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Editorial

With this issue of the International Journal of Sanitary Engineering **Research** we all, readers, publisher the Institute of the Sanitary Engineering and editorial of the journal can be satisfied. The first goal is achieved; the journal is regularly published twice in the year. The next task which concerns all of us is to raise the quality of the papers. The researchers, experts and policy makers are invited to contribute to this endeavor. The editorial of he journal is preparing serial of technical papers regarding developing the scientific an technical papers from the idea through the research and finally to the preparation of the structure of the paper and writing he paper ready for the assessment by the editors. The papers will be published in both languages in English and in Slovene ones. The editorial hopes that this will influence to the quality of your research, efficiency and of course papers sent to be published in this journal. If our readers have ideas and suggestions from this field they are invited to send it to the editorial to be included in the next issues.

We also need to mention that the editorial's activities are directed to the field of including our journal to the certain data bases which offer us the opportunity to receive the impact factor and science citation index.

This volume of the journal deals with several topics such as environmental protection, food safety, medicine research, and reports on international projects co-financed by EU commission. Beside original scientific and technical papers there are two contribution marked by the *Actual*. One is technical paper deals with the consumer's responsibility to food safety and another is Odziv potrošnikov na gensko spremenjena živila.

The editorial encourages you – **readers** to report on successful cases studies and international projects in the scope of the journal. Sharing ideas, knowledge, experience and research results contributes to the sharing information, to transferring research results from universities to industry and vice versa and therefore strengths the development and research at all levels by using this information in our research and professional work.

Sincerely,

Janez Petek Editor-in-Chief

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Collection and analysis of particulate matter deposition around the Port of Koper

Gregor **JEREB**^{*1,5}, Boris **MARZI**², Franka **CEPAK**³, Sidney A. **KATZ**^{4,5}, Borut **POLJŠAK**¹

ABSTRACT:

Particulates are tiny particles of solid or liquid suspended in a gas. Sources of particulate matter can be anthropogenic or natural. Increased levels of fine particles in the air are linked to health hazards such as heart disease, altered lung function and lung cancer. Human activities, such as burning of fossil fuels in vehicles, power plants and various industrial processes also generate significant amounts of particulates.

Results of epidemiological studies show an influence of polluted air on health status of inhabitants of Municipality of Koper. There are many different sources of air pollution in the region, mainly warehouse of petroleum products, chemical industry, waste incinerator, ironworks, Port of Koper, traffic pollution, elevated ground-level ozone in summer months, etc. The degree of each pollutant on total air pollution and health risk is difficult to establish. For this reason, a study was conducted in an effort to determine the extent of dust emissions from iron and coal ore manipulation at the Port of Koper on total particulate matter deposition in the surrounding area.

The results indicate that the Port of Koper has a partial impact on total dust emissions. During the sampling period from October 2005 to October 2006, the total amount of particulate matter deposition at all sampling sites in the area of the municipality of Ankaran and surroundings exceeded nine times the emission limit value of 350 mg/m² a day. The highest monthly values were measured in the period from 15. 9. 2006 to 15. 10. 2006. During this period, the monthly emission limit value was exceeded at nearly all of the sampling sites. A similar increase of particulate matter deposition values was seen also in the period from 15. 2. 2006 to 15. 3. 2006 and in the period from 15. 6. 2006 to 15. 7. 2006. New strategies with which the emissions of dust at Port of Koper will be reduced should be considered.

KEY WORDS:

Air Pollution, Particulate Matter Deposition, Iron Ore and Coal Dust Emissions

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INTRODUCTION

The quality of ambient air in residential districts is influenced mostly by air emissions of substances from nearby sources. The pollution results from the transport of these substances over large distances. Aerosols are small solid and liquid particles suspended in a gas phase. They originate as a result of processes on the surface of the earth and in the atmosphere. They vary in shape, size, density and chemical composition [1]. Primary particles in the air are the result of direct emissions from traffic, industry and combustion installations, agriculture, while secondary particles are the result of various physical-chemical processes in polluted atmosphere [1]. In addition to the anthropogenic sources, a portion of dust particles is also a result of natural processes in the environment (volcano eruptions, forest fires, wind erosion, plants pollen, etc.). Particles that arise from different processes have consequently a different chemical structure, shape and physical characteristics. Various factors influence the elevated concentration of pollutants, such as climatic characteristics, meteorological phenomena, physical-chemical processes of transformation of substances in the air and the topographical structure of the area [2].

Particulate matters can impact the health merely because of their presence, or because of a specific chemical composition or morphological structure that has additionally harmful influences on health. In most cases, the most susceptible organs are the lungs and the respiratory tract. Some air pollutants, such as fine particles, can also damage other organs. Coal particles belong by categorization of the International Agency for Research on Cancer (IARC) in group 3 – the agent is not classifiable as to its carcinogenicity to humans, which means that there were not enough studies made to confirm carcinogenicity, however it is not excluded [3]. Coal particles are, due to their chemical structure and their morphology, capable of binding with diverse pollutants, causing synergistic effects between various substances. Few if any studies regarding these phenomena have been reported. When a particle such as coal gets into the lungs, it reacts with the pulmonary cells, irritates them and releases the pollutants bound to it. Coal particles can cause allergic reactions in upper respiratory tract and lungs. That is why its toxicity is connected mainly to the size of the particles and the presence of other pollutants in the air. Kodavanti et al. [4] established a connection between metals in small particulate matters (particularly zinc) and the phenomenon of pulmonary inflammation in laboratory animals. Pulmonary inflammation in most cases resulted in the damage of the cardiac muscle. Authors concluded that the fine particles in outdoor air contain important concentrations of various metals, in most cases also an important concentration of zinc. Beside zinc in particulate matter deposition a considerable portion of other metals, such as lead and iron is present [5]. Iron oxide is a potentially dangerous substance because it is a catalyst for the reaction according to Fenton's chemistry [6]. In this reaction hydrogen peroxide, which is a normal metabolite in pulmonary cells in the presence of iron forms a hydroxyl radical, which is the most active and most aggressive free radical. Hydroxyl radicals directly Particles that arise from different processes have consequently a different chemical structure, shape and physical characteristics.

Coal particles are, due to their chemical structure and their morphology, capable of binding with diverse pollutants, causing synergistic effects between various substances. When a particle such as coal gets into the lungs, it reacts with the pulmonary cells, irritates them and releases the pollutants bound to it. The most exposed group are children, because the particles penetrate deeper in the lungs as compared to adults, they spend more time outdoor and are more active, subsequently they also breathe deeper and faster, the polluted air slows down the development of pulmonary functions in children.

One of the major sources of dust in the area of the Port of Koper is the activities at the EET. It is important to emphasize that the emission of particles results from the manipulation of ore (off- or on- loading). destroy cells, degrade cellular membranes, proteins and cellular deoxyribonucleic acid (DNA) and cause mutations. In the legislation iron oxide is not yet declared as toxic, however the latest studies support the above mentioned mechanism of action [6,7]. Also in the case of iron oxide the size of the particles has the main role in determining its danger for health.

The presence of particles in the air is associated with asthma, chronic bronchitis and reduction of pulmonary function [5,8]. Various epidemiological studies [9,10,11] showed a connection between the elevated concentration of particles in the air and the increase of diseases of the respiratory tract and cardiovascular diseases, regardless of the chemical structure of the particulate matter. Exposure to particles in air can affect health, while elevated concentration of fine particle has a direct influence on morbidity and mortality [9,10,12,13]. The most exposed group are children, because the particles penetrate deeper in the lungs as compared to adults, they spend more time outdoor and are more active, subsequently they also breathe deeper and faster [14], the polluted air slows down the development of pulmonary functions in children [15,16]. Senior citizens, especially those with a weakened cardiovascular and respiratory system, are a high risk group too [5]. Another risk group is patients with chronic pulmonary emphysema, asthma or cardiovascular diseases [5].

Also in Slovenia there is a problem of elevated air pollution. Results of epidemiological studies show an influence of polluted air on health. Analysis of the study made by the Institute of Public Health Celje [17] on the health status of the residents in the Municipality of Koper show an increase of chronic respiratory diseases and allergic illnesses among children. Also an increase in the number of women with lung cancer, which is 1.6 times higher than the Slovenian average, a result of the pollution load on the discussed area, was detected.

The basic activities performed in the Port of Koper are cargo handling and warehousing. At the European Energy Terminal (EET) in the Port of Koper, large amounts of coal and iron ore are handled and warehoused. The storage capacity of the landfill is 450,000 t for coal ore and 350,000 t for iron ore. The area covered by the coal and iron ore is 108,500 m². Unloading daily capacity of coal is 17,000 t (cape size vessel) or 15,000 t (panamax vessel) and for iron ore (cape size vessel) is 25,000 t. One of the major sources of dust in the area of the Port of Koper is the activities at the EET. It is important to emphasize that the emission of particles results from the manipulation of ore (off- or onloading). A big source of emission represents also the landfill, particularly during strong winds. Dust control measures already introduced include construction of an 11 m high anti-dust emission wall, spraying the body of the landfill with water and wet cleaning the transportation roads around the landfill at least twice per day. However, when a strong wind blows unexpectedly, the dusting is not suppressed completely. Because the landfill is not completely closed, it is still exposed to the weather conditions.

For many years the inhabitants of Ankaran (distance from the iron ore and coal storage sites is approximately 1800 m) and its surroundings (the closest residential area is 1000 m from the storage sites) have been pointing out the problem of the pollution of their residential area with emissions from the terminal EET in the Port of Koper. Particulate matter depositions are visibly perceived on their yards, places of residence, linen and vegetables (Figure 6). The results of the study of environment pollution in Ankaran [18] made by the Environment Agency of the Republic of Slovenia (ARSO) show the 24 hour limited value for particles PM_{10} was exceeded. With respect to wind direction, the distribution showed an increased concentration of particles during the mistral wind. Consequently, we can assume, regarding the location of the sampling site, a direct influence of the Port of Koper, specifically, the landfill EET. The same study ascertained a very strong correlation between the particles PM_{10} and the presence of iron. The correlation factor is 0.91.

The focus of the study was to collect and analyze the particulate matter deposition around the port of Koper. Standard and modified methods of particulate matter deposition collection were employed. Six sampling sites at different locations of Ankaran and its surrounding were established.

The basis of the Slovene legislation in the field of outdoor air quality is the Environmental Protection Act [25] and its subsequent Decrees. The particulate matter deposition and its limit values were regulated by the Decree on limit values, alert thresholds and critical emission values for substances into the atmosphere [26]. However, it was annulled on July 27th 2007 [27]. The change of the Slovene regulations is a consequence of the harmonization of the Slovene legislation with the EU one. The decree on sulfur dioxide, nitric oxides, particles and lead in the atmosphere [28], which regulates the area of emission of particles into the atmosphere, applies to only the supervision of particles PM₁₀ and PM_{2,5}. It does not supervise particulate matter deposition.

METHODS

Location of sampling sites

The choice for sampling sites location on the micro level was based on the anticipated influence of emissions in the nearby area. Location of the measurement spot and influences on it by location (distance between the nearby buildings, trees etc.) was meant by the term micro location. The sampling gauge was placed 1.5 - 2.0 meters above the ground (the height of breathing of an adult person). In close proximity of the sampling sites were no obstacles that could have influence on the air flow passing the sampling gauge. The micro-location of individual sampling sites was well ventilated. The study included six sampling sites in the area of Ankaran and its surrounding (Figure 1). Some sites were chosen for their proximity to potential dust sources, and other sites were located downwind from a dust source. The first five sampling sites were placed in the prevailing direction of the wind or in the direcThe results of the study of environment pollution in Ankaran made by the Environment Agency of the Republic of Slovenia (ARSO) show the 24 hour limited value for particles PM₁₀ was exceeded.

The same study ascertained a very strong correlation between the particles PM₁₀ and the presence of iron. The correlation factor is 0.91. tion of the residential area, all in closed proximity to the Port of Koper. The sixth sampling place was used as a control sample of the background (located in the residential area of Hrvatini, where due to distance from Port of Koper and the height above sea level a negligible influence of the terminal of strewn cargo of the Port of Koper was expected).

Figure 1:

Geographical location of the sampling sites.



The study included six sampling sites in the area of Ankaran and its surrounding.

The same method was applied also in the proposition of guidelines of the Association of clean air societies of Yugoslavia n. 201. The sampling design for this study was not statistically based; rather, sites were chosen to provide data on dust flux at the selected sites and to answer specific questions about the relation of port activities with the total dust deposition-emission regarding the distance from source, climate influence and other. Samples were collected and analyzed (gravimetrically) on the 15th day of each month for a one year period from October 2005 to October 2006.

Collection of particulate matter deposition

The collection of particulate matter deposition was conducted with the standard Bergerhoff sedimentators according to Commission Reinhaltung der Luft im VDI und DIN Guideline VDI 2119 part 2 (1996) – Measurement of Particulate Precipitations Determination of dust precipitation with collecting pots made of glass [19]. The same method was applied also in the proposition of guidelines of the Association of clean air societies of Yugoslavia n. 201 [20]. The stated method is used as the standard method for measuring the particulate matter deposition in Slovenia.

Modified method for collection of particulate matter deposition

The method for collection of particulate matter deposition is not perfect, because it does not entirely include the wind contribution since when wind blown through the sampling gauge, majority of particles are not deposit on the ground or in the sampling gauge but blown away with the wind and precipitate when wind stop blowing or when hits the obstacle. That why metal screen as obstacle directed toward ore depot was added. That is the reason the method was modified by adding a metal screen (20 cm x 30 cm – sampling sites marked with letter B), which intercepted particulate matter spread horizontally. The flow chart of sampling sites is shown in Figure 2. Distillate water was also added to prevent the wind blowing away the particulate matter deposition from the containers. The main purpose of the modification was to establish and quantify the particulate matter brought also by the wind from the observed directions. Distilled water was added to improve the method.



Three sample collectors were placed at each sampling site (Figure 3). The mass of material deposited in the first container, container A in Figure 2., was determined monthly. The mass of material deposited in the



The main purpose of the modification was to establish and quantify the particulate matter brought also by the wind from the observed directions.



Figure 3: View from sampling site No. 5

The samples were analyzed by the gravimetric method and the amount of dust deposition was determined in mg/m² a day. second container, container C, was meant to be determined annually. The mass of material deposited in the third container, container B, from both the horizontal and vertical directions was determined monthly. The yearly sample from container C was unusable because of the presence of decayed insects and plant leaves and contamination with algae. For this reason the yearly value of particulate matter deposition was calculated by the monthly values.

GRAVIMETRICAL ANALYSES

Each month, the glass collecting bottles from each sampling site were taken to the laboratory. When insects or parts of insects, parts of leafs, etc. were observed to be present, they were removed prior to the analysis. The samples were analyzed by the gravimetric method and the amount of dust deposition was determined in mg/m² a day. The samples were filtered through a cellulose nitrate membrane filter of porosity size of 3 μ m (ME 29 Scheilcher and Schuell) using a vacuum filtering device. A modified procedure ISO 11923 [21] was used. Three blank filters were used prior to analyzing real samples. A part of filtrate was evaporated in an evaporating dish. Before weighting the samples and blank filters were thermostated for 60 minutes at 105 °C ± 5 °C in both procedures (Binder FED 53, Tuttlingen, Germany). At the end filter paper and evaporating dishes were weighted and the total dust amount was calculated. An analytical balance, capable of weighing with a precision of at least ± 0,1 mg, was used (Mettler Toledo AT 261, USA).

Analysis of the amount of different metals

The depositions of aluminum, cadmium, chromium, copper, iron, lead, nickel and zinc were quantified as mg/m² a day by atomic absorption spectrometry of the material collected from the combined one year samples at each site (Shimatzu AA 6701 instrumentation). These determinations were performed according to methods EPA 7380 [22], EN ISO 15586 [23], ISO 8288 [24]. Samples were prepared for atomic absorption spectrometry by acid microwave-assisted digestion (Milestone 1200 Mega unit, Milestone Ethos plus, Rome, Italy). A multi-element Standard Reference Material SPS-WW2, Wastewater Level 2 (SPS 2002) was used for calibration and Quality Assurance/ Quality Control (QA/QC) procedures. The analysis was performed in the laboratory of the Public Health Institute of Koper.

Results and discussion

The monthly particulate matter deposition was collected from the 15th of each month for a one year period from October 2005 to October 2006. Figure 4 and Figure 5 represent the measured monthly emission of particulate matter at each sampling sites (in mg/m² a day). Vertical deposition is differentiated from vertical plus horizontal deposition as A and B, respectively.

During the sampling period from October 2005 to October 2006, the total amount of particulate matter deposition at all sampling sites in the

The analysis was performed in the laboratory of the Public Health Institute of Koper.



Figure 4:

Monthly mass of particulate matter – vertical deposition (collected by standardized method).



area of the municipality of Ankaran and surroundings exceeded nine times the emission limit value of 350 mg/m² a day. The highest monthly values were measured in the period from 15. 9. 2006 to 15. 10. 2006. During this period, the monthly emission limit value was exceeded at nearly all of the sampling sites. A similar increase of particulate matter deposition values was seen in the period from 15. 2. 2006 to 15. 3. 2006 and in the period from 15. 6. 2006 to 15. 7. 2006.

Figure 5:

Monthly mass of particulate matter – vertical plus horizontal deposition (collected by modified method). The highest values of dust deposition were measured particularly in the period 15. 9. 2006 to 15. 10. 2006. These could be attributed to strong wind, which blew from the direction of the sea across the ore storage pile toward the residential area for a short time (in a few hours). The consequences were reported also by the local residents as dust deposition seen on yards, gardens and facades. Figure 6 (a and b) were taken in the proximity of the sampling site 4 in October 2006, in the period when the values of particulate matter deposition was extremely high.



With the modification of the sampling device by adding a metal screen (20 cm x 30 cm) oriented towards the iron ore and coal storage piles, more dust was obtained in samples. The amount of the collected dust and its frequency was higher than that obtained with the standard method (Figure 4 and Figure 5). With this screen also the horizontal transport of the dust with wind could be obtained. However, with the addition of distilled water, the loss of dry dust sample from the glass by the action of strong wind was prevented.

Figure 6: Rožnik residential area (approx. 1000 m from landfill) – after strong wind, October 2006.

a) clean gauze and b) gauze after wiping the floor.

The amount of the collected dust and its frequency was higher than that obtained with the standard method. The average particulate matter deposition per year (Figure 7) was calculated from the results of the monthly sampling intervals. Only sampling sites A (vertical deposition) were comparable with limited value since limiting value was set for standardize method. In any individual month as well as on an annual basis, the highest amounts were collected at the sampling site 4 – Rožnik, which lies approx. 1000 m north – north east from the European Energy Terminal storage piles. These results can be explained by the morphology of the terrain and the position of sampling site regarding the proximity of the coal and iron ore depot.



The results from the determinations of aluminum, cadmium, chromium, copper, iron, nickel and zinc in the one – year samples of particulate matter show that the limit value of the metals Zn, Cd and Pb [26] were not exceeded. The only exception was the concentration of zinc at the sampling site 5 A. The current regulations do not specify limits for Al, Cr, Cu, Fe and Ni. Results also show increased values of Fe, Al, Cu, Zn and Pb at the sampling site 4. High values of metals in the deposition are present also on the control location (sampling site 6 is located in the residential area Hrvatini, where influences from Trieste and its surroundings as well as those from the Gulf of Koper are possible. In comparison with the control (6a) it is possible to note the presence of particulate matter depositions also from other sources and not only as a consequence of the coal and iron ore depot from the Port of Koper.

It was not possible to distinguish the coal and iron ore originating at the Port of Koper from other organic sources of carbon (burning of fossil fuel, traffic, pollen, etc.) and iron compounds in the soil on the basis of the results from the metal determinations. Figure 7: Yearly particulate matter depositions

on sampling sites A – vertical deposition.

In comparison with the control (6a) it is possible to note the presence of particulate matter depositions also from other sources and not only as a consequence of the coal and iron ore depot from the Port of Koper.

Та	ble	1:

Values of metals in the yearly sample of particulate matter deposition (μ g/m² a day).

	1a	2a	3a	4a	5a	6a	Limit value (µg/m² a day) [26]
Fe	3,054	7,740	14,433	29,450	6,892	13,081	/
AI	2,584	7,263	16,805	31,942	6,110	8,748	/
Cu	66	93	93	371	80	172	/
Zn	146	239	278	861	93	324	400
Cd	<0.3	<0.3	<0.3	1.2	<0.3	<0.3	2
Pb	25	35	40	87	24	60	100
Cr	27	39	90	98	28	46	/
Ni	14	26	35	50	14	42	/

/ - the limited value was not determined.

The results show significant differences between sampling sites and monthly and seasonal levels.

The results indicate periodical extremely elevated values of particulate matter deposition around the Port of Koper. According to gravimetrical results the highest measured amounts of particles were observed during time period from 15. 9. 2006 to 15. 10. 2006 at sampling site 4. With standardize method of collection (sampling site A) 4,283 mg/m² a day was measured; this value exceeded the limited value (350 mg/m² a day) for the factor 12. Furthermore, on sampling site B, 5,743 mg/m² a day was measured, the limited value was exceeded for the factor 16.5, most probably due to wind contribution. During this time period also in all other sampling sites elevated values were observed. During entire study time period also two other elevations of particulate matter deposition were observed (15. 2.-15. 3. and 15. 6.-15. 7.); however, measured values were lower and limited value was not exceeded in all sampling sites. The results show significant differences between sampling sites and monthly and seasonal levels. Increased values are noticed in the hot dry periods, mainly in summer and autumn months due to the dryness of the terrain and strengthening of the wind, which blew from the sea across the storage piles of ore toward the sampling sites. During winter, a north wind, which blows across the storage piles towards the sea, prevails in the study area. Due to only one year sampling period more accurate conclusions can not be made due to unpredictability of weather and variations of climate. It is suggested that monitoring should continue for longer time period before accurate conclusion can be done.

Several conclusions can be drawn from this study. (1) Monthly monitoring intervals may be too large to correlate the influence of cargo manipulation on the quantity of the particulate matter deposition in the environment. (2) The off-loading, on-loading and storage of the iron ore has a small influence on the dustiness of the environment. (3) The main influence is the sudden strong wind, which blows from the sea crosses the storage piles and continues towards the residential area [29]. (4) The quantity of particulate matter depositions depends mostly on weather conditions, especially the strength and direction of the wind and also the quantity of rainfall, which determined the dryness of the terrain and also dryness of coal and iron ore. Building the 11 m high anti-dust emission wall and the spraying of the landfill with water lowers the emissions of dust particles in the environment. Unfortunately no data on emission from the period before the anti-dust emission wall was build are available. Thus, it is not possible to evaluate how much this measure decreased the emission in the environment from coal and iron ore depot. However the Port of Koper is considering the adoption of additional new strategies with which the emissions of dust will be reduced even more in the future.

In the area of the municipality of Koper are various point and dispersed sources of emissions of various air pollutants. In this area there are located a large storage depot of petroleum products (emissions of benzene, toluene), chemical industry (emissions of formaldehyde, acetaldehyde), waste incinerator and the ironworks in Trieste (dioxins, furans, dust particles). Additionally heavy traffic pollution, ground-level ozone in summer months, transport of polluted air with the western winds from the Po river basin, etc is present. For these reasons, it is necessary to take measures to reduce the environmental burden with various pollutants from diverse sources. By no means should additional emissions into the environment, which would burden the air, be allowed.

The results indicate that Port of Koper has some impact on particulate matter emissions. For this reason further studies should be carried out to estimate the percentage ratio of particulates origin from the Port of Koper. For future work analyze of samples with electron microscopy will be performed to determined the shape, the size and also the chemical composition of the individual particle. Additionally the ¹³C/¹²C ratio in samples will be analysed. Results from both method will be used to determinate the origin of particulate matter. Additionally it would be reasonable to make risk assessment, specially focused on respiratory disease incidence. According to the results of epidemiological study [17] additional epidemiologic research on respiratory problems in the impact area would also be reasonable to be made.

Clean air is one of the necessities for healthy life now and in the future. Achieving this goal requires the active participation of legislators, experts from various fields and all those who burden the air with their activities.

Acknowledgements

Financial support to the study from Port of Koper is gratefully acknowledged. Thus, it is not possible to evaluate how much this measure decreased the emission in the environment from coal and iron ore depot.

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Temperature and time impact on food safety in domestic refrigerator

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ABSTRACT:

The domestic refrigerator is indispensable kitchen device and very important link for food safety in food supply chain. Various studies of consumers' food safety knowledge and practices show consumers' insufficient knowledge about the importance of cold chain maintaining and carelessness by handling with foodstuffs. By consideration of fact that recommended time in which chilled foodstuffs are stored at required temperatures in case of electric power failure vary from two to six hours, it is necessary to gain information about internal temperatures of selected foodstuffs after power failure of domestic refrigerator at different environmental conditions. Measurements of selected foodstuffs internal temperatures in domestic refrigerator in case of electric power failure were done. The research includes different refrigerator exterior and interior conditions. The results have shown impact of exterior ambient temperatures and different degrees of refrigerator loading on internal temperatures of selected perishable foodstuffs' while different frequencies of door openings did not correlate with internal temperatures of selected perishable foodstuffs. The biggest internal temperature difference after six hours of electricity cut-out appears by cream in case of empty refrigerator and high ambient temperatures. The opposite situation appears by frankfurters in case of full refrigerator and low ambient temperatures. Results pointed out the need upon reorganization of methods used for consumers' education and better cooperation between consumers' and producer of cold appliances.

KEY WORDS:

Food safety, Good housekeeping practise, Domestic refrigerator, Power failure

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INTRODUCTION

The refrigerator is a common and one of the most popular household device in the developed world and very few households do not own a refrigerator or fridge-freezer for the storage of chilled foodstuffs [1,2]. There are now about 1 billion domestic refrigerators worldwide and this is twice as many as 12 years ago [3]. Jackson and others [4] reported that foodstuffs which are stored at low temperatures (chilled and frozen foodstuffs) and products, which can be consumed without further heat treatment, represent more than 60 % of typical shopping basket of an average European consumer. Consumer food safety partly depends on temperature control throughout all stages in the food supply chain: production, transport, storage, retail display and domestic refrigeration. Maintaining a cold chain is one of important preventive steps for ensuring food safety and temperature is one of key parameters, which effect growth of microorganisms and their survival in food. If perishable foodstuffs are not stored at required temperature the process of spoiling starts and if these foodstuffs are also secondary contaminated, pathogen microorganisms, which are causing food borne illness, can grow. Various studies [5,6,7,8,9,10] show consumers' insufficient knowledge about the importance of cold chain maintaining and carelessness by handling with foodstuffs. Food safety risk is also presented at the last step before foodstuffs reach consumers in retail when the cold chain is frequently broken [11,12].

Numerous domestic refrigerators are increasing electric energy consumption. Worldwide energy consumption has been increasing rapidly, in fact almost exponentially, since the industrial revolution; and this increasing trend of energy consumption has been accelerated by the improvement of the quality of life, that almost directly relates to the amount of energy consumption [13]. One of reasons for more frequent cut-outs of a circuit in near future could be sustained energy demand, uncertainty over future fossil fuel reserves, and increasing dependency on a few geopolitically unstable regions, cause serious concerns over energy security [14]. Another reason for cut-outs of circuit are extreme climate conditions which can also interrupt electricity supply almost unannounced. There exist different recommendations from different institutions like Institutes for public health, government authorities, food inspection agencies, nongovernmental associations and after all from producers of cooling appliances, which warn consumer about safety of foodstuffs in domestic refrigerator in case of electric power failure. The recommended time in which, chilled foodstuffs are stored at required temperatures in case of power failure vary e.g. from 2 to 4 hours [15], 4 hours [16,17], from 4 to 6 hours [18]; by consideration of some preventive actions e.g. avoid opening the refrigerator door. Majority of these recommendations do not take into consideration all other factors like ambient temperature, frequency of door openings, degree of loading and product arrangement in the refrigerator.

The aim of the research was to observe the internal temperatures of four selected perishable foods in domestic refrigerator at different internal and external controlled conditions after the six hour cut-out of a circuit.

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METHODS

A static household refrigerator (single-door) without ventilation and freezer compartment, with three glass shelves and vegetable storage tray was used (width x height x depth: 60 cm x 85 cm x 60 cm; net volume: 156 L; number of shelves: 3; year of manufacturing: 2006). The thermostat setting before cut-out of circuit was set on position four on seven point scale during the research, which included different refrigerator interior and exterior conditions (Table 1).

Table 1:

Refrigerator interior and exterior conditions during the research.

Refrigerator interior conditions	Door openings	Refrigerator exterior conditions
Freedown feinersten with each 4 colored	Without	
Empty retrigerator with only 4 selected	Every two hours	
	Every hour	
	Without	
Partially full refrigerator	Every two hours	$T[S] = 30.6 \pm 0.9 ^{\circ}\text{C}$
	Every hour	$T[W] = 18.5 \pm 0.4 ^{\circ}\text{C}$
	Without	
Full refrigerator	Every two hours	
	Every hour	

Legend:

T – average value of ambient air temperature; [S] – simulation of summer temperatures; [W] – simulation of winter temperatures.

Refrigerator was placed in experiment room (Length: 470 cm x width: 225 cm x height: 310 cm) on fourth floor of four-floor building. It was placed 10 cm from the wall. In the experiment room any other source of heat was not present. Selected perishable foodstuffs were: cottage cheese (250 g) in original plastic container, cream (250 mL) in original tetra Pac package, hash meat (800 g) in polyvinyl bag and frankfurters (350 g) in original plastic foil. Cottage cheese was placed on the top shelf, cream on the middle shelf and hash meat on the bottom shelf. All three were placed as close as possible to the refrigerator door with intention to observe the most critical parts of refrigerator, regarding temperature conditions. The frankfurters were placed in the vegetable storage tray with the intention to observe the internal temperature of food in this area of refrigerator. By different degrees of loading, refrigerator was filled with packages, where single package was filled with mixture of water (940 g) and gelatine (60 g). The mixture was first placed in models (width: 15 cm x height: 20 cm x depth: 4 cm) and then after 12 hours in the refrigerator putted in plastic bags (simulation of packing material) which were vacuumed. The packages were used to simulate different degrees of refrigerator load. Their function was to provide thermal ballast and fill up space. At partially full refrigerator 14 of this packages were used, four on each shelf and two in vegetable storage tray. At

The investigator did the door openings manually. In all cases the time from cut-out of a circuit until the end of the measurement was 6 hours. full refrigerator 28 of these packages were used, eight on each shelf and four in vegetable storage tray. Before cut out of circuit, selected foodstuffs and packages, which were used to simulate different degrees of loading spent 24 hours in refrigerator by its normal operation. Different ambient temperatures were assured with air condition system. Air condition system was placed under the room ceiling and had no direct impact (air flow) on the refrigerator. During the research different internal and external parameters were observed (Table 2).

Table 2:

Internal and external parameters observed during the research.

Parameter	Unit	Time interval
Ambient temperature in experiment room	°C	30 min
Internal temperature of selected foodstuffs	°C	5 min
Time of single door opening	S	10s (90 ° angle)
Time interval of simulated power failure	h	6h

The investigator did the door openings manually. In all cases the time from cut-out of a circuit until the end of the measurement was 6 hours. For ambient temperature measurements Testo 605-H1 and for food internal temperature measurements Testo 177-T4 calibrated thermometers were used. Testo 605-H1 is a temperature & humidity instrument, with measurement range -20 °C to +50 °C, resolution 0.1 °C, accuracy ± 0.5 °C. Testo 177-T4 thermocouple data logger has up to 4 external temperature probe connections for simultaneous temperature measurement range is -200 °C to +400 °C, resolution 0.1 °C, accuracy by -100 °C to +70 °C is ± 0.3 °C. By internal temperature measurement thermocouples were placed in the center of foodstuffs.

RESULTS

Different ambient temperatures and different degrees of loading have shown impact on internal temperatures of selected foodstuffs while different frequencies of door openings did not (Figure 1). The biggest internal temperature difference after six hours of electricity cut-out appears by cream in case of empty refrigerator and *[S]* ambient temperatures. The opposite situation appears by frankfurters in case of full refrigerator and *[W]* ambient temperatures.

There was no significant difference by different frequencies of door openings which is evident in Figure 1. If we look at worst possible conditions (empty refrigerator, foodstuffs as close as possible to the refrigerator door and high [S] ambient temperatures) there is less than 0.5 $^{\circ}$ C difference

There was no significant difference by different frequencies of door openings which is evident in Figure 1.



between zero door openings and door openings every hour. The situation is very similar by lower ambient temperatures and by different degrees of load. In some cases, like cream, temperature differences by door openings are some tenths lower than in case without door opening.

Differences according to different degrees of refrigerator load can be explained with physical characteristic of water (thermal conductivity), which represents 94 % of packages used to provide thermal ballast and to fill up space in refrigerator. By all observed foodstuffs the smallest difference of internal temperature in case of full refrigerator and by lower [W] ambient temperatures can be observed (Figure 1). The opposite situation can be observed in case of empty refrigerator and by higher [S] ambient temperatures (Figure 1). It should be noted, that frankfurters were placed in vegetable storage tray, which is physically separated from the rest of refrigerator interior.

In Figure 2 six hours movement of internal temperatures by different ambient temperatures in case of partially full refrigerator without door openings can be observed. Results presented in Figure 2 clearly show the impact of external ambient temperature on internal temperatures of selected foodstuffs after cut-out of circuit. Gentler sloped curve by lower *[W]* ambient temperatures then by higher *[S]* ambient temperatures can be observed. In spite of same conditions also differences between selected foodstuffs can be observed. Smaller internal temperature difference between *[S]* and *[W]* case in Figure 2b (3.9 °C) in comparison to Figure 2a (4.9 °C) after six hour cut out of circuit, could be result of different structure of foodstuffs used. Even smaller internal temperature difference between *[S]* and *[W]* case after six hour cut out of circuit in Figure 2c (2.3 °C) could be the result of bigger amount of hash meat compared to other foodstuffs used. The smallest temperature difference

Figure 1:

Internal temperature differences (°C) of selected foodstuffs after 6h cut-out of circuit by different ambient (*[S]* and *[W]*) temperatures, different frequencies of door openings and different degrees of loading.

Legend:

/ – zero door openings; 2h – door openings every two hours; 1h – door openings every hour; $[S] = 30.6 \pm 0.9 \text{ °C};$ $[W] = 18.5 \pm 0.4 \text{ °C}.$

Even smaller internal temperature difference between [S] and [W] case after six hour cut out of circuit in Figure 2c (2.3 °C) could be the result of bigger amount of hash meat compared to other foodstuffs used.



Figure 2:

Comparison of internal temperatures movement of (a) cottage cheese (b) cream (c) hash meat and (d) frankfurters in case of partially full refrigerator without door openings by high [S] and low [W] ambient temperatures after 6h cut-out of circuit. **Legend:**

[S]= 30.6 ± 0.9 °C; [W]= 18.5 ± 0.4

(2.2 °C) between [S] and [W] case after six hour cut out of circuit can be observed in Figure 2d (frankfurters), which were placed in vegetable storage tray. The vegetable storage tray is the lowest part of the refrigerator but it is physically separated from the rest of refrigerator interior with plastic container and that barrier as it can be observed soothes temperature oscillations. In some cases like foodstuffs by lower [W] ambient temperatures presented in Figure 2 it can be seen that internal temperatures after the cut-out of circuit first drop and then after one hour start to grow. That could depend on working program of refrigerator (compressor "on" and "off" cycles). If electricity is cuted-out at time when refrigerator is cooling (compressor "on") then internal temperature is still dropping. If electricity is cuted-out at time when compressor is not working (standby position) the internal temperature starts to grow earlier. This phenomenon occurs because at the moment before the compressor starts, the evaporator is at its highest temperature [19].

The very similar situation like presented in Figure 2 can be observed by different door openings. There is some difference according to different degrees of loading regarding to higher (empty refrigerator) or lower (full refrigerator) final temperature after 6 h cut-out of circuit.

DISCUSSION

We estimated that in case of power failure ambient temperature and degree of loading affects internal temperature of selected foodstuffs while frequency of door openings does not. James and Evans [20] assert that the ambient temperature does not exert an effect on the refrigerator temperature. That may be true in case of working refrigerator, but as it can be seen from results in this research, that is no longer true in case of power failure. Most people maintain a temperature of 17 °C - 23 °C in their homes and refrigerators are designed for use in these conditions [20], but in case of cut-out of circuit beside refrigerator also heating or air condition system stop to operate. In such case ambient temperature depends on climate conditions. Different ambient temperatures in this research simulate different time of season or different parts of the world. As shown by Nauta and others [21] in northern European countries refrigerator operating temperatures are usually lower than in southern countries and so consumers in southern parts of Europe take much more microbiological risk for their health than consumers in northern parts of Europe.

Parameter with the least impact on internal temperatures of selected foodstuffs is frequency of door openings. Reason for such situation could be to small interval (10 s) of single door opening. By choosing interval of single door opening test standards for household refrigerating appliances were considered. By chosen time intervals between door openings greater impact on internal temperature of selected foodstuffs was expected. No impact of door opening frequency is maybe not surprising, since the door openings were so infrequent. As shown by Laguerre and others [22] in everyday life frequency of door openings is <10 times/day in 19 %, 10 to 20 times/day in 43 % and over 20 times/day in 38 % (N = 119), but that considers normal operating of domestic refrigerator. We have avoided more frequent door opening while recommendations for consumers handling in case of electric power failure are recommending to avoid acts like door opening during electric power failure.

High internal temperatures before the cut-out of the circuit (Figure 2) can be attributed to the position of selected foodstuffs (as close as possible to the refrigerator door), to the thermostat setting and as shown by Baar and others [23] to the horizontal temperature gradient (2 °C - 2,3 °C) which appears in refrigerators without air circulation and without built-in freezing box at the top. Thermostat setting on position four on seven point scale was chosen while foodstuffs were freezing by lower settings downwards from seven in empty refrigerator. In refrigerators without ventilation like in this case, strong temperature heterogeneity is often observed with warm zones, which cause microbiological risk and cold zones, which cause freezing risk. So it is very important that consumers know the position of this zones, so that foodstuffs can be placed correctly [24]. But as shown by James and others [1] surveys about consumer handling and also survey done by Ovca and Jevšnik [10] show that consumers usually believe that the coldest location is the top shelf. Beside the bottom also along the back wall cold zone appears. When the refrigerator is loaded, the temperatures of products located near the evaporator are lower than As shown by Nauta and others in northern European countries refrigerator operating temperatures are usually lower than in southern countries and so consumers in southern parts of Europe take much more microbiological risk for their health than consumers in northern parts of Europe.

We have avoided more frequent door opening while recommendations for consumers handling in case of electric power failure are recommending to avoid acts like door opening during electric power failure. We have to consider microorganisms as described by Jay and others, which grow well at refrigerator temperatures and cause spoilage already at 5 °C – 7 °C, relative humidity of refrigerator environment and also eventually presence of other micro-organisms that are either inhibitory or lethal to pathogens.

Here institutions like Institutes of Public health should give their contribution or this topic should be regulated on national or even better on international level. located near the door [24]. So it is more proper to store perishable foodstuffs near the back wall. From results obtained in this research it is difficult to predict how long foodstuffs in case of electric power failure are still safe from microbiological point of view. We have to consider microorganisms as described by Jay and others [25], which grow well at refrigerator temperatures and cause spoilage already at 5 °C – 7 °C, relative humidity of refrigerator environment and also eventually presence of other micro-organisms that are either inhibitory or lethal to pathogens. As shown by James and others [1] it is clear that many refrigerators throughout the world are already running at higher temperatures then recommended. Also in our case internal temperatures before cut-out of circuit were higher than recommended. To get exact prove of food safety after electric power failure of domestic refrigerator, microbiological analysis should be than in the future.

CONCLUSIONS

Because of different recommendations for consumers presented at introduction part and many variations in domestic refrigerators, which were presented in this research, reorganization of methods used for consumer education is proposed. In spite of creating recommendations for consumers how long foodstuffs are still safe after the electric power failure of domestic refrigerator, it is better to educate them when and in which cases they should reject foodstuffs from domestic refrigerators especially perishable foodstuffs. One from lot of different possibilities is incorporation of this topic in to refrigerator producer manuals. The content of this topic should not be abandoned to the producers of cooling appliances only. The attitudes of consumers towards food safety and their practices concerning food are themes of interest to food producers and retailers, public authorities and health educators [26.]. Here institutions like Institutes of Public health should give their contribution or this topic should be regulated on national or even better on international level.

In this research only one type of domestic refrigerator was used and therefore it may not be appropriate to generalize the results. There are a lot of possibilities of future research within this type of refrigerator (different choice of foodstuffs, different arrangement of selected foodstuffs, different arrangement of test packages, different thermostat settings, different positions of thermocouples, etc.) and also examination of different refrigerator types (different volumes, with ventilation, with freezer compartment, etc.). Consumers' knowledge about issue discussed in our article is also of interest.

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Nutritional behaviours of slovenian soldiers

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ABSTRACT:

Healthy nutrition is one of the most important conditions to maintain physical abilities of professional soldiers. We aimed to evaluate dietary behaviours, nutritional knowledge and body mass index of Slovenian professional soldiers with regard to selected biological and socioeconomic determinants.

This cross-sectional study included one contingent (n=84) of Slovenian professional soldiers who were sent on a peacekeeping mission to Bosnia and Herzegovina from July 2004 to January 2005. Their mean age was 27.3 years, 92 % of them were males, 61 % of them were single. A self-report questionnaire (n = 45 items) was used to obtain information on their biological and socioeconomic determinants and dietary behaviours. They completed the questionnaire after starting the peacekeeping mission in July 2004.

Statistical analyses revealed fruits were consumed by 40 % of the soldiers each day; vegetables were consumed by 38 % of each day. Single soldiers consumed fruit on a daily basis at a higher level than married soldiers, 53 % versus 22.2 %. Similar results were identified with vegetable consumption, 49 % daily consumption for single soldiers versus 22.2 % for married soldiers. Fried foods were consumed by 2 % of soldiers daily and soft drinks by 56 %. There is a need for further nutritional assessment studies, initiation of nutritional educational programs into military trainings, and new organizational

KEY WORDS:

policies to support healthy eating.

Dietary behaviours, Nutritional knowledge, Biological and socioeconomic determinants, Slovenian soldiers.

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INTRODUCTION

Healthy dietary behaviours have a protective role and increase longevity and quality of life, whereas unhealthy dietary behaviours are an important health risk factor, which can contribute to increased morbidity and mortality [1,2].

The aim of nutritional education policies is to prevent unhealthy dietary behaviours and diseases [3,4]. This can be achieved through education and via policies that support healthy eating [5]. In Slovenia, children learn the basics about foods and nutrition in primary schools in the subject called "home economics". The home economics curriculum includes basic information about food and nutrition, hygiene, mechanical and thermal processing of foods as well as about the understanding the information contained on the food labels. In high school this knowledge is systematically upgraded only at those schools which have nutrition programmes as part of their curricula. Currently, there are no nutrition programmes for life-long learning.

The first dietary behaviours are acquired already during infancy and childhood, and are later modified during the educational process and by the environment [6,7]. Nutritional knowledge has an important impact on dietary behaviours and acceptability of certain foods till adulthood. It is important to maintain a well-balanced diet with adequate energy intake as well as micro- and macronutrient intake. Additionally it is important to restrict unhealthy dietary behaviours such as too high intakes of: total fat, trans and saturated fats, cholesterol, salt, sugar, alcohol as well as active or passive smoking [8,9,10,11].

The dietary behaviours of soldiers should aim at maintaining a healthy well-balanced diet to best satisfy their physiological needs. In the nutrition educational programmes in the United States Army some time is devoted to informing the soldiers about healthy dietary behaviours and the prevention of unhealthy dietary behaviours [12,13].

In Slovenia several studies have shown that dietary behaviours of adults Slovene are not optimal (adequate). They are characterized by insufficient number of daily meals, excessive energy and fat intakes, and insufficient carbohydrates and dietary fibres intakes [14,15,16,17]. The National Survey on Health Status and Health Behaviour of Slovenian adults, published in 2004, has shown that Slovenia has already reached epidemic levels of excessive food intake and obesity [14].

Slovenia became a full member of the NATO in 2004 and from this year forward maintains a professional army. Before that the Slovenian army consisted of military recruits. A few studies concerning dietary behaviours of Slovenian recruits exist, but there has been no research about dietary behaviours of Slovenian professional soldiers [18,19].

This study is the first study in the field of Slovenian military medicine with participation of professional soldiers. The aim of the study was to evaluate dietary behaviours and nutritional knowledge of Slovenian professional soldiers and to investigate the relationships between unhealthy nutrition, nutritional knowledge and body mass index and selected bioNutritional knowledge has an important impact on dietary behaviours and acceptability of certain foods till adulthood. It is important to maintain a well-balanced diet with adequate energy intake as well as micro- and macronutrient intake.

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This study is the first study in the field of Slovenian military medicine with participation of professional soldiers.

logical (age) and socioeconomic determinants (education and marital status). The results of our study can serve as a basis for working out the development of the nutritional educational programmes and policy change for military personnel of the Slovenian army which will promote healthy lifestyle. These preventive measures can contribute to the reduction of high mortality in Slovenia, attributable to non-communicable disease.

METHODS

Study population

Our study included 84 professional soldiers who served in one contingent of the Slovenian units of the Stabilization Forces in Bosnia and Herzegovina (SFOR BiH) from July 2004 until January 2005. The soldiers were active-duty soldiers, employed by the Ministry of Defence of the Republic of Slovenia. They were selected by their commanding officers in April 2004 based on their physical and mental abilities and went through the military training program for a period of a few weeks.

The study protocol was approved by the Ethical Committee of the Medical Faculty of the University of Ljubljana and included informed consent from the soldiers.

Study protocol and data collection

To asses the dietary behaviours and nutritional knowledge of Slovenian professional soldiers was used **a multiple-choice questionnaire which consisted of three parts**. The questionnaire was given to the soldiers to be completed on their own after their arrival into BiH in July 2004. They were divided into four groups. Each group was tested separately. The testing session lasted about 45 minutes and the soldiers could return to other tasks as soon as they completed the questionnaire. All 84 soldiers returned the questionnaire and all were completely answered.

In Part I data were collected the data about biological (date of birth and gender) and socioeconomic determinants such as education, rank in the Slovenian army, marital status and number of children. A soldier's marital status was categorized as: a.) I am single; b.) I have a partner but I don't live with him/her; c.) I cohabit with partner d.) I am married and e.) I am divorced. The soldiers were asked to record their body weight and height. Body mass index (BMI) was calculated as weight divided by height squared (kg/m²). The equivalent World Health Organization (WHO) BMI standards were used as reference: normal range BMI between 18.50 and 24.99; overweight BMI between 25.00 and 29.99; obese BMI=30.00 and more [20]. Since no underweight persons were identified, there was no need to identify this group.

In Part II dietary behaviours of the soldiers was investigated by the food frequency questionnaire (FFQ). Our FFQ was created based on the questionnaire used in the previous large extend Slovenian cross-sectional study (CSS) that was conducted in late spring 2001 [14]. The Slovenian CSS represented a part of a wider World Health Organization project of Countrywide Integrated Noncomunicable Disease Intervention Health

The study protocol was approved by the Ethical Committee of the Medical Faculty of the University of Ljubljana and included informed consent from the soldiers. Monitor (WHO CINDI – CHM). The WHO – CHM project is mostly aiming at monitoring, assessing and comparing the trends of health behaviour in CINDI countries with different political and economic systems [21]. The project is coordinated by Finland, due to its rich tradition and great experience and successes in preventing non-communicable disease [22]. The FFQ reflected dietary behaviours of the soldiers over a period during the previous one year while they were serving in the army at home in Slovenia. Namely, soldiers work in the army but they live at home.

Part III included the questions related to the nutritional knowledge of the soldiers. Questions were adopted from the research which was made on the active-duty Navy personnel in the United States Army [12]. This part included 40 true/false items (22 true and 18 false) which concerned nine nutrition content domains: calories/food intake, fats, cholesterol, vitamins/minerals, fibre, sodium, carbohydrates, protein, and weight loss diet. The true/false items were scored as follows: "+1" for correct response, "0" for incorrect response or no response and were summed together. In addition, items written to tap similar content domains were clustered together into nine priori scales as mentioned above; the scales were computed as the mean value (from 0.00 to 1.00) of all the items in the cluster. Content validity of the questions has been done by the author of research [12].

Statistical methods

Statistical data analyses were performed with Statistical Package for Social Science, version 13.0 (SPSS Inc., Chicago, IL, USA). Numeric variables are presented as mean \pm standard deviation (SD) and categorical variables as percentage (relative numbers).

Unhealthy behaviors in nutrition were defined on the basis of Slovenian CSS [20]. Behaviors classified in our study as unhealthy were: consumption of three daily meals or less, consumption of fruit 4-6-times per week or less, consumption of vegetable 4-6-times per week or less and consumption of fish and sea food 1- 3-times per week or less. All components were considered as equally important, and the number of unhealthy components was calculated for every participant. The participants were classified into two groups on the basis of the number of unhealthy components as follows: healthy (0 components) and unhealthy (1-4 components). Taking into account the nutritional knowledge, the soldiers were divided into two groups, namely soldiers with adequate nutritional knowledge if they achieved 21 or more scores (i.e. if they answered at least 21 items correctly) and soldiers with inadequate nutritional knowledge if they achieved 20 or less scores (i.e. if they answered 20 or less items correctly).

The observed outcomes were examined in relation to numerous covariates including: age: <25; 25-30 and >30 years; level of education: vocational or high school and college or university; marital status: single and married. The strength of the association between observed outcomes and selected biological and socioeconomic determinants was estimated using chi-square test (χ^2 test) and Contingency coefficient (C). The level of significance was set at P<0.05 [23]. Behaviors classified in our study as unhealthy were: consumption of three daily meals or less, consumption of fruit 4-6-times per week or less, consumption of vegetable 4-6-times per week or less and consumption of fish and sea food 1- 3-times per week or less. The soldiers consumed most often white bread (31.4 %), followed by different sorts of bread (26.5 %) and by wholemeal bread (8.4 %).

RESULTS AND DISCUSSION

Characteristics of the study population

General characteristics of the participating soldiers are shown in table 1. They were on average 27.3 years old (SD=3.77), predominantly males, finished vocational or high school education, primarily single and without children. Less than half of the soldiers had normal BMI, 45.2% of the soldiers were overweight and 6 % were obese.

Table 1:

Biological, socioeconomic and anthropometrical data of the participating soldiers in percentages (n=84).

Variable		Percentage /%
Age (y)		
	<25	13.1
	25-30	69.0
	>30	17.9
Sex		
	Males	91.7
	Females	8.3
Rank		
	Soldier	79.8
	Officer	20.2
Education		
	Vocational/high school	90.5
	College or university	9.5
Current marital s	tatus	
	Single	60.7
	Married	39.3
Children		
	No	81.1
	Yes	18.9
Body mass index		
	18.0 – 24.99 (normal)	48.8
	25.00 – 29.99 (overweight)	45.2
	≥ 30.00 (obese)	6.0

Dietary behaviours and nutritional knowledge of the soldiers

The mean number of meals consumed by the soldiers per day was 3.32 (SD=1.05). Only 35.3 % of soldiers ingested 4 or more meals per day; 45.2 % of the soldiers consumed breakfast every day, 75.0 % lunch and 44.0 % dinner. Morning and afternoon snack were consumed by 20.0 % of the soldiers every day.

Ordinary margarine was mainly used by 38.2 % of soldiers in the form of bread spread and 40 % of soldiers used margarine with less fat content. Full-fat milk was mainly consumed by 34.5 % of soldiers, milk with low fat (1.6 % fat or less) by 50.0 % and skimmed milk (0.5 % fat) by 3.6 % of soldiers.

The soldiers consumed most often white bread (31.4 %), followed by different sorts of bread (26.5 %) and by wholemeal bread (8.4 %).

Table 2 shows the frequency of consumption of some food items, fast foods and soft drinks by soldiers.

Table 2:

Frequency of intake of food items, fast foods and soft drinks by Slovenian soldiers (n=84) in percentages.

	Every day	1-3 times a week	1-3 times a month	Never
Milk and milk products	29.8	63.0	4.8	2.4
Fruits	40.5	55.0	1.2	2.4
Vegetables	38.1	52.3	3.6	6.0
Poultry	6.0	71.4	20.2	2.4
Read meat	1.2	71.5	23.7	3.6
Fish and seafood	7.1	29.7	52.4	10.8
Potatoes, rice, pasta	4.8	88.0	6.0	1.2
Cereals and cereal products	6.0	40.5	29.7	23.8
Fried food	2.4	54.8	42.8	0.0
Sandwiches, hot dogs, pizza	9.5	46.4	42.9	1.2
Canned food, instant soups	0.0	7.1	51.2	41.7
Sweets, confectionery	8.4	45.2	45.2	1.2
Soft drinks (Coca Cola, Fanta, Sprite)	56.0	35.7	8.3	0.0

In the part of the questionnaire concerning nutritional knowledge of the soldiers the mean number of correct items was 21.2 (SD = 2.85). The mean scale scores range from 0.66 to 0.38. Soldiers achieved the highest scores on the questions about fibres, proteins and sodium (0.66, 0.61 and 0.60), whereas the lowest scores for the questions related to carbohydrates and calories/food intake (0.39 and 0.38). Questions related to vitamins and minerals, fats and cholesterol resulted in similar response means (0.55 for questions referring to vitamins and minerals, 0.53 for questions about fats and 0.52 for questions on cholesterol). For the questions concerning weight loss diet the soldiers achieved mean value 0.59.

Dietary behaviours, nutritional knowledge and body mass index related to selected biological and socioeconomic determinants

Table 3 shows the influence of some socioeconomic and biological determinants on dietary behaviours and nutritional knowledge of the soldiers. There were differences in food intake according to age. In response to the question do you consume fish and sea products at least 1-3 times per week: a positive response was received by 75 % of the soldiers aged more than 30 years; 34.8 % of soldiers aged from 25 to 30 years; and 22.7 % of soldiers aged under 25 years ($\chi^2 = 8.067$, C = 0.296, P<0.05). Responses also varied according to educational level attained. In response to how often fish and sea products were consumed, those whit higher education consumed more; 75 % of the soldiers with college or university degree, and 32.9 % of soldiers with vocational or high school ($\chi^2 = 5.511$, C = 0.248, P<0.05). There were statistical correlations between marital status of the soldiers and frequency of fruits and vegetables consumption. Fruits were eaten by 53.0 % of single and only 22.2 % of married soldiers ($\chi^2 = 8.372$, C = 0.301, P<0.05) whereas

vegetables were eaten by 49.0 % of single and 22.2 % of married ($\chi^2 = 6.570$, C = 0.269, P<0.05) every day.

There was no statistical significance between nutritional knowledge (i.e. number of scores) and selected biological and socioeconomic determinants.

Table 3:

Impact of some biological and socioeconomic determinants on dietary behaviours and nutritional knowledge of participating soldiers (n=84).

	Age	Education	Marital status
	P -value ^a	P -value ^a	P-value ^a
Dietary habits:			
N° of daily meals intake	0.859	0.677	0.270
Frequency consumption of:			
Fruits	0.686	0.348	0.004
Vegetables	0.916	0.971	0.010
Fish and sea foods	0.018	0.019	0.704
Nutritional knowledge	0.776	0.857	0.455

a: c² test

Table 4 shows the influence of some socioeconomic determinants on BMI. Among soldiers with vocational or high school were more than half of those with normal BMI and soldiers with college or university had normal BMI only in 25.0 % ($\chi^2 = 6.461$; C = 0.267; P<0.05). In the married group of soldiers were 60.8 % of those with normal BMI and in the group of single only 30.3 % ($\chi^2 = 7.551$; C = 0.287; P<0.05).

Table 4:

Statistical significance among the groups classified to BMI and selected socioeconomic determinants (n=84).

	BMI groups (%)			Dualua
_	18.50-24.90	24.91-29.90	>29.90	P-value"
Age groups (y)				
<25	60.9	39.1	0.0	0.362
25-30	47.8	43.5	8.7	
>30	33.3	60.0	6.7	
Education				
Vocational/high school	51.3	44.7	3.9	0.040
College/university	25.0	50.0	25.0	
Marital status				
Married	60.8	35.3	3.9	0.023
Single	30.3	60.6	9.1	

a: c² test

There are few published studies on soldier's nutrition therefore our data was compared with data based on adult population. Our study is the first that investigated dietary behaviours and nutritional knowledge of the Slovenian professional soldiers. This group of Slovenian soldiers which was investigated is not representative of the entire Slovenian military. Slovenian soldiers are to a great extent overweight and obese and have quite unhealthy dietary behaviours. The dietary behaviours of Slovenian soldiers in our study did not differ extensively from the dietary behaviors that were reported for the Slovenian adult population [14,15,16]. On average, soldiers in our study ate only three meals per day. Less than half of them ate breakfast regularly. The national health study conducted in late spring 2001 showed, two thirds (67 %) of Slovenian adults consumed three meals per day or less; 49 % had breakfast every day, 98 % lunch and 54 % dinner [14]. Soldiers in our study had too long time intervals between the meals. They reported to consume morning and afternoon snacks only 20 % of the time, whereas the Slovenian adults in 37 % and 30 % respectively [14]. From present study, the causes can not be explained, but the assumption is that this is due to the fact that the majority of soldiers drives to work from distance places and usually do not have time to eat.

It was found previously that in order to achieve healthy dietary behaviours of Slovenian soldiers, we need to ensure an adequate nutritional knowledge at the first place [18,19]. The soldiers who participated in our research did not have any nutritional educational programs when they joined the Slovenian army not even later when they served in the army, so their answers reflected only their basically acquired knowledge during childhood and educational process. Comparing the knowledge of Slovenian soldiers in our study with the literature data on American soldiers, the average achievements during the test in Slovenian soldiers were lower compared to the American soldiers [12].

Cross sectional evidence show that married persons have healthier lifestyle than unmarried persons [14,27]. There are several underlying mechanisms by which change in marital status affect healthy behaviours. It has been hypothesised that the marital relationship provides social control over healthy behaviours. Social support from a spouse may also be a key mediating factor in the establishment and maintenance of a healthy lifestyle [27]. In contrary to the literature our data have shown that married soldiers consumed fruits and vegetables less often than single. This is interesting compared with the Slovenian study about health and state of health, conducted in November 2007, where this is opposite; namely 82 % of married and 63 % of single inhabitants ate fruits every day; 81 % of married and 65 % of single ate vegetables every day [28]. Further studies are required to clarify this inversely findings that we found in our study.

Many studies have found that individual with lower education have higher BMI than individual with higher education [29,30]. In our study this findings were contrary, possibly because of the fact, that Slovenian soldiers with vocational/high education had a rank of professional soldier and soldiers with college/university had a rank of an officer. Professional soldiers have different physical trainings every day, because of maintaining adequate psychophysical condition. On the contrary officers have higher position that is they have more desk work and not so many physical trainings anymore. Several, but not all cross-sectional studies have shown that married or cohabiting subjects have a higher BMI than subjects living alone [31,32,33,34]. Our data have shown that in the group of married soldiers there were more of those with normal BMI than in the group of single soldiers. On average, soldiers in our study ate only three meals per day.

From present study, the causes can not be explained, but the assumption is that this is due to the fact that the majority of soldiers drives to work from distance places and usually do not have time to eat.

Professional soldiers have different physical trainings every day, because of maintaining adequate psychophysical condition. On the contrary officers have higher position that is they have more desk work and not so many physical trainings anymore. In summary our study showed that dietary habits of Slovenian soldiers markedly deviated from healthy dietary habits and reflected dietary habits of adult Slovenian population.

Based on our research findings, the Ministry of Defence of the Republic Slovenia will be able to form suitable and focused preventive programmes with the aim for improving dietary behaviours and nutritional knowledge of Slovenian professional soldiers and in particular to promote physical activity between them. In our study, soldiers reported height and weight by themselves. Selfreports of height and weight are considered valid for the population studies, with correlations between measured and self-reported weight averaging from 0.97 to 0.99 [35]. The BMI is a simple, easy- to- use, and cost effective screening tool, because it is highly correlated with various measures of body fat. In our study only 49 % of soldiers had normal BMI. According to the Slovenian national health survey there was 44 % of adult Slovene with normal BMI (33 % of men and 53 % of woman) [14]. Because of increasing obesity rates in the United States, the Army's standards now disqualify a large percentage of the population. A study conducted by Army researchers found that 27 % of the 18-year-olds who applied to join the military in 2006 were overweight-up from 23 % in 1993 [36]. Body habits, as described by BMI, is related to skeletal size, muscle mass and adiposity. It was reported that overweight and obesity were not significant predictors of discharge from United States Air Force [37]. It is possible that some of individuals classified as the overweight or obese in our sample had higher BMI's due to increased muscle mass and not due increased body fat.

In summary our study showed that dietary habits of Slovenian soldiers markedly deviated from healthy dietary habits and reflected dietary habits of adult Slovenian population. 51,2 % of Slovenian professional soldiers were overweight or obese. Their knowledge about the nutrition was poor. In the view of socioeconomic status, we found that healthier dietary habits prevailed among older, single and among higher educated soldiers. In contrary normal BMI prevailed among less educated, as well as among married soldiers. Nevertheless it must be emphasized that our study offers the basic information about dietary behaviours, nutritional knowledge and BMI of Slovenian professional soldiers, which all need to be improved. Changing the traditional lifestyle is one of the most important elements in health but extremely difficult, and a process of very long duration, tightly bound to the economy and politics of a country [38]. The results of the present study clearly show the need for a greater emphasis of the benefits of regular and adequate nutritional education early in military training to encourage soldiers to adopt healthier behaviours. Intervention programs should be targeted at younger and less educated soldiers and physical activity (especially among overweight and obese soldiers and officers) encouraged. Based on our research findings, the Ministry of Defence of the Republic Slovenia will be able to form suitable and focused preventive programmes with the aim for improving dietary behaviours and nutritional knowledge of Slovenian professional soldiers and in particular to promote physical activity between them. Further detailed investigations of health status, anthropometric measures, dietary habits as well as physical activities of representative sample of Slovenian soldiers should be performed in the future. The investigations should be performed periodically (i. e. every 4 years) in order to follow the trends in dietary habits as well as to follow the efficacy of the education process.

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Inflammatory bowel disease, intestinal microflora, prebiotics and probiotics

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ABSTRACT:

Inflammatory bowel disease (IBD) is a group of intestinal conditions characterized by chronic relapsing course of uncontrolled inflammation within the gastrointestinal tract. The two main types of IBD are ulcerative colitis (UC) and Crohn's disease (CD).

Both genetic and environmental factors are involved in etiopathogenesis. According to most recent theories, IBD is probably a consequence of abnormal mucosal immune response to antigens of gut bacterial microflora in genetically susceptable individuals. If tolerance to commensal bacteria is lost, an immune response may be elucidated against non-pathogenic bacteria, leading to increased production of inflammatory cytokines and chemokines. Consequently different subsets of inflammatory cells are activated.

Growing knowledge about implication of gut microflora into the pathogenesis of IBD encouraged scientific word to search for new therapeutic strategies concentrated on changing the microenvironmental factors. Nutritional therapy has been advocated in CD patients, especially for children and adolescents.

The rationale behind prebiotic use is to elevate the populations of certain beneficial bacteria and thereby quantitatively changing the composition of microflora. Although several prebiotic compounds possess promising properties to have beneficial effect on IBD, only few of them (Plantago ovata, germinated barley foodstuff) have been clinically tested.

Multiple mechanisms of action have been suggested to explain the effect of probiotics in IBD. A great number of basic, animal model and human studies have revealed the great potential of probiotic use in treatment of IBD patients. However, clinical use of probiotics has been proved effective only in a therapy of pouchitis and maintenance of remission in ulcerative colitis, while their effectiveness in a therapy of Crohn's disease is not firmly proved.

KEY WORDS:

Inflammatory bowel disease, Crohn's disease, Ulcerative colitis, Pouchitis, Intestinal microflora, Prebiotics, Probiotics

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INTRODUCTION

Inflammatory bowel disease (IBD) is a group of idiopathic intestinal conditions characterized by chronic relapsing course of uncontrolled inflammation within the gastrointestinal tract. The two main types of IBD are ulcerative colitis (UC) and Crohn's disease (CD). UC and CD share many characteristics, but they also differ in certain aspects. UC is characterised by mucosal inflammation of large bowel while small bowel mucosa is spared. Rectal mucosa is regularly inflamed and inflammation spreads continuously to the proximal parts of the colon. On the contrary in Crohn's disease not only colon, but any part of gastrointestinal tract from mouth to anus can be involved. Inflammation spreads through all layers of intestinal wall and inflamed and healthy parts of intestine can follow each other (so called skip lesions). Moreover, CD can present not only with intestinal inflammation but also with penetrating lesions such as intestinal fistulas to other parts of intestine, adjacent hollow organs such as urinary bladder or vagina and skin, and with fibrozating course resulting in intestinal stenoses.

Abdominal pain and tenderness, diarrhoea often containing blood and mucus in the stools, as well as fatigue, low grade fever, weight loss and growth retardation in the case of early onset disease in the childhood are most frequent symptoms and signs of IBD. Although IBD are primary diseases of the intestinal tract, involvement of many extraintestinal organs such as joints, skin, eyes, liver, biliary tract or urinary organs is not unusual.

The prevalence of IBD varies in different populations across the world. The prevalence of CD and UC are high in the most developed countries of Northern and Western Europe and North America, reaching 214 and 243, and 198 and 229 per 10⁵, respectively, but they are low in the developing countries of Asia, Africa and South America [1]. Moreover, the incidences of UC and CD are still growing. IBD manifests during childhood and adolescence in approximately 25% to 40% of all patients [2,3].

Etiopathogenesis of IBD is not completely understood. Both genetic and environmental factors are involved. Increased sanitation and the lifestyles within developed countries appear to increase the risk of IBD. It has been proposed that the exposure to unhygienic conditions during childhood can prime the intestinal environment which will lead to optimal mucosal immune development and regulation, preventing a future immune response [4]. According to most recent theories, IBD is probably a consequence of abnormal mucosal immune response to antigens of gut bacterial microflora in genetically susceptible individuals.

Previous studies focused on identifying specific pathogenic infections responsible for IBD. *Mycobacterium paratuberculosis, Mycobacterium kansasii*, paramixovirus, *Listeria monocytogenes, Chlamydia trachomatis*, RNA reovirus and *Pseudomonas multophilia* infection were regarded associated with Crohn's disease, and *Escherichia coli*, diplostreptococcus, *Fusobacterium necrophorum, Shigella, Helicobacter hepaticus* and RNA viruses were linked to ulcerative colitis [5]. However, further studies have not confirmed the role of specific infections in the pathogenesis of IBD. Moreover, there is a growing evidence that normal bacterial microflora Moreover, CD can present not only with intestinal inflammation but also with penetrating lesions such as intestinal fistulas to other parts of intestine, adjacent hollow organs such as urinary bladder or vagina and skin, and with fibrozating course resulting in intestinal stenoses.

According to most recent theories, IBD is probably a consequence of abnormal mucosal immune response to antigens of gut bacterial microflora in genetically susceptible individuals. Several observations in humans implicate microbial factors in the pathogenesis of IBD. Bowel lesions in IBD occur predominantly in areas with highest bacterial counts like terminal ileum and colon.

In the case of IBD however, genetically predisposed individuals appear to lose the normal tolerance to commensal bacteria, leading to elevated inflammatory response. can trigger harmful immune reactions in susceptible hosts. The most convincing evidence supporting the role of enteric microflora in the pathogenesis of IBD comes from animal models. Animals with genetically engineered dysregulation of the immune response develop spontaneous colitis when grown in normal conditions resembling IBD in humans. However, they do not develop intestinal inflammation when grown in germ free environment, indicating that bacterial exposure and colonisation are essential for the development of colitis [6-8]. Interleukin-10 deficient mice displayed a significant higher number of mucosal adherent bacteria and lower levels of protective bacteria like Lactobacillus compared with healthy mice. Both the proportion of mucosal adherent bacteria and the development of colitis were significantly decreased by nutritional supplementation with lactose or enema delivery of Lactobacillus reuteri [9]. Similarly, Lactobacillus plantarum when given in feedings to the IL-10 knock-out mice attenuated established colitis, corresponding to reduction in intestinal permeability and anti-endotoxin core antibody levels [10,11].

Several observations in humans implicate microbial factors in the pathogenesis of IBD [12]. Bowel lesions in IBD occur predominantly in areas with highest bacterial counts like terminal ileum and colon [13]. Diversion of faecal stream is associated with distal improvement in patients with CD and relapse occurs after restoration of faecal stream [14]. UC patients who undergo ileal pouch-anal anastomosis surgery develop mucosal inflammation after bacterial colonisation of the pouch [15]. Early IBD lesions can be induced in susceptible individuals by the direct installation of faecal material into non-inflamed excluded loops of intestine [16]. Antibiotic treatment appears to provide clinical benefit in patients with CD and inflammation of ileal pouch [17].

Pathogenic events in IBD may be associated with different alterations in the intestinal flora in the ileum and colon. More bacteria were detected on the mucosal surface of IBD patients than on those of healthy controls and bacterial invasion of mucosa was evident in up to 83% of biopsies from IBD patients but no bacteria were detected in tissue of controls [18]. Moreover, IBD patients have altered composition of commensal enteric bacteria with increased *Bacteroides*, adherent/invasive *Escherichia coli, Enterococci,* and decreased *Bifidobacterium* and *Lactobacillus* species [19].

INTESTINAL MICROFLORA, IMMUNE SYSTEM, GENES AND INFLAMMATION

Optimal development of intestinal immune system is determined in part by environmental contact with the commensal gut microflora [20]. Changes in the microbial flora may alter mucosal immune development. In the healthy gut, there is a symbiotic relationship between the host and the commensal bacteria in which exposure leads to down-regulation of inflammatory genes, inhibiting the immune response of the gut (4). In the case of IBD however, genetically predisposed individuals appear to lose the normal tolerance to commensal bacteria, leading to elevated inflammatory response. The microbiota then provides a constant stimulus for the host immune system [21]. Majority of genes found to be associated with the increased risk for development of IBD are encoding proteins functioning in preservation of mucosal barrier function or in regulation of mucosal immune system. The major breakthrough of understanding a linkage between genetic predisposition and IBD development was made in 2001 by 3 independent groups which reported the identification of the first Crohn's disease susceptibility gene, NOD2, subsequently renamed CARD15 by the International Nomenclature Committee, on chromosome 16q12 [22-24]. The CARD15 gene encodes a protein that contains 2 caspase rich domains (CARDs), a central nucleotide-binding domain (NBD), and a leucine-rich repeat region [25]. There are 3 common genetic variants of CARD15 associated with CD, Arg702Trp, Gly908Arg, and Leu1007fsinsC, and many other less common putative variants [22,26]. 10% to 30% of CD patients are heterozygotes for one of these common mutations, whereas 3% to 15% of patients are either homozygotes or compound heterozygotes [27]. The relative risk of development CD associated with carriage of one CARD15 variants is between 1.5 and 3, increasing to 20 to 40 in people carrying two mutations [27]. CARD15 belongs to the family of pattern-recognition receptors which are responsible for microbial recognition, induction of anti-microbial genes and control of adaptive immune response [28]. While CARD15 is an intracellular pattern-recognition protein capable of recognizing peptidoglycans from gram-negative and gram-positive bacteria through the detection of muramyl dipeptide, the minimal motif in all peptidoglycans [29,30], other pattern-recognition receptors, named toll-like receptors (TLRs), recognize other microbial components like lipoproteins (TLR1, 2, 6), double-stranded RNA (TLR3), lipopolysaccharide (TLR4), flagellin (TLR5), single-stranded RNA (TLR7, 8), and CpG DNA (TLR9) [28,31]. Mutations in genes for tolllike receptors as well as for CARD4/NOD1 receptor may be also associated with increased susceptibility for IBD [32-35].

Binding of specific microbial components to the pattern-recognition receptors in most cases, including CARD15, leads to intracellular signalling pathways which result in most occasions to nuclear factor- κ B (NF- κ B) activation [30,36,37]. NF- κ B is a key intracellular signalling molecule in a variety of inflammatory pathways and its elevated in IBD tissues [38]. However, common mutations of CARD15 gene lead to a decrease in NF- κ B activation and not to its overactivation [30]. It has been proposed that failure to trigger protective pathways by bacterial components mediated by CARD15 results in defective bacterial eradication [39] resulting in a NF- κ B activation by CARD15 independent mechanisms. CARD15 receptors originally thought to be confined to monocytic and dentritic cells [40] have been recently found also in intestinal epithelial cells [41] and Paneth cells [42]. Paneth cells play an important antibacterial role in the gut, secreting potent antimicrobial substances such as lyzozyme, phospholipase A2 and α and β defensins [27,43].

Pattern-recognition receptors are required to discriminate between pathogenic and commensal microorganisms. If tolerance to commensal bacteria is lost, an immune response may be elucidated against non-pathogenic bacteria, leading to increased production of inflammatory cytokines and chemokines (4). Consequently different subsets of inflammatory cells are activated. Mucosa of patients with CD is dominated by CD4+ lymphocytes with a type 1 helper-T-cell (Th 1) phenotype, characterized by Mutations in genes for tolllike receptors as well as for CARD4/NOD1 receptor may be also associated with increased susceptibility for IBD.

Paneth cells play an important antibacterial role in the gut, secreting potent antimicrobial substances such as lyzozyme, phospholipase A2 and α and β defensins.

the production of interferon- γ and interleukin-2, while the mucosa of patients with UC is dominated by CD4+ lymphocytes with an atypical type 2 helper-T-cell (Th 2) phenotype, characterized by production of transforming growth factor β (TGF- β) and interleukin-5 [44]. Th1 cytokines activate macrophages to produce a potent mix of broadly active cytokines as interleukin-12, interleukin-18, macrophage migration inhibitor factor, tumour necrosis factor (TNF), interleukin-1, and interleukin-6 [45].

In conclusion, intolerance to intestinal microorganisms because of genetical susceptibility in addition to possible dysbiosis of gut microflora may together lead to broad spectrum inflammation of the gut.

INFLUENCING GUT MICROFLORA – THERAPEUTIC OPTION IN IBD

Traditionally, therapy of IBD has been directed against inflammatory response of gut immune system. Corticosteroids, 5-aminosalicylates, immunosuppressive and immunoregulatory agents have been used for over a half of a century to treat active disease and to maintain remission. In the last decade new biologic agents, such as anti-TNF antibodies, emerged as therapeutical options targeting immune system components most important for intestinal inflammation. However, growing knowledge about implication of gut microbial environment into the pathogenesis of IBD encouraged scientific word to search for new therapeutic strategies concentrated on changing the microenvironmental factors that play an important role in the pathogenesis.

Not surprisingly, nutritional therapy has been advocated in IBD patients, especially for children and adolescents, for many years, as the disease often results in weight lost, poor growth and development and numerous nutrient deficiencies [46-49]. However, early studies using enteral nutrition with so called elemental formulas, in which proteins were degraded to amino acids, showed that they were not efficacious only in restoring patient's nutritional status but also in reducing activity of intestinal inflammation in CD patients [50-52]. Because of their bitter taste and high cost. elemental formulas have been gradually replaced by semielemental formulas, using short peptides instead of amino acids, and by polymeric formulas containing whole protein molecules. Interestingly, several meta-analyses revealed that therapy with enteral nutrition has efficiency comparable of that of corticosteroid treatment in active CD, especially in childhood population and that polymeric formulas are as efficient as the elemental ones [53-57]. Although several mechanisms of an action could be important in therapeutical use of enteral nutrition, its influence on the intestinal microflora may play a crucial role [58].

Experimental evidence of the central role of the luminal flora as an essential factor for the development of IBD provided an impetus to the development of alternative strategies to manipulate the intestinal flora by prebiotics and probiotics.

PREBIOTICS AND IBD

Prebiotics are non-digestible food constituents that beneficially affect the host by selectively stimulating the growth or activity of one, or limited

However, growing knowledge about implication of gut microbial environment into the pathogenesis of IBD encouraged scientific word to search for new therapeutic strategies concentrated on changing the microenvironmental factors that play an important role in the pathogenesis. number of bacterial species in the gut, thus improving host health [59]. The rationale behind prebiotic use is to elevate the populations of certain endogene beneficial bacteria such as *Lactobacillus* and *Bifidobacterium* and thereby quantitatively changing the composition of microflora. This change may act beneficially by causing luminal production of short chain fatty acids (SCFA), which are important nutrients for the intestinal cells and induce acidic environment, by preventing of pathogenic bacteria adherence and by production of anti-bacterial substances [60]. Some examples of prebiotics are dietary fiber and some types of oligosaccharides. Although several prebiotic compounds possess promising properties to have beneficial effect on IBD, only few of them have been clinically tested.

Inulin and oligofructose are composed of multiple saccharide units, which are indigestible by the human enzymes. They stimulate the growth of lactic acid bacteria and the generation of SCFA [61]. In dextran sodium sulphate-induced colitis animal model, inulin attenuated gut inflammation [61].

Similarly, fructooligosaccharide was shown to decrease the severity of damage in experimental model of rat trinitrobenzene sulfonic acid induced colitis [62].

Psyllium, also called Ispaghula husk or Plantago ovata, is a water soluble dietary fiber [63]. Hallert and co-workers reported that Ispaghula husk significantly attenuates symptoms in patients with UC [64] and Spanish Group for the Study of Crohn's Disease and Ulcerative Colitis (GETECCU) found it as efficient as sulphasalazine in maintaining remission [65].

Germinated barley foodstuff (GBF) is derived from aleurone layer and scutellum fractions of germinated barley and consists mainly of dietary fiber and glutamine-rich protein [66]. It induces intestinal microflora to produce SCFA [67]. Treatment of rat experimental colitis with GBF led to improvement of the clinical and pathological signs of colitis and decrease serum IL-8 and alpha 1-acid-glycoprotein. GBF was comparable effective against mucosal inflammation and more effective against diarrhoea when compared with sulphasalazine [68]. The same Japanese group proved the effectiveness of GBF in several studies on patients with active UC and UC in remission [69,70]. Patients revealed both clinical and endoscopic improvement of colitis. Therefore, GBF is registered as a special foodstuff for UC by Japan's Ministry of Health, Labour and Welfare.

PROBIOTICS AND IBD

Probiotics are defined as live microorganisms which, when consumed in adequate quantities, confer a health benefit on the host [21]. Multiple mechanisms of action have been suggested to explain the effect of probiotics in IBD. Potential mechanisms include suppression of growth or epithelial binding and invasion by pathogenic bacteria, production of antimicrobial substances, improved epithelial barrier function, and immunoregulation [60,71]. A great number of basic, animal model and human studies have revealed the great potential of probiotic use in treatment of IBD patients.

The effects of probiotic are probably both strain-dependent and dose dependent. For example, probiotic *Lactobacillus rhamnosus* GG (LGG) atThe rationale behind prebiotic use is to elevate the populations of certain endogene beneficial bacteria such as *Lactobacillus* and *Bifidobacterium* and thereby quantitatively changing the composition of microflora.

Treatment of rat experimental colitis with GBF led to improvement of the clinical and pathological signs of colitis and decrease serum IL-8 and alpha 1-acid-glycoprotein.

The effects of probiotic are probably both straindependent and dose dependent. The effect of probiotics on barrier function was studied in T84 cell monolayers. It was demonstrated that VSL#3 prevented the decrease in trans-epithelial resistance following incubation with pathogenic bacteria.

The reviewers concluded that probiotics in combination with conventional therapy do not improve overall remission rates in patients with mild to moderate ulcerative colitis however; they may provide some benefits in terms of reduction of disease activity. tenuated the TNF- α induced IL-8 production at doses 10⁶⁻⁸ by the Caco-2 intestinal cell line, but on the contrary, at higher doses LGG actually increased IL-8 levels [72]. This finding indicated that determining the correct dose of probiotic for treatment is vital. The same study demonstrated that heat-killed LGG was also able to decrease IL-8 production, conflicting the paradigm that viability of probiotics is essential for their efficacy. Similarly, bacterial DNA from VSL#3, a high dose mixture of three strains of Bifidobacteria, four strains of Lactobacilli, and one strain of Streptococcus salivarius ssp. thermophilus, was able to decrease IL-8 secretion, delay NF-KB activation and stabilise IKB levels [73]. However, in another study using Lactobacillus reuteri on HT-29 and T84 cells only live but not deactivated bacteria reduced TNF- α induced IL-8 production and induced production of anti-inflammatory factors [74]. The effect of probiotics on barrier function was studied in T84 cell monolayers. It was demonstrated that VSL#3 prevented the decrease in trans-epithelial resistance following incubation with pathogenic bacteria [75].

The most convincing evidence of probiotic efficacy and mechanisms came from animal studies. More than 20 different animal models of IBD are available [76]. In IL-10 knockout mice, L. plantarum 299V [77], L. salivarius subspecies salivarius 433118 and UCC118 [78,79], B. infantis 35624 [78], and VSL#3 (80) have been shown to attenuate intestinal inflammation [76]. In HLA-B27 transgenic rats LGG prevented recurrent colitis after antibiotic treatment, whereas L. plantarum had no effect (81). Amelioration of inflammation was observed after administration of L. salivarius subspecies salivarius CECT5713 [82] and L. plantarum NCIMB8826 [83] in trinitrobenzene sulfonic acid-induced colitis, and after L .reuteri and oat fiber application in acetic acid and methotrexate induced colitis in rats [84,85]. Similarly, VSL#3 and LGG significantly improved sulphhydril-blocker iodoacetamide-induced colitis in rats, but had no effect on dinitrobenzene sulfonic acid-induced colitis [86]. These experiments clearly demonstrated that the effect of probiotic treatment depends both on probiotic strain and on type of inflammation.

Not only live bacteria, but also soluble bacterial antigens extracted from *E. coli* in dextran sulphate sodium-induced colitis (DSS) model [87] and bacterial DNA from VSL#3 preparation in DSS and IL-10 knockout mouse models [73,88] showed the ability to reduce inflammation. Therefore, viability of probiotic bacteria was not proven to be a prerequisite for their effect.

Many clinical trials have been performed to investigate the efficacy of probiotics in achieving and maintaining remission of different forms of IBD. However, only four which investigated the efficacy of probiotics for induction of remission in ulcerative colitis met the criteria of randomised controlled trials and were reviewed in recent Cochrane Collaboration review [89]. The reviewers concluded that probiotics in combination with conventional therapy do not improve overall remission rates in patients with mild to moderate ulcerative colitis however; they may provide some benefits in terms of reduction of disease activity.

Rembacken et al. [90] reported that probiotics (*E. coli* Nissle 1917) with steroids had similar effectiveness to mesalazine with steroids in achieving

remission, however relapse rate was slightly higher in the mesalazine group compared to probiotic group (73% vs. 67%, P < 0.05).

Kato et al. [91] reported that probiotics (*Bifidobacterium breve, B. bifidum, Lactobacillus acidophilus*) with 5-aminosalicilates (5-ASA) were as effective as placebo with 5-ASA when they compared the proportion of patients achieving the remission. However, when they took into account not only remission data but also clinical improvement data, clinical activity index (CAI) was found to be significantly lover in the probiotic group compared to placebo group after 12 weeks of treatment (3.7+/-0.4 vs. 5.8+/-0.8, P < 0.05). They also found that mean endoscopic index score and mean histological score was significantly reduced in the probiotic group (P < 0.01) but not in the placebo group after therapy.

Although Tursi et al. [92] did not found statistical significant difference in proportion of patients who achieved remission in probiotic (VSL#3)+5-ASA group in compare with placebo+5-ASA group, the mean time to remission was significantly shorter for probiotic group (4 vs. 7 days, P < 0.01).

In the trial of Furrie and colleagues [93] no significant differences were found between synbiotic (probiotic *Bifidobacterium longum* and fructooligosaccharide) group and placebo group in numbers of patients improved, CAI, endoscopy and histology scores. However, synbiotic treatment in conjunction with standard therapy caused significant reduction in TNF- α and IL-1 α compared to standard therapy and placebo.

Several controlled studies showed that some probiotics can be used in the maintenance therapy of UC. Rembacker et al. [94] randomized UC patients who entered remission with conventional therapy to receive mesalazine or probiotic (*E. coli* Nissle 1917) for maintenance treatment for one year. At the end of the trial 73% of 5-ASA-treated patients relapsed as compared with 67% of those assigned to the probiotic. Authors concluded that the two strategies were of equivalent efficacy.

Same can be concluded from the studies of Kruis et al. They found that 11.3% of patients treated with 5-ASA relapsed in 12-week follow-up period as compared with 16% treated with *E. coli* Nisssle 1917 [95]. In their later study they found that during one year follow-up relapse occurred in 36.4% of the *E. coli* group and 33.9% of the mesalazine group [96].

Ishikawa et al. found out that bifidobacteria-fermented milk supplemented as a dietary adjunct was effective in maintaining remission of UC. After 1 year treatment, exacerbation of symptoms was observed in 27% of probiotic group and in 90% of the control group [97].

Zocco et al. [98] compared three groups of therapeutic regimen for maintenance of remission in UC patients. They found no significant difference in relapse rate at 6 and 12 month between the groups that received *Lactobacillus rhamnosus* GG or 5-ASA or both of them.

In an open uncontrolled study Venturi et al. [99] with VSL#3, 75% of patients with UC remained in remission during one-year follow-up.

Pouchitis, chronic inflammation of ileal pouch created after proctocolectomy, is usually treated by antibiotics. However, several controlled trials have revealed that probiotic use can be highly effective. Gionchetty et al. Several controlled studies showed that some probiotics can be used in the maintenance therapy of UC.

In contrast to these results, probiotic *Lactobacillus rhamnosus* GG was ineffective in preventing relapses of chronic pouchitis.

In conclusion, clinical use of probiotics has been proved effective in a therapy of pouchitis and maintenance of remission in ulcerative colitis, while their effectiveness in a therapy of Crohn's disease is not firmly proved. [100] compared the efficacy of VSL#3 with placebo in maintenance of remission of pouchitis. The patients in the probiotic group relapsed in 15% as compared with 100% in the placebo group.

These results were practically replicated by Mimura et al. [101], who found that the relapse rate in one year after beginning of the therapy was 15% for VSL#3 group versus 94% for the placebo group.

Gionchetti et al. [102] studied prophylactic role of probiotic treatment in patients undergoing colectomy and pouch surgery. During the first year after operation only 10% of the patients in VSL#3 group but 40% of the patients in placebo group developed pouchitis.

In contrast to these results, probiotic *Lactobacillus rhamnosus* GG was ineffective in preventing relapses of chronic pouchitis [103].

Efficacy of probiotics for preventing recurrence of active disease has been studied in patients who rich the remission after medical or surgical therapy of CD. Malchow [104] who treated small number of patients with colonic CD in remission with either *E. coli* Nissle 1917 or placebo for 3 months, observed relapse rate 33% in the probiotic group and 63% in the placebo group.

Campieri et al. [105] reported that VSL#3 in combination with 5-ASA were more efficient than 5-ASA alone in prevention of post-operative recurrence of CD. They observed endoscopic recurrence in 40% of patients treated with 5-ASA alone but only in 10% of patients on combined therapy with 5-ASA and probiotics.

However, in two clinical studies using probiotic *Lactobacillus rhamnosus* GG, no differences were found in compare to placebo in CD relapse prevention [106,107]. French GETAID group [108] reported slightly lower endoscopic recurrence in a group of patients that was treated post-operatively with probiotic *Lactobacillus johnsonii* strain LA1 than in the placebo group (49% vs. 64%), but the difference was not statistically significant.

Guslandi et al. [109] reported that maintenance therapy with Saccharomyces boulardii and 5-ASA was significantly more effective in preventing relapse of CD than 5-ASA alone.

In the meta-analysis by Rolfe et al. for Cochrane Collaboration [110] seven studies were identified to reach the inclusion criteria for review of maintenance therapy in CD. The authors concluded that there was no statistical significant benefits of *E. coli* for reducing the risk of relapse compared to placebo (RR 0.43, 95% CI 0.15 to 1.20), or *Lactobacillus* GG after surgically-induced remission (RR 1.58, 95% CI 0.30 to 8.40) or medically-induced remission (RR 0.83, 95% CI 0.25 to 2.80). There were no statistically significant benefits for reducing the risk of relapse compared to maintenance therapy employing aminosalicylates or azathioprine (RR 0.67, 95% CI 0.13 to 3.30). In children, there was no statistically significant difference between *Lactobacillus* GG and placebo for reducing the risk of relapse (RR 1.85, 95% CI 0.77 to 4.40).

In conclusion, clinical use of probiotics has been proved effective in a therapy of pouchitis and maintenance of remission in ulcerative colitis, while their effectiveness in a therapy of Crohn's disease is not firmly proved.

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Higiena živil v primarni proizvodnji Food hygine in primary production

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POVZETEK:

Varnost živil je področje, ki mora slediti hitremu in intenzivnemu gospodarskemu razvoju ter zahtevam regulative. Namen prispevka je prikazati pomen in razumevanje področja varnosti živil v primarni proizvodnji. Uporabljena je bila kvalitativna metoda dela. Tekstovno gradivo za kvalitativno analizo sestavljajo predpisi sedmih delno strukturiranih intervjujev (s predstavniki vladnih in nevladnih organizacij) in 78 odgovorov kmetovalcev (prejemnikov denarnih sredstev iz programa SKOP za leto 2005) na odprto vprašanje iz anketnega vprašalnika. Rezultati kažejo, da je po mnenju predstavnikov vladnih in nevladnih organizacij odgovornost ključni pojem pri zagotavljanju varnosti živil. Močno je izpostavljena problematika deljenih pristojnosti. Za zagotavljanje učinkovitega in sledljivega sistema dobrih praks za proizvodnjo kakovostne in varne hrane je potrebno vzpostaviti ustrezne mehanizme tako na ravni države kot na ravni posameznika. Zavedati se je potrebno, da je varna hrana rezultat čistega okolja in takšne proizvodnje, ki pripomore k varovanju in ohranjanju zdravja ljudi.

KLJUČNE BESEDE:

varnost živil, primarna proizvodnja, kmetijska politika, kvalitativna analiza

ABSTRACT:

Food safety is an area that has to keep pace with the quick and intense economic development and regulatory requirements. The puropse of the contribution is to show the meaning and understanding of novelties of food safety in the field of primary production. Qualitative and quantitative methods of approach were used. Textual material for the qualitative analysis was acquired from two sources, namely the seven partly structured interviews (with representatives of governmental and non-governmental organizations) and 78 ansvers of one open-ended question in a questionnaire survey relayed to farmers (the recipients of finances form the SKOP programme for the year 2005). In representatives' of governmental and nongovernmental organizations opinion, the key factor for ensuring food safety is the very responsibility. The problem of shared competence is strongly highlighted. For provision of effective and traceable system of good practices with the intention of production of qualitative and safe food, appropriate mechanisms need to be established at the state as well as individual level. One should be aware of the fact that safe food is the result of the clean environment and such a production which contributes to safeguarding and maintaining of the health of people.

KEY WORDS:

food safety, primary production, agricultural politics, qualitative analyses

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Proizvodnja hrane je doživela pozitivne kot tudi negativne spremembe, ki so se odražale tudi skozi spremembe zdravstvenega stanja določene populacije.

Večanje kapacitet proizvodnje hrane močno vpliva na zagotavljanje biološke raznovrstnosti, kar predstavlja resen vpliv na dobro počutje in zdravje ljudi.

Živila so v osnovi kmetijski pridelki, zato se zagotavljanje varnosti in kakovosti začne že na kmetiji.

UVOD

Družbeni razvoj je z svojim vplivom posegel tudi na področje proizvodnje hrane. Uporabljati smo začelei metode dela, ki temeljijo na intezifikaciji. Proizvodnja hrane je doživela pozitivne kot tudi negativne spremembe, ki so se odražale tudi skozi spremembe zdravstvenega stanja določene populacije [1]. Pozitivne strani razvoja se kažejo skozi povečano kapaciteto proizvodnje hrane, izboljšanje materialnega stanja populacije in prehranjenosti otrok. Vse to je omogočil dostop do zdrave in v prehranskem smislu bogate hrane. Dostopnost le te je omogočila intenziteta proizvodnje, transport in odprtje številnih trgovin, ki so potrošnikom omogočile dostop do varne hrane [1]. Vzporedno s pozitivnimi stranmi tovrstnega sistema, pa so se pojavila tudi tveganja za zdravje. V zadnjem obdobju je bilo nekaj zelo odmevnih prehranskih afer (Bovine spongiform encephalopathy (BSE) [5], E. coli, dioksin [3], ostanki pesticidov [15] in drugih škodljivih snovi v hrani [9] ter dvomov v tehnološki razvoj (gensko spremenjena živila [14,11], ionizirajoče sevanje hrane itd.). Večanje kapacitet proizvodnje hrane močno vpliva na zagotavljanje biološke raznovrstnosti, kar predstavlja resen vpliv na dobro počutje in zdravje ljudi. Rezultat intezifikacije je tudi moteno prehajanje snovi v naravi. Prav tako poraba fosilnih virov energije za proizvodnjo hrane in krme za živali ter živinoreja, ki temelji na moderni tehnologiji, povzročata znaten del emisij toplogrednih plinov [12].

Živila so v osnovi kmetijski pridelki, zato se zagotavljanje varnosti in kakovosti začne že na kmetiji. Iz tega izhaja, da prehrana ljudi predstavlja interakcijo z okoljem, pri kateri okolje predstavlja osnovni vir prehransko pomembnih elementov. Istočasno pa okolje predstavlja vir infekcij, toksičnih in farmakoloških agensov [3]. Evropski svet je v sklopu "Agende 2000" sprejel reformo skupne kmetijske politike, katere cilj je znižanje stroškov na tem področju in ohranjanje konkurenčnosti evropskega kmetijstva. Odtlej je bil glavni cilj spodbujati kmetovalce, da bi se preusmerili v proizvodnjo visokokakovostnih proizvodov, in sicer v količinah, ki bi nekoliko bolj sledile povpraševanju in da bi opustili intenzivne metode pridelave, ki so škodljive za okolje [14,11]. Varna in kakovostna živila se mora zagotavljati skozi vso prehrambeno verigo "od polja do mize". Omenjen pristop posega na področje zagotavljanja kvalitete v primarni proizvodnii in na področie sistema celovitega obvladovanja kakovosti (Total quality Manegment) pridelovalcev, živilske industrije, trgovine in na koncu tudi potrošnika (slika 1). S tem namenom se na področju primarne proizvodnje v zadnjem času uveljavljajo dobre prakse, kot je dobra kmetijska praksa [16,17] in dobra higienska praksa. Živila so v osnovi kmetijski pridelki, zato se zagotavljanje varnosti in kakovosti začne že na kmetiji. Prvo in osnovno načelo za doseganje slednjega je dobra higienska praksa (slika 2).





Slika 3:

Opredelitev pojma "dobra kmetijska praksa (DKP)" [2].

Namen prispevka je prikazati pomen in razumevanje področja varnosti živil v primarni proizvodnji. Organizacije, kot sta FAO in Codex Alimentarious Commission, dobro kmetijsko prakso opisujejo kot enega izmed sistemov zagotavljanja kakovosti. Dobra kmetijska praksa v Sloveniji pomeni, da mora kmet izvajati vsaj minimalne tehnološke postopke obdelave, ki bi jih izvajal razumen kmet ob upoštevanju kmetijske zakonodaje (slika 3). To pa pomeni, da bo ravnal gospodarno brez dolgoročnih negativnih vplivov kmetovanja na okolje, zlasti na tla, vodo in rastline [16].

Namen prispevka je prikazati pomen in razumevanje področja varnosti živil v primarni proizvodnji. S tem namenom so v raziskavo bili vključeni pridelovalci v primarni proizvodnji in predstavniki nekaterih vladnih in nevladnih institucij.

METODOLOGIJA IN INŠTRUMENTI

Članek vsebuje delne rezultate raziskave "Higiena živil v primarni proizvodnji", ki je bila izvedena leta 2005. Uporabljeni sta bili kvalitativna in kvantitativna metoda dela. Podatke smo zbirali z anketnim vprašalnikom in delno strukturiranim intervjujem. V začetni fazi je bil opravljen prvi del kvalitativne analize z namenom, da sestavimo vprašalnik. Vzorec za izvedbo ankete je predstavljalo 0,4 % (N = 242) prejemnikov denarnih sredstev iz programa SKOP za leto 2005. Vprašalnik je izpolnilo 37,2 % (N = 90) respondentov. V analizo smo vključili 32,2 % (N = 78) pravilno izpolnjenih anketnih vprašalnikov. Vprašalnik je bil obdelan s kvantitativno – statistično analizo. Temu je sledil drugi del analize, ki je omogočil bolj poglobljen pogled na obravnavano problematiko. V tem sestavku je predstavljen le kvalitativni del raziskave. Tekstno gradivo za kvalitativno analizo je bilo pridobljeno iz dveh virov. Prvi vir je sedem delno strukturiranih intervjujev z predstavniki vladnih in nevladnih institucij. Intervjuji so potekali na sedežu posamezne organizacije. Izvedeni so bili v časovnem obdobju od 24. 8. 2005 do 16. 11. 2005. Intervjuji so bili posneti z diktafonom, povprečni čas trajanja intervjuja je bil 18 minut. V prepisih so bila spremenjena imena in podatki, ki bi lahko vplivali na prepoznavnost intervjuvanca. S tem je bila zajamčena zaupnost osebnih podatkov.

Drugi vir tekstovnega gradiva je predstavljalo 78 odgovorov kmetov na odprto vprašanje "Kaj razumete pod pojmom *"dobra kmetijska praksa"* in zakaj je pomembna". Vprašanje je del anketnega vprašalnika "higiena živil v primarni proizvodnji".

Tekstovno gradivo je bilo analizirano s kvalitativno tematsko analizo vsebine z nekaterimi osnovnimi elementi utemeljene teorije [13, 18]. Poudarek je bil na odkrivanju ponavljajočih se značilnih dogodkov na podlagi katerih lahko odkrili ključni pojmi. Na ta način smo dobili osnovne dogodke in aktivnosti, ki smo jih povezali v vzročno posledično zaporedje, prav tako pa smo upoštevali še rekurzivne odnose, pri katerih se odnos in posledica nenehno izmenjavata [13].

REZULTATI IN RAZPRAVA

Skozi analizo kvalitativnega dela raziskave smo dobili zanimive ugotovitve. Iz izjav predstavnikov vladnih in nevladnih organizacij je bilo razvidno, da so pridelovalci v primarni proizvodnji najbolj odgovorni za zagotavljanje varnosti kmetijskih pridelkov in izdelkov. Ta odgovornost se po njihovem mnenju kaže skozi odgovornost do izobraževanja in izvajanja ukrepov, določenih s strani države oziroma EU zaradi uveljavitve skupne kmetijske politike. Eden izmed osnovnih ciljev slednjega je tudi zagotovitev varnih in kakovostnih kmetijskih pridelkov in izdelkov. Na pomembnost zagotavljanja varnosti in kakovosti živil v primarni proizvodnji opozarja tudi Doménech s sod. [7]. Afere, ki so v zadnjih letih zaznamovale evropski prostor na področju zagotavljanja varnosti živil so narekovale potrebo po strožji zakonodajni ureditvi. Tako je Evropska komisija leta 2000 izdala Belo knjigo o varnosti živil. Na podlagi te je bila oblikovana nova EU zakonodaja, t. i. higienski paket, iz katerega izhaja, da je zagotavljanje varnih in kakovostnih kmetijskih pridelkov in izdelkov pomembna naloga vseh, ki so vključeni v verigo "od vil do vilic" oziroma "od polja do mize". Torej so za varnost živil primarno in zakonsko odgovorni nosilci živilske dejavnosti [8,19], medtem ko pristojni organi države članice preverjajo, ali so pravila pravilno izvedena [8]. Njihova odgovornost je, da dejavnost izvajajo skladno s predpisi in na ta način zagotavljajo, da so živila, ki jih dajo v promet, varna. Vse to pa velja tudi za tiste, ki s svojim primarnim produktom ali predelanim živilom vstopajo prvi v distribucijsko verigo (promet s primarnimi kmetijskimi proizvodi ali živili, ki so predelana v okviru dopolnilne dejavnosti) [19]. Proizvodnja naj temelji na dobri higienski praksi in načelih sistema HACCP, pri proizvodih pa je potrebno izpolnjevati mikrobiološke kriterije in vzdrževati temperaturne omejitve [8].

Iz izjav predstavnikov vladnih in nevladnih organizacij je bilo razvidno, da so pridelovalci v primarni proizvodnji najbolj odgovorni za zagotavljanje varnosti kmetijskih pridelkov in izdelkov.

Afere, ki so v zadnjih letih zaznamovale evropski prostor na področju zagotavljanja varnosti živil so narekovale potrebo po strožji zakonodajni ureditvi.



Slika 4:

Relacije razumevanja dobre kmetijske prakse [2].

O izredno nizki podpori gensko spremenjenim (GS) živilom nakazuje Evrobarometer, kjer je Slovenja med državami Evropske Unije, ki so med najmanj naklonjenimi uporabi GSO. Mnenje in stališče intervjuvancev do gensko spremenjenih organizmov (GSO) je deljeno in sicer variira med popolnim naprotovanjem in nevtralnim stališčem. Stališče intervjuvancev je v veliki meri odvisno od področja njihovega delovanja. Intervjuvanci so mnenja, da v Sloveniji GSO so, in sicer v krmi in živilih, ki pa seveda morajo biti ustrezno označena. Prav tako so izrazili dvom, ki se nanaša na soobstoj GSO in konvencionalnih rastlin. Dvom, ki so ga intervjuvanci izrazili glede soobstoja, so navezali naprej na odgovornost do zagotavljanja varnosti živil, ki jo pripisujejo državi, in sicer v smislu vzpostavitve zakonodaje in usmeritve kmetijske politike. Prav tako so intervjuvanci v povezavi z GSO izpostavili ovire, in sicer deljene pristojnosti, informacije, izobraževanje, možnost izbire, odgovornost in nadzor. O izredno nizki podpori gensko spremenjenim (GS) živilom nakazuje Evrobarometer [10], kjer je Slovenja med državami Evropske Unije, ki so med najmanj naklonjenimi uporabi GSO.

Gospodarski razvoj v zadnjih 20-ih letih je med drugim tudi na področju kmetijstva povzročil korenite spremembe, ki se odražajo skozi zahteve

glede kmetovanja, predelovanja, distribucije kar dodatno nakazuje na kompleksnost in širokost področja zagotavljanja varnosti in kakovosti hrane. Zaradi tega je pomembno vzpostaviti sodelovanje med kmetovalci in strokovnimi službami. Dolžnost stroke in kmetijske politike je, da pridelovalce v primarni proizvodnji seznanjata z najboljšimi možnimi razpoložljivimi načini kmetovanja. Pri tem imajo poleg rednega izobraževanja pomembno vlogo kodeksi dobre kmetijske prakse. Kodeksi dobre kmetijske prakse se med seboj razlikujejo. Imamo svetovalne kodekse, ki seznanjajo kmete s priporočljivimi načini kmetovanja in obvezne, katerih izvajanje je obvezno za vse kmete ali za posamezne skupine [20], kar so izpostavili tudi predstavniki vladnih in nevladnih organizacij. Četrta skupina SKOP-a predstavlja izobraževanje in promocijo, kar pogojuje obvezno sodelovanje kmetovalcev, ki so vključeni v okoljske programe [16].

Rezultati kvalitativne analize odgovorov na odprto vprašanje o razumevanju pojma "dobra kmetijska praksa" se ujemajo z načeli smernic in veljavne regulative. Izkazalo se je, da način razlage omenjenih terminov izhaja iz načina pridelave in se kaže kot rezultat relacij dobljenih skozi analizo odprtega vprašanja "Kaj razumete pod pojmom *"dobra kmetijska praksa"* in zakaj je pomembna" (slika 4).

Pojmovanje se deli na dva nivoja: pozitivno in negativno. Zanimivo je, da so kmetje pri pozitivnem pojmovanju povezali termina DKP in DHP z sonaravnim načinom kmetovanja, upoštevanjem norm, znanjem ter izkušnjami, gospodarnostjo, medtem ko se pri negativnem pojmovanju terminov pojavita intenzifikacija in slab odnos bo okolja. Oba nivoja poimenovanja pa se nanašata na zagotavljanje varnih in kakovostnih kmetijskih pridelkov in izdelkov. To nakazuje na povezovanje izvajanja dobre kmetijske prakse kot nenehno interakcijo med varovanjem okolja in zagotavljanjem varnih in kakovostnih živil. Predstavniki vladnih in nevladnih organizacij kot ključni dejavnik pri zagotavljanju varne in kakovostne hrane poleg izvajanja DKP izpostavljajo še odgovornost, ki se nanaša na pridelovalce (slika 5). Odgovornost v prvi fazi povezujejo z zakonodajo, v drugi fazi pa odgovornost navežejo na osebno raven. Intervjuvanci trdijo, da naj bi osnovna odgovornost po zagotavljanju varne in kakovostne hrane izhajala iz dejstva, da kmetovalci v prvi vrsti zagoDolžnost stroke in kmetijske politike je, da pridelovalce v primarni proizvodnji seznanjata z najboljšimi možnimi razpoložljivimi načini kmetovanja.

Predstavniki vladnih in nevladnih organizacij kot ključni dejavnik pri zagotavljanju varne in kakovostne hrane poleg izvajanja DKP izpostavljajo še odgovornost, ki se nanaša na pridelovalce.







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Slika 6:
Relacije glede odgovornosti.
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V tem primeru lahko govorimo, da gre za t. i. "začaran krog", pri katerem posledica (zagotavljanje varnih in kakovostnih kmetijskih pridelkov in izdelkov) povratno vpliva na vzrok (ovire). tavljajo varno in kakovostno hrano zase in svojo družino. Nadaljnja analiza odprtega vprašanja in delno strukturiranih intervjujev pokaže, da je kot dejavnik za izvajanje DKP in DHP zelo pomembna izobrazba v smislu potrebe po sprotnem izpopolnjevanju. Kmetje izrazijo potrebo po dodatnem izobraževanju, medtem ko predstavniki vladnih in nevladnih organizacij izpostavijo, kako zagotoviti boljši sistem izobraževanja. Intervjuvanci so izobraževanje v tem kontekstu označili kot oviro. Izobraževanje označijo dokaj kritično, za neučinkovito, kar se nanaša tako na strokovne službe kot izvajalce in pridelovalce v primarni proizvodnji, ki se izobraževanj premalo udeležujejo. Kmetje izpostavijo tudi ovire, ki se v tem kontekstu navezujejo na organiziranost in usmerjenost države, medtem ko predstavniki vladne in nevladne organizacije izpostavijo oviro, ki se nanaša na sodelovanje, in sicer v smislu deljenih pristojnosti in s tem izvajanja ukrepov, določenih s strani regulative.

Ugotovitve skozi analizo nakazujejo na dopolnjevanje in ujemanje ovir, ki so bile navedene s strani predstavnikov vladnih in nevladnih organizacij in kmetov. Glede na podane rezultate je vzrok vseh ovir prav slabo sodelovanje in komunikacija.

Tako kmetje kot predstavnikov vladnih in nevladnih organizacij se zavedajo odgovornosti in dolžnosti (slika 6). Izpostavi se namreč seznanjenost s področji svojega delovanja. S strani predstavnikov vladnih in nevladnih organizacij so bile podane številne ovire tako na področju zagotavljanja varnosti in kakovosti hrane kot ravnanja z GSO. Istočasno pa so bile podane tudi rešitve. Vse opisane rešitve izhajajo iz odgovornosti, ki se kaže na ravni države in na ravni posameznika. V tem primeru lahko govorimo, da gre za t. i. "začaran krog", pri katerem posledica (*zagotavljanje varnih in kakovostnih kmetijskih pridelkov in izdelkov*) povratno vpliva na vzrok (*ovire*). Izpostaviti velja še prepoznani pasivni odnos kmetov do sistema zagotavljanja varne in kakovostne hrane, ki ga pogojuje negativizem. Ta negativizem lahko pripišem neza-upanju respondentov v sistem, ki se nanaša na primarno proizvodnjo.

ZAKLJUČEK

Intenzifikacija v kmetijstvu je rezultat industrijskega in kapitalističnega pristopa, ki se je uvedel v tradicionalno dejavnost primarne proizvodnje. Na sistem zagotavljanja varnosti živil, ne glede na razvitost države vplivajo dejavniki, kot so demografske značilnosti, globalizacija trga, spremembe, ki jih je deležna predelovalna industrija, večanje svetovne populacije, večja urbana središča ter vse večje intenzitete pri sami pridelavi hrane in vse večja poraba energije. To dejstvo se kaže tako v izjavah predstavnikov vladnih in nevladnih organizacij kot tudi skozi pregled strokovne literature. Najbolj osnovna pravila zagotavljanja kakovosti in varnosti živil izhajajo iz DHP in se nanašajo na živilsko-prehransko verigo. Z namenom globalne varnosti živil v proizvodnji in prometu živil je postal v državah EU in drugod obvezen sistem HACCP.

Ključni dejavnik za zagotavljanje varnosti živil je po mnenju predstavnikov vladnih in nevladnih organizacij odgovornost. Odgovornost pa povezujejo z odgovornostjo, ki izhaja iz zakonodaje in osebnostne ravni pridelovalcev (*kmetov*). V sistemu zagotavljanja varnosti živil sebi pripisujejo manjšo pomembnost. Močno se izpostavi problematika deljenih pristojnosti.

V zadnjih nekaj letih je bilo podpisanih in predlaganih veliko strateških dokumentov, ki govorijo o varovanju zdravja potrošnika, varni in kakovostni hrani, varovanju okolja, zagotavljanju dobrobita živali itd. Kljub temu, da se veliko govori o izobraževanju in informiranju slehernega potrošnika, ostaja dilema glede "odgovornosti", ki si jo vsak člen živilsko-prehranske verige razlaga po svoje. Razdeljene pristojnosti, iskanje najšibkejšega člena v primeru napak je prav tako problem. Zato bo v prihodnje za zagotavljanje učinkovitega in sledljivega sistema varnosti živil potrebno vzpostaviti celovitejše mehanizme, tako na ravni države kot na ravni vsakega posameznika. Zavedati se je potrebno, da je varna hrana rezultat čistega okolja in takšne proizvodnje, ki pripomore k varovanju in ohranjanju zdravja ljudi. Ključni dejavnik za zagotavljanje varnosti živil je po mnenju predstavnikov vladnih in nevladnih organizacij odgovornost.

Zato bo v prihodnje za zagotavljanje učinkovitega in sledljivega sistema varnosti živil potrebno vzpostaviti celovitejše mehanizme, tako na ravni države kot na ravni vsakega posameznika.

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Odziv potrošnikov na gensko spremenjena živila

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Gensko spremenjeni organizmi so že vstopili v prehrambeno verigo v večini predelov sveta. Veliko mikroorganizmov, predvsem pa bakterije in glive so bile spremenjene z namenom povečanja produkcije proteinov, aminokislin in komercialnih kemikalij. Pionirska dela na področju GSO so povezana z odkritjem naravnega pojava mutageneze, s pomočjo katerega so se razvile različne vrste mikroorganizmov. Takšni mutanti so bili prva biološka orodja za znanost na tem področju, hkrati pa so za živilsko industrijo predstavljali komercialni dobiček. Med prvimi generacijami GSO so bile rastline, ki pa so se pojavljale le na redkih trgih. Z razvojem znanosti, s tem pa tudi genskih informacij široke palete organizmov, se je trg z GSO sprostil. Posledična ekspanzija GSO je prinesla paralelno povečanje skrbi o uporabi genskega inženirstva za živilsko industrijo na eni strani in dvome o morebitnih škodljivih učinkih na človekovo zdravje in okolje.

Kot vse nove tehnologije tudi tehnologija genskega inženiringa sproža določena vprašanja in dileme, ki se nanašajo na človeško in okoljsko varnost, označevanje živil in potrošnikovo izbiro, na pravice intelektualne lastnine, etiko, varnost živil, večanje moči biotehnoloških podjetij in vplive na okolje. Na vsa porajajoča se vprašanja in dileme, ki se pojavljajo danes, še ni odgovora. Zato so in bodo potrebne dodatne raziskave tako na področju biotehnologije kot tudi na področju antropologije.

Težko je na splošno opisati odnos in reakcije potrošnika do gensko spremenjenih živil, ki so na trgu že prisotna. V nekaterih državah Evropske unije so bile že izvedene različne javnomnenjske raziskave o odzivu potrošnika do gensko spremenjenih živil.

Zaradi vedno aktualnejše tematike tako z raziskovalnega kot političnega vprašanja pa je bila težnja, da bi po poenoteni metodologiji ugotovili odnos evropskega potrošnika do gensko spremenjenih živil in njihovo razširjenost v državah Evropske unije.

V ta namen je bila izvedena obsežna raziskava v okviru evropskega projekta: **"Do European consumers buy GMO foods? (Consumerchoice)"**

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Ocena javnega mnenja je bila izvedena s pomočjo kvalitativnih in kvantitativnih metod. Potrošniki so podali svoje mnenje do GSO, vplivih na zdravje, pomenu uradnega nadzora, pripravljenosti nakupa tovrstne hrane in načina informiranja. Project no. 518435, ki ga je vodil profesor Vivian Mosess (King's College London) v obdobju od 2006 do 2008. Celotno poročilo je dostopno v angleškem jeziku na spletni strani: http://www.kcl.ac.uk/schools/biohealth/research/nutritional/consumerchoice/.

Namen projekta, v katerega je bilo vključenih deset evropskih držav (Češka, Estonija, Nizozemska, Poljska, Španija, Velika Britanija, Nemčija, Grčija, Švedska), med katerimi je bila tudi Slovenija, je bil:

- ugotoviti odnos potrošnikov Evropske unije do živil z gensko spremenjenimi organizmi,
- preveriti ponudbo gensko spremenjenih živil v trgovinah in način obvladovanja le-teh, vključno s preverjanjem načina označevanja, cen in lokacije izdelkov ter način informiranja potrošnikov,
- ugotoviti odziv medijev in politični odziv na gensko spremenjena živila,
- zagotoviti verodostojne informacije vsem udeležencem živilsko/prehransko/oskrbovalne verige o odnosu evropskih potrošnikov do gensko spremenjenih živil.

V maloprodaji so bile pregledane deklaracije živil, kje na policah se pojavljajo živila deklarirana kot GSO ali "GSO free", ali so potrošnikom omogočene dodatne informacije in kako so prodajalci informirani o živilih z GSO. Povprečni potrošnik prejme večino informacij s pomočjo različnih medijev javnega obveščanja, zato so bili tematsko analizirani prispevki v dnevnem, tedenskem in regionalnem časopisju. Raziskovalo se je politično ozadje stališč do uporabe GSO med vladnimi in nevladnimi organizacijami. Ocena javnega mnenja je bila izvedena s pomočjo kvalitativnih in kvantitativnih metod. Potrošniki so podali svoje mnenje do GSO, vplivih na zdravje, pomenu uradnega nadzora, pripravljenosti nakupa tovrstne hrane in načina informiranja.

Čeprav se rezultati po posameznih državah v nekaterih poglavjih precej razlikujejo, predvsem glede na tradicijo uporabe GSO (v šestih državah je mogoče kupiti izdelek, ki je deklariran kot GSO) lahko zaključimo, da je predvsem priložnost tista, ki bo odločila ali bo potrošnik kupil živilo deklarirano kot GSO ali ne.

Povzetek glavnih ugotovitev projekta:

- Evropski potrošniki kupujejo gensko spremenjene živilske izdelke.
 Edini omejujoč dejavnih pri tem je razpoložljivost le-teh v trgovinah.
- Večina potrošnikov ni dovolj pozorna na označbe na živilih in tako tudi ne prepoznajo živil z GSO. Njihove reakcije so različne od popolnoma ambivalentnih do zelo zaskrbljenih potrošnikov, ki pa jih begajo dvomi o koristnosti GSO in vplivih na zdravje.
- Potrošniki želijo svobodo izbire, ko nakupujejo hrano in nekateri izmed njih odobravajo živila z GSO, ko jim je ta svoboda ponujena. V več državah so potrošniki izrazili etično zaskrbljenost in pokazali na okoljsko in zdravstveno tveganje.
- Pri opazovanju potrošnikov med nakupom so se le-ti obnašali drugače, kot so rekli, da bi se ob nakupu gensko spremenjene hrane. Ena

tretjina anketiranih se je motila v svojih predvidevanjih o nakupu gensko spremenjene hrane, medtem ko druga tretjina ni vedela kaj je pravzaprav kupila.

 Poljaki, ki živijo v Severni Ameriki in prebivalci Velike Britanije, ki so obiskali Severno Ameriko, so bili večinoma brezbrižni do pojava GSO v hrani, ko so bili v ZDA in Kanadi.

Rezultati kažejo, da v slovenskem prostoru prevladuje negativno stališče do pridelave, prodaje in nakupa gensko spremenjenih živil. Potrošniki in trgovci so nezadostno informirani o tovrstnih izdelkih. Vladne in nevladne organizacije spodbujajo predvsem trajnostni razvoj, tradicionalno in ekološko pridelavo hrane. Do gensko spremenjenih živil gojijo odklonilen odnos. V medijih se redko pojavljajo članki z obravnavano vsebino. Glede tematskih skupin prevladuje politični vidik, ki se prepleta z zdravstvenim in okoljsko ekološkim vidikom. Tiskani mediji imajo veliko moč pri informiranju ljudi in oblikovanju javnega mnenja. Strokovno znanje poročevalca je zelo pomembno, saj lahko v nasprotnem primeru prihaja do posploševanja in podajanja neverodostojnih informacij, kar se odraža na odzivu javnosti do uporabe GSO v živilih. Slovenski potrošniki glede vira in verodostojnosti informacij veliko bolj zaupajo strokovnja-kom in znanstvenikom kakor medijem.

Tudi v prihodnje je potrebno spremljati trende javnega mnenja do GSO in zagotoviti primerne in učinkovite načine informiranja javnosti. Le dobro informiran in osveščen potrošnik lahko oblikuje realno mnenje o raziskovani tematiki. Zato sta vrsta in vir informacij pomembna elementa pri oblikovanju odnosa do novosti, še posebej tistih, ki so povezani z zdravjem ljudi. Rezultati kažejo, da v slovenskem prostoru prevladuje negativno stališče do pridelave, prodaje in nakupa gensko spremenjenih živil.

Tudi v prihodnje je potrebno spremljati trende javnega mnenja do GSO in zagotoviti primerne in učinkovite načine informiranja javnosti. Le dobro informiran in osveščen potrošnik lahko oblikuje realno mnenje o raziskovani tematiki.

INSTRUCTIONS FOR AUTHORS

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- [2] American college of physicians. Clinical Ecology. An Int Med 1989; 111:168-78.
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- [5] Evaluation of the European Agency for Safety and Health at Work: http://osha.europa.eu/publications/ other/20010315/index_1.htm (20. 12. 2006).

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