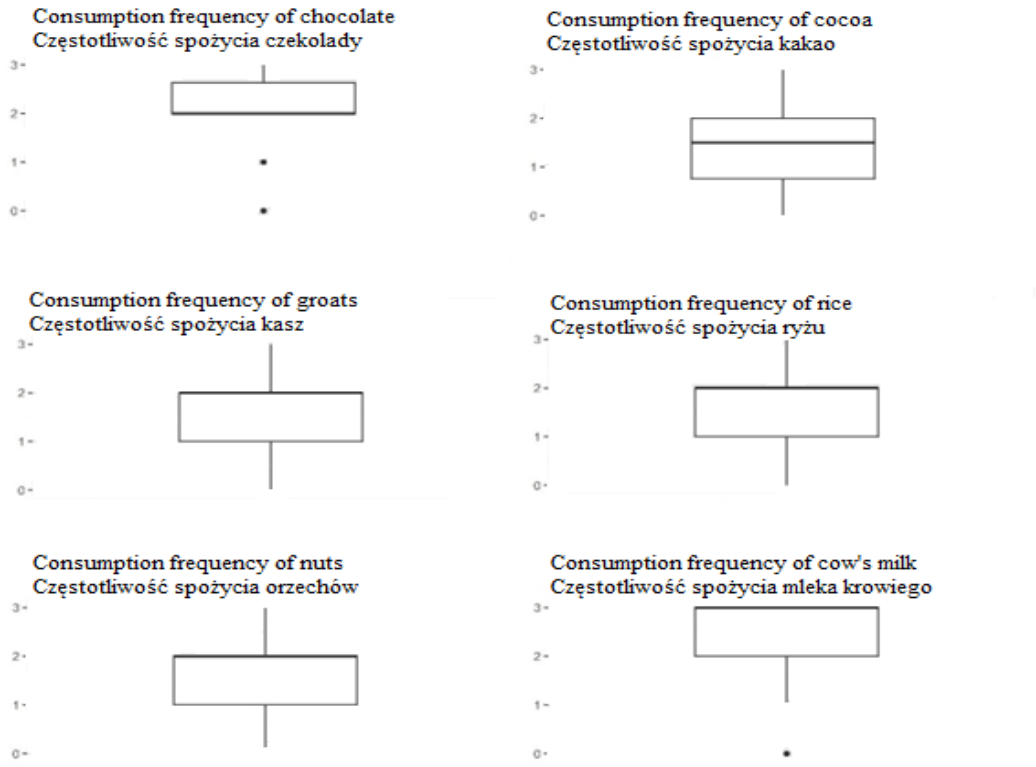

Figure 3. Distribution of values of the variable "frequency of consumption": chocolate, cocoa, cereals, rice, nuts, cow's milk

Rycina 3. Rozkład wartości zmiennej "częstotliwość spożycia": czekolada, kakao, zboża, ryż, orzechy, mleko krowie

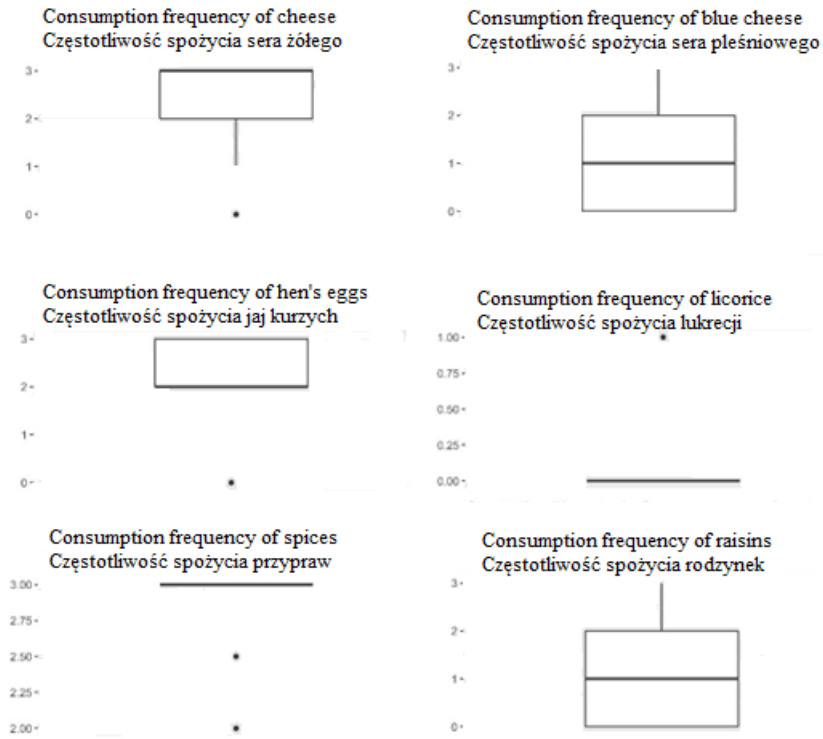

Figure 4. Distribution of values of the variable "frequency of consumption": cheese, blue cheese, hen's eggs, licorice, spices, raisins

Rycina 4. Rozkład wartości zmiennej "częstotliwość spożycia": ser, ser pleśniowy, jaja kurze, lukrecja, przyprawy, rodzynki

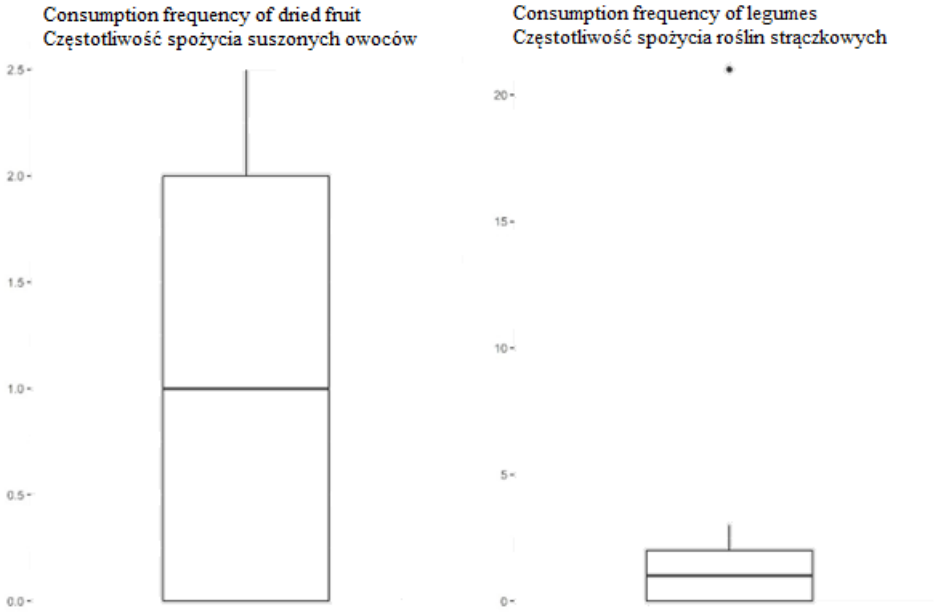

Figure 5. Distribution of values of the variable "frequency of consumption": dried fruit, legumes
Rycina 5. Rozkład wartości zmiennej "częstotliwość spożycia": suszone owoce, rośliny strączkowe

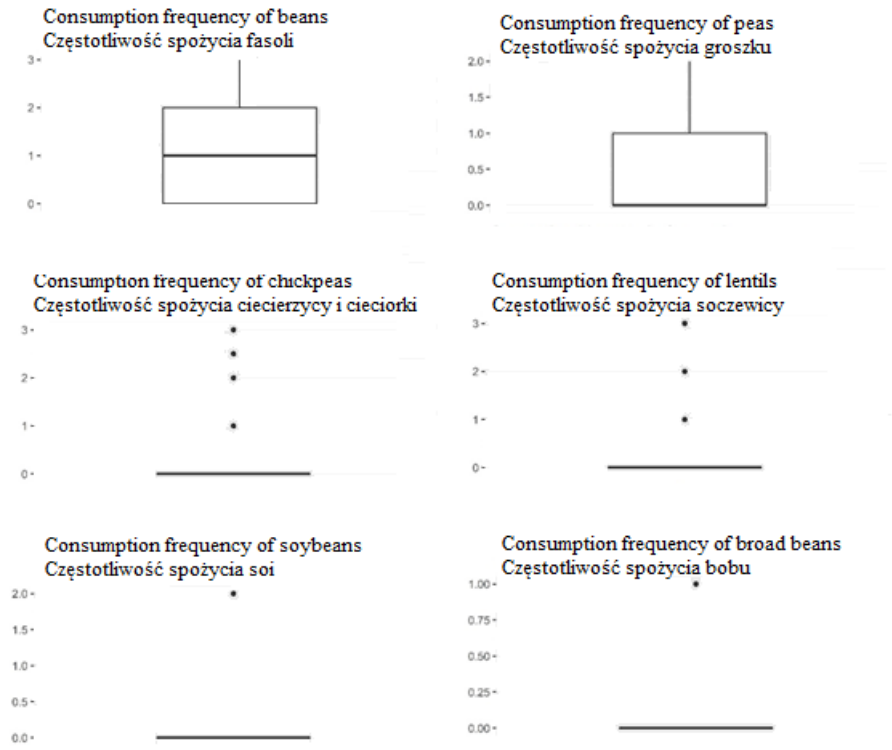

Figure 6. Distribution of values of the variable "frequency of consumption" of legumes: beans, peas, chickpeas, lentils, soybeans, broad beans

Rycina 6. Rozkład wartości zmiennej "częstotliwość spożycia" roślin strączkowych: fasola, groch, ciecierzyca i cieciorka, soczewica, soja, bób

Discussion

In tests carried out in Morocco, 45 out of 82 (maximum value 10.04 ng/cm^3) tested samples turned out to be contaminated with OTA, and in our own research - 4 out of 60 samples (maximum value 0.018 ng/cm^3). Cherkani-Hassani et al. [5] showed that the level of ochratoxin A in human milk was related to the frequency of consumption of legumes, dried fruit and dairy products. In the results of own research, the frequency of consumption of dried vine fruit significantly affected the level of ochratoxin A in breast milk.

A few years earlier, a similar study was conducted in Italy, which examined the effect of the diet of breastfeeding women on the levels of ochratoxin A in their milk. The research group consisted of 82 women. Ochratoxin A was detected in 61 samples with a maximum level of 405 ng/dm^3. The results showed that women who ate more bread, confectionery and cold cuts had higher levels of ochratoxin A in their milk [11].

Similar research results were published in 2004 by Turconi et al. [29], who studied the effect of a diet on the level of ochratoxin in the breast milk of Italian women. This research team found that ochratoxin A was present in 198 of 231 milk samples with a maximum value of 57 ng/dm^3, with bread consumption having a statistically significant effect on ochratoxin A levels.

Studies on the effect of the diet on the occurrence of ochratoxin A were conducted as early as 2001 in Norway by Skaug et al. [24] There were 17 of 80 samples tested which contained ochratoxin A with a maximum level of 182 ng/dm^3. Women with the most ochratoxin A-contaminated milk consumed more cakes, processed meat, cereals (corn flakes, oat flakes, muesli) and cheese.

Navas et al. [18] conducted a study on the presence of ochratoxin A in human milk in relation to women's eating habits, however, too small number of positive samples - 2 out of 50, made it impossible to examine the correlation associated with the presence of ochratoxin A and the diet.

In 2010, Biasucci et al. [4] published the results of a study on the correlation between the occurrence of ochratoxin A and the dietary habits of lactating women. Ochratoxin A was detected in 45 of 57 milk samples tested, with peak levels above 75.1 ng/dm^3. Based on data collected in a women's nutrition survey, the authors showed a significant correlation between the presence of ochratoxin A in milk and the mother's diet. That diet included sweets, seed oils and soft drinks (not including fruit juices), and the authors showed that fish consumption was inversely associated with milk ochratoxin A levels.

In addition to studying the effect of a diet on the occurrence of ochratoxin A in milk, researchers from all around the world are studying the effect of this diet on the occurrence of ochratoxin A in urine. This type of study was conducted in Bangladesh in 2016 by Ali et al., [1] moreover, pregnant women between 18 and 36 years of age participated in the study. The results of the study showed the presence of ochratoxin A in 50 of 54 urine samples, noting that the occurrence of higher concentrations of ochratoxin A was correlated with more frequent consumption of rice (based on a dietary survey). Additionally, the study showed that higher levels of ochratoxin A were present in urine samples of women living in the suburbs. In turn, Gilbert et al. [12], in the United Kingdom, observed a significant relationship between the diet and the presence of ochratoxin A in the urine of 50 volunteers tested, however, this correlation was not suitable for use in practice. Among this study group, 46 samples were contaminated with ochratoxin A with a maximum value of 0.058 ng/cm^3. These results, despite the lower maximum value of ochratoxin A in the urine, are similar to our own research, in which no connection between the diet and the presence of ochratoxin A in the urine of the examined women was found.

Another type of study was conducted by Ali et al. [2] Several samples were taken from two volunteers. A survey of their eating habits was also conducted - the subject who ate higher amounts of products potentially contaminated with ochratoxin A - bread, breakfast cereals, coffee - had lower levels of ochratoxin A in both plasma and blood. Moreover, the plasma levels of this mycotoxin were measured in the volunteers. It turned out that the analytes determined in plasma were higher than in urine. This may be related to the affinity of ochratoxin A to albumin, which is one of the blood components.

Ochratoxin A in serum and plasma is analyzed by many authors. One of the studies looked at the relationship between the presence of ochratoxin A and the diet of volunteers who underwent testing. Pregnant women turn out to be a frequent research group. This type of research was conducted by Biasucci et al. in Italy in 2010 [4]. Given the results obtained, 129 out of 130 serum samples were contaminated with ochratoxin A between 84 ÷ 4.835 ng/dm^3. Based on a survey of food intake, the authors determined a significant correlation between the presence of ochratoxin A in serum and the consumption of sweets (especially those containing chocolate and cocoa) and red wine.

In a study by Thuvander et al. [28], they showed a strong correlation of the occurrence of ochratoxin A in the blood of women, brown bread and beer, and a weaker correlation for ham, corn snacks and dark crisp bread. The study covered 166 female blood bank donors, and all samples showed the presence of ochratoxin A. The previously mentioned study by Gilbert et al. [12] also included the determination of the correlation between plasma ochratoxin A and a diet. Gilbert's research team was unable to determine a significant correlation. Ochratoxin A was determined in all 50 tested samples (in own work in 60 samples).

Conclusions

1. Comparing data from various authors with the data presented in this paper, the following conclusions can be made: the concentration of ochratoxin A in the milk, urine and serum of women tested is not high. Milk is safe for a nutritional therapy.
2. Compared to the results of studies published by other scientists, the author's findings do not differ significantly from them. As the frequency of consumption of dried grape fruit increases, the level of OTA in breast milk increases. As the frequency of beer consumption increases, the level of OTA in maternal urine increases. As the frequency of consumption of dried fruit and legumes increases, the level of OTA in maternal serum increases. As the frequency of consumption of cow's milk and spices increases, the level of OTA in maternal serum decreases.
3. Despite the foregoing, research on the occurrence of ochratoxin A in human bodies is extremely important, not only because of their adverse effects, but also due to

the fact that in the case of milk, they can be transferred with it into the bodies of children. These, in turn, due to their lower mass, have a harder time removing toxins from their bodies.

4. As a result, they may be exposed to and suffer from the negative effects of mycotoxins from an early age. The present study examined women who may be (or are) donors of breast milk. This milk can go to the neediest children, who are often sick, hence they should not be further exposed to consuming contaminated food.
5. The research conducted in this work clearly shows that an appropriately selected diet can affect the presence or absence of ochratoxin A in breast milk, while it does not show the effect of the diet on the presence of ochratoxin A in urine and blood serum.

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WPŁYW DIETY NA WYSTĘPOWANIE OCHRATOKSYNY A W PŁYNACH USTROJOWYCH

S t r e s z c z e n i e

Wprowadzenie. Ochratoksyna A (OTA), wtórny metabolit grzybów, jest wytwarzana przez grzyby z rodzajów *Aspergillus* i *Penicillium*. Dostaje się ona do organizmu człowieka wraz ze spożywaną żywnością. Celem tego badania było określenie występowania ochratoksyny A w ludzkich płynach ustrojowych w zależności od diety. Część grupy badanej składała się z kobiet, które były dawczyniami z banku mleka kobiecego. Celem pracy było określenie korelacji występowania ochratoksyny A w płynach ustrojowych (mleko, mocz, surowica) oraz diety prowadzonej przez badane kobiety. Materiał badamy składał się z 60 próbek moczu, 60 próbek mleka i 60 próbek krwi. Ochratoksynę A w mleku i moczu oznaczono metodą HPLC z detekcją fluorescencyjną. Do oznaczania ochratoksyny A w surowicy krwi zastosowano metodę chromatografii cieczowej ze spektrometrem masowym (LC - MS/MS).

Wyniki i wnioski. W wyniku analizy stwierdzono, że wraz ze wzrostem częstotliwości spożywania suszonych owoców winogron następuje znaczny wzrost poziomu OTA w mleku matki. Ponadto wykazano, że wraz ze wzrostem częstotliwości spożywania piwa następuje znaczący wzrost poziomu OTA w moczu matki. Dodatkowo wykazano, że znaczący wzrost poziomu OTA w surowicy matki występuje wraz ze wzrostem częstotliwości spożywania suszonych owoców. OTA stwierdzono w 4 z 60 próbek mleka, w 40 z 60 próbek moczu i w 60 z 60 próbek surowicy. Badane kobiety miały średnio 31 lat (od 22 do 41 lat). 46 mieszkało w mieście,14 na wsi. 35 ankietowanych było czynne zawodowo, 22 nie, 3 przebywały na urlopie macierzyńskim. Średnio posiadały 2 dzieci (od 1 do 4). 35 karmionych dzieci było płci męskiej, 25 płci kobiecej. Poród nastąpił średnio w 37 tygodniu ciąży (między 27tc a 42tc).

Słowa kluczowe: mikotoksyny, ochratoksyna A, dieta, płyny ustrojowe