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1. PRIMARNA PROIZVODNJA I PRERADA HRANE  
PRIMARY PRODUCTION AND PROCESSING OF FOOD



## Comparative overview of milk composition in different animal species

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### Abstract

Milk, an essential source of nourishment, varies significantly in its composition among different species and even between breeds of the same species. Chemically, milk is a complex physiological fluid containing over 100,000 distinct molecules and chemical compounds, the concentration of which depends on the type of milk. Regardless of the species, milk is composed of the following main components: fat, protein, lactose, and water. Cow's milk is the most commonly consumed, accounting for 85% of worldwide milk production. Buffaloes contribute 11% of global milk production, followed by other species with lower production levels. This paper aims to compare the composition of milk from various animal species and highlight their respective benefits. The composition of the compared milk species differs significantly in calorie content, with buffalo milk having noticeably higher calorie content compared to others, while donkey milk has the lowest. Regarding protein content, buffalo and cow milk have the highest levels, while donkey milk has the lowest. The lactose content is highest in milk from donkeys and lowest in milk from buffaloes. The greatest fat content is found in buffalo milk, while the lowest fat content is found in milk from donkeys and camels.

*Keywords: milk composition, buffalo milk, doe milk, donkey milk, camel milk*

### Introduction

Milk is a natural secretion of the mammary gland, obtained through regular and continuous milking of one or more healthy lactating animals that are properly fed and kept, with nothing added or removed. The composition of milk varies among species and even between breeds of the same species, influenced by factors such as lactation stage, diet, season, water availability, and other variables (Togo, Dufour, Lagier, 2019). Milk comprises water-soluble vitamins, proteins (casein and whey), carbohydrates (lactose), fats (phospholipids, short-chain saturated fatty acids, long-chain mono - and

polyunsaturated fatty acids), and mineral salts (calcium, phosphorus, iron, potassium, magnesium, selenium, etc.). Given the diversity of animals, it is essential to highlight the benefits and differences between the various types of milk. This article will focus on the differences in the composition of milk from donkeys, buffalo, camels and doe.

### ***Experimental***

Articles and studies were searched in Google Scholar, Pubmed and the Archives of Veterinary Science.

### ***Results and Discussion***

#### ***Water***

All components of milk (dry matter) are either dissolved or suspended in water. The proportion of free water in milk is determined by the species and breed of the milking animal and averages between 85.4% and 89.2%. A small portion of water (<2%) in milk constitutes bound (hydrated) water in the composition of proteins, salts, and lactose (Chandan, White, Kilara, 2006.)

#### ***Milk Protein***

Proteins are essential for key physiological processes in both humans and animals, including tissue growth and repair, enzyme and hormone regulation, immune function, and cellular transport and storage. The two primary proteins found in milk are casein and whey protein. Camel milk (77.50%) and cow milk (77.23%) have the highest casein contents. Buffalo milk contains approximately 68.93% casein, while donkey milk contains 45%. Among the three main types of casein—alpha ( $\alpha$ ), beta ( $\beta$ ), and kappa ( $\kappa$ )—the alpha fraction exhibits polymorphism and is denoted as  $\alpha$ s1 and  $\alpha$ s2 casein. The  $\alpha$ s1 fraction is prevalent in cow's and buffalo's milk, and it is a key factor in causing milk protein allergies (MPA) in humans (Hassanin, Osman, Atallah, 2022). Similar to humans, animals can also experience allergic reactions to proteins in milk. Reactions can manifest as gastrointestinal issues (diarrhea, vomiting), skin problems (itching, rashes), respiratory issues (coughing, wheezing), and even more severe systemic reactions. Whey protein, a significant component of milk, is mainly made up of  $\alpha$ -lactalbumin and  $\beta$ -lactoglobulin.  $\alpha$ -Lactalbumin is an essential protein involved in lactose synthesis, serving a key role in the milk production process. It is present in every species within the Mammalia group, while  $\beta$ -lactoglobulin is predominantly found in ruminant milk (Hettinga and Bijl, 2022). Other whey proteins include immunoglobulins, serum proteins, enzymes, hormones, and growth factors.  $\beta$ -lactoglobulin is another source of milk protein allergy; however, its lower levels in other animals, such as doe's, contribute to making their milk less allergenic than that of cows



(Yasmin, Iqbal, Liaqat, et al., 2020). Camel milk lacks  $\beta$ -lactoglobulin, providing it with an anti-allergenic advantage over other types of milk (Hassen, 2020). Camel milk is further distinguished by the presence of immunoglobulins and antimicrobial compounds, including lysozyme, lactoferrin, and insulin equivalent to human insulin. This composition makes it a suitable choice for diabetic individuals, as they can consume it to manage their blood sugar levels (Mohammadabadi, 2020).

### *Milk Fats*

Lipids or fats, present in the form of globules, consist of a triglyceride core surrounded by a natural biological membrane that contains cholesterol, enzymes, glycoproteins, and glycolipids. This composition significantly enhances the nutritional value of milk (Lopez, 2020). The diameter of fat globules varies among different species, with buffalo having the largest diameter, while camel milk has the smallest, which contributes to the easier digestibility of camel milk. Cow milk is reported to have the highest fat content, while camel milk has lower fat content, and donkey milk has the lowest (Table 1.) (Maryam, Maqsood, Asia, 2023).

### *Cholesterol*

Cholesterol is a natural component of milk, albeit at relatively low levels, ranging between 0.01 and 0.02% depending on the animal species. Among them, doe milk exhibits the lowest levels, while buffalo milk has the highest. The milk fat globule membrane contains cholesterol, constituting approximately 95% of the sterols found in milk. In terms of cholesterol content, camel milk has the highest, followed by cow milk, with buffalo milk having the lowest (Table 1.) (Maryam et al., 2023).

### *Milk Sugar*

Lactose, commonly known as milk sugar, is a disaccharide composed of glucose and galactose. It is present in all species with slight variations among them, being highest in donkey milk. While the lactose content is high in donkey (Table 1.), doe and cow milk, buffalo and camel milk are more suitable for individuals with lactose intolerance (Suri, Kumar, Prasad., 2019). Symptoms of lactose intolerance, including bloating, gas, and diarrhea, typically indicate a lack of lactase in the body (Silberman and Jin, 2019). A high lactose content promotes the processes of osteogenesis, facilitates intestinal absorption of calcium and phosphorus, and influences the accumulation of minerals in the bone structure, which contributes to the prevention of osteoporosis (Maryam et al., 2023.).

### *Milk Minerals*

Milk is a valuable source of essential nutrients and serves as a reservoir of calcium and phosphorus, which, in conjunction with casein, facilitates milk digestion within the body (Guantario, Giribaldi, Devirgiliis, 2020). Additionally, milk contains various minerals such as salt, potassium, chloride, iodine, magnesium, and iron. The presence of lactoferrin has a significant impact on the iron content of milk. Camel milk stands out for its richness in iron, zinc, and copper. (Kandhro, Kazi, Affridif, 2022).

**Table 1.** Physical-chemical components comparison: concentrations in 100g of milk across different animal species (Maryam et al., 2023.)

<b>Animals</b>	<b>Cow</b>	<b>Buffalo</b>	<b>Doe</b>	<b>Donkey</b>	<b>Camel</b>
<b>Calories (cal/100g)</b>	66	100	70	40.5-49	49
<b>Water (%)</b>	86	83	80.5	90.4	85
<b>Proteins (g)</b>	8	9	3.6	1.3-1.8	2.5
<b>Casein (g)</b>	2.6	4.0	2.81	0.6-1.0	2.21
<b>Fats</b>	3.9	8	3.5	0.3-1.8	2.9-3.7
<b>Cholesterol (mg)</b>	25.6	6.5	16.6	8.6	37.1
<b>Sugar (g)</b>	5	4.4	5.1	5.8-7.4	4.8
<b>Minerals (ash%)</b>	0,64	0.88	1.04	0.36	0.62
<b>Calcium (mg)</b>	122	112	132	68.9	116
<b>Phosphorus (mg)</b>	119	99	97.7	41	87.4

### *Milk Vitamins*

Vitamins, encompassing both water-soluble (B and C) and fat-soluble (A, D, E, and K) varieties, constitute another crucial component of milk. Doe milk, in particular, stands out for their exceptional richness in vitamin A. The beta-carotene, a precursor or inactive form of vitamin A, present in doe milk, converts to retinol, contributing to the milk's white coloration.

### *Conclusion*

This article highlights the nutritional diversity of milk from donkeys, buffalo, camels, and doe, showcasing their unique benefits. The composition of milk varies among species and even between breeds of the same species. Donkey milk, with its high levels of essential nutrients, supports bone health and overall well-being, and is considered the easiest to digest among milks,

characterized by an optimal ratio of casein to whey proteins. The enzymes contained in donkey milk have bactericidal properties. Recently, it has been used in cosmetics due to its high concentration of polyunsaturated fatty acids, and the proteins in the milk are regarded as active natural ingredients for hydration and prevention of skin aging. Buffalo milk has a higher protein content than cow's milk, which is essential for muscle growth, repair, and the production of enzymes and hormones. It is rich in vitamin D and minerals, supporting bone health, improving gut health and nutrient absorption, and strengthening the immune system. Its low cholesterol content and high levels of unsaturated fats promote heart health. Additionally, it has a favorable calcium-to-phosphorus ratio (1:80) and lower levels of sodium and potassium, making it a good nutritional supplement for children. Camel milk, rich in vitamins and minerals, especially calcium and potassium, offers numerous health benefits, including suitability for individuals who are lactose intolerant, thanks to its diverse protein profile and anti-diarrheal properties. The presence of antioxidants in camel milk provides cellular protection against oxidative damage, which may reduce the risk of diseases such as cancer, diabetes, and cardiovascular disorders. It is known for its similarity to human milk and is used in some regions of the world as a therapeutic approach for individuals with autism. Doe milk is rich in proteins, calcium, phosphorus, potassium, and vitamins A and D, which play a vital role in promoting bone health and supporting immune function. The protein structure in doe milk and donkey milk differs from that in cow's milk, which may reduce the likelihood of triggering allergic reactions in people, making them suitable alternatives to cow's milk.

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## Komparativni prikaz sastava mlijeka kod različitih vrsta životinja

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### Sažetak

Mlijeko, kao esencijalni izvor ishrane značajno se razlikuje u sastavu među različitim vrstama, pa čak i između pasmina iste vrste. Hemijski, mlijeko je složena fiziološka tečnost koja sadrži više od 100.000 različitih molekula i hemijskih jedinjenja čija koncentracija ovisi o vrsti mlijeka. Bez obzira na vrstu, mlijeko se sastoji od sljedećih glavnih komponenti: masti, proteina, laktoze i vode. Najčešće se konzumira kravlje mlijeko koje čini 85% ukupne proizvodnje mlijeka u svijetu. Bivolice doprinose 11% globalne proizvodnje mlijeka, a slijede ih druge vrste s nižom razinom proizvodnje. Cilj ovog rada je prikazati komparaciju sastava mlijeka kod različitih vrsta životinja i prikazati njihove benefite. Sastav upoređivanih vrsta mlijeka značajno se razlikuje u kalorijskom sadržaju, pri čemu bivolje mlijeko ima primjetno viši kalorijski sadržaj u poređenju s ostalima, dok magareće mlijeko ima najniži. Što se tiče sadržaja proteina, bivolje i kravlje mlijeko imaju najviše nivoe, dok magareće mlijeko ima najniži. Sadržaj laktoze je najviši u mlijeku od magarica, a najniži u mlijeku od bivolica. Najveći sadržaj masti se nalazi u bivoljem mlijeku, dok se najniži sadržaj masti nalazi u mlijeku od magarica i kamila.

*Ključne riječi: sastav mlijeka, bivolje mlijeko, srneće mlijeko, magareće mlijeko, devino mlijeko*

1-O-2

## The impact of cold stress on physiological parameters in Romanov sheep

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### Abstract

The production and reproductive capabilities of domestic animals are directly influenced by the climatic conditions of their environment. Since the mid-20th century, increasingly frequent and intense climate changes have brought on discussions about their causes and effects on both humans and animals. This study aimed to determine how extreme climatic circumstances, specifically very low temperatures, impact the overall condition of Romanov sheep. The research was conducted on the Hacienda de la Vida farm during January and February 2024. The primary physiological parameters (rectal temperature, heart rate, and respiratory rate) were assessed in twenty sheep, initially in harsh cold conditions (-6.8 °C) and subsequently in thermoneutral conditions (10.9 °C). Based on climatic data from the Federal Hydro-Meteorological Institute of Bosnia and Herzegovina, the thermal index (THI) was calculated, confirming that the sheep were exposed to cold stress during the low-temperature measurements. Statistically significant differences, between the two assessments were found only in respiratory rate. These results indicate a minor impact of low temperatures on physiological parameters, highlighting the resilience and adaptability of this sheep breed to harsh climatic conditions.

*Keywords: climate change, physiological parameters, cold stress, Romanov sheep*

### ***Introduction***

Climate and weather have a substantial impact on livestock growth and production. Combined meteorological elements that operate in average conditions in the long term are expressed as climate, while short-term oscillations are regarded as weather (Bianca, 1976). Since the middle of the twentieth century, changes in the Earth's climate have been more and more prominent, and the main cause is considered to be an increase in the

production of greenhouse gases which in turn leads to the warming of the atmosphere. The United Nations Framework Convention on Climate Change (UNFCCC) defines climate change as "a change of climate which is attributed directly or indirectly to human activity that alters the composition of the global atmosphere and which is in addition to natural climate variability observed over comparable time periods" (Bodansky, 1993).

Climate change manifests through the shifts in meteorological variables (air temperature and humidity, intensity of solar radiation, wind speed, quantity of precipitation), and scientists predict that they will consequently affect animal well-being, production, and reproduction through the rise in competition for natural resources, a decrease of quality and amount of fodder, disease occurrence, loss of biodiversity, and the occurrence of thermal stress (Thorton, 2010).

Thermal stress occurs when the ambient temperature exceeds the upper or lower critical points of the internal temperature of domestic animals (Collier, Baumgard, Zimbelman, et al., 2019). Meteorological elements can affect the organism individually or synergistically in different combinations; e.g. low ambient temperature combined with rapid air movement (the feeling of cold) or high ambient temperature combined with high air humidity and solar radiation (the feeling of warmth) (Bianca, 1976).

In correlation with its physiological state, each species, breed, or category of animal, has its own comfort zone, in which energy consumption is minimal, constant, and independent of the ambient temperature (Nardone, Ronchi, Lacetera, et al., 2006). Outside this zone, the animal invests additional energy in order to maintain the homeothermy of the organism and prevent the occurrence of thermal stress, thus less energy is available for production processes.

Exposure to cold during the winter season is considered an important stressor for ruminants and is reflected in behavioral changes, particularly in food consumption, and changes in physiological and metabolic functions, as a result, the quantity and quality of production and reproduction are altered (Nardone et al., 2006; Kang, Lee, Piao, et al., 2016). Research has shown that cold stress, experienced at temperatures below 0 °C to a maximum of 3 °C, leads to a 25% decrease in milk yield in sheep (Peana, Dimauro, Carta, et al., 2007).

For many years, primary physiological and thermoregulatory parameters (rectal temperature, skin temperature, respiration, perspiration, heart rate) were used as very reliable factors in assessing the adaptive physiology of livestock. While rectal temperature provides insight into the distinct thermal state, other thermoregulatory variables (skin temperature, breathing,

sweating, heart rate) reflect the animal's thermolytic state (Dalcin, Fischer, Daltro, et al., 2016; Minka and Ayo, 2016).

The study was conducted on twenty Romanov sheep, a sheep breed of combined production characteristics originating from Russia. They are distinguished by seasonal polyestricity, high fertility and large litters, early maturity, excellent wool quality, and high resistance to harsh weather conditions (Marzanov, Devrishov, Ozerov, et al., 2023).

The goal of the research was to explore the previously mentioned characteristic resistance to low temperatures. Namely, to determine in which way, if at all, cold stress impacts the change in primary physiological parameters (rectal temperature, breathing, and heart rate) in these sheep.

## ***Experimental***

### *Climatic Data of the Research Site*

Climatic data for the researched area were provided by the Federal Hydrometeorological Institute of Bosnia and Herzegovina (FHMZBIH). The area of Kiseljak, according to FHMZBIH, is characterized by a mountainous continental climate, with micro-local variations affected by river valleys and altitude. The temperature-humidity index (THI), which indicates the combined effect of temperature and air humidity on the animal, was calculated using the climatic data in order to determine whether sheep were exposed to any thermal stress. The following formula by Marai, El-Darawany, Fadiel, et al. (2007) was used for the calculations:

$$\text{THI} = T - \{(0.31 - 0.31 \times \text{RH})(T - 14.4)\}$$

The THI threshold value for cold stress is 9.8, where anything below this value confirms cold stress conditions (Carabaño, Pineda-Quiroga, Ugarte, et al., 2021). Climatic data of the researched area and THI are presented in Table 1, where the presence of cold stress is confirmed on the first day of measurement but is absent during the second measurement in thermoneutral conditions.



**Table 1.** Climatic parameters of the area at the time of the research

Climate parameters	Days of research			
	Three days before the 1st measurement (10.1.2024.)	On the day of the 1st measurement (13.1.2024.)	Three days before the 2nd measurement (12.2.2024.)	On the day of the 2nd measurement (15.2.2024.)
Air temperature	-1.88 °C (daily average)	-6.8 °C (at the time of measurement)	6.8 °C (daily average)	10.9 °C (at the time of measurement)
Relative air humidity	88%	96%	92%	82%
Wind speed	1.1 m/s	1.1 m/s	1.68 m/s	1.31 m/s
Amount of precipitation	0 mm	0 mm	3.06 mm	0 mm
THI	-1.27	-6.54	6.99	11.09

### *Animals and Experimental Design*

The research was conducted in January and February of 2024, at the Hacienda de la Vida farm in the village of Podastinje near Kiseljak (GPS coordinates: 43.9557394, 18.0823022). This research was carried out in two phases: the first measurement of the physiological parameters was carried out on 13.1.2024. in the early morning hours - low-temperature conditions, and the second measurement on 15.2.2024. in the afternoon - thermoneutral conditions. The same 20 sheep were used both times, therefore every measurement was taken twice per sheep.

Sheep selection was based on breed, age, average body weight, and pregnancy status. The physiological parameters were tested on female Romanov sheep, aged 6-8 months, weighing an average of 41 kg. The weight of the examined sheep was measured with an electronic livestock scale (Kerbl GmbH, Germany). The animals participating in the experiment have never been sheared and were not pregnant at the time of research. Following the practices of intensive farming, the sheep were kept indoors, fed total mixed ration (TMR) meals, and drank water from the farm's well.

The following physiological parameters were examined in the study: rectal temperature, respiratory rate, heart rate, and shivering index. Rectal temperature was determined using a digital thermometer (Microlife AG, Switzerland) while respiratory and heart rate were measured via a single-

sided stethoscope (Littmann, USA). Namely, the heart rate was measured on the left side of the chest at the height of the elbow joint, while the respiratory rate was measured on the chest along with the observation of abdominal muscle movement on the rib cage. The shivering index, determined by observing the animal while placing a hand on its body, was evaluated on a scale from 1 to 5, where 1 represented the absence of shivering, and 5 representing extreme shaking and noticeable movement of the entire body (Nel, Cloete, Kruger, et al., 2021). Descriptions of shivering index values are shown in Table 2.

**Table 2.** Descriptions/meanings of tremor index values (Nel et al. 2021)

Shivering index	Description/meaning of the index
1	Shivering is not present
2	Slight tremors, hardly noticeable when the hand is placed on the body
3	The animal shivers considerably, with a distinct feeling of shivering when the hand is placed on the body
4	Pronounced shivering, noticeable without touching the body
5	Extreme shaking, moving the whole body

### *Statistical analysis*

Statistical data processing was performed in Microsoft Excel. All data on physiological parameters were analyzed using the method of descriptive statistics to get a better insight into the obtained results. Afterward, a t-test for dependent samples was performed to determine the existence of statistically significant differences between the two measurements. The statistical level of significance used in the research is  $p < 0.05$ .

### *Results and Discussion*

Table 3. shows the results of descriptive statistics for the physiological parameter of rectal temperature in the examined sheep and the results of the t-test for dependent samples. The mean value of the rectal temperature measured in low-temperature conditions was 39.66 °C and in thermoneutral conditions 39.51 °C. No statistically significant difference in the values of the investigated parameter was found between these two measurements.

**Table 3.** Descriptive statistics and t-test of rectal temperature values in Romanov sheep breed (n=20) in low-temperature and thermoneutral conditions

Parameters	Rectal temperature (°C) in low-temperature conditions	Rectal temperature (°C) in thermoneutral conditions
Mean	39.66	39.51
Standard Error	0.068	0.069
Median	39.75	39.55
Standard Deviation	0.307	0.313
Minimum	39	30
Maximum	40.1	40
Degrees of freedom (df)	19	
T test	1.344	
P(T<=t) two-tail	0.195	
T critical two-tail	2.093	

The mean value of heart rate in low-temperature conditions was 133.5 beats per minute, and in thermoneutral conditions 121.3 beats per minute, with no statistically significant differences between the two measurements. Other descriptive statistical data are presented in Table 4.

**Table 4.** Descriptive statistics and t-test of heart rate values in Romanov breed sheep (n=20) in low temperature and thermoneutral conditions

Parameters	Heart rate (beats/minute) in low-temperature conditions	Heart rate (beats/minute) in thermoneutral conditions
Mean	133.5	121.3
Standard Error	5.917	6.249
Median	133	121
Standard Deviation	26.46	27.949
Minimum	92	78
Maximum	176	188
Degrees of freedom (df)	19	
T test	1.619	
P(T<=t) two-tail	0.122	
T critical two-tail	2.093	

Table 5. shows the results of variations in breathing frequency between thermoneutral and low-temperature conditions. A statistically significant difference ( $p < 0.00036$ ) was found in the mean values of this parameter between measurements at low temperatures and thermoneutral conditions. In conditions of low temperatures, the mean value of breathing frequency is 71.9 breaths per minute, while in thermoneutral conditions the mean value is 102.3 breaths per minute.

**Table 5.** Descriptive statistics and t-test values of breathing frequency in Romanov sheep breed (n=20) in low temperature and thermoneutral conditions

Parameters	Breathing frequency (breaths/minute) in low-temperature conditions	Breathing frequency (breaths/minute) in thermoneutral conditions
Mean	71.9	102.3
Standard Error	4.214	5.316
Median	71	100
Standard Deviation	18.845	23.773
Minimum	40	58
Maximum	96	144
Degrees of freedom (df)	19	
T test	-4.331	
P(T<=t) two-tail	0.00036	
T critical two-tail	2.093	

The shivering index for all sheep in both the low-temperature and thermoneutral conditions was determined as 1 (shivering was not present). THI acts as a reliable indicator of thermal stress in sheep, where higher THI is directly related to increased values of physiological parameters (rectal temperature, heart rate, respiratory rate) and vice versa (Rathwa, Vasava, Pathan, et al., 2017). THI showed that, when conducting measurements in low-temperature conditions, the sheep were indeed exposed to cold stress, while in thermoneutral conditions they were not (Table 1).

Homeothermy is a key factor for the development of all physiological, reproductive, and productive functions of the organism. Deviation from the referent physiological value of rectal temperature ( $38.5^{\circ}\text{C} - 40^{\circ}\text{C}$ ) (Jackson, Cockcroft, Elmhurst, 2002) is considered an adequate indicator of the presence of thermal stress in sheep. The difference between rectal temperature values measured in thermoneutral conditions and low-

temperature conditions, during the conduct of this research, proved to be statistically negligible (Table 3). In other words, research has shown that Romanov sheep manage to maintain a constant body temperature, even when exposed to cold stress.

Heart rate is an essential physiological parameter whose variations can be influenced by age, vigor, blood pressure, and respective hormone concentration, but also various external factors such as exposure to stress or extreme temperatures, which can lead to tachycardia or bradycardia. The referent values of heart rate in sheep are 70 to 90 beats per minute (Jackson et al., 2002), but due to many factors affecting this parameter, certain deviations often occur. During the research, it was determined that there were no statistically significant differences in heart rate values between measurements in low-temperature and thermoneutral conditions, although the values during both measurements were elevated when compared to physiological limits (Table 4). The reason for this is most likely stress brought upon by the handling of the sheep in the pursuit of measuring the parameters. When exposed to cold, heart rate increases and vasoconstriction of the peripheral blood vessels ensues in order to minimize and prevent heat loss (Manou-Stathopoulou, Goodwin, Patterson, et al., 2015). During the measurements in low-temperature conditions (when the sheep were under the influence of cold stress), higher heart rate values were recorded compared to the values measured in thermoneutral conditions, although the difference did not prove to be statistically significant (which could have contributed to the relatively high-value standard deviations). Such results suggest the hypothesized tolerance of the tested breed of sheep to harsh temperatures.

The respiratory rate is considered the most sensitive of the primary physiological parameters and is an important line of homeostatic defense, having the ability to either prevent heat loss during cold stress (slowing of respiration) or increase it during cold stress (accelerating respiration) (Habibu, Yaqub, Dzenda, et al., 2019). The normal resting respiratory rate in sheep is 20 to 30 respirations per minute (Jackson et al., 2002). During the measurement, probably due to stress, the breathing frequency was significantly elevated compared to the reference values. However, statistically significant differences in breathing frequency were observed between measurements in thermoneutral and low-temperature conditions (Table 5). The mean value of this parameter when sheep were exposed to cold stress is significantly lower than the mean value in thermoneutral conditions. The reason for this could be that homeothermic animals initially react to cold stress by increasing thermoregulatory mechanisms, such as heart and respiratory rate, in order to avoid an unwanted increase in body temperature. Similar observations were made by Maurya, Sejian, Naqvi

(2012) who investigated the effects of cold stress on Malpura lambs. Slee, Alexander, Bradley, et al. (1991) and Muller and McCutcheon (1991) also reported the same findings while working on newborn Merino lambs.

### ***Conclusion***

Selecting a suitable breed, taking into account the geographical and climatic characteristics of the area, is crucial for the ultimate success and productivity of cultivation. Farmers' lack of education and insufficient understanding of different breeds of sheep and their characteristics represent a significant problem in the selection process. This study established that cold stress does not significantly affect the change of physiological parameters in Romanov sheep, thus confirming the resistance and adaptability of this breed to harsh climatic conditions.

Consequently, the results of this study could provide some insight and result in the popularization of the Romanov sheep in the areas of continental and mountainous climates that are also present in Bosnia and Herzegovina. Further research, which would include the influence of cold stress on biochemical and hematological parameters, as well as on reproductive and production abilities, could additionally ensure these findings.

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## Utjecaj hladnog stresa na fiziološke parametre kod ovaca pasmine Romanovska

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### Sažetak

Proizvodne i reproduktivne sposobnosti domaćih životinja pod direktnim su utjecajem klimatskih uslova sredine u kojoj borave. Od sredine dvadesetog stoljeća, sve češće i intenzivnije promjene u Zemljinoj klimi izazivaju rasprave o njihovim uzrocima i efektima, kako na ljude, tako i na životinje. Ovo istraživanje ispituje da li i na koji način ekstremni klimatski uslovi, u ovom slučaju uslovi niskih temperatura, utječu na opće stanje ovaca pasmine Romanovska. Istraživanje je sprovedeno na farmi Hacienda de la Vida tokom janura i februara 2024. godine. Osnovni fiziološki parametri (rektalna temperatura, frekvencija rada srca i disanja), kao pokazatelji općeg stanja organizma, ispitivani su na ukupno dvadeset ovaca i to prvo u uslovima niskih temperatura (-6,8 °C), a zatim u termoneutralnim uslovima (10,9 °C). Na osnovu klimatskih podataka, preuzetih iz Federalnog hidrometeorološkog zavoda Bosne i Hercegovine (FHMZBIH), izračunat je toplotni indeks (THI), kojim se potvrđuje da su ovce tokom merenja u uslovima niskih temperatura bile izložene hladnom stresu. Ipak statistički signifikantne razlike, između termoneutralnih uslova i uslova hladnog stresa, uočene su jedino na parametru frekvencije disanja. Ovakvim rezultatima pokazan je neznatna utjecaj niskih temperatura na promjenu vrijednosti fizioloških parametara, čime se ukazuje na izdržljivost i adaptiranost ove pasmine ovaca na uzgoj u oštrim klimatskim uslovima.

*Ključne riječi: klimatske promjene, fiziološki parametri, hladni stres, Romanovska ovca*

1-O-3

## **Analysis of physiological parameters in Bosnian – Herzegovinian Pramenka sheep as a response to cold stress**

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### **Abstract**

Global warming poses a challenge for animals like sheep, potentially impacting their health and productivity. Sheep, such as the indigenous breed – Pramenka in Bosnia and Herzegovina, demonstrate some adaptability to extreme climatic conditions. However, there is a lack of detailed understanding of their adaptation to cold stress. This study analyzes physiological parameters to understand their behavior and responses to cold stress compared to thermoneutral conditions. The results show statistically significant changes in parameters such as body temperature, respiratory rate, and shivering index, indicating adaptive mechanisms in the studied individuals in cold conditions. This research contributes to a better understanding of sheep adaptation to cold stress and has important implications for improving their welfare and productivity in changing climatic conditions.

*Keywords: sheep, cold stress, physiological parameters, adaptation, climate change*

### ***Introduction***

Global warming presents a significant risk to animal and human health, as it affects the natural world in numerous ways. According to the National Oceanic and Atmospheric Administration (NOAA), 2022 was the sixth warmest year since 1880, with a temperature of 0.86°C above the 20th-century average of 13.9°C, which led to record-breaking high temperatures across nearly all continents and triggered extreme weather events (e.g., heat waves, droughts, severe wildfires, and tropical storms) worldwide (Astuti, Fajardo, Wanjala, et al., 2023).

Heat stress is one of the most damaging factors contributing to reductions in growth, production, reproduction, milk quantity, quality, and natural immunity, making animals more vulnerable to diseases and even death (Berihulay, Abied, He, et al., 2019). Despite this, small ruminants have adapted to these extreme environments and exhibit unique adaptive traits based on behavioral, morphological, physiological, and primarily genetic principles.

However, complete information on how these animals can adapt and survive in new and changing environments is still lacking (Berihulay et al., 2019). Sheep, in general, demonstrate high adaptability and resilience to harsh environmental conditions. However, physiological and cognitive changes in response to warm or cold environments impact the productivity of small livestock (Li, Yang, Jenna, et al., 2018).

The thermoneutral zone for sheep typically ranges between 12 and 27°C (Marai, El – Darawany, Fadiel, et al., 2007; Seijan, Kumar, Naqvi, 2017). Activating compensatory and adaptive mechanisms allows sheep to endure temperatures above or below their thermoneutral zone without severely compromising their productivity (Pérez, Macías Cruz, Avendaño Reyes, et al., 2020). In today's context of global climate change, understanding the impact of environmental changes on organisms and the adaptive mechanisms they possess to cope with these changes is essential (Henry, Eckard, Beauchemin, 2018).

Most research on farm animals has focused on the adverse effects of heat stress, often overlooking cold stress. However, exposure to cold temperatures is one of the animals' most significant stress factors during winter. The indigenous sheep breed in Bosnia and Herzegovina is the Pramenka, with various subspecies originating from different geographic and macroclimatic conditions. Sheep, which serve multiple purposes (meat, wool, and milk), play an essential role in the agricultural economy of Bosnia and Herzegovina (Hrković – Porobija, Hodžić, Ohran, et al., 2018).

This research aimed to assess the resilience of the Bosnian Pramenka sheep to cold stress by analyzing several basic physiological parameters and comparing them with measurements taken under thermoneutral conditions.

### ***Experimental***

The research was conducted on a family estate in Stupni Do, Vareš municipality, Zenica-Doboj Canton, Federation of Bosnia and Herzegovina, Bosnia and Herzegovina (44°07'20.1"N 18°19'44.6"E, elevation 1000 m).

The analysis included a flock of 25 sheep of the indigenous Bosnian breed – Pramenka, with 18 females and seven males.

The analysis was conducted twice, under cold conditions (January 2024) and thermoneutral conditions (February 2024). It was determined that all selected animals were healthy, in lactation, and, on average, 2.5 years old. All female individuals were pregnant at the time of the research. The breeding method is semi-open; the sheep spend most of the year grazing, except from November to March when they are housed.

The animals roam freely over large expanses, mostly covered with grass, so their diet is based exclusively on grazing. During the colder part of the year, the diet is based on hay and small amounts of concentrated nutrients, and they are provided with water 2 to 3 times a day.

The purpose of breeding, or the flock's production aim, is primarily for personal use – meat and wool. All individuals were sheared in May 2023, meaning eight or nine months before the research was conducted. The body weight of the sheep ranged from 50 to 60 kilograms, with an average of 55.8 kg, showing normal condition with no fat or thin sheep. The sheep are not used for milking, so there is no data on milk production.

Meteorological data for the estate location was obtained from the Federal Hydrometeorological Institute (FHMZBIH), which monitored air temperature (°C), relative humidity (%), and precipitation (mm) for the five days before the measurements, as well as on the day the physiological parameters were measured. The average daily temperature for the mentioned period in January was -0.9°C; in February, it was 5.4°C. The average temperature on the day of parameter measurement in January was -5°C; in February, it was 6.6°C. The area had an average relative humidity of 98.5% in January and 92.2% in February.

The average precipitation for the mentioned period was 0.02 mm in January, while in February, it was 4.4 mm. The parameters monitored were rectal temperature, respiratory rate, pulse, and shivering score. Rectal temperature was measured with a digital thermometer (MT 400, Microlife, Widnau, Switzerland). Respiratory rate was measured using the standard method of observing the rise and fall of the flanks and palpating the animal itself, using a stopwatch to count breaths per minute (BPM).

We differentiated between typical panting and occasional deep breaths, which satisfy the animal's need for gas exchange. Pulse was measured using a stopwatch, palpating the *a. femoralis* on the inner side of the animal's thigh for one minute. A stethoscope (08.811.00, Fazzini Srl, Italy) was used in both previous procedures. The shivering score was determined by observing the

animal with a hand placed on its body and evaluated by the relevant authors (Nel, Cloete, Kruger, et al., 2021). All efforts were made to minimize discomfort during the research.

The collected data was analyzed using IBM SPSS Statistics software (version 29.0.2.0) and Microsoft Office Excel 365 Data module. Differences between the mean values of the measured parameters were tested using the t-test and were considered statistically significant based on the correlation coefficient ( $p < 0.05^*$ ). Descriptive statistics were also used in the evaluation. Heat load was described using the THI (Temperature-Humidity Index), which was calculated using the formula commonly applied for sheep (Marai et al., 2007; Carabaño, Quiroga – Pineda, Ugarte, et al., 2021)

$$\text{THI} = T - \{(0.31 - 0.31 \times \text{RH}) (T - 14.4)\}$$

where T = average temperature for the observed measurement period (°C) and RH = average air humidity (%).

### ***Results and Discussion***

The research results show that the average THI (Temperature-Humidity Index) value is below the reference value of 9.8 (Carabaño et al., 2021), confirming cold stress conditions during both measurements of physiological parameters, considering the average temperature and humidity, except for the period immediately before the second sampling, which confirms thermoneutral conditions, desired for the second sampling.

Significant temperature differences were observed between the five days before the parameter measurements and the day of the measurements due to prevailing fluctuations and temperature inversion. The obtained results are described in more detail in Table 1.

**Table 1.** Results of parameters during the experimental periods (January – February 2024) with corresponding THI indices

Period	Temperature (°C)	Relative humidity (%)	Precipitation (mm)	THI
First measurement (January 6th-10th, 2024)*	-0.9	98.5	0.02	-0.83
Second measurement (February 21st-26th, 2024)*	5.4	92.2	4.4	5.62
On the day of the first measurement (January 10th, 2024)**	-5	99.6	0	-4.98
On the day of the second measurement (February 26th, 2024)**	6.6	93	0	6.77
Immediately before the first measurement (in the time interval from 1:00 PM to 3:00 PM)	-2.4	96	0	-2.19
Immediately before the second measurement (in the time interval from 1:00 PM to 3:00 PM)	15	92	0	14.99

\*five (5) days before the parameter measurements (which are included in the analysis of meteorological data)

\*\*average values – on the day of the parameter measurements

Rectally measured body temperature varies statistically significantly ( $p < 0.05$ ) during the measurement period. Lower body temperatures were recorded under cold stress conditions, which can be attributed to low temperatures increasing energy needs by up to 20 percent (Nenadović, Janković, Dimitrijević, et al., 2021). Sheep are often more exposed to cold weather compared to other domestic animals. Due to their exceptional fleece insulation, they are considered well-adapted to cold climates (Bugarija, Ostović, Pavičić, et al., 2014). This is also the case with the Bosnian Pramenka breed.

Physiological responses such as respiratory rate and shivering score were consistently higher in sheep exposed to cold stress, which may be because homeothermic animals initially respond to cold stress by enhancing their thermoregulatory mechanisms, such as increasing the above parameters to avoid undesirable temperature drops (Maurya, Seijan, Naqvi, 2013).

From this, the difference is statistically significant at a significance level of 0.05 in the respiratory rate measurement. Sheep exposed to cold conditions had a significantly higher respiratory rate than those in thermoneutral conditions. On the other hand, there is no statistically significant difference in the measured pulse values. The lack of statistical significance in pulse rates between the mentioned conditions suggests that changes in pulse frequency were not large or consistent enough to be considered statistically significant in this sample.

The results indicate that sheep responded more to temperature changes through changes in respiration. At the same time, the pulse was not as sensitive to these changes, and this may have implications for understanding how sheep regulate their bodily functions in different climatic conditions and how their behavior can be adapted to maintain homeostasis (Ljubičić, Vince, Shek Vugrovečki, et al., 2022; Čukić, Rakonjac, Djoković, et al., 2023). Additionally, the subjectivity of the examiner in manual pulse measurement should also be considered.

For the shivering score, statistically significant differences in measured values were recorded. This index can help assess the level of stress or discomfort in sheep, especially in situations such as exposure to cold temperatures, lack of food or water, the presence of predators, or any other situations that may cause stress in sheep. Measuring the shivering score can provide valuable information for assessing sheep welfare and identifying potential health and well-being issues. The obtained results are summarized in Table 2.

**Table 2.** Respiratory rates, pulse, and shivering indices in the first and second measurements (analyzed using descriptive statistics)

Parameter	First measurement (N = 25)	Second measurement (N = 25)
Respiratory rate/min	Min: 12, Max: 38, Mean: 21.16, Std. Dev: 5.836	Min: 11, Max: 34, Mean: 17.88, Std. Dev: 3.432
Pulse rate/min	Min: 66, Max: 98, Mean: 82.84, Std. Dev: 10.294	Min: 72, Max: 101, Mean: 85.32, Std. Dev: 8.335
Shivering Score 1 (No tremor present)	44% (11 cases)	72% (18 cases)
Shivering Score 2 (Mild tremor)	36% (9 cases)	28% (7 cases)
Shivering Score 3 (Animal trembles significantly)	16% (4 cases)	/
Shivering Score 4 (Severe tremor)	4% (1 case)	/

The mean values of physiological parameters in the examined individuals are summarized in Table 3.

**Table 3.** Average values of physiological parameters (mean  $\pm$  SD) in the examined individuals

Parameter	First measurement (January 2024)	Second measurement (February 2024)	P (* / ns)
Rectal temperature	37.74 $\pm$ 1.41	39.02 $\pm$ 0.90	*
Respiratory rate/min	21.16 $\pm$ 5.84	17.88 $\pm$ 3.43	*
Pulse rate/min	82.84 $\pm$ 10.29	85.32 $\pm$ 8.33	ns
Shivering Score	1.80 $\pm$ 0.87	1.28 $\pm$ 0.46	*

\*p < 0.05; ns: non-significance (p > 0,05);



## ***Conclusion***

Several important conclusions can be drawn based on the research findings. The data indicates that the sheep studied experienced cold stress during the research period, given the average air temperature and humidity, except right before the second sampling, when thermoneutral conditions were achieved. These temperature fluctuations, along with the occurrence of temperature inversions, had a notable impact on the physiological responses of the sheep. Secondly, during periods of cold stress, body temperature significantly dropped, which aligns with the increased energy demands caused by low temperatures in these animals.

The sheep demonstrated an elevated respiratory rate and shivering score in response to cold conditions, suggesting a heightened thermoregulatory effort. However, no significant differences were observed in pulse rate between the cold-stress and thermoneutral conditions. Thirdly, the shivering score proved to be an effective indicator of the level of stress or discomfort in sheep, offering valuable insights for evaluating their well-being and identifying potential health risks.

The data also indicated that the sheep responded more to temperature changes through respiratory adjustments, while their pulse rates remained stable, hinting at different thermoregulatory strategies. These findings are essential for understanding the adaptive mechanisms sheep use to cope with fluctuating climates and for developing future strategies to safeguard their health and welfare.

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## **Analiza fizioloških parametara kod bosanskohercegovačke ovce Pramenke kao odgovor na hladni stres**

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### **Sažetak**

Globalno zagrijavanje predstavlja izazov za životinje poput ovaca, s potencijalom da utiče na njihovo zdravlje i produktivnost. Ovce, posmatrajući autohtonu vrstu – Pramenku u Bosni i Hercegovini, pokazuju određenu prilagodljivost na ekstremne klimatske uslove, ali nedostaje detaljno razumijevanje njihove adaptacije na hladni stres. Ovo istraživanje fokusira se na analizu fizioloških parametara kako bi se razumjelo njihovo ponašanje i reakcije na hladni stres u poređenju s termoneutralnim uslovima. Rezultati pokazuju statistički značajne promjene u parametrima poput tjelesne temperature, frekvencije disanja i indeksa drhtanja, što ukazuje na adaptivne mehanizme ispitivanih jedinki u hladnim uslovima. Ovo istraživanje doprinosi boljem razumijevanju adaptacije ovaca na hladni stres i ima važne implikacije za poboljšanje njihove dobrobiti i produktivnosti u promjenjivim klimatskim uslovima.

*Ključne riječi: ovca, hladni stres, fiziološki parametri, adaptacija, klimatske promjene*

## Expression of the IGF-1 gene in Pramenka sheep under conditions of thermal stress

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### Abstract

Pramenka, the autochthonous sheep breed of Bosnia and Herzegovina, is well-known for its resilience to harsh external conditions. This makes it the most prevalent sheep breed in Bosnia and Herzegovina, despite modest production performances. With increasingly frequent extreme climate events and record-high temperatures threatening agricultural productivity and animal well-being globally, Bosnia and Herzegovina faces similar challenges due to climate change. Therefore, it is crucial for the country to invest in research on its indigenous breeds like Pramenka to adapt breeding and production practices to new environmental conditions. One reliable method for assessing the adaptability of animals to environmental stressors involves analyzing genes associated with desired traits. Stress, including exposure to extreme temperatures, often results in changes in gene expression within hours of exposure. This study focuses on the IGF-1 gene and its expression in Pramenka sheep under thermal stress conditions, using the relative gene expression RT-PCR method.

The goal is to improve livestock production, assess resilience, and explore the genetic potential of this breed under varying climate conditions. Additionally, the study aims to promote and protect our indigenous breed. Our results reveal significant differences in IGF-1 gene expression between the Dubska and Herzegovinian breeds under thermal stress. In the Herzegovinian breed, individuals exposed to severe to extremely severe heat stress exhibited significantly higher IGF-1 gene expression compared to the Dubska breed, suggesting a potential adaptation to heat.

Conversely, the consistent IGF-1 expression in the Dubska breed can point to its potential adaptability to cold conditions, particularly exposure to severe to extremely severe cold stress.

*Keywords: Pramenka, thermal stress, IGF-1, relative gene expression*

## ***Introduction***

Climate change has globally led to record-high temperatures over the past few decades (WMO, 2023), and represents one of the greatest challenges to global agricultural production. Numerous compensatory mechanisms make sheep one of the most resilient species to extreme climate conditions. One of the consequences of stress, including exposure to extreme temperatures, is a change in the expression pattern of certain genes, which occurs within a few hours of exposure to adverse temperatures. Among the potential candidates, is the gene that encodes the synthesis of the IGF-1 hormone. As one of the key elements of the somatotrophic axis, IGF-1 regulates anabolic processes essential for postnatal growth and metabolism in mammals through glycogenesis, gluconeogenesis, and protein synthesis. Although considered a primitive breed, Pramenka sheep are the most prevalent in Bosnia and Herzegovina, and a large part of the country's production of sheep milk, meat, and wool is based on the breeding of this breed (Važić, Rogić, Drinić et al., 2017). Their name is derived from its characteristic sparse and long wool (bos. *pramen* – lock), which, depending on the breed, is mostly white with black spots on the head and legs.

Numerous studies have been conducted to identify potential markers for thermal stress in small ruminants (Angel, Bagat, Sejian et al., 2018); however, similar research on Pramenka has only recently been conducted (Hrković-Porobija, Vegara, Hodžić et al., 2019; Ohran et al., 2023). This study aims to analyze the expression of the IGF-1 gene using the relative gene expression RT-PCR method under thermal stress conditions in Pramenka sheep.

The goal is to contribute to the improvement of livestock production in Bosnia and Herzegovina, to assess the resilience and genetic potential of Pramenka under different climate conditions, and to promote and protect this indigenous breed.

## ***Experimental***

This research included the Dubska and Herzegovinian Pramenka breeds. In total, 96 samples were collected of individuals from two different flocks at two different locations for each breed. The locations were selected based on data regarding altitude and above-average summer and winter temperatures. Samples for the Dubska breed were taken from two farms on the Vlašić Mountain, in the village of Mudrike, and two farms in the village of Zvirnjača on the Kupres plateau. For the Herzegovinian breed, sampling was

conducted at two farms in the village of Kružanj in Podveležje and at two farms in the village of Žiljevo near Nevesinje. To assess the impact of weather conditions on IGF-1 gene expression, data on ambient temperature, relative humidity, and the temperature-humidity index (THI) were used. The THI is typically employed as an indicator of the degree of weather-related stress in animals. Temperature and relative humidity were measured throughout the summer and winter periods, five days before sampling, and on the day of blood sampling. The THI value was calculated using the formula from Finocchiaro, van Kaam, Portolano et al. (2005).

The research analyzed the IGF-1 gene as a potential candidate gene and the endogenous reference gene GAPDH. The analysis of relative gene expression included four steps.

(1) Total genomic RNA was isolated from the samples using the commercial NucleoSpin™ RNA kit (Macherey-Nagel™, Germany) according to the manufacturer's instructions.

(2) After RNA extraction, reverse transcription was performed using the High-Capacity cDNA Reverse Transcription kit (Applied Biosystems™, USA). Through this process, single-stranded RNA was translated into double-stranded cDNA.

(3) Primer optimization is necessary to determine the exact annealing temperature during the annealing phase. The temperature varies depending on the composition of the primers (in terms of G and C nucleotide ratio), their size, and the template to which they bind. Therefore, the exact annealing temperature must be determined for each primer pair, which must match the annealing temperature of the reference gene.

Optimization was done using PCR Mix Plus Green (A&A Biotechnology®) and a gradient PCR machine (PCRmax Alpha Thermal Cycler, Cole-Palmer®). Primers were designed using the National Center for Biotechnology Information Primer-BLAST tool. Primers were tested at six different annealing temperatures, in a temperature gradient from 51 to 61°C. A 50 bp protein marker from Blirt (Poland) was used.

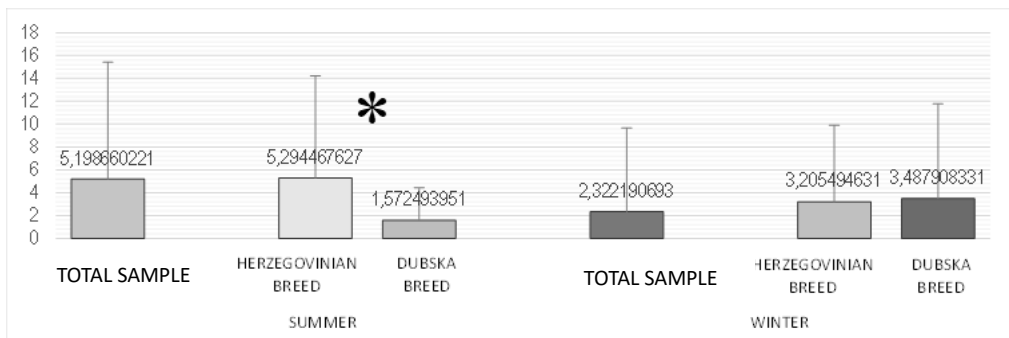
(4) For the RT-PCR analysis, the Applied Biosystems 7300 Real-Time PCR System and Applied Biosystems QuantStudio 5 Real-Time PCR System were used. The analyses were performed using the SYBR-Green method. The cDNA generated earlier was used as the sample for analysis. Each reaction contained 9 µl of master mix and 1 µl of cDNA for a final volume of 10 µl. The thermal profile for the PCR program was as follows: polymerase activation at 95°C for five minutes, followed by 40 cycles of denaturation at 95°C for 40 seconds, primer annealing for 30 seconds at 57.5°C, and

elongation at 72°C for 30 seconds. This was followed by gradual heating and cooling of the primers (melting curve analysis) to check the efficiency of simultaneous amplification of both primer pairs, for IGF-1 and the reference gene. In this step, samples were heated to 95°C for 15 seconds, cooled to 54°C for one minute, and then heated to 95°C. The efficiency of PCR was determined by the amplification of a standardized series of cDNA dilutions. After the analysis, the threshold cycle (Ct) values of the reference gene and the IGF-1 gene were used to calculate the relative expression of the IGF-1 gene.

For the purpose of comparing the relative expression of the IGF-1 gene between breeds, locations, and seasons, the Pfaffl method (Pfaffl, 2001) was used, in which Ct values of gene expression between two compared groups are translated into the level of relative expression. Subsequently, the T-test was used to determine statistically significant differences in the level of relative gene expression between the compared groups. Differences were considered statistically significant at  $P < 0.05$ . All statistical analyses were performed using Microsoft® Excel® 2019.

### Results and discussion

A statistically significant difference in the relative expression of the IGF-1 gene was observed during the summer period between the Herzegovinian and Dubaska breeds. The IGF-1 gene expression in the Herzegovinian breed during the summer period was significantly higher than in the Dubaska breed, while in the winter period, the relative expression in the Dubaska breed was only slightly higher, without statistical significance (Graph 1.).

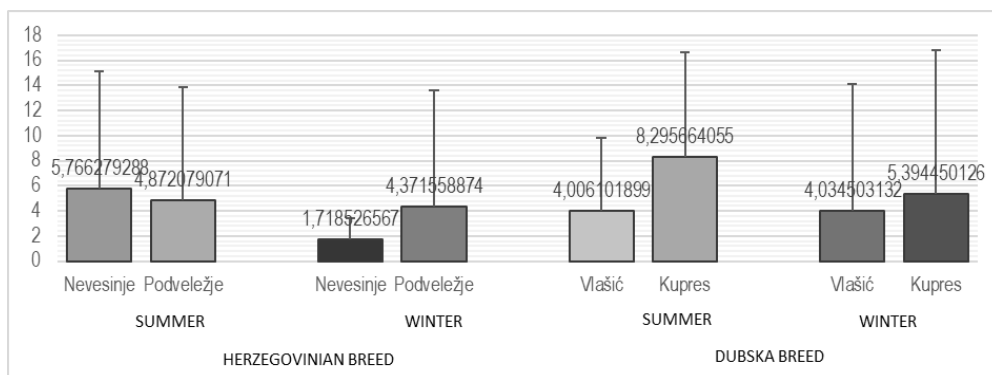


**Graph 1.** Relative expression of the IGF-1 gene in the total sample, and in samples from both breeds, by research period. All values are presented as  $x \pm SD$ ; \*Statistically significant difference ( $P < 0.05$ ).



Comparing the total sample of both breeds between the two sampling periods, the relative gene expression was higher during the summer than in the winter period, but without statistically significant differences.

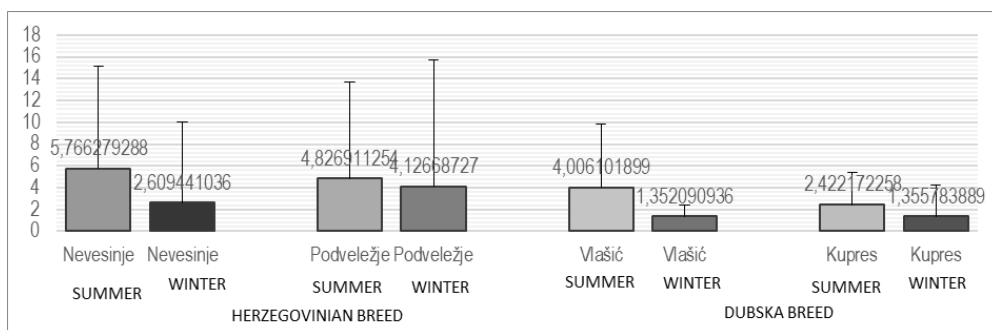
Within the Herzegovinian breed, a higher level of relative gene expression was recorded in Nevesinje compared to Podveležje during the summer period, while during the winter period, the opposite was observed – lower expression levels in Nevesinje compared to Podveležje (Graph 2.).



**Graph 2.** Relative expression of the IGF-1 gene between locations within the same breeds by sampling periods. All values are presented as  $x \pm SD$ .

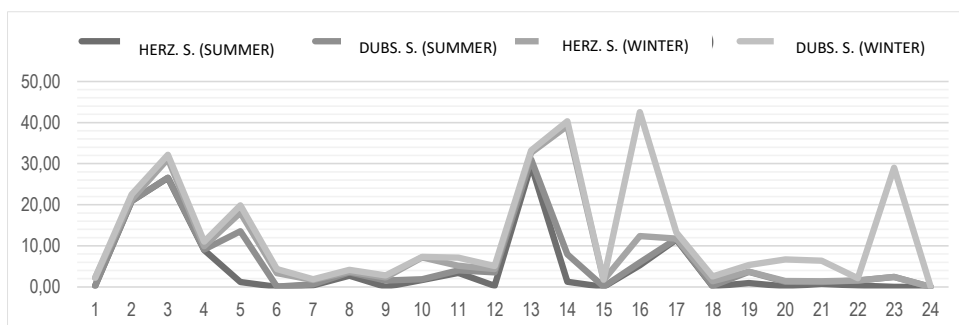
Regarding the locations where the Dubska breed was tested, a consistent pattern in the IGF-1 gene expression was observed – during both periods of the study, a higher level of expression

was recorded in individuals at the Kupres location compared to Vlašić. However, none of these differences in relative gene expression were statistically significant (Graph 3.).



**Graph 3.** Relative expression of the IGF-1 gene at the examined locations by sampling periods. All values are presented as  $x \pm SD$ .

Individuals of the Dubska breed showed the greatest fluctuations in IGF-1 gene expression during the winter period, while fluctuations were relatively small in the same individuals during the summer period (Graph 4.).



**Graph 4.** Individual values of relative IGF-1 gene expression by breeds and sampling periods.

According to climatological data, sheep in Podveležje and Nevesinje were exposed to conditions of strong to extreme heat stress during the summer period. One possible explanation for the significantly higher relative gene expression in the Herzegovinian breed during the summer period can be found in the study by Onzima, Upadhyay, Doekes et al. (2018), where genes of six indigenous goat breeds in Uganda were analyzed. The results showed that goat breeds exposed to warmer climates had increased expression of genes associated with heat resistance, including the IGF-1 gene.

The authors believe that the increased gene expression in these individuals may have resulted from increased selective pressure where high environmental temperatures favored the expression of certain genes, which influenced the development of thermoresistant mechanisms. This could also explain the higher gene expression in the Dubska breed compared to the Herzegovinian breed during the winter period. This hypothesis is supported by the results of the study by Pragna, Sejian, Bagath et al. (2018), who measured the growth rate of three goat breeds under heat stress conditions. They recorded increased IGF-1 mRNA expression in liver tissue of Salem Black goats, while in the other two breeds, expression was either reduced or unchanged compared to control groups. The authors suggest that this could serve as evidence of the adaptation and ability of this breed, which is the most widespread in the tropical parts of the Indian subcontinent, to maintain a stable growth rate even in unfavorable temperature conditions.

It should also be considered that individuals of the Herzegovinian breed are generally smaller in size compared to those of the Dubska breed, which according to some sources may be another mechanism of adaptation to heat stress and food scarcity in ruminants, thereby influencing IGF-1 gene

expression (Collier and Gebremedhin, 2015; Pacifici, Visconti, Butchart et al., 2017). THI values for the Nevesinje location during the winter sampling period were drastically lower than the threshold for cold stress, confirming that the individuals were in a state of cold stress.

Comparing expression at the locations between sampling periods, we observe that at all locations, IGF-1 gene expression was higher during the summer than in the winter. In examining IGF-1 concentrations in blood plasma and liver tissue of pigs under heat stress conditions over a 21-day period, Xin, Zhang, Sun et al. (2018) obtained similar results. The authors believe that the increase in plasma IGF-1 levels could be a thermoprotective mechanism that, along with insulin, stimulates glucose clearance from the bloodstream and helps pigs acclimate to thermal stress (Xin et al., 2018).

Noticeable individual variations in the relative gene expression of the animals were observed. It is important to emphasize that our research was conducted under natural conditions, and given the climate in Bosnia and Herzegovina, the animals were often exposed to large fluctuations in external temperature, which may explain the individual differences in relative expression of the examined gene. Physiological conditions of individuals, such as pregnancy and lactation, also have an effect on the relative expression of the IGF-1 gene.

### ***Conclusion***

The results of this study indicate that there are differences in IGF-1 gene expression between the two examined breeds of Pramenka under thermal stress conditions. In individuals of the Herzegovinian breed, higher IGF-1 gene expression compared to the Dubska breed may indicate a potential adaptation of this breed to heat stress conditions. The relative consistency of the IGF-1 gene expression pattern in the Dubska breed may support the thesis about the adaptation of individuals of the Dubska breed to harsh conditions and low temperatures to which they are exposed. Large individual variations in IGF-1 gene expression could also be a consequence of large fluctuations in external temperatures at the locations of sampling.

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***Conflicts of interest:*** The authors declare no conflicts of interest.

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## **Ekspresija gena IGF-1 kod pramenke u uvjetima termalnog stresa**

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### **Sažetak**

Pramenka, autohtona pasmina ovaca Bosne i Hercegovine, poznata je po svojoj otpornosti na surove vanjske uslove. Zbog toga je ona najzastupljenija pasmina ovaca u Bosni i Hercegovini, iako su njeni proizvodni učinci skromni. Sa sve učestalijim ekstremnim klimatskim događajima i rekordno visokim temperaturama koje globalno ugrožavaju poljoprivrednu produktivnost i dobrobit životinja, Bosna i Hercegovina se suočava sa sličnim izazovima zbog klimatskih promjena. Stoga je ključno da zemlja ulaže u istraživanje svojih autohtonih pasmina, poput Pramenke, kako bi prilagodila prakse uzgoja i proizvodnje novim okolišnim uvjetima. Jedna od pouzdanih metoda za procjenu sposobnosti prilagodbe životinja na stresne uvjete okoline uključuje analizu gena povezanih sa željenim osobinama. Stres, uključujući izloženost ekstremnim temperaturama, često uzrokuje promjene u ekspresiji gena unutar nekoliko sati nakon izlaganja.

Ova studija se fokusira na gen IGF-1 i njegovu ekspresiju kod ovaca pasmine Pramenka pod uvjetima toplinskog stresa, koristeći metodu relativne ekspresije gena RT-PCR. Cilj je poboljšati stočarsku proizvodnju, procijeniti otpornost i istražiti genetski potencijal ove pasmine pod različitim klimatskim uvjetima. Također, studija ima za cilj promicanje i zaštitu naše autohtone pasmine. Naši rezultati otkrivaju značajne razlike u ekspresiji IGF-1 gena između dva soja Pramenke pod toplinskim stresom. Kod hercegovačkog soja, jedinke izložene jakom do ekstremno jakom toplinskom stresu pokazale su znatno višu ekspresiju IGF-1 gena u poređenju s dubskoj sojem, što ukazuje na moguću prilagodbu na vrućinu. S druge strane, dosljedna ekspresija IGF-1 gena kod dubskoj soja može ukazivati na njegovu potencijalnu prilagodljivost na hladne uvjete, naročito izloženost jakom do ekstremno jakom hladnom stresu.

*Ključne riječi: Pramenka, toplinski stres, IGF-1, relativna ekspresija gena*

1-0-5

### **3D (3-dimensional) printed meals - for or against?**

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#### **Abstract**

3D food printing uses 3D printers to produce meals from various food ingredients. This technology can optimize nutrition and reduce waste, but also brings challenges in terms of safety and health effects. The aim of this paper was to investigate the safety of 3D printed food and assess its advantages and disadvantages in terms of consumer health and safety. The work was conducted by reviewing scientific and professional literature from relevant databases (EBSCO, PubMed, Scholar) using the keywords: "3D", "food", "development", "health". 3D printing of food allows the customization of nutritional profiles and the reduction of waste, but also brings challenges. Thermal processes during printing can affect the nutritional value of food, while additives can negatively impact health and the gut microbiome. There is also a risk of contamination through contact with the printer components and the release of toxic particles. Excessive use can affect the quality of the food as it is ultra-processed. 3D food printing offers significant benefits in customizing nutrition and reducing waste, but requires additional research and the development of standards to ensure safety and quality in the food industry.

*Keywords: 3D, food, development, health*

#### **Introduction**

3D printing is a digitally controlled robotic process that can be used to construct solid objects with complex structures by depositing materials layer by layer. Like other 3D printing processes, food printing relies on the conversion of 3D data into a sequential layer-by-layer deposition to produce an object, in this case food (Eswaran, Ponnuswamy, Kannapan, 2023). 3D printing has been around for some time, but it was only in 2007 that it was first used to produce food. Currently, 3D printing is being used in the development of food for the military, astronauts, the elderly and children (Silva, Pereira, Mendes, Gordo, Gil). The process of 3D printing food is complex and involves many aspects, such as easy and homogeneous flow

through the nozzle, optimal adhesion of the food layers and the ability of the printed structure to withstand the weight of the overlying layers. Overall, the successive phases during material deposition can be divided into the initiation of flow, continuous extrusion, recovery phase and final deposition (Derossi, Corradini, Caporizzi, Oral, Severini, 2023).

### ***Experimental***

The research was conducted through a review of available literature from relevant databases, such as: Science direct, EBSCO and Google Scholar, dealing with 3D printing of food, with particular reference to the advantages and disadvantages of 3D printed food. Articles dealing with material safety, nutritional value, equipment hygiene, microbiological risks and the impact of the technology on food safety were analyzed. Keywords used for the search: "3D", "food", "development", "health".

### ***Results and discussion***

3D printing of food has its advantages, such as the ability to personalize meals and reduce food waste, but also its disadvantages, such as potential health risks related to material and equipment hygiene.

The most common 3D printing processes for food printing are: Powder Fusion, Binder Injection, Material Injection and Material Extrusion (Meijers, Han, 2024).

The studies conducted by Bebek Markovinović and colleagues with wheat starch (6%, 8% and 10%) and different 3D printing programs showed a significant impact on the content of bioactive compounds, antioxidant capacity, color parameters, rheological and sensory properties of food. Furthermore, the results confirmed that 3D printing can support the concept of "zero waste" in the food industry (Bebek Markovinović, Bosiljkov, Janči, 2024).

The researchers believe that potential applications for 3D printing could play a useful role in the food and nutrition industry, including texture-modified diets and the development of personalized nutrition, as a potential solution to food insecurity and as a way to introduce new ingredients into the diet (Zhu, Iskandar, Baeghbali, Kubow, 2023; Oral, Derossi, Caporizzi, Severini, 2021). 3D printing of food can improve the appearance of soft or pureed foods, which can help people with swallowing difficulties and prevent aspiration pneumonia from ingestion, as well as improve patients' appetite and improve malnutrition. This technology enables the production of personalized foods based on individual nutritional needs, including personalized dietary supplements, personalized consumer-directed diets, and personalized probiotics and nutrients in functional foods through microencapsulation

technology (Zhu et al, 2023; Severini, Derossi, 2016; Sun, Peng, Zhou, et al, 2015).

The technical aspects of 3D food printing and the safety issues surrounding 3D printed meals are of great concern. As many 3D printed foods are produced in the form of a paste, their shelf life may be limited compared to original products, that have a shelf life of several months to several years. Although 3D printing food allows precise ingredient dosing and portion optimization, which can help reduce food waste, the shorter shelf life of 3D printed products can negatively impact these benefits. When food spoils faster, the risk of it being thrown away increases, reducing the overall potential of the technology to reduce waste.

Most food 3D printing processes require heating during the extrusion process and cooling after deposition. In most cases, the 3D printed meals also need to be reheated. These heating and cooling or reheating processes can favor the growth of microorganisms and affect food safety. In addition, there is direct contact between the components of the 3D printer (e.g. nozzles, trays) and the raw materials used in food preparation, which increases the risk of microbiological contamination (Ekonomou, Kageler, Stratakos, 2024). Since 3D printed foods require pre- and post-processing of food materials and processing in a 3D printer and may contain food additives, they can be classified as ultra-processed foods, which can have a negative impact on health (Monteiro, Moubarac, Cannon, Ng, Popkin, 2013).

### ***Conclusion***

3D printing technology has significant potential to revolutionize food production by individualizing production, reducing food waste and incorporating valuable bioactive substances. However, there are still some challenges, particularly in terms of food safety and the risks associated with the printing process. The shorter shelf life of 3D-printed food, the possibility of microbiological contamination through direct contact with printer components and the classification of 3D-printed food as ultra-processed raise concerns that need to be addressed to ensure consumer safety. Despite these limitations, with further advances in hygiene protocols, material handling and additional processing, 3D food printing could play a crucial role in improving food sustainability and personalization, and offer a promising future for both food production and public health.

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### **3D (3-dimenzionalni) printani obroci – za ili protiv?**

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#### **Sažetak**

3D printanje hrane koristi 3D printere za kreiranje obroka iz različitih prehrambenih sastojaka. Ova tehnologija može optimizirati ishranu i smanjiti otpada, ali donosi i izazove u pogledu sigurnosti i uticaja na zdravlje. Cilj oog rada je bio istražiti sigurnost 3D printane hrane i ocijeniti njene prednosti i nedostatke u vezi s zdravstvenom ispravnošću i sigurnošću za potrošače. Rad je urađen pregledom naučne i stručne literature iz relevantnih baza podataka (EBSCO, PubMed, Scholar) koristeći ključne riječi: „3D“, „hrana“, „razvoj“, „zdravlje“. 3D printanje hrane omogućava prilagodbu nutritivnih profila i smanjenje otpada, ali donosi izazove. Termički procesi tokom printanja mogu utjecati na nutritivnu vrijednost hrane, dok aditivi mogu negativno utjecati na zdravlje i crijevni mikrobiom. Također, postoji rizik od kontaminacije zbog kontakta s komponentama printera i oslobađanja toksičnih čestica. Prekomjerno korištenje može smanjiti prehrambenu kvalitetu zbog ultra-prerađenog statusa. 3D printanje hrane nudi značajne prednosti u prilagodbi ishrane i smanjenju otpada, ali zahtijeva dodatna istraživanja i razvoj standarda za osiguranje sigurnosti i kvalitete u prehrambenoj industriji.

*Ključne riječi: 3D, hrana, razvoj, zdravlje*

## Physiological parameters under heat stress conditions in Pramenka sheep

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### Abstract

Heat stress is increasingly present throughout the world and it is associated with reduced productivity and animal welfare, especially during the summer months. Although the reactions of animals to their thermal environment are very different, it is clear that the thermal environment affects the health, productivity and welfare of sheep. The aim of this research was to analyse how heat stress in the summer months affects physiological parameters in sheep: rectal temperature and pulse rate. The research was conducted in August 2023 in two locations: Miševići (Canton Sarajevo) and Klokočnica (Tuzla Canton). The sample included 44 sheep. The parameters were measured on the same individuals in thermoneutral conditions (15 - 20°C) and high temperature conditions (over 30°C). The research results show that the presence of mild and severe heat stress during the research period significantly affects the rise in rectal temperature and pulse frequency acceleration. The obtained results point to the need for further and more extensive empirical research, and to find an option to minimize the negative impact of heat stress. One of the ways to do this is to adapt the diet during periods of high temperatures.

*Keywords: heat stress, sheep, temperature and humidity index (THI), rectal temperature, pulse rate*

### Introduction

Climate change refers to the long-term imbalance of typical weather conditions as temperature, wind, and precipitation characteristics in a given region. Climate models predict an increase in global average temperature by 2100 of between 2.2. and 3.5°C (IPCC, 2023). Anthropogenic climate change is expected to have significant impacts on livestock, including increased incidence of heat stress in intensive and extensive livestock systems. Sheep and cattle are capable of regulating their body temperature withing a narrow range of air temperatures. The thermoneutral zone for sheep is approximately between 5 and 25°C, but it also depends on individual factors and the

environment (Schutz, 2022). Signs indicating heat stress include high body temperature, increased water intake, panting, loss of appetite, lethargy, open mouth breathing, and salivation. In some cases, there can be coordination disorders, trembling and collapse. Heat stress can also adversely impact productive and reproductive performance with a suppressive effect on reproductive hormones (Al-Dawood, 2017).

Environmental variables directly related to heat stress include temperature, humidity, wind (air movement), solar radiation and access to shade. Increased temperature and humidity reduce the animals' ability to dissipate heat. The thermoregulatory mechanisms of sheep are compromised when air temperatures rise above 31°C (Le Gallou, 2021). This effect worsens when heat stress is accompanied by high humidity (Kandemir et al., 2013).

The impact of solar radiation can be a major determinant of the environmental conditions in which livestock are kept, especially concerning pastures. For this reason, livestock on pasture are more susceptible to heat stress (Herbut et al., 2018). Access to shade is also an important factor (Masters et al., 2013), but due to operationalization issues, it is rarely taken into account. Numerous bio-metrological indices have been developed to predict when livestock begin to experience heat stress.

The Temperature-Humidity Index (THI) is unique value that represents the combined effects of air temperature and humidity related to the level of thermal stress. Rectal temperature is a reliable indicator of heat stress. The normal range of rectal temperature in sheep is between 38.3 – 39.9°C (Robertshaw, 2004). Measuring pulse rate is an additional method to evaluate the impact of heat stress on animals. Under heat stress conditions, the pulse rate significantly increases. The reference range is 70 to 90 beats per minute for adult individuals, while under heat load, values rise above 100 beats per minute (Wojtas et al., 2014).

Srikandakumar et al. (2003) studied the effect of heat stress on changes in respiratory rate, rectal temperature and biochemical parameters in the blood of mature black-fleeced Omani sheep and white-fleeced Australian Merinos. Both respiratory rate and rectal temperature were significantly increased when the animals were under heat stress ( $p < 0.01$ ). The Omani sheep were more tolerant to heat stress than the Merino sheep.

In the study by Wojtas et al. (2014), 15 sheep were placed in a climate chamber and exposed to high temperatures (30°C). Air movement was then induced to examine its calming effect on heat stress-induced responses. Physiological responses such as respiratory functions and heart rate were monitored, along with morphological, biochemical parameters, and cortisol levels in the blood. A significant increase in respiratory rate and heart rate

was observed. Increased air movement resulted in a calming effect on heat stress.

Heat stress is a limiting factor for sheep breeding and according to Xu et al. (2022), it is necessary to screen sheep breeds that tolerate heat better. Their study compared serum metabolites of Hu and Dorper sheep and identified biomarkers associated capacity and glutathione peroxidase in Hu sheep were significantly higher while the concentration of malondialdehyde was lower.

Antioxidant and energy-related metabolite levels were higher in the serum of hu sheep than in Dorper sheep; however, lipid catabolism and inflammation levels were higher in the serum of Dorper sheep. These results indicate that Hu sheep had better resistance to heat stress than Dorper sheep. The findings revealed that respiratory rate, heart rate and rectal temperature in Dorper sheep were significantly higher than those in Hu sheep.

Considering the importance of this topic and the lack of empirical data, the aim of this study is to investigate the impact of heat stress on rectal temperature values and pulse frequency in Pramenka sheep at two different locations in Bosnia and Herzegovina.

### ***Experimental***

The study included a total of 44 sheep and was conducted on August 25 and 26, 2023, at two locations:

- Miševići, municipality of Hadžići, Sarajevo Canton: 17 randomly chosen crossbred Pramenka sheep weighing 40 to 70 kg, aged 2 to 8 years, including only one male;
- Klokotnica, municipality of Doboj Istok, Tuzla Canton: 27 Pramenka sheep weighing 50 to 60 kg, consisting of 19 males and 8 males.

At the Klokotnica location, the flock was fed on pasture while at the Miševići location, the diet was combined. The sheep were sheared approximately three months before the measurements for this study. The purpose of the animals at the Klokotnica location was meat production while at Miševići location it was combined breeding type. According to lactation status, the females at Miševići were dry.

Measurements at Klokotnica were conducted on August 25, while at Miševići, the experiment was carried out in duplicate on the same animals on August 25 and 26, with the average of the two measurements taken.

The physiological parameters monitored in the study were rectal temperature and pulse frequency. These parameters were measured in the same animals under thermoneutral conditions (15-20°C) and under high-temperature

conditions (over 30°C). Rectal temperature was measured using a digital thermometer (Microlife AG, Switzerland). To obtain an accurate rectal temperature, the tip of the thermometer was held directly against the rectal wall. Pulse frequency was determined by palpation of the femoral artery on the inner side of the hind leg.

For the days before, during, and after measurements, climate data were collected from the Hydrometeorological Institute of FBiH (location Miševići) and the Republic Hydrometeorological Institute of RS (location Klokotnica) regarding environmental conditions: air temperature in °C (TZ) and relative humidity in % (RV%), as well as wind direction and speed. These institutions do not have data on solar radiation. Using the formula from Hahn et al. (1999), based on air temperature (TZ) and relative humidity (RVZ=RVZ%100), the THI index was calculated:

$$THI = 0.81 * TZ + RVZ * (TZ - 14.4) + 46.4$$

THI values are evaluated as follows: THI of 74 or lower – absence of heat stress; 75 to 78- warning status (mild heat stress); 79 to 83- danger status (severe heat stress); THI equal to or greater than 84 – extreme heat stress (Habeeb et al., 2018).

To analyse the significance of differences between the means of the observed variables for measurements on the same individuals or on the same days under thermoneutral conditions and high-temperature conditions, statistical test for dependent (paired) samples were used: the parametric option of the t-test for dependent samples was applied if “normality” was satisfied and the non-parametric option of the Wilcoxon test for dependent samples was used if “normality” was not satisfied.

To analyse the significance of differences between the means of the observed variables for measurements at different locations under the same conditions, statistical tests for independent samples were used: the parametric option of the t-test for independent samples was applied if “normality” was satisfied and the non-parametric of the Mann-Whitney test for independent samples was used if “normality” was not satisfied. The assumption of “normality” was checked using the Shapiro-Wilk test, as the samples were smaller than 50 elements. Statistical data processing was performed in Microsoft Excel and SPSS (IBM SPSS Statistics for Windows, version 36, IBM Corp., Armonk, NY, USA).

### ***Results and Discussion***

In table 1, the results of the statistical analysis for climate parameters are presented, including air temperature, relative humidity, and the THI index during the research period.

**Table 1.** Descriptive statistics and statistical tests for climate indicator variables.

t test for dependent samples	Wilcoxon test		Shapiro-Wilk test of normality		SD	Median	Average	Max	Min	Location/climate data Statistical data
	p	z	p value	SW statistics						
<b>0.000</b>	21.4	<b>0.011</b>	-2.55	0.005	0.748	20	<b>19.33</b>	20	18	AT TN
				0.613	0.943	29	<b>29.56</b>	35	24	AT HTC
<b>0.000</b>	21.4			0.13	0.864	82.5	<b>79.62</b>	88	64	RH TN
				0.073	0.839	51.5	<b>48.75</b>	56	33	RH HTC
		<b>0.012</b>	-2.52	0.132	0.864	66.52	<b>66.23</b>	67.25	63.97	THI TN
				0.007	0.775	77.74	<b>76.47</b>	79.27	70.06	THI HTC
<b>0.000</b>	-18.7			0.338	0.913	21	<b>20.56</b>	22	19	AT TN
				0.585	0.940	32	<b>31.89</b>	35	27	AT HTC
<b>0.000</b>	21.0			0.752	0.954	82.5	<b>81.62</b>	90	70	RH TN
				0.814	0.96	41.5	<b>42.12</b>	52	29	RH HTC
		<b>0.012</b>	-2.52	0.323	0.905	68.63	<b>68.15</b>	69.54	65.93	THI TN
				0.003	0.749	79.99	<b>79.54</b>	81.69	73.31	THI HTC

M I Š E V I Ć I  
K L O K O T N I C A

AT – air temperature in °C, RH – relative humidity, TN – termoneutral conditions, HTC – high-temperature conditions

For both locations, air temperature was statistically significantly higher ( $p < 0.05$ ) under high-temperature conditions compared to thermoneutral conditions. It is also evident that air temperatures were consistently higher at the Klokotnica site compared to the Miševiči site (table 1).

At both locations, relative humidity was statistically significantly lower ( $p < 0.05$ ) under high-temperature conditions compared to the thermoneutral conditions. The THI index shows a rising trend from thermoneutral to high-temperature conditions.

The maximum THI value under thermoneutral conditions never exceeded 74 on any day, indicating the absence of heat stress. The average THI value under high-temperature conditions for the Klokotnica site was above 79, indicating a danger status, while for Miševiči it ranged between 75 and 78, indicating a warning status. The results suggest the presence of mild (Miševiči) and severe (Klokotnica) heat stress during the research period.

Table 2 presents the results of descriptive statistics and significance tests for the measured physiological parameters of sheep from the analyzed sample ( $n=47$ ).



**Table 2.** Descriptive statistics and statistical tests for the variables rectal temperature and puls rate of sheep. (RT – rectal temperature, TN – thermoneutral conditions, HTC – high-temperature conditions)

t test for dependent samples		Shapiro-Wilk test of normality		Standard deviation	Median	Average	Max	Min	Location/Physiological parameters Statistical data
		P value	SW statistics						
<b>0.000</b>	-4.427	0.054	0.894	0.43	39.4	<b>39.49</b>	41.1	38.5	RT TN
		0.285	0.937	0.37	40	<b>39.99</b>	41	38.9	RT HTC
<b>0.000</b>	-5.854	0.98	0.983	14.08	72	<b>71.32</b>	97	42	Puls TN
		0.054	0.894	14.78	94	<b>98.65</b>	128	79	Puls HTC
<b>0.000</b>	-10.530	0.324	0.987	0.39	39.4	<b>39.35</b>	40	38.6	RT TN
		0.203	0.949	0.34	39.9	<b>39.87</b>	40.5	39.3	RT HTC
<b>0.007</b>	-2.904	0.180	0.947	21.92	100	<b>101.92</b>	132	68	Puls TN
		0.308	0.957	18.32	116	<b>112.59</b>	144	80	Puls HTC

The average rectal temperature values were 0.5° C higher for Miševići and 0.52° C higher for Klokočnica under high- temperature conditions compared to thermoneutral conditions. The median values for rectal temperature were higher by 0.5 °C (for Klokočnica) to 0.6 °C (for Miševići) under high temperature conditions compared to thermoneutral conditions. According to statistical test, this difference is statistically significant at level of  $p < 0.05$  at both locations (Table 2). To test the difference between the mean values of rectal temperature at different locations, a parametric t-test for independent samples with equal variances was applied: under thermoneutral conditions,  $t=1,027$ ,  $p=0,312 > 0.05$ ; under high-temperature conditions,  $t=1.009$ ,  $p=0,321 > 0.05$ . Therefore, our study did not find a statistically significant difference in rectal temperature values at different locations.

The average pulse rate values under high-temperature conditions were higher by 10.67 (for Klokočnica) to 27.33 (for Miševići) compared to thermoneutral conditions. The median values for pulse rate under high-temperature conditions were higher by 16 (for Klokočnica) to 22 (for Miševići) compared to thermoneutral conditions. According to statistical test, this difference was statistically significant at both locations at the level of  $p < 0.05$  (table 2). At the Klokočnica site, higher pulse rate values were observed both in thermoneutral conditions and in high-temperature conditions, along with more pronounced variability. To test the difference in pulse rate values between the two examined locations, a parametric t-tests for independent samples with equal variances was applied: under thermoneutral conditions,  $t= -5.638$ ,  $p=0.000 < 0.05$ ; under high-temperature conditions,  $t=2.774$ ,  $p=0.004 < 0.05$ . Therefore, there is statistically significant difference in pulse rate values between the examined locations, and it is evident that the pulse rate is significantly higher in sheep at Klokočnica site in both thermoneutral and high-temperature conditions.

According to our research findings, the rectal temperature under high-temperature conditions averaged above 39.8°C, reaching levels as high as 41°C, which is beyond the reference values for rectal temperature in sheep (the reference interval is 38.3 to 39.9), with an average increase of 0.5°C for Miševići and 0.52°C for Klokočnica. Similarly, other studies have shown that sheep exposed to high-temperatures exhibited increased rectal temperature values ranging from 39.5-39.8°C (Marai et al., 2000; Sirkandakumar et al., 2003), and even up to 40.5°C (Xu et al., 2021).

According to our results, the presence of mild heat stress at the Miševići site or severe heat stress at the Klokočnica site significantly affects the acceleration of pulse rate (Table 2). The pulse rate was predominantly above 94, reaching values of up to 144 beats per minute under high-temperature conditions, which is beyond the reference values of sheep (the reference

value is 70 to 90 beats per minute for adult individuals), with an average acceleration of pulse ranging from 10.67 for Klokočnica to 27.33 for Miševići. The increase in pulse rate is one of the first physiological responses to high temperatures. It ensures enhanced blood flow from the interior of the body to peripheral parts, thereby increasing heat dissipation to the environment. In the study by Wojtas et al. (2014), it was found that under heat stress, the respiratory rate increased to 96.43 breaths per minute, and the pulse rate rose to 107.79 beats per minute. According to Xu et al. (2021), by the end of the experimental period, the pulse rate reached values exceeding 200 beats per minute. Like our study, this research demonstrated that heat stress leads to significant changes in physiological parameters in sheep.

### ***Conclusion***

Climate change encompasses various dramatic events, among which exposure to heat stress is the most prevalent. The negative effects of heat stress on animal welfare are complex, particularly with significant financial burdens for the livestock sector. The results of our study indicate that the presence of mild and severe heat stress in sheep in the region of Bosnia and Herzegovina during the summer months significantly affects the rise in rectal temperature. According to that, it is essential to continuously work on reducing the effects of heat stress on physiological parameters in sheep. One way to achieve this is through targeted feeding.

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### **Fiziološki parametri u uslovima toplotnog stresa kod ovaca Pramenka**

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#### **Sažetak**

Toplotni stres je sve više prisutan u cijelom svijetu i povezan je sa smanjenom produktivnošću i dobrobiti životinja, posebno tokom ljetnih mjeseci. Iako su reakcije životinja na njihovo toplinsko okruženje vrlo različite jasno je da toplinska okolina utiče na zdravlje, produktivnost i dobrobit ovaca. Cilj ovog istraživanja bio je da se sagleda kako toplotni stres u ljetnim mjesecima utiče na fiziološke parametre kod ovaca: rektalnu temperaturu i puls. Istraživanje je rađeno u avgustu 2023. godine na dva lokaliteta: Miševići (Kanton Sarajevo) i Klokotnica (Tuzlanski kanton). Uzorak je obuhvatio 44 jedinke. Mjerenje parametara se vršilo na istim jedinkama u termoneutralnim uslovima (15 - 20°C) i uslovima visokih temperatura (preko 30°C). Rezultati istraživanja ukazuju da prisustvo blagog ili jakog toplotnog stresa u periodu istraživanja signifikantno utiče na porast rektalne temperature i ubrzanje pulsa. Dobijeni rezultati ukazuju na potrebu za daljim i opsežnijim empirijskim istraživanjima, te pronalaskom opcija da se minimizira negativni uticaj toplotnog stresa. Jedan od načina da se to uradi je prilagođena ishrana u periodima visokih temperatura.

*Ključne riječi: toplotni stres, ovce, THI, rektalna temperatura, frekvencija pulsa*

1-O-7

## Nutritional strategies to reduce methane emissions in ruminants

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### Abstract

Methane (CH<sub>4</sub>) is the most powerful greenhouse gas that pollutes the environment and atmosphere. Animal production, especially cattle breeding, has the greatest impact on the total amount of methane emissions. Methane production in cattle is a result of their specific digestive system because they can obtain food that other mammals are unable to efficiently utilize. During the digestion process and with the help of microorganisms and enzymes, methane is created in the rumen, which is expelled from the body and over time pollutes the environment. It should be noted that methane emissions from cattle vary in different parts of the world. Various factors affect the emission such as the type of feed used to feed the cattle, the way they are kept, the climatic conditions, and other factors. This paper aims to show different feeding strategies that can affect the reduction of methane emissions in ruminants.

*Keywords: methane emission, ruminants, nutrition*

### Introduction

Climate change is one of today's biggest challenges related to long-term changes in temperatures and weather conditions. For decades, human activities have been the main drivers of climate change, primarily due to the burning of fossil fuels such as coal, oil and gas. The greenhouse gases that cause climate change are carbon dioxide, methane, and nitrous oxide, and sharp increases in their concentration have been recorded since the 1800s until today.

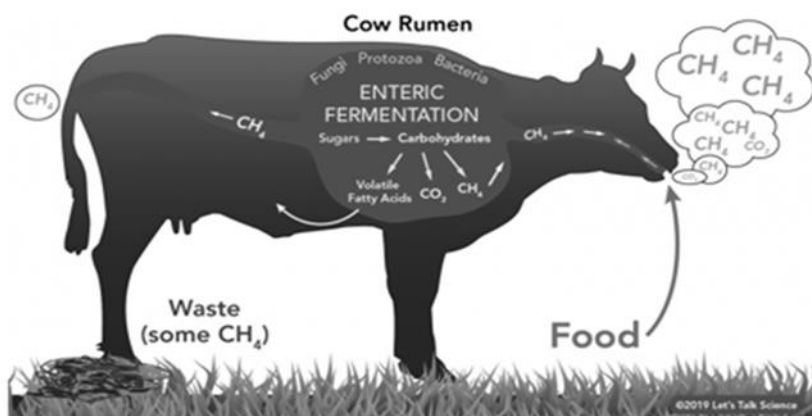
Although it breaks down relatively quickly in the atmosphere, methane is a more potent greenhouse gas than carbon dioxide. Methane comes from natural and anthropogenic sources. About a third of methane emissions come from animal production, mostly cattle, which is produced in their digestive systems due to the way they eat.

Cattle production emits 65% of greenhouse gases, with 45% coming from meat production and 20% from milk production. The contribution of other types of domestic animals to the total gas emissions is significantly lower and ranges between 7 and 10%, with pig farming contributing 9%, buffalo meat and milk production 8%, chicken meat and egg production 8% and small ruminants 6% (Čengić -Dzomba and Dzomba, 2021).

Due to the specificity of the structure of the digestive system in ruminants, large quantities of gases are produced, which the animal expels into the external environment, and these gases have a negative effect on the environment. In order to preserve the environment, wherever possible, human efforts try to achieve a reduction in the production of greenhouse gases, and with the help of various strategies science wants to reduce the emission of methane in animal production. To help reduce methane emissions, animals should be provided with optimal conditions for housing and feeding conditions to increase their productivity and reduce methane emissions.

#### *Methane production in ruminants*

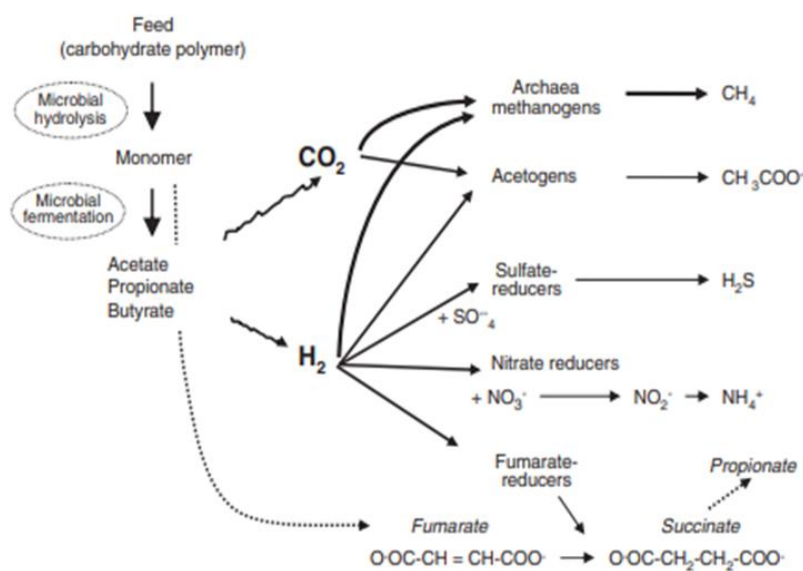
Methane is produced as a by-product during the decomposition of plant material in the rumen and large intestines and is mainly excreted from the body through belching (Figure 1). Another significant source of methane is the anaerobic microbial decomposition of manure. Its amount is significantly smaller and depends on the method of animal husbandry and manure storage (Čengić-Džomba and Džomba, 2021).



**Figure 1.** Fermentation process in the digestive system of cows (Source: <https://letstalkscience.ca/educational>)

The primary products of fermentation in the rumen include hydrogen and carbon dioxide. There is an excess of carbon dioxide in the rumen, as a

significant part is created in the buffering reaction of acidic fermentation products with bicarbonates of saliva. A large part of carbon dioxide reacts with hydrogen to produce methane, which represents the basic methanogenic reaction of the rumen (Figure 2). Bacteria that produce methane form a special class of microorganisms responsible for the regulation of the entire fermentation process in the rumen. They remove hydrogen from the contents of the rumen by reducing carbon dioxide with hydrogen to produce methane. In this way, they maintain the hydrogen concentration at a low level and contribute to the homeostasis of the rumen content, thereby enabling the growth of other types of bacteria and contributing to higher fermentation efficiency. So far, two types of active methanogenic bacteria have been identified: *Methanobacterium ruminantium*, a Gram-positive coccobacillus, and *Methanobacterium mobilis*, a Gram-negative ciliated coccidial rod. Both bacteria require a highly reduced medium for isolation and both use only hydrogen plus carbon dioxide or formic (methanoic) acid. In this way, formic acid, which is formed in primary fermentation, is converted into methane (Hamamdžić and Hodžić, 2010).



**Figure 2.** Methanogenesis – production of methane in the rumen (Morgavi et al., 2010)

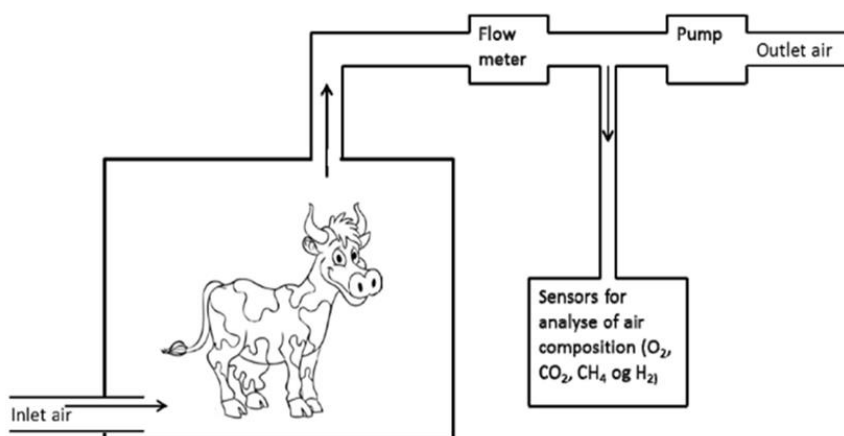
### *Determination of methane emissions*

In order to develop strategies to reduce methane emissions, it must be possible to determine the quantities of methane produced. For this purpose, various measurement methods have been developed, which have both their advantages and limitations. Open or closed breathing chambers, chambers



with controlled temperature and air humidity, simple chambers in which the microclimate is not controlled, mini chambers with methane sensors in which only the head of the animal is fixed, etc. are used to determine methane emissions. (CengiĆ-Džomba and Džomba, 2021).

Various chamber systems have been used over the last 100 years to study the energy balance of animals. The amount of methane produced by the animal is determined as the difference in the concentration of methane in the air entering the chamber and the concentration of methane leaving the chamber (Figure 3).



**Figure 3.** Open-type respiratory chamber (Storm et al., 2012)

In addition to the described *in vivo* methods, *in vitro* methods simulating the rumen fermentation of food in laboratory conditions are also used to measure methane production. The food is incubated with natural rumen fluid, buffers and minerals for a specified period at a temperature of 39°C. After this process, the amount of emitted gas and methane is measured, as well as the degree of food degradation in the rumen (ĆengiĆ-Džomba and Džomba, 2021).

## **Results**

### *Food strategies to reduce methane emissions*

1. Strategies aimed at improving feed quality and changing the structure of meals

Bulky forages - high quality forages, for example young plants, can reduce methane production as these forages contain higher amounts of easily fermentable carbohydrates and lower levels neutral detergent fiber (NDF), leading to higher digestibility and faster passage of food. Different types of

feed can also affect methane emissions due to differences in their chemical composition. The processing and preservation of feed also affects methane emissions. Grinding or pelleting forage can reduce methane emissions per unit of product because smaller particles require a shorter breakdown time in the rumen. Methanogenesis tends to be lower in silage because silage has already been partially fermented during the ensiling process. Also, methane emissions from animals fed grass silage are likely to be higher. However, by replacing grass silage with corn silage, the effect of reducing methane emissions is achieved. Maize silage or other small grain silage usually provides a higher dry matter content with easy digestibility carbohydrates, e.g. starch, which also reduces methane production in animals (Haque, 2018).

Concentrated feeds - increasing the proportion of concentrate feeds in diets tends to reduce methane production per unit of product. A high proportion of concentrate feed in meals increases the consumption of food and non-structural carbohydrates, the volume of fermentation in the rumen and the passage of food. During this change, there is also a change in the microbial population in the rumen in terms of an increase in the number of microorganisms that participate in starch fermentation, when there is an increased production of propionic acid. These fermentative processes in the rumen lead to a decrease in the amount of free hydrogen that enters the methanogenesis process and thus methane production is reduced (Čengić-Džomba and Džomba, 2021).

Fats -The addition of fats to feed has long been used to increase energy in rations to meet the energy needs of high-producing dairy cows. More recently, fats has been used to reduce methane emissions. If energy supplementation in ruminant diets is changed from carbohydrates to fats, then there will be less fermentation and methane production. By analyzing the results of a large number of studies, where the influence of fats and oils on methane production in the rumen of dairy cows, beef cattle, sheep and lambs examining 67 meals supplemented with different fats and oils, Martin et al. (2009) came to the following conclusions:

- the average reduction in methane production was 3.8% for each percentage of fat added;
- the source and length of the fatty acid chain have a significant impact on the level of methane production in the rumen. The largest decrease in methane production was determined by the addition of coconut oil with a high content of medium-chain fatty acids;
- a similar effect is achieved by adding, individually or in combination, myristic and palmitic acids;

- by adding soybean and sunflower oil as a source of polyunsaturated linoleic acid and linseed oil as a source of linolenic acid, methane production in the rumen decreased by 4.1% and 4.7%, respectively, for each percentage of added oil;
- by adding pork fat to meals, the average drop in methane production was 3.5% for each percentage of added fat, so adding pork fat in the amount of 12% reduced methane production by 30%;
- the addition of fish oil significantly reduced methane production in *in vitro* experiments, while in *in vivo* experiments such an effect was absent;
- in addition to the type of fat, the structure of the meal has an impact on the level of methanogenesis. A lower production of methane was found when animals were fed predominantly concentrated meals compared to predominantly voluminous rations;
- the mechanism of action of fatty acids on methanogenesis is multiple. Medium-chain fatty acids directly affect the number of methanogens.

In addition to the above strategies, the use of *Asparagopsis taxiformis*, which is a type of red algae, can significantly affect the reduction of methane. Research conducted on feedlot cattle with the addition *A. taxiformis* algae showed reductions in methane by 80%. The longest exposure to *A. taxiformis* algae was shown in bulls, and the study was completed after a 90-day period. To date, only three *in vivo* experiments using *Asparagopsis spp* have been published. in order to reduce methane emissions in the feedlot of Brangus cattle, lactating dairy cows and sheep. Experiments show significant but variable reductions in methane emissions. Differences in efficacy are likely due to the level of seaweed inclusion, diet formulation, and differences in seaweed quality based on bromoform concentrations (Roque et al., 2021).

## 2. Application of various supplements to meals

Ionophores that have a positive effect on milk and meat production, and also reduce methane production in the rumen. Recombinant bovine somatotropin indirectly reduces methane emissions by increasing animal productivity. The addition of growth hormone to meals increases milk production, but due to the possible development of clinical signs of mastitis, laminitis and shortened expression of estrus, somatotropins are banned in the European Union, Canada, Japan, Australia and New Zealand. Essential oils are also used, which inhibit the growth of some Gram-positive and Gram-negative bacteria and reduce the extent of deamination and methanogenesis in the rumen and methane production.

Organic acids such as fumarate, malate, citrate, succinate, etc. have a suppressive effect on methane production in the rumen. Probiotics that stimulate the growth of certain groups of microorganisms direct fermentation in the rumen towards the production of propionic acid. The mechanism of action of condensed tannins on rumen methanogens is still not fully understood. By feeding ruminants with feed rich in tannins, it is possible to reduce methane production by up to 55%.

Saponins have a suppressive effect or completely eliminate protozoa populations in the rumen. It has been found that saponins can reduce methane production by up to 50%. Chemical inhibitors of methanogenesis, such as bromochloromethane, 2-bromoethanesulfonate and chloroform, effectively inhibit methanogenesis (Čengić-Džomba and Džomba, 2021).

### 3. Application of different biotechnological methods

In recent years, in order to reduce methane emissions, research has been focused on the application of various biotechnological strategies (Table 1), which include:

**Table 1.** Different strategies for reducing methane emissions and the expected reduction in ruminants (Cherdthong, 2012)

Methane emission reduction strategy	Reduction mechanism	Reduction efficiency
Nutritional management Ratio of voluminous/concentrate food	By increasing the digestibility of voluminous feeds By replacing grass silage with corn silage Reduction of rumen pH	7 - 90%
Herbal composition Condensed tannins, saponins, essential oils	By limiting the availability of hydrogen	10 - 96%
Organic acids - malate, citrate, succinate	By adding more different organic acids, methanogenesis is more effectively inhibited	3 - 75%
Ionophores	Change of bacterial population from Gram positive to Gram negative bacteria	4 - 76%
Defaunation	Reduction of protozoa population	20 - 60%
Immunization and biological control Application of probiotics	Administration of vaccines that exclude certain types of methanogens	7 - 50%
Genetic selection	Genetic selection of animals to reduce methane emissions	

- immunization - includes the use of vaccines that target specific types of ruminal methanogens, which also reduces methane production;
- defaunation- refers to the reduction of the population of protozoa in the rumen and the number of methanogens related to protozoa, which ultimately results in reduced methane production;
- application of probiotics - adding microorganisms to animal meals has been practiced for many years, with the aim of producing lactic, acetic and propionic acid;
- genetic selection - animals that have the same nutritional conditions can show significant individual variations in methane production;
- genetic manipulation of the rumen microbiome – recently, efforts have been focused on characterizing the rumen microbiome and its function in order to implement nutritional and selective breeding strategies to alter it.

Both host microbiota and ruminal microbiota influence livestock traits such as efficiency and viability, including methane production, and are partially controlled by host genotype;

- genetic bioengineering of ruminants-production of transgenic domestic animals through genetic engineering to gain or lose certain gene functions.

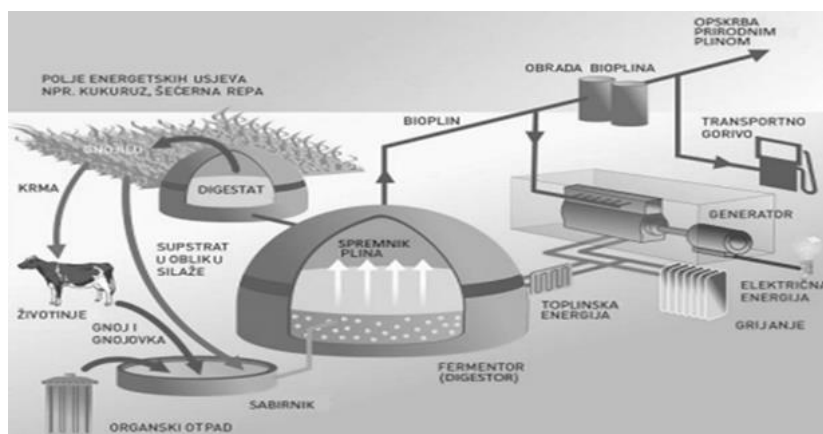
In the future, genetic engineering technologies will enable the understanding of the genetic traits of cattle and easily adapt them for biomedical applications in cattle (ČengiĆ-Džomba and Džomba, 2021).

**Biogas** - Biogas is a mixture of methane, carbon dioxide, nitrogen, hydrogen and hydrogen sulfide gases. The most important and to predominant component is methane, which also gives the energy value to biogas. Biogas is produced by the process of anaerobic digestion, in which various microorganisms break down complex organic compounds into simpler elements under anaerobic conditions. The final products of anaerobic digestion are:

- biogas – a high-value energy source that can be used to obtain other forms of energy (heat, electricity) and
- digestate – a by-product of digestion that can be used as a substitute for mineral and other organic fertilizers.

By producing biogas from manure, cattle farms can become significant energy producers and reduce greenhouse gas emissions by preventing methane from escaping into the atmosphere (Figure 4). Production begins with the process of anaerobic decomposition of biomass, where it is decomposed by bacteria (Tilhof, 2020). The decomposition process takes

place in four basic phases: hydrolysis, acidogenesis, acetogenesis and methanogenesis (Majkovčan, 2012).



**Figure 4.** Scheme of the biogas plant on the farm (Kuljak, 2022)

If farms, regardless of production capacity, had built biogas plants, sustainable waste management would be ensured for the purpose of cycle production processes and the conditions of animal husbandry, working environment and environmental protection would be improved.

### ***Conclusion***

Greenhouse gas emissions, especially methane, from animal production have a negative impact on the environment, and efforts are being made to reduce their concentrations to a minimum. The paper lists feeding strategies, the application of which leads to significant reductions in methane emissions. All of the mentioned strategies focus on improving the quality of meals, individualizing nutrition with precise determination of the needs of each animals, and adding additives, which directly affects the reduction of methane emissions. In addition to strategies related directly to nutrition, various biotechnological methods are also applied to achieve similar effects, such as immunization, genetic manipulation of the rumen microbiome, genetic selection and genetic bioengineering of ruminants. All the above-mentioned strategies will show their best potential through combination, working together in animals, with the fact that they must not compromise the health and productivity of the animals.

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# Nutritivne strategije za smanjenje emisije metana kod preživara

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## Sažetak

Metan (CH<sub>4</sub>) je najsnažniji staklenički plin koji zagađuje okolinu i atmosferu. Animalna proizvodnja, prvenstveno govedarstvo, imaju najveći uticaj na ukupnu količinu emisije metana. Proizvodnja metana kod goveda je rezultat njihovog specifičnog probavnog sistema, jer su u stanju da probavljaju hranu koju drugi sisari nisu u mogućnosti efikasno iskorištavati. Tokom procesa varenja i posredstvom mikroorganizama i enzima u buragu se stvara metan koji se izbacuje iz organizma i time zagađuje okolinu. Emisije metana od preživara razlikuju se u različitim dijelovima svijeta. Postoje i različiti faktori koji utiču na emisiju metana, kao što su vrsta hrane koja se koristi za hranjenje preživara, način držanja, klimatski uslovi i drugi faktori. Cilj ovoga rada jeste prikazati različite hranidbene strategije koje mogu uticati na smanjenje emisije metana kod preživara.

*Ključne riječi: emisija metana, preživari, ishrana*



**Cultivation of lettuce (*lactuca sativa l.*) in a vertical farming system**

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Corresponding author: [mirza.valjevac@ppf.unsa.ba](mailto:mirza.valjevac@ppf.unsa.ba)**Abstract**

The concepts of vertical farming are being developed in response to global urbanization and the need for sustainable urban development. Lettuce, due to its morphological and biological characteristics, is the most commonly grown vegetable using various vertical farming methods. The aim of this study was to examine, observe, and measure the differences in the morphological, productive, and qualitative characteristics of two popular lettuce hybrids in Bosnia and Herzegovina. The experiment was conducted in a vertical farming system on an area of 2.4 m<sup>2</sup>. The research included the green head lettuce hybrid *Tonale* and the red lettuce hybrid *Bacchus Vilmorin*. Sampling for analysis was carried out at the technological maturity stage of the lettuce. Differences in morphological parameters between the hybrids were not statistically significant. However, for productive parameters, the differences between the hybrids were statistically significant for average head weight (0.261–0.372 kg) and yield (3.56–5.948 kg/m<sup>2</sup>). The examined parameters and established differences in qualitative analyses did not have a statistically significant impact, except for dry matter content, which ranged from 5.986% to 7.126%.

*Keywords: lettuce, variety, yield, quality*

**Introduction**

Lettuce (*Lactuca sativa L.*) is an annual plant from the *Asteraceae* family, classified among yellow-green vegetables rich in biologically important substances, such as minerals, vitamins, and organic compounds with antioxidant effects. It is abundant in phosphorus, potassium, magnesium, zinc, iron, vitamins C and E, beta-carotene, and pigments. Organic acids, such as malic and citric acid, provide a pleasant flavour to the lettuce, while the bitter taste is due to *lactucin*, which has a calming effect. Incorrect application of nitrogen fertilisers and low light levels during winter can lead

to the accumulation of harmful nitrate (NO<sub>3</sub>), which may pose health risks (Shii *et al.*, 2022). In terms of economic significance, lettuce holds a prominent position among vegetable crops. It is cultivated through various methods, including soil-based, hydroponic, and aquaponic production, with vertical farming systems becoming increasingly popular in recent times.

Lettuce is particularly valuable during the winter months when fresh vegetables are scarce, making it a financially lucrative crop. It is grown from seedlings as the main, preceding, or subsequent crop, and in heated facilities, it can be cultivated year-round through successive planting (Filho *et al.*, 2018). Vertical farming is one of the most innovative and sustainable plant cultivation systems, although its origins date back to ancient Babylon and the famed Hanging Gardens (Ptak *et al.*, 2024). While this practice was neglected for a time, vertical farming experienced a revival in the early 21st century, witnessing significant growth and now being widely adopted globally, especially in urban areas of Africa, Europe, and the United States. This concept has addressed challenges posed by rapid urbanisation and the need to develop sustainable cities for the future. Faced with global issues such as environmental pollution, climate change, diminishing arable land, and increasing urbanisation, researchers and innovators have focused on developing systems that are environmentally friendly, energy-efficient, and sustainable. Vertical farming reduces water usage, optimises space, and significantly decreases the need for food transportation, thus directly reducing carbon dioxide emissions.

Furthermore, vertical farming systems play an important socio-ecological role. Not only do they contribute to biodiversity preservation and environmental protection, but they can also play a crucial role in promoting healthy eating habits, reducing hunger, and ensuring access to fresh food for the most vulnerable populations. These systems can be integrated into schools, communities, and urban farms, thereby strengthening local food production (Fasciolo *et al.*, 2024). Vertical farming is increasingly becoming a key factor in shaping a sustainable future, providing innovative solutions to the most pressing challenges faced by modern society. For successful lettuce production in vertical systems, it is crucial to ensure high-quality seedlings, an appropriate growing substrate, proper nutrition and irrigation, as well as other agronomic measures. The aim of the research was to explore the feasibility of lettuce cultivation in vertical systems and to determine whether it is possible to produce the same or a larger quantity of lettuce in a smaller space compared to traditional soil-based cultivation. Additionally, the study examined the influence of variety/hybrid on morphological and productive traits, as well as the content of vitamin C and nitrates in the edible parts of lettuce.

## *Experimental*

The materials used in this study consisted of two lettuce varieties: *Tonale* and *Bacchus* (Figure 1.) *Tonale* is a green lettuce variety, short-day type, suitable for winter and spring production in protected environments. It is classified as an early variety and does not form a head and has an attractive appearance with lighter green leaves and is resistant to cold. *Bacchus* is a *Lollo rosso*-type variety, with a mild flavour. Its leaves are dark red, making this variety highly decorative. Additionally, the leaves are thin, glossy, and form large heads. *Bacchus* is resistant to diseases such as downy mildew and is tolerant to lettuce aphid (*Nasonovia ribisnigri*), reducing the risk of lettuce mosaic virus infection.



**Figure 1.** *Lettuce varieties: Tonale and Bacchus*

The experiment was conducted on a vertical system mounted on the wall of the Faculty of Agriculture and Food Science, University of Sarajevo (2.5 m wide and 1.08 m high). Lettuce was produced from seedlings. The sowing was done in polystyrene containers using a sowing substrate. The seedlings were grown following standard vegetable-growing practices and preventive care measures. The lettuce seedlings were transplanted when they reached the 4-6 true leaf stage. Planting was done in pots of a size corresponding to

the holders of the vertical system. The total number of plants examined was 78 (46 *Tonale* plants and 32 *Bacchus* plants). The morphological, productive, and qualitative characteristics of the two varieties, *Tonale* and *Bacchus*, were studied in this experiment.

During the vegetation period, the diameter of the head was measured and recorded in centimetres. After harvest, quantitative parameters were observed, including the average head mass in grams, yield per square meter in kilograms, and the percentage of waste during harvest. In the laboratory of the Faculty of Agriculture and Food Sciences, the percentage of dry matter was also analysed.

**Dry Matter Content**—was determined by drying the lettuce leaves in an oven at 105°C until constant mass was achieved. The sample (3-5 g) was placed in a dry container with 20 g of quartz sand, dried for one hour, then cooled for 30 minutes in a desiccator and reweighed. The drying process lasted for 3 hours, and the cooling and weighing process was repeated until a constant mass was obtained.

$$\% \text{ dry matter} = \frac{c - a}{c - b} \times 100\%$$

- *a* – mass of the container, stick, and sand,
- *b* – mass of the container, stick, and sand with the sample,
- *c* – mass of the container and sample after drying.

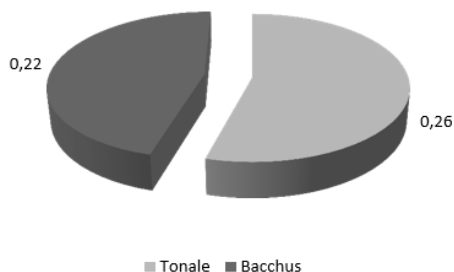
**Vitamin C Content in Lettuce Heads** – was determined using the titrimetric method with the indicator 2,6-dichlorophenolindophenol, known as Tillman’s reagent (AOAC, 2002). The method is based on the strong reducing properties of L-ascorbic acid, which reduces 2,6-dichlorophenolindophenol. This is visually observed by the colour change of the blue indicator to a colourless reduced form, which, in an acidic medium, turns pink, enabling the reaction to be tracked.

**Statistical Data Analysis** – was performed using the SPSS software package. Data were analysed using an independent t-test at a significance level of 0.05.

### ***Results and discussion***

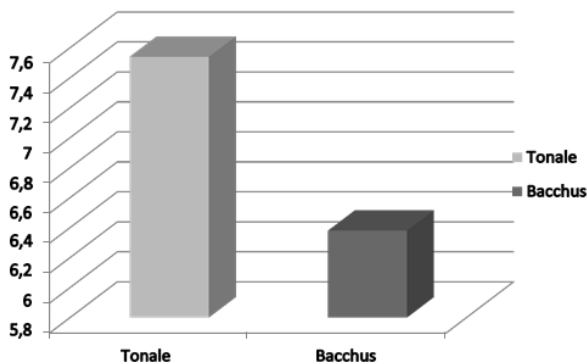
**Head Mass** – The average head mass represents the mean value of 30 mature heads measured at the technological maturity stage. The highest average mass was recorded in the *Tonale* variety, while the *Bacchus* hybrid had a

lower head mass. The average head masses for both varieties are shown in Graph 1.



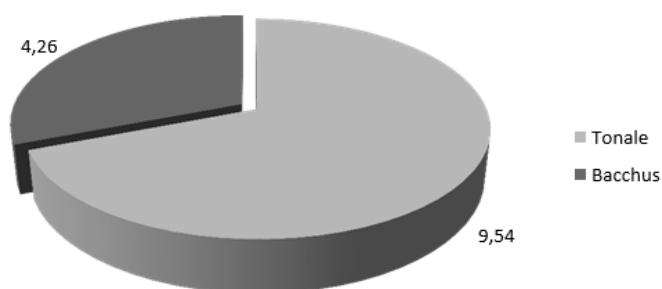
**Graph 1.** Average head mass expressed in kg

*Yield* – The yield of the studied varieties and hybrids was calculated based on the average head mass of the lettuce and the number of plants per square meter. The *Tonale* variety had a yield of 7.54 kg/m<sup>2</sup>, while the *Bacchus* hybrid had a slightly lower yield of 6.38 kg/m<sup>2</sup> (graph 2). The yields obtained from the lettuce grown in the vertical system are consistent with results from numerous scientific studies, where values for this parameter did not differ significantly from those in our research (Govedarica – Lučić and Perković, 2013; Custic *et al.*, 1994; Todorović *et al.*, 2012). In the vertical growing system, slightly higher yields were recorded, which can be attributed to the dense planting rather than the head size. There are studies that have shown significantly lower yields compared to ours, such as Franquera’s study (2016), where the yields of lettuce grown on mulch ranged from 0.67 to 1.49 kg/m<sup>2</sup>.



**Graph 2.** Lettuce yield in kg/m<sup>2</sup>

*Waste Amount During Harvest (%)* –The outer leaves of the lettuce that are damaged, diseased, or overripe are considered waste and must be removed after harvest before the product is offered on the market. The average waste amount (%) for the tested varieties is shown in graph 3. Although significance testing of differences was not conducted, averages per replication are provided. The *Bacchus* variety had a lower average waste amount (4.26%), while the *Tonale* variety had a significantly higher amount (9.54%). According to Toth (2006), losses during the preparation of lettuce on PE foil ranged from 16% to 27%, which are higher losses compared to this study. Therefore, the reduction in waste leaves can be considered an advantage of the vertical growing system.



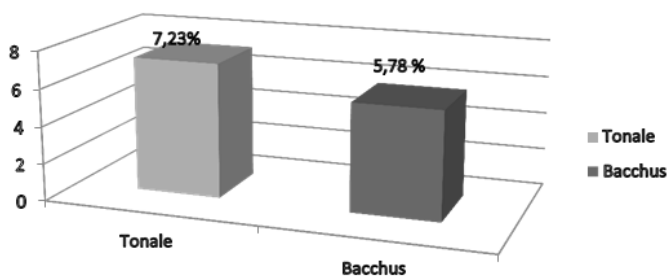
**Graph 3.** Average waste amount (%) by variety

*Head Diameter* – During the research, the head diameter of the studied lettuce varieties and hybrids ranged from 32.5 to 35 cm, as shown in table 1. The largest diameter was observed in the *Tonale* variety, averaging 35 cm, while the *Bacchus* hybrid had the smallest diameter of 32.5 cm (table 1). Results from other authors regarding this trait generally align with our analyses (Sharma et al., 2009; Toth, 2006). However, there are studies that recorded significantly larger or wider head diameters (Castoldi et al., 2014).

**Table 1.** Head diameter of the studied varieties/hybrids (cm)

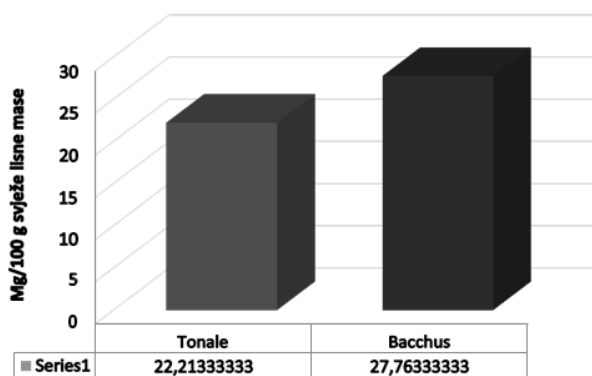
Measurement type (cm)	<i>Tonale</i>	<i>Bacchus</i>
I	40	35
II	37	31
III	31	34
IV	32	30
<b>Average</b>	<b>35</b>	<b>32.5</b>

*Dry Matter Content* – Shown in graph 4. The average dry matter content of the tested varieties ranged from 5.78% to 7.23%. The *Tonale* variety had the highest content, 7.23 g/100 g of fresh leaf mass (72.3 g/kg), while the *Bacchus* hybrid had a lower content of 5.78 g/kg. The results of this study are consistent with values from similar studies (Koudela and Petříková, 2008; Castoldi, 2014).



**Graph 4.** Dry matter content in %

*Vitamin C content* – the average values of vitamin C content in the analyzed varieties *Tonale* and the *Bacchus* hybrid are shown in graph 5. According to the data in graph 5, the *Bacchus* hybrid had the highest vitamin C content, averaging 27.76 mg per 100 g of fresh leaves, while the *Tonale* variety had a lower content of 22.21 mg per 100 g. Aćamović-Đoković et al. (2011) recorded significantly lower vitamin C values in green lettuce compared to our results.



**Graph 5.** Vitamin C content

## **Conclusion**

Based on the research objectives and the obtained results, it can be concluded that the variety had a significant impact on the quantitative parameters of lettuce, such as yield and average head weight, while its influence on qualitative traits was less pronounced. The *Tonale* variety demonstrated superiority in terms of average head weight (0.26 kg) and total yield (7.8 kg/m<sup>2</sup>), whereas the *Bacchus* hybrid, despite having a lower head weight (0.22 kg) and yield (6.35 kg/m<sup>2</sup>), recorded a smaller percentage of waste leaves (4.26%) compared to *Tonale* (9.54%). These findings suggest that, although *Bacchus* has a lower yield, it may be more economically favorable due to the reduced waste during harvest.

Head diameter also showed significant differences between the varieties, with *Tonale* having a larger average diameter (35 cm) compared to the *Bacchus* hybrid (32.5 cm). The examination of qualitative parameters, such as dry matter content and vitamin C levels, revealed variability between the varieties, with *Tonale* exhibiting a higher dry matter content (7.23 g/100 g), while the *Bacchus* hybrid had a higher vitamin C content (27.76 mg/100 g) compared to *Tonale* (22.21 mg/100 g). These results indicate that, although the *Tonale* variety performed better in terms of quantitative traits, *Bacchus* may offer advantages in terms of reduced harvest losses and nutritional composition.

**Author contributions:** Conceptualization: S.B., M.V., A.Š. and Dž.F.; methodology, S.B., M.V., A.Š.; investigation, S.B., M.V., A.Š. and Dž.F.; writing—original draft preparation, S.B., M.V.; writing—review and editing, S.B., M.V., A.Š., Dž.F.; visualization, S.B., A.Š. and Dž.F.; supervision, Dž.F.

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## Uzgoj salate (*Lactuca sativa L.*) u vertikalnom sistemu

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### Sažetak

Koncepti vertikalnog uzgoja hrane razvijaju se kao odgovor na globalnu urbanizaciju i potrebu za održivim razvojem gradova. Zelena salata, zahvaljujući svojim morfološkim i biološkim osobinama, najčešće je uzgajano povrće primjenom različitih metoda vertikalnog uzgoja. Cilj rada bio je ispitivanje, uočavanje i mjerenje razlika u morfološkim, produktivnim i kvalitativnim karakteristikama dva popularna hibrida salate u Bosni i Hercegovini. Ogled je postavljen u vertikalnom sistemu uzgoja na površini od 2.4 m<sup>2</sup>. U istraživanju su obuhvaćeni hibridi glavičaste zelene – *Tonale* i crvene salate *Bacchus Vilmorin*. Uzorkovanje za analize provedeno je u fazi tehnološke zrelosti salate. Razlike između hibrida u pogledu morfoloških parametara nisu bile statistički značajne. Kod produktivnih parametara, razlike među hibridima bile su statistički značajne za prosječnu masu glavice (0.261–0.372 kg) i prinos (3.56–5.948 kg/m<sup>2</sup>). Ispitivani parametri i utvrđene razlike u kvalitativnim analizama nisu imale značajan statistički utjecaj, osim sadržaja suhe materije koji se kretao u rasponu od 5.986 % do 7.126 %.

*Ključne riječi: salata, sorta, prinos, kvalitet*

2. TOKSIKOLOGIJA I SIGURNOST HRANE I OKOLIŠA  
FOOD AND ENVIRONMENT TOXICOLOGY AND SAFETY



## Intake Assessment of Nitrate and Nitrite Food Additives

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### Abstract

Nitrates and nitrites are added to processed meats to improve color, flavor, and shelf life. This research aimed to evaluate exposure to these additives in Bosnia and Herzegovina, considering demographic factors and associated health risks. An online survey was conducted among individuals aged 18 and older, with parental input for children. Additive intake was assessed through two scenarios: the first using maximum permitted concentrations and the second based on actual concentrations from literature. In Scenario 1, 94% of adults exceeded the acceptable daily intake (ADI) for nitrites, while nitrate intake was within limits. All children exceeded the ADI for nitrites, but their nitrate levels were safe. In Scenario 2, 4% of adults and 80% of children exceeded the ADI for nitrites. Significant differences in intake were noted based on sex and age, while other factors had minimal impact. The findings underscore the need for stricter regulation of these additives to mitigate health risks and highlight the necessity for further research into the factors affecting nitrate and nitrite intake.

*Keywords: additives, nitrates and nitrites, exposure, demographic factors, health risks*

### Introduction

Nitrates and nitrites play a crucial role as preservatives and antimicrobial agents in food preservation, particularly in meat and meat products. Nitrites (E249, E250) inhibit the growth of pathogenic microorganisms, such as *Clostridium botulinum*, while nitrates (E251, E252) act as a reservoir for nitrites, providing long-lasting protection. (Zhang, Zhang, & Jia, 2023) In addition to their antimicrobial action, nitrites preserve the colour of meat, prevent the oxidation of lipids and proteins, and contribute to the flavour of cured meat products. (FCEC, 2016) Due to potential health risks, their use is strictly regulated. The aim of this research is to assess the intake of nitrates and nitrites in the population of Bosnia and Herzegovina, as well as the relationship between socio-demographic factors and health risks associated with this intake.

## ***Experimental***

Market research in hypermarkets in Bosnia and Herzegovina identified meat products containing nitrates and nitrites, in accordance with relevant regulations. The research was conducted as a descriptive cross-sectional study using an online questionnaire. The assessment of intake was based on monitoring the consumption of meat products over a period of 7 days, allowing participants to accurately report quantities. Each question regarding meat products was accompanied by a photograph of a standardized portion of approximately 100 g to facilitate portion size estimation. Exposure to the additives was assessed according to two scenarios: (1) maximum permitted concentrations as outlined in the Food Additives Regulation. (Službeni glasnik BiH, 33/18, 2018) i (2) average concentrations from the literature. (Kovačević, Mastanjević, & Ćosić, 2016) When literature data were not available, assumptions were made regarding the reduction of concentrations. The calculations were based on the daily food intake, taking into account the body weight of the respondents and the conversion of nitrates to nitrites. The quantities of food items were expressed in ranges, with means used to calculate daily intake. The estimated daily intake (EDI) of nitrites was calculated by dividing the total intake by 7, and then multiplying by the maximum permitted concentrations. EDI was expressed per kilogram of body weight for a clearer picture of respondents' exposure. The analysis employed t-tests and ANOVA with post hoc Tukey tests, along with Bonferroni correction for multiple comparisons, using OpenEpi and GraphPad software.

## ***Results and Discussion***

The study involved 564 respondents, comprising 394 individuals aged 18 and older and 170 younger (<18 years) participants. Assessment of nitrate and nitrite intake revealed significant exposure among both adults and children.

### ***Scenario 1:***

Among adults, 94% exceeded the acceptable daily intake (ADI) of nitrites (0.07 mg/kg bw/day), with a mean intake of 0.1892 mg/kg bw/day, representing an increase of 270% to 500% above the ADI. In children, ADI was consistently exceeded across all age groups, with the highest intake in those aged 1-3 years, exceeding the ADI by up to 1700% (1.7682 mg/kg bw/day). Nitrates contributed less than 1% to total nitrite equivalents, and no significant difference was observed between the levels of exposure to nitrates and nitrites. These results were compared with studies conducted by EFSA

(EFSA, 2017a) and Dutch National Institute for Public Health and the Environment (RIVM) (Sprong, Niekerk, & Beukers, 2017), which also indicated high nitrite intake in certain groups, although the mean exposure values were lower compared to our study. EFSA specifically highlighted that sausages and canned meats are key sources of nitrites, which aligns with our findings. The contribution of nitrates in EFSA studies was also marginal. (EFSA, 2017b) Similarities were also observed in the results of other studies, (Mancini, Paul, & Gauvreaz, 2015) which also recorded exceedances of the ADI in young children. These results point to the need for cautious consideration of the intake of these additives in the diet, particularly among children, although it should be noted that maximum permitted concentrations were used, which may overestimate actual exposure.

#### *Scenario 2:*

In the second scenario, which utilized literature data on actual concentrations of nitrates and nitrites in meat products, a more realistic depiction of exposure was achieved compared to the first scenario. The estimated mean and high EDI were 0.0364 mg/kg bw/day and 0.0644 mg/kg bw/day, with 16 respondents exceeding the ADI. The highest recorded intake was 0.1213 mg/kg bw/day (approximately 170% of the ADI) among those consuming the tested foods several times a week. Total exposure to nitrites constituted 52% and 92% of the ADI for mean and high estimates, respectively, while nitrate intake remained within permitted limits. Statistical analysis revealed that all age groups of children exceeded the ADI for nitrites, although nitrate intake was acceptable. When analyzing nitrites together with nitrates, the number of respondents exceeding the ADI increased, except in the 6-12 age group. Mean and high levels of exposure to nitrates in children were 0.0779 mg/kg bw/day (111% ADI) and 0.1886 mg/kg bw/day (270% ADI). This suggests that while nitrate intake remains acceptable, it may contribute to higher nitrite intake. Data from EFSA (EFSA, 2017a; EFSA, 2017b) indicate higher mean and high exposure levels, likely due to methodological differences and a broader data range, while our study shows lower intake values, potentially reflecting market specificity and a limited data sample. Additionally, various European studies indicate higher nitrate intakes than those recorded in our research. Studies from Sweden (Larsson, Dernerud, & Ilback, 2011) have shown that the intake of nitrites from processed meat products can be a health concern, particularly among the youngest, highlighting a significant contribution from certain products, such as chicken sausages. The intake of nitrites from meat products among children in Serbia (Milešević, Vranić, & Gurinović, 2022) was lower compared to our results. While the average intakes were similar, a higher proportion of younger

children exceeded the recommended intake levels, which may be explained by their lower body weight.

### *Socio-demographic factors*

The analysis revealed significant differences in nitrite exposure between men and women, with men exhibiting nearly 1.5 times higher mean and high intakes. The age groups of women did not show significant differences in additive intake, while significant differences were found among men with the highest intakes observed in the 18-40 age group. Other socio-demographic factors, including monthly income, employment status, education level, marital status, and household size, did not have a significant impact on additive intake. Heat-treated meat products dominated as the largest contributors across all demographic groups, reflecting the dietary habits of the surveyed population in Bosnia and Herzegovina.

### *Health implications*

The use of nitrites and nitrates in processed meat raises concerns due to potential health risks, with some studies suggesting a link to certain forms of cancer. (Farvid, Sidahmed, & Spence, 2021) believe they may pose a health risk. Additionally, the results indicate that the majority of the population does not exceed the safety limits of the ADI, although 16 respondents exceeded the doses, potentially increasing the risk of health issues. Long-term exposure to these additives may be linked to the development of diseases, including colorectal cancer, (Zhu, Wang, & Green, 2014), gastric cancer (Song, Wu, & Guan, 2015) and pancreatic cancer. (Aschebrook-Kilfoy, Cross, & Stolzenberg-Solomon, 2011) It is also concerning that 70 children exceeded the ADI, further increasing the risk. While nitrates and nitrites are essential for health preservation and have potential benefits in regulating blood pressure and reproductive function, it is crucial to ensure their safe use to minimize harmful effects.

### *Conclusion*

Our research indicates that respondents may be exposed to excessive amounts of nitrates and nitrites, particularly when maximum concentrations are used, while actual concentrations provided a more realistic picture. Heat-processed meat products are the primary source of these additives. There is notable concern among respondents regarding their intake, although an understanding of the purpose of additives is present. There is a need to intensify education on the safe use of additives and clarify the difference between perception and reality regarding consumption. Differences in intake were observed based on gender and age, while other socio-demographic



factors had a lesser impact. It is important to ensure the safe use of nitrates and nitrites, despite their necessity for meat preservation, and to conduct further research for a better understanding of their health effects. Comprehensive epidemiological studies are needed to elucidate the complex interaction between these additives and health.

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## **Procjena dijetarnog unosa aditiva nitrata i nitrita**

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### **Sažetak**

Nitrati i nitriti se dodaju prerađenim mesima kako bi se poboljšali boja, ukus i trajnost. Ova istraživanja imala su za cilj procjenu izloženosti ovim aditivima u Bosni i Hercegovini, uzimajući u obzir demografske faktore i povezane zdravstvene rizike. Anketa je provedena online među osobama starijim od 18 godina, uz roditeljski doprinos za djecu. Unos aditiva procijenjen je kroz dva scenarija: prvi se oslanjao na maksimalno dozvoljene koncentracije, a drugi na stvarne koncentracije iz literature. U Scenariju 1, 94% odraslih je prekoračilo prihvatljivi dnevni unos (ADI) za nitrite, dok je unos nitrata bio unutar dozvoljenih granica. Sva djeca su prekoračila ADI za nitrite, ali su njihovi nivoi nitrata bili sigurni. U Scenariju 2, 4% odraslih i 80% djece prekoračilo je ADI za nitrite. Značajne razlike u unosu uočene su na osnovu pola i starosti, dok su drugi faktori imali minimalan uticaj. Nalazi naglašavaju potrebu za strožom regulacijom ovih aditiva kako bi se smanjili zdravstveni rizici i ističu neophodnost daljnjih istraživanja o faktorima koji utiču na unos nitrata i nitrita.

*Ključne riječi: aditivi, nitrati i nitriti, izloženost, demografski faktori, zdravstveni rizici*

## 2-O-2

### **The effect of hormones in meat and milk on human health**

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#### **Abstract**

Meat and milk are products of animal origin and the favorite food of most people. However, the use of growth promoters and artificial hormones calls into question their nutritional value and positive effect on human health. Cow's milk naturally contains steroid hormones, including glucocorticoids and sex hormones, and protein hormones, such as prolactin, insulin growth factor and bovine somatotropin. Growth promoters, including hormonal substances, are used legally and illegally all over the world in animals, and serve to accelerate their growth, as well as to increase the value of meat and reduce the cost of raising animals. Hormonal substances used to promote growth in cattle include natural steroids such as  $17\beta$ -estradiol, progesterone and testosterone, as well as synthetic compounds such as zeranol, trenbolone acetate and melengestrol acetate. Many of these substances have an extremely carcinogenic effect (breast, prostate and colon cancer), and lead to an early onset of puberty in children. Therefore, the aim of this paper was to present a link between the increased intake of meat, milk and dairy products and the increasingly frequent occurrence of the previously mentioned human health disorders. Since this kind of effect of the mentioned substances on human health has not been proven, further research is necessary to confirm or deny their harmful effects.

*Keywords: human health, hormones, meat, milk, breast and prostate cancer, early puberty*

#### **Introduction**

Since the 1960s, meat production across the world has grown five to six fold and milk production has increased almost three fold, which is disproportional to the human population. Therefore, the consumption of food of animal origin has been steadily increasing in the recent decades. In the same period, an incidence of prostate and breast cancer and a decrease of semen quality was reported (Snoj, 2019). This is why many studies correlate exposure to

hormones in meat and milk with these disorders. In this paper we aimed to review the effects of hormones in meat and milk on human health. As sex hormones are part of animal metabolism, any product deriving from an animal source will contain these hormones. On the other hand, hormones are extensively used in veterinary medicine, and there are three main purposes of their administration to animals: to cure sick animals, in reproductive disorders, synchronization, and control of oestrus and as growth promoters to increase milk production and improve animals' growth rate (Qaid and Abdoun, 2022).

Growth promoters are substances added to feed or implanted in animals to increase meat and milk production, which allows producers to meet population needs in a short time (Kamaly and Sharkawy, 2023). Besides hormones, growth promoters include antibiotics and hormone-like anabolics. They reduce the average amount of feed required by an animal and increase overall efficiency and profitability of meat and dairy industries (Hirpessa, Ulusoy and Hecer, 2020). The opinions among European countries and the United States towards hormonal usage is different.

The European Economic Community (EEC) banned the use of all hormones and hormone-like substances as growth promoters; on the other hand, the United States Food and Drug Administration (USFDA) has permitted the limited use of some natural origin hormones in animal fattening (Kamaly and Sharkawy, 2023). Hormones such as steroidal, nonsteroidal, semisynthetic, and synthetic drugs are used as growth promoters. However, this can lead to the occurrence of residues or metabolites of those hormones in the food of animal origin which can cause public health problems.

Much higher residue levels may appear in animal products when used unintentionally in overdoses or due to noncompliance with withdrawal periods (Hirpessa et al., 2020). Although relatively low doses of exogenous hormones enter the body by food, constant exposure to these compounds might induce cancer development (Snoj, 2019). The exposure and effect of hormone residues vary individually according to different factors, such as the consumer's eating habits, age, sex, and the concentration of hormone residues in food of animal origin as noted by Kamaly and Sharkawy (2023).

### ***Experimental***

Scirus, Google Scholar, Pubmed and Research Gate were searched for research articles and studies related to potential effects which milk, meat and their products can have on human health.

## ***Results and Discussion***

### *Hormones in milk and dairy products and their effects on human health*

There is a difference between cow's milk nowadays and the milk 100 years ago, which is mostly related to the length of lactation. Nowadays, standard lactation in dairy cows is 305 days, and at the end of lactation cows are in late pregnancy. During that period, the production of estrogens by the placenta is on a high level; consequently, milk contains a high concentration of estrogens (Zoppi, 2024). In contrast, 100 years ago, milk production was highly seasonal. Peak milk volumes occurred in the spring after calving and cows were not milked during the winter months, but 4 to 5 months after parturition when they were in an early stage of pregnancy or even not pregnant (Melnik, John and Schmitz, 2023). It cannot be established how much estrogens milk contained 100 years ago, but, due to shorter lactation, the concentration of the estrogens in milk was most likely lower than nowadays (Zoppi, 2024).

In pregnant cows, the predominant estrogen is estrone sulfate, which passes into milk by diffusion. Heat treatment does not affect estrone sulfate concentrations compared to unprocessed raw milk, as stated by Melnik et al. (2023). Cow's milk naturally contains a number of steroid and protein hormones in minute amounts. Steroid hormones include the glucocorticoids (cortisol and corticosterone) and the sex hormones – progesterone, 17 $\beta$ -estradiol, estrone, and estradiol. Protein hormones in milk include prolactin, gonadotropin-releasing hormone, insulin-like growth factor-1 (IGF-1) and bovine somatotropin (Rath, Sahoo and Chattopadhyay, 2018). The consumption of milk has been linked with an elevated risk of various cancers, particularly breast cancer and prostate cancer (Gao, Gong, Liu et al., 2024).

### *Bovine somatotropin*

Bovine somatotropin or bovine growth hormone (BGH) is a natural occurring protein produced in cows, whereas recombinant bovine growth hormone (rBGH) is a genetically engineered, synthetic version of the hormone (Venkatraman, Rajan, Divya et al., 2018). Bovine IGF-1 in milk is identical to human IGF-1 (Rath et al., 2018). rBGH is injected into dairy cattle to increase milk production. It increases the blood flow through the mammary gland, leads to production of large number of mammary cells and improves the ability of existing cells to synthesize more milk. Venkatraman et al. (2018) state that rBGH has been used in the United States for the past 50 years, and due to this the incidence of breast cancer in U.S. women was increased to one in eight women from one in twenty. rBGH may also cause the decreasing in average age of girls in the United States showing first signs

of puberty and menstruation. The use of rBGH in dairy cows is questionable from the ethic point of view since cows treated with rBGH tend to develop more health disorders such as clinical and subclinical mastitis, lameness, ketosis, and hypocalcaemia (Rath et al., 2018; Snoj, 2019). However, rBGH does not directly cause any side effects, but stimulates liver that initiates the release of IGF-1 which causes health issues in human beings (Venkatraman et al., 2018).

#### *Insuline-like growth factor 1 (IGF-1)*

IGF-1 can contribute to the development of tumors through anti-apoptosis effects and by stimulating cell proliferation. High levels of IGF-1 have been associated with increased risk of cancer of the colon, pancreas, endometrium and breast and prostate in particular (Zoppi, 2024). Prostate cancer has become the third most common cancer in men. Dairy products raise the concentrations of IGF-1, which is connected to the risk of developing prostate cancer (Sargsyan and Dubasi, 2021). A study was conducted showing a seven-time increased risk of breast cancer in pre-menopausal woman with high levels of IGF-1 in their blood and a four time increase of prostate cancer in men with high levels of IGF-1 in their blood (Venkatraman et al., 2018). Neither estradiol nor progesterone can act in the absence of IGF-1. Elevations of plasma IGF-1 concentrations by cow's milk intake may thus deviate sebaceous gland, eye growth, and bone homeostasis during puberty and displaying acne, early-onset juvenile myopia, pubertal skeletal overgrowth, as well as disturbing mammary gland maturation and potentially increasing the risk of breast cancer (Melnik et al., 2023).

#### *Estrogens*

Due to their strong biological activity and presence in several types of food of animal origin, estrogens are the most important agents in hormonal disruption (Snoj, 2019). The concentration of naturally occurring estrogens in food varies from species to species along with its age, gender and physiological status. Milk is considered to be one of the potent sources of steroids including estrogens. Estrogens are utilised in the body in the form of estradiol ( $17\alpha$  and  $17\beta$ -estradiol) and because it is more potent than other two physiologic estrogens, estrone and estriol. Rath et al. (2018) noted that the main estrogen in cow's milk is the biologically inactive  $17\beta$ -estradiol which is classified as a group I human carcinogen, according to the International Agency for Research on Cancer (IARC). It is followed by  $17\alpha$ -estradiol, which was evidenced as a mammary carcinogen and acts both as an initiator and promoter of breast carcinogenesis (Rath et al., 2018). As a promoter, estradiol binds estrogen receptors and elicits both cell proliferation and inhibition of apoptosis (Nachman and Smith, 2015). Many types of cells,

such as glandular mammary cells, ovary, uteri, and cervical cells, can respond to estrogen stimulation with differentiation and proliferation. It was observed that in 2020, 2.3 million women were diagnosed with breast cancer with 685,000 deaths globally (Melnik et al., 2023). A study has shown that more developed countries which have a higher intake of milk and dairy products, have a higher incidence of breast and uteri cancer compared to the developing countries, but it cannot be concluded that the western diet causes more risk for cancer, since data from developing countries are incomplete (Snoj, 2019). Estrogens may cause some disorders in the male reproductive system as well, but there isn't enough evidence to confirm this (Malekinejad and Rezabakhsh, 2015). Low-fat milk intake is related to a higher sperm concentration and progressive motility. Full-fat milk and cheese consumption is associated with lower sperm motility, since they contain relatively high amounts of steroid hormones which can affect hormonal homeostasis as was described by Snoj (2019).

Estrogen oxidative metabolites can be directly or indirectly genotoxic because they can bind and damage DNA directly and by other metabolites that can elicit DNA damage via oxidative stress (Rath et al., 2018). Potential exposure to exogenous estrogens is especially significant in regard to the prenatal and prepubertal development of the reproductive system in children, which leads to a decrease in the age of onset of pubertal development, especially in western countries during this century (Snoj, 2019; Andersson and Skakkebaek, 1999). If a child isn't yet producing growth hormones themselves, even small amounts of exogenous estrogens could interact with their hormonal system and disrupt its balance, and entering the child into puberty seven months earlier (Snoj, 2019; Zoppi, 2024). However, this isn't certain since early puberty can be caused by a number of other reasons, ranging from obesity and lack of exercise, to the consumption of a lot of processed food (Zoppi, 2024).

#### *Hormones in meat and meat products and their effect on human health*

Growth promoters are used legally and illegally worldwide in meat-producing animals for accelerating their growth, as well as to increase the worth of the meat and make animals cheaper to raise (Zoppi, 2024; Rath et al., 2018). These hormones are administered to cattle mainly as an ear implant. The hormonal substances used for growth promotion in cattle are the naturally occurring steroids such as 17 $\beta$ -estradiol, progesterone, and testosterone, as well as synthetic compounds such as zeranol, trenbolone acetate and melengestrol acetate which are classified as anabolic agents (Rath et al., 2018). Since steroids are highly fat-soluble, they are accumulated in tissues with high fat content (Snoj, 2019). However, the use of hormones in such animals can impact human health. Growth hormones



found in meat could have an effect on prepubescent children, in the same way as described in the previous section “Hormones in milk and dairy products and their effects on human health”.

### *Estrogens*

Diethylstilbestrol (DES) is a synthetic estrogen. In animals, DES was used as a growth promoter to improve feed conversion ability, growth rates, increased protein metabolism leading to daily gain and reduced fat. However, it was discovered that it has mutagenic, carcinogenic and teratogenic properties which have raised a widespread public health concern. Its use as a growth promoter in veterinary food products has been banned since 1979 in USA and 1981 in EU, as Rath et al. (2018) pointed out. More details on the effects of estrogen on human health can be found in the previous section “Hormones in milk and dairy products and their effects on human health”.

### *Progesterone*

Qaid and Abdoun (2022) observed that residue levels of progesterone in milk and tissues of administrated animals are under the daily production of the hormone, and are not considered to be a risk to human health. However, it was reported that progesterone increased the incidences of ovarian, uterine, and mammary tumors in mice as well as mammary gland tumors in dogs, which might indicate that progesterone could be considered as one of the potential causes of public health problems. On the other hand, in another study progesterone has shown no evidence of genotoxicity (Hirpessa et al., 2020). Melengestrol acetate (MGA) is a synthetic progesterone that is used as an animal feed additive to improve feed efficiency, increase the rate of weight gain, and suppress oestrus in beef heifers (Rath et al., 2018). It was discovered that it stimulates tumour formation in mammary glands (Venkatraman et al., 2018). MGA possesses reproductive toxicity as several research findings revealed mammary and endometrial hyperplasia in rats fed MGA at certain doses, and mammary carcinogenicity was found as well in studies of captive wild felids in which MGA was used as a contraceptive (Rath et al., 2018). Rath et al. (2018) remarks that prepubertal exposure to MGA accelerates the onset of puberty in the beef heifer.

### *Testosterone*

In cattle, testosterone propionate is metabolized to a form that is indistinguishable from endogenous testosterone. Endogenous testosterone may play a role in the occurrence of prostate cancer. However, testosterone might be aromatized to estradiol, which was found to be genotoxic as noted by Venkatraman et al. (2018). Studies have shown a connection between

circulating levels of testosterone and breast cancer risk, especially among postmenopausal women (Kamaly and Sharkawy, 2023). Conversely, an inverse relationship between circulating levels of the testosterone precursor dehydroepiandrosterone and risk of breast cancer has been reported in premenopausal women (Nachman and Smith, 2015). Nachman and Smith (2015) report that androgens antagonize estrogen-dependent cell growth in premenopausal women via one mechanism but stimulate cell growth in postmenopausal women via a different mechanism.

### *Zeranol*

Zeranol ( $\alpha$ -zearalanol) is a nonsteroidal, estrogenic mycotoxin produced by several *Fusarium* species which is used in livestock as a growth promoter (Rath et al., 2018; Lee, Jung and Han, 2018). It mimics estrogen activity through binding affinity to the estrogen receptor and thus may have endocrine-disrupting effects (Lee et al., 2018). Zeranol is a mammary carcinogen (Nachman and Smith, 2015). In the adult female, it can also induce alteration or suppression of ovarian cycles and endometrial hyperplasia. In the adult male, an increase in the plasma level of zeranol administered by ingestion or implants induces a decrease in testis, seminal vesicle, prostate weights and alterations of spermatogenesis as noted by Venkatraman et al. (2018).

### *Trenbolone*

Trenbolone is a synthetic androgen used for rapid muscle development in cattle (Lee et al., 2018). In cattle, trenbolone acetate (TBA) is metabolized to its most active form, 17 $\beta$ -trenbolone (TB), and then further metabolized to 17 $\alpha$ -trenbolone, which is also biologically active, and trendione, which is not biologically active (Nachman and Smith, 2015). TB exerts androgenic effects by binding to androgen receptors. The androgen receptor plays a critical role in the progression of prostate cancer. A study indicates that 17 TB promotes proliferation of the human androgen-dependent prostate cancer cells line (Lee et al., 2018). TBA appears not to be a genotoxic substance (Qaid and Abdoun, 2022). However, hazardous effects of TBA exposure were reported in the reproduction of both male and female mammals of various species (Venkatraman et al., 2018). In the adult male, TBA in direct administration induces a decrease in testis, seminal vesicle and prostate weights with alterations in spermatogenesis. In the adult female, such treatments induce virilization and alteration or suppression of ovarian cycles. In a study involving women volunteers given TBA showed disturbances of the menstruation cycle, as reported by Venkatraman et al. (2018).

## ***Conclusion***

In conclusion, the use of both natural and synthetic growth-promoting hormones in food-producing animals presents risks to both human and animal health since excessive levels of anabolics in food of animal origin might be harmful to the consumer. It can have carcinogenic effects, cause early puberty in children or decrease semen quality. This is why their use should be strictly controlled or completely forbidden, especially improper or illegal use of these hormones. However, since many of these hormones found in meat, milk and dairy products are normally present in animals and humans, it's quite difficult to differentiate the synthetic hormones from the natural ones. Also, the effects of these hormones which are ingested through meat and milk products can take a long time to present themselves, as they are consumed in very small amounts. Due to this, there is no definitive proof that hormones found in meat and dairy products can cause these human health disorders. Further research is needed to confirm or deny the role of exogenous hormones in public health, but also in farm animal health.

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## **Utjecaj hormona u mesu i mlijeku na zdravlje ljudi**

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### **Sažetak**

Meso i mlijeko su produkti animalnog porijekla i omiljena namirnica većine ljudi. No, upotreba promotora rasta i vještačkih hormona dovodi u pitanje njihovu nutritivnu vrijednost i pozitivan učinak na zdravlje ljudi. Kravlje mlijeko prirodno sadrži steroidne hormone gdje spadaju glukokortikoidi i spolni hormoni, te proteinske hormone, kao što su prolaktin, insulinski faktor rasta i goveđi somatotropin. Promotori rasta, uključujući i hormonske supstance koriste se legalno i ilegalno diljem svijeta kod životinja, te služe za ubrzavanje njihovog rasta, kao i za povećanje vrijednosti mesa i smanjenje cijene uzgoja životinja. Hormonske tvari koje se koriste za poticanje rasta kod goveda su prirodni steroidi kao što su  $17\beta$ -estradiol, progesteron i testosteron, kao i sintetski spojevi kao što su zeranol, trenbolon acetat i melengestrol acetat. Mnogi od ovih ovih tvari imaju izuzetno kancerogeno djelovanje (rak dojke, prostate i kolona), te dovode do ranog početka puberteta kod djece. Stoga je cilj ovog rada bio predstaviti poveznicu između povećanog unosa mesa, mlijeka i mliječnih proizvoda i sve češće pojave pomenutih poremećaja ljudskog zdravlja. Budući da ovakav uticaj navedenih supstanci na ljudsko zdravlje nije sigurno dokazan, neophodna su daljnja istraživanja da se potvrdi ili opovrgne njihovo štetno djelovanje.

*Ključne riječi: zdravlje ljudi, hormoni, meso, mlijeko, rak dojke i prostate, rani pubertet*

2-O-3

## **The influence of prenatal and postnatal exposure to heavy metals: transfer through the placenta and breast milk**

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### **Abstract**

Heavy metals like lead, mercury, cadmium, and arsenic are toxic elements that pose significant health risks, particularly during pregnancy and breastfeeding. These metals are pervasive in the environment and can enter the human body through contaminated food, water, air, and soil. Once in the body, they accumulate over time, making them difficult to eliminate. For pregnant and breastfeeding women, this is particularly concerning as heavy metals can cross the placenta during pregnancy and be passed through breast milk, exposing the fetus and infant to harmful substances at crucial stages of development. The placenta, though it serves as a barrier, cannot fully prevent heavy metals from reaching the fetus due to their chemical similarity to essential nutrients. Similarly, during breastfeeding, these metals can be transferred through breast milk, posing risks to newborns. Prenatal and postnatal exposure to heavy metals is linked to developmental issues, including neurological impairments, cognitive deficits, and weakened immune systems. Understanding the mechanisms of this transfer is critical for reducing exposure and improving health outcomes for children. The placenta, although a barrier, allows heavy metals to pass by mimicking essential minerals. Lead and cadmium use pathways typically reserved for calcium and zinc, resulting in harmful prenatal exposure. This can impair brain development, reduce IQ, and lead to attention and behavioral problems. Postnatally, breast milk can also transmit metals like mercury and lead, causing anemia, respiratory issues, and motor development delays. Additionally, studies show increased risks of congenital heart defects (CHDs) and autism spectrum disorder (ASD) in children exposed to heavy metals during early development.

*Keywords: heavy metals, maternal nutrition, diseases, placenta and breast milk*

## ***Introduction***

Heavy metals, such as lead, mercury, cadmium, and arsenic, are naturally occurring elements that pose significant health risks when humans are exposed to them in high concentrations. These metals are persistent in the environment and can enter the human body through various sources, including contaminated food, water, air, and even soil. Unlike other toxins, heavy metals do not break down easily in the body and tend to accumulate over time, which increases their potential for causing long-term damage. (Pajewska-Szmyt, M., et al. 2019.)

For pregnant and breastfeeding women, this is particularly concerning, as these metals can cross the placenta and be passed through breast milk, thereby exposing the developing fetus or infant to toxic substances at critical stages of growth. During pregnancy, the placenta, which functions as a protective barrier, can unfortunately allow some heavy metals to pass through due to their chemical similarity to essential nutrients. (Michael, T., et al., 2022). Similarly, postnatal exposure through breastfeeding can occur if a mother has accumulated these metals in her body. Exposure to heavy metals during these vulnerable periods is associated with a range of developmental issues in children, including cognitive deficits, neurological impairments, and weakened immune systems. Understanding the mechanisms by which heavy metals are transferred from mother to child is crucial for assessing the potential risks and implementing strategies to minimize exposure, thereby promoting healthier outcomes for both mother and child. (Samiee, F., & Vahidinia, A., 2019).

## ***Experimental***

This study was conducted as a bibliographic review, aiming to gather, analyze, and synthesize existing scientific literature on the influence of prenatal and postnatal exposure to heavy metals through maternal nutrition on children's development. The main focus was on understanding the mechanisms of heavy metal transfer through the placenta and breast milk and the associated health risks for children. The research was carried out by systematically searching for peer-reviewed articles, books, and relevant scientific reports published in reputable journals. Databases such as PubMed, ScienceDirect, and Google Scholar were used to retrieve studies that covered heavy metal exposure during pregnancy and lactation, the mechanisms of metal transfer to the fetus and infant, and the developmental outcomes in children. The keywords used for the search included "heavy metals," "prenatal exposure," "postnatal exposure," "placenta," "breast milk," "child development," "lead," "mercury," "cadmium," and "arsenic." The findings from the literature review were used to draw conclusions about the risks of

prenatal and postnatal heavy metal exposure and to identify gaps in current research that warrant further investigation.

### ***Results and Discussion***

The placenta, which acts as a barrier between the mother and the fetus, allows essential nutrients to pass through but, unfortunately, also permits toxic substances such as heavy metals. The mechanisms by which heavy metals cross the placenta involve transport proteins that normally carry essential minerals.

Due to similarities between heavy metals and essential elements like iron and zinc, heavy metals often use the same transport pathways. For instance, lead can use the same pathways as calcium, while cadmium may substitute for zinc in transport processes. Lead and mercury, for example, are well-known for disrupting brain and nervous system development, leading to cognitive impairments, reduced IQ, behavioral problems, and attention deficits (Wang, C., et al., 2022).

Cadmium can harm the kidneys and respiratory system, while arsenic is associated with genetic mutations and immune system dysfunction. In the postnatal period, breastfeeding is one of the primary routes through which a child can be exposed to heavy metals. While breastfeeding is crucial for the infant's growth and development, breast milk may contain traces of heavy metals that accumulate in the mother's body through food, water, and environmental exposure. For example, mercury can end up in breast milk due to the consumption of contaminated fish, while cadmium and lead can enter breast milk as a result of exposure to polluted air or water (Abadin HG, et al., 1997). Exposure to metals, specifically mercury and lead, during pregnancy significantly increased the risk of congenital heart defects (CHDs) in newborns. Mercury, in particular, played a major role in this effect, especially when the metal mixture concentrations were higher than the 40th percentile. The interaction between mercury and lead was also noted in lot of studies.

Mercury in particulate matter (PM<sub>2.5</sub>) between 10–25 weeks post-birth showed an increased risk of developing autism spectrum disorder (ASD). The risk of ASD is also higher among children with low birth weights, suggesting a compounding effect of early-life exposure to heavy metals. These metals can negatively affect the newborn's immune system, causing anemia, respiratory problems, and hindering brain and motor development. (Lin, H et al., 2023).

### ***Conclusion***

Exposure to heavy metals during both the prenatal and postnatal periods poses a significant threat to child development, with long-term consequences



for physical, neurological, and cognitive health. The ability of heavy metals like lead, mercury, cadmium, and arsenic to cross biological barriers, such as the placenta and mammary glands, allows these toxic substances to directly impact the developing fetus and newborn. The placenta, while a critical barrier, is not impermeable to these metals due to their chemical similarity to essential nutrients like calcium and zinc, which they mimic to gain access to the fetus. Similarly, breast milk, despite its numerous health benefits, can be a source of exposure to heavy metals if the mother has accumulated these elements through environmental sources such as contaminated food, water, or air. It is crucial to raise awareness among pregnant and breastfeeding women about the sources and dangers of heavy metal exposure. Public health interventions should focus on reducing environmental contamination, monitoring food and water quality, and providing nutritional advice that can help mitigate the absorption of heavy metals. Pregnant and lactating women should be encouraged to avoid high-risk sources of contamination, such as polluted environments and certain foods like mercury-laden fish.

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## **Utjecaj prenatalne i postnatalne izloženosti teškim metalima: prijenos kroz placentu i majčino mlijeko**

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### **Sažetak**

Teški metali kao što su olovo, živa, kadmij i arsen su toksični elementi koji predstavljaju značajne zdravstvene rizike, posebno tokom trudnoće i dojenja. Ovi metali su svuda prisutni u okolišu i mogu ući u ljudsko tijelo kroz zagađenu hranu, vodu, zrak i tlo. Jednom kada se nađu u tijelu, akumuliraju se tokom vremena, što otežava njihovo izbacivanje. Za trudnice i dojilje, ovo je posebno zabrinjavajuće jer teški metali mogu preći placentu tokom trudnoće i biti preneseni kroz majčino mlijeko, izlažući fetus i novorođenče štetnim supstancama u ključnim fazama razvoja. Placenta, iako služi kao barijera, ne može u potpunosti spriječiti prolazak teških metala do fetusa zbog njihove hemijske sličnosti sa esencijalnim nutrijentima. Slično tome, tokom dojenja, ovi metali se mogu prenositi kroz majčino mlijeko, predstavljajući rizik za novorođenčad. Prenatalna i postnatalna izloženost teškim metalima povezana je sa razvojnim problemima, uključujući neurološke poremećaje, kognitivne deficite i oslabljen imuni sistem. Razumijevanje mehanizama ovog prenosa je ključno za smanjenje izloženosti i poboljšanje zdravstvenih ishoda za djecu. Placenta, iako predstavlja barijeru, omogućava prolazak teških metala oponašajući esencijalne minerale. Olovo i kadmij koriste puteve koji su obično rezervisani za kalcijum i cink, što rezultira štetnom prenatalnom izloženošću. To može ometati razvoj mozga, smanjiti IQ i dovesti do problema s pažnjom i ponašanjem. Postnatalno, majčino mlijeko takođe može prenositi metale poput žive i olova, uzrokujući anemiju, respiratorne probleme i kašnjenja u motorčkom razvoju. Dodatno, istraživanja pokazuju povećane rizike od kongenitalnih srčanih mana (CHDs) i poremećaja iz spektra autizma (ASD) kod djece izložene teškim metalima tokom ranog razvoja.

*Ključne riječi: teški metali, prehrana majke, bolesti, placenta i majčino mlijeko*

## **Importance of antibiotic consumption monitoring in the veterinary medicine from One health perspective of antimicrobial resistance**

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### **Abstract**

The widespread use of antimicrobial veterinary medicine products (AMVMPs) drives antimicrobial resistance (AMR), global public health problem, with consequences for animal and human health. Global monitoring, as well as collecting of data on the consumption of antibiotics is essential to track progression of AMR. Furthermore, the very complex problem represents critically important antimicrobials (CIAs), given and listed by public and animal health. The aim of this research was to create quantitative review of antimicrobial sale compared to total VMPs used in animal therapy in Serbia in 2022, with special attention of the use of CIAs for human use in total AMVMPs. In Republic of Serbia, antimicrobial usage was estimated at 868.325 kg (35.34%), which is not acceptable from the standpoint of AMR. On the other side, the ratio between individual groups of CIAs and total AMVMPs is encouraging compared to the previous percentage, with the highest consumption of gentamicin (2.153%). Key strategies to fight against AMR are raising public awareness and rational use of antibiotics, both in human and veterinary medicine.

*Keywords: antimicrobials, antimicrobial resistance, global health, One health*

### **Introduction**

Antimicrobial resistance (AMR) represent global public health issue since around 2.8 million human cases of infections is caused by antimicrobial-resistant bacteria and 700,000 deaths are reported annually, and this number could reach 10 million by 2050. if AMR is not reduced (Conceição, Queiroga and Laranjo, 2023). The main drivers of spread and development of AMR are inappropriate and excessive use of antimicrobials, as well as gene transmission between humans, animals and environment (Dadgostar, 2019). With the intention to acquire the information on the progression of resistance, since there is well entrenched connection between antibiotics use and resistance both in humans and food-producing animals (Kovačević,

Samardžija, Horvat, et al., 2022), (Rahman and Hollis, 2023), so collection of data on the consumption of antibiotics is essential (Mugoša, Vračar, Kovačević, et al., 2021). Furthermore, public and animal health organizations have created competing lists of critically important antimicrobials (CIAs) where the drugs are classified according to their importance in human and veterinary medicine (Scott, Acuff, Bergeron, et al., 2019). Across the European Union (EU), The European Surveillance of Veterinary Antimicrobial Consumption (ESVAC) project gathers information of antimicrobial exploiting in animals (European medicines agency, 2020). Since Serbia is not part of ESVAC, the authority to fulfil collecting and processing data on consumption of veterinary medicine products (VMPs), has ALIMs (Medicines and Medical Devices Agency of Serbia).

Therefore, the aim of this study was to create quantitative evidence of antimicrobial veterinary medicine products (AMVMPs) sale used in animal treatment in Serbia in 2022, compared to total VMPs sales in the same period, with special attention of the use of CIAs for human use in total of AMVMPs.

### ***Experimental***

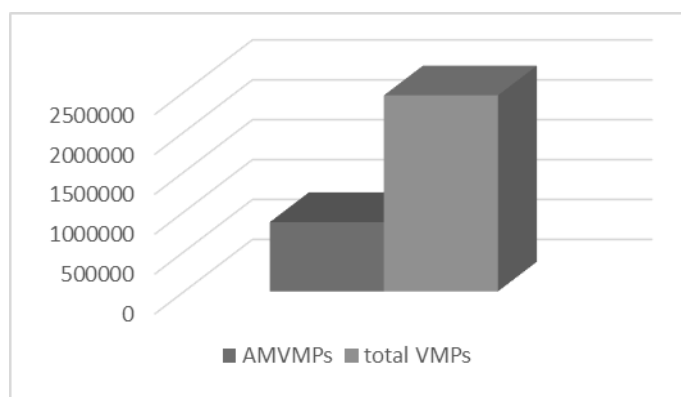
Information about the consumption of AMVMPs, was acquired from the annual reports of the ALIMs for a one year period (from 2021 to 2022). In the database of the ALIMs, veterinary drugs are classified according to the classification presented by WHO (World Health Organization) by anatomic-therapeutic-chemical veterinary groups (ATCvet), as well as by international non-protected names (INNs). In order to monitor the consumption of antibiotics and quantitative evidence from the provided reports, the following types of data were used: sales of veterinary medicines according to the ATCvet classification from publication “Trade of veterinary medicines 2021-2022” (ALIMs, 2022) and the contribution of therapeutic-pharmacological groups achieved in 2022. Based on the previous reports, the data on total consumption of VMPs were expressed as a total amount in kilograms (kg).

### ***Results and Discussion***

There are gaps in monitoring antimicrobial usage in veterinary medicine in analysis of its impact on emergence of AMR in Serbia. Analyzing the reports “Trade of veterinary medicines for the period from 2021 to 2022” (ALIMs, 2022) it was observed that the consumption of AMVMPs in the Republic of Serbia is substantial, estimated at 868.325 kg. It represents 35.34% of overall VMPs sales in kilograms in 2022 (Figure 1). From the perspective of AMR, this data is not encouraging, as a reduction in the consumption of AMVMPs could contribute to its decrease. The data gathered in this way are crucial for

risk analysis, providing better oversight and evaluating the effectiveness of rational antibiotics use principles (WHO, 2001).

Special attention in this study was given to CIAs for human medicine since in some cases antibiotics intended for use in humans are used to treat pets („off-label“ use), while the reasons for this choice are not only the absence of a VMPs, but also the packaging size and formulation of the drug (Hölsö, Rantala, Lillas, et al., 2005). Some human approved antimicrobials are critical for preventing or treating life-threatening infections in humans (Tomanic, Stojanovic, Belić, et al. 2021). Beside, CIAs for human medicine are used for treatment of animals.

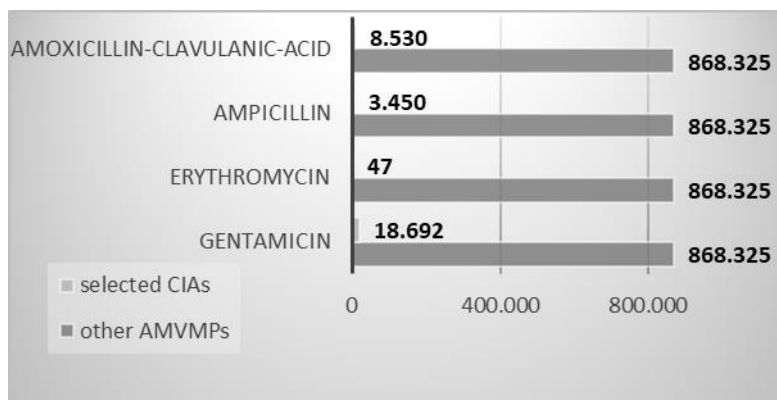


**Figure 1.** The ratio of AMVMPs in the overall veterinary medicines products sales in kilograms (kg) in 2022

**Table 1.** CIAs for human medicine in 2022. presented as quantity of sold medicines in 2022. from „Trade of veterinary medicines 2021-2022“ (ALIMS, 2022)<sup>10</sup>

Pharmacological group	ATCvet	International Nonproprietary Name (INN)	(kg)	(%)
Aminoglycosides	QJ01GB03	gentamicin	18692	2.153
Macrolides and ketolides	QJ01FA01	erythromycin	47	0.005
Penicillins (aminopenicillins)	QJ51CA01	ampicillin	3450	0.397
Penicillins (aminopenicillin with beta-lactamase inhibitors)	QJ01CR02	amoxicillin-clavulanic-acid	8530	0.982

Analyzing the share of CIAs in relation to the total trade of VMPs, the results showed that individual groups of antibiotics used in human medicine, although utilized in veterinary practice, exhibit a low percentage. The largest share is in the sales of gentamicin, with a percentage of 2.153%, while erythromycin amount is 0.005% (Table 1). These results might seem harmless, but it is still a pose threat since frequent use of human antibiotics for animals further complicate the fight against AMR (Figure 2).



**Figure 2.** The ratio of selected CIAs in the overall AMVMPs sales in kilograms (kg) in 2022

From all of the above, it is clear that a complete insight into antibiotic consumption in the VMPs in the Republic of Serbia could be achieved only if we could monitor not only the trade of AMVMPs but also the antibiotic utilization data by animal species, prescribing habits of veterinarians, particularly the use of antibiotics from the human medicine for the treatment of animals.

### ***Conclusion***

In conclusion, the use of CIAs in both human and veterinary medicine poses a significant risk for the development of AMR. One of the key strategies to combat AMR recommended by organizations that are dealing with human and animal health worldwide is the reduction and potential limitation of antimicrobial use in veterinary medicine since humans, animals and environment are interconnected under the One health approach. Achieving rational antibiotic use and raising public awareness about AMR are key strategies to mitigate this global health threat in the future.

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## Važnost praćenja potrošnje antibiotika u veterinarskoj medicini iz perspektive Jednog zdravlja antimikrobne rezistencije

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### Sažetak

Široka upotreba antimikrobnih veterinarskih proizvoda (AMVMPs) dovodi do nastanka antimikrobne rezistencije (AMR), globalnog zdravstvenog problema koji ima posledice za zdravlje životinja i ljudi. Globalno praćenje, kao i prikupljanje podataka o potrošnji antibiotika, od suštinskog je značaja za praćenje napredovanja AMR-a. Ovaj složen problem obuhvata "kritično važna antimikrobna sredstva" (CIAs), koja se navode od strane javnih i veterinarskih organizacija. Cilj ovog istraživanja bio je da se izradi kvantitativni pregled prodaje antimikrobnih sredstava u poređenju sa ukupnim VMPs koji se koriste u terapiji životinja u Srbiji 2022. godine, sa posebnim osvrtom na upotrebu CIAs za ljudsku upotrebu u ukupnim AMVMPs. U Republici Srbiji, procenjeno je da je potrošnja antimikrobnih sredstava iznosila 868.325 kg (35,34%), što nije prihvatljivo sa stanovišta AMR-a. S druge strane, odnos između pojedinačnih grupa CIAs i ukupnih AMVMPs je ohrabrujući u poređenju sa prethodnim procentima, pri čemu je najviša potrošnja gentamicina (2,153%). Ključne strategije u borbi protiv antimikrobne otpornosti uključuju podizanje svesti javnosti i racionalnu upotrebu antibiotika, kako u ljudskoj, tako i u veterinarskoj medicini.

*Ključne reči: antimikrobni proizvodi, antimikrobna rezistencija, globalno zdravlje, Jedno zdravlje*



3. ISHRANA TOKOM ŽIVOTNOG CIKLUSA  
NUTRITION THROUGH THE LIFECYCLE



## Assessment of vitamin B<sub>2</sub> intake in the adult population of Sarajevo Canton

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### Abstract

Riboflavin is a water-soluble member of the B-vitamin family. Research on the assessment of vitamin B<sub>2</sub> intake among the adult population of Sarajevo Canton aims to provide a comprehensive insight into the dietary habits and nutritional status of respondents. The main objectives include the estimation of the average intake of vitamin B<sub>2</sub> and the identification of the main sources of this vitamin in the diet of the subjects. Variations in vitamin B<sub>2</sub> intake according to gender, age, and lifestyle will be analyzed, along with investigating possible connections between dietary habits and vitamin B<sub>2</sub> levels. This study also focuses on assessing awareness of the importance of vitamin B<sub>2</sub> in the diet and recognizing the need for education about dietary habits, especially if the results indicate an insufficient intake of this vitamin. The research reveals a lack of awareness about the symptoms of vitamin B<sub>2</sub> deficiency and varying knowledge about its benefits among respondents. Promoting the use of food supplements and regular consumption of vitamin B<sub>2</sub>-rich foods are suggested to improve overall intake. Strategies to enhance awareness and proper intake of vitamin B<sub>2</sub> in the daily diet of the adult population of Sarajevo Canton are recommended based on the findings.

*Keywords: riboflavin (vitamin B<sub>2</sub>), Sarajevo Canton, diet, intake, sources*

### Introduction

Riboflavin (vitamin B<sub>2</sub>) was first documented as a yellow pigment found in milk. (Suwannasom, 2020) Milk and other dairy products make the greatest contributions of riboflavin. Other common dietary sources include almonds, organ meats, whole grain, wild rice, mushrooms, soybeans, yogurt, eggs, broccoli, brussels sprouts and spinach. Most dietary riboflavin is ingested as food protein. In the stomach, gastric acidity cleaves most of the coenzyme forms of riboflavin (FAD and FMN) from the protein. The coenzymes are then hydrolyzed to riboflavin by pyrophosphatases and phosphatases in the

upper intestine. Primary absorption of riboflavin occurs in the proximal small intestine via a rapid, active and saturable transport system (Ccd, 2022). Riboflavin plays an important role in the antioxidant status inside cell systems as well as being part of the glutathione reductase and xanthine oxidase system. Oxidative stress plays a crucial functional role in the pathogenesis of various human disease states including ischemia, diabetes, angina pectoris and degenerative diseases. In addition, the modulation of blood pressure is linked to this vitamin. As a proteasome inhibitor, riboflavin suppresses the production of TNF -  $\alpha$  and NO.

In recent years, there has been much interest in the antinociceptive and anti-inflammatory effects of riboflavin. Therefore, riboflavin might represent a promising new therapeutic strategy for the treatment of sepsis and septic shock. There have been several articles reporting the results of randomized controlled trials of riboflavin on the risk of cancer incidence. (Suwannasom, 2020)

Research has shown that riboflavin deficiency causes: anaemia, cataracts, cleft lip and palate deformities, fatigue, migraines, malignancies, night blindness, peripheral neuropathy and red, itchy eyes. (Mahabadi, 2023) From the aspect of etiology, primary riboflavin deficiency results from inadequate intake of fortified cereals, milk and other animal products.

Secondary, riboflavin deficiency is most commonly caused by the following: chronic diarrhea, malabsorption syndromes, liver disorders, hemodialysis, peritoneal dialysis, long-term use of barbiturates and chronic alcohol use disorder. Diagnosis of riboflavin deficiency can be confirmed by a therapeutic trial or laboratory testing, usually by measuring the urinary excretion of riboflavin. Riboflavin 5 to 30 mg orally once daily in divided doses is given until recovery. Other water- soluble vitamins should also be given to patients. (Johnson, 2024)

Research on the assessment of vitamin B<sub>2</sub> intake among the adult population of Sarajevo Canton aims to create a comprehensive insight into the dietary habits and nutritional status of the respondents. The main objectives include the estimation of the average intake of vitamin B<sub>2</sub> and the identification of the main sources of this vitamin in the diet of the subjects. We also want to analyze variations in vitamin B<sub>2</sub> intake according to gender, age and lifestyle, and investigate possible connections between dietary habits and vitamin B<sub>2</sub> levels.

In addition, the research will focus on assessing the respondents' awareness of the importance of vitamin B<sub>2</sub> in the diet and recognizing the need for education about dietary habits, especially if the results indicate an insufficient intake of this vitamin. All this information can serve as a basis

for recommendations and interventions aimed at improving the eating habits and health of the population in Sarajevo Canton.

### ***Experimental***

In order to achieve the stated goals, literature data from relevant scientific - research works, as well as professional books, manuals and relevant articles were used. Medical databases (Medline) and Internet browsers (Google Scholar, PubMed, etc.) were used for the search. Research materials and methods included the adult population of Sarajevo Canton.

The research participants voluntarily agreed to fill out an online survey, which was available in the form of Google Forms. Distribution of the survey was conducted through different locations to ensure sample diversity. In order to preserve the anonymity and privacy of the respondents, all information collected through the survey was treated confidentially.

The analysis of the collected data was carried out with the help of appropriate statistical methods, and the results were interpreted to gain insight into the dietary habits and assessment of vitamin B<sub>2</sub> intake among the respondents.

### ***Results and discussion***

122 adults from Sarajevo Canton participated in the survey. Demographic data included gender and age. Therefore, 83.3% of respondents were female and 16.7% were male. The largest percentage of respondents were aged 18 - 25 (79.2%), and only 3 people answered that they were over 50 years old.

The following question in the survey related to the number of meals during the day. About half of the respondents answered that they have three meals a day, 37.5% of the respondents that they have two meals a day, and 8.3% of the respondents have more than three meals a day. The next question is about how often they consume milk and dairy products during the week. Today, we rightly say that milk is the richest source of vitamin B<sub>2</sub>.

*Table 1. Assessment of vitamin B<sub>2</sub> intake in adult population of Sarajevo*

<b>Assessment of vitamin B<sub>2</sub> intake in the adult population of Sarajevo Canton</b>				
<b>Total number of participants</b>				
122				
<b>Female</b>		<b>Male</b>		
83.3%		16.7%		
<b>The number of meals during one day</b>				
<b>Two meals</b>		<b>Three meals</b>		<b>More than three meals</b>
37.5%		54.2%		8.3%
<b>Weekly consumption of milk and dairy products</b>				
<b>1 – 2 × a week</b>	<b>2 – 3 × a week</b>	<b>4 – 5 × a week</b>	<b>Every day</b>	<b>Not consume</b>
33.3%	25.0%	20.8%	16.7%	4.2%
<b>Weekly consumption of eggs</b>				
<b>1 – 2 × a week</b>	<b>2 – 3 × a week</b>	<b>4 – 5 × a week</b>	<b>Every day</b>	<b>Not consume</b>
37.5%	29.2%	20.8%	8.3%	4.2%
<b>Consumption of supplementation</b>				
<b>Yes</b>		<b>No</b>		
41.7%		58.3%		
<b>Supplements containing vitamin B<sub>2</sub></b>				
<b>Yes</b>		<b>No</b>		
32.8%		67.2%		
<b>Knowledge about benefits of vitamin B<sub>2</sub></b>				
<b>Informed about benefits</b>		<b>Partially informed about benefits</b>		<b>Not informed about benefits</b>
58.3%		25.0%		16.7%
<b>Symptoms of vitamin B<sub>2</sub> deficiency</b>				
<b>Yes</b>		<b>No</b>		
58.2%		41.8%		
<b>Knowledge about symptoms were actually a sign of vitamin B<sub>2</sub> deficiency</b>				
<b>Informed about symptoms</b>		<b>Not informed about symptoms</b>		
62.5%		38.5%		

From the Table 1, it can be seen that 33.3% of the respondents consume milk 1 – 2 times a week, 25.0% of the respondents 2 – 3 times a week, and 20.8% of the respondents consume milk 4 – 5 times a week. 16.7% of respondents consume milk every day, but 4.2% of respondents do not consume milk. The next question was about the consumption of eggs during the week, where 37.5% of respondents answered that they consume eggs once or twice a week, 27.2% of respondents answered that they consume eggs 2 - 3 times a week, then 20.8% of respondents 4 - 5 times a week, and only 8.3% of respondents consume eggs daily. 4,2 % of respondents do not consume eggs. The following questions related to the use of nutritional supplements, including vitamin B<sub>2</sub>. When asked whether they use any nutritional supplements, 58.3% of the 122 respondents responded negatively. 41.7% of



respondents answered that they consume food supplements, but only 32.8% of respondents out of 122 answered that they consume food supplements that contain vitamin B<sub>2</sub>. The other respondents answered negatively.

The last three questions in the survey were about knowledge about riboflavin. Therefore, 58.3% of the respondents answered that they know what the benefits of this vitamin are, 25.0% of them know something about the said vitamin, and 16.7% of the respondents are not familiar with the benefits of taking this vitamin.

Then the respondents answered the question “Have they had the symptoms mentioned so far, such as a general feeling of weakness, pain in the throat or tongue, cracks on the edge of the lips, irritable skin and the like”. 58.2% of respondents gave a positive answer to this question, and 41.7% answered 'No'. Despite the visible symptoms of vitamin B<sub>2</sub> deficiency, as many as 62.5% of respondents did not know that the listed symptoms were actually a sign of riboflavin deficiency.

### ***Conclusion***

The research conducted on the adult population of Sarajevo Canton on the assessment of vitamin B<sub>2</sub> intake reveals important information about the awareness, knowledge and behavior of the respondents regarding this vitamin. Based on the conducted survey, it is observed that there is a lack of awareness about the symptoms of vitamin B<sub>2</sub> deficiency among the respondents, considering that 62.5% of the respondents did not recognize the mentioned symptoms as a sign of riboflavin deficiency. Different knowledge among respondents about the benefits of vitamin B<sub>2</sub> is also noticed. This result indicates the need for education to improve understanding of the importance of vitamin B<sub>2</sub>.

Regarding the use of food supplements, the results indicate the possibility of promoting the use of food supplements to meet the recommended daily doses. Analyzing the consumption of eggs and milk, one notices the variation in habits among the respondents. Regarding the distribution of meals during the day, diversity is observed among respondents, with half of them having three meals a day. Promoting the proper distribution of meals can support the balance of vitamin B<sub>2</sub> intake.

In conclusion, this research highlights the importance of educating the population about vitamin B<sub>2</sub>, and the need to promote regular consumption of foods rich in this vitamin. It also suggests the need for strategies that will improve awareness and proper intake of vitamin B<sub>2</sub> in the daily diet of the adult population of Sarajevo Canton.

**Author contributions:** conceptualization, M. A. and L. D.; methodology, A. K.; investigation, M. K.; writing – preparation of original draft, M. A.; writing review and editing, E. M.; visualisation, S. M.; supervision, M. A. and L. D. All authors have read and agreed to the published version of the proceeding.

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## **Procjena unosa vitamina B<sub>2</sub> u odrasloj populaciji Kantona Sarajevo**

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### **Sažetak**

Riboflavin je vodotopivi član porodice B - vitamina. Istraživanje o procjeni unosa vitamina B<sub>2</sub> među odraslom populacijom Kantona Sarajevo ima za cilj stvoriti sveobuhvatan uvid u prehrabene navike i nutritivni status ispitanika. Glavni ciljevi uključuju procjenu prosječnog unosa vitamina B<sub>2</sub> i identifikaciju glavnih izvora ovog vitamina u ishrani ispitanika. Analizirat će se varijacije u unosu vitamina B<sub>2</sub> prema spolu, dobi i načinu života, uz istraživanje mogućih veza između prehrabnenih navika i nivoa vitamina B<sub>2</sub>. Studija se također fokusira na procjenu svijesti o važnosti vitamina B<sub>2</sub> u ishrani i prepoznavanje potrebe za edukacijom o prehrabnenim navikama, posebno ako rezultati ukazuju na nedovoljan unos ovog vitamina. Istraživanje otkriva nedostatak svijesti o simptomima nedostatka vitamina B<sub>2</sub> i različita znanja o njegovim prednostima među ispitanicima. Predlaže se promoviranje upotrebe dodataka prehrani i redovna konzumacija hrane bogate vitaminom B<sub>2</sub> kako bi se poboljšao ukupni unos. Na osnovu rezultata istraživanja preporučuju se strategije za jačanje svijesti i pravilnog unosa vitamina B<sub>2</sub> u svakodnevnu prehranu odrasle populacije Kantona Sarajevo.

*Ključne riječi: riboflavin (vitamin B<sub>2</sub>), Sarajevo Kanton, dijeta, unos; izvori*

### 3-O-2

## **Assessment of dietary intake of n-3 polyunsaturated fatty acids In Bosnia and Herzegovina adult population**

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### **Abstract**

The purpose of the research is to assess the intake of omega-3 fatty acids in the population, in order to gain insight into the quantities and how often foods that are rich or enriched with this nutrient are consumed. The results were compared with the results of the last research on the assessment of the intake of omega-3 fatty acids in the territory of Bosnia and Herzegovina. The intake of fish, walnuts, flax, chia seeds, linseed oil, rapeseed oil, and margarine was tested. The results show that only 6.5% of respondents consume fish in accordance with the recommendations of 2-3 meals a week. Dietary intake of EPA+DHA meets the recommended amount in 56.3% of man and 64.8% of women participants. An insufficient intake of alpha-linolenic acid (ALA) has been recorded in men, while more than half of women meet an adequate intake for ALA. Use of omega-3 fatty acids as a dietary supplement are more prevalent in women, while men consume them less.

*Keywords: omega-3 fatty acids, EPA, DHA, ALA*

### **Introduction**

The Adequate Intake (AI) for men is 1.6g ALA/day, while for women it is 1.1g ALA/day at the age of 19 to 50 years. Up to 10% AI for ALA can provide EPA (eicosapentaenoic acid) or DHA (docosahexaenoic acid) (Gebauer Sarah K et al, 2006). Pregnant and lactating women should additionally consume 100-200 mg of preformed DHA with the aim of compensating for oxidative losses of dietary DHA in the mother and accumulation of DHA in fat in the fetus or toddler.

Children older than 6 months should consume 100 mg of DHA per day to achieve adequate intake, and the dose is the same for small children (under 2 years). For children in the age group of 2-18 years, the same diet as for the adult population is advised. So, 1-2 servings of oily fish on a weekly basis,

or ~250 EPA+DHA on a daily basis (EFSA, 2017). Sources of  $\alpha$ -linolenic acid are predominantly nuts and seeds such as flax, perilla seeds, chia, walnut and hemp seeds, while EPA and DHA are predominantly present in fish and other seafood (Duo Li, 2019). Fish is a food that is very rich in EPA and DHA, and is therefore the primary source of these omega-3 fatty acids in humans. The reason for this is that fish mainly feed on algae that are rich in EPA and DHA (Cholewski et al, 2018). The main goal of this research was to determine whether the intake of omega-3 fatty acids in the population of Bosnia and Herzegovina is in accordance with the guidelines and recommendations given by the competent organizations. In addition, the representation of different dietary sources of n-3 PUFA was examined, as well as the frequency of use of supplements containing n-3 PUFA. A comparison was also made of the intake of ALA, EPA, DHA of the subjects who participated in this study with the subjects of the last study conducted in 2017 in the territory of Bosnia and Herzegovina, where the intake of EPA and DHA was at an unsatisfactory level, while ALA was adequately taken in by women, but not in men (Gićević et al, 2017).

### ***Experimental***

The research was conducted during December-March 2024 using an online questionnaire. The questionnaire was distributed via the Internet and social networks. The survey was approved by the Ethical Committee of the University of Sarajevo-Faculty of Pharmacy (0101-6031/23, 07.12.2023). The online questionnaire included 27 questions related to the patient's general characteristics (gender, age), physiological condition of the subjects (gestation and lactation), health condition, diet (intake of fish, seafood, products rich in omega-3 fatty acids such as walnuts, flax, rapeseed oil etc.), as well as questions related to the intake of omega-3 fatty acids through dietary supplements.

A previously published validated questionnaire was (Aeberli et al, 2019), which was modified by addition of three new questions. The first two questions related to the age and gender of the patient, then the physiological state of the female respondents, and further questions related to the health status of the respondents (presence of certain diseases, as well as the presence of diseases of their family members). The intake of omega-3 fatty acids is particularly important when it comes to subjects suffering from certain diseases such as hypertension, diabetes, cancer, depression, rheumatoid arthritis and hyperlipidemia.

In order to assess whether the respondents consume enough omega-3 fatty acids through their diet, questions were also related to the intake of fish and seafood (as one of the richest sources of EPA and DHA), but also other foods of plant origin, which are the main source of ALA. Respondents were asked about how often they consume certain foods, as well as their quantity, and the answers were offered in the survey itself. The last part of the survey refers to questions related to the intake of certain food supplements containing n-3 fatty acids, their dosage, as well as the manufacturer (if the respondent knows which manufacturer it is).

For each respondent, the daily intake of these three fatty acids was calculated on the basis of answers about the frequency of consumption and the amount of food per portion (g), as well as literature data on the content of ALA, EPA and DHA in a given food. The obtained values were compared with previous similar researches published in relevant sources as well as recommendations for daily intake for a healthy population given by EFSA.

In the calculation for the daily intake of ALA, EPA and DHA for each respondent, foods for which they did not give an answer regarding the amount or frequency of consumption of the same food were excluded. Out of the 201 subjects, for 8 of them who gave incomplete answers, walnuts were excluded when calculating the daily intake of ALA, for 39 subjects rapeseed oil was excluded, for 16 subjects flax seeds, for 17 chia seeds, for 17 subjects margarine, and for 22 subjects flax oil. For the calculation of the daily intake of EPA and DHA, 11 subjects out of 201 subjects were excluded due to incomplete answers given to the questions regarding the type, amount or frequency of fish or seafood consumption.

Also, supplementation was not taken into account when calculating the total daily intake of EPA and DHA, given the insufficient number of answers about the dose of omega-3 capsules used by the respondents. The computer program Microsoft Office Excel was used for data entry. The results obtained on the basis of statistical data processing are presented in the form of graphs and tables.

### ***Results and Discussion***

Only 6.5% of respondents consume fish and seafood in the quantity recommended by the American Heart Association, 2-3 times a week. The results were comparable to those reported for Zagreb County in 2022 where about 7-8% of respondents consumed fish or seafood (Pavazza, 2022).

The most common amount of fish consumed is 150g for men (47.5%) and women (51.4%), which corresponds to recommendations for fish intake of  $\geq 130$ g per serving. Also, a study from Zagreb recorded that around 60% of respondents consume fish in this same amount (Pavazza, 2022). The most

preferred form of fish among women is canned (27.4%), followed by fresh fish (24.3%) and frozen fish (22.0%), while among men it is fresh fish (33.8%), canned food (29.9%), and frozen fish (20.8%). Of the types of fish, the most represented in the respondents' diet is tuna (69.2%). The results are similar to studies from 2022, where hake and tuna are also the most consumed (Pavazza, 2022). Consumption of flounder, turbot, crab, caviar and oysters was not reported by any of the male respondents in the last six months, while for women it was flounder, turbot and perch. Looking at men, 56.3% consume adequate amounts of EPA and DHA. When it comes to women, 64.8% consume adequate amounts of EPA+DHA primarily through fish and nuts. If we look at the results of the research conducted in 2017 in the territory of Bosnia and Herzegovina, the intake of total PUFA was generally low among men and women. Also, none of the respondents consumed adequate amounts of ALA, EPA, DHA (Gićević et al, 2017). The obtained results overlap with the results of the study from 2017, where women consumed a higher percentage of energy from ALA (0.04%) compared to men (0.02%) (Gićević et al, 2017). The intake of nuts in the 2017 study was relatively low, which also coincides with the results of this study, where individually consumed amounts of nuts would not meet the needs for daily intake of alpha-linolenic acid (ALA).

Furthermore, only 12.1% of men consume ALA in the recommended doses of 1.6g ALA/day, while the majority (87.9%) consume less than the recommended amount. Omega-3 fatty acids as a dietary supplement are used more by women (51.6%) than by men (38.1%). Of the total number of respondents, 48.5% use nutritional supplements, which compared to the results from the study in Zagreb County (29.0%) is a much higher percentage, where women also took more nutritional supplements rich in omega-3 fatty acids in comparison on men (30.0% vs. 9.0%) (Pavazza, 2022).

### ***Conclusion***

From the results of the research, it can be concluded that women's diets are rich in plant-based foods with a high ALA content, as 53.13% of female participants meet the recommended intake for this fatty acid. On the other side, men consume less ALA-rich foods, with adequate intake observed in only 12.12% of male participants. About 56.25% of men adequately consume EPA and DHA through fish and seafood, while 64.76% of women meet the recommended intake of these fatty acids. A total of 48.5% of respondents use omega-3 fatty acid supplements, with 51.6% of women and 38.1% of men reporting supplement use. Among the respondents who take omega-3 supplements, 50.0% meet the recommended intake of EPA and

DHA through their diet, while the remaining 50.0% do not achieve adequate EPA and DHA intake through diet alone.

When it comes to the intake of alpha-linolenic acid, the intake of ALA in more than half of the women (53.1%) is in accordance with the guidelines for the intake of 1.1g ALA/day, while 46.9% of women do not consume ALA as recommended. Nuts, fish and functional foods enriched with omega-3 fatty acids should definitely be found more often in an individual's menu, in order to achieve an adequate intake of ALA, EPA and DHA.

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## **Procjena unosa n-3 polinezasićenih masnih kiselina u odrasloj populaciji Bosne i Hercegovine**

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### **Sažetak**

Svrha istraživanja je da se procijeni unos omega-3 masnih kiselina kod stanovništva, kako bi se dobio uvid u to u kojim količinama i koliko često se konzumiraju namirnice koje su bogate ili obogaćene ovim nutrijentom. Dobiveni rezultati su upoređeni sa rezultatima posljednjeg istraživanja procjene unosa omega-3 masnih kiselina na području Bosne i Hercegovine. Ispitivan je unos ribe, oraha, lana, chia sjemenki, lanenog ulja, ulja repice, margarina. Dobiveni rezultati pokazuju da svega 6.5% ispitanika konzumira ribu u skladu sa preporukama od 2-3 obroka sedmično. Unos EPA+DHA hranom zadovoljava preporučenu količinu kod 56,3% ispitivanih muškaraca i 64,8% ispitivanih žena. Zabilježen je nedovoljan unos alfa-linolenske kiseline (ALA) kod muškaraca, dok više od polovine žena zadovoljava adekvatan unos za ALA. Omega-3 masne kiseline kao dodatak prehrani zastupljenije su kod žena, dok ih muškarci slabije konzumiraju.

*Ključne riječi: omega-3 masne kiseline, EPA, DHA, ALA*

### 3-O-3

## Antimicrobial effect of toothpaste on selected microorganisms

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### Abstract

Microbiota is present in the oral cavity, which consists of a large number of Gram-positive and Gram-negative microorganisms. Due to the presence of a large number of microorganisms in the oral cavity, the formation of a dental biofilm can occur, the formation of which can be prevented by regular oral hygiene and tooth brushing. Therefore, it would be desirable if the toothpaste, in addition to maintaining dental health, oral hygiene, and tooth mineralization, also exhibits an antimicrobial effect. The aim of this work was to examine the antimicrobial effect of toothpastes of different composition on selected microorganisms *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Enterococcus faecalis* and *Escherichia coli*. Twelve samples of toothpastes, which are present on the market, were analyzed in this work. The following microorganisms were used as test microorganisms: *Staphylococcus aureus* (ATCC 25923), *Pseudomonas aeruginosa*, *Enterococcus faecalis* and *Escherichia coli* (ATCC 25922). The antimicrobial effect of toothpaste in three dilutions (1:2, 1:4 and 1:8) on the test microorganisms was examined by the agar diffusion method. The pH was measured in toothpastes. Antimicrobial activity of toothpastes on test microorganisms ranged from 0 to 35 mm, absence of inhibition was observed with *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Escherichia coli*. The largest zone of inhibition was shown by the paste 4. The pH value of the paste ranged from 6.27 to 9.18. Toothpastes with plant extracts showed a greater antimicrobial effect against the test microorganisms.

*Keywords: toothpaste, oral cavity, Staphylococcus aureus, Pseudomonas aeruginosa, Enterococcus faecalis, Escherichia coli.*

### Introduction

Food, especially food of animal origin, occupies an important place in human nutrition, as it is a source of biologically valuable proteins, fats, carbohydrates, vitamins and minerals. Digestion of food begins in the mouth,

primarily carbohydrates, polysaccharides to disaccharides under the influence of alpha amylase originating from saliva (Stojić et al., 2007). According to the FAO (Food and Agriculture Organization) report (2023), food of animal origin is essential for human nutrition and health. This report is the first of four documents highlighted by the FAO Committee on Agriculture as important in 2020. On the other hand, due to its chemical composition, food represents a good environment for the growth and reproduction of microorganisms. Foods of animal origin, especially dairy products and fermented products, are the main natural sources of lactic acid bacteria (LAC).

These microorganisms are known for their antimicrobial properties, role in the occurrence of organoleptic changes in food, antioxidant activity, digestibility of nutrients, release of peptides and polysaccharides, decarboxylation of amino acids and production and degradation of biogenic amines (Miranda et al., 2021). On the other hand, there is a microbiota in the oral cavity, which consists of a large number of Gram-positive and Gram-negative microorganisms (Rooparawathi et al., 2015).

Several studies have shown that the oral cavity, due to humidity, temperature and the presence of nutrients, represents an ideal environment for the growth and reproduction of microorganisms (Kanchanakamol et al., 1995). *Streptococcus mutans* is an opportunistic microorganism of the microbiota of the oral cavity, which plays a central role in the fermentation of carbohydrates, creating acid, which causes demineralization of tooth enamel. *Candida albicans* is the most common fungus isolated from the oral cavity. *Escherichia coli* and *Candida albicans* are also reported to cause caries (Rooparawathi et al., 2015).

The presence of a large number of microorganisms in the oral cavity can lead to the formation of a dental biofilm, the formation of which can be prevented by regular oral hygiene and tooth brushing (Arweiler et al., 2002; Davies et al., 2008). The most common diseases of the oral cavity, which appear globally, are caries, gingivitis and periodontitis, and their frequency is increasing due to changes in people's eating habits as well as increased consumption of simple sugars.

Toothpastes are primarily used to maintain dental health and oral hygiene, but also to whiten, heal wounds and mineralize teeth. Toothpaste formulations usually contain a combination of fluoride and detergents, whose role is primarily aimed at controlling the formation of biofilm. (Davies et al., 2008, Marsh et al., 2010). However, the main goal and challenge in both veterinary and human medicine is the choice of paste, which, in addition to maintaining hygiene, can also exhibit an antimicrobial effect.

The addition of certain antimicrobial active components represents a potential method for reducing, controlling and preventing the formation of deposits of cariogenic and periodontogenic microorganisms (Prasanth et al., 2011). However, the antimicrobial ability of such substances in combination with fluoride-containing toothpastes has not yet been effectively investigated (Aakankshya et al., 2021).

A relatively small number of published works in the literature compare the effect of toothpaste on microorganisms that participate in caries formation (Serdar et al., 2021).

Accordingly, it would be desirable if the preparations used for brushing teeth contain in their composition certain antimicrobial components in order to prevent the occurrence of dental diseases. Although many products on the market claim to have antimicrobial properties, insufficient research has been conducted to confirm this, and all of this considering that the antimicrobial effectiveness of chemical substances can be reduced or inactivated when combined with other toothpaste ingredients. (Serdar et al., 2021).

Therefore, the aim of this research was to examine the antimicrobial effect of commercially available toothpastes, of different composition and active components, on selected microorganisms *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Enterococcus faecalis* and *Escherichia coli*.

### ***Experimental***

The material consisted of twelve toothpaste samples. The brands of pastes, commercial names and composition of the pastes are shown in Table I. Before the beginning of the research, samples of toothpastes, which are available on the market, of different manufacturers and compositions, were collected from free sale and delivered to the laboratory.

A total of twelve toothpastes were selected, from which serial dilutions were made by diluting 1g of the paste in sterile saline solution in order to obtain three different dilutions 1:2 (1g of paste in 2ml of saline), 1:4 (1g paste in 4 ml of physiological solution) and 1:8 (1 g of paste in 8 ml of physiological solution). The negative control was sterile distilled water, while 0.2% chlorhexidine digluconate (Curasept, Italy) was used for the positive control.

**Table 1. Toothpaste marks and their composition**

No	The name of toothpaste	Toothpaste composition (taken from the declaration of product)	Herbal components
1	<i>Colgate Propolis</i>	Calcium Carbonate, Water, Sorbitol, Sodium Lauryl Sulfate, Silica Hydrated, Sodium Monofluorophosphate, Flavouring, Cellulose Gum, Xanthine Gum, Sodium Carbonate, Benzyl Alcohol, Sodium Saccharin, Sodium Bicarbonate, Eucalyptol, Propolis Extract, Peppermint Oil	<i>Propolis extract and Mentha spicata plant oil.</i>
2	<i>Himalaya dental cream with clove oil</i>	Calcium carbonate, water, sorbitol, glycerin, hydrated silica, sodium lauryl sulfate, flavor, xanthine gum, sodium silicate, phenoxyethanol, silicon dioxide, clove bud oil, PVM/MA copolymer, caramel, sodium saccharin, vanillin butyrate, titanium dioxide, CI 12490, CI 77266, CI 77492, eugenol	Clove bud oil ( <i>Eugenia caryophyllus</i> ).
3	<i>Himalaya dental cream with neem and pomegranate</i>	Water, glycerin, hydrated silicon dioxide, sodium lauryl sulfate, titanium dioxide, aroma, xanthan gum, sodium saccharin, menthol, calcium fluoride, sodium benzoate, sodium sulfite, pomegranate peel extract	Plant extracts <i>Punica granatum</i> , <i>Zantoxylum Alatus</i> , <i>Acacia arabica</i> , <i>Terminalia chebula</i> , <i>Terminalia bellerica</i> , <i>Emblica officinalis</i> , <i>Embelia ribes</i> , <i>Melia azadirachta</i> , <i>Vitex negundo</i>
4	<i>Himalaya ultra whitening with carbon powder</i>	Water, hydrated silica, sorbitol, glycerin, bromelain, flavor, xanthan gum, cocamidopropyl betaine, sodium lauroyl sarcosinate, tetrasodium pyrophosphate, carbon powder, sodium saccharin, phenoxyethanol, menthol 2, carbonate 6, hydroxide 6, hydroxide 6,7	<i>Seed oils Nigela sativa and Prunus amygdalus dulcis.</i>
5	<i>Colgate Whitening</i>	Water, Calcium Carbonate, Hydrated Silica, Sodium Lauryl Sulfate, Flavor, Cellulose Gum, Sodium Monofluorophosphate, Xanthan Gum, Sodium Carbonate, Benzyl Alcohol, Sodium Saccharin, Sodium Bicarbonate, Eugenol, Cinnamon, Limonene	Without herbal ingredients
6	<i>DENTility with eight herbs</i>	Water, Silica Hydrated, Sorbitol, Sodium Lauryl Sulfate, Flavor, Cellulose Gum, Titanium Dioxide, Sodium Saccharin, Propylene Glycol, Methylparaben, 2-Bromo-2nitropropane-1,3-diol, CI 19140, CI 42051	Extracts of plants <i>Thymus serpyllum</i> , <i>Chamomilla recutite</i> , <i>Urtica dioica</i> , <i>Salvia officinalis</i> , <i>Mentha piperita</i> , <i>Camellia sinensis</i> .

**Table 1.** Toothpaste marks and their composition, cont.

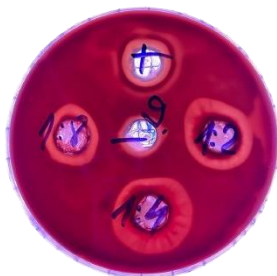
No	The name of toothpaste	Toothpaste composition (taken from the declaration of product)	Herbal components
7	<i>Signal herbal fresh</i>	Calcium carbonate, water, sorbitol, Hydrated silicon dioxide, sodium lauryl sulfate, sodium, monofluorophosphate, flavoring, cellulose gum	Plant oils <i>Eucalyptus globulus</i> , <i>Mentha atvensis</i> , <i>Mentha piperita</i> , <i>Salvia officinalis</i> , <i>Anthemis nobilis</i> , <i>Commiphora myrrha</i> , <i>Melaleuca alternifolia</i> .
8	<i>Signal cavity protection</i>	Calcium carbonate, water, sorbitol, hydrated silicon dioxide, sodium lauryl sulfate, sodium monofluorophosphate, flavoring, cellulose gum, potassium citrate, trisodium phosphate, sodium saccharin, phenylcarbinol, glycerin, limonene.	Without herbal ingredients.
9	<i>DENTility Parodont White</i>	Water, hydrated silicon dioxide, sorbitol, glycerin, cellulose gum, sodium lauryl sulfate, aroma, sodium monofluorophosphate, titanium dioxide, phenylpropanol, saccharin, sodium hydroxide, glycol, lacroflavin, CI 42090	Extracts of the plants <i>Salvadora persica</i> , <i>Salvia officinalis</i> , <i>Zingiber officinale</i> , <i>Commiphora abyssinica</i> , <i>Echinacea purpurea</i> .
10	<i>Splat BIOCALCIUM</i>	Water, hydrated silica, hydrogenated starch hydrolyzate, PEG-8, sodium coco sulfate, cellulose gum, flavoring, calcium lactate, CI 77891 sodium bicarbonate, sodium methylparaben, hydroxyapatite, PVP, sodium saccharin, fish oil, papain. Without fluoride.	Without herbal ingredients
11	<i>MISWAK gold</i>	Calcium carbonate, sorbitol, silicon dioxide, sodium lauryl sulfate, carrageenan sodium silicate, cellulose gum, copolymer, CI 77891, water	Pure extract of Arak plant
12	<i>Elmex</i>	Arginine, calcium carbonate, water, sorbitol, bicarbonate, sodium lauryl sulfate, na-monofluorophosphate, flavoring, sodium silicate, cellulose gum, sodium bicarbonate, K-acesulfame, xanthan gum, sucralose	Without herbal ingredients

**Microorganisms-**The following microorganisms were used as test microorganisms: *Staphylococcus aureus* (ATCC 25923), *Pseudomonas aeruginosa*, *Enterococcus faecalis* and *Escherichia coli* (ATCC 25922), which are from the collection of the Department of Hygiene and Food Technology of Animal Origin. During the research, the microorganisms were

stored on nutrient agar in a refrigerator at 4 °C, and were activated by seeding onto the nutrient broth, which was incubated at 37 °C for 24 h.

*Substrates* - The medium used in the research was prepared from the powder of the commercial *Mueller-Hinton* agar medium (Himedia, India) (MH) with the addition of 10% bovine blood. A measured amount of dehydrated powder was dissolved in distilled water according to the manufacturer's instructions. After dissolution and thawing, the substrate was sterilized in an autoclave at 121 °C for 15 minutes. Given that bovine blood was used for the preparation of MH agar, ethical permission was obtained for the use of bovine blood by the Ethics Committee of the Faculty of Veterinary Medicine, University of Belgrade (opinion 01-02/2024).

Composition of *Mueller-Hinton* agar: Beef infusion 300 g, acid casein hydrolyzate 17.5 g, starch 150 g, and agar 17 g per 1000 ml of distilled water. The measured pH of the substrate was  $7.3 \pm 0.2$ . The substrate was aseptically poured into Petri plates (Spektar, Čačak) with a diameter of 8.5 cm, so that the thickness of the substrate was 5 mm. The antimicrobial activity of the pastes was tested for each microorganism, whereby 3 different dilutions of the same paste, as well as a positive and a negative control, were inoculated onto each plate (Picture 1).



**Picture 1.** Zones of inhibition of test microorganism on *Mueller–Hinton* agar

#### *Examination of the antimicrobial effect of the paste on microorganisms*

The antimicrobial effect of toothpaste at different concentrations on selected microorganisms was tested using the agar diffusion method (Rooparawathi et al., 2015). From the overnight culture (24 h) of microorganism isolates, suspensions were made in physiological solution to obtain a 0.5 McFarland solution. An amount of 0.1 ml of the microorganism suspension was applied to the surface of the MH agar and the inoculum was distributed on the substrate with a sterile glass rod. Petri plates were left in a horizontal position at room temperature for the substrate to absorb, and then small pools were made using a sterile test tube ( $r=8$  mm) whose opening was lit on a

burner. The excess of the substrate was removed with an ezo so that a small pool remained in which the positive control (0.2% chlorhexidine), the negative control (distilled water) and three dilutions (1:2, 1:4 and 1 :8) of each paste. Petri plates so seeded were placed in the refrigerator for 1 hour so that the paste could diffuse into the medium. Then the plates were transferred to a thermostat for incubation at 37 °C for 24 hours. The results were read after 24 hours by measuring the diameter of the zone of inhibition using a ruler around the pool for each dilution of the paste. The method used is in accordance with the recommendations of the European Committee for Antimicrobial Susceptibility Testing (EUCAST, 2024).

#### *Determination of pH in toothpaste samples*

Determination of pH in toothpaste samples was performed in the original substance of the paste using a pH meter (Testo 205, China). Before the measurement, the instrument was calibrated with two known solutions.

#### **Results and Discussion**

The results of testing the antimicrobial action of toothpaste on *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Enterococcus faecalis* and *Escherichia coli* are shown in Table 2, 3, 4 and 5, respectively. The obtained results were processed with descriptive statistics parameters, of which the maximum value (x max), minimum value (x min), arithmetic mean (x) and standard deviation (SD) are tabulated (Table 6). The processing of statistical data was carried out in the statistical program Excel.

**Table 2.** Results of testing the antimicrobial action of toothpaste on *Staphylococcus aureus* expressed as the diameter of the zone of inhibition in mm

Toothpaste mark	Dilutions		
	1:2	1:4	1:8
1	18	18	16
2	0	0	0
3	25	30	31
4	35	20	24
5	20	19	16
6	30	26	25
7	22	25	24
8	25	25	18
9	20	23	24
10	25	22	14
11	19	18	13
12	18	14	13



**Table 3.** Results of testing the antimicrobial action of toothpaste on *Pseudomonas aeruginosa* expressed as the diameter of the zone of inhibition in mm

Toothpaste mark	Dilutions		
	1:2	1:4	1:8
1	19	18	18
2	20	25	25
3	19	19	19
4	23	21	14
5	21	23	20
6	18	17	19
7	24	23	20
8	19	18	18
9	18	0	0
10	18	23	15
11	15	18	16
12	19	18	16

**Table 4.** Results of testing the antimicrobial action of toothpaste on *Enterococcus faecalis* expressed as the diameter of the zone of inhibition in mm

Toothpaste mark	Dilutions		
	1:2	1:4	1:8
1	22	20	20
2	20	33	25
3	16	20	20
4	15	31	21
5	20	19	18
6	27	29	25
7	25	28	22
8	27	20	21
9	22	25	18
10	23	26	19
11	21	21	17
12	21	23	19

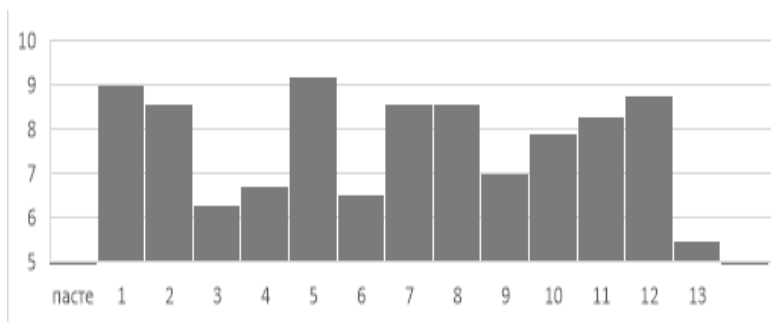
**Table 5.** Results of testing the antimicrobial action of toothpaste on *Escherichia coli* expressed as the diameter of the zone of inhibition in mm

Toothpaste mark	Dilutions		
	1:2	1:4	1:8
1	19	18	15
2	19	30	23
3	19	22	20
4	14	25	25
5	18	20	23
6	18	18	17
7	22	21	19
8	25	20	0
9	21	24	13
10	21	21	17
11	19	20	16
12	23	20	20

**Table 6.** Results of the summary statistical analysis of the antimicrobial action of toothpaste on microorganisms

Statistical parameters	Type of microorganism			
	<i>Staphylococcus aureus</i>	<i>Pseudomonas aeruginosa</i>	<i>Enterococcus faecalis</i>	<i>Escherichia coli</i>
Xmin	0	0	15	0
Xmax	35	25	33	30
$\bar{x}$	19,86	18,2	22,19	19,58
SD	7,96	5,23	4,11	4.74

The results of the pH measurement in the toothpaste samples, as well as the pH value of the positive control (number 13) are shown in Graph 1.



**Graph 1.** Results of determination of pH values in toothpaste samples

*Staphylococcus aureus* is a Gram-positive ubiquitous microorganism that inhabits the skin and mucous membranes of humans and animals. It is believed that almost one third of healthy people are carriers of staphylococci on the mucous membrane of the nasopharynx. From the aspect of food hygiene, the importance of this microorganism is reflected in its ability to create enterotoxins, which can cause alimentary intoxication in people after consuming food containing enterotoxin. According to Frank et al. (2010) as many as 50% of healthy adults are constant or occasional carriers of *S. aureus* on the nasal mucosa, while in 50% of people this microorganism is rarely or never isolated from the nasal mucosa. Although this microorganism does not represent the normal microbiota of the oral cavity, we examined the antimicrobial activity of the toothpaste, because data from the literature speak of its finding in the oral cavity (Obiazi et al., 2018) and because of its proven ability to form a biofilm on solid surfaces, especially methicillin-resistant ones. *S. aureus*, which are resistant to antimicrobial therapy (Wang and Ren, 2017).

From the obtained results, it can be seen that the largest inhibition zones for *S. aureus* were shown by pastes labeled 4 and 6, which gave an inhibition zone of 35 and 30 mm in a 1:2 dilution. Paste 4 has activated charcoal in its composition and measured pH 6.27, while paste 6 is a herbal paste with extracts of six plants (Table 1) and its pH was 6.57. Also, paste 3 showed inhibition zones of 25 to 31 mm in all three dilutions, while the measured pH was 6.27. In addition to chemical compounds, Paste 3 contains nine herbal ingredients, the main of which are pomegranate and *Terminalia chebula* and *Terminalia bellerica*, wild plum species that grow in India and have a tradition of use in Ayurvedic medicine due to their beneficial effects on the gums and diseases of the oral cavity. Our results agree with those of Kengadaran et al. (2020), who proved the greater effectiveness of pastes containing plant extracts compared to pastes containing only chemical substances. The zones of inhibition obtained by these authors for Gram-positive microorganisms ranged from 17 to 24 mm, but they used higher dilutions of 1:5, 1:10, and 1:15. No zone of inhibition for *S. aureus* was paste 2 with clove bud extract in all three dilutions (Table 2). The measured pH was 8.57 (Graph 1), and at this value *S. aureus* can grow.

*Enterococcus faecalis* belongs to Gram positive cocci and is an indicator of poor hygiene, improper cleaning, washing and disinfection of surfaces. Its finding in food indicates contamination of fecal origin. It is most often isolated from food originating from the sea, in swabs from surfaces, hands of staff and utensils (Teodorović et al. 2015). This microorganism does not belong to the normal microbiota of the oral cavity, but can be found as an infectious agent after unsuccessful dental treatment. Data from the literature

supports the fact that *E. faecalis* has been proven in biofilm (Kouidhi et al., 2011). We decided to investigate the antimicrobial action on this microorganism because of its antimicrobial resistance to vancomycin, ciprofloxacin and erythromycin. The World Health Organization (WHO) lists *E. faecalis* as a high-priority pathogen for the development of antimicrobial resistance. Our results differ from those of Serdar et al. (2021), who did not prove inhibition zones for pastes with propolis and Arak plant extract, while we obtained inhibition zones for paste 1, which contains propolis, and paste 11 with Arak plant extract, in a 1:2 dilution (22 mm), 1:4 and 1:8 (20 mm) and 1:2 and 1:4 (21 mm), and for 1:8 (17 mm) (Table 4). Zones of inhibition can be explained by measured alkaline pH values in pastes 1 and 11 (Graph 1).

*Pseudomonas aeruginosa* and *E. coli* are Gram-negative rod-shaped bacteria. In the literature, there are data on the finding of these two microorganisms in the oral cavity and saliva (Al Jader et al., 2022). The results of testing the antimicrobial activity of the pastes on *P. aeruginosa* showed that pastes 4 and 7 gave the largest zones of inhibition in dilutions 1:2 (23 mm), 1:4 (21 mm), and 1:8 (14 mm), and for paste 7 in 1:2 (24 mm), 1:4 (23 mm), and for 1:8 (20 mm) (Table 3). The smallest zone of inhibition was given by paste 11 (Table 1) in 1:2 (15 mm), 1:4 (18 mm), and 1:8 (16 mm) and paste 9, which in dilutions 1:4 and 1:8 did not show a zone of inhibition.

For *E. coli*, the largest zones of inhibition were given by pastes 8 and 12 without plant extracts, however with fluorine compounds in dilutions 1:2 (25 mm), 1:4 (20 mm), and 1:8 (0 mm) and for the paste 12 in 1:2 (25 mm), 1:4 (20 mm), and 1:8 (0 mm) (Table 5). Smaller dilutions gave a larger zone of inhibition, which can be explained by a higher concentration of the active substance of the paste. Approximately the same pH value was measured in these pastes (8.55 and 8.75). The antimicrobial effect cannot be attributed to the pH value, because at these values *E. coli* can grow. Our results are in accordance with the results of Kengadaran et al. (2020), who proved the greatest antimicrobial effect in pastes with plant extracts (16 to 18 mm), while for our plant pastes the zones of inhibition were from 15 to 30 mm. Higher values than ours were obtained by Rooparawathi et al. (2015), where the zones of inhibition were from 24 to 37 mm. Contrary to our results for the antimicrobial activity of paste 2 against *S. aureus*, which contains clove, this paste showed greater antimicrobial activity against Gram-negative microorganisms (*P. aeruginosa* and *E. coli*) (Table 4 and 5) where inhibition zones of 19 up to 30 mm. Our results are in full agreement with those of Rooparawathi et al. (2015) regarding pastes containing neem (a tree also known as Indian lilac), papain and fluoride (Table 5). These authors proved the highest zone of inhibition (37 mm) for pastes, where the main ingredients

are triclosan and zinc sulfate. However, the main inhibitory action cannot be attributed to triclosan, because the same authors did not prove antimicrobial action for three pastes containing this active ingredient. The results of Kengadaran et al. (2020) showed that pastes with herbal ingredients showed a better effect compared to triclosan. Triclosan was not listed as an ingredient in any of the paste samples we examined.

Our results showed that the pH value of the toothpaste ranged from 6.27 in toothpaste 3 to 9.18 in toothpaste 5 (Graph 1). Since the test microorganisms used in this research can grow at these pH values, the antimicrobial activity does not depend exclusively on this physicochemical parameter. It is difficult to delineate the inhibitory action of chemical compounds and plant extracts.

Larger zones of inhibition of test microorganisms, which were given by pastes with plant extracts, can be explained by the presence of secondary metabolites from plants, which have long been recognized as sources of molecules with antimicrobial potential (Demir et al., 2021).

### ***Conclusion***

Based on the obtained results, it can be concluded that the pastes with plant extracts showed a greater antimicrobial effect against the test microorganisms. Future *in vitro* studies could clarify which components show the greatest antimicrobial effect alone or in combination with other chemical constituents that influence the physicochemical characteristics of toothpaste.

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## Antimikrobni efekat pasti za zube na odabrane mikroorganizme

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### Sažetak

U usnoj duplji je prisutna mikrobiota koja se sastoji od velikog broja gram-pozitivnih i gram-negativnih mikroorganizama. Zbog prisustva velikog broja mikroorganizama u usnoj duplji može doći do stvaranja zubnog biofilma čije se stvaranje može sprečiti redovnom oralnom higijenom i pranjem zuba. Iz tog razloga poželjno da pasta za zube, osim što omogućava zdravlje zuba, oralnu higijenu i mineralizaciju zuba, ispoljava i antimikrobno dejstvo. Cilj ovog rada je bio da se ispita antimikrobni efekat pasti za zube različitog sastava na odabrane mikroorganizme *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Enterococcus faecalis* i *Escherichia coli*. U materijalu je predstavljeno dvanaest uzoraka pasta za zube, koje su prisutne na tržištu. Kao test mikroorganizmi korišćeni su sledeći mikroorganizmi: *Staphylococcus aureus* (ATCC 25923), *Pseudomonas aeruginosa*, *Enterococcus faecalis* i *Escherichia coli* (ATCC 25922). Antimikrobni efekat pasti za zube u tri razblaženja (1:2, 1:4 i 1:8) na ispitivane mikroorganizme ispitivan je metodom agar difuzionom metodom. U pastama za zube izmerena je i pH vrednost. Antimikrobna aktivnost pasti za zube na test mikroorganizme kretala se u rasponu od 0 do 35 mm, a odsustvo inhibicije uočeno je kod *Staphylococcus aureus*, *Pseudomonas aeruginosa* i *Escherichia coli*. Najveću zonu inhibicije pokazala je pasta 4. pH vrednost pasti se kretala od 6,27 do 9,18. Paste za zube sa biljnim ekstraktima pokazale su veći antimikrobni efekat prema ispitivanim mikroorganizmima.

*Ključne reči:* pasta za zube, oralna šupljina, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Enterococcus faecalis*, *Escherichia coli*



## Assessment of n-3 and n-6 Fatty Acid Intake Among the Adult Population of Sarajevo Canton

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### Abstract

This research paper focuses on the importance of n-3 and n-6 polyunsaturated fatty acids, which the body cannot synthesize, making their dietary intake essential. A key question is how informed the population of Sarajevo Canton is about the impact of these fatty acids on the functioning of the organism. The research was conducted using the Google Forms application, with the survey questionnaire consisting of thirteen questions and an estimated completion time of five minutes. To ensure data credibility, the survey was conducted anonymously. Data analysis revealed that respondents are aware of the benefits of omega fatty acids, such as their effects on heart health, but their intake is insufficient and inadequate. These results indicate a need for education on the proper intake of these nutrients and promote a balanced diet that includes various sources of fatty acids. In conclusion, the findings have important implications for changes in dietary habits and collaboration between healthcare professionals and the community.

*Keywords: polyunsaturated fatty acids, nutrition, awareness, nutrients, education*

### **Introduction**

The aim of this research is to assess the intake of omega-3 and omega-6 fatty acids among the adult population of the Sarajevo Canton. Omega-3 and omega-6 fatty acids are essential polyunsaturated fatty acids that play a critical role in maintaining human health, particularly cardiovascular, brain, and immune functions. Given that the body cannot synthesize these fats independently, they must be acquired through diet. Previous studies emphasize the importance of balanced consumption of these fatty acids, as

an imbalance, especially excessive intake of omega-6 compared to omega-3, can lead to chronic inflammation and health complications (Simopoulos, 2002; Calder, 2006). This study investigates dietary habits and awareness regarding the benefits of these essential nutrients in the Sarajevo Canton, contributing to the existing body of knowledge on public health nutrition.

### ***Experimental***

A survey-based study was conducted to meet the research objectives. An anonymous online questionnaire, consisting of 13 questions, was distributed to classify participants, evaluate their dietary habits, and assess their knowledge of omega-3 and omega-6 fatty acids. The survey was created using Google Forms and took approximately five minutes to complete. Standard statistical methods were applied to analyze the data, including classification by age, gender, and dietary intake. The participants were classified into age groups above and below 18 years, with only data from adult participants being analyzed. Survey data were collected anonymously to ensure the reliability of the responses, and a total of 452 responses were analyzed after excluding invalid entries.

### ***Results and Discussion***

The survey results revealed that 75.4% of participants were aware of the health benefits associated with adequate omega fatty acid intake, while 24.6% were unaware. Despite this awareness, a significant portion of participants expressed dissatisfaction with their dietary intake of these fatty acids. Only 9.3% were highly satisfied, while 30.3% reported being dissatisfied with their intake. The imbalance in omega-3 and omega-6 consumption was evident, as the majority (53.5%) consumed fish and related products less than once per week, which is below the recommended levels for maintaining adequate omega-3 intake (Kris-Etherton et al., 2002). Furthermore, 34.1% of participants frequently consumed seeds rich in omega fatty acids, while a larger proportion did not incorporate such sources into their diet regularly. The findings suggest a need for increased public education on achieving a balanced intake of these essential nutrients. Comparison with international studies (Meyer et al., 2003; Shen et al., 2019) indicates similar patterns of deficiency in omega-3 intake across different populations. The results emphasize the importance of dietary interventions to promote healthier habits and the use of supplements where necessary.

### ***Conclusion***

This research highlights the gaps in omega-3 and omega-6 fatty acid intake among the adult population in Sarajevo Canton. Although there is a general awareness of the benefits of these fatty acids, dietary practices do not reflect

sufficient intake, particularly of omega-3 sources. To address these shortcomings, further public health efforts should focus on education and encouraging the consumption of foods rich in omega-3, such as fish and seeds, as well as the appropriate use of supplements when needed.

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## **Procjena unosa n-3 i n-6 masnih kiselina kod odraslog stanovništva Kantona Sarajevo**

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### **Sažetak**

Ovaj istraživački rad fokusira se na značaj unosa n-3 i n-6 polinezasićenih masnih kiselina, koje tijelo ne može sintetizirati, stoga je njihov unos putem prehrane od esencijalne važnosti. Ključno pitanje je koliko je stanovništvo Kantona Sarajevo informisano o uticaju ovih masnih kiselina na funkcionisanje organizma. Istraživanje je sprovedeno korišćenjem aplikacije Google Forms, a anketni upitnik je sadržavao trinaest pitanja, s procijenjenim vremenom popunjavanja od pet minuta. Radi osiguranja vjerodostojnosti, anketa je bila anonimnog karaktera. Analiza podataka pokazala je da su ispitanici svjesni dobrobiti omega masnih kiselina, poput njihovog uticaja na zdravlje srca, ali je njihov unos nedovoljan i neadekvatan. Ovi rezultati ukazuju na potrebu za edukacijom o pravilnom unosu ovih nutrijenata i promovišu uravnoteženu prehranu koja uključuje raznovrsne izvore masnih kiselina. Zaključno, rezultati imaju važne implikacije za promjene u prehrambenim navikama i suradnju između zdravstvenih radnika i zajednice.

*Ključne riječi: polinezasićene masne kiseline, prehrana, informisanost, nutrijenti, edukacija*

## **Consumption of cow's milk and the impact on human health - benefits and potential risks**

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### **Abstract**

It is known that cow's milk is a significant source of nutrients, such as proteins, minerals and vitamins, which are necessary for the normal functioning of the human body. Conversely, for a certain population, milk can represent a health risk due to lactose intolerance and the occurrence of allergies. The public is progressively talking about the negative impact of milk on the health of consumers. The aim of this research was to examine the awareness and attitudes of consumers in the Republic of Serbia about the potential health benefits, but also the risks associated with the consumption of cow's milk. The majority of respondents believe that milk is essential for the growth and development of children, that as an important source of calcium, it is a big factor in the prevention of osteoporosis, but other foods also play an important role. Conversely, it is clear that most respondents are not sufficiently informed about the impact of milk and dairy products on the occurrence of malignant diseases, asthmatic attacks and cardiovascular diseases. The research indicates that, despite certain media and modern trends, which propagate the harmfulness of cow's milk to health, people still believe that milk brings more benefits than risks.

*Keywords: cow's milk, nutrition, consumer attitudes, Republic of Serbia*

### ***Introduction***

Milk is a product of the mammary gland of female mammals, intended for feeding the young. Nowadays, in Serbia and the region, people consume different types of milk: cows, sheep, goats, buffaloes, mares, and donkeys. The question of how milk consumption affects human health is increasingly being asked. The public is increasingly talking about the negative impact of milk on human health. Lactose intolerance is becoming more and more common (Lomer, Parkes, Sanderson, 2007), as well as milk protein allergies

(Allen, Davidson, Day, 2009), and milk consumption is also associated with the occurrence of malignant diseases (Davoodi, Esmaeili, Mortazavian, 2013; Parodi 2012). It is also discussed whether the milk consumption contributes to the increasing occurrence of obesity in children (Berkey, Rockett, Willett, 2005) and to the occurrence of cardiovascular diseases (Fontecha, Calvo, Juarez 2019) and asthmatic attacks (Song, Xie, Guo, 2023). The role of milk in the prevention of osteoporosis is also being discussed (Caroli, Poli, Ricotta, 2011). The aim of this study was to examine the awareness and attitudes of consumers in the Republic of Serbia about whether milk carries more benefits or risks to human health, because there are many doubts and conflicting opinions about it.

### ***Experimental***

The research was conducted through an online questionnaire that contained a total of 19 questions, the first five of which related to the socio-demographic characteristics of the respondents. The other 14 questions were related to respondents' attitudes and their personal experiences and opinions. The respondents were residents of the Republic of Serbia, and they filled out the questionnaire anonymously and voluntarily via an online Google questionnaire. The questions are filled in according to the principle of the offered answers with the possibility of writing an additional answer.

### ***Results and discussion***

The questionnaire was filled out by 300 respondents, 24% men and 76% women, between the 25<sup>th</sup> and 29<sup>th</sup> of September, 2024. The average age of the respondents was 37 years. The same percentage of respondents (39%) completed secondary school, i.e. higher school or university, while 17% completed post-graduate studies, and 5% of respondents completed primary school. The largest percentage of respondents spent their lives so far exclusively in urban areas (38%). The majority of respondents (71.6%) consume cow's milk once a week or more often, 20.3% less often than once a week, while 8% do not consume milk at all.

Respondents rated their general health as excellent or good (79%), average (19%) and poor (2%). More than half of the respondents (52%) believe that calcium intake through milk is important, but milk is not the only source of this micronutrient, so it is not necessary for optimal calcium intake, which is in line with the findings of the authors from Brazil (Bueno, Czepielewski, 2008). The role of milk in the prevention of osteoporosis is significant (Caroli, 2011), which is what the majority of respondents believe (61.7%). Consumers believe that the main benefits associated with consuming cow's milk are that it strengthens bones (57.3%), helps with growth and development (61.6%) and is a source of protein (49.3%).

Of the total number of respondents, 10.7% stated that they have an allergy and/or intolerance to lactose, which does not agree with other data from the literature in which the percentage is much higher when it comes to lactose intolerance (Lomer, 2007), but as stated in this study, there is a possibility that people are not sufficiently informed that certain symptoms can be associated with lactose intolerance.

There are various studies on hormones in cow's milk, by increasing the lactation period in dairy cows, the concentration of estrogen in the milk can increase, which has caused concern among many people (72.3% of respondents state that the presence of hormones in milk worries them). Estrogen has been linked to the occurrence of breast, ovarian, endometrial and prostate cancer, but as stated in the Australian study (Parodi, 2012), epidemiological data show that estrogen from milk cannot affect the occurrence of cancer in women, while in men it can be associated with prostate cancer, although it is not considered a primary etiological factor. More than 50% of the respondents believe that milk cannot be linked to the occurrence of malignant diseases. As many as 23% of respondents believe that weight gain is related to milk consumption, but in a study conducted on this topic (Berkey, 2005), milk fat did not contribute to weight gain in children.

Respondents were uncertain/not sure about the impact of milk consumption on the cardiovascular system (49%) and asthmatic attacks (53%). To date, it has not been proven that there are harmful effects on the cardiovascular system, regardless of the amount of milk and milk products consumed and regardless of the fat content (Fontecha, Calvo, Juarez 2019), nor is there a link between the consumption of dairy products and risk of asthma (Song, 2023).

### ***Conclusion***

The study findings can indicate that respondents generally consider milk to be an important source of nutrients. It can be assumed that consumers are not adequately informed about the symptoms of lactose intolerance. Concern about the presence of hormones in milk is present in a significant part of respondents (72.2%). More than half of respondents (51%) associate milk consumption with the risk of cancer. In addition, views on the relationship between milk consumption and cardiovascular diseases, as well as asthmatic attacks, are uncertain, indicating the need for additional information on this topic. Overall, the results of this research show that, despite the modern trends of healthy eating and the growing popularity of replacing milk and milk products with products of plant origin and milk substitutes, most people in the Republic of Serbia still think that the consumption of cow's milk is

healthy. This indicates the need for additional research and consumer information to remove all ambiguities related to the risks and benefits of consuming cow's milk.

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## **Konsumiranje kravljeg mleka i uticaj na ljudsko zdravlje - prednosti i potencijalni rizici**

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### **Sažetak**

Poznato je da je kravlje mleko značajan izvor hranljivih materija, kao što su proteini, mineralne materije i vitamin koji su neophodni za normalno funkcionisanje ljudskog organizma. Sa druge strane, za određenu populaciju mleko može predstavljati zdravstveni rizik zbog laktosne intolerancije i pojave alergija. U javnosti se sve više govori o negativnom uticaju mleka na zdravlje potrošača. Cilj istraživanja bio je da se ispituju informisanost i stavovi potrošača u Republici Srbiji o potencijalnim zdravstvenim koristima, ali i rizicima povezanim sa konzumiranjem kravljeg mleka. Većina ispitanika smatra da je mleko esencijalno za rast i razvoj dece, da je kao važan izvor kalcijuma bitan faktor u prevenciji osteoporoze, ali da pored njega značajnu ulogu imaju i druge namirnice. Sa druge strane je jasno da većina ispitanika nije dovoljno informisana o uticaju mleka i mlečnih proizvoda na pojavu malignih oboljenja, astmatičnih napada i kardiovaskularnih bolesti. Iz rezultata istraživanja može se zaključiti da i pored određenih medija i modernih trendova, koji propagiraju štetnost kravljeg mleka na zdravlje, ljudi ipak smatraju da mleko donosi više prednosti nego rizika.

*Ključne reči: kravlje mleko, ishrana, stavovi potrošača, Republika Srbija*

### 3-O-6

#### **Assessment of dietary polyphenol intake in the adult population of Sarajevo Canton**

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#### **Abstract**

The aim of this research was to analyze the perception and intake of polyphenols among the population of Sarajevo Canton (KS). The study was conducted through an online survey with a sample of 131 respondents, mostly younger individuals. Key findings show that 70.2% of respondents are unfamiliar with the term polyphenols, and only 17.6% use supplements rich in these compounds. The average intake of polyphenols, assessed using the Food Frequency Questionnaire method, is 649.51 mg, which is close to the lower limit of the recommended intake (500 mg - 1 g daily). Polyphenols, found in fruits, vegetables, tea, coffee, and dark chocolate, play a significant role in the prevention of chronic diseases (cardiovascular diseases, diabetes, neurodegenerative diseases) due to their antioxidant properties. Despite their health benefits, most respondents are unaware of the importance of polyphenols in the diet. It is recommended to increase public education about polyphenols through various information channels to improve knowledge and consumption of these beneficial compounds.

*Keywords: polyphenols, diet, antioxidants*

#### ***Introduction***

This research aims to gain insight into the perception of polyphenols among the population in KS, increase awareness of consumer attitudes regarding polyphenols in their diet, enhance knowledge about the polyphenols present in foods, and familiarize individuals with the concept of "polyphenols." Polyphenols are a large and heterogeneous group of phytochemicals characterized by a phenolic ring. They can be classified in various ways, most commonly based on their chemical structure (Manach, Scalbert, Morand, Rémésy and Jiménez, 2004). Produced by plants as secondary metabolites, polyphenols are essential functional foods found in fruits, vegetables, teas, chocolate, plant oils, grains, and legumes (Tsao, 2010; Pandey and Rizvi, 2009; Cantero, 2006; Sano, Tabata, Suzuki, Degawa,

Miyase, and Maeda-Yamamoto, 2001; Wollgast i Anklam, 2000; Ren et al., 2003). In European countries, the estimated daily dietary intake of polyphenols is approximately 1 g, with significant contributions from vegetables and fruits (Wisnuwardani, R.H., Stefaan, A., Odysseas, F., et al., 2019). These compounds play a vital role in protecting the body from external stressors and repairing damage caused by free radicals (Bhat, Azmi and Hadi, 2006). They exhibit various biological activities, including antioxidant, antiallergic, antimicrobial, anti-inflammatory, and regenerative effects. Additionally, dietary polyphenols help improve lipid profiles, regulate blood pressure, enhance insulin sensitivity, and reduce systemic inflammation. Research has also shown that polyphenols can positively influence cardiovascular health by reducing the risk of atherosclerosis and other heart-related conditions. Their ability to limit the formation of LDL cholesterol and support healthy blood circulation makes them an important dietary consideration (Clarkson et al, 2000; Del Rio et al., 2013). Furthermore, polyphenols have been linked to improved metabolic health, aiding in the management of diabetes by enhancing glucose metabolism and insulin response (Scalbert et al., 2005). Preclinical and epidemiological studies suggest that polyphenols may be effective in limiting neurodegenerative conditions and preventing age-related declines in cognitive function. Some studies indicate that flavonoids, a subclass of polyphenols, may enhance memory and learning abilities by protecting neurons and promoting neuronal regeneration. Osteoporosis is among the most common diseases, and a diet rich in polyphenols is believed to offer protective benefits against its onset and progression. The regenerative properties of polyphenols can support bone health by enhancing the activity of osteoblasts, the cells responsible for bone formation (Del Rio et al., 2013). Given their widespread presence in various foods, polyphenols are likely part of the daily diet for most people. This review highlights the importance of incorporating polyphenol-rich foods into everyday meals, emphasizing their potential health benefits and their role in disease prevention. Encouraging greater consumption of these beneficial compounds can lead to improved overall health and wellness. By increasing awareness and understanding of polyphenols, this research aims to foster healthier dietary choices within the community, ultimately contributing to better health outcomes and a higher quality of life.

In summary, polyphenols represent a crucial aspect of nutrition that warrants further exploration and promotion. Their diverse health benefits make them indispensable components of a balanced diet, and their presence in everyday foods underscores the importance of mindful eating habits. As research continues to uncover the myriad ways polyphenols contribute to health, it

becomes increasingly clear that they should be a staple in dietary guidelines and public health initiatives.

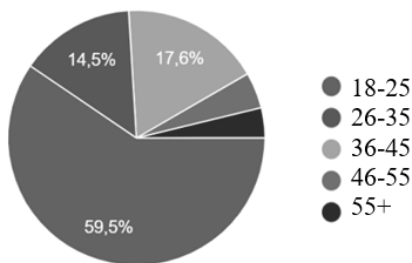
### ***Experimental***

This paper includes experimental work in the form of research/a survey conducted in Sarajevo Canton. The research was carried out by students at the Faculty of Pharmacy in Sarajevo. The questionnaire was created as a survey using Google Forms and conducted online. The questions were designed clearly and concisely to be understandable to a broad audience, including citizens of various age groups and educational levels. For the realization of this survey online databases like "Google Scholar" were used, along with valid regulations and laws of Bosnia and Herzegovina. Methods for the estimation of polyphenol consumption include either measuring the intake of specific food items or measuring biomarkers in urine or plasma.

### ***Results and Discussion***

Surveying the population of Sarajevo Canton was conducted with the aim of assessing dietary polyphenol intake. To evaluate food consumption, the FFQ (Food Frequency Questionnaire) method was employed. This method is used to highlight the frequency of consumption of polyphenol-rich foods. Data on food intake from the survey were compared with available information on the polyphenol content of these foods using the Phenol-Explorer database.

The survey was conducted among the adult population of Sarajevo Canton. Of the total number of respondents (131), 64.1% are female, while 35.9% are male. The ranking of respondents was conducted according to the standard statistical classification by age for the adult population: 18-25 years, 26-35 years, 36-45 years, 46-55 years, and 55 years and older. Of the total number of respondents, those aged 18-25 make up 59.5%, those aged 26-35 account for 14.5%, those aged 36-45 represent 17.6%, those aged 46-55 comprise 4.5%, and those aged 55 and older make up 3.9% of the respondents. (Figure 1.)

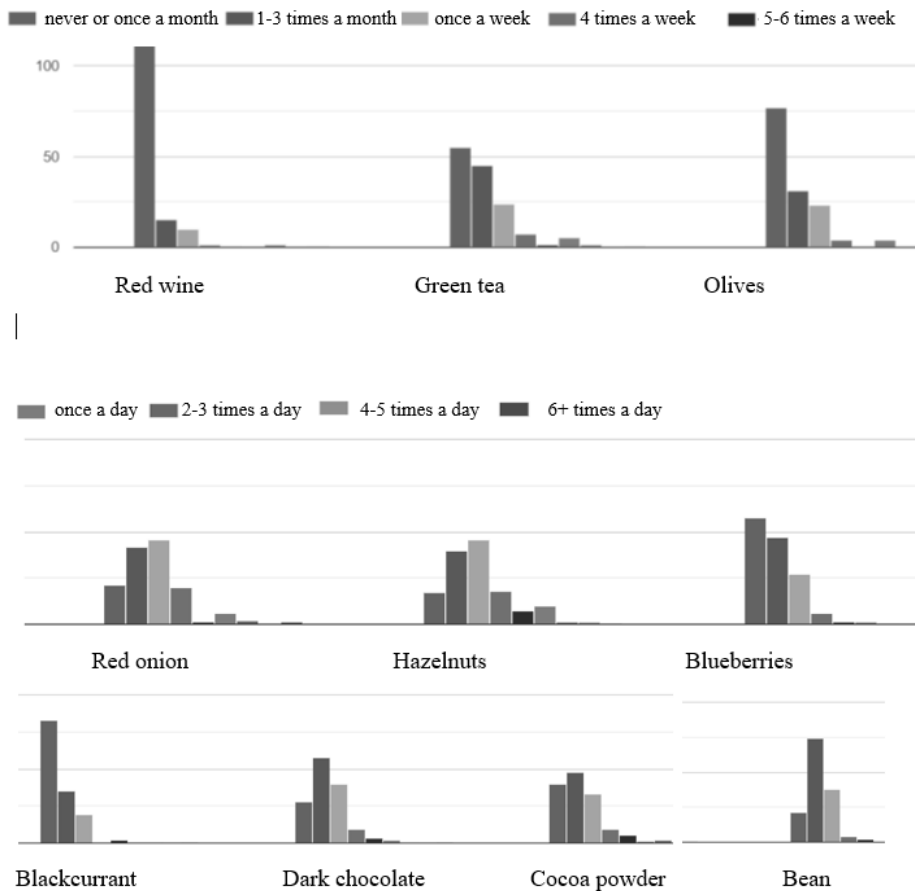


**Figure 1.** Age profile of participants

Of the total number of respondents, 29.8% are familiar with the term polyphenols, while 70.2% are not aware of the term and the benefits of polyphenols. The question referred to the use of supplements rich in polyphenols. Of the total number of respondents, 17.6% reported using supplements, while 82.4% do not use supplements.

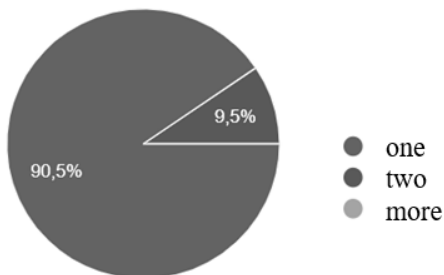
*Investigation of the frequency of consumption of foods containing polyphenols*

Ten foods rich in polyphenols are listed, and for each food, a frequency of consumption is established (never or once a month, 1-3 times a month, once a week, 2-4 times a week, 5-6 times a week, once a day, 2-3 times a day, 4-5 times a day, and 6+ times a day). The results of the frequency of consumption of these foods are presented in Figure 2.



**Figure 2.** Average portions of consumed foods

The question pertained to the number of servings consumed in a single instance of food intake. Figure 3. displays the results of the survey. The question was conducted to calculate the average portion sizes consumed in order to provide a more accurate representation of the food intake.



**Figure 3.** Number of portions per one meal

### *Determination of average polyphenol intake for an individual*

According to the polyphenol database (Phenol-Explorer), the polyphenol content, determined by the Folin method (Singleton, V.L. and Rossi, J.A., 1965), for the following foods is as follows: red wine contains 215.48 mg/100 ml of polyphenols, black tea contains 104.48 mg/100 ml of polyphenols, olives contain 117.17 mg/100 g of polyphenols, red onions contain 102.83 mg/100 g of polyphenols, hazelnuts contain 671.80 mg/100 g of polyphenols, blueberries contain 656 mg/100 g, black currants contain 820.64 mg/100 g, dark chocolate contains 1859.88 mg/100 g, cocoa powder contains 5624.23 mg/100 g, and beans contain 207.67 mg/100 g of polyphenols.

From the results in Figure 2, it has been statistically calculated that the average intake of red wine is 29.25 ml/person/day, black tea 46.32 ml/person/day, olives 22.5 g/person/day, red onions 103.15 g/person/day, hazelnuts 19.47 g/person/day, blueberries 18.05 g/person/day, black currants 8.94 g/person/day, dark chocolate 13.68 g/person/day, cocoa powder 2.8 g/person/day, and beans 6.5 g/person/day.

Based on the average intake of these foods, the amounts of polyphenols have been further calculated using the database. The calculated amounts of polyphenols for the targeted foods are: 63 mg from red wine, 48.39 mg from black tea, 26.36 mg from olives, 106.07 mg from red onions, 130.8 mg from hazelnuts, 11.84 mg from blueberries, 73.36 mg from black currants, 18.73 mg from dark chocolate, 157.47 mg from cocoa powder, and 13.49 mg from beans. The total amounts to 649.51 mg of polyphenols, meaning that an individual from the Sarajevo Canton consumes an average of this total polyphenol amount. According to recommendations, the intake of polyphenols is approximately 500 mg to 1 g. The results indicate that the polyphenol intake among the citizens of the Sarajevo Canton is close to the lower recommended limit. It appears that the population is not familiar with the term polyphenols, as most respondents do not use supplements rich in polyphenols.

### **Conclusion**

Through our research, we concluded that participants are often unaware or only partially aware of the polyphenols present in dietary products. To change this, it is necessary to educate consumers about their significance and presence in the diet (e.g., through leaflets, TV and radio programs, the internet). Foods containing polyphenols represent an exceptionally broad area suitable for discussion. Polyphenols should be key components of daily

nutrition due to their bioactive properties, disease prevention, health improvement, and functional benefits. According to a study conducted in the Sarajevo Canton, many people are not aware of the recommended daily intake of polyphenols. Younger populations show greater interest in raising awareness about the importance of adequate polyphenol consumption. It is important to emphasize that the intake of polyphenols positively affects cardiovascular, metabolic, and neurodegenerative diseases due to their antioxidant, anticancer, anti-allergic, and anti-inflammatory activities. Educating the population about the benefits of consuming foods high in polyphenols is crucial. Their wide availability and specific physiological effects position them as unique dietary compounds. The effects of polyphenols aid in various physiological conditions during and after physical activity. Significant roles have been demonstrated in the protection and recovery of muscles and bones, prevention of metabolic diseases, and maintenance of mental health in athletes and recreational participants. In summary, analyses from this systematic review indicate that further research is needed to develop tools specifically designed to measure polyphenol intake. Improvements in existing food composition databases are also key to providing more reliable, detailed, and up-to-date data. Future research should focus on developing faster, more efficient, sensitive, and accurate analytical methods for measuring concentrations of phenolic metabolites in biological fluids. Understanding the various measurement methods and their advantages and limitations is an important step toward developing a standardized approach to measuring and reporting dietary polyphenol intake. This will enable comparisons between studies and facilitate future data collection in systematic reviews to strengthen the evidence base.

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### **Procjena dijetetskog unosa polifenola kod odraslog stanovništva Kantona Sarajevo**

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### **Sažetak**

Cilj ovog istraživanja bio je analizirati percepciju i unos polifenola među stanovništvom Kantona Sarajevo (KS). Istraživanje je provedeno putem online ankete na uzorku od 131 ispitanika, većinom mlađih osoba. Ključni nalazi pokazuju da 70,2% ispitanika nije upoznatone s pojmom polifenola, a samo 17,6% koristi suplemente bogate ovim spojevima. Unos polifenola, procijenjen metodom Food Frequency Questionnaire, iznosi prosječno 649,51 mg, što je blizu donje granice preporučenog unosa (500 mg - 1 g dnevno). Polifenoli, koji se nalaze u voću, povrću, čaju, kafi i tamnoj čokoladi, imaju značajnu ulogu u prevenciji hroničnih bolesti (kardiovaskularne bolesti, dijabetes, neurodegenerativne bolesti) zahvaljujući svojim antioksidativnim svojstvima. Unatoč njihovim zdravstvenim benefitima, većina ispitanika nije svjesna važnosti polifenola u ishrani. Preporučuje se povećanje edukacije stanovništva o polifenolima kroz različite informacijske kanale kako bi se poboljšalo znanje i potrošnja ovih korisnih sastojaka.

*Ključne riječi: polifenoli, ishrana, antioksidansi*

## Nutrition during the menstrual cycle

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### Abstract

The menstrual cycle is the period from the first day of menstrual bleeding to the first day of the next bleeding (21-32 days on average), during which a coordinated sequence of hormonal secretions prepares the body for a possible pregnancy. The complex interaction of estrogen, progesterone, follicle-stimulating hormone (FSH) and luteinizing hormone (LH) represents the hormonal regulation of the menstrual cycle. The cycle consists of two phases: the follicular phase, which begins on the first day of menstrual bleeding and ends with ovulation and the luteal phase, which starts with ovulation and ends with menstruation. The study aims to investigate and explain the importance of proper nutrition according to the phases of the menstrual cycle. For the research, the specialist literature was searched in relevant databases using the keywords "menstrual cycle", "diet" and "hormone balance". In 2017, 11 women with a normal cycle, divided into luteal and follicular groups, took part in the study by Heni et al. They found an increased sensitivity of the brain to insulin in the follicular phase when resistance characterized by hyperphagia occurs. In further studies, they found that the aforementioned changes occur at the level of the hypothalamus, the regulatory center of hunger and metabolism. As more and more women of reproductive age suffer from menstrual cycle disorders caused by hormonal imbalance, the need to correct diet and lifestyle to start and end this cycle healthily is emphasized. Emotional stress, poor diet, lack of sleep and physical activity have a major influence.

*Keywords: menstrual cycle, nutrition, hormonal balance*

### Introduction

A regular menstrual cycle comprises a certain number of days (21-35) with a normal change in the hormones estrogen, progesterone, FSH and LH, depending on the phase of the cycle. For more and more women, modern lifestyles are throwing this whole process out of its natural rhythm by

causing a deficit/excess of a particular hormone in a phase when it should not be. Mostly, women experience difficulties in regulating estrogen and progesterone levels. Initially, they first resort to drug therapy, which often causes additional hormonal disturbances and puts the whole organism into a vicious circle of a series of disturbances. The first and most important step, which should be given the most attention, is the correction of diet, physical activity, and sleep quality, i.e. taking care of the lifestyle as a whole to achieve a healthy menstrual cycle in the long term.

### ***Experimental***

In preparing this article, the electronic databases of medical literature were thoroughly searched using keywords such as "menstrual cycle", "nutrition" and "hormonal balance". The aim of the article is to explain the mechanisms by which hormones cause certain changes in the female organism, their consequences and how to control them through proper nutrition and lifestyle hygiene.

### ***Results and discussion***

The most important hormones in the menstrual cycle are insulin, estrogen and progesterone. A 2017 study showed a slight but significant difference in the body's sensitivity to insulin depending on the phase of the cycle. Eleven women with a regular cycle, divided into follicular and luteal phases, took part. The euglycemic clamp method enabled the rapid delivery of a significant insulin dose directly to the brain, where it crosses the hematoencephalic barrier to regulate certain body processes. It was found that the brain is more sensitive to the action of insulin in the follicular phase and this sensitivity decreases with the onset of the luteal phase, i.e. it turns into insulin resistance characterized by polyphagy. Resistance at the brain cell level leads to a reduced ability to produce total energy (Heni, Wagner, Kullman, 2017). Estrogen rises in the follicular phase and falls in the luteal phase, i.e. after the onset of ovulation. This drop triggers the hypothalamus to release noradrenaline, which in turn causes a drop in acetylcholine, dopamine and serotonin, which is associated with fatigue, insomnia and feelings of depression, the classic symptoms of PMS (Gudipally, Sharma, 2023). In addition, women in the late follicular phase and during ovulation have been shown to consume less energy due to increased estrogen levels suppressing appetite than in the luteal phase when they consume more energy (Rogan, Black, 2023). During menstruation, when estrogen levels are low, it is advisable to consume foods that contain phytoestrogens, such as apples, plums, pears, berries, garlic, legumes, nuts and seeds. When seed cycling, eat linseed and pumpkin seeds during the follicular phase. Sunflower and sesame seeds are introduced during the luteal phase. They

contain lignans, which are phytoestrogens and can help increase estrogen levels (Hajdinjak, 2022). They are also rich in fiber, which binds estrogen in the intestine and thus helps to excrete it in excess. Omega-3 and Omega-6 fatty acids have anti-inflammatory effects, reducing menstrual pain, while magnesium does the same by relaxing the muscles. The zinc contained in pumpkin and sesame seeds supports progesterone production and the formation of the corpus luteum, which better prepares the uterus for pregnancy. Vitamin E, with its anti-inflammatory function, plays a role in supporting follicular function, and selenium, as part of the antioxidant enzyme superoxide dismutase (SOD), helps to detoxify estrogen in the liver when progesterone levels need to rise.

During chronic stress, progesterone becomes a precursor to cortisol, causing progesterone levels to decrease while cortisol levels rise (Herrera, Nielsen, Mather, 2016). In addition to lifestyle factors, overtraining can also contribute. A progesterone deficiency can be recognized by brown spots on the laundry, shortened luteal phases, impossibility of conception and miscarriages. Vitamin B6 is necessary for progesterone synthesis, so increasing the intake of foods rich in this vitamin is recommended. It is also essential for the synthesis of the neurotransmitters serotonin and dopamine, which become undersupplied due to a drop in estrogen. Additionally, it is important to consume foods rich in tyrosine, which aids in dopamine synthesis, as well as tryptophan and salts involved in serotonin synthesis and transport (Šaravanja, 2021). The chocolate craving can be explained by its components, such as phenethylamine, which is converted into dopamine and tryptophan, as well as its ability to increase the level of endorphins. Endorphins are substances that raise the pain tolerance threshold and help manage pain (Vukoje, 2014).

Penaforte and colleagues point out that regular consumption of sweets leads to a reduction in the brain's reward system response, requiring the need for an ever-increasing amount to make it respond (Penaforte, Minelli, Rezende, 2019), while Matsuura and colleagues associate this pattern of consumption with the onset of depression, anger and sleepiness, and increased consumption of fast food with depression and irritability (Matsuura, Inoue, Kidani, 2020). Instead, it is beneficial to increase the consumption of foods with a low glycemic index (rich in fiber), which help regulate blood sugar and reduce inflammatory markers (Kaushik, Sharma, Ajmera, 2017). Regular physical activity, regardless of type and intensity, has similar benefits, with aerobic activities such as cycling, walking and swimming recommended to reduce body stress (Saglam, Orsal, 2019). Premenstrual psychological changes will also stabilize with these lifestyle adjustments.

## ***Conclusion***

Foods rich in phytoestrogens, consumed during the follicular phase and menstrual bleeding, help to maintain adequate estrogen levels, while foods rich in vitamin B6 and zinc are important for maintaining progesterone levels during the luteal and bleeding phases. It is important to learn time seed cycling according to hormonal phases for balancing these hormones. Serotonin and dopamine levels can be increased through regular exercise and consuming foods rich in their precursors. The body and mind must be freed from stress. The main contributors to this are relaxing activities that a particular person likes and where they find peace, followed by aerobic exercise, vitamin B6, tyrosine, dark chocolate and other tryptophan-rich foods, as well as reduced consumption of refined sugar and fast food. Foods rich in antioxidants, omega-3 and omega-6 fatty acids, vitamin E, selenium and magnesium are effective in relieving menstrual pain.

***Author's contribution:*** conceptualization, S.Š.S.; methodology, S. I., A. Ž., Research: S.Š. S., F. C.; preparation of the original draft, S. I., A. Ž., F. C.; revision and editing: S.Š.S; visualization, S. I.; supervision, S.Š.S; all authors agreed with the final version of the paper and contributed to its quality and accuracy.

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## Ishrana tokom menstrualnog ciklusa

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### Sažetak

Menstrualni ciklus predstavlja period od prvog dana menstrualnog krvarenja do prvog dana slijedećeg krvarenja (u prosjeku 21 – 32 dana) gdje postoji usklađeni slijed izlučivanja hormona kojima se tijelo priprema za moguću trudnoću. Složena interakcija između estrogena, progesterona, foliklostimulirajućeg (FSH) i luteinizirajućeg (LH) hormona predstavlja hormonsku regulaciju menstrualnog ciklusa. Ciklus čine dvije faze: folikularna koja započinje prvim danom menstrualnog krvarenja i završava ovulacijom i lutealna faza koja počinje ovulacijom i završava menstruacijom. Cilj rada jeste detaljnije istražiti i objasniti važnost pravilne ishrane po fazama menstrualnog ciklusa. Istraživanje je sprovedeno pregledom stručne literature u relevantnim bazama podataka korištenjem ključnih riječi „*menstrual cycle*“, „*diet*“ i „*hormonal balance*“. U istraživanju Henija i saradnika 2017. godine sudjelovalo je 11 žena s normalnim ciklusom podijeljenih u lutealnu i folikularnu grupu, te je utvrđena povećana osjetljivost mozga na inzulin u folikularnoj fazi, kada se javlja rezistencija karakterizirana hiperfagijom. U dodatnom istraživanju ustanovili su da se navedene promjene dešavaju na nivou hipotalamusa, regulatornog centra gladi i metabolizma. Zbog sve većeg broja žena reproduktivne dobi s poremećajima menstrualnog ciklusa uzrokovanih hormonalnim disbalansom, ističe se potreba za korekcijom ishrane i životnog stila kako bi ovaj ciklus zdravo započeo i tako završio, a velik utjecaj imaju emocionalni stres, nepravilna ishrana, nedostatak sna kao i fizičke aktivnosti.

*Ključne riječi: menstrualni ciklus, ishrana, hormonska ravnoteža*



## Attitudes of consumers of municipality of Zrenjanin on the consumption of chicken eggs

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### Abstract

Chicken eggs are a part of many people's daily meals, and today, the egg industry represents an important part of animal husbandry. Consumer preferences for different characteristics of this food are changing, so in recent years, more emphasis has been placed on production conditions. At the same time, the safety of the eggs is often mentioned as a problem. Also, the impact of eggs and their components on health has been questioned. The aim of this research was to examine the attitudes of consumers of the municipality of Zrenjanin on these topics and to determine which factors are important to them when it comes to egg consumption. The respondents emphasize the production and the laying hens welfare, while the physical characteristics of the eggs are somewhat less important. They consider eggs a generally healthy food and are unaware of an increased risk of heart disease caused by their consumption, which has also been increasingly discussed in recent years. When it comes to the safety of eggs, many respondents didn't understand this term. These results indicate the need for better education of the population and spreading awareness, both about the positive sides and the potential risks of consuming chicken eggs.

*Keywords: eggs, Zrenjanin municipality, consumers, laying hens welfare*

### Introduction

Eggs are a low-cost and nutrient-dense whole food. Due to its high biological value, almost all of it is used by the body and it is shown as a sample protein source along with breast milk. (Park, Jung, Choi, et al., 2018) In Serbia, testing of laying hens for *Salmonella* is mandatory as part of the producer's self-control every two weeks. They also mention that the facilities where table eggs are produced for independent needs are not subjected to this legal obligation to test for *Salmonella*. In their study, Vidaković Knežević, Babić, Knežević (2017) analyzed salmonellosis infections in humans during 2016 in the territory of AP Vojvodina, and explained the importance of

regular egg checks for this bacteria. The Zrenjanin Veterinary Specialist Institute regularly conducts tests on *Salmonella spp.* in eggs and egg products. However, it should be noted that the entity in the food business is responsible for the control and regular delivery of eggs for testing. Animal welfare is the state of the organism, which shows how animals adapt to the living conditions provided for them by the humans who use them. (Vučinić, 2016) Bonnefous, Collin, Guilloteau Laurence et al. (2022) state that conventional cages for laying hens were developed after World War II in order to increase production. In 2012, conventional cages were banned in the EU and only enriched cages and cage-free systems were allowed. The enriched cage system provides 750 cm<sup>2</sup> of space per animal. In addition to enriched and conventional cages, there are also floor, free-range and organic systems for raising laying hens. (Nielsen, Alvarez, Bicout, et al., 2023)

The aim of the work is to examine the opinion of the population of Zrenjanin about preferences for buying eggs related to the organoleptic characteristics and different ways of keeping laying hens, as well as how their consumption affects human health, in order to give certain recommendations and guidelines for further research and education on this topic.

### ***Experimental***

The questionnaire consisted of 63 questions, and it was anonymous and voluntary. At the very beginning of the survey, there were questions about the socio-demographic characteristics of the respondents (sex, age, living area, professional qualifications). The following questions had three answers offered and the possibility of correspondence. In the questions, there was also a part with a Likert scale, where the respondents were supposed to mark how much they agree with the given statements (1–do not agree at all; 5–completely agree), as well as a part of the questions where they were asked to mark with a number from 0 to 5 how important certain factors are to them when buying eggs. (0–unimportant factors, 5–extremely important). Descriptive statistics methods such as the median were used during data processing. (Mutavdžić, Nikolić Đorić, 2018)

### ***Results and discussion***

When it comes to the habits and affinities of consumers related to the purchase and use of eggs on the territory of Zrenjanin municipality, the majority buy eggs once a week and consume eggs two or more times. Eggs are most often purchased in supermarkets. In the mentioned questions, it was noted that mostly women are the ones who procure the eggs. Quality components for raw chicken eggs include primarily external characteristics related to the cleanliness and quality of the shell, color, size, and then to the quality of the internal contents. (Mitrović, 2022) Based on the answers

received, it has been concluded that the size and shape of the eggs are less important items when choosing eggs for purchase. However, when it comes to colour, consumers buy light brown eggs more often.

**Table 1.** Socio-demographic characteristics of the respondents

	N %	Average age of respondents (min.-max.)	Living area		Degree of professional qualification	
			City N, %	Village N, %	IV N,%	V or> N, %
<b>Female</b>	41 59.4%	39 years (19-82)	29 60.4%	2 57.1%	26 72.2%	15 45.4%
<b>Male</b>	28 40.6%	41 years (20-84)	19 39.6%	9 42.5%	11 28.2%	17 51,5%
<b>Total</b>	69 100%	40 years (19-84)	48 69.6%	21 30.4%	36 52.2%	33 47.8%

One of the biggest problems when it comes to the safety of table eggs is contamination with *Salmonella spp.*, which can be vertical or horizontal. Vertical contamination of eggs occurs when it invades the reproductive tract and then the egg. (Vidaković Knežević et al., 2017)

Horizontal contamination occurs via the shell from feces, equipment, rodents or workers. (Holt, 2021) 36.2% of respondents believe that soiling eggs is a risk of transmitting pathogens to humans, and 30.4% of them do not know the meaning of the term egg safety. Trust in institutions is high when it comes to controls on *Salmonella spp.* which is considered by 71% of respondents, and 63.8% respondents believe that the safety of eggs is the egg producer's responsibility

Regarding the welfare of the laying hens, for many consumers, it is important that the law on animal welfare is respected during egg production (52.2%) and that they know the conditions under which the laying hens were kept (30.4%). 30.4% of respondents believe that free housing systems do not represent a greater risk of pathogens, but 36.2% of respondents believe that animal welfare and egg safety are directly related.

Fossum, Jansson, Etterlin et al. (2009) report a higher occurrence of parasitic diseases in free-range laying hens, as well as an increased risk of infectious diseases (*Pasteurella*), but Tuytens, Heyndrickx, De Boeck et al. (2008) note that the condition of the limbs in free-range hens was better than in cage systems.

**Table 2.** The importance of certain factors when buying eggs scored by respondents (0–not important at all, to 5–extremely important)

	Price	Package	Local economy support	Hygiene of workers	Shell hardness	Free range eggs	Housing systems of hens
<b>Average score</b>	2.83	2.87	4.23	4.46	3.13	3.61	3.75
<b>Median</b>	3	3	5	5	3	3	4
<b>Min.-max. score</b>	0-5	0-5	0-5	2-5	0-5	0-5	0-5
<b>Less important to respondents %</b>	37.6	36.2	7.2	5.8	30.4	14.5	14.5
<b>More important to respondents %</b>	62.3	63.7	92.7	94.2	69.6	85.5	85.5

Consumers mostly agreed with the claims that eggs contribute to increasing muscle mass (34.8%), and that eggs are an irreplaceable source of nutrients and vitamins. (30.4%). Bioactive components found in eggs such as phospholipids, cholesterol, lutein, zeaxanthin and proteins have pro- and/or anti-inflammatory characteristics that play a role in the pathophysiology of various chronic diseases. (Sanlier, Ustun 2021) Consumers generally do not believe that consuming large amounts of eggs poses a risk to human health (23.2%), that egg consumption directly affects the increase in blood cholesterol, nor that excessive egg consumption increases the risk of heart disease (21.7%). The American Dietary Guidelines have confirmed that, regardless of other dietary factors, one egg per day does not cause increased daily blood cholesterol levels and does not increase the risk of cardiovascular disease in healthy people. (Haytowitz, D., Lemar, L., Pehrsson, P. 2011)

### **Conclusion**

Based on the results of the survey, it can be concluded that consumers of the Zrenjanin municipality do not view the physical characteristics of the eggs as an important factor when buying eggs, while the conditions of egg production and keeping of laying hens are important. Based on the answers regarding the safety of eggs, it can be concluded that the consumers of the municipality of Zrenjanin usually (30.4%) do not understand the meaning of that term. However, one of the most important factors for buying eggs is the hygiene of workers with a score of 5 given by 65 respondents (94.2%). Eggs are considered by consumers to be a generally healthy food, without excessive risks for cardiovascular diseases that are often associated with this

food. When it comes to the production process and keeping conditions, the majority (52.2%) of respondents stated that they believe that free-range systems are the future of egg production, and that cage systems should be completely abandoned. Further research on this topic in different territories is desirable in order to improve the egg production process, as well as educate consumers on these topics and meet their needs.

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**Conflict of interest:** The author declares no conflicts of interest.

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## Stavovi potrošača opštine Zrenjanin o konzumiranju kokošijih jaja

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### Sažetak

Kokošija jaja predstavljaju deo svakodnevnih obroka mnogih ljudi, a industrija proizvodnje konzumnih jaja danas predstavlja bitnu granu stočarstva. Preference potrošača za različitim karakteristikama ove namirnice se menjaju, pa je tako poslednjih godina stavljen veći akcenat na uslove proizvodnje, dok se i bezbednost konzumnih jaja često pominje kao problem. Takođe, uticaj jaja i njihovih komponenti na zdravlje doveden je u pitanje. Cilj ovog istraživanja bio je da se ispituju stavovi potrošača zrenjaninske opštine o ovim temama i da se utvrdi koji su im to faktori bitni kada je u pitanju konzumacija jaja. Ispitanici ističu važnost uslova proizvodnje i dobrobiti koka nosilja, dok su fizičke karakteristike jaja nešto manje značajne. Jaja smatraju za generalno zdravu namirnicu, i ne vide povećani rizik od nastanka srčanih bolesti zbog njihove konzumacije, o čemu se takođe poslednjih godina sve više diskutuje. Kada je reč o bezbednosti jaja, kod većeg broja ispitanika primećeno je nerazumevanje tog pojma. Ovi rezultati ukazuju na potrebu za boljom edukacijom stanovništva i širenjem svesti, kako o pozitivnim stranama, tako i o potencijalnim rizicima konzumiranja kokošijih jaja.

*Ključne reči: jaja, zrenjaninska opština, potrošači, dobrobit koka nosilja*

**3-O-9**

## **Advantage of use food fortification and future for it**

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### **Abstract**

Food fortification is defined as the addition of an essential substance or more of them to food: the goals are the prevention and the correction of the deficiency of one or more nutrients in the population or in a part of it (e.g. children, pregnant women etc). Two basic conditions are the use of widely consumed food and that it is cheap and easily accessible. Some cases of food fortification have been analyzed in this work: flour and rice with iron and folate acid and dairies with vitamin D in different countries all over the world. Also, a local problem has been analyzed: the shortage of selenium and some possible solutions in the Balkan region. All the results of this research confirm that food fortification is a useful tool to fight many health problems.

*Keywords: food fortification, iron, vitamin D, selenium*

### **Introduction**

Diseases caused by lack of micronutrients represent a serious social and health problem: every third person in the world is affected by or is at risk of having a micronutrient deficiency. Anemia is endemic throughout the world. In 2011, 29% of nonpregnant women, 38% of pregnant women, and 43% of children aged 6-59 months were anemic in low-income and middle-income countries. Maternal anemia has serious consequences: maternal mortality (anemia caused by iron deficiency is cause of death for 111.000 pregnant women each year), adverse birth outcomes, poor mental health and fatigue, and delayed child development (Martorell, et al., 2015). The most common cause of anemia is iron deficiency, and worldwide, the proportion of all anemia amenable to iron supplementation is estimated to be ~ 50% in nonpregnant end pregnant women and 42% in children. Another cause of anemia is folate deficiency, but folate deficiency doesn't cause just anemia, it is correlated to most neuronal tube defect in infant (NTD) too. Low red cell folate levels may be associated with an increased risk of cardiovascular disease and stroke, in every tenth person who died because of an ischemic disease is recorded a low folate level (United Nation Children's Fund, 2004). Recent large observational data have suggested that 40% of Europeans are



vitamin D deficient and 13% are several deficient (Cashman, et al., 2016). Vitamin D deficiency is associated with unfavorable skeletal outcomes, including fractures and bone loss; severe vitamin D deficiency dramatically increases the risk of excess mortality, infections and many other diseases. In contrast to many other micronutrients, the intake of selenium varies hugely worldwide, ranging from deficient to toxic concentration. Low plasma selenium is associated with cancer mortality in elderly individuals (Rayman, 2012). Selenium is important for its immune function, its effects on the brain (during selenium depletion, brain selenium is maintained at the expense of other tissues whereas selenium deficiency causes irreversible brain injury-importance of Selenoprotein P SEPP1). Micronutrient deficiency can be prevented by a proper diet when it's possible, supplements are a good option too, but food fortification represents a solution for a large number of people.

### ***Experimental***

For the realization of this research, literature review was conducted, namely PubMed, Google Scholar, reviewed scientific literature, publicly available information using the keywords: food fortification, iron (ferrum), vitamin D, folic acid and selenium.

### ***Results and Discussion***

*Codex Alimentarius* defines fortification as the addition of one or more essential substances to food, whether they are a part of food, for the prevention or lack correction one of them in the population or in a part of the population (FAO, 1996). Due to high cereals consumption, they are the most used in food fortification followed by sugar, dairies, juices, food for children, depending on the range population we are interested to involve with. The most common used micronutrients in food fortification are iodine, iron, folate, vitamin D, vitamin A etc. Fortification can be divided into mandatory fortification (legally binding), voluntary fortification and target fortification (directed towards a specific population group). The concept of fortification developed during the XX century in Europe and USA and the first example was salt iodization in Switzerland. Another example was the mandatory fortification of cereals (white flour, rice etc.) with iron in USA from 1938; with this fortification, pellagra (a vicious disease from which annually 3000 people died) has been completely eradicated (Lazarević, Nikolić, Mitrović, 2006). Another example of food fortification with cereals has been used in Costa Rica. Costa Rica has been a pioneer and a model for mass fortification. It is distinguished by fortifying several staples. Salt has been fortified with iodine since 1972 and sugar with vitamin A during 1974 through 1981 and then reinitiated in 2003. Wheat flour was fortified with reduced iron starting

in 1958 since 2000 a more bioavailable fortifiant, ferrous fumarate, has been used instead. Ferrous bisglycinate was added to maize flour and to milk; powered milk is also fortified with ferrous bisglycinate. It is really interesting to analyze the effect of iron food fortification in Costa Rica because a significant anemia reduction was observed in the population: at the national level anemia declined in children from 19,3% to 4,0% and in women from 18,4% to 10,2%; in children, iron deficiency declined from 26,9% to 6,8% and iron deficiency anemia, which was 6,2% at baseline, could no longer be detected at the endline (Martorell, et al., 2015). The Chilean Ministry of Health legislated to add folic acid to wheat flour to reduce the risk of neural tube defects (NTD), beginning in January 2000. They identified folic acid fortification of wheat flour as a promising strategy for increasing folic acid intake on population. This assumption was based on several reasons: wheat flour is a staple food in Chile; milling for bread-making corresponds to 90% of the total wheat flour consumed, and more than 70% of the wheat flour is used for making *marraquetas* and *hallulla*, the types of bread that are typically consumed by Chileans; mills are technologically developed and the quality assurance systems for the fortification process are already in place. This policy (fortification with folic acid) resulted in a significant increase in serum and red blood cell folate in women of childbearing age 1 year after fortification, the NTD rate was significantly reduced by 43% (Hertrampf, Cortes, 2008). This kind of fortification has proven to be an effective and cost saving strategy (\$1.8million cost saving). At the beginning of XX century dairy food fortification with vitamin A and D eradicated rachitis in Europe and USA, however there was an increased incidence of hypercalcemia due to massive intakes of vitamin D from various food fortification. In some cases, hypercalcemia was associated with drinking D-fortified milk, revealing fortification of up to 232,565 IU instead of standard 400IU/quart, and consequently, prohibition of milk fortification (Jacobus, et al., 1992). However current evidence suggests that vitamin D fortification prevents deficiency safely and effectively, which is good to know because a low vitamin D status is emerging as a very common condition worldwide, and several studies from basic science to clinical applications have highlighted a strong association with chronic diseases, as well as acute conditions. Moreover, the large amount of observational data currently available are also accompanied by pathophysiological associations of vitamin D with energy homeostasis, and regulation of the immune and endocrine systems (Amrein, et al., 2020). Meta-analyses of randomized controlled trials have shown that vitamin D supplementation reduces cancer mortality by 13%, current vitamin D fortification is estimated to prevent approximately 11.000 in the EU and 27.000 cancer deaths in all European countries considered per year. If all countries in Europe would implement

adequate vitamin D fortification of foods, an estimated additional 129.000 cancer deaths could be prevented, corresponding to almost 1.2 million prevented years of life lost or approximately 9% of cancer deaths (Niedermaier, et al., 2022). In contrast to many other micronutrients, the intake of selenium varies highly worldwide, ranging from deficient (associated with selenium-deficiency diseases) to toxic concentrations (Rayman, 2012). Low selenium concentration in the soil has been recognized in Serbia when they noticed that domestic animals suffered from muscular dystrophy. This can have an implication on humans, because they eat domestic animal meat and agricultural products from that same soil, so they will have low selenium concentration in their bodies. The solution was easy to find: increase selenium in domestic animals (with fortified animal food) and indirectly increase it in human (Lazarević, et al., 2006). An example of that model is provided by eggs enriched with selenium using biotechnological solutions in the diet of laying hens, where in each egg we would have 20-25 micrograms that represents ~30% daily selenium intake (Šefer, et al., 2023).

### ***Conclusion***

Using of food fortification has showed excellent results from mitigation to complete eradication of certain diseases. Food fortification and supplements should be used more to achieve results on a large scale and in this way reduce healthcare costs and increase life quality.

***Conflict of Interest:*** Authors declares no conflict of interest

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## **Prednost upotrebe obogaćivanja hrane i njena budućnost**

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### **Sažetak**

Obogaćivanje hrane se definiše kao dodavanje esencijalne supstance ili više njih prehrambenim proizvodima: ciljevi su prevencija ili korekcija nedostatka jednog ili više hranljivih materija u populaciji ili njenom delu (npr. deca, trudnice itd). Dva osnova uslova su upotreba hrane koja se široko konzumira i koja je jeftina i lako dostupna. U ovom radu su analizirani neki slučajevi obogaćivanja hrane: brašno i pirinač sa gvoždem i folnom kiselinom kao i mlečnih proizvoda sa vitaminom D u različitim zemljama širom sveta. Analiziran je takođe i jedan lokalni problem: nedostatak selena i neka moguća rešenja na području Balkana. Svi rezultati ovog istraživanja potvrđuju da je obogaćivanje hrane korisno oružje u borbi protiv mnogih zdravstvenih problema.

*Ključne reči: obogaćena ishrana, gvožđe, vitamin D, selen*

## Assessment of vitamin C intake in the adult population of Sarajevo Canton

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### Abstract

This work is focused on the assessment of vitamin C intake in the adult population in Sarajevo Canton. The method of nutritional assessment was a 24-hour questionnaire. The obtained results showed that 33 male persons participated with their estimated intake of vitamin C being 187.7 mg, which is significantly more than the recommended 90 mg, but does not exceed the maximum allowed amount of 2 g. The highest intake was 996.5 mg, and the lowest was 11 mg, which is below the recommended amount. The sample included 35 females whose average value was 128.7 mg, which is more than the recommended 75 mg, but also does not exceed the maximum 2 g. The maximum intake of vitamin C in women was 351.2 mg, while the lowest intake was only 7 mg. Also, it was carried out a comparison of our results with the results of the previously a year long research on the territory of Federation of Bosnia and Herzegovina, where a high prevalence of insufficient intake of vitamin C was recorded (66.9% among women and 81.5% among men).

*Keywords: vitamin C, nutritional assessment, recommended intake*

### Introduction

Vitamin C, ascorbic acid, belongs to the group of water-soluble vitamins, and acts as a reducing agent and an electron donor. Vitamin C plays an extremely important role as an antioxidant reducing free radicals.

Vitamin C deficiency is mainly caused by malnutrition or an unbalanced diet, however it can also be caused by other risk factors such as pregnancy, genetic predisposition, age and conditions such as diabetes, obesity or hypertension. Chronic vitamin C deficiency leads to scurvy, a disease whose symptoms include bone weakness, tooth loss, impaired wound healing, hyperkeratosis, and anemia caused by decreased iron absorption. Vitamin C deficiency symptoms such as dizziness or lethargy are mostly associated

with improper biosynthesis of L-carnitine which consequently leads to reduced transport of fatty acids, reduced beta oxidation in mitochondria which is necessary for ATP synthesis. Recommended daily intake for different groups according to the National Institutes of Health (2021) is given in the table 1.

**Table 1. Recommended Dietary Allowances for vitamin C**

Age	Male	Female	Pregnancy	Lactation
0–6 months	40 mg*	40 mg*		
7–12 months	50 mg*	50 mg*		
1–3 years	15 mg	15 mg		
4–8 years	25 mg	25 mg		
9–13 years	45 mg	45 mg		
14–18 years	75 mg	65 mg	80 mg	115 mg
19+ years	90 mg	75 mg	85 mg	120 mg
Smokers	Individuals who smoke require 35 mg/day more vitamin C than nonsmokers.			

Therefore, the aim of this paper was to monitor and assess the intake of vitamin C on a sample of Sarajevo Canton adult population and compare the results with previous research at the level of Federation of Bosnia and Herzegovina conducted by Gicevic et al. (2019) which was based on a two-stage cluster sample of 980 households with groups based on age, gender and place of residence – urban and rural. Results for vitamin C included 853 adults. In this sample, women were on average less active than men, suffered more from chronic diseases, had a higher prevalence of using supplements and a lower prevalence of current smoking and alcohol consumption, but men had a higher prevalence of high blood pressure. More than two-thirds of participants were categorized as obese or morbidly obese. A high prevalence of insufficient intake of vitamin C was recorded - in 66.9% women and 81.5% men. According to the data obtained in this study low intake occurred even in food-rich environments due to modern lifestyles such as low intake of fruits and vegetable.

### **Experimental**

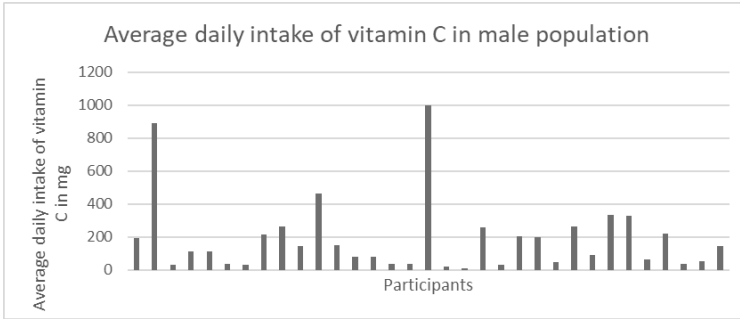
In this study a questionnaire based on 24-hour recall as a method of nutritional assessment was used. The main advantage of this method is a possibility to obtain answers on a large number of subjects in a short period of time, it is not an expensive method, it is a simple, objective method, there is little burden on the subjects and it is possible to estimate the nutrients

within the group. This is precisely one of the reasons why this method was chosen for monitoring the nutritional assessment of vitamin C in Sarajevo. An electronic survey was created which was accessed by 90 people, mostly university students from Canton Sarajevo. Considering the validity of the answers, a total of 80 responses to the survey were analyzed as some participants did not list specific groceries. However, the questionnaire is based on 24-hour recall, and the ability to recall can decrease significantly even during the day. This lowers the accuracy and representativeness of the obtained data (Block et al, 1982). Furthermore, participants in the survey may have intentionally or unintentionally excluded some of the foods in order to leave an impression or due to forgetfulness. Based on this, it is possible to distinguish between two terms: 'disappeared' food and 'phantom' food. 'Missing' food would be defined as food, which the respondent consumed, but did not mention, and 'phantom' food is food, which the respondent did not consume, but mentioned (Crawford et al, 1994). There are other disadvantages accompanying this method, namely: one recall is not enough to estimate a representative intake, participants can overestimate or underestimate their intake, and computer processing can be demanding (Block et al, 1986).

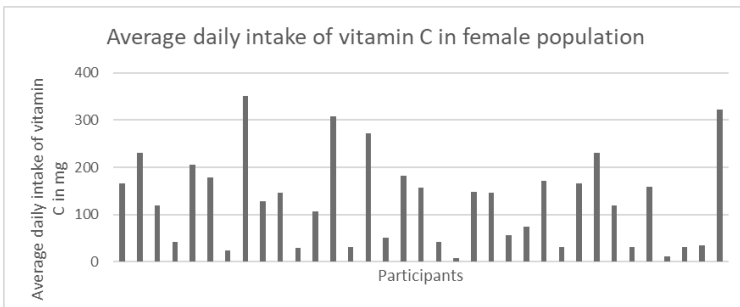
### ***Results and Discussion***

Considering the detail of the responses, 80 out of 90 responses to the electronic survey were analyzed as certain participants did not specify the exact groceries, but included their daily intake under general terms, for example 'fruit'. Of the 80 analyzed answers, 68 of them mentioned foods rich in vitamin C. Therefore, we can conclude that 85% of the participants consumed vitamin C during 24 hours.

Out of the analyzed 68 responses that included vitamin C in their daily diet, 35 women and 33 men took part in the survey. The average value of vitamin C intake in men was 187.7 mg, which is significantly more than the recommended 90 mg, but does not exceed the maximum allowed amount of 2 g. The highest intake was 996.5 mg, and the lowest intake was 11 mg which is far below the recommended amount. For women, the average value was 128.7 mg, which is more than the recommended 75 mg, but also does not exceed the maximum of 2 g. The maximum intake in women was 351.2 mg, while the lowest intake was only 7 mg. Less variations in the response occurred in the female participants, which is easily visible from the results for all respondents shown in Graphs 1 and 2.

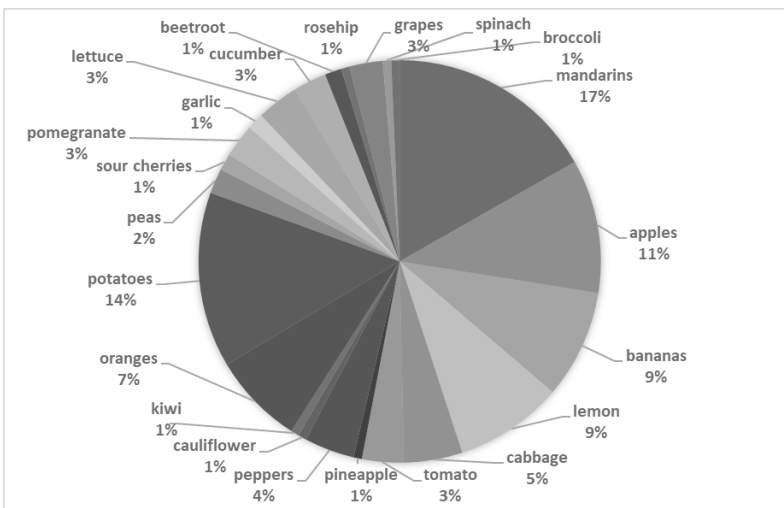


**Graph 1.** Average daily intake of vitamin C in male population



**Graph 2.** Average daily intake of vitamin C in female population

The largest number of participants satisfied their daily intake of vitamin C by consuming citrus fruits, mostly mandarins, which was expected considering the research was conducted in autumn, and apples and portions of potatoes. The intake of vitamin C through different foods is shown in Graph 3.



**Graph 3.** Intake of vitamin C in participants through specific groceries



This research has reached different results than the research by Gicevic et al. (2019). That study has found low intakes of vitamin C in most participants, especially pronounced in women, most likely due to lack of fruits and vegetables in the diet. This research has analyzed amounts of vitamin C intake, with men having a significantly higher average value than the recommended 90 mg, while women also exceeded the recommended values. However, Gicevic et al. (2019) included more participants from different demographic groups, whereas this research included only 80 valid responses from mainly young student population of a smaller area. Therefore, this research's participants are not as representative of the general population. Gicevic et al. (2019) study lasted an entire year, collecting enough data for all periods of the year giving a more precise insight in the change of levels of consumption of food rich in vitamin C during the year. On the other hand, this research only collected data for one day during autumn when participants were more likely to consume seasonal citrus fruits. Therefore, the results of this research cannot be generalized on a larger population, but provides insight into specific group that matches recommended intake, and gives an idea in which groups action to improve are needed.

### ***Conclusion***

The results of previous research highlight significant differences between men and women in levels of physical activity, chronic disease and supplement use, providing a basis for targeted interventions. The high prevalence of obesity indicates the need for public health measures aimed at promoting healthy lifestyles. The prevalence of vitamin C deficiency, especially among women, highlights the need to improve access to fruits and vegetables. This research has found satisfactory levels of vitamin C intake among participants albeit on a smaller sample of a specific demographic and social group in Canton Sarajevo. Even if the results of this study cannot be generalized, it gives an idea what population groups are more vulnerable to vitamin C deficiency and need attention compared to the one included in this research. A continual monitoring is required in order to obtain realistic data in all populations. It is important to encourage and promote a diet rich in vitamin C in vulnerable groups in Federation of Bosnia and Herzegovina.

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## **Procjena unosa vitamina C kod odraslog stanovništva Kantona Sarajevo**

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### **Sažetak**

Ovaj rad fokusiran je na procjenu unosa vitamina C kod odraslog stanovništva u Kantonu Sarajevo. Metoda nutricionističke procjene je bio 24-satni upitnik, te su dobiveni rezultati pokazali da su u anketi učestvovale 33 osobe muškog spola, a njihov procijenjeni unos vitamina C iznosio je 187.7 mg što je znatno više od preporučenih 90 mg, ali ne prelazi maksimalnu dozvoljenu količinu od 2 g. Od toga je najveći unos iznosio 996.5 mg, a najmanji 11 mg što je ispod preporučene količine. Također, u anketi su učestvovale 35 osobe ženskog spola čiji je prosječni unos vitamina C iznosio 128.7 mg što je više nego preporučenih 75 mg, ali također ne prelazi maksimalna 2 g. Maksimalni unos kod žena je iznosio 351.2 mg, dok je najmanji iznosio samo 7 mg. Također, izvršena je komparacija dobivenih rezultata sa rezultatima prethodnog istraživanja na području Federacije Bosne i Hercegovine u toku jedne godine gdje je zabilježena visoka prevalenca nedovoljnog unosa vitamina C (66.9% među ženama i 81.5% među muškarcima).

*Ključne riječi: vitamin C, nutricionistička procjena, preporučeni unos*

**3-O-11**

## **Galactagogue foods - efficacy in supporting lactation maintenance**

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### **Abstract**

For infants, human milk provides natural nutrition and is associated with short- and long-term health benefits for both infants and mothers. The World Health Organization recommends exclusive breastfeeding for the first six months of a child's life, with continued breastfeeding for at least two years. Most mothers face the problem of an insufficient amount of breast milk, which affects the health and well-being of the infant and is often cited as a reason for the introduction of supplementary feeding and premature termination of breastfeeding. Where insufficient lactation persists, galactagogues—substances thought to promote or increase breast milk production – may be used. Commonly reported galactagogues include dietary or herbal supplements, for example, oats or fenugreek, and drugs such as domperidone. Although various non-pharmacological options have anecdotal evidence of their ability to improve breast milk production, reliable studies confirming their effectiveness are lacking. Due to a lack of evidence, healthcare professionals are challenged to recommend or prescribe galactagogues, and more studies are needed to evaluate the effects of available galactagogues on breast milk production. The aim of this paper is to analyze the literature on used galactagogues including lactation cookies and non-alcoholic beer in mothers.

*Keywords: galactagogues, lactation, breastfeeding, self-efficacy in breastfeeding*

### ***Introduction***

Since 2001, the World Health Organization has recommended exclusive breastfeeding for the first six months of life, with continued breastfeeding for at least two years. While the health benefits of breastfeeding are well-known and initiation rates have risen in recent decades, many mothers stop breastfeeding before the recommended six months due to difficulties rather than personal choice. Low milk supply is the most common reason for

breastfeeding failure, often caused by factors like preterm birth, maternal or infant illness, separation from the baby, relactation after a break, and induced lactation. Anxiety, fatigue, stress, inverted nipples, oral clefts, infections, pain, and poor latch are also significant inhibitors of lactation. (Yasuda, Fukuda, Toba, et al., 2022) Additionally, the perception of insufficient milk (PIM), where a mother believes her milk is inadequate in quantity or quality, is a major reason for discontinuing breastfeeding and is common across various cultures and demographics. (Robert, Coppieters, Swennen, et al. 2014)

Galactagogues are synthetic or herbal molecules used to induce and increase milk production, involving complex interactions between physical and physiological factors, with hormones like prolactin being the most important. Oral galactagogues are categorized into pharmacological and natural (non-pharmacological) types. The choice between these two often depends on local customs and the level of awareness. Evidence regarding the benefits and potential side effects of galactagogues is crucial for making an informed decision about their use. Given the many documented benefits of continued breastfeeding for both the infant and mother, various measures are taken to enhance breast milk production.

Domperidone is often the preferred pharmacological galactagogue due to its safety for mother and infant, despite not being approved for this use in many countries and concerns about rare cardiac side effects, while metoclopramide and sulpiride carry a higher risk of adverse effects. For mothers who prefer non-pharmacological methods, there are numerous natural galactagogues that have been used for generations to boost milk production, primarily derived from plants and food sources. In many traditional cultures, specific foods and herbs play a key role in supporting lactation. The mechanisms of action of galactagogues are diverse and can be understood through three different paradigms: those that directly enhance milk synthesis, those that improve milk synthesis by addressing lactation issues (hormonal or receptor-related problems), and those that stimulate lactation by strengthening the milk ejection reflex. (Foong, Tan, Foong 2020)

The aim of this study is to investigate the nutritional context of galactagogue foods, focusing on specific nutrients that promote lactation; to systematize the mechanisms of action of galactagogue foods; to evaluate their safety and potential side effects for mothers and infants; and to formulate guidelines and recommendations for the practical application of galactagogue foods to optimize the lactation process and enhance the health of mothers and infants during breastfeeding.

## ***Experimental***

This work is descriptive and bibliographic in nature and does not include original experimental data. To achieve the research objectives, data and information were sourced from professional and scientific journals, published studies, available clinical trials, and relevant academic literature. The search was conducted using PubMed and Google Scholar. The literature reviewed spans from 2000 to 2024, providing a detailed and comprehensive overview of the topic. The search was carried out in English. The literature review process took place from March to July 2024. The analysis focused on studies assessing the effectiveness of galactagogues in enhancing breast milk production.

## ***Results and Discussion***

Lactation cookies are named for their inclusion of galactagogues, substances that enhance human milk production. Common ingredients thought to boost milk production include oats, flaxseed meal, fenugreek, brewer's yeast, and others. Palacios, Cardel, Parker, et al. (2023) aimed to compare lactation cookies containing oats, brewer's yeast, flax, and fenugreek with similar cookies lacking these galactagogues in a randomized, double-blind study. The participants were women (n = 176) breastfeeding infants aged two months, who were instructed to consume one bag of cookies (56.5 g) daily for 30 days. The milk production rate was 5.8 mL/hour in the control group and 5.5 mL/hour in the group consuming the galactagogue cookies, which was not a statistically significant difference. The authors noted potential deviations in the protocol due to remote implementation during the COVID-19 pandemic, which may have led to data entry errors. For example, participants were advised to consume one bag of cookies daily for 30 consecutive days, but some may not have eaten all the cookies or may have shared them with others. It is also important to note that the study used a specific brand of cookies, so it cannot be concluded that other lactation cookies have no effect on milk production.

In another experimental study, the galactagogic effect of cookies enriched with galohgor powder on the volume of maternal milk and lactose concentration in mothers during the early postpartum period was analyzed. Galohgor is a traditional nutraceutical made from 56 types of plants that contains  $\beta$ -carotene, bioactive compounds, and various other nutrients.  $\beta$ -carotene is a key carotenoid compound present in galohgor nutraceuticals. Within cells, retinol is oxidized into active derivatives such as retinaldehyde (retinal) and retinoic acid, which are important for regulating cell differentiation and embryonic development of the mammary gland through

nuclear receptors RAR $\alpha$ ,  $\beta$ , and  $\gamma$ , which are widely expressed in the breasts. The study involved twenty mothers, with one group consuming cookies with galohgor nutraceutical powder (NLC, n=9), while the other group consumed original cookies (ORC, n=11) without the galohgor addition. Each mother consumed four cookies daily for fourteen days postpartum. The volume of produced maternal milk was measured using an indirect method by weighing the baby on a digital scale, while lactose concentration in maternal milk was determined through high-performance liquid chromatography. The results showed a statistically significant increase in maternal milk volume in the NLC group compared to the ORC group ( $P < 0.05$ ), with an average volume of 557.0 mL/day for the NLC group and 435.9 mL/day for the ORC group. Additionally, the lactose concentration in maternal milk among mothers consuming NLC was significantly higher than in the ORC group ( $P < 0.05$ ). Based on the findings, Roosita, Ma'rifah, Nurdin, et al. (2022) concluded that NLC containing galohgor nutraceutical powder can be recommended to promote an increase in maternal milk volume and lactose concentration during the early postpartum period.

Moringa is a fast-growing, drought-resistant tree from the *Moringaceae* family, rich in vitamins, minerals, and essential amino acids. It contains a variety of glycosides and is used as a galactagogue in South and Southeast Asia. Studies have found no harmful effects associated with the consumption of moringa leaves by breastfeeding mothers (Fungtammasan and Phupong 2022). In an open-label, non-randomized study, 23 postpartum mothers consumed cookies (125 g/day) containing moringa, iron, and vitamin A, while another group of 23 mothers received only iron and vitamin A for 14 days starting from the first day after delivery. The moringa cookies, developed in the Nutrition Laboratory at Poltekkes Kemenkes Semarang with the help of nutritionists, contained 86% wheat flour and 14% moringa leaf flour, along with margarine, sugar, egg yolks, skim milk powder, baking soda, salt, and banana flavoring. Results showed that in the experimental group, the average weight of the infants increased from 3206.96 g before the intervention to 3390.96 g after seven days (Post 1) and to 3756.70 g after 14 days of treatment (Post 2). Statistical analysis using a paired t-test revealed a significant difference in average weight post-intervention ( $p = 0.000 < 0.05$ ). According to Pujiastuti, Salsabila and Anwar (2022), the moringa leaf cookies effectively increased breast milk production, as indicated by infant weight gain. The cookies contained flavonoids and polyphenols, with flavonoid content at 0.1626% and polyphenol content at 0.3635% per 100 g of cookies. The hypothesis that flavonoids and polyphenols contribute to increased milk production is based on their known biological properties, particularly as antioxidants. These compounds may influence hormonal processes, including the stimulation of prolactin, although the precise

mechanisms remain to be fully elucidated. This is supported by earlier research by Aliyanto and Rosmadewi (2019), which indicated that the consumption of moringa leaves as vegetables could enhance milk production compared to those who do not consume them. Another study showed that a combination of fenugreek seed flour and banana flower, rich in flavonoids and polyphenols, also increases milk production based on indicators of milk volume (Okinarum, Lestariningsih, and Dewi 2020). It is important to note that not all dietary sources of polyphenols have the same effect, as plants contain different types of polyphenols and flavonoids with potentially varying biological effects. Specific compounds present in moringa leaves may have unique lactogenic effects that other polyphenol sources lack. Further research is needed to explore these effects in detail and to compare different sources of polyphenols in the context of lactation.

Beer can increase serum prolactin levels during breastfeeding due to polysaccharides from barley and hops. Non-alcoholic beer is considered a traditional galactagogue in many cultures, although its prolactogenic mechanism has not been fully clarified in clinical studies. During the malting process, where grains are soaked to germinate and then rapidly dried to stop germination, bioactive components are released from barley, including those that may enhance milk production, such as malt extract. A double-blind study compared a relatively new commercial galactagogue product (Femaltiker, Nutropharma LLC, Poland) containing malt extract, 70% barley  $\beta$ -glucan, and powdered lemon balm (*Melissa officinalis* L.) with a placebo in mothers of premature infants. The aim of this research was to assess the safety and efficacy of this unique galactagogue composition based on barley malt with  $\beta$ -glucan and lemon balm. The study involved 117 mothers of preterm infants, randomly divided into galactagogue and placebo groups, with forty mothers in each group completing the study. Mothers took one packet of the product twice daily for fourteen days, starting from the third day postpartum. Milk volume was measured by the mothers after pumping with an electric breast pump. The total milk volume over the two-week period was greater in the active group compared to the placebo group (an average of 6036 mL vs. 4209 mL). On the last day of the study (Day 14), mothers in the barley group produced more milk than those taking the placebo (an average of 95 mL vs. 62.5 mL). Mothers of preterm infants with inadequate milk supply who received the galactagogue produced 34% more milk by Day 14 of lactation and 30% more milk overall compared to the placebo group. Additionally, the average daily volume of milk expressed in the experimental group exceeded 500 mL by the seventh day, whereas this was not observed in the placebo group, even at the end of the study. No adverse effects were reported in mothers during the study, but caution is advised for individuals with celiac disease due to the presence of gluten in



barley. In clinical practice, barley malt-based products like Femaltiker® may be used in the first two weeks of lactation to support milk supply in cases of inadequate production, considering their safety and efficacy. However, recommendations for their long-term use to maintain milk supply should be evaluated in future studies. (Wesolowska, Pietrzak, Kociszewska-Najman, et al., 2021)

### ***Conclusion***

Based on the analyzed studies in this paper, galactagogues such as lactation cookies enriched with galohgor powder, lactation cookies enriched with moringa powder and non-alcoholic beer can be recommended, with consultation from a physician, to support the optimal lactation process and improve the health of mothers and infants during breastfeeding. The research findings presented in this paper indicate a positive effect of the mentioned galactagogues on increasing the volume of maternal milk, as well as the fact that they are still insufficiently studied. Therefore, the main conclusions of the paper are as follows:

- There is a need for better, evidence-based information on the efficacy and safety of non-pharmacological galactagogues.
- It is necessary to adequately educate breastfeeding mothers about issues such as inadequate frequency and duration of breastfeeding, as well as the proper and safe use of galactagogue foods in practice.
- Health professionals should be prepared to assist consumers in understanding the use of galactagogue preparations, exploring and considering all available options, from traditional to those based on scientific evidence.

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***Conflicts of Interest:*** The author declare no conflicts of interest.

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## **Galaktogoga hrana – efikasnost primjene u održavanju dojenja**

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### **Sažetak**

Za dojenčad, humano mlijeko pruža prirodnu prehranu i povezano je s kratkoročnim i dugoročnim zdravstvenim prednostima za njih i majke. Svjetska zdravstvena organizacija preporučuje isključivo dojenje u prvih šest mjeseci života djeteta, uz nastavak dojenja najmanje dvije godine. Većina majki se suočava s problemom nedovoljne količine majčinog mlijeka što utiče na zdravlje i dobrobit dojenčeta i često se navodi kao razlog za uvođenje dohrane i prijevremeni prekid dojenja. Tamo gdje insuficijencija laktacije traje, mogu se koristiti galaktagogi – tvari za koje se smatra da pospješuju ili povećavaju proizvodnju majčinog mlijeka. Često prijavljeni galaktagogi uključuju dijetetske ili biljne suplemente, na primjer, zob ili piskavicu, i lijekove kao što je domperidon. Iako razne nefarmakološke opcije imaju anegdotske dokaze o njihovoj sposobnosti poboljšanja proizvodnje majčinog mlijeka, nedostaju pouzdane studije koje bi potvrdile njihovu efikasnost. Zbog nedostatka dokaza, zdravstveni radnici se suočavaju s izazovom preporučivanja ili propisivanja galaktagoga, te je potrebno više studija da bi se procijenili efekti dostupnih galaktagoga na proizvodnju majčinog mlijeka. Cilj ovog rada je analizirati literaturu o korištenim galaktagogima kao što su laktacijski keksići i bezalkoholno pivo kod dojilja.

*Ključne riječi: galaktagogi, laktacija, dojenje, samoučinkovitost u dojenju*

**3-O-12**

**Effects of BCAA & Pre-Workout in dietary of “Brazilian Jiu Jitsu” practitioners**

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**Abstract**

Brazilian Jiu Jitsu (BJJ) is a demanding combat sport which requires high durability, strenght and stamina. In order to perform better, BJJ athletes often consume supplements such as Pre-Workout and BCAA. The aim of this work is to present side effects and potential health risks that these supplements can produce in certain situations when they're not used properly. To investigate this, participants from a selected BJJ academy completed a questionnaire designed to gather information about their dietary habits and supplement usage, as well as any positive or negative effects they experienced. These findings were compared with results from existing research on the topic. Symptoms suggestive of acute pancreatitis were reported, potentially linked to pre-workout supplement intake. Additionally, another study associated high concentrations of BCAAs with oxidative stress and inflammation. Several symptoms observed in the work, which indicate these issues, may also be attributed to BCAA supplementation. Therefore, it is crucial for athletes to use these supplements cautiously, prioritize verified products, and seek professional guidance to avoid potential health complications.

*Keywords: Pre-Workout, BCAA, acute pancreatitis, oxidative stress, participants*

***Introduction***

Branched-chain amino acids represent essential amino acids for the human organism. BCAA supplements like ActivLab or Nutrend are often used in BJJ. Main reasons of BCAA consumption involve muscle growth, muscle recovery, better exercrise performance in terms of stamina, durability etc. Pre-Workout is a mainstream supplement which consists of mainly non-

essential amino acids such as beta-alanine, l-arginine, l-citrulline combined with creatine monohydrate and caffeine, even though, some formulations contain BCAA. The benefits include more energy and better focus during intense training drills, delay of muscle fatigue by buffering acid in the muscles, endurance, amplifying muscle gains etc. Although widely popular, data from the literature suggest that these supplements are not entirely safe.

The effects of certain ingredient combinations have not been fully tested, leading to reports of side effects and adverse reactions, such as decreased insulin levels, abdominal pain, insomnia, and edema. (Ridha, Rivera Gonzalez, Seenivasagam;2023) The aim of this work is to record frequency of toxic effects potentially caused by ingredients of these supplements in BJJ students.

### ***Experimental***

This research was conducted among BJJ students from the BJJ academy "Brotherhood" from Sarajevo. Participants were required to be adults (18 years or older) who provided informed consent, agreeing that their responses could be used for research purposes. Additionally, participants needed to be consumers of one or both of the supplements. Participants' (n=21) daily intake of dietary proteins and supplements was assessed by questionnaire as a research instrument.

The questionnaire consists of two parts for each supplement (Pre-workout and BCAA). First part is general (demographic data, dietary and supplemental daily protein intake). The second part is the main one containing question on any symptoms noticed by participant, from the list based on literature data on known or suspected toxic effects of selected supplements and/or their ingredients.

Estimation of daily protein intake is crucial for the assessment of possible toxic effects while adding extra amino acids. It's been asked whether the person had consulted a health professional before taking supplements or not. They were also asked if they use medication along with BCAA which would be contraindicated such as levodopa, thyroid gland hormones, hypoglycemic drugs and corticosteroids.

The participants report on noticed symptoms were compared to similar reports found in previously published papers.

## ***Results and discussion***

There were 23 candidates (12 amateurs, 9 competitors and two professionals) that filled the form, but 21 of them fulfilled conditions to participate. None of the participants reported on previous health conditions or use of any medications contraindicated in the combination with BCAA. Participants age ranged from 18 to 50 years (mean  $25.43 \pm 7.35$  years).

13 participants took Pre-Workout, two of which consulted a health professional (No. 6 and 22). Two participants were excluded (No.10 and 22). Six of supplement users take it half an hour pre-training, seven take it shortly pre-training and two use supplements an hour before training (Table 1.).

Dose intake varies from 5-20 g where almost half of them takes 5g. Most of the students consume the supplement once a day, and a minor of them once in two days. Five of them consume it consistently over one month, the other five from time to time and the rest at least few times per week in a month.

Three consumers (No. 5, 14 and 19) registered abdominal pain in the higher region. Other symptoms that occurred during their consumption were: exhaustion (No.5), diarrhea (No.14) and dehydration (No.19). These symptoms could be linked with acute pancreatitis.

Acute pancreatitis is a disorder of the exocrine pancreas and is associated with acinar cell injury with local and systemic inflammatory responses with pancreatic edema. It appears suddenly and generally lasts a short time. This was proven to be triggered by non-essential amino acids (L-arginine and L-citrulline) which are a major part of Pre-Workout. (Nirula;2000) L-arginine is synthesized by our organism and is important for the muscle metabolism, tissue regeneration etc.

In this situation, with high daily protein intake and using pre-workout, it converts in nitric oxide (NO) which has a vasodilatory effect increasing vascular permeability improving blood flow. L-citrulline boosts nitric oxide production by converting into arginine. (Wu, Morris;1998) With so much water inside the pancreatic tissue it causes edema and cell necrosis.

The first clinical case report linked a healthy 16-year-old to pancreatitis induced by L-arginine causing effects which were mentioned. Investigating further, the patient reported that he started consuming supplements a month ago. (Saka, Tüzün, Ateş et al.;2004) Arginine directly affects pancreatic beta cells which will stimulate excess secretion of insulin. With that in the way, excess levels of insulin lead to hyperinsulinemia, where hyperinsulinemia results from insulin resistance, which happens when cells in muscles, fat and liver don't respond as they should to insulin. (Freeman, Pennings; 2022)

**Table 1.** Performance effect and side effect related to supplementation with pre-workout or BCAA reported by participants

Participant AMATEUR			Protein intake (g/day)		Pre-workout		BCAA	
N	Age year	Sex F/M	Food	PS	Performance effect	Side effect	Performance effect	Side effect
1	24	M	n.r.	n.a	n.a.	n.a.	d.t.faster heartbeat and stronger; blood pressure decrease; fatigue p.t.bigger heart rate	n.r.
2	34	M	n.r.	n.a	d.t.: feeling stronger and faster p.t.: feeling like before consumption	n.r.	d.t. more focused	a.p.t. exhausted p.t.
3	22	M	100 - 150	n.a	n.r.	n.r.	n.a.	n.a.
4	40	M	n.r.	n.a	n.r.	n.r.	n.r.	n.r.
5	18	M	80	20	d.t: feeling stronger and faster p.t.: feeling like before consumption	a.p.t.: <b>I had abdominal pain, specifically higher region</b> p.t.: exhausted	d.t. more focused	p.t. I had stomachache a.p.t. <b>sometimes I couldn't sleep</b>
6	20	M	90	30	d.t.: more energy, feel stronger and faster	p.t.:urinated a lot a.p.t.:dehydrated	d.t. more focused	a.p.t.: felt exhausted post-training
7	21	M	85	n.a	d.t.:feel stronger and faster p.t.:feel like before consumption	p.t.:urinated a lot a.p.t.:felt dehydrated	n.r.	a.p.t.: felt better d.t. but p.t. I'm <b>in pain</b>

**Table 1.** Performance effect and side effect related to supplementation with pre-workout or BCAA reported by participants-cont.

Participant AMATEUR			Protein intake (g/day)		Pre-workout		BCAA	
N	Age year	Sex F/M	Food	PS	Performance effect	Side effect	Performance effect	Side effect
8	21	M	70	n.a.	d.t.: feeling stronger and faster	n.r.	n.r.	n.r.
9	24	M	54	40	n.a.	n.a.	d.t.: feeling stronger p.t.: I feel better	a.p.t.: <b>I couldn't sleep</b>
11	19	M	70	n.a.	d.t.: feeling stronger and faster	n.r.	d.t.: feeling stronger p.t. : I feel better	n.r.
12	22	M	95	n.a.	d.t: more energy, feeling stronger and faster	n.r.	n.r.	n.r.
Participant COMPETITORS & PROFESSIONALS			Protein intake (g/day)		Pre-workout		BCAA	
N	Age year	Sex F/M	Food	PS	Performance effect	Side effect	Performance effect	Side effect
13	47	M	n.r.	n.a.	d.t.: feeling stronger and faster p.t.: feeling like before consumption	a.p.t.: feeling sleepy if there's too much caffeine	n.r.	n.r.
14	22	M	150	40	d.t. : feeling stronger	p.t. diarrhea a.p.t.: <b>I had abdominal pain, specifically higher region</b>	n.a.	n.a.
15	32	M	100	20-40	n.a.	n.a.	n.r.	n.r.
16	27	M	n.r.	n.a.	n.a.	n.a.	n.r.	n.r.



**Table 1.** Performance effect and side effect related to supplementation with pre-workout or BCAA reported by participants-cont.

Participant COMPETITORS & PROFESSIONALS			Protein intake (g/day)		Pre-workout		BCAA	
N	Age year	Sex F/M	Food	PS	Performance effect	Side effect	Performance effect	Side effect
17	30	M	n.r.	n.a.	d.t.:feeling stronger and faster p.t.: feeling like before consumption	a.p.t.:I felt exhausted post-training	n.r.	n.a.
18	22	M	61.6	n.a.	n.r.	n.r.	n.a.	n.a.
19	22	M	60-70	n.a.	n.r.	p.t.: tired a.p.t.: <b>I had abdominal pain, specifically higher region;</b> dehydrated	n.r.	d.t.: faster heartbeat and stronger; blood pressure decrease; fatigue p.t.: <b>couldn't sleep</b> a.p.t.: <b>insomnia</b>
20	21	M	50	n.a.	d.t.: more strenght	p.t.: bloating	n.r.	a.p.t.: <b>chest asphyxiation</b>
21	22	M	n.r.	n.a.	n.a.	n.a.	d.t.: more focus; decreases over time	n.r.
23	24 P.	M	100	20	n.r.	n.r.	n.r.	n.r.

Legend: P. – professional; PS – protein supplement; n.r. – not reported; n.a. – not used; d.t. – during training; p.t.-post-training; a.p.t. – after a period of time

Another clinical case with the same health effect is a 35-year-old patient with abdominal pain, who denied any use of steroids, and admit use of mainly Pre-Workout, and Whey protein. He was using supplementation as directed on the label and did not overuse or double any servings.

Other potential side reaction participants reported is frequent urination and sometimes dehydration. This can be explained with the diuretic effects of caffeine which is one of the frequent ingredients in this kind of supplements, but it can be also connected with prediabetic hyperglycemic state. Other

amino acids, such as beta-alanine, have been linked to pancreatic beta cell desensitization, resulting in decreased insulin release. (Ridha et al.) Similar symptoms that appeared with consumers (No. 5, 14 and 19) could be mostly due to their high protein intake combined with this supplementation containing high levels of non-essential amino acids. Although supplement intake was not assessed as a causal factor in the three detected cases, it could indeed be one since these were generally healthy subjects without other known expositions that would result in such health damage. Arginine, alanine and citrulline most definitely are helpful in normal conditions, but they could most likely be a huge issue for a person of average weight with high protein intake.

The rest of participants reported mainly positive effects of supplementation, which was expected due to caffeine and creatine monohydrate effects. Caffeine is a methylxanthine which stimulates the central nervous system. It affects the blood vessels by vasodilation, causing their relaxation, and also increase focus. Besides that, the presence of creatine will increase creatine-phosphate storages. (Rang, Dale, Ritter et al.;2016) So, it justifies energy boost. 19 students take BCAA, and only three of them consulted a health professional before using supplementation (No. 6, 7 and 22). 12 participants take BCAA post-training, five use BCAA 30 minutes pre-training, and two use supplements an hour pre-training. 13 of them consume 5g of BCAA, where 7 of them consume it as a ActiveLab 330 ml drink and 6 as a powder, as for the rest, three of them use 10 g, one use 20 g and one practitioner use half a dose of 5g (2.5g). Five students (No. 20, 19, 9, 7 and 5) recorded several health issues such as insomnia (No.5, 9 and 19), pain (No.7) and chest asphyxiation (No.20). These symptoms could be associated with oxidative stress and inflammation caused by high concentration of BCAA.

It was discovered that isoleucine, leucine and valine (BCAA) create superoxide anions ( $O_2^-$ ) by NADPH oxidase and the mitochondria which causes oxidative stress and they promote pro-inflammatory factors which cause inflammation and pain in the body, potentially initiating creation of cancer cells in some areas. (Mescka, Guerreiro, Donida et al; 2015)

An experiment was done to prove whether this is true or not by following these actions in peripheral blood mononuclear cells (PBMCs) that were obtained by healthy donors. In cultured PBMCs, BCAA increased the production of reactive oxygen species (ROS) and activated Akt-mTOR signaling. Researchers used different inhibitors and activators of this molecular pathway and deduced that several mTOR activation by BCAA is linked to ROS production and mitochondrial dysfunction. BCAA stimulated the activation and release of pro-inflammatory molecules, which are cytokines such as interleukin-6, tumor necrosis factor- $\alpha$ , intracellular adhesion molecule-1 or CD40L, and the migration of PBMCs.

In conclusion, elevated BCAA blood levels can promote the activation of circulating PBMCs. These data suggest that high concentrations of BCAA can do serious damage. This is a big issue for BJJ students due to their extensive workouts where the body uses a lot of oxygen, and this results in disbalance between oxidative radicals and the synthesis of antioxidants especially when they add BCAA to their diet with a potentially higher protein intake. So, symptoms that they could feel after some times are pain, tiredness, insomnia because of the oxidative stress itself, and of course inflammation. Taking medical drugs with BCAA could therefore cause serious health problems. (Zhenyukh, Civantos, Ruiz-Ortega et al;2017). Corticosteroids can increase muscle breakdown and taking BCAAs might be seen as a way to counterbalance these effects by supporting muscle protein synthesis. (Holecek; 2018) Hypoglycemic medication used with BCAA who promotes insulin secretion would cause a problem with glucose levels and cause hypoglycemia. (Nie, He, Zhang et al.; 2018) Using thyroid gland hormones with BCAA could affect hormonal gastrointestinal resorption and metabolic rate in person with hypothyroidism. (Zhang, Guo, LeBlanc, et al.; 2007) When BCAAs are present in high concentrations, they can compete with levodopa for transport into the brain. This competition can reduce the amount of levodopa that enters the brain, potentially diminishing its effectiveness in managing Parkinson's symptoms. (Cumming & Nair; 1978)

### ***Conclusion***

In this survey some health problems were reported by participants, and they can be connected to the use of supplements even though there are some limitations of this study (the sample size was small). This suggests that prevalence of side effects and negative health effects could be higher. Summarizing answers showed that many participants don't realize what potential side effects these supplements could cause especially if the protein intake is high or if they combine some drugs with BCAA. Therefore, an initiative focused on education regarding rational dosage and proper dietary intake is necessary to improve training outcomes. Additionally, consulting with healthcare professionals is essential to minimize the risk of side effects.

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## **Uticaj BCAA i Pre-Workouta u ishrani sportista vještine “Braziliska Jiu Jitsa”**

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### **Sažetak**

Braziliska Jiu Jitsa (BJJ) je zahtjevan borilački sport koji zahtijeva visoku kondiciju spremu, snagu i izdržljivost. Kako bi poboljšali svoje performanse, BJJ sportisti često koriste dodatke prehrani kao što su Pre-Workout i BCAA. Cilj ovog rada je da predstavi nuspojave i potencijalne zdravstvene rizike koje ovi dodaci mogu uzrokovati u određenim situacijama kada se ne koriste pravilno. Kako bi se istražili efekti, učesnici iz odabrane BJJ akademije ispunili su upitnik dizajniran tako da prikupi informacije o njihovim prehrambenim navikama i upotrebi dodataka, kao i o bilo kojim pozitivnim ili negativnim efektima koje su primjetili. Ovi rezultati su uspoređeni sa rezultatima postojećih istraživanja na ovu temu. Prijavljeni su simptomi koji upućuju na akutni pankreatitis i moguća je njihova povezanost sa primjenom suplementa Pre-Workout. Pored toga, drugo istraživanje je povezalo to da visoke koncentracije BCAA dovode do pojave oksidativnog stresa i upale. Za prijavljene simptome u radu, koji ukazuju povezanost sa ovim problemima, postoji šansa da se mogu pripisati uticaju BCAA suplementacije. Stoga, ključno je da sportisti koriste ove dodatke oprezno, prioritiziraju verifikovane proizvode i da se posavjetuju sa zdravstvenim stručnim licem kako bi izbjegli potencijalne zdravstvene komplikacije.

*Ključne riječi: Pre-Workout, BCAA, akutni pankreatitis, oksidativni stres, učesnici*

## Consumer attitudes towards donkey milk in Vojvodina

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### Abstract

In previous years donkey milk has attracted increasing attention due to its beneficial effects on the human health, as well as its great similarity in composition to human milk. Donkey milk is popularly considered to be one of the healthiest and the most healing products of animal origin. What makes this milk very attractive for consumers is its effect on the immune system (due to the high content of immunoglobulins), the lower percentage of fat, lactose, and rare occurrence of allergic reactions during its consumption. Through a survey conducted among different demographic groups in the territory of Vojvodina, we analyzed consumer attitudes about the prices, quality, availability and benefits of donkey milk. The results indicate that most consumers recognize donkey milk as a healthier alternative to cow's milk, but they think it's expensive and difficult to find on the market. This research shows that the respondents have insufficient information about the benefits of donkey milk, as well as its role in the treatment of certain diseases and conditions. Based on these results, it is recommended to organize various educational campaigns and to take measures to increase the availability of donkey milk.

*Keywords: donkey milk, health, consumer attitudes*

### *Introduction*

Milk as a product for human nutrition has been used since ancient times. In recent years, as lactose intolerance grows, alternatives are increasingly being used, i.e. yam and cow's milk (Aspri, Economou, Papademas, 2016). What further contributes to the reduced allergic reaction to milk proteins is the reduced amount of casein in donkey milk than in cow's milk (Martini, Licitra, Salari, 2017). The increasing consumer interest in alternative dairy products has placed donkey milk in the spotlight, especially within regions where access to conventional types of milk is restricted or contraindicated for health-related issues (Murgia, Scano, Contu, 2016). Donkey milk has high nutritional and therapeutic values and a rich cultural history with various

civilizations having consumed it for centuries. Donkey milk examined in earlier studies showed that it has a higher amount of lysozyme than cow's and human milk, which contributes to better conservation of milk and dairy products (Pilla, Daprà, Zeconi, 2010). The other scientific studies have unveiled its nutrient-rich profile that is filled with vitamins, minerals, and bioactive components that seem to confer promising effects on human health (Martini, et al. 2017). In addition to the effect on the immune system and its hypoallergenic properties, certain studies have even shown that by activating lymphocytes and macrophages, donkey milk kills lung cancer tumor cells (Martini, et al. 2017). The present study seeks to explore the consumers' knowledge, attitudes, perceptions, and behavior towards donkey milk using a mixed-methods research design in order to pinpoint the determinants that underpin consumer interest and acceptance of this unique product category.

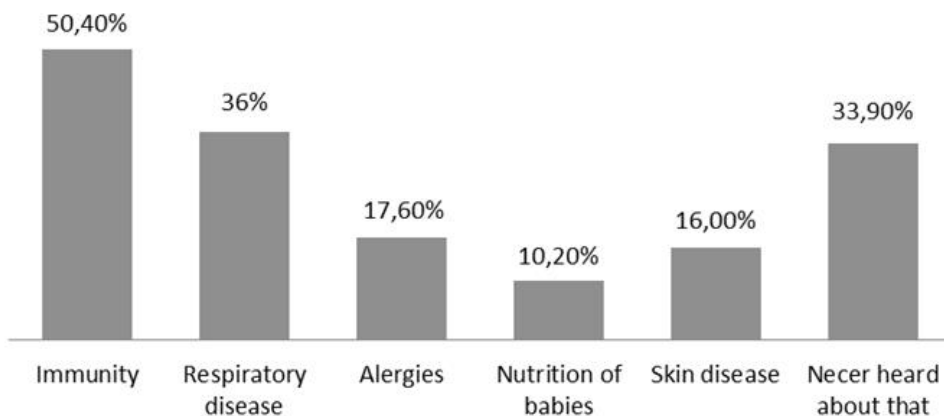
### ***Experimental***

This study use a survey method to investigate consumer attitudes towards donkey milk. Survey research is used in this study as it provided an overview of opinions and behavior of participants. A quantitative surveys interviews was undertaken to achieve greater depth of insight into consumer perceptions, preferences, and purchasing decisions. The target population for this study were consumers 18 years or older from a variety of demographic backgrounds. Overall 381 respondents were individuals who currently reside in urban and rural locations in Vojvodina with large representation percentage for health conscious consumers through convenience sampling method. The survey and interview responses were the major source of data. An on-line survey via social media (Facebook) was facilitated over the course of ten days. Informed consent was collected from potential respondents prior to the start of this study. Subjects and their answers will remain anonymous and confidential.

### ***Results and discussion***

The majority of respondents were women (66.4%), while men made up 33.6%. Half of the respondents (50.4%) have completed university and/or postgraduate studies, while the other half have completed high school (46%). The majority of respondents answered that their place of residence is in an urban environment (73.3%). As for consumer habits and affinities regarding the purchase and consumption of milk and milk products in the territory of Vojvodina, the majority of people answered that they consume cow milk (78.7%). When it comes to donkey's milk, only a small percentage of respondents have consumed it (11%), while even a third of the total number of respondents said that they had never heard of dairy products from donkeys in general (32.5%). Despite the fact that donkey's milk is an excellent

alternative to cow's milk, the small number of farmers who breed donkeys makes this product a luxury. This is supported by the fact that only a small percentage of respondents have tried this type of milk (11%).



*Chart 1. The respondent's opinion about the therapeutic effects of donkey milk*

Respondents in the territory of Vojvodina answered the question of what they think donkey's milk is most used for. Even a third of respondents answered that they had never heard of donkey milk as a food product (33,90%), while another third answered that it is used for the purpose of treating respiratory symptoms (36%). Half of the respondents (50.4%) declared that they think it is used to strengthen immunity in immunocompromised and healthy people (Chart 1.). What none of the interviewees mentioned was the effect of donkey milk on lung tumor cells. Antiproliferative and antitumor effects of milk enable the reduction of cell viability of A549 tumor cells (Mao, Gu, Sun, 2009). The fact that even a third of respondents have never heard of this type of milk tells us about the low availability of the product on the market, as well as the low awareness of people about the impact of donkey milk on the body and its chemical composition. What may have contributed to the fact that people consume this milk to a lesser extent is the fact that the yield of donkey milk is 100-150g in the entire lactation, which is extremely small (Li, Liu, Guo, 2017). Today, there are no acceptance criteria for donkey milk, so it is necessary to set standards for market standardization and strengthening of quality inspections. On the other hand, lately as the environmental awareness of people in Serbia is growing, they are increasingly buying alternative products from local markets and farms, nurturing the organic origin of animal products (Pajić, Čobanović, Erdeljan, 2023). When making a potential purchase, the most important thing for respondents was hygiene



correctness of milk and the way and conditions of keeping animals from the aspect of animal welfare and hygiene of the animal itself.

### ***Conclusion***

The study findings suggest that while the population in Vojvodina seems to be interested in other dairy products, there continues to be great impediments when it comes to acceptance of donkey milk. Some participants understood that donkey milk probably contain some health benefits especially for the lactose intolerant or people who prefer natural foods, but there were stated barriers: misconceptions about taste, limited access, and a higher price. In the end, the conclusion of this research is that respondents in the territory of Vojvodina have a positive attitude about donkey milk, but there is also a lack of knowledge about it. Through building greater awareness and understanding, producers in the dairy industry can position the donkey milk more effectively in the increasing pool of consumers who are becoming more conscious of their health.

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## **Stav potrošača u Vojvodini prema magarećem mleku**

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### **Sažetak**

Prethodnih godina sve veću pažnju privlače netradicionalni proizvodi, kao što je magareće mleko, zbog svog blagotvornog dejstva na ljudsko zdravlje, kao i zbog velike sličnosti u sastavu sa majčiniim mlekom. Magareće mleko u narodu važi za jedno od najlekovitijih i najzdravijih proizvoda životinjskog porekla. Ono što čini ovo mleko veoma atraktivnim za potrošače jeste njegovo dejstvo na imuni sistem (zbog velike količine imunoglobulina), manji procenat masti, manja količina laktoze nego u kravljem mleku i smanjena pojava alergijskih reakcija pri kozumaciji. Kroz anketu sprovedenu među različitim demografskim grupama na teritoriji Vojvodine, analizirali smo stavove potrošača o cenama, kvalitetu, dostupnosti i koristi magarećeg mleka. Rezultati ukazuju na to da većina potrošača prepoznaje magareće mleko kao zdraviju alternativu kravljem mleku, ali se suočava sa preprekama poput visoke cene i ograničene dostupnosti na tržištu. Ovo istraživanje pokazuje da ispitanici nemaju dovoljno informacija o benefitima magarećeg mleka, kao i ulozi u lečenju određenih bolesti i stanja. Na osnovu ovih rezultata, preporučuje se organizovanje različitih edukativnih kampanja, kao i preduzimanje mera u cilju povećanja dostupnosti magarećeg mleka.

*Ključne reči: magareće mleko, zdravlje, stavovi potrošača*

**Diluted milk: health threat or harmless practice?**

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**Abstract**

The objective of this research was to assess consumer attitudes and knowledge in Republic of Serbia regarding the term "milk adulteration", specifically focusing on the practice of diluting milk with water. The study aimed to explore both consumer awareness and perceptions about this issue. A structured questionnaire was distributed to respondents, revealing that a majority were aware of the practice of milk dilution. However, most respondents were unfamiliar with the term "milk adulteration", despite being aware of the act itself. More than 60% of the respondents indicated that they would avoid consuming diluted milk, citing concerns about nutritional value and possible health risks. Furthermore, 47% expressed moderate concern about the potential health effects of consuming diluted milk. These findings suggest a notable gap between awareness of the practice and understanding of its implications, pointing to the necessity for targeted public education initiatives. Such efforts could improve consumer knowledge about the risks associated with adulterated milk and enhance food safety standards in the region.

*Keywords: milk adulteration, consumer awareness, food safety, milk dilution*

***Introduction***

Milk is a staple in human nutrition due to its rich nutritional value, including proteins, calcium, vitamins, and minerals. In Serbia, as in many other countries, milk is frequently consumed as part of daily diets, especially by children and the elderly (Gavojdian, Janku, Krasic, 2016). However, the practice of milk adulteration by dilution with water has become a serious concern. This practice not only reduces the nutritional value of milk but may also pose health risks. Problems arise particularly when inadequate water is used, which can contain harmful microorganisms, or when substances like melamine are added to cover up adulteration. Melamine, a plasticizer used in the manufacture of plastic products, can mimic high-quality proteins in quality control tests. The consumption of food contaminated with melamine

can lead to serious health issues, including kidney stones, kidney damage, bladder problems, and in severe cases, hospitalization or even death, particularly in children (Şerbancea, Belc, Stănescu, 2018). The aim of this research was to examine the awareness of consumers in Serbia regarding the concept of milk adulteration, particularly dilution with water, and their attitudes towards the safety and quality of such products in order to provide valuable insights into the need for increased consumer education and stricter regulations in the dairy sector.

### ***Experimental***

The questionnaire consisted of 20 questions in total, the first five of which focused on the socio-demographic characteristics of the respondents. The remaining 15 questions were aimed at personal attitudes, experiences, and opinions. The respondents were residents of the Republic of Serbia, and the survey was completed anonymously and voluntarily, with sampling conducted through social networks. The questions were multiple-choice, and respondents selected one option per question.

### ***Results and Discussion***

A total of 300 respondents completed the questionnaire, with 24% being male and 76% female. The average age of respondents was 37 years. An equal percentage of respondents (39%) had completed high school or higher education (college or university), while 17% had completed postgraduate studies, and 5% had only completed primary school. The majority of respondents (38%) had lived exclusively in urban areas. Regarding milk consumption, 71.6% of respondents consume cow's milk once a week or more, while 20.3% consume it less than once a week, and 8% do not consume milk at all. In terms of where they purchase milk, respondents indicated they most often buy milk from large supermarkets (58.7%), small local stores (20.3%), or directly from producers or farms (17.3%), reflecting a trend toward urbanization and product availability. The remaining 3.7% stated that they buy milk from other sources or do not prioritize where they buy it.

Milk origin is highly significant to respondents, with 48.3% considering it very important, 41% occasionally checking labels, and 32% always doing so. This awareness of origin aligns with global food consumption trends, where increasing attention is paid to product quality and transparency. Accordingly, further consumer education on the importance of label information is recommended. When asked about powdered milk, 70.7% of respondents stated they never buy it, while 47% believe that "regular" milk is healthier

than reconstituted milk. These results suggest that consumers prefer fresher, more natural products, which may have implications for market and production strategies. Manufacturers may need to invest more in marketing that emphasizes the freshness and naturalness of their products.

The survey revealed that 61.7% of respondents had heard of milk dilution with water, but 39.7% were unfamiliar with the term "milk adulteration." Around 35.7% of respondents understood the term and its implications, while 24.7% had heard of it but were unsure of its exact meaning. When asked what they would do if they found out the milk they buy had been diluted with water, 60.3% said they would stop purchasing it, indicating high concern about product quality. This concern may stimulate a stronger demand for quality certification and control, which could be implemented by retailers and producers. A significant percentage of respondents (47%) expressed concern about diluted milk, and 76% agreed that stricter quality control measures for milk should be in place. These results highlight the need for greater regulation in the dairy industry to protect consumers. Only 57.7% of respondents had partial trust in existing controls, raising questions about the effectiveness of current regulations. This lack of confidence in the system may lead to increased demand for alternative sources of milk and local producers.

In Serbia, milk quality is regulated by the Food safety law (2019), the Regulation on the quality of raw milk (2017) and the Regulation on the quality of dairy products and starter cultures (2014). These regulations establish standards for the production, processing, and control of milk and dairy products, including requirements for quality and safety. Additionally, specific aspects of milk quality are governed by regulations derived from these laws. These include detailed guidelines on acceptable levels of contaminants, such as microorganisms and chemical additives, to ensure that products meet both national and international safety standards.

The Food safety law requires regular inspections and testing of milk and dairy products to prevent adulteration and contamination, while the Veterinary law focuses on the health and safety of animals used in milk production. Together, these laws provide a comprehensive framework aimed at protecting consumer health. However, enforcement can be inconsistent, and as shown in this study, consumer trust in these controls is not fully established. To strengthen consumer confidence, it is recommended that additional resources be allocated to stricter enforcement of these laws and greater transparency in the milk production process. Increased collaboration between regulatory bodies and industry stakeholders can improve compliance with safety standards. Additionally, consumer education campaigns about the importance of food safety regulations, how to identify certified products, and the potential health risks of adulterated milk could further enhance trust and

promote public health. Consumers and producers are encouraged to consult the most up-to-date versions of the Food safety law and Veterinary law through official government websites, such as the Ministry of agriculture, forestry, and water management or the Ministry of health. These resources provide crucial information on food safety requirements, helping both consumers and businesses stay informed and compliant with current regulations.

### ***Conclusion***

The research on milk dilution and adulteration in Republic of Serbia reveals a significant gap between consumer awareness and attitudes on the subject. While most respondents recognize the importance of milk quality, there is a clear need for increased education about the risks associated with adulteration, particularly those related to health. The results indicate that although a large percentage of respondents regularly consume milk, only 35.7% are fully aware of the issue of milk adulteration, while 39.7% are unfamiliar with the term. This situation suggests a need for enhanced consumer education to raise awareness about potential health risks and the quality of dairy products. Moreover, the majority of respondents (76%) believe that stricter milk quality controls are necessary, highlighting general concern for food safety. This provides a basis for policy recommendations, which could include strengthening regulations and implementing consumer education campaigns. In conclusion, this study underscores the importance of collaborative efforts in protecting consumers and improving the quality of dairy products in Serbia. Increased consumer awareness and education, along with appropriate quality control measures, can significantly reduce the occurrence of milk adulteration and protect public health. Further research in this field can shed more light on consumer attitudes and behaviors and help identify effective strategies to prevent milk adulteration.

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Șerbancea F, Belc N, Stănescu A. Risk Factors in the Assessment of the Conformity of Falsified Dairy Products. Quality-access to success. 2018 Apr 1;19(163).

## **Razblaženo mleko: zdravstvena pretnja ili bezopasna praksa?**

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### **Sažetak**

Cilj ovog istraživanja bio je da se ispituju stavovi i znanja potrošača u Republici Srbiji u vezi sa pojmom „falsifikovanja mleka“, sa posebnim fokusom na praksu razblaživanja mleka vodom. Studija je ispitala informisanost i stav potrošača o ovoj temi. Upitnik je podeljen ispitanicima, od kojih je većina (preko 70%) navela da je upoznata sa praksom razblaživanja mleka. Međutim, oko 65% učesnika nije bilo upoznato sa terminom „falsifikovanje mleka“, uprkos tome što su znali za samu praksu. Više od 60% ispitanika izjavilo je da ne bi konzumiralo razblaženo mleko, navodeći zabrinutost zbog smanjene nutritivne vrednosti i potencijalnih zdravstvenih rizika. Dodatno, 47% ispitanika je izrazilo umerenu zabrinutost zbog mogućih zdravstvenih posledica konzumiranja razblaženog mleka. Ovi rezultati ukazuju na značajan jaz između svesti o praksi i razumevanja njenih posledica, što ukazuje na potrebu za ciljanom edukacijom javnosti. Takve inicijative bi mogle poboljšati znanje potrošača o rizicima povezanih sa falsifikovanim mlekom i unaprediti standarde bezbednosti hrane u regionu.

*Ključne reči: falsifikovanje mleka, svest potrošača, bezbednost hrane, razblaživanje mleka*

## Periodontitis and diabetes mellitus

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### Abstract

Periodontitis and diabetes mellitus are complex, interrelated diseases that significantly impact each other, as well as oral and overall health. Diabetes mellitus is a chronic disease characterized by hyperglycemia and leads to a range of health complications. It develops either due to an autoimmune reaction against insulin-producing pancreatic cells (type 1) or as a result of insulin resistance (type 2). Diabetes can affect all organ systems and is now recognized as a risk factor for periodontitis in both adults and children. On the other hand, periodontitis, a disease of the tooth-supporting structures, raises inflammatory markers in the body and may adversely affect glycemic control in diabetic patients. Current research focuses on uncovering the mechanisms involved in the interaction between diabetes and periodontitis.

The aim of this paper was to highlight the bidirectional relationship between diabetes and periodontitis.

Diabetes affects periodontitis through various mechanisms, including impaired collagen metabolism, reduced neutrophil function, and increased periodontal tissue destruction. Although the impact of periodontitis on diabetes is not yet fully understood, studies have shown that it negatively affects glycemic control in diabetic patients and may contribute to the development of insulin resistance.

*Keywords: diabetes mellitus, periodontitis, periodontal therapy, complications*

### **Introduction**

Periodontitis and diabetes mellitus are complex diseases that are interconnected and have a significant impact on each other, as well as on the oral health and general well-being of the individual (Hong, Kim, Seok, et al., 2016). Diabetes mellitus is a common chronic disease manifested by the



presence of hyperglycemia and causes various health complications. It results either from an autoimmune reaction which targets pancreatic cells that produce insulin (type 1) or results from insulin resistance (type 2) (Hong, et al., 2016; Naiff, Carneiro, Carmo Guimarães, 2018). It can affect all organs of the body and today diabetes is recognized as a risk factor for periodontitis in both adults and children. On the other hand, periodontitis as an inflammation of the tooth supporting tissue can increase the serum levels of pro-inflammatory mediators and have an adverse effect on the glycemic control in diabetic patients. Today, research is directed towards discovering the overall mechanisms involved in the interaction between diabetes and periodontitis (Hong, et al., 2016; Nascimento, Leite, Vestergaard, et al., 2018; Baeza, Morales, Cisterna, et al., 2020).

The aim of the work was to point out the two-way connection between diabetes and periodontitis.

### ***Experimental***

The study utilized numerous research articles published in relevant citation databases, such as PubMed and Google Scholar.

### ***Results and Discussion***

Patients with diabetes mellitus are at a higher risk of developing periodontal disease, with periodontitis recognized as the sixth complication of diabetes (Preshaw, Alba, Herrera, et al., 2012). Studies indicate that patients with type 2 diabetes are three times more likely to develop periodontitis compared to healthy individuals. Both diseases are prevalent, multifactorial, and increase pro-inflammatory mediators (Wu, Xiao, Graves, 2015; Taylor, Preshaw, Lalla, 2013). Diabetes disrupts collagen metabolism, impairs neutrophil function, and accelerates periodontal tissue destruction, while periodontitis negatively affects glycemic control and may lead to insulin resistance (Sanz, Ceriello, Buyschaert, et al., 2018).

Many diabetic patients are unaware of the link between diabetes and periodontal health. Better communication between healthcare providers and dental practitioners is needed to address this. Regular dental visits should be encouraged, and education for both medical and dental professionals must be improved (Bascones-Martínez, Muñoz-Corcuera, Bascones-Ilundain, 2015; Mattson and Cerutis, 2001).

Managing diabetes and periodontitis requires patient motivation. While diabetes treatment involves medication and lifestyle changes, patient engagement in oral health is crucial for successful periodontal therapy. Quitting smoking can enhance control of both conditions (Matthews, Perio, 2002). Additionally, periodontal therapy has been shown to reduce inflammatory markers, improve glycemic control, and lower the risk of

diabetic complications, especially in well-controlled diabetic patients (Kumar, Mishra, Mohanty, et al., 2014).

### ***Conclusion***

Diabetes mellitus is a disease that requires an interdisciplinary approach treatment, and the competent doctors should highlight the need of patients for regular check-ups doctor of dental medicine. Doctors of dental medicine must be well acquainted with the medical history of patients, but also with the specifics of their illness in order to know how to correctly approach the treatment of such patients. Constant education and literature study on the importance of periodontal therapy in patients patients with diabetes mellitus will give us new knowledge about the connection between these two diseases that will maybe change the course of disease progression and improve the quality of life.

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***Conflicts of Interest:*** The authors declare no conflicts of interest.

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## **Parodontalna bolest i diabetes mellitus**

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### **Sažetak**

Parodontitis i diabetes mellitus su kompleksne, međusobno povezane bolesti i imaju značajan uticaj kako jedna na drugu, tako i na oralno i opće zdravlje. Dijabetes melitus je hronična bolest koja se očituje prisutnošću hiperglikemije i uzrokuje niz zdravstvenih komplikacija. Nastaje ili zbog autoimune reakcije na ćelije gušterače koje proizvode inzulin (tip 1) ili kao posljedica razvoja inzulinske rezistencije (tip 2). Može izazvati posljedice na svim organskim sistemima, a danas se navodi i kao rizični faktor za razvoj parodontitisa kod odraslih, ali i kod djece. S druge strane, parodontitis kao bolest potpornih struktura zuba uzrokuje povišenje upalnih parametara u organizmu i može imati štetno djelovanje na kontrolu glikemije kod pacijenata oboljelih od dijabetesa. Istraživanja su danas usmjerena prema otkrivanju sveukupnih mehanizama koji sudjeluju u interakciji između dijabetesa i parodontitisa.

Cilj rada je bio ukazati na dvosmjernu vezu između dijabetesa i parodontitisa. Dijabetes različitim mehanizmima utiče na parodontitis, uključujući poremećaj metabolizma kolagena, smanjenu funkciju neutrofila i pojačanu destrukciju parodontalnih tkiva. Uticaj parodontitisa na dijabetes još uvijek nije do kraja razjašnjen, ali istraživanja su pokazala da kod pacijenata oboljelih od dijabetesa nepovoljno utiče na kontrolu glikemije i može doprinijeti razvoju inzulinske rezistencije.

*Ključne riječi: dijabetes melitus, parodontitis, parodontološka terapija, komplikacije*

**Nutritional status of patients treated with radiotherapy**

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**Abstract**

Oncology patients undergo a range of therapeutic treatments, including radiotherapy. Such therapies lead to impaired body functions and numerous side effects such as vomiting, nausea and weight loss. To improve quality of life, nutrition is very important in this type of patient. The aim of this article is to show how important it is to maintain the nutritional status of oncology patients during radiotherapy. A review study was conducted by analyzing the available literature in the relevant databases (ScienceDirect, PubMed and Google Scholar). The study showed that nutritional measures had a positive effect on the results and that counseling had a similar or even more favorable effect. After RT function scores improved ( $p < 0.003$ ) in terms of improvement in food intake and nutritional status in group 1 / group 2 ( $p < 0.05$ ) and worsened in group 3 ( $p < 0.05$ ). This information suggests that the nutritional status of patients contributes to the prevention and subsequent treatment of oncologic diseases. Significant weight loss in oncology patients during radiotherapy remains a problem despite improvements in radiotherapy technology and a multidisciplinary approach. Selecting high quality foods in the diet of oncology patients helps maintain muscle mass and improves treatment outcomes.

*Keywords: nutritional status, radiotherapy, nutritional therapy, personalized approach*

**Introduction**

Cancer is a disease characterized by uncontrolled cell division and their abnormal growth. Oncology patients undergo a range of treatments, including radiotherapy. This type of therapy leads to impaired bodily functions and numerous side effects such as vomiting, nausea, weight loss, etc. To improve the quality of life, nutrition is very important for this type of patient (Rahelić, 2023). It is very important that they consume all the necessary macro- and micronutrients as part of their overall diet. The nutritional status of patients treated with radiotherapy is a key factor influencing the success of treatment

and quality of life (Vranešić Bender, Krznarić, 2008). Radiotherapy, which is used to treat various malignancies, often causes side effects such as nausea, vomiting, loss of appetite, changes in taste and inflammation of the mucous membranes. These symptoms can lead to reduced food intake, which can result in malnutrition and weakened immunity in patients (Grdelj Šimec, Planinc-Peraica, Ostojić Kolonić, 2013). Nutritional status and general health will affect the patient's ability to tolerate the therapy or prescribed treatment. In addition, with appropriate nutritional therapy and maintenance of adequate nutritional status, it is possible to influence prognosis, quality of life and functional status, as well as improve response and tolerability of therapy. In cancer patients, it is not recommended to follow strict, restrictive diets that would lead to a reduction in nutrient intake or to consume high doses of vitamins and minerals if their deficiency has not been proven by biochemical tests (Antunović, 2019). When we talk about the diet of oncology patients in general, an adequate diet is recommended: balanced, varied and moderate. The most common problem in oncology patients is loss of appetite. During this time, despite the possible side effects, it is important to eat regularly and choose foods that provide enough calories and protein to support recovery. Oncology patients require an increased calorie intake while paying attention to the quality of food, which should contain adequate amounts of proteins, minerals, vitamins, electrolytes, trace elements and fiber. Nutrition plays a key role in supporting the health of cancer patients, as it can improve the body's resilience, reduce the side effects of treatments and improve quality of life (Mišlov, 2018). Proteins are particularly important for tissue repair and maintaining muscle mass, while carbohydrates provide the necessary energy. Fats, especially healthy fats such as omega-3 fatty acids, reduce inflammation and support the function of the immune system. Vitamins and minerals such as vitamin C, D, zinc and selenium also help to boost immunity and support the body during recovery. A diet rich in fiber from fruits, vegetables and whole grains improves digestion and can help prevent constipation, which is common in patients undergoing treatment. Patients are also advised to avoid processed foods, refined sugars and saturated fats as these can exacerbate inflammation and have a negative impact on overall health. A diet plan tailored to the patient's needs and condition can significantly contribute to recovery and better tolerance of therapies (Vukić, 2023).

Objective of this work was to define the fundamental role of nutrition and its importance in oncology patients and to investigate how nutrition affects the body during radiotherapy.

### ***Experimental***

An analytical study was conducted by searching the available scientific literature in the relevant databases (ScienceDirect, PubMed, Medline and

Google Scholar) published in the period from January 2019 to October 2024, using the keywords: "radiotherapy", "nutrition", "side effects", "oncology patients" and "nutritional status".

### ***Results and Discussion***

The nutritional status is of great importance for the survival of patients suffering from a tumor. Malnutrition is common in cancer patients and has a negative impact on clinical outcomes. Moderate and severe malnutrition was found in 76% and 12% of cancer patients, respectively. The prevalence of malnutrition in cancer patients is 60.2%. However, only 28.4% of malnourished patients and 57.6% of malnourished patients have a balanced diet. Radiotherapy is crucial for tumor control, but it further increases the risk of malnutrition. Studies show that 74% of patients are malnourished at the end of radiotherapy. The 2002 Nutritional Risk Score (NRS-2002), body weight, BMI, hemoglobin, lymphocyte, total protein and albumin levels were significantly reduced during radiotherapy (Wang, Lu, Zho, et al., 2019). Oncology patients exhibit intense catabolic activity and are prone to higher nutritional risk and clinical complications. It is important to analyze the nutritional prognosis of patients undergoing radiotherapy and relate it to outcomes and complications. Prognostic results can be obtained by a combination of prognostic nutritional index (PNI) and subjective global assessment (SGA) (Irigaray, Santana, Pott, et al., 2024). A prospective multi-institutional study conducted in Seoul (Korea) to assess the nutritional status of 1000 patients undergoing radiotherapy for the treatment of head and neck, lung or gastrointestinal cancers found that 39.2% of subjects were malnourished. Their malnutrition is associated with: Loss of subcutaneous fat or muscle wasting (odds ratio [OR], 11.473), increased metabolic burden (OR, 8.688), sacral edema or ascites (OR, 3.234), and weight loss  $\geq 5\%$  (OR, 2.299). The results of this study serve as a basis for the implementation of nutritional interventions in patients treated in radiation oncology departments (Koom, Ahn, Song, et al., 2012). Researchers Paul Ravasco, Isabel Monteiro-Grillo, Pedro Marques Vidal and Maria Ermelinda Camilo (2005) conducted a prospective, randomized, controlled trial in patients with head and neck tumors undergoing radiotherapy. Seventy patients with head and neck tumors referred for radiotherapy were randomly assigned to the following groups: Group 1, patients who received nutritional counseling with a normal diet; Group 2, patients who maintained their usual diet plus nutritional supplements; and Group 3, patients who maintained their preferred dietary intake. Dietary intake (determined by dietary history) and nutritional status (determined by the Ottery subjective global assessment) and quality of life (determined by the European Organization for Research and Treatment of the Cancer Quality of Life Questionnaire version 3.0 [EORTC QLQ-C30]) were

assessed at baseline, at the end of RT and after 3 months. The study showed that nutritional measures had a positive effect on the results and that counseling had a similar or even more favorable effect. Counseling had a significant effect on patient outcomes in that Group 1 had fewer symptoms compared to Group 2 and Group 3 ( $p < 0.07$ ). At 3 months, the study showed a 90% reduction in severity scores 1+2 for anorexia, nausea, vomiting, xerostomia and dysgeusia in group 1 compared to 67% in group 2 and 51% in group 3 ( $p < 0.0001$ ). After RT, QOL function scores improved ( $p < 0.003$ ) in terms of improvement in food intake and nutritional status in group 1 / group 2 ( $p < 0.05$ ) and worsened in group 3 ( $p < 0.05$ ). After 3 months, patients in group 1 had maintained or improved their overall quality of life, while patients in groups 2 and 3 maintained or worsened their overall quality of life (Ravasco, et al., 2005). Another prospective study included 207 patients referred to the Department of Radiotherapy. Patients were classified according to the location of the tumor (head/neck, breast, lung, stomach or colon). Patients' nutritional status was assessed at baseline, at the end of radiotherapy and 3 and 6 months after radiotherapy by subjective global assessment (SGA). All patients were supported with additional meal portions or normal enteral nutrition during and after radiotherapy if they belonged to the moderately or severely malnourished groups. The study showed that 31 of the patients were malnourished at the start of radiotherapy, which increased to 43 after radiotherapy. After 6 months of following a nutritional plan set up by experts, the number decreased by 8% and the patients' nutritional status improved during this period (Diclehan, Bulent, Muge, et al., 2006). The third study included 87 patients with non-small cell lung cancer (NSCLC) who underwent curative thoracic radiotherapy (CTRT). The aim of the study was to evaluate the prognostic value of changes in the prognostic nutrition index before and after radiotherapy in patients with stage III NSCLC. The study showed that the change in the prognostic nutritional index PNI is a suitable parameter to assess the patient's immune-nutritional status at the end of CTRT and that a decrease in the PNI value of more than 22% can predict a poor therapeutic prognosis. For the entire group, median overall survival (OS) and progression-free survival (PFS) were 18.7 (95% confidence interval (CI): 15.3–22.16) and 13.4 (95% CI: 12.07–14.8) months, respectively. The 2-year OS was reported as 38.2% (Kaymak, Ozkan, 2021). As a glimmer of hope in the diagnostic treatment of tumor patients, proton radiation therapy can improve the nutritional status of cancer patients (Menglin, Yue, Yinghui, et al., 2023).

### ***Conclusion***

Nutrition is an important aspect in the treatment and recovery of oncology patients, especially during radiotherapy, where there are a number of side



effects that can affect nutritional status. An adequate diet, which includes a balanced intake of macro and micronutrients, helps to reduce side effects such as nausea, vomiting and loss of appetite, while supporting immunity and tissue repair. Poor dietary habits have been linked to the development of several types of cancer, further emphasizing the importance of prevention through nutrition. In industrialized countries, a significant percentage of cancers are due to poor diet, highlighting the need for a proactive approach to disease prevention. A diet rich in fruits, vegetables, whole grains and healthy fats can significantly reduce the risk of malignancies while improving the quality of life for patients already facing a diagnosis. Tailored diet plans that take into account the individual needs of patients are key to maintaining nutritional status during treatment. It is important to avoid strict diets and high-dose supplements without prior medical supervision, as they can worsen the patient's condition. In summary, nutritional support for oncology patients not only improves the tolerability of treatment, but also contributes to a better recovery and quality of life. Education about proper nutrition should be an integral part of oncology care so that patients can take an active role in their fight against the disease. A healthy diet as a preventative measure is an important step in reducing cancer incidence and improving health.

**Author Contributions:** conceptualization: M. M. M.; methodology: N. M., L. S., M.V.; research: N. M., L. S., M. M. M., M. V.; original draft preparation: N. M. and L. S.; review and editing: M. M. M. and M. V., vizualization: M. V.; supervision: M. M. M. All authors have read and agreed to the published version of the proceeding.

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## Nutritivni status pacijenata tretiranih radioterapijom

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### Sažetak

Onkološki pacijenti prolaze kroz niz terapijskih tretmana, uključujući radioterapiju. Takve terapije, dovode do narušavanja tjelesnih funkcija, i mnogobrojnih nuspojava kao što su: povraćanje, mučnina, i gubitak tjelesne težine. U cilju poboljšanja kvalitete života, kod takve vrste pacijenata je veoma važna ishrana. Cilj rada je prikazati značaj održavanja nutritivnog statusa onkoloških pacijenata kod primjene radioterapijskog tretmana. Provedena je pregledna studija analizom dostupne literature u relevantnim bazama podataka (ScienceDirect, PubMed i Google Scholar). Studija je pokazala da su prehrambene mjere imale pozitivan učinak na rezultate i da je savjetovanje imalo sličan ili čak povoljniji učinak. Nakon radioterapije rezultati su se poboljšali ( $p < 0,003$ ) u smislu poboljšanja unosa hrane i nutritivnog statusa u grupi 1 / grupi 2 ( $p < 0,05$ ) i pogoršali u grupi 3 ( $p < 0,05$ ). Takva informacija donosi zaključak da nutritivni status pacijenata doprinosi prevenciji, i kasnijem liječenju onkoloških stanja. Značajan gubitak težine onkoloških pacijenata u toku radioterapijskog tretmana ostaje problem, uprkos poboljšanju tehnologije radioterapije i multidisciplinarnom pristupu. Odabir kvalitetnih namirnica u ishrani onkoloških pacijenata, pomaže u održavanju mišićne mase i poboljšava ishod liječenja.

*Ključne riječi: nutritivni status, radioterapija, nutritivna terapija, personalizirani pristup*

### 3-O-17

## Significance of Magnetic Resonance in Assessing Nutrition in School-aged Children

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### Abstract

In terms of association with cardiometabolic risk, magnetic resonance imaging is superior to other anthropometric measurements. Magnetic resonance imaging (MRI) is the gold standard for examining and quantifying the distribution of abdominal fat in young children. The aim of this article is to demonstrate the importance of magnetic resonance imaging in the assessment of body composition and metabolism in children. A review study was conducted by screening the available scientific literature in relevant databases (PubMed, Medline and Google Scholar). The primary outcome of the case-controlled study was visceral fat volume. Secondary outcomes were subcutaneous fat volume, proton density fat fraction (PDFF) of visceral and subcutaneous fat and hepatic PDFF. Visceral and subcutaneous fat volume as well as PDFF and hepatic PDFF were higher in the obese group than in the control group. MRI measures of adiposity and hepatic PDFF were strongly correlated with anthropometry, especially with the waist circumference. Quantification of adipose tissue volume is time-consuming. Advances in MRI technology, including high-speed multichannel imaging sequences for data acquisition with advanced coil designs and motion artifact corrections, will improve the capabilities of magnetic resonance imaging for use in young children.

*Keywords: visceral fat, children, MRI imaging, imaging techniques*

### Introduction

Body composition and psychological changes determine nutrient requirements and behavioral variability in diet and physical activity (Pietrobelli, Malavolti, Fuiano, 2007). Body composition regulates metabolism in children and adolescents, with differences between genders and age groups (Ly, Armstrong, Yeh, et al., 2019). Overweight and obesity in children and adolescents have become a global public health problem with increasing prevalence (Simoni, Guglielmi, Aparisi Gómez, 2020). They are

also considered strong risk factors for the development of cardiovascular disease, diabetes mellitus, depression and cancer in adulthood (Marunowski, Świętoń, Bzyl, et al., 2021). Anthropometric measurements usually determine cardiometabolic risk, but also by magnetic resonance imaging (MRI). MRI allows a more accurate quantification of abdominal fat, especially visceral fat, which carries a higher health risk. MRI enables more precise visualization, which is crucial for the early detection of cardiometabolic risks.

This study aims to discuss recent advances in measurements to quantify total body and regional adiposity and map adipose tissue distribution to assess metabolic risk factors in children. The aim is to provide a research-oriented overview of magnetic resonance imaging methods for the study of pediatric body composition and metabolism (Pietrobelli, et al., 2007). Although the increasing prevalence of childhood obesity was noted nearly two decades ago, childhood obesity continues to increase at an alarming rate. Childhood obesity is not only associated with health risks such as metabolic syndrome and insulin resistance, but also often leads to obesity in adulthood, which is associated with morbidity and mortality. Accurate measurement of obesity in phenotyped children is critical for modern genetic and clinical research (Shen, Liu, Punyanitya, et al., 2005). Quantitative magnetic resonance imaging (qMRI) provides precise measurements of body composition by quantifying visceral adipose tissue (VAT), subcutaneous fat (SAT) and brown adipose tissue (BAT) as well as lean mass. In addition, qMRI provides further meaningful estimates such as proton density and fat percentage of adipose tissue (Simoni, et al., 2020).

### ***Experimental***

A review study was conducted by screening the available scientific literature in relevant databases (PubMed, Medline and Google Scholar).

### ***Results and discussion***

In the human body, the main function of white adipose tissue is to contribute to energy homeostasis by absorbing and storing lipids and preventing ectopic lipid deposition. Deposits of white adipose tissue are mainly found in the subcutaneous areas of the upper and lower half of the body and in the visceral area. In recent decades, there has been increasing evidence that the amount of visceral adipose tissue (VAT) is associated with various metabolic disorders such as insulin resistance, hyperinsulinemia, dyslipidemia and hypertension. In clinical practice, some anthropometric indices have been proposed for rapid and reliable assessment, such as waist circumference, hip circumference, waist-to-hip ratio, skinfold thickness, and body mass index (BMI), although none of them can distinguish between the distribution

between the layers of fat content and muscle mass (Pescatori, Savarino, Mauri, et al., 2019). Thus, in children, VAT does not affect glucose metabolism until after puberty. In adolescents, VAT is associated with insulin sensitivity and high-density lipoprotein cholesterol levels. Furthermore, unlike in adults, insulin sensitivity in overweight and obese prepubertal children is associated with subcutaneous adipose tissue (SAT) but not with VAT (Simoni, et al., 2020). MRI is a new technique for assessing body composition in children and adolescents. MRI has been shown to have comparable accuracy to computed tomography (CT) in quantifying SAT and VAT in obese adults and adolescents. MRI does not require radiation, which is the major limitation of using CT in children and adolescents. MRI is particularly suitable for quantifying abdominal fat in children as it only takes a few minutes without compromising the quality of the imaging. MRI can also be used to determine the proportion of brown adipose tissue (BAT), which is inversely related to VAT (Simoni, et al., 2020). The primary outcome of the observational study was visceral adipose tissue volume. Secondary outcomes were subcutaneous adipose tissue volume, PDFF of visceral and subcutaneous adipose tissue, and PDFF of the liver. Two groups were recruited: healthy and overweight children. As expected, visceral and subcutaneous fat volume as well as PDFF and hepatic PDFF were larger in the obese group than in the control group (Ly et al., 2019). MRI measures of adiposity and hepatic PDFF were strongly correlated with anthropometry, particularly waist circumference. Although waist circumference is a known surrogate for visceral fat, it measures visceral and subcutaneous fat and does not provide information on fat PDFF. Inclusion criteria for the control group included age 6–17 years and normal weight (body mass index (BMI) 5th to 85th percentile). Inclusion criteria for the obese group included age 6–17 years, obesity (BMI  $\geq$ 85th percentile) and suspected NAFLD (Ly, et al., 2019). Quantitative MRI also revealed gender and ethnic differences in fat distribution. In a population of 382 children aged 5 to 18 years, including African American and Caucasian children, total body fat, i.e., total abdominal adipose tissue (total volume and L4–L5 cross-sectional area), including SAT and VAT, and age-adjusted total body fat were greater in African Americans ( $P = 0.017$ ) and females ( $P < 0.0001$ ) compared with Caucasians and males, respectively. However, VAT volume was greater in Caucasians ( $P < 0.0001$ ) and men ( $P < 0.0001$ ) than in African Americans and women. Similar differences were observed for SAT and VAT at the L4–L5 level (Simoni, et al., 2020).

### ***Conclusion***

MRI machines are not easily accessible for all patients, children have to sit still in confined spaces, and MRI produces loud, repetitive noises. In

addition, the quantification of fatty tissue is time-consuming. Limitations also include the small sample size and that the overweight group was older than the control group. In this study, a free-breathing magnetic resonance technique was used to measure not only the volume of adipose tissue and hepatic PDFF, but also the PDFF of visceral and subcutaneous adipose tissue. Increased visceral and subcutaneous adipose tissue PDFF was observed in obese children with elevated liver function tests compared to their healthy peers, and visceral adipose tissue PDFF correlated strongly with hepatic PDFF. MRI analysis of body composition is now also possible in very young children without sedation by using free-breathing radial sequences (Ly, et al., 2019). Recently, efficient, time-saving software for three-dimensional segmentation has been developed and is available as a free online resource, making qMRI analysis accessible to a wide audience. This could contribute to an early expansion of clinical application in pediatrics. It is likely that quantitative MRI will soon completely replace CT due to radiation dose concerns (Simoni, et al., 2020).

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## **Značaj magnetne rezonance u procjeni uhranjenosti kod djece školske dobi**

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### **Sažetak**

U smislu povezanosti sa kardiometaboličkim rizikom magnetna rezonanca je superiornija od drugih antropometrijskih mjera. Magnetna rezonanca je zlatni standard za proučavanje i kvantifikaciju raspodjele abdominalne masti kod male djece. Cilj rada je prikazati značaj magnetne rezonance u procjeni sastava tijela i metabolizma u dječijoj dobi. Provedena je pregledna studija pregledom dostupne naučne literature u relevantnim bazama podataka (PubMed, Medline i Google Scholar). Primarni ishod studije, zasnovane na kontroli slučajeva, bio je volumen visceralne masti. Sekundarni ishodi bili su volumen potkožne masti, *Proton Density Fat Fraction* (PDFF) visceralne i potkožne masti i jetreni PDFF. Zapremine visceralne i potkožne masti i PDFF i jetreni PDFF bili su veći u grupi sa prekomjernom težinom u odnosu na kontrolnu grupu. MRI mjerenja adipoznosti i jetreni PDFF bili su u snažnoj korelaciji s antropometrijom, posebno sa obimom struka. Kvantifikacija zapremine masnog tkiva je dugotrajna. Napredak u tehnologiji MRI, uključujući brze sekvence snimanja s više kanala za prikupljanje podataka s naprednim dizajnom zavojnica i korekcijama artefakata kretanja, poboljšat će mogućnosti magnetne rezonance za upotrebu u procjeni kod male djece.

*Ključne riječi: visceralna mast, djeca, MRI snimanje, tehnike snimanje*



## Nutrition of the student population at the University of Sarajevo

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### *Abstract*

The student population develops specific eating habits under the influence of stress, tight schedules and numerous obligations. During their studies, students make their own dietary choices and often form unhealthy habits that persist later in life. The aim of the study was to investigate the eating habits of students at the University of Sarajevo. A standardized questionnaire on food frequency (Food Frequency Questionnaire) was used in a cross-sectional study. 240 students from 13 faculties participated in the study. The results show that 50.8% of the students eat breakfast every day, while 35% add more salt to their diet than is likely. Only 51.2% eat vegetables several times a week and 25.8% once or more a day. 44.2% of students eat fruit several times a week and 31.7% eat fruit daily. 44.6% of respondents eat sweets once or more a day, while 48.3% of students drink coffee every day. The students at the University of Sarajevo have unhealthy eating habits. Therefore, education is the key to solving the problem in order to protect the health of this population.

*Keywords: student, habits, nutrition*

### *Introduction*

According to the World Health Organization (WHO), "a healthy diet is the foundation for health, well-being, optimal growth and development" (World Health Organisation, 2023). The definition of a healthy diet is constantly changing to better understand how different foods, essential nutrients and other components of the diet contribute to health. (Cena, Calder, 2020). A proper diet, in conjunction with other healthy habits, can reduce the risk of many chronic diseases, digestive problems and mental illnesses and allow individuals to stay healthy in the long term (Willett, Skerrett, Giovannucci,

2017). Immediate benefits of a healthy diet include better performance in daily activities, a stable mood throughout the day and overall better general health (Willett et al, 2017; James, Lawrence, O'Connor, 2022). The components of a healthy diet can be adjusted according to individual needs, locally available foods, habits, cultural norms, age, gender, weight, activity level and general health, as well as many other circumstances (World Health Organisation, 2023; Gush, Shah, Gilani, 2021). The macronutrients in the diet provide calories or energy and are needed in large amounts to maintain body functions and perform daily activities. These are: Proteins, carbohydrates and fats (World Health Organization - Regional Office for the Eastern Mediterranean, 2023). The intake of macronutrients and micronutrients depends on the body's needs, age, circumstances and environment (Passarelli, S., Free, C.M., Alon Shepon, Beal, T., Batis, C. and Golden, C.D. 2024). The student population has a different lifestyle than the general population. (Choi J. 2020). Studying influences the formation of habits, including the type of diet that is continued in later life (Benaich S, Mehdad S, Andaloussi Z, Boutayeb S, Alamy M, Aguenau H. 2020; Elmskini FZ, Bouh A, Labyad A, Elghoulam N, Iraqi H, Mehdad S. 2024). Environmental factors and social influence on the student population can lead to negative eating habits, e.g. students often skip meals and consume fast food instead of meals cooked at home. The results showed that psychological factors such as poor strategies for coping with stressful situations, including college exams, have a negative influence on the type of food consumed (Alolabi H, Alchallah MO, Mohsen F, Marrawi M, Alourfi Z. 2022). A large percentage of college students are transitioning from a family lifestyle to an independent lifestyle, and this has certain implications for nutrition. By leaving the family environment and the emotional and psychological changes they are exposed to, students begin to make independent decisions about their diet during this time. In doing so, they may make mistakes in their diet and focus on unhealthy foods (Benaich S et al., 2020; Bárbara R, Ferreira-Pêgo C. 2020). The aim was to investigate the eating habits of students at the University of Sarajevo.

### ***Experimental***

The study was conducted in the form of an electronic questionnaire in the period from 2023 to 2024. 240 students from 13 different faculties (Faculty of Health Studies, Faculty of Veterinary Medicine, Faculty of Philosophy, Faculty of Dentistry, Faculty of Medicine, Faculty of Pharmacy, Faculty of Electrical Engineering, Faculty of Architecture, Faculty of Civil Engineering, Faculty of Natural Sciences and Mathematics, Faculty of Economics, Faculty of Islamic Studies, Faculty of Education) of the University of Sarajevo

participated in the study. The questionnaire consisted of 23 questions, 6 demographic and 17 nutrition-related questions.

### ***Results and discussion***

240 students from the University of Sarajevo participated in the survey. The results show that a large number of students have unhealthy eating habits. 50.8% of students eat breakfast regularly every day. 35% of students add extra salt to their food without tasting it first. Vegetables and fruit are insufficiently represented in the diet, only 51.2% of respondents eat vegetables several times a week and 25.8% eat vegetables once or more a day. In addition, 44.2% of respondents stated that they eat fruit several times a week and 31.7% of respondents eat fruit once or several times a day. 44.6% of students stated that they eat sweets once or several times a day. The study shows that coffee is an important beverage for students: 48.3% of respondents drinking coffee once or several times a day. 52.5% of students consume nuts once a week or less. Fish is consumed once a week or less in 61.7% of cases, while 20.4% of students have no fish in their overall diet, seafood is not consumed at all in 66.7% and 27.9% refer to a consumption once a week or less. The results from the study conducted among students at the University of Sarajevo reveal the presence of unhealthy dietary habits, with irregular breakfast consumption and high salt intake emerging as significant challenges. Although 50.8% of students report having breakfast regularly, it is concerning that 35% add salt to their food without tasting it first. Milošević Georgiev (2020), in her dissertation, identifies similar patterns among students at the University of Belgrade, highlighting the frequent consumption of high-calorie snacks as a potential risk factor for developing hypertension. Regarding fruit and vegetable intake, both studies indicate concerningly low levels. At the University of Sarajevo, only 31.7% of students consume fruit daily, and just 25.8% consume vegetables. A similar study from Belgrade confirms a low intake of these food items, with somewhat better results recorded for vegetables, potentially due to cultural dietary patterns or the availability of health education programs among students (Milošević Georgiev, 2020). Consumption of fish and nuts, both known for their beneficial nutritional properties, is also low. In the Sarajevo sample, as many as 61.7% of students report rare fish consumption, while a similar trend is seen in the Belgrade study, where most students report limited fish intake (Milošević Georgiev, 2020). Such dietary patterns could increase the risk of cardiovascular diseases, particularly when combined with high salt intake. Despite the importance of physical activity for health, both studies report low engagement among students. In the Belgrade sample, only 6.4% of students participate in organized physical activities, which could have long-term health implications for young people (Milošević Georgiev,

2020). Collectively, these findings highlight the need for educational programs to raise awareness of the importance of proper nutrition and physical activity. The findings from both studies indicate a need for a strategic approach involving preventive and educational programs that would support students in adopting healthier eating and exercise habits. Such programs could play a vital role in reducing the risk of lifestyle-related diseases, including hypertension and other cardiovascular disorders.

### ***Conclusion***

Based on the research conducted, we conclude that the dietary habits of students at the University of Sarajevo are largely unhealthy. Although more than half of the surveyed students eat breakfast, adding salt to food is an unhealthy habit. The imbalance is also reflected in the lower consumption of fruit and vegetables, while on the other hand, the consumption of sweets as one of the most common eating habits is increasing significantly. A major problem, however, is the extremely high consumption of coffee, which most students consume on a daily basis. The results show that a large percentage of respondents consume less nuts, fish and seafood. These results show that there is a need to raise and spread awareness about the unsatisfactory lifestyle in terms of students' diet.

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## Ishrana studentske populacije Univerziteta u Sarajevu

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### *Sažetak*

Studentska populacija razvija specifične prehrambene navike pod uticajem stresa, gustog rasporeda i brojnih obaveza. Tokom studija, studenti samostalno donose odluke o ishrani, često formirajući nezdrave navike koje ostaju i kasnije u životu. Cilj rada bio je ispitati prehrambene navike studenata Univerziteta u Sarajevu. Za izradu rada korišten je standardizirani upitnik za učestalost konzumacije hrane (Food Frequency Questionnaire) u studiji poprečnog presjeka. U istraživanju je učestvovalo 240 studenata sa 13 fakulteta. Rezultati pokazuju da 50,8% studenata doručkuje svaki dan, dok 35% dodatno soli hranu prije nego što je proba. Samo 51,2% jede povrće nekoliko puta sedmično, a 25,8% jednom ili više puta dnevno. Voće nekoliko puta sedmično jede 44,2% studenata, a 31,7% ga konzumira dnevno. Slatkiše jednom ili više puta dnevno jede 44,6% ispitanika, dok 48,3% studenata pije kafu svaki dan. Studenti Univerziteta u Sarajevu imaju nezdrave prehrambene navike, te je edukacija ključna u rješavanju problema kako bi se doprinijelo zaštiti zdravlja ove populacije.

*Ključne riječi: studenti, navike, ishrane*

## **The role of the intestinal microbiota in the maintenance of reproductive health in women**

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### **Abstract**

The intestinal microbiota comprises more than a thousand species of bacteria, viruses, fungi and parasites and represents a unique feature of each of us. Infants inherit their first gut microbes during vaginal delivery or breastfeeding, and later our diet and other environmental influences introduce new microbes into the gut. Many studies have emphasized the essential role of the gut microbiome in human health and especially in women's reproductive health. Given the reported correlations between gut microbiome dysbiosis and various diseases, the female gut microbiome is worth investigating. The aim of this paper is to define the role of the gut microbiome in women's reproductive health and their endocrine system. For this work, the available scientific literature published in the relevant databases was reviewed using key words: "intestinal microbiota", "phytoestrogen"; "reproductive health". The human intestinal microbiota regulates estrogen metabolism through the "oestrobolome", a collection of bacterial genes encoding enzymes such as  $\beta$ -glucuronidase and  $\beta$ -glucosidase. These enzymes deconjugate and reactivate estrogen and thus influence its circulating levels. Changes in the composition of the gut microbiota and the function of the oestrobolome are associated with estrogen-related diseases such as breast cancer, endometrial cancer and polycystic ovary syndrome. Phytoestrogens ingested with food are also metabolized by bacteria to active metabolites such as equol. The intestinal microbiota is an essential regulator of estrogen status, which is of great clinical importance for women's health and numerous hormonal disorders.

*Keywords: intestinal microbiota, reproductive health, phytoestrogen*

### **Introduction**

The intestinal microbiota comprises over a thousand species of bacteria, viruses, fungi and parasites and is a unique feature of each of us. Under

physiological conditions, the maintained integrity of the intestinal microbiota barrier regulates metabolism, the immune system, nutrition and, crucially, the prevention of various diseases (Singh, Pal, Shubham et al., 2023). Intestinal dysbiosis could mediate inflammatory processes via intestinal permeability, which subsequently manifest in the development of morbidity. Considering that the intestinal microbiota regulates and develops the function of the immune system, activates it in the protection against infections and ensures the energy balance of the body, it is quite understandable that a disturbed integrity of the same develops dysfunctional metabolic and hormonal profiles. The protective role of the intestinal microbiota is based on the elimination of pathogens through antimicrobial markers, and the structural role ensures that the contents of the intestinal lumen do not enter the bloodstream and consequently regulates the immune system by initiating the synthesis of immunoglobulin A. If we consider the intestinal microbiota as a regulator of metabolic functions, its involvement in carbohydrate metabolism, the synthesis of short-chain fatty acids, the synthesis of biotin and vitamin K, folic acid metabolism and iron absorption is emphasized (Pieczyńska-Zajac, Malinowska, Pruszyńska-Oszmałek et al, 2024).

The metabolic potential of intestinal bacteria is indicated by the presence of an extremely large number of genes in the microbiota, referred to in one word as the microbiome. A significant sequence of changes in the intestinal microbiota is defined by long-term adopted eating habits, which, depending on the type of eating habits, can regulate the intestinal microbiota or create an environment for its dysbiosis. Therefore, the modern and fast-paced lifestyle is the most important factor that stimulates the modulation of the intestinal microbiota. In this context, the authors clearly emphasize that a proper diet ensures the health of the microbiota. Considering that the intestinal microbiota potentially influences changes in hormone levels, its importance is particularly evident in the regulation of estrogen levels and its functionality (Kasprzak-Drozd, Oniszczyk, Stasiak et al., 2021).

### ***Experimental***

The available literature for non-experimental qualitative research was screened electronically across a broad spectrum through the available relevant databases. The search was conducted using keywords related to the topic and aims of this thesis in English: "intestinal microbiota", "reproductive health" and "phytoestrogen". The aim of this work is to define the role of the intestinal microbiota in women's reproductive health and their endocrine system.



## ***Results and discussion***

Changes in a woman's menstrual age cause certain fluctuations in the secretion and composition of hormones, which contribute to changes in the composition and distribution of microorganisms in the gut. Estrogen, because it is metabolized under the influence of intestinal microbiota, is thought to be a hormone that links the digestive and reproductive systems. In a study by Ram et al. investigating the effects of diet and estradiol levels on the structural composition of the intestinal microbiota, the results showed a protective role of estradiol in regulating body weight and the ratio of Firmicutes and Bacteroidetes in the intestinal microbiota, independent of a high-fat diet. On the other hand, decreased activity of this enzyme associated with dysbiosis can lead to lower levels of circulating estrogen, which can cause weight gain and the development of cardiovascular comorbidities. On the other hand, increased enzyme activity can lead to excessive estrogen levels, which are associated with the development of pathological conditions such as endometriosis and cancer (Ata, Yildiz, Turkgeldi, et al., 2019).

In addition, the intestinal microbiota produces short-chain fatty acids, which have an influence on the synthesis of sex hormones such as progesterone and oestradiol. Butyrate, one of the most important products of the microbiota, can stimulate or inhibit the secretion of progesterone, depending on its concentration.

Recent research also supports the hypothesis of a link between an altered testosterone/estrogen profile and gut dysbiosis in women with polycystic ovary syndrome (PCOS), highlighting changes in alpha and beta diversity and their interaction with obesity and insulin resistance. In examining the causal relationship between polycystic ovary syndrome and the integrity of the intestinal microbiota, the study finds that differences and structural modulations of the intestinal microbiota in women with PCOS may be under the influence of insulin resistance, obesity and sex hormone levels. The authors point to significant changes in the biodiversity of the intestinal composition, noting a lower abundance of the commensal bacteria *Lactobacilli* and *Bifidobacteria* and an increase in the pathogens *Escherichia coli* and *Shigella* in women with PCOS. The lower species diversity of changes in bacterial taxa is explained by an increased penetration of lipopolysaccharides from Gram-negative bacteria of the colon into the bloodstream, which subsequently triggers immune system activity to reduce the sensitivity of the receptor for insulin by increasing serum insulin levels and thus the production of androgens and the consequent disruption of folliculogenesis (Lindheim, Bashir, Münzker et al., 2017)

Extensive research indicates a link between endometriosis and microbial dysbiosis in the gut. In their study, Ata et al. (2019) found that test subjects in the third and fourth stages of endometriosis have a higher proportion of

bacteria of the genera *Shigella* and *Escherichia* in their intestinal microbiota than other control groups.

In addition, a study by Svensson, Brunkwal, Roth et al. (2021) involving 66 participants with endometriosis and 198 healthy controls showed that both alpha and beta diversity were higher in the control group. In a large cohort study, 12 bacterial species were identified that differed significantly between subjects with endometriosis and controls (Chadchan, Singh, Kommagani, et al., 2022). For example, *Bacteroides* and *Parabacteroides* were more common in patients with endometriosis, while *Paraprevotella* and an unidentified bacterium were more common in healthy controls (Lu, Li, Lei, et al., 2017). The focus of today's research within the concept of studying gut microbiota functionality is on intervention programs aimed at supplementation and application of probiotics, prebiotics and synbiotics which have been shown to improve and ensure the health of the gut and thus the whole organism with a focus on metabolic functionality (Lu, Wei, Zhong, et al. 2022). Research has shown that controlled supplementation with probiotics improves the metabolic profile of PCOS, as emphasized by Singh et al. (2023) in a study in which supplementation with *L. acidophilus*, *L. casei* and *B. bifidum* was carried out over 12 weeks. In addition, a significant decrease in plasma glucose levels, serum insulin, triglycerides and cholesterol was observed in subjects with PCOS after supplementation with the probiotics *L. casei*, *L. acidophilus*, *L. rhamnosus*, *L. bulgaricus*, *B. breve*, *B. longum* and *Streptococcus thermophiles*. The meta-analysis identified the regulation of hormonal and inflammatory markers with a significant reduction in the free androgen index, the homeostatic model for the assessment of insulin resistance (HOMA-IR), very low density lipoprotein (VLDL), cholesterol, total cholesterol and triglycerides.

### **Conclusion**

Dysbiosis of the intestinal microbiota is increasingly recognized as a factor that may contribute to the development of reproductive disorders such as endometriosis, PCOS, gynecological cancers and infertility. Although there is clear evidence that certain changes in the intestinal microbiota can affect reproductive function, further research is needed to confirm whether these changes cause disease or are a consequence of its progression. Key to this is the identification of bacterial species and their metabolites that could serve as biomarkers for the early detection of metabolic and reproductive disorders.

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S. Š. S. All authors agreed with the final version of the paper and contributed to its quality and accuracy.

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## Uloga crijevne mikrobiote u očuvanju reproduktivnog zdravlja žene

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### Sažetak

Crijevna mikrobiota uključuje preko hiljadu vrsta bakterija, virusa, gljivica i parazita, te predstavlja jedinstvenu karakteristiku svakoga od nas. Dojenčad nasljeđuju svoje prve crijevne mikrobe tokom vaginalnog porođaja ili dojenja, kasnije naša prehrana i druga izloženost okolišu uvodi nove mikrobe u njega. Mnoštvo studija je naglasilo bitnu ulogu crijevnog mikrobioma u ljudskom zdravlju, a naročito reproduktivnom zdravlju žena. S obzirom na prijavljene korelacije između disbioze crijevnog mikrobioma i različitih bolesti, mikrobiom ženskog crijeva je vrijedan istraživanja. Cilj ovoga rada je definisati ulogu crijevnog mikrobioma u reproduktivnom zdravlju žene i njenog endokrinog sistema. Rad je urađen pregledom dostupne naučne literature objavljene u relevantnim bazama podataka upotrebom ključnih riječi : „crijevni mikrobiota“, „fitoestrogen“, „reproduktivno zdravlje“. Ljudska crijevna mikrobiota reguliše metabolizam estrogena kroz „estrobolom“, kolekciju bakterijskih gena koji koodiraju enzime poput  $\beta$ -glukoronidaze i  $\beta$ -glukozidaze. Ovi enzimi dekonjugiraju i reaktiviraju estrogen, utičući na njegov nivo u cirkulaciji. Promjene u sastavu crijevne mikrobiote i funkcije estroboloma povezani su sa bolestima povezanim sa estrogenom kao što su rak dojke, rak endometriuma i sindrom policističnih jajnika. Dijetalni fitoestrogeni takođe prolaze kroz bakterijski metabolizam i prelaze u aktivne metabolite kao što je equol. Crijevni mikrobiota je integralni regulator statusa estrogena koji ima klinički veliki značaj u zdravlju žena i brojnim hormonalnim poremećajima.

*Ključne riječi: crijevna mikrobiota, reproduktivno zdravlje, fitoestrogen*

## Challenges of feeding newborn infants with clefts

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### Abstract

Cleft lip and palate (CLP) are primarily structural malformations, but due to their nature they also affect certain developmental functions and may cause speech and language difficulties, hearing impairments, voice disorders, feeding and swallowing difficulties, dental deviations as well as psychosocial issues. Feeding children with the cleft lip and palate is one of the first challenges parents encounter, demonstrated that 97 % of parents thought that one of the most important things to discuss were feeding challenges. The main goal of this research was to get an insight into different feeding methods of children with different type of CLP and to explore if there is a connection between different types of CLP and parental concerns. In the paper, we will also present the importance of mother's milk and natural nutrition (breastfeeding).

*Keywords: cleft lip and palate, feeding difficulties, parental cocncer, breastfeeding*

### Introduction

One of the most important functions of the first postnatal period is breastfeeding. The sucking reflex, according to Pavlov, is an innate unconditional reflex, but the existence of this reflex does not necessarily guarantee successful breastfeeding, which depends on several factors. If there is no medical contraindication, breastfeeding should be forced, because mother's milk is a nutritionally perfect food adapted, both in terms of composition, purity and temperature. Due to rapid digestion and absorption, mother's milk does not burden the still immature digestive tract, so the infant has no colic, no diarrhea, which leads to optimal development of the child (Lorencin-Bulic, 2024). Breastfeeding in children without clefts is a certain challenge, especially for the first newborn. Mothers can have a problem with sore nipples, then lack of milk, stress, fatigue of the mother herself, as well as bonding problems in the first days, which can have a very emotional effect on the mother herself (Burianova, Kulihova, Vitkova, et al., 2017). Parents of children with orofacial clefts face an even more stressful situation

considering the diagnosis itself. They go through a great emotional and psychological burden, therefore the midwife's role is important to give parents clear explanations on how to feed their child. Feeding, apart from being extremely important in the life of every newborn, is the time when a special bond is established between mother and child, and feeding is the biggest challenge for parents of children with orofacial clefts (Goldstein, O'Riordan, Robin, et al., 2001). Cleft lip and palate in most parents cause concern due to suffocation, inability to breastfeed, dehydration of the child, difficulty in coordinating breathing and swallowing during feeding and prolonged feeding. However, with the application of appropriate guidelines, feeding itself can be simplified and made easier for parents. Of course, it is important, considering the severity of the cleft itself, to adapt to the needs of each child (Radić, 2023).

The aim of the paper is to describe the challenges that parents face when breastfeeding children with clefts, as well as to describe the way in which children with clefts can be fed, depending on the severity of the case.

### ***Experimental***

Systematic search and literature analysis by combining keywords: orofacial clefts, feeding, newborns, breastfeeding, in the following databases: PubMed, Google Scholar; ResearchGate, ScienDirect, Elsevier. The criteria for the inclusion of works in the analysis were the availability of the work in full text, and works in English and native language.

### ***Results and Discussion***

Cleft lip and palate are congenital defects involving an opening in the upper lip and/or palate. The severity varies, from minor clefts to more serious forms like bilateral clefts affecting the lip, alveolar process, and palate. Beyond aesthetic concerns, these defects can lead to functional issues, such as difficulties with feeding, breathing, speech, hearing, and jaw or dental development. Children may also face emotional and social challenges due to changes in their facial appearance and function (Zorić, Knežević, Aras, 2014). One of the biggest challenges for parents of newborns with orofacial clefts is feeding. Due to the communication between the mouth and nasal cavity, babies cannot create the necessary suction for sucking. Treatment for orofacial clefts is a long-term process, starting just days after birth. Providing psychological support to parents from the beginning is essential. They need to be informed about all stages of therapy, have their questions answered, and receive help in overcoming doubts and fears (Gailey, 2016). In the first days of life, ensuring adequate nutrition is a priority for infants with orofacial clefts. Babies with a partial cleft lip may be able to breastfeed with proper positioning, so it's important to educate mothers on techniques that can help.

However, newborns with a complete cleft lip and palate cannot breastfeed because they cannot generate the necessary suction. In these cases, specially designed bottles and pacifiers are used—some specifically for children with cleft palate and others for those with cleft lip—to assist in feeding (Lindberg and Berglund, 2014). Specially shaped pacifiers have a tip that is wide and rounded and with each sip it clings to the palate, closing the cleft and thus allowing milk to flow. This prevents the milk from flowing into the respiratory system and possibly suffocating the child. By using such pacifiers, the natural sucking reflex is encouraged (Peterson-Falzone, Trost-Cardamone, Karnell, et al., 2016). In order to help the feeding of children, considering the difficulty of sucking, instead of classic hard bottles for feeding, softer, plastic bottles can be used that can be gently pressed during feeding and help the milk flow (Marković, Rakanović-Todić, Burnazović-Ristić, et al., 2021). Parents must be careful to recognize signs of discomfort in babies with cleft lip and palate during feeding, as these signs may indicate difficulties that require adjustments in the way of feeding. If formula feeding is used, the proper approach involves mixing the powdered formula instead of shaking the formula, to avoid creating air bubbles that can cause gas and discomfort in the baby. It is important to pay attention to signs such as pulling the head back, stretching the neck, pauses with milk coming out of the nose, coughing, or "wet" raspy breaths. These manifestations may indicate problems with feeding and the need to slow down or adjust the position of the bottle. Also, physical signs like waving your arms, raising your fists, or blinking frequently can be indicators of stress or fatigue (Miller, 2011). Keeping feedings between 30 and 40 minutes apart is key to preventing baby exhaustion and ensuring optimal energy intake. Longer feedings can result in more energy expenditure than intake, which can slow the baby's growth. The goal is for the newborn to regain birth weight in the first two weeks of life and to achieve a normal rate of growth and weight gain compared to peers without a cleft (Lorencin-Bulic, 2024). In cases of unilateral or bilateral cleft lip and palate where bottle feeding is insufficient, a neonatologist consults with a maxillofacial surgeon and orthodontist to create an orthodontic appliance. This marks the start of pre-surgical orthodontic therapy, the first phase in treating orofacial clefts. Initiated immediately after birth, this therapy aims to ensure proper feeding and prepare for later surgery. Orthodontic appliances help close the clefts, allowing the baby to swallow food more effectively, preventing milk from leaking into the nasal cavity, and reducing the risk of aspiration (Gailey, 2016). At the same time, orthodontic appliances direct the separated segments of the lip and palate into a more correct position, which facilitates future surgical reconstruction. This early treatment also has a positive psychological impact on parents, giving them the feeling that crucial steps are



being taken for their child's health and development (Burianova, et al., 2017) The devices are adapted to the growth and development of the child, which enables gradual tissue shaping and optimal preparation for surgery. An orthodontic appliance, known as a "stimulator" or "obturator", closes the communication between the mouth and the nasal cavity, making it easier for a newborn with a cleft to eat, swallow and breathe. This function of the device allows the baby to feed without the risk of food or liquid leaking into the nasal cavity, which can cause breathing complications. After the device is properly placed and the child gets used to it, reflex sucking often develops quickly, which is a positive sign for further progress. When feeding, the newborn is positioned at an angle of 45° or more, in order to establish control over nasal regurgitation, i.e. the return of liquid through the nose, and ensure safe feeding (Gailey, 2016). When feeding a newborn with an orofacial cleft, the head and neck should be level with the shoulders or slightly bent towards the chest, to facilitate swallowing and breathing. It is recommended to put more milk in the bottle than the baby can drink, as this helps prevent the head from being thrown back, which could make feeding difficult. The teat opening should be wide enough for the milk to flow slowly, but not too quickly, to allow for a one-way flow of milk (Miller, 2011). The flow should be fast enough to keep the baby interested in feeding, but at the same time light enough to allow breathing between sips. In cases where milk leaks into the nasal cavity, feeding should be stopped, and the child should be raised so that he can cough or sneeze, which clears the airways. It is important to note that there is no universal method of feeding a child with a cleft. Each mother, in cooperation with the medical team, will find the best way of feeding that corresponds to the specific needs of her child (Lindberg and Berglund, 2014). In the first months of life, the growth and development of the orofacial complex is extremely intense, which requires regular replacement of the orthodontic appliance. As the newborn develops, it is necessary to adapt the appliance to the new dimensions and shapes of the mouth. A new device is usually made every three to four weeks, and the process is the same as when making the first device. Presurgical orthodontic therapy usually involves making five to six such appliances, which are regularly replaced as the child grows (Gailey, 2016). After this phase of orthodontic therapy is completed, the first surgical intervention for cleft correction follows. This method prepares orofacial structures and facilitates the surgical process, while also improving feeding ability during early development (Marković, et al., 2021).

### ***Conclusion***

Newborns with a partial cleft can come with a proper position on the breast, so educating mothers about breastfeeding techniques is the most important task of neonatal counseling. Infants with a complete cleft can be successfully

fed using bottles and specially designed pacifiers. In the case of the most complex forms of clefts, pre-surgical orthodontic therapy is necessary immediately after birth in order to enable adequate feeding and facilitate later surgical intervention.

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## **Izazovi hranjenja novorođenčeta sa rascjepima**

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### **Sažetak**

Orofacijalni rascjepi primarno su strukturalna malformacija, no zbog svoje prirode utječu i na određene razvojne funkcije i mogu uzrokovati teškoće u području jezika, govora, sluha, glasa, hranjenja i gutanja, dentalne probleme, kao i psihosocijalne teškoće. Upravo je hranjenje jedan od prvih izazova s kojim se roditelji djece s orofacijalnim rascjepima susreću, a čak 97 % roditelja informacije o hranjenju smatraju najvažnijima u razdoblju odmah nakon poroda. Stoga je cilj ovog rada dati uvid u načine hranjenja kod djece s raznim vrstama rascjepa ispitati povezanost između vrste rascjepa i roditeljske zabrinutosti. U radu ćemo takođe predložiti kolika je važnost majčinog mlijeka, te prirodne ishrane (dojenja).

*Ključne riječi: orofacijalni rascjepi, teškoće pri hranjenju, roditeljska zabrinutost, dojenje*

## **Knowledge, attitude and behavior regarding dietary salt intake among adult population of Sarajevo canton**

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### **Abstract**

Table salt is an essential component of daily nutrition, contributing not only to flavor enhancement but also to vital physiological functions, including thyroid hormone production through its iodine content. The World Health Organization recommends a maximum daily salt intake of five grams; however, many individuals consume two to three times this amount, often due to hidden salts in processed foods. Excessive salt intake is linked to serious health issues such as hypertension, cardiovascular diseases, and other chronic conditions. This paper aims to assess salt consumption and awareness among the adult population in Sarajevo Canton. A survey among 257 respondents revealed that a significant portion lacks knowledge about recommended salt levels and the health risks associated with overconsumption. Findings indicate that many individuals habitually add salt during meals and are unaware of the salt content in common foods, particularly bakery items. The results underscore the need for public education on salt intake to mitigate health risks, particularly cardiovascular diseases, which are prevalent in the region.

*Keywords: salt, diseases, food*

### **Introduction**

Table salt is an inevitable ingredient in everyday nutrition, key for adding taste, normal function of the human body and various physiological processes. In addition, iodized table salt is an important iodine source for public health. Iodine is vital for the normal function of thyroid hormones and other important metabolic processes. "There are two fundamental purposes of table salt use in food: as a spice which improves the taste of the food, and as a preservative". However, it is important to use it moderately when preparing meals. "The amount of salt that is naturally found in food is sufficient for the body's needs and normal and healthy life, but people do not consider it

enough (Kohlmeier M., 2015; Filipović-Hadžiomerađić et al., 2017). According to the recommendation made by the World Health Organisation, daily intake of table salt should not exceed five grams, but in reality, it is often two to three times the recommended amount that is consumed (WHO, 2023). Hidden salt present in meat products, canned vegetables, and other products is often left unrecognized. „Exceeding the recommended amount of table salt intake has serious consequences for human health, including high blood pressure and increased risk of various chronic diseases, such as heart and lung diseases, osteoporosis, diabetes, stroke, and even certain forms of cancer, which can shorten one's lifespan“ (Pubchem, 2023). Results of our survey show that salt intake is higher than recommended, the population is not sufficiently educated about the health consequences of consuming too much salt.“It is believed that reducing salt intake could have a protective effect on cognitive functions., (Koščak J., 2014).

### ***Experimental***

We conducted an online survey in October 2023. There were 257 respondents 18 years old or older. The survey was made available online via a link and it consisted of 11 multiple-choice questions regarding dietary salt intake (in processed food and by addition in home food preparation) and one open-ended question in which the respondents were given the option to leave their comments about whether they think that the population is informed well enough about the consequences of the excessive salt intake, and in which way could the possible insufficient information be improved. All of the respondents were from the Sarajevo canton area.

### ***Results and Discussion***

Of the total number of respondents (257), who participated in the survey, 62.6% of them belong to the age group of 18-25 years old, 8.6% belong to the age group 26-35 years old, 11.7% belong to the 36-45 age group, 8.9% belong to 46-55 age group, and 8.2% of the respondents belong to the age group older than 55.

Based on the survey results, it has been concluded that 35.8% of the respondents are not familiar with the information related to the recommended daily salt intake. According to the World Health Organisation, the recommended amount of daily salt intake is less than five grams a day. The results of the survey show that 25.7% of the respondents are familiar with the information, while 3.9% of the respondents think that the recommended daily intake is higher than five grams a day., „Compared to the multicenter study conducted in 2019 on 2,459 university students from 4 European countries (Germany, Greece, Poland, and Slovenia) and 2 Asian countries (Sri Lanka and Taiwan), it was found that the residents of Sarajevo Canton are more

familiar with the recommended daily salt intake, given that only 6.5% (Marakis et al., 2021) of respondents in the aforementioned study were aware of this information. Based on the survey results, only 8.9% of the respondents check the content of table salt on product declarations. Much higher is the percentage of respondents who do not check the content of table salt on product declaration when shopping (91.1%). This data shows a lack of interest among the population when it comes to the amount of table salt in individual products.

Out of the total number of respondents, 19.1% said that they feel increased thirst after having a meal. This piece of information tells us that there is a potential higher amount of salt ingested daily. By ingesting a higher amount of salt, the body requires more liquids to enable the muscles and other organs to function properly. Only 5.1% of the respondents claim that they never feel an increased thirst after having a meal. The survey shows that 58.8% of the respondents add even more salt during the meal, while a smaller percentage (41.2%) does not add more salt. It can be concluded that there is a habit of adding more salt when consuming food to improve the organoleptic properties of food, not taking into account the recommended daily intake of salt nor the consequences that too much intake can cause.

Among those respondents who add salt after meal preparation majority (70,5%) do so after tasting the food, while 29.5% of the respondents add salt before even tasting it.

There is a possibility that, at least in the latter group, it is just a case of an unhealthy habit. The recommendation is, of course, to add salt in normal amounts to improve the organoleptic properties of food. 90.7% of the respondents negated having high blood pressure, while 9.3% of the respondents had been diagnosed with hypertension. This result can be connected to the fact that the majority of the respondents fall in the 18-25 age group and that hypertension develops and is diagnosed mostly later in life, as a consequence of genetic predisposition and lifestyle choices which include increased table salt intake, inadequate physical activity, and obesity.

Excessive intake of table salt is one of the risk factors for high blood pressure and other cardiovascular diseases, and it is also one of the factors that can be affected by self-discipline and additional health care. Patients with hypertension are always advised to reduce table salt intake because it can act synergistically with antihypertensive therapy and consequently lead to general improvement of the patient's health. According to the survey results, 75.5% of respondents are familiar with the benefits of proper salt intake, while 24.5% are not. The respondents' thoughts on which diseases are potentially connected to higher salt intake reveal interesting insights. The largest percentage of respondents (92.6%) believe that kidney disease is connected to higher salt intake, which is correct. Nutrition with large

amounts of sodium increases the risk of kidney stones forming because calcium levels increase in the urine. As more calcium is excreted, bones weaken, so osteoporosis can also appear. 48.6% of the respondents believe that increased salt intake can cause a heart attack, and a minor part of the respondents believe that stroke, obesity, and diabetes can also develop.

Bakery products, especially bread, are a significant source of table salt in daily nutrition. Besides improving the taste of bakery products, table salt, also affects gluten development, dough rheology, and fermentation speed. However, our respondents were largely unaware of these facts. When asked, “Are you familiar with the fact that two slices of store-bought white bread contain more than 1.5 grams of table salt (which represents one-third of the total daily need for salt intake)?” only 15.2% of respondents are familiar with salt content in bakery products. “Considering the average daily intake of salt in Europe, Croatia ranks the third highest consumer (after Turkey and Hungary) with an average of 13–16 g (Delaš Aždajić et al., 2019), mostly consumed through bread and bakery products. Comparing this with Sarajevo Canton, we see that the awareness of salt content in bread and bakery products is not at a satisfactory level in either location. The majority of the respondents (81.3%) consider it necessary to inform the population more about the optimal salt intake. This finding is further supported by the fact that a significant proportion (35.8%) of respondents were not informed about the recommended daily salt intake.

### ***Conclusion***

Table salt is a key ingredient in daily nutrition. It enhances the flavor of food and plays a vital role in physiological processes. According to research conducted in Sarajevo Canton, a significant number of people are not aware of the recommended amount of salt intake and the risks of excessive intake. However, the younger population shows more interest in checking salt content on food declarations and the awareness of these risks. It is important to emphasize the fact that salt intake affects the development of cardiovascular diseases, which are frequent in Bosnia and Herzegovina and contribute to many deaths, especially among the elderly and those with family predispositions for these diseases. Hypertension, as one of those diseases, is directly connected to salt intake. Therefore, it is important to educate the population about the risks of excessive salt intake, food items with high salt content, and the means of reducing salt intake to prevent long-term harmful health effects.

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supervision, J. Đ.; All authors have read and agreed to the published version of the proceeding.

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## **Znanje, stav i ponašanje u vezi s unosom soli za ishranu među odraslom populacijom Kantona Sarajevo**

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### **Sažetak**

So je osnovna komponenta svakodnevne ishrane, koja doprinosi ne samo poboljšanju ukusa, već i vitalnim fiziološkim funkcijama, uključujući proizvodnju hormona štitne žlijezde putem sadržaja joda. Svjetska zdravstvena organizacija preporučuje maksimalan dnevni unos soli od pet grama; međutim, mnogi pojedinci unose dva do tri puta više od ove količine, često zbog skrivene soli u prerađenoj hrani. Prekomjieran unos soli povezan je s ozbiljnim zdravstvenim problemima kao što su hipertenzija, kardiovaskularne bolesti i druge hronične bolesti. Ovaj rad ima za cilj procijeniti unos soli i svijest među odraslom populacijom u Sarajevskom kantonu. Istraživanje sa 257 ispitanika pokazalo je da značajan dio populacije nema znanje o preporučenim nivoima soli i zdravstvenim rizicima povezanim s prekomjernom konzumacijom. Saznanja ukazuju na to da su mnogi pojedinci navikli da dodaju so tokom obroka i nisu svjesni sadržaja soli u uobičajenim namirnicama, posebno pekarskim proizvodima. Rezultati naglašavaju hitnu potrebu za javnom edukacijom o unosu soli kako bi se smanjili zdravstveni rizici, posebno kardiovaskularne bolesti, koje su rasprostranjene u ovom regionu.

*Ključne riječi: so, bolesti, ishrana*

### 3-P-4

#### **Assessment of iron intake among the adult population of Sarajevo Canton**

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#### **Abstract**

The presence of iron in the body is crucial for a variety of biological processes, from oxygen transport to participation in metabolic activities. Iron deficiency can cause anemia, especially in women, children and vegetarians, and on the other hand, it can also cause hemochromatosis, which is an excessive accumulation of iron. Therefore, the primary aim of this research is to gather relevant information on iron intake from various sources, including food, supplementation, and the importance of a balanced daily diet. This study provides a deeper understanding of dietary habits and awareness surrounding this essential nutrient, with the goal of identifying educational needs and promoting healthy dietary practices. The data analyzed in this study were collected from 326 participants, aged between 18 and over 50 years. Survey analysis reveals that a significant portion, specifically 60.4% of respondents, limit their iron intake solely to dietary sources. On the other hand, 39.6% of respondents recognize the importance of additional efforts to maintain optimal iron levels and have thus incorporated iron supplements into their daily routine. The survey results highlight the need for further education on iron intake, supplementation, and the correct use of supplements to ensure optimal health and well-being among the population of Sarajevo Canton in terms of iron intake. There is a strong need to raise awareness about the critical role of iron in maintaining human health and vital functions, as well as the importance of voluntary blood donation.

*Keywords: iron, nutrient, anemia, hemochromatosis, diet, respondents*

#### **Introduction**

Iron is an essential micronutrient that is needed for many vital functions in the body such as hemoglobin production and oxygen transport (Kim and Wessling-Resnick, 2014). Given the importance of iron in normal body function its deficiency can cause some alarming problems. One example is anemia that can commonly occur in women, children and vegetarians.

Anemia caused by lack of iron can manifest with symptoms, such as pale skin and fatigue but it can be treated with iron rich foods and supplementation (Felson, 2020) Malabsorption, which further decreases absorption of iron from food can significantly greater the chances of deficiency developing among population (Saboor, Zehra and Qamar, 2015). On the other hand hemochromatosis can be described as an issue where iron is accumulated in the body which can lead to serious health issues. It can be treated with diet that includes reduced intake of iron or venipuncture to remove excess (NHS, 2019). The primary goal of our research was to gather relevant information about iron intake among the adult population in Sarajevo Canton. At the same time, we aimed to assess the general level of knowledge about iron intake from various sources, including food, supplementation, and awareness of risk factors associated with iron deficiency.

### ***Experimental***

In this study, a quantitative approach was used to gather and analyze data on iron intake and dietary habits among 326 residents of Sarajevo Canton, aged 18 to over 50. The primary data were collected through a structured survey, distributed online using Google Forms. The survey included a series of closed-ended questions aimed at assessing participants' dietary habits, specifically focusing on their sources of iron intake, awareness of iron supplements, and general nutritional practices. Participants were recruited voluntarily, and the survey was conducted during November 2023. Responses were exported into Microsoft Excel for initial data organization. To support the findings and provide context for the study, a comprehensive literature review was conducted using the following databases: PubMed, WebMD, and Google Scholar focusing on recent studies related to iron intake and supplementation, with keywords such as „iron intake“, „iron supplements“ and „dietary habits“. Articles were filtered based on relevance, date of publication and inclusion of human population studies. While standard survey and literature review methods were utilized, modifications were made in the design of the survey to tailor it to the Sarajevo Canton population. This was necessary to ensure the relevance and accuracy of the data collected.

### ***Results and Discussion***

Our survey revealed that 60.7% of participants were aged 18-30 years, with 34.4% between 30-50 years, and a minimal response from those over 50. The gender distribution was significantly skewed, with 96.3% female

respondents. Educational levels showed that 66% had higher education, and 53.7% were in the medical field, which may have contributed to their prior knowledge about iron intake and its health implications. These demographic trends align with similar studies, where younger, educated women are often more engaged in health-related surveys. Regarding dietary habits, 35.3% followed the recommended three meals a day, while 32.5% consumed only two meals, reflecting busy lifestyles similar to those found in other urban populations. The intake of iron-rich foods like nuts, legumes, and red meat was moderate, with red meat being the least consumed. This finding is consistent with other research that points to a decline in red meat consumption due to dietary shifts towards plant-based foods. Iron supplementation habits varied, with 39.6% of participants using supplements, mainly based on healthcare recommendations (35.8%). The reliance on dietary sources for iron (60.4%) indicates a need for further education, as many individuals may not meet their iron requirements through diet alone, especially those following restrictive diets. Most respondents obtained supplements from pharmacies (69.9%), underscoring the importance of proper pharmaceutical guidance in supplementation. This survey also explored the co-consumption of vitamin C with iron, a critical factor in enhancing iron absorption. A significant 60.7% of participants reported consuming vitamin C alongside iron, which likely improves iron bioavailability in their bodies. However, 39.3% believed they didn't have sufficient vitamin C intake, which could hinder iron utilization, both from dietary sources and body stores. Regarding blood donation awareness, 42.6% of respondents wanted to donate but were ineligible due to health reasons, while 35.9% had never donated, and 14.4% were regular donors. Given the high female participation, these results were expected, as conditions like iron deficiency may limit eligibility.

In comparison to relevant literature, the findings highlight the importance of targeted nutritional education, especially for younger populations and women, who are at greater risk of iron deficiency. The overall results suggest that while awareness exists, consistent dietary and supplementation habits need improvement to ensure optimal iron levels.

## **Conclusion**

The survey results highlight the need for further education on iron intake, supplementation, and the correct use of supplements to ensure optimal health and well-being among the population of Sarajevo Canton in terms of iron intake. There is a strong need to raise awareness about the critical role of iron

in maintaining human health and vital functions, as well as the importance of voluntary blood donation.

**Author contributions:** conceptualization, E.H. and A.H.; methodology, E.H.; investigation, S.N.; writing—original draft preparation, A.H.; writing—review and editing, A.H.; visualization, E.H.; supervision, Š.K. and M.B.; All authors have read and agreed to the published version of the proceeding.

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## **Procjena unosa željeza kod odraslog stanovništva Kantona Sarajevo**

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### **Sažetak**

Prisutnost željeza u organizmu je glavna za većinu bioloških procesa, sve od transporta kisika, pa do učestvovanja u metaboličkim procesima. Nedostatak željeza može izazvati anemiju posebno kod žena, djece i vegeterijanaca, a s druge strane može izazvati i hemohromatozu što predstavlja prekomjerno nakupljanje željeza. Stoga osnovni cilj predstavljenog istraživačkog rada jeste prikupiti relevantne informacije o unosu željeza iz različitih izvora, uključujući namirnice, suplementaciju i bitnost ujednačene svakodnevne ishrane. Ovim istraživanjem omogućeno je dublje razumijevanje prehranbenih navika i svijesti o ovom ključnom nutrijentu, s ciljem identificiranja potreba za edukacijom i promicanjem zdravih prehranbenih praksi. U sklopu istraživanja analizirani su podaci prikupljeni od 326 ispitanika s dobnim rasponom od 18 do preko 50 godina. Analizom ankete zaključujemo da značajan broj, tačnije 60.4% ispitanika, svoj unos željeza ograničava isključivo na prehranbene proizvode. S druge strane, zbog različitih potreba, 39.6% ispitanika prepoznaje važnost dodatnih napora u održavanju optimalnog unosa željeza. Shodno tome, prisutno je uvođenje suplemenata željeza u dnevnu rutinu. Rezultati ankete potiču razmišljanje o važnosti edukacije o unosu željeza, suplementaciji i pružanju informacija o pravilnom korištenju suplemenata kako bi se osigurala optimalna dobrobit i zdravlje populacije Kantona Sarajevo u kontekstu unosa željeza. Potrebno je mnogo više podizati svijest o važnosti željeza za očuvanje ljudskog zdravlja i vitalnih funkcija, a samim tim i o važnosti dobrovoljnog darivanja krvi.

*Ključne riječi: željezo, nutrijent, anemija, hemohromatoza, prehrana, ispitanici*

4. DIJETOTERAPIJA  
DIETOTHERAPY





## **The role of the Mediterranean diet in the regulation of cortisol and metabolic balance**

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### **Abstract**

Cortisol, as a key glucocorticoid hormone, plays an important role in the regulation of physiological processes, including metabolic and immune functions. Its dysregulation is associated with the development of metabolic abnormalities, such as insulin resistance and type 2 diabetes mellitus. Extensive research highlights the importance of a Mediterranean diet, rich in antioxidants and polyphenols, in reducing the negative effects of cortisol on metabolic functions while simultaneously preserving the intestinal microbiota. The aim of this review is to investigate the role of cortisol in metabolic regulation and to analyze the effectiveness of the Mediterranean diet in achieving the optimal level of this hormone in the body. The search was performed in the relevant databases using the keywords "cortisol", "metabolism" and "Mediterranean diet". Abnormal cortisol rhythms, associated with disruption of glucose homeostasis, can affect the development of inflammatory processes by activating inflammatory cytokines. High cortisol values were positively correlated with glycemic status, detected by fasting plasma glucose (FPG) and glycosylated hemoglobin (HbA1c) results, in the third tertile of waist circumference. Literature data emphasize the importance of the Mediterranean diet in optimizing the level of free cortisol in the serum. After 18 months, subjects showed a decrease in fasting cortisol (FMC) values with improvements in FPG, HbA1c, C-reactive protein (hs-CRP), thyrotropin, and testosterone. Therefore, monitoring the variability of the daily cortisol level is a predictive risk factor for the development of abnormalities in glucose metabolic activities. Long-term adherence to the Mediterranean diet implies protection of cardiometabolic health regardless of nutritional status.

*Keywords: cortisol, metabolism, Mediterranean diet*

### **Introduction**

Cortisol, a glucocorticoid hormone, regulates physiological processes such as lipid, protein and glucose metabolism as well as immune functions. Its

dysregulation is often associated with the development of metabolic abnormalities such as insulin resistance and type 2 diabetes. Data from the literature indicate that subclinical hypercortisolism may initiate the redistribution of subcutaneous adipose tissue into visceral fat via glucocorticoid receptors in visceral fat through adipocyte differentiation and proliferation. Subclinical hypercortisolism, manifested by elevated cortisol levels in the evening, also contributes to the increased incidence of type 2 diabetes, indicating the complex influence of a dysfunctional hypothalamic-pituitary-adrenal (HPA) axis on the body's metabolism (Hackett, Kivimäki, Kumari et al., 2016). These pathophysiological conditions also manifest themselves in an increase in lipolytic responses leading to damage to glucose homeostasis. Given the growing evidence of the link between cortisol and metabolic health, a cross-sectional study by Kamba, Daimon, Murakami et al., (2016) found a positive correlation between elevated morning cortisol levels and decreased pancreatic beta cell functionality, leading to the development of insulin resistance. Despite scientific findings suggesting that cortisol levels influence the development of cardiovascular comorbidities and dyslipidemia, research is not sufficiently focused on the pathogenesis of metabolic diseases. Furthermore, extensive research emphasizes the role of diet in modulating cortisol levels, particularly the Mediterranean diet, which is rich in antioxidants and polyphenols and has been shown to reduce the negative effects on metabolic function while preserving the gut microbiota. In this context, the Mediterranean diet is recognized as a potential regulator of hormonal and metabolic activities and should be the focus of future research (Ortiz, Kluwe, Odei et al., 2018).

### ***Experimental***

The available literature for the need of non-experimental qualitative research was broadly reviewed electronically through the available relevant databases. The search was conducted using keywords related to the topic and aims of this paper in English: "cortisol", "metabolism" and "Mediterranean diet". The aim of this review is to determine the role of cortisol in the regulation of metabolism and to analyze the effectiveness of the Mediterranean diet in achieving optimal cortisol levels in the body.

### ***Results and discussion***

Elevated cortisol levels in the evening pose a significant risk for the development of type 2 diabetes as they reduce insulin sensitivity (Lee, Baranowski, Sakremath et al., 2023; Liav, Gal, Ehud et al., 2023)

Abnormal cortisol rhythms associated with impaired glucose homeostasis may promote the development of inflammatory processes by activating inflammatory cytokines. Although obesity may influence changes in evening

cortisol levels, this association remains significant even after adjustment for body mass index (BMI) (Choi, Koo, 2020).

Stress provides a further link between cortisol secretion and the risk of type 2 diabetes, as psychosocial stress activates the hypothalamic-pituitary-adrenal axis, which stimulates cortisol secretion. The research findings suggest that hypercortisolism is an important risk indicator for diabetes and emphasize the need for further research into the mechanisms linking the HPA axis to glucose homeostasis (Petra, Panagiotidou, Hatzigelaki et al., 2015) Research by Robin et al. found an association between morning serum cortisol and glucose metabolism in African American subjects, with results stratified specifically by waist circumference (Min, 2016). High cortisol levels were positively correlated with blood glucose status as determined by FPG and HbA1c, with correlations found in the third tertile of waist circumference. In addition, cortisol was inversely correlated with  $\beta$ -cell functionality in subjects without diabetes. The strongest association between cortisol levels and the homeostatic model of insulin resistance assessment (HOMA-IR) was found in the highest tertile of waist circumference. These results suggest that changes in  $\beta$ -cell function may precede the development of hyperglycemia (Tsang, Hodgson, Bussu et al., 2019). The literature emphasizes the importance of the Mediterranean diet for regulating the level of free cortisol in the serum and thus for metabolic functions (Mendola, Chen, Gu et al., 2016). A study in which the effectiveness of a Mediterranean diet (MED) and a variant with a high polyphenol content (Green-MED) on serum free cortisol (FMC) levels was investigated included obese subjects, 88% of whom were male. The average body mass index (BMI) of the subjects was 31.3 kg/m<sup>2</sup>, and the FMC level was 304.07 nmol/L. Of the subjects, 11% had confirmed type 2 diabetes, while 38% had prediabetes. Before the intervention, FMC was significantly higher in men than in women, and the authors found correlations with age, visceral mass, FPG, hs-CRP, testosterone, progesterone and TSH. After 18 months, subjects in the MED and Green MED groups showed a significant decrease in FMC levels, with improvements in FPG, HbA1c, hsCRP, thyroid-stimulating hormone and testosterone levels (Liav et al., 2023).

## ***Conclusion***

Monitoring the diurnal variability of cortisol levels represents an important predictive risk factor for the development of metabolic abnormalities in glucose activities. Long-term adherence to the Mediterranean diet, particularly its polyphenol-rich variant, therefore effectively lowers free cortisol levels, suggesting potential protection of cardiometabolic health, regardless of nutritional status.

**Author's contribution:** conceptualization, S. Š. S.; methodology, N. B., A. A.; research: S. G., A. B.; preparation of the original draft, A. A., S. G., A. B.; revision and editing: S. Š. S., N. B.; visualization, A. B.; supervision, S. Š. S.; all authors agreed with the final version of the paper and contributed to its quality and accuracy.

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## **Uloga mediteranske ishrane u regulaciji kortizola i metaboličke ravnoteže**

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### **Sažetak**

Kortizol, kao ključni glukokortikoidni hormon, ima važnu ulogu u regulaciji fizioloških procesa, uključujući metaboličke i imunološke funkcije. Njegova disregulacija povezana je s razvojem metaboličkih abnormalnosti, poput inzulinske rezistencije i dijabetesa melitusa tipa 2. Opsežna istraživanja ističu značaj mediteranske dijeta, bogate antioksidansima i polifenolima, koja smanjuje negativne efekte kortizola na metaboličke funkcije uz istovremeno očuvanje crijevne mikrobiote. Cilj ovog preglednog rada je istražiti ulogu kortizola u metaboličkoj regulaciji i analizirati efikasnost mediteranske ishrane u postizanju optimalnog nivoa ovog hormona u organizmu. Pretraživanje je izvršeno u relevantnim bazama podataka korištenjem ključnih riječi „cortisol“, „metabolism“ i „mediterranean diet“. Abnormalni ritmovi kortizola, povezani s poremećajem homeostaze glukoze, mogu utjecati na razvoj inflamatornih procesa aktiviranjem inflamatornih citokina. Visoke vrijednosti kortizola pozitivno su korelirane sa statusom glikemije, detektovanim putem rezultata glukoze u plazmi natašte (FPG) i glikoliziranog hemoglobina (HbA1c), u trećem tertilu obima struka. U literaturnim podacima naglašava se značaj mediteranske prehrane u optimizaciji nivoa slobodnog kortizola u serumu. Nakon 18 mjeseci, ispitanici su pokazali smanjenje vrijednosti kortizola natašaste (FMC) uz poboljšanje FPG, HbA1c, C-reaktivnog proteina (hsCRP), tireostimulirajućeg hormona i testosterona. Stoga, praćenje varijabilnosti dnevnog nivoa kortizola predstavlja prediktivni faktor rizika za razvoj abnormalnosti u metaboličkim aktivnostima glukoze. Dugotrajno pridržavanje mediteranske dijeta implicira zaštitu kardiometaboličkog zdravlja neovisno o stanju uhranjenosti.

*Ključne riječi: kortizol, metabolizam, mediteranska dijeta*

## **The importance of nutritional therapy in the prevention and treatment of iron deficiency anemia**

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### **Abstract**

Sideropenic anemia, the most common form of anemia caused by iron deficiency, affects about one-third of the world's population, particularly women of reproductive age, pregnant women, and children. Around 40% of pregnant women suffer from anemia during pregnancy, which can have serious consequences for both the mother and the child. Analyzing scientific papers with the keywords „sideropenic anemia“ , „iron intake“, „iron deficiency“, „nutrition“ has shown that vegetarians and vegans are especially susceptible to this form of anemia due to low heme iron intake. Nutritional therapy can identify and correct deficiencies contributing to the development of sideropenic anemia, and taking iron supplements in combination with foods rich in heme and non-heme iron can improve hemoglobin levels. Taking iron supplements in conjunction with a diet rich in heme iron (found in meat, fish and poultry) and non-heme iron (found in plant sources such as legumes and nuts) can improve hemoglobin levels. Supplements with vitamins such as vitamin C, which improves iron absorption, can also contribute to the effectiveness of the therapy. Adequate nutrient intake enhances cognitive functions, emotional health, and physical endurance, thereby contributing to the overall well-being of patients.

*Keywords: sideropenic anemia, iron intake, iron deficiency, nutrition*

### **Introduction**

Sideropenic anemia is a serious public health problem, especially among populations at increased risk such as pregnant women, women of reproductive age and children. Iron deficiency not only affects physical health, but also has far-reaching consequences for mental function and emotional well-being. In pregnant women, anemia can increase the risk of complications during pregnancy and negatively affect fetal development (Kumar, Arnipalli, Mehta et al., 2022). Health intervention through nutritional therapy can be crucial in the prevention and treatment of this

condition. A proper diet, which includes heme iron (from red meat, fish and poultry) and non-heme iron (from legumes, green vegetables and nuts), is essential for optimizing hemoglobin levels. Heme-iron is more easily absorbed in the intestines, which makes it extremely important for people who follow vegetarian and vegan diets, because they often consume mostly non-heme-iron from plant sources, which is poorly absorbed (only 2-20%). Because of this, vegetarians and vegans are more susceptible to developing anemia and must consume 1.8 times more iron to meet their needs (Lombardi, Carfora, Tarallo et al., 2022). According to a study by Lombardi et al. (2022), vegans have specific nutritional needs, including an increased need for iron intake, due to the lower bioavailability of non-heme iron from plant sources. Compared to omnivores, who consume food of both plant and animal origin, vegans are often exposed to a higher risk of micronutrient deficiencies such as iron, vitamin B12 and vitamin D. Research has shown that vegans should pay special attention to the intake of foods rich in vitamin C, which can improve the absorption of non-heme iron and reduce the risk of anemia. In addition to iron, essential vitamins such as B12 and folic acid play a key role in hematopoiesis. Sideropenic anemia, which occurs due to the lack of iron necessary for the synthesis of hemoglobin, can be further complicated by the lack of essential vitamins B12 and folic acid, which are crucial for normal hematopoiesis; therefore, it is important that people on restrictive diets, such as a vegan diet, regularly consume these nutrients through carefully planned meals or supplementation in order to prevent the development of anemia and ensure optimal blood health, especially in pregnant women for whom iron, folic acid and vitamin B12 are key micronutrients for health of pregnant women and normal hematopoiesis. Iron deficiency can lead to anemia, while folic acid is necessary for the development of the neural tube and the prevention of defects. Vitamin B12 is essential for DNA synthesis and red blood cell formation (Mayasari, Bai, Chao et al., 2023). Optimal absorption of iron can be significantly improved by consuming foods rich in vitamin C together with iron-containing meals, thereby enabling more efficient utilization of non-heme iron. It is recommended to include foods rich in vitamin C, such as oranges, lemons, peppers, strawberries, broccoli and tomatoes, in meals in order to maximize the bioavailability of iron and improve the general state of health. During periods of rapid growth, such as childhood and adolescence, careful monitoring of iron intake is essential. Regular blood count checks, along with potential supplementation, can help identify and prevent anemia. It is important to note that children on a vegan diet often have a higher risk of nutrient deficiency, given their limited access to foods of animal origin that are rich in iron (Kaur, Agarwal, Sabharwal, 2022; Hojsak, Niseteo, Kolacek et al., 2018).



## ***Experimental***

For the purpose of researching sideropenic anemia, a review and analysis of available scientific papers published in the relevant databases was carried out. The study focused on key words such as "sideropenic anemia", "iron absorption" and "diet", in order to collect relevant data on the prevalence, causes and consequences of sideropenic anemia.

## ***Results and discussions***

Adapted nutritional interventions play a key role in the prevention and treatment of sideropenic anemia, which affects a significant part of the population, especially 25% of preschool children and 37% of women of reproductive age. The research emphasizes that iron deficiency significantly contributes to the prevalence of anemia, and suggests that simple interventions, such as increasing iron intake through supplementation and food fortification, can significantly reduce the prevalence of anemia. However, in populations with a high prevalence of inflammatory conditions or infections, the effects of nutritional therapy are limited, indicating the need for a combined approach that includes other treatment strategies (Petry, Olofin, Hurrell et al. 2016). Additionally, research conducted by Hojsak et al. 2018, emphasizes the importance of nutritional monitoring in children who eat vegetarian and vegan diets. A vegan diet appears to be particularly risky for the development of sideropenic anemia due to insufficient intake of key nutrients such as iron, calcium and vitamin B12. Proper meal planning, together with supplementation, becomes essential for the prevention and treatment of anemia in these groups. Meal planning is also essential in the context of a vegetarian diet, to ensure an adequate amount of nutrients, given that vegans and vegetarians may be more prone to mineral deficits (Leitzmann, 2014). Despite decades of public health measures, the global problem of nutritional iron deficiency remains widespread, affecting between 1.5 and 2 billion people (Lynch, 2011). Reasons for the persistence of this problem include low availability of bioavailable iron in the diet, poverty that limits dietary diversity, as well as technical barriers to adding iron to food products. Studies by Kauret al. (2022) indicate that mass food fortification, as well as supplementation, may be necessary to reduce the prevalence of anemia and improve health in affected populations. In Costa Rica, the implementation of a fortification program resulted in a reduction in the prevalence of anemia from 20% to 9.9%. Innovative strategies, such as "food-to-food fortification", contribute to improving the bioavailability of iron, especially by using rich plant sources of iron such as amaranth, sesame and moringa. Research in Cameroon, Ethiopia and Bangladesh confirmed the effectiveness of fortified foods such as rice and bread in significantly

reducing the level of anemia in children and women (Kaur, Agarwal, Sabharwal, 2022). Research by Travali et al. shows that innovative supplements based on microencapsulated and micronized iron sugar, together with vitamins B6, B12, folic acid and vitamin C, lead to a significant improvement in hemoglobin, ferritin and transferrin saturation percentage in women with sideropenic anemia. This supplementation, during 120 days, resulted in an increase in hemoglobin levels from 10.8 g/dL to 12.6 g/dL, while the symptoms associated with anemia, such as fatigue and dyspnea, were significantly reduced (E. Travali, N. Travali, Garo et al., 2023). In India, a study by Kumar et al. emphasizes that iron supplementation alone is insufficient due to the multifactorial causes of anemia, including cultural dietary habits, infections, and genetic predispositions. The prevalence of anemia among young women, especially in rural areas, suggests the need for comprehensive intervention programs that include improved nutrition and prevention strategies. A diet rich in rice and millet, which contains phytates and oxalates, can reduce the body's ability to absorb iron, while vitamin C from fruits improves the absorption of nonheme iron (Kumar, Arnipalli, Mehta et al., 2022).

### ***Conclusion***

Nutritional therapy plays a key role in the prevention and treatment of sideropenic anemia, especially in at-risk populations. A properly planned diet rich in heme and non-heme iron, in combination with iron supplementation and vitamins, can significantly reduce the incidence of this form of anemia. Research shows that mass food fortification and public health programs that focus on increasing iron availability can be extremely effective. Although the effect of nutritional therapy may vary depending on the condition of the organism and the presence of inflammatory processes, it is clear that combined treatment approaches that include nutrition and supplementation can significantly improve quality of life and reduce the incidence of sideropenic anemia worldwide. Integrated approaches that include dietary measures and nutritional supplements have been shown to be highly effective, especially in cases where diet alone is not sufficient to compensate for iron deficiency.

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## **Značaj nutritivne terapije u prevenciji i liječenju sideropenične anemije**

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### **Sažetak**

Sideropenična anemija, najčešći oblik anemije uzrokovane nedostatkom željeza, pogađa oko jedne trećine svjetske populacije, posebno žene reproduktivnog uzrasta, trudnice i djecu. Oko 40% trudnica pati od anemije tokom trudnoće, što može imati ozbiljne posljedice i za majku i za dijete. Analizom naučnih radova s ključnim riječima „sideropenična anemija“, „unos željeza“, „nedostatak željeza“, „ishrana“ utvrđeno je da su vegetarijanci i vegani posebno podložni ovom obliku anemije zbog niskog unosa hem željeza. Nutricionistička terapija može identificirati i ispraviti nedostatke koji doprinose razvoju sideropenične anemije, a uzimanje suplemenata željeza u kombinaciji s hranom bogatom hem i nehem željezom može poboljšati nivo hemoglobina. Uzimanje suplemenata željeza zajedno s prehranom bogatom hem željezom (koje se nalazi u mesu, ribi i peradi) i nehem željezom (koje se nalazi u biljnim izvorima kao što su mahunarke i orašasti plodovi) može poboljšati nivo hemoglobina. Suplementi s vitaminima, kao što je vitamin C, koji poboljšava apsorpciju željeza, takođe mogu doprinijeti efikasnosti terapije. Adekvatan unos hranljivih sastojaka poboljšava kognitivne funkcije, emocionalno zdravlje i fizičku izdržljivost, čime doprinosi opštem blagostanju pacijenata.

*Ključne riječi: sideropenična anemija, unos željeza, nedostatak željeza, ishrana*

## **The significance of nutritional and biopsychosocial factors for chronic musculoskeletal pain**

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### **Abstract**

Chronic musculoskeletal pain (CMP), defined as persistent discomfort in musculoskeletal tissues persisting for over 3 months, afflicts an estimated 1.71 billion people globally, leading to significant functional impairments and psychological distress, thereby detrimentally affecting individuals' quality of life. The role of nutritional and biopsychosocial factors in the treatment of CMP is often overlooked. Adopting a healthier diet may have significant implications for managing chronic pain and reducing inflammation. The aim of the paper is to review the available literature to indicate the importance of biopsychosocial and nutritional factors in the development of CMP and the importance of their combined efforts in the treatment of CMP. The research was conducted based on the review and analysis of literature from relevant databases (PubMed, NLM). In one of the studies, the impact of psychosocial factors on chronic lumbar pain syndrome was analyzed. Personalized pain medicine emphasizes the importance of viewing pain as a dynamic interaction between and within biological, psychological, and social factors unique to each individual patient, with the goal of optimizing treatment outcomes.

*Keywords: biopsychosocial, nutrition, musculoskeletal pain, chronic pain*

### **Introduction**

The biopsychosocial (BPS) model aims to represent the integrated and indivisible nature of the biological, psychological and social domains that cannot be separated and that all three levels, biological, psychological and social, must be taken into account in any health care task. A biopsychosocial approach was developed with the awareness that physical and mental always act together and that in addition to biological and psychological factors, numerous external socioeconomic factors affect the experience of pain. The association between nutrition and CMP has become a subject of increasing interest in recent years. Adequate nutritional intake plays a crucial role in modulating pain perception, managing inflammation, and promoting overall well-being. Unhealthy dietary behaviors and a poor diet have been implicated

in predicting, perpetuating, or contributing to CMP. For instance, individuals with chronic pain often exhibit high levels of obesity, excessive calorie consumption, and diets rich in sugar, fat, sodium, and caffeine. Adopting a healthier diet may have significant implications for managing chronic pain and reducing inflammation. Further, we can think of musculoskeletal care as a team effort with many possible interventions across the person-environment system at different points in time and potential for mutually reinforcing effects. Any model or approach to pain that focuses exclusively on just one of these dimensions would be incomplete, inadequate, and consequently ineffective in terms of pain treatment outcomes.

The aim of the paper is to review the available literature to indicate the importance of biopsychosocial factors in the development of pain syndromes and the importance of the biopsychosocial and nutritional factors in their treatment.

### ***Experimental***

The research was conducted based on the review and analysis of literature from relevant databases (PubMed, NLM).

### ***Results and Discussion***

One of the reviewed studies by Svedmark, Björklund and Häger (2018.) the aim was to evaluate if pain, disability and work productivity are influenced by physical and psychosocial work exposures as well as by stress, up to 1 year after a randomized controlled trial treatment intervention, and to determine whether any such association differed between treatment and control groups. Physical and psychosocial exposures, as well as perceived stress, were assessed after the treatment intervention. High perceived stress was associated with more neck pain, more neck disability, and decreased work productivity in both cross-sectional and longitudinal analyses.

In a systematic review and meta-analysis by Uvelli, Ribaldo and Gualtieri (2018.) the aim was to contribute to the clarification of the literature on the association between violence against women and chronic pain, considering all types of abuse verified only in adulthood (abuse suffered after 18 years old), and the major chronic pain diagnoses found in the literature. In this review, 37 studies were evaluated on women both with and without a history of sexual, physical, and emotional abuse perpetrated by a partner, a family member, or a stranger. The results show that women who have experienced violence during adulthood have 2 times greater odds of developing chronic pain.

Cherkin, Sherman and Balderson (2016.) conducted a randomized, interviewer-blind, clinical trial in an integrated health care system in

Washington State of 342 adults aged 20 to 70 years with chronic low back pain and randomly assigned to receive MBSR, CBT, or usual care. CBT (training to change pain-related thoughts and behaviors) and MBSR (training in mindfulness meditation and yoga) were delivered in 8 weekly 2-hour groups. These findings suggest that MBSR may be an effective treatment option for patients with chronic low back pain. An analysis by Dai, Jafarzadeh and Niu (2018.) suggested that this association between fiber intake and OA risk was partly influenced by BMI and C-reactive protein (CRP) levels. In this study, the authors found that a higher BMI, associated with lower fiber intake, leads to higher CRP levels, which could induce symptomatic OA according to the data analysis results, utilizing the Osteoarthritis Initiative database, revealed a significant reduction in the risk of worsening pain and symptomatic knee OA with higher adherence to the Mediterranean diet. Sears (2015.) in his study concludes that nutrition plays a pivotal role in supporting tissue repair, reducing inflammation, and optimizing overall physical and mental well-being. Poor nutrition is a recognized precursor to dysbiosis and sarcopenia, both of which may predispose individuals to the onset and exacerbation of CMP.

In A cross-sectional study by Elbinoune Amine and Shyen (2016.) there were 80 patients with neck pain lasting for more than 3 months, seen in rheumatology consultations. The aim of this review was to synthesize the evidence on the potential relationship between psychosocial work factors from the Areas of Worklife model (workload, job control, social support, reward, fairness, and values) and chronic low back pain (CLBP; unspecific pain in the lumbar region lasting 3 months or longer). The results support employees' workload, job control, and social support as predictors of CLBP.

### ***Conclusion***

The overarching aim of an enactive biopsychosocial and nutritional factors approach is to help a patient to better attune to their environment by helping them view and experience their body and world in positive ways. In relation to chronic pain, this may mean helping a person to become “unstuck.” Over time, the goal is to help the patient regularly perceive opportunities for meaningful action based on self-identified goals. (Re)engagement in activities may result in reduced pain and disability, or at the very least guide the person toward engagement in personally valued activities. There are many ways to achieve this, including helping patients make sense of their pain and to see new opportunities for action, intervening at the level of the body to reduce pain and improve movement, or changing the environment so that the patient is afforded new opportunities for action and taking into consideration nutritional factors. While there seems to be a general awareness of the

biopsychosocial and nutritional factors and their importance, clinicians describe a lack of knowledge and a wavering attitude, feel they lack the ability and utilization of necessary skills, and have difficulty integrating a BPS approach in clinical reasoning. Committing to an in-depth appreciation of nutrition's influence in CMP, coupled with a patient-centric stance, will lay the groundwork for more adept and all-encompassing pain management protocols. More research needs to be conducted on how to target these barriers and facilitators in implementation, the relevance for pre- and post-graduate education, and the role of the health care system.

**Author contributions:** conceptualization and methodology was developed by Adna Borić; investigation was done by Lejla Čoralić; writing—original draft preparation, review and editing was done by Edvina Vukalić; all under the supervision of Demir Džaferović. All authors have read and agreed to the published version of the proceeding.

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## **Značaj nutritivnih i biopsihosocijalnih faktora za hroničnu koštanomišićnu bol**

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### **Sažetak**

Hronični koštanomišićni bol, definiran kao trajna nelagoda u mišićno-koštanom tkivu koja traje više od 3 mjeseca, pogađa oko 1,71 milijardu ljudi širom svijeta, što dovodi do značajnih funkcionalnih oštećenja i psihičkog stresa, čime štetno utiče na kvalitet života pojedinaca. Uloga nutritivnih i biopsihosocijalnih faktora u liječenju hroničnog muskuloskeletnog bola se često zanemaruje. Usvajanje zdravije prehrane može imati značajne implikacije za upravljanje kroničnim bolom i smanjenje upale. Istraživanje je sprovedeno na osnovu pregleda i analize literature iz relevantnih baza podataka (PubMed, NLM). U jednoj od studija analiziran je uticaj psihosocijalnih faktora na sindrom hronične lumbalne boli. Personalizirana medicina boli naglašava važnost gledanja na bol kao na dinamičku interakciju između i unutar bioloških, psiholoških i društvenih faktora jedinstvenih za svakog pojedinačnog pacijenta, s ciljem optimizacije ishoda liječenja.

*Ključne riječi: biopsihosocijalni, ishrana, mišićno-skeletni bol, kronični bol*

#### 4-O-4

### **The effect of intermittent fasting on inflammatory biomarkers in autoimmune diseases**

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#### **Abstract**

Intermittent fasting (IF) is a pattern based on limited food intake during certain periods of time. IF is gaining increasing importance in health and nutrition research due to its potential effects on the control of autoimmune diseases. The aim of this study is to illustrate the importance of intermittent fasting and its effects on inflammatory biomarker levels in autoimmune diseases. In order to conduct a non-experimental, qualitative study, a selection of scientific papers was made by using relevant databases such as Medline (PubMed) and Google Scholar with keywords such as *intermittent fasting*, *type 1 diabetes*, *rheumatoid arthritis* and *systemic lupus erythematosus*. According to the available research, IF has different effects on inflammatory biomarker levels in autoimmune diseases, particularly systemic lupus erythematosus (SLE), rheumatoid arthritis (RA) and type 1 diabetes mellitus (T1DM). In RA, significantly lower levels of C-reactive protein (CRP) were found three months after a one-month, 16-hour IP. In SLE and T1DM, fasting minimizes interleukin (IL) levels such as IL-1, IL-1 $\beta$ , IL-6, tumor necrosis factor alpha (TNF- $\alpha$ ) and total leukocyte count. There is a growing need for detailed assessment and application of IP to clarify its long-term impact on the value of inflammatory biomarkers in patients with autoimmune diseases.

*Keywords: intermittent fasting, autoimmune diseases, inflammatory biomarkers*

#### **Introduction**

Intermittent fasting (IF) is gaining increasing attention in health and nutrition research, particularly due to its potential effects on inflammatory processes in the body. This pattern, based on reducing food intake during certain periods

of time, can have a significant impact on the immune system and levels of inflammatory biomarkers, which is particularly important for individuals with autoimmune diseases. IF is best known in the context of Ramadan fasting in Islam, where believers abstain from food, drink and other things that invalidate fasting according to Islam for a certain number of hours a day, depending on the time of year and place where they live (Aazza, Aazza, Adawi, et al. 2017; Ben Nessib, Maatallah, Ferjani, et al. 2020).

### ***Experimental***

In order to conduct a non-experimental, qualitative study, a selection of scientific papers was made by using relevant databases such as Medline (PubMed) and Google Scholar with keywords such as *intermittent fasting*, *type 1 diabetes*, *rheumatoid arthritis* and *systemic lupus erythematosus*. The selected articles relevant to the topic of this review were analyzed and critically reviewed and presented in a format that includes basic information about the study, the research objectives, the selected methods, the main results and the conclusions drawn. Only scientific papers not older than 10 years were considered. On this basis, the author's own reflections and conclusions on the current status and prospects of the topic are presented.

### ***Results and Discussion***

Adawi et al. 2017, analyzed data from 45 studies on the effects of IF on the immune system and inflammatory biomarkers, particularly in autoimmune diseases. The subjects were between 18 and 70 years old. The fasting time varied between 8-17h per day for 30 days, depending on the place of residence. In systemic lupus erythematosus (SLE), C3 levels increased during fasting but returned to baseline levels after three months, suggesting that Ramadan fasting may have minimal and harmless effects on SLE patients.

In rheumatoid arthritis (RA), a decrease in circulating immune complex (CIC) levels was found in 70% of patients after Ramadan. In type 1 diabetes mellitus (T1DM), fasting lowers levels of pro-inflammatory cytokines such as interleukins (IL) IL-4 and IL-6. Fasting has been shown to help lower CRP, such as IL-1, IL-1 $\beta$ , IL-6, tumor necrosis factor alpha (TNF- $\alpha$ ) and total leukocyte count. A study by Ben Nessib et al. 2020, investigated the effects of Ramadan fasting on RA and spondyloarthritis (SpA). The fasting time varied from 15.5 hours to 16.5 hours.

Specifically, 84.3% of patients had a decrease in ESR levels, while 48.5% of patients had a decrease in CRP levels. The values before Ramadan were  $36.9 \pm 24.5$  for ESR (mm) and  $7.9 \pm 16.1$  for CRP (mg/dL), while the values after Ramadan were  $22.4 \pm 16.2$  for ESR and  $3.9 \pm 5.1$  for CRP, from which we can conclude a decrease in the values of inflammatory markers during fasting.

Although the research conducted by Siddique, Yaser, Afzal et al. 2020, was based more based on the determination of the DAS28 SCORE, they also showed in their work that fasting in people suffering from RA reduces the ESR and the inflammatory cytokines TNF- $\alpha$  and IL-6, which has a positive effect on the function of the patient's immune system. The authors Dell'Oro, Fischer, Häupl et al. 2022, conducted a randomized, controlled study with 50 RA patients aged 18 to 79 years. They found that both values increased on day 7 and in week 6 of the research, but that CRP and ESR values decreased in week 12.

Research by Barati, Ghahremani, Namdar Ahmadabad et al. 2023, showed that IF is high risk for people with T1DM and can cause metabolic complications. IF in RA patients resulted in a decrease in ESR and CRP levels and inhibition of inflammatory cytokine production. The study showed that C3 complement levels were increased compared to the pre-fasting period.

### ***Conclusion***

Current research emphasizes the positive effects of IF on inflammatory biomarker levels, especially in autoimmune diseases such as RA, SLE and T1DM. There is an increasing need for detailed assessment and application of IP to clarify its long-term effects on inflammatory biomarkers in patients with autoimmune diseases.

***Author contributions:*** conceptualization, S.G. and N.Ć.; methodology, S.G.; investigation N.Ć.; writing—original draft preparation, S.G.; writing—review and editing, V.D.; visualization, I.B.; supervision, E.P.; All authors have read and agreed to the published version of the proceeding.

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***Conflicts of Interest:*** The authors declare no conflicts of interest.

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## Uticaj intermitentnog posta na vrijednost inflamatornih biomarkera u autoimunim oboljenjima

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### Sažetak

Intermitentni post (IP) je obrazac koji se zasniva na ograničenom unosu hrane u određenim razdobljima. U istraživanjima o zdravlju i ishrani, IP dobiva sve veći značaj zbog potencijalnih efekata u kontroli autoimunim oboljenjima. Cilj istraživanja je predstaviti značaj intermitentnog posta i njegov uticaj na vrijednosti inflamatornih biomarkera u autoimunim oboljenjima. Za potrebe izrade ne-eksperimentalnog kvalitativnog tipa istraživanja pregledane su relevantne baze podataka kao što su Medline (PubMed) i Google Scholar kombinacijom ključnih riječi *intermittent fasting*, *type 1 diabetes*, *rheumatoid arthritis* i *systemic lupus erythematosus*. Prema dostupnim rezultatima istraživanja, uočen je različit uticaj IP na vrijednost inflamatornih biomarkera u autoimunim oboljenjima kod sistemskog lupus eritematozusa (SLE), reuma-toidnog artritisa (RA) i diabetes mellitus tip 1 (DMT1). U većini autoimunih oboljenja, a osobito kod RA, tri mjeseca nakon IP u trajanju od jednog mjeseca i 16 sati dnevno, zabilježene su značajno niže vrijednosti C-reaktivnog proteina (CRP). Kod SLE i DMT1, IP doprinosi sniženju vrijednosti interleukina (IL) IL-1, IL-1 $\beta$ , IL-6, faktora nekroze tumora alfa (TNF- $\alpha$ ) i ukupnog broja leukocita. Međutim, zbog ograničenog broja istraživanja, raste potreba za detaljnom procjenom i primjenom IP kako bi se razjasnili njegovi dugoročni efekti na vrijednost inflamatornih biomarkera kod pacijenata s autoimunim oboljenjima.

*Ključne riječi: intermitentni post, autoimuna oboljenja, inflamatorni biomarkeri*

## Most common questions about food and nutrition addressed to pharmacists

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### Abstract

In the last few decades, the role of pharmacists in healthcare has surpassed mere "pharmaceutical care," which traditionally involved ensuring appropriate, effective, safe, and convenient therapy for patients. Since pharmacists are often the first point of contact with patients, they should be able to provide adequate, evidence-based health advice and answer patients' questions, including those related to food and nutrition. This means that aligning the professional field with pharmaceutical education is the best solution. To better understand the significance and importance of pharmacists in this area, a survey was conducted in the Western Balkans (Bosnia and Herzegovina, Croatia, Montenegro, Serbia, and Kosovo). The aim was to identify the most common questions about food and nutrition that pharmacists encounter daily while working in pharmacies and assess their ability to respond to these inquiries. It is crucial to highlight the importance of proper education and continuous knowledge updates in all areas addressed to healthcare professionals in the pharmaceutical field, with the goal of maintaining and improving public health and ensuring the highest possible quality of life for patients.

*Keywords: food and nutrition, health advice, pharmaceutical education, training*

## ***Introduction***

Pharmacists are the most accessible health workers in the community with a broad education in the field of biomedicine and health. Furthermore, certain postgraduate and life-long education programs (courses, specializations etc) are offered to pharmacist in order to maintain and further improve their professional competences, which is both their ethical and legal obligation. Since the global burden of non-communicable diseases (NCDs) are recognized as a major public health challenge in a modern society, and the main risk factors for majority of NCDs are unhealthy diet and lifestyle (WHO, 2015), there is a need for all health professionals to actively participate in health promotion and preventive actions within their communities and globally (DiMaria-Ghalili et al., 2014). To fulfill this role adequately, health professionals should be adequately prepared through their professional education, both graduate and postgraduate (Tommelein, De Boevre, Vanhie, et al., 2021). This significant counseling role of pharmacists is specifically stated in the International Pharmaceutical Federation document on the pharmacists role in beating non-communicable diseases in the community (FIP, 2019). Still, the preparedness and involvement of pharmacists in such counseling is largely unknown in the Western Balkan region. The aim of this survey was to shed some light on this important topic which could direct further actions in this area.

## ***Experimental***

The survey was conducted in a form of online posted questionnaire during December to September 2023. The eligible respondents were professionals in the field of pharmacy, including pharmacists, pharmacy technicians, and pharmacy students currently attending internship in community pharmacy. The survey was anonymous and consists of 15 questions, of which 4 were mandatory.

## ***Results and Discussion***

Over a period of six months, a scientific study was conducted in the form of an anonymous survey on the topic "Most Common Questions About Food and Nutrition Addressed to Pharmacists." The number of respondents who completed this survey was 128.

Of the 128 respondents who completed the survey, 34% work in Bosnia and Herzegovina, 38% in Serbia, 7% in Croatia, 20% in Montenegro, and 1% in Kosovo.

Seventy-four percent of the respondents hold the title of Master of Pharmacy/Doctor of Pharmacy, 24% are pharmacy technicians, and 2% are students in practice.



The age range of 20 to 30 years encompasses 47% of the respondents, the range of 31 to 40 years accounts for 36%, the range of 41 to 50 years makes up 12%, and those over 50 years old comprise 5%.

Ninety-two percent of the respondents were women, while 8% were men.

Regarding years of work experience in a pharmacy, 44% work for less than 5 years, 29% work for 5 to 10 years, 20% work for 11 to 20 years, and 7% work for more than 20 years.

Of the 128 survey respondents 33.6% have gotten less than 5 food related questions, 26.5% have gotten 5-9 food related questions, 14.1% have gotten 10-15 and nearly 25% have gotten more than 15 food related questions in the previous month.

The most frequently asked questions are about infant formulas, nutritional supplements, questions about breastfeeding, general questions about nutrition and food allergy questions.

20% of the survey respondents have said that they don't often need to inform themselves beforehand to answer these questions, nearly 43% have often informed themselves, 32% always inform themselves before giving an answer and only 3% stated that they were always able to answer the question without any additional information.

When collecting additional information, the most commonly used data sources were: professional literature (producer's brochure, professional or scientific papers, books, etc.) in printed or electronic edition (40%); data from product packaging or accompanying declaration (41%); databases (6%); internet - websites of professional organizations and associations (9%); internet - websites of manufacturers (11%); internet - other (2%); other - associates who promote baby food (0%).

Only 3.9% of the 128 survey respondents have said that although they have informed themselves, they didn't have the adequate answer for the patient/client.

In order for the survey participants to evaluate their knowledge regarding food and nutrition, a scale from 1 (I have absolutely no knowledge in this field without consulting or checking) to 5 (I have excellent knowledge) was used. The most frequently chosen rating was 5 (I have excellent knowledge), with 71 responses accounting for 55.5% of total 128 responses. The rating of 4 follows with 37 responses (28.9%). The rating of 3 with 15 responses (11.7%). The rating of 2 follows with 4 responses (3.1%) and only 1 response for rating 1 (0.8%).

Regarding the question of acquired knowledge, 23.4% of survey participants acquired their food and nutrition-related knowledge through university, 16.4% through regular education after obtaining a degree (e.g. courses, specialization, etc.), and 60.2% mainly through work.

To allow participants in the survey to assess the significance of food and nutrition-related education, a scale from 1 (completely unimportant) to 5 (very important) was provided. Out of 128 responses, 69.5% rated the significance of education a 5, 21.9% a 4, 3.9% a 3 and 2, only 0.8% with 1. Out of 128 respondents 78.13% showed their interest in additional training in the next 3 to 6 months, while 21.83% responded with negatively.

Those who showed interest in additional trainings suggested some interesting topics such as: Nutritional guidance for specific medical conditions, Nutrition for specific patient categories, Allergies and food intolerance, Interactions between food and medication, Supplements and additives, Nutrition in Early childhood, Caloric and nutritional value of food.

### ***Conclusion***

To understand the importance and significance of pharmacists in the field of food and nutrition, the presented scientific study was conducted. The most common questions pertain to general nutrition, with a particular emphasis on infant formulas. Pharmacists in pharmacies frequently encounter such questions on a daily basis, highlighting the importance of education in this area. These questions are predominantly directed toward women, who make up the majority of survey respondents. The respondents' age and desire for education vary significantly among participants. Most respondents had to seek additional information before providing answers. Given the importance of adequate education and the continuous updating of knowledge in all areas of inquiry directed at healthcare professionals with pharmaceutical training—aimed at preserving and improving public health and ensuring the highest possible quality of life for patients—there is a need for better alignment of pharmacy curricula with the demands of practice. Assessment of food and nutrition-related modules within the university curricula of the studies in pharmacy in the Western Balkan is warranted.

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## Najčešća pitanja o hrani i prehrani upućena farmaceutima

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### Sažetak

U nekoliko posljednjih decenija uloga farmaceuta u zdravstvu je prevazišla samu 'farmaceutsku njegu' koja je podrazumijevala osiguravanje prikladne, efikasne, sigurne i pogodne terapije za pacijente. Budući da je farmaceut često i prvi kontakt sa pacijentom, mora biti sposoban da pruži adekvatne zdravstvene savjete zasnovane na dokazima, kao i da odgovori na pitanja pacijenata, uključujući pitanja vezana za hranu i ishranu. Što znači da je usklađivanje stručne oblasti i samog farmaceutskog obrazovanja najbolje rješenje. U cilju samog razumijevanja značaja i važnosti farmaceuta u ovom polju, sprovedeno je naučno istraživanje na prostoru Zapadnog Balkana (Bosna i Hercegovina, Hrvatska, Crna Gora, Srbija i Kosovo) u vidu ankete, čiji cilj je bio upoznavanje sa najčešćim pitanjima o hrani i prehrani s kojim se farmaceuti pri radu u apotekama svakodnevno susreću i njihova sposobnost da na ista odgovore. Potrebno je naglasiti poseban značaj adekvatne edukacije i stalne nadogradnje znanja iz oblasti svih pitanja upućenih zdravstvenim radnicima farmaceutskog smjera obrazovanja u cilju očuvanja i unaprijeđenja zdravlja stanovništva te osiguranja što kvalitetnijeg života pacijenta.

*Ključne riječi: hrana i ishrana, zdravstveni savjeti, farmaceutsko obrazovanje, edukacija*

## **The effects of physical activity and supplementation on children with attention deficit hyperactivity disorder (ADHD)**

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### **Abstract**

Attention deficit hyperactivity disorder (ADHD) is one of the most common neuropsychiatric disorders in children and is characterized by complex symptoms. Children with ADHD often experience motor and cognitive difficulties. While drug therapy is often used to alleviate symptoms of ADHD, many medical professionals prefer the nonpharmacologic approach. This study analyzes the effects of physical activity and supplementation on children with ADHD. Based on a review of scientific literature from relevant databases such as Google Scholar, PubMed and Web of Science, the research, though still limited, generally indicates that regular or occasional physical activity and supplementation can help to reduce ADHD symptoms. Studies prove that physical activity improves cognitive function in general, especially exceptional functions essential for executing complex tasks, which indirectly supports the hypothesis that physical activity and supplementation can positively impact cognitive symptoms in children with ADHD. Except cognitive, behavioral and physical symptoms of ADHD are in most cases diminished and it is considered that the best effects come from exercise programs that combine various exercises of moderate intensity. According to research studies, harmful effects of physical activity with supplementation on children with ADHD haven't been reported, suggesting that physical activity is a highly positive nonpharmacologic intervention for these individuals.

*Keywords: ADHD, physical activity, supplementation, children, health*

### **Introduction**

Attention Deficit Hyperactivity Disorder (ADHD) is one of the most common childhood disorders, characterized by inattention, hyperactivity, and impulsivity, affecting 2% to 14% of school-aged children. The disorder includes three subtypes: inattentive, hyperactive-impulsive, and combined types. The causes of ADHD include genetic and neurological factors, with a higher prevalence among individuals with a family history of the disorder. Children with ADHD often exhibit deficits in executive functions, which

include inhibitory control, working memory, and cognitive flexibility. Impaired inhibitory control is considered a primary cause of ADHD symptoms, leading to slower reactions and increased errors in tasks due to attention difficulties. Current treatment mainly involves medication; however, many children do not respond well or experience side effects. As a result, there is growing interest in the potential benefits of physical activity as an intervention for children with ADHD. Some evidence suggests that supplements in the form of polyunsaturated fatty acid formulations and minerals such as iron and zinc may be effective in improving ADHD symptoms. Alternative non-pharmacological approaches, such as mindfulness training, physical activity, and yoga, have also shown positive effects on the behavior of children with ADHD. Recent studies emphasize that physical activity, particularly moderate to high-intensity aerobic exercise, can have a significant positive impact on ADHD symptoms. When children engage in such exercises, their bodies respond by increasing the production of neurotransmitters and brain-derived neurotrophic factor (BDNF). These components enhance mood and neuroplasticity, allowing the brain to adapt and function better. The objective of this study is to review available literature to demonstrate the impact and importance of physical activity on individuals with ADHD.

### ***Experimental***

The research was conducted by reviewing and analyzing scientific articles from relevant databases (Google Scholar, PubMed, and Web of Science) using keywords such as “ADHD,” “physical activity,” “children,” and “health.”

### ***Results and Discussion***

Ziereis and Jansen (2015.) conducted a study involving 43 children with ADHD (32 boys and 11 girls), aged 7 to 12, divided into two experimental groups (EG1, n = 13; EG2, n = 14) and a control group (CG, n = 16). Children in EG1 participated in training focused on ball handling, balance, and manual dexterity, while those in EG2 trained without specific goals. The control group had no physical activity. Working memory (WM) and motor performance assessments were conducted before the intervention, after the first week, and one week after training ended. After 12 weeks, significant improvements were observed in both EG1 and EG2 compared to the CG. This study indicates that long-term physical activity positively affects executive functions in children with ADHD and can be considered an alternative non-pharmacological treatment.

An initial meta-analysis by Simopoulos (1991) involving ten trials including 699 children with ADHD demonstrated a significant benefit of Polyunsaturated Fatty Acids (PUFA) supplementation compared to placebo. The benefits of PUFA supplementation were small (compared to the effect sizes observed for conventional pharmacological treatments for ADHD) but statistically significant.

Konofal Lecendreux and Deron (2008) conducted a small, pilot, randomized, placebo-controlled trial involving 23 children with ADHD and abnormally low serum ferritin levels. The trial demonstrated a significant improvement in ADHD symptoms in children randomized to receive ferrous sulfate (80mg/day) compared to placebo. Further trials are needed to establish whether iron supplementation has any clinical utility beyond ADHD patients with evidence of iron deficiency.

A completer's analysis by Bilici, Yildirim and Kandil (2004) found that of 193 (out of 400 randomized) Turkish children with ADHD, demonstrated a significant benefit of 150mg of zinc sulfate per day compared to placebo after 12 weeks of treatment.

Silva, Prado and Scardovelli (2015) examined how physical activity influences children's attention. The study involved 28 volunteers, 14 of whom had ADHD, while the other 14 did not. After 5 minutes of running, participants played a computer game, and their response times to tasks were recorded. The group that exercised showed a 30.52% improvement in attention compared to those who did not exercise, achieving similar results to the group without ADHD symptoms.

Liu, Sun and Tse (2023) conducted a meta-analysis on sleep problems, which are common among children with ADHD, and explored the impact of physical activity on sleep quality. One study examined 33 children diagnosed with ADHD (average age = 10.12 years) and assessed four specific sleep parameters. Results revealed significant improvements in sleep efficiency, sleep onset latency, and waking after sleep onset in the intervention group, but not in the control group.

Seal, Vu and Winfield (2023) conducted a survey-based research showing that the coronavirus disease 2019 (COVID-19) pandemic reduced physical activity across all population groups, especially children. This study used a qualitative approach to understand how the pandemic affected physical activity among children with ADHD and their caregivers. Between October 2020 and January 2021, 33 interviews were conducted. Content analysis revealed that physical activity decreased among children and caregivers, with significant barriers such as social isolation, reduced self-confidence, low energy levels, and mental health challenges. These factors further distanced families from physical activity, diminishing its positive effects on ADHD symptoms and mental well-being. Physical activity is deemed essential

during stressful situations, particularly for families dealing with ADHD, to prevent symptom deterioration.

### ***Conclusion***

ADHD presents significant challenges for children, parents, and educational institutions, with a prevalence ranging from 2% to 14% among school-aged children. The disorder stems from genetic and neurological factors, and traditional medical treatments often yield unsatisfactory results, necessitating the inclusion of non-pharmacological interventions, especially physical activity, in treatment plans. Research has confirmed the positive effects of physical activity on children with ADHD, covering various age groups and testing methods. Moderate to high-intensity aerobic exercises improve motor performance, working memory, attention, and sleep quality. Studies have shown that children who participate in structured physical activities exhibit significant improvements in executive functions compared to control groups without physical activity.

Recent research highlights that physical activity can reduce ADHD symptoms and improve mental health, which is particularly important during stressful situations like the COVID-19 pandemic. In addition to alleviating ADHD symptoms, physical activity and supplementation contribute to children's overall well-being by providing opportunities for self-expression and social skill development. Based on the reviewed research, integrating physical activity and supplementation into children's daily lives should become a part of ADHD treatment alongside other therapeutic methods. Such an approach can help reduce symptoms, enhance quality of life, and improve mental health, fostering a positive environment for children and their families.

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## **Utjecaj fizičke aktivnosti i suplementacije na djecu s poremećajem hiperaktivnosti i poremećajem pažnje (ADHD)**

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### **Sažetak**

Poremećaj pažnje i hiperaktivnosti (ADHD) jedan je od najčešćih neuropsihijatrijskih poremećaja kod djece, sa složenom simptomatologijom. Djeca sa ADHD-om vrlo često imaju značajne poteškoće u motoričkim i kognitivnim sposobnostima. Iako se medikamentozna terapija često koristi za ublažavanje simptoma ADHD-a, mnogi medicinski stručnjaci preporučuju i nefarmakološke pristupe. Cilj ove studije je analizirati efekte fizičke aktivnosti na djecu sa ADHD-om. Na osnovu pregleda naučne literature iz relevantnih baza podataka Google Scholar, PubMed i Web of Science, istraživanja, iako još uvijek ograničena, općenito ukazuju da redovna ili povremena fizička aktivnost i suplementacija mogu pomoći u ublažavanju simptoma ADHD-a. Istraživanja su dokazala da fizička aktivnost uz adekvatnu suplementaciju poboljšava kognitivnu funkciju uopšte, a posebno izvršne funkcije koje su ključne za obavljanje složenih zadataka, što indirektno podržava hipotezu da fizička aktivnost može uticati na kognitivne simptome kod osoba s poremećajem hiperaktivnosti i poremećajem pažnje. Osim kognitivnih simptoma, bihevioralni i fizički simptomi ADHD-a su u većini slučajeva umanjeni, a smatra se da su najbolji efekat pokazali programi vježbanja koji kombinuju različite vrste vježbi umjerenog intenziteta. Prema istraženim studijama štetni efekti fizičke aktivnosti i suplementacije na djecu sa ADHD-om nisu prijavljeni, što sugerira da je fizička aktivnost uz suplementaciju izrazito pozitivna nefarmakološka intervencija kod ovih osoba.

*Ključne riječi:* ADHD, fizička aktivnost, suplementacija, djeca, zdravlje

## Detox diets – types and promoted health effects

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### Abstract

Detox diets involve various dietary regimes aimed at eliminating toxins from the body, improving physiological processes, and promoting weight loss. These diets can last from one to several days, with a focus on strict food restriction. The premise is that toxins accumulated from the environment need to be removed to avoid harmful health effects.

This paper describes different types of detox diets, including those based on the consumption of fruits and vegetables, alkaline foods, anti-inflammatory foods, and fasting. Each of these has its own benefits and potential risks. For example, consuming fruits and vegetables is beneficial, but there is no convincing evidence that commercial detox diets, as such, actually remove toxins from the body. Additionally, some regimes, like the lemon detox diet, may lead to weight loss but can also have unwanted side effects, such as muscle loss and nutritional deficiencies.

This study also includes the results of a survey conducted among students at the University of Sarajevo about their attitudes and experiences with detox diets. The aim of the study is to understand the effectiveness of detox diets, their types and potential consequences, and to provide recommendations for consulting a doctor before adopting any dietary regime.

*Keywords: detox diet, toxins, benefits, risks*

### Introduction

Detox diet includes nutritional regimes that lead to the elimination of toxic substances that have accumulated in the body. They enable optimal biochemical and enzymatic functioning of physiological processes in the body and accelerate the process of body weight loss (Allen, Montalto, Lovejoy & Weber, 2011). It is believed that if they are not removed, these toxins accumulate in the body, which eventually leads to tissue damage and the development of diseases, such as cancer. Our bodies are extraordinary systems, which have the ability to continuously filter toxins, but these

mechanisms can be saturated, which leads to the accumulation of toxins and harmful consequences (Khalil, 2017).

Our main goal is to define detox diets, the types of diets mentioned, and possible benefits, but also unwanted consequences of practicing them. The goal is also to present the results of a survey conducted among students of the University of Sarajevo regarding the assessment of information, attitudes and possible previous experiences with the use of detox diets. It is recommended to consult a doctor before establishing a new diet, because not all individuals react equally to individual diets.

### ***Experimental***

Relevant scientific journals and databases were used for research and information gathering: Web of Sci and SCOPUS. When it comes to the conducted survey, the sample consists of 100 respondents, of which the largest number belongs to the younger population, i.e. the age ranges from 18 to 25 years. The survey was conducted online in December 2023, and it contained 9 questions, to which the respondents voluntarily gave their answers.

### ***Results and Discussion***

We have conducted a survey among participants, trying to find out more about the type of detox diet they have used, as well as the positive and negative effects they have felt while being on a certain diet.

Out of the 100 people who completed the survey, 81% of them were female and 19% were male. The largest percentage of responders, 95%, is in the age group of 18 to 25 years, 4% in the age group of 25 to 30 years and 1% in the age group of 30 to 35 years.

76 % of them are familiar with the term "detox diet" and have heard of it before, while 24 % of them are not familiar with this term. This shows us that detox diets are known to the general public and that today we encounter this term more and more often.

Only 9% of respondents used some type of detox diet at some point in their lives. The other 91% of respondents have not used a detox diet.

83% of respondents used a detox diet for health reasons, such as high cholesterol, insulin resistance, polycystic ovarian syndrome, thyroid insufficiency and many others. 17% of respondents used a diet to lose body weight. The use of a detox diet for health reasons and weight loss are closely related. Excess body mass is primarily a health problem, which can result in some disease or shorter life expectancy. It is very important to emphasize the importance of body weight control. Doctors express concern if a person loses at least 5% of their body weight in less than 6 months.

78% of respondents used detox juices, 22% of respondents used various types of teas as a detox diet. Detox juices and teas are the most promoted these days. Precisely because of this, we should be careful when choosing those that will truly provide us with the desired effect, and at the same time will not damage our health.

Out of 100 respondents, 68 of them believe that detox diets have more benefits, while 32 believe that they have more disadvantages.

The positive effects of a detox diet can be numerous. As many as 46% of respondents noticed a loss of body mass, 16% a better lipid status, which is a laboratory check that includes four basic parameters: total cholesterol, LDL cholesterol, HDL cholesterol and triglycerides, from which the risk factor and atherosclerosis index can be determined. This is crucial for patients who are at risk of developing atherosclerosis and coronary heart disease. 16% of respondents noticed an increase in energy, a decrease in fatigue and muscle pain. 15% noticed less bloating and better digestion. 8% of respondents noticed an acceleration of metabolism (Khalil, 2017)

94% of respondents who used the detox diet did not experience side effects from it, while the other 6% noticed unwanted effects of the diet itself. Research has shown that sudden weight loss leads to an increase in the blood concentration of potentially toxic substances such as pesticides and polychlorinated biphenyls (PCBs) in obese subjects. Severe energy restriction and nutritional inadequacy can lead to protein and vitamin deficiency, electrolyte imbalance, lactic acidosis, and even death (Clemens, Pressman, 2005).

The last question related to the harmful effects experienced by users of detox diets. Almost 50% of them felt faint, headache and general weakness of the body occurred somewhat less often. Anemia also occurred in 14% of respondents, which indicates that detox diets are not as harmless as they seem at first glance.

## ***Conclusion***

The detox diet is most commonly used for weight loss, health reasons, and overall cleansing of the body. But detox diets can have negative consequences for the human body. Certain groups of people should not follow this type of diet, including children of all ages, pregnant women, and individuals with diabetes. This type of diet is easily accessible, so one should be cautious when choosing one that will provide the desired effects without compromising health. The positive effect of this type of diet in obese individuals is that it leads to a reduction in body mass and cholesterol levels, as well as accelerating metabolism. Considering the popularity and availability of detox diets, this is an area that deserves attention so that

consumers can be informed about the potential benefits and risks of detoxification.

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## **Detox dijete – tipovi i promovisani zdravstveni efekti**

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### **Sažetak**

Detox dijete uključuju različite režime ishrane usmjerene ka eliminaciji toksina iz organizma, poboljšanju fizioloških procesa i pospješivanju gubitka tjelesne mase. Ove dijete mogu trajati od jednog do nekoliko dana, s naglaskom na strogo ograničenje unosa hrane. Premisa je da se toksini akumulirani iz okoliša moraju ukloniti, kako bi se izbjegle štetne posljedice po zdravlje.

Kroz rad su opisane različite vrste detox dijeta, uključujući one koje se temelje na konzumaciji voća i povrća, alkalnim namirnicama, protuupalnim hranama, te postu. Svaka od njih ima svoje prednosti i potencijalne rizike. Na primjer, konzumacija voća i povrća je korisna, ali ne postoji uvjerljiv dokaz da komercijalne detox dijete, kao takve, stvarno uklanjaju toksine iz tijela. Također, neki režimi, poput limunske dijete, mogu dovesti do smanjenja tjelesne mase, ali mogu imati i neželjene efekte, poput smanjenja mišićne mase i nutritivnih nedostataka.

Rad također obuhvata rezultate ankete provedene među studentima Univerziteta u Sarajevu o njihovim stavovima i iskustvima sa detox dijetama. Cilj rada je razumjeti efikasnost detox dijeta, njihove vrste i moguće posljedice, te pružiti preporuke za konsultacije s liječnikom prije primjene bilo kakvih dijetnih režima.

*Ključne riječi: detox dijeta, toksini, prednosti, rizici*

## **Inflammatory processes in the gut and their role in the development of mental disorders**

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### **Abstract**

Inflammatory bowel diseases (IBD) include ulcerative colitis and Crohn's disease. They can cause a variety of extraintestinal manifestations, including mental disorders. Research shows that there is a link between the gut and the central nervous system known as the "gut–brain axis". Epidemiological studies show that patients with IBD are more likely to suffer from mental disorders than the general population, which poses a diagnostic and therapeutic challenge. In this article, the relationship between inflammatory processes in the gut and mental disorders is analyzed and approaches for the treatment of patients with IBD and mental health problems are discussed. Using available scientific papers from relevant databases, it was found that the frequency of anxiety (19.1% vs. 9.6%) and depression (21.2% vs. 13.4%) was higher in patients with active IBD. Risk factors for depression included age, disease severity, exacerbations and socioeconomic status. About 6% of IBD patients have suicidal thoughts. Changes in the composition of gut bacteria have been linked to anxiety, depression, schizophrenia and bipolar disorder. In particular, studies have shown that patients with these disorders often experience a decrease in anti-inflammatory bacteria such as *Faecalibacterium*, while pro-inflammatory bacteria such as *Eggerthella* increase. The results show that gut health is crucial for mental health.

Keywords: *inflammatory bowel disease, gut-brain axis, mental disorder, microbiota*

### **Introduction**

Inflammatory processes in the intestines have a significant impact on mental health, and more and more research is being done on how they are connected through the gut-brain system. This system represents a complex way of communication between the gut and the brain, including various mechanisms that can affect our emotional state. This axis encompasses a complex two-way communication pathway between the gut and the brain, involving neural,



immune and endocrine mechanisms. The health of the gut reflects on the functioning of the brain, and an imbalance in the gut microbiota, known as dysbiosis, is associated with the development of various mental disorders such as depression, anxiety, and even neurodegenerative diseases (Cryan, O'Riordan, Cowan, 2019; Clapp, Aurora, Herrera, et al., 2017). Research has shown that changes in the composition of intestinal bacteria can affect mental health by increasing the level of proinflammatory cytokines that disrupt the function of the blood-brain barrier (Merlo, Bachtel, Sugden, 2024). In addition, modern research indicates that nutrition has a significant influence on the composition of the intestinal microbiota, and therefore on inflammatory processes in the intestines. Nutritional therapy, which includes properly balanced meals rich in probiotics, prebiotics, fiber and omega-3 fatty acids, has become a potential strategy in the prevention and treatment of inflammatory disorders and mental conditions associated with them. Research has shown that proper nutrition can improve the quality of life of people with chronic inflammatory bowel diseases, reducing not only physical symptoms but also improving mental health. Understanding the impact of nutrition on intestinal processes and mental health is becoming more and more important, because it is shown that eating habits can significantly affect the emotional state of an individual. This topic highlights the importance of integrating proper nutrition into daily life, especially when it comes to mental health (Xiong, Li, Cheng, et al., 2023; Ni, Xu, Yan, et al., 2022; Appleton, 2018).

### ***Experimental***

A review and analysis of the available scientific and technical literature in relevant scientific databases was carried out. The keywords used to search for articles on inflammatory bowel disease and mental health were "inflammatory bowel disease", "mental health", "gut-brain barrier" and "microbiota". This review includes studies investigating the effects of inflammatory bowel disease on the development of mental disorders.

### ***Results and Discussion***

Inflammatory processes in the gut are increasingly recognized as a key factor in the development of mental disorders such as depression, anxiety, and bipolar disorder. This phenomenon can be explained through the concept of the gut-brain axis, which represents a complex communication system between the gut and the brain via immune, endocrine, and neurological mechanisms. Dysbiosis, or an imbalance in the composition of gut bacteria, can lead to inflammatory processes that affect brain function and,

consequently, mental health. Research shows that a reduction in beneficial bacteria such as *Faecalibacterium* and an increase in pro-inflammatory bacteria such as *Eggerthella* contribute to the development of depression and anxiety. These bacterial changes, combined with inflammatory processes in the gut, can negatively impact the production of neurotransmitters like serotonin and dopamine, which are crucial for mood regulation. Clinical trials have indicated that the *Bacteroides* family is associated with depression. Elevated levels of serum immunoglobulin (Ig)M and lipopolysaccharide antagonists IgA have been observed. In healthy individuals, milk fermented with probiotic bacteria has shown a positive effect on emotional centers (Nikolova, Smith, Hall et al., 2021). Berk et al. (2013) reported that low-grade chronic inflammation is present in depression and may be associated with intestinal permeability disorders. They suggested that anti-inflammatory therapies, including nonsteroidal anti-inflammatory drugs (NSAIDs), statins, omega-3 fatty acids, and lifestyle changes such as a healthier diet and increased exercise may have a positive effect on reducing depressive symptoms in patients with elevated inflammatory markers. The microbiota is considered a key factor in the relationship between unhealthy diets and depression (Evrensel, Ceylan, 2015). The diet can significantly impact on mental health and emotional state, whereby an improper diet with high quantities of processed food and saturated fats can increase the risk for depression and anxiety disorders (Grajek, Krupa-Kotara, Białek-Dratwa, et al., 2022). Nutritional therapy has proven to be a significant method for reducing intestinal inflammation and improving mental health. Proper nutrition can influence the composition of the gut microbiota and help restore its balance. For example, a fiber-rich diet, including whole grains, fruits, and vegetables, improves the diversity of gut bacteria and reduces inflammatory processes in the gut. Fibers are fermented in the intestines, producing short-chain fatty acids such as butyrate, which have strong anti-inflammatory effects (Clapp, Aurora, Herrera et al., 2017; Fu, Zheng, Gao et al., 2022). Low levels of vitamin D are associated with a higher risk of schizophrenia and depression. Supplementation with vitamin D over a three-month period (4000 IU/day for one month and 2000 IU/day for two months) has been shown to significantly reduce the severity of depression, irritability, fatigue, mood swings, sleep difficulties, weakness, and concentration ability in adolescents diagnosed with depression. Zinc deficiency is linked to the severity of depressive symptoms, and its supplementation, included in antidepressants, plays a role in stabilizing mood. Zinc modulates cytokine activity and affects neurogenesis by influencing levels of brain-derived neurotrophic factor. B vitamins play a role in the proper functioning of nervous tissue. A deficiency in folic acid (vitamin B9) is associated with depressive symptoms and has been identified in subjects with a moderate

response to antidepressants (Grajek et al., 2022). Probiotics hold particular significance in the prevention of mental illnesses. In a 30-day study, healthy volunteers without prior depressive symptoms were given either probiotics or antidepressants. Those receiving probiotics showed reduced cortisol levels and improved psychological effects similar to those reported by participants receiving diazepam, a commonly used anxiety medication. Similar studies have revealed that probiotic therapy reduces depressive symptoms and improves HPA axis functionality, comparable to citalopram and diazepam (Clapp et al., 2017).

### ***Conclusion***

Inflammatory processes in the gut and the imbalance of the gut microbiota play a crucial role in the development of mental disorders such as depression, anxiety, and bipolar disorder. The communication between the gut and the brain, known as the gut-brain axis, clearly demonstrates how dysbiosis can impact mental health through immune, endocrine, and neural mechanisms. A reduction in beneficial bacteria and an increase in pro-inflammatory species may contribute to the development of depressive symptoms. Research suggests that proper nutrition, particularly one rich in fiber, omega-3 fatty acids, vitamins, and probiotics, can reduce inflammation, restore the balance of the gut microbiota, and improve mental health. The Mediterranean and MIND diets show significant positive effects in reducing the risk of neurological disorders. In addition to dietary interventions, supplementation with vitamins and minerals, such as vitamin D and zinc, may also play a key role in the prevention and treatment of mental disorders. Probiotics have proven effective in reducing depressive symptoms and improving HPA axis functionality. All this information highlights the importance of nutritional therapy as a significant part of a holistic approach to treating mental disorders, emphasizing the need for further research to understand the complex interactions between diet, microbiota, and mental health.

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## Upalni procesi u crijevima i njihova uloga u razvoju mentalnih poremećaja

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### Sažetak

Upalna bolest crijeva (IBD) obuhvata ulcerativni kolitis i Crohnovu bolest te može izazvati različite vancrijevne manifestacije, uključujući mentalne poremećaje. Istraživanja pokazuju da postoji povezanost između crijeva i centralnog nervnog sistema, poznata kao "Gut – Brain axis". Epidemiološke studije pokazuju da pacijenti s IBD-om češće imaju poremećaj mentalnog statusa u odnosu na opću populaciju, što stvara dijagnostičke i terapijske izazove. Ovaj rad analizira vezu između upalnih procesa u crijevima i mentalnih poremećaja, te razmatra pristupe u upravljanju pacijentima s IBD-om i mentalnim problemima. Koristeći dostupne naučne radove iz relevantnih baza, primijećeno je da je učestalost anksioznosti (19,1% naspram 9,6%) i depresije (21,2% naspram 13,4%) veća kod pacijenata s aktivnim IBD-om. Rizik za depresiju uključuje starost, težinu bolesti, pogoršanja i socioekonomski status. Oko 6% pacijenata s IBD-om ima suicidalne misli. Promjene u sastavu crijevnih bakterija povezane su s anksioznošću, depresijom, šizofrenijom i bipolarnim poremećajem. Konkretno, studije su pokazale da pacijenti s ovim poremećajima često imaju smanjenje antiinflamatornih bakterija poput *Faecalibacterium*, dok su proinflamatorne bakterije poput *Eggerthella* povećane. Rezultati pokazuju da je zdravlje crijeva od ključnog značaja za mentalno zdravlje.

*Ključne riječi: upalna bolest crijeva, crijevno-mozgovna osovina, mentalni poremećaj, mikrobiota*

**Association of hypomagnesemia with anxiety and depressive disorders**

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**Abstract**

Anxiety and depressive disorders are widespread in the population and impair quality of life. They are often associated with an inadequate intake of magnesium (Mg), which plays an important role in regulating the activity of a large number of enzymes and neurotransmitters in the central nervous system. The aim of this study was to demonstrate the importance of laboratory monitoring of hypomagnesemia in anxiety and depressive disorders. As part of a qualitative, non-experimental study, relevant databases such as Medline (PubMed) and Google Scholar were searched using the keywords: *hypomagnesemia*, *anxiety*, *depression* and *mental disorders*. Hypomagnesemia is associated with an increased incidence of anxiety and depressive disorders, and laboratory monitoring is based on the determination of serum Mg. Considering the fact that serum Mg levels do not correlate with total body concentrations, the determination of the ionized form is gaining diagnostic importance. It has been observed that symptoms of depressive and anxiety disorders correlate with lower concentrations of ionized Mg. Since the prevalence of hypomagnesemia, anxiety and depressive disorders in the population varies according to age and gender, it is important to develop effective strategies for laboratory monitoring of Mg levels, nutrition and supplementation of this mineral in the population.

*Keywords: hypomagnesemia, anxiety, depression, mental disorders*

**Introduction**

Magnesium (Mg) is an important mineral and the second most common intracellular cation. It is involved in numerous enzymatic reactions and processes in the central nervous system (Opanković, Milovanović, Radosavljević et al. 2022). As the fourth most abundant mineral and second most abundant cation in the cell, Mg is a cofactor for more than 300 enzymes and is required for anaerobic and aerobic energy production, glycolysis,

oxidative phosphorylation in the mitochondria and potassium and calcium regulation (Bhatia, Nair, Choudhri et al. 2020). Anxiety and depressive disorders are common conditions in the population that affect quality of life and are associated with inadequate Mg intake. The results of numerous studies indicate a connection between reduced Mg concentrations and symptoms of depressive and anxiety disorders. Also, Mg supplementation may play a therapeutic role in the treatment of these disorders, but the results are largely inconclusive and require additional research (Botturi, Ciappolino, Delvecchio et al. 2020). In recent years, the role of hypomagnesemia in the pathophysiology of anxiety and depressive disorders has been increasingly investigated, implying that Mg concentration could be a potential new biomarker for anxiety and depression.

### ***Experimental***

As part of a qualitative, non-experimental study, relevant databases such as Medline (PubMed) and Google Scholar were searched using the keywords: hypomagnesemia, anxiety, depression and mental disorders. The selected scientific papers relevant to the topic of this review were analyzed and critically examined and tabulated by presenting the basic data of the work, the research objective, the methods chosen, the main results and the conclusions drawn. Only scientific papers not older than 10 years were considered. On the basis of all this information, our own considerations and conclusions on the problem posed are presented.

### ***Results and Discussion***

Although the exact mechanism underlying the association between hypomagnesemia in the blood and depressive disorders is not known, (Abdelmoneam, Khafagy, Elbeh et al. 2024), state that the possible reasons could be oxidative stress pathways in the central nervous system. The subjects in the depressed group had lower average Mg levels. The value of Mg for predicting depression in both groups studied was 104.5 ng/dL, with a sensitivity of 64%. In a cross-sectional study by Bhatia et al (2020), it was observed that the mean value of serum Mg was lower in depressives compared to the control group ( $1.72 \pm 0.33$  mg/dL versus  $2.1 \pm 0.26$  mg/dL). The results of the study by Altunöz, Baykan, Baykan (2023), showed that serum Mg levels were below the reference values (1.9-2.7 mg/dl) in 12.9% of women and 9.1% of men. As in the previous study, no statistically significant difference was found between the Mg level and the age of the patients. Slightly different research results were reported by Rotter, Wiatrak, Rył et. al (2021), where a correlation was found between serum Mg levels and the presence of comorbidities such as diabetes, hypertension and depressive symptoms in older men compared to the control group. In the study by



Opanković et al. (2022) a statistical significance was found between the control group and patients with anxiety and depressive disorders in the levels of ionized Mg. This indicates that the laboratory determination of ionized Mg indicates the actual availability of Mg in the body compared to the determination of serum levels. The growing need for personalized treatment is evidenced by a study by Tarleton, Kennedy, Rose et al. (2023), involving 3604 adults in which hypomagnesemia was associated with depressive symptoms, supporting the potential use of Mg in a therapeutic approach. Although conventional treatment for depression and anxiety disorders relies on the use of antidepressants and other medications, modern treatment is increasingly focusing on the use of supplements such as Mg, which may play an important role in preventing and suppressing symptoms. In a study by Rajizadeh, Mozaffari-Khosravi, Yassini-Ardakani et al. (2017), 60 depressed subjects with hypomagnesemia were divided into two groups. The research results showed that at the end of the intervention, 88.5% of the subjects taking Mg and 48.1% of the subjects taking the placebo had normal Mg levels and that the symptoms of depression decreased. In a randomized study, 126 adult subjects with depressive disorders took part in the study. The efficacy of Mg supplementation for anxiety and depressive disorders is shown for mild to moderate depression in adults.

### ***Conclusion***

Although the serum concentration of Mg is primarily determined in laboratory diagnostics, it has been observed that the symptoms of depressive and anxiety disorders are also correlated with the concentrations of ionized Mg, with lower concentrations being associated with an exacerbation of these disorders. Since the prevalence of hypomagnesemia, anxiety and depressive disorders in the population varies according to age and gender, it is important to develop effective strategies for laboratory monitoring of Mg levels, nutrition and supplementation of this mineral in the population.

***Author contributions:*** conceptualization, A.LJ. and B.Č.; methodology, B.Č. and A.Ć.; investigation, E.T.; writing—original draft preparation, A.Lj., A.Ć.; writing—review and editing, A.Lj., B.Č., E.T.; visualization, A.LJ.; supervision, E.P.; All authors have read and agreed to the published version of the proceeding.

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## **Povezanost hipomagnezijemije sa anksioznim i depresivnim poremećajima**

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### **Sažetak**

Anksiozni i depresivni poremećaji predstavljaju česta stanja u populaciji koja negativno utječu na kvalitet života. Nerijetko su povezani s neadekvatnim unosom magnezija (Mg) koji ima važnu ulogu u regulaciji aktivnosti velikog broja enzima i neurotransmitera u centralnom nervnom sistemu. Cilj ovog istraživanja bio je predstaviti značaj laboratorijskog praćenja hipomagnezijemije u anksioznim i depresivnim poremećajima. Za potrebe kvalitativnog ne-eksperimentalnog istraživanja, pretražene su relevantne baze podataka kao što su *Medline (PubMed)* i *Google Scholar* upotrebom ključnih riječi: *hypomagnesiemia, anxiety, depression* i *mental disorders*. Hipomagnezijemija se dovodi u vezu s povećanom incidencom anksioznih i depresivnih poremećaja, a laboratorijsko praćenje iste zasniva se na određivanju serumskog Mg. S obzirom na to da vrijednosti serumskog Mg ne koreliraju s ukupnom koncentracijom u organizmu, sve se veći dijagnostički značaj pridaje određivanju ioniziranog oblika, odnosno aktivne forme. Uočeno je da simptomi depresivnih i anksioznih poremećaja koreliraju s koncentracijama ioniziranog Mg, pri čemu se niže koncentracije istog dovode se u vezu s pogoršanjem ovih poremećaja. Uzimajući u obzir da učestalost hipomagnezijemije, anksioznih i depresivnih poremećaja u populaciji varira u odnosu na dob i spol, važno je uspostaviti efikasne strategije za laboratorijsko praćenje nivoa Mg, ishranu i suplementaciju ovog minerala u populaciji.

*Ključne riječi: hipomagnezijemija, anksioznost, depresija, mentalni poremećaji*

**Planning and implementation of nutritional interventions for patients with cardiovascular diseases**

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**Abstract**

Planning and implementing nutritional interventions for patients with cardiovascular disease is critical to improving cardiovascular health. Various diets such as the DASH diet, the Mediterranean diet and a diet rich in omega-3 fatty acids have been shown to be effective in reducing the risk of hypertension, elevated cholesterol levels and inflammatory processes. This study is based on a review and analysis of scientific papers published in relevant databases using the keywords "nutrition", "heart disease", "healthcare" and "cardiovascular disease". Research shows that coronary heart disease, hypertension and dyslipidemia require an integrative approach that includes nutritional therapy. Reducing the intake of saturated fats and trans fats and increasing the intake of fruits, vegetables, whole grains and fish significantly improves the condition of patients with coronary heart disease. High blood pressure can be regulated by increasing the intake of potassium, magnesium, calcium and vitamin D, while limiting salt intake. For dyslipidemia, reducing the intake of saturated fats and increasing the intake of omega-3 fatty acids helps to regulate blood lipid levels and thus reduce the risk of complications. Educating patients about the importance of nutrition and working with healthcare professionals are key to establishing healthy eating habits that can improve quality of life and reduce the need for medication. Through appropriate nutritional interventions, it is possible to reduce the risk of complications and improve public health.

*Keywords: nutrition, heart disease, public health, cardiovascular disease*

***Introduction***

The planning and implementation of nutritional interventions for patients with cardiovascular disease are critical to improving health outcomes and preventing complications. According to the World Health Organization (WHO), nutrition is one of the most important factors in maintaining health and preventing chronic diseases, including cardiovascular disease (WHO

2020). A poor nutrition rich in saturated fats, sodium and sugar contributes to the development of hypertension, dyslipidemia and obesity. A proper diet with an increased intake of fruits, vegetables, whole grains and unsaturated fats improves cardiovascular health and reduces the risk of heart attacks and strokes. Dietary strategies such as the DASH diet, the Mediterranean diet and a diet rich in omega-3 fatty acids are effective in reduce the risk of high blood pressure and inflammatory processes (WHO 2019). Healthcare professionals play a key role in this, as they are responsible for educating patients, adapting dietary plans and supporting patients. Nutritional interventions are becoming an integral part of healthcare, enabling patients to adopt and maintain healthy eating habits that reduce the long-term risk of complications and improve quality of life. Ultimately, such approaches not only strengthen patients' physical health, but also contribute to their emotional well-being and quality of life. In addition, healthier eating habits can lead to a reduced need for drug therapy and thus lower healthcare costs. Regular monitoring and evaluation of patients' progress further strengthens healthcare strategies and makes them more effective. In addition, collaboration between healthcare professionals, patients and their families is key to successful outcomes in this process. Finally, educating and informing the population about the importance of proper nutrition can help create a healthier society and reduce the burden of cardiovascular disease at the population level (Podvorica, Bekteshi, Oruqi, Kalo, (2021)).

### ***Experimental***

A review and analysis of available scientific and professional literature were conducted in relevant scientific databases. The keywords used to search for studies related to nutritional interventions and cardiovascular diseases were "nutrition" "heart disease", "health care", "cardiovascular disease". This review includes studies investigating the impact of various diets on risk factors associated with cardiovascular diseases.

### ***Results and Discussion***

Analysis of different nutritional approaches for patients with cardiovascular disease highlights the importance of tailored nutritional interventions in the treatment of conditions such as hypertension. A review of several studies conducted from 2014 to 2023 shows that the DASH (Dietary Approaches to Stop Hypertension) diet, the Mediterranean diet and a diet rich in omega-3 fatty acids significantly reduce cardiovascular risks by targeting the main factors that contribute to the development of the disease, such as elevated blood pressure, cholesterol and inflammatory processes. Based on the study by Filippou, Tsioufis, Thomopoulos et al. (2022), who conducted a

systematic review and meta-analysis of randomized controlled trials, it was confirmed that the DASH diet significantly lowers blood pressure in adults with and without hypertension. This diet, which emphasizes fruits, vegetables, whole grains and a reduction in salt, led to a significant reduction in systolic and diastolic blood pressure. Compared to a standard diet, blood pressure decreased by up to 11 mmHg in participants on the DASH diet, indicating a significant contribution of this diet to controlling high blood pressure and reducing the risk of cardiovascular disease. A Mediterranean diet rich in unsaturated fats from olive oil and fish has also been shown to lower LDL cholesterol levels and improve the overall lipid profile in patients with cardiovascular disease (Estruch R, Ros E, Salas-Salvadó J, et al. 2013). In a study conducted by Liyanage and Ninomiya, it was found that the Mediterranean diet was associated with a significant improvement in cardiovascular outcomes. This systematic review and meta-analysis included numerous clinical trials and found that adherence to the Mediterranean diet was associated with a lower risk of cardiovascular disease, including heart attack and stroke. The study showed that participants who regularly consumed foods typical of the Mediterranean diet had a lower mortality rate from cardiovascular disease. These results reconfirm the importance of the Mediterranean diet as a preventive tool for maintaining heart health and emphasize the need to promote this type of diet to improve public health (Liyanage, Ninomiya, Wang et al. 2016). Regular consumption of fish and plant sources rich in omega-3 fatty acids is recommended to reduce the risk of deficiency and prevent cardiovascular disease. Adults are recommended to eat at least two meals of fish per week (Shahidi, Ambigaipalan, 2018). Increased intake of potassium, magnesium, calcium and vitamin D can contribute significantly to the regulation of high blood pressure. Studies have shown that a high intake of these minerals can help lower blood pressure. A 2011 study suggests that an increased potassium intake reduces the risk of high blood pressure, especially in people with a low sodium intake. Magnesium also plays an important role in regulating blood pressure, and an increased intake of this mineral may help lower high blood pressure. (Houston, 2011). Adherence to dietary changes is crucial for patients with cardiovascular disease, as proper nutrition can significantly reduce the risk of complications such as worsening heart disease or hospitalization. The study found that educating patients about the importance of nutrition led to a reduction in body weight, improved diabetes control and lipid profile, as well as a reduction in blood pressure. The study suggests that ongoing education and support from healthcare professionals plays a key role in the adoption of healthy habits that can significantly improve the quality of life of patients with heart disease (Podvorica, Bekteshi, Oruqi, Kalo, 2021).

## **Conclusion**

Given the growing problem of cardiovascular disease and its link to nutrition, it is essential to adopt effective nutritional measures. These studies confirm that a proper diet, including approaches such as the DASH diet, the Mediterranean diet and a diet rich in omega-3 fatty acids, can significantly reduce the risk of heart disease. By continually monitoring and adjusting dietary plans, physicians can help their patients adopt healthy habits and overcome obstacles that prevent them from achieving optimal health. This integrative approach not only improves clinical outcomes, but also empowers patients to take control of their own health.

**Author Contributions:** conceptualization: A. N.; methodology: A. N. and N. B.; research and preparation of the original draft: A. N., S. Š. S. and N. B.; revision and editing: S. Š. S. and N. B.; visualization: A. N. and N. B.; supervision: N. B. All authors agreed with the final version of the manuscript and contributed to its quality and accuracy.

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## **Planiranje i implementacija nutritivnih intervencija za pacijente sa kardiovaskularnim oboljenjima**

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### **Sažetak**

Planiranje i implementacija nutritivnih intervencija za pacijente s kardiovaskularnim oboljenjima ključno je za poboljšanje zdravlja srca i krvnih sudova. Različite dijetete, poput DASH dijetete, mediteranske dijetete i prehrane bogate omega-3 masnim kiselinama, pokazale su se efikasnim u smanjenju rizika od hipertenzije, povišenog holesterola i inflamatornih procesa. Ovo istraživanje je sprovedeno na osnovu pregleda i analize naučnih radova koji su objavljeni u relevantnim bazama podataka upotrebom ključnih riječi „nutrition“, „heart disease“, „health care“, „cardiovascular diseases“. Istraživanja pokazuju da koronarna bolest srca, hipertenzija i dislipidemija zahtijevaju integrativni pristup koji uključuje dijetoterapiju. Smanjenje unosa zasićenih masti i trans masti te povećanje unosa voća, povrća, integralnih žitarica i ribe značajno poboljšava stanje pacijenata sa koronarnom bolešću srca. Hipertenzija se može regulisati povećanim unosom kalija, magnezija, kalcija i vitamina D, dok se ograničava unos soli. Kod dislipidemije, smanjenje unosa zasićenih masti i povećan unos omega-3 masnih kiselina pomažu u regulaciji nivoa lipida u krvi, čime se smanjuje rizik od komplikacija. Edukacija pacijenata o važnosti ishrane, kao i saradnja sa zdravstvenim radnicima, ključni su za usvajanje zdravih prehrambenih navika, što može poboljšati kvalitet života i smanjiti potrebu za medikamentoznom terapijom. Kroz pravilne nutritivne intervencije, moguće je smanjiti rizik od komplikacija i poboljšati javno zdravlje.

*Ključne riječi: prehrana, srčane bolesti, zdravstvena njega, kardiovaskularne bolesti*

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## Therapeutic Properties of Bitter Melon: A review

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### Abstract

Bitter melon (*Momordica charantia L.*), belonging to the Cucurbitaceae family, is cultivated widely in Asia and valued for its therapeutic properties. It contains bioactive compounds like charantin and polypeptides, which contribute to its anti-diabetic, anti-cancer, anti-ulcer, and antioxidant effects. Bitter melon is particularly effective in managing diabetes by lowering blood glucose levels and enhancing insulin secretion. Its anticancer potential is evidenced by its ability to inhibit cell growth in breast, colon, and prostate cancers. Furthermore, it demonstrates protective effects against gastric ulcers and oxidative stress. Despite its benefits, excessive intake may result in adverse effects, including toxicity, highlighting the need for controlled use. This review emphasizes the importance of further clinical research to validate bitter melon's efficacy and safety as a therapeutic agent.

*Keywords: Bitter melon, anti-diabetic, anticancer, antioxidant, therapeutic*

### Introduction

Bitter melon (*Momordica charantia L.*) belongs to the Cucurbitaceae family. It is a multifunctional plant cultivated worldwide, especially in Asia, where it is an integral part of the local diet. There are several varieties of bitter melon, differing in origin and harvest time, which affects the composition of its chemical constituents. It is also known by other names, such as karela, balsam pear, or foo gwa, depending on the culture and country where it is grown and used. Bitter melon has the highest nutritional value among squashes and contains over 30 nutrients, including carbohydrates, proteins, fibers, vitamins, and other bioactive substances. It has a characteristic bitter taste that varies depending on the fruit's color and variety. The bitter taste, after which the fruit is named, arises from the alkaloid momordicin. The main constituents of bitter melon are alkaloids charantin, momordicin I, II, and III, karavilagenin A, B, C, D, and E, and kuguacins A-S. In folk medicine, it is recognized for its therapeutic properties. Bitter melon is a natural product capable of overcoming or delaying the aging process due to the presence of

bioactive molecules, and it is known for its assistance in combating various diseases. Bitter melon is most well-known for its high rate of diabetes prevention, but it also exhibits anticancer, anti-ulcer, anti-inflammatory, and antimicrobial effects. Despite all its positive attributes, consuming large quantities of bitter melon can lead to adverse effects, some of which can be toxic. The aim of this work is to highlight the significance and therapeutic properties of bitter melon through a review of scientific papers and studies conducted worldwide on humans and animals..

### ***Experimental***

In this study, we employed the methodology known as systematic review to comprehensively evaluate the therapeutic properties of bitter melon (*Momordica charantia* L.) and its effectiveness in managing diseases such as diabetes and cancer. This review aims to address the following questions:

1. What therapeutic properties are associated with bitter melon (*Momordica charantia* L.)?
2. How effective is bitter melon in the management of diseases such as diabetes and cancer?

A systematic search was conducted across multiple electronic databases, including PubMed, Scopus, and Google Scholar, using keywords such as “bitter melon,” “*Momordica charantia*,” and “therapeutic effects.” The search focused on peer-reviewed articles published in English between 2000 and 2023. Inclusion criteria encompassed studies that investigated the health benefits of bitter melon in humans and animal models, while exclusion criteria eliminated non-peer-reviewed articles, reviews, and studies not specifically addressing therapeutic applications. The relevant studies were reviewed, and key findings related to the therapeutic effects of bitter melon were summarized. A qualitative synthesis of the findings was conducted to highlight the role of bitter melon in diabetes management and cancer prevention.

### ***Results and Discussion***

This systematic review reveals significant therapeutic properties of bitter melon (*Momordica charantia* L.), particularly its anti-diabetic, anti-cancer, anti-inflammatory, and antioxidant effects. Bitter melon is widely recognized for its ability to lower blood glucose levels and enhance insulin sensitivity. Studies indicate that compounds such as charantin and polypeptides in bitter melon mimic insulin, thereby facilitating glucose uptake in cells (Ali et al., 1993; Dans et al., 2007). Clinical trials have shown that consumption of bitter

melon can effectively reduce hemoglobin A1c levels in individuals with Type 2 diabetes (Abascal & Yarnell, 2005).

Emerging evidence suggests that bitter melon possesses anticancer effects, inhibiting cell proliferation in various cancers, including breast, colon, and prostate cancers (Sur & Ray, 2020). The bioactive compounds in bitter melon, such as triterpenoids and flavonoids, have been shown to induce apoptosis in cancer cells while sparing normal cells, demonstrating its potential as a therapeutic agent (Hsiao et al., 2013; Ray et al., 2010).

The anti-inflammatory properties of bitter melon are attributed to its ability to reduce pro-inflammatory cytokines and oxidative stress markers (Kobori, 2003; Saeed et al., 2018). Research indicates that bitter melon extracts can significantly lower the expression of TNF- $\alpha$  and IL-6, contributing to its overall health benefits. Additionally, its antioxidant activity is linked to high levels of phenolic compounds, which help combat oxidative damage in cells (Ekici, 2019).

Despite its numerous benefits, excessive consumption of bitter melon can lead to adverse effects, including gastrointestinal issues and hypoglycemic episodes (Lucas et al., 2010). It is essential to establish safe dosage guidelines to mitigate these risks, particularly in populations with underlying health conditions. In summary, bitter melon presents a multifaceted approach to disease prevention and management, especially for diabetes and cancer. Continued research is needed to further elucidate its mechanisms and therapeutic potential while ensuring safe consumption practices (El Batran et al., 2006).

## ***Conclusion***

In conclusion, bitter melon (*Momordica charantia L.*) emerges as a valuable plant with significant therapeutic properties. This systematic review highlights its potential benefits, particularly in the management of diabetes, where it effectively reduces blood glucose levels and enhances insulin secretion. Additionally, bitter melon demonstrates promising anticancer effects, showing the ability to inhibit the growth of various cancer cells. Its anti-inflammatory, anti-ulcer, and antioxidant properties further underscore its role in promoting overall health. Despite the numerous advantages associated with bitter melon, it is crucial to acknowledge the risks of excessive consumption, which can lead to adverse effects, including toxicity. Therefore, further research is essential to establish optimal dosages and safety profiles for therapeutic use in humans. As the body of evidence surrounding bitter melon continues to grow, it holds promise not only as a functional food but also as a complementary treatment in managing chronic diseases. This review emphasizes the need for more comprehensive clinical

studies to fully understand its mechanisms of action and enhance its application in modern medicine.

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### **Terapeutska svojstva gorke dinje: pregledni rad**

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### **Sažetak**

Gorka dinja (*Momordica charantia* L.), koja pripada porodici Cucurbitaceae, široko se uzgaja u Aziji i cijenjena je zbog svojih terapeutskih svojstava. Sadrži bioaktivne spojeve poput charantina i polipeptida, koji doprinose njenim antidijabetičkim, antikancerogenim, antiulkusnim i antioksidativnim efektima. Gorka dinja je posebno efikasna u upravljanju dijabetesom, jer snižava nivo glukoze u krvi i poboljšava lučenje inzulina. Njeni antikancerogeni potencijali su potvrđeni sposobnošću inhibicije rasta ćelija raka dojke, debelog crijeva i prostate. Osim toga, pokazuje zaštitna svojstva protiv želučanih ulkusa i oksidativnog stresa. Uprkos benefitima, prekomjeren unos može dovesti do neželjenih efekata, uključujući toksičnost, što naglašava potrebu za kontroliranom upotrebom. Ovaj pregledni rad ističe važnost daljih kliničkih istraživanja kako bi se potvrdila efikasnost i sigurnost gorke dinje kao terapeuskog agensa.

*Ključne riječi:* Gorka dinja, antidijabetički, antikancerogeni, antioksidativni, terapeutski

**The effect of an alkaline diet on the pH of saliva and urine**

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**Abstract**

Unbalanced pH values in the body can cause various health problems. The aim of this study was to examine the influence of a predominantly alkaline diet on the pH values of saliva and urine. The project involved 14 respondents, aged 21 to 31, who voluntarily followed a seven-day, predominantly alkaline diet, followed by a seven-day predominantly acidic diet. Subjects independently, following the instructions, measured and recorded (on Monday, Thursday and Sunday) the pH of saliva and first morning urine, fasting and before brushing their teeth. We followed the linear trend of pH changes throughout the experiment. A drop in saliva pH was recorded during the period of consumption of acidic foods, while the drop in urine pH was more pronounced. Also, there is a slight increase in the pH of saliva during the period of alkaline diet, while the pH of urine does not change. This pilot study with a small number of samples indicates the existence of a connection between the consumption of alkaline or acidic food and the pH values of saliva and urine. It is necessary to conduct research with a larger sample.

*Keywords: food, pH, saliva, urine*

***Introduction***

One of the basic rules of maintaining health - which we often forget - is the balance of acidity and alkalinity in our body, the pH-balance. Today's typical diet consists mostly of acid-producing foods (proteins, sugar, grains). Alkaline food, eg. vegetables, is less digestible. Stimulants such as nicotine, coffee, tea and alcohol are consumed in large quantities, and they are highly acidic. Even stress and exercise (insufficient as well as excessive) can cause acidification. Most foods are alkaline or neutral in nature, but processed foods advertised in the media and available for sale are mostly acidic. The diet should contain at least 60% alkaline food to maintain the balance in our body. A state of imbalance can result in numerous health problems, both physical and psychological. The pH level in the human body has the ability to

affect every cell. When the blood has a slightly alkaline pH, it has a positive effect on bodily functions. The brain, bloodstream, nerves, muscles, respiratory system, digestive system, and reproductive function normally only when the pH value is appropriate. On the other hand, when the pH of the body is too low, the organism is subject to many irregularities in its work and even diseases. If the pH is not balanced, vitamins, minerals and supplements can not be used, which means that pH is one of the basic pillars of health. Some of the diseases associated with acidosis: problems of the locomotor system - painful back, osteoporosis, rheumatic problems, problems of the digestive system - gastritis, intestinal inflammation, spasms, disturbed intestinal flora, chronic fatigue, sleepiness, lack of energy, depression, nervousness, heart disease and circulatory system, hypotension, cardiac arrhythmias, headache, migraine, caries, periodontal disease, renal imbalance, gout, decreased endurance and muscle stiffness, low pain threshold - pain intolerance. (Manz, Remer, 1995) The aim of this study was to examine the influence of a predominantly alkaline diet on the pH value of saliva and urine. The body is slightly alkaline, and the cells create acidic products of physiological metabolism and activity. Acidic waste is converted to carbon dioxide and water, which can be safely excreted from the body. However, what is not used and metabolized from the consumed food is called "ash". The pH of the food does not have to be the same after it has been metabolized. Lemon is metabolized as sugars to CO<sub>2</sub> and water leaving a base ash consisting of mineral salts such as Na, K, Ca. Lemon tastes sour (low pH), but its metabolized residue is alkaline. On the other hand, proteins leave an ash rich in phosphates, sulfates and nitrates, which produce acids. Minerals that have a negative charge attract the H<sup>+</sup> ion and are called acidic minerals. These are chlorine (Cl<sup>-</sup>), sulfate (S<sup>-</sup>), phosphorus (P<sup>-</sup>), and they form acids: hydrochloric (HCl), sulfate (H<sub>2</sub>SO<sub>4</sub>), and phosphoric (H<sub>3</sub>PO<sub>4</sub>). Minerals with a positive charge are attracted by the negatively charged OH<sup>-</sup> ions. These minerals are called alkaline and include calcium (Ca<sup>+</sup>), potassium (K<sup>+</sup>), magnesium (Mg<sup>+</sup>) and sodium (Na<sup>+</sup>). (Reich, 1996)

Urine pH is an indicator of the body's ability to regulate and maintain the appropriate blood pH. The pH of urine varies from 4.5 to 8.0, and the ideal is 6.0 to 7.5. Values are lower in the morning and higher in the evening, but wide variations are possible. The pH value of urine is also affected by the chemicals that the body eliminates, including minerals, vitamins and metabolic products as well as drugs, toxins, etc. The pH of urine is not influenced by digestive enzymes like the pH of saliva. The pH of urine is influenced by: preservatives in the diet, pollutants we inhale, stress we are exposed to, food and water, the amount of pathogens in the body, how much we rest and all the biochemical processes that take place in the body. (Natural Health School, 2024)



pH of saliva immediately after waking up in the morning is 6.4. Values less than 6.1 indicate the possible existence of pathological processes. The pH value of unstimulated saliva should not be less than 6.3, while salivary values increase with greater production stimulated by food and smells. The ideal pH value is 7.2, 6.8-7.2 before a meal, and 7.2-8.5 after a meal or drink of an alkaline nature. A lower pH than 6.5 is an indicator of calcium deficiency, especially due to aging and bad lifestyle habits. (Reich, 1996)

### ***Experimental***

The project included 14 respondents, aged 21 to 31, who were voluntarily on the diet:

- gradual introduction to an alkaline diet - reduced intake of acidic foods for 3 days;
- predominantly alkaline diet - 7 days without animal proteins, sweets, coffee and sweet drinks;
- predominantly acidic diet - 7 days (animal proteins, sweets, coffee, sweet drinks).

The time of conducting both diets and measuring pH was during the period without exams, excessive physical activity and everything that can contribute to the appearance of greater stress. Subjects independently, following the instructions, measured and recorded (on Monday, Thursday and Sunday) the pH of saliva and first morning urine, fasting and before brushing their teeth, with pH strips (Figure 1). We followed the linear trend of pH changes throughout the experiment.

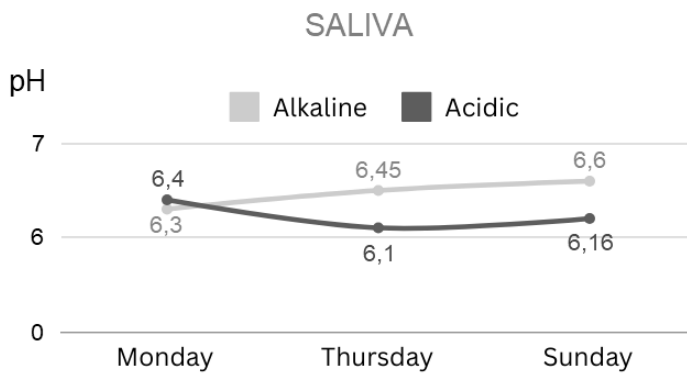


***Figure 1. pH strips***

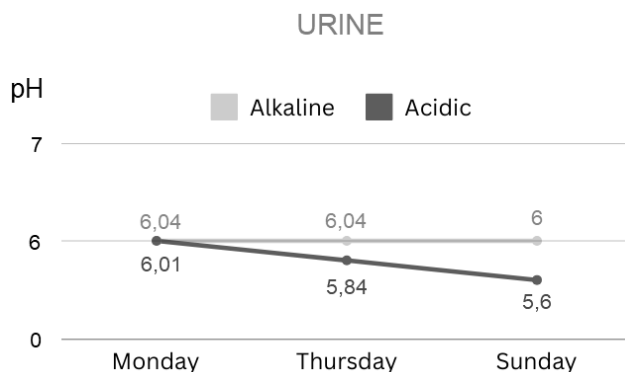
### ***Results and discussion***

A drop in salivary pH was noted, from a mean value of 6.4 to 6.16, in the period of consumption of acidic foods, while a slight increase in salivary pH

was observed in the period of an alkaline diet, from a mean value of 6.3 to 6.6 (Graph 1.). The drop in urine pH during an acid diet was more pronounced, from a mean value of 6.01 to 5.6, while urine pH during an alkaline diet does not change and maintains the ideal value of 6 (Graph 2.). As already stated, the physiological pH values of urine are 4.5-8, and the ideal is 6.0-7.5. Therefore, the acid diet shows us a drop in that value below the ideal values that were present before the start of the diet. Also, it has already been mentioned that the morning value of saliva should not be less than 6.3, which is what this value was, as can be seen on Graph 1., at the beginning of the alkaline diet, and by the end of the week it was 6.6. On the other hand, during the acid week, the pH value of saliva, from the appropriate 6.4, decreases to 6.16.



**Graph 1.** pH values of saliva in periods of alkaline and acid diet



**Graph 2.** urine pH values in periods of alkaline and acid diet

## ***Conclusion***

This pilot study with a small number of samples indicates the existence of a connection between the consumption of alkaline or acidic food and the pH value of saliva and urine. For greater conclusions and more precise views, it is necessary to conduct research with a larger sample.

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***Informed Consent Statement:*** Informed consent was obtained from all subjects involved in the study. The study was conducted in accordance with the provisions of the Declaration of Helsinki.

***Conflicts of interest:*** The authors declare no conflicts of interest.

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## Uticaj alkalne dijeta na pH pljuvačke i urina

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### Sažetak

Neuravnotežene pH vrijednosti u organizmu mogu izazvati razne zdravstvene probleme. Cilj ove studije je bio ispitati uticaj pretežno alkalne ishrane na pH vrijednosti pljuvačke i urina. Projekat je uključio 14 ispitanika, u dobi od 21 do 31 godine koji su dobrovoljno bili na sedmodnevnoj, pretežno alkalnoj ishrani, a zatim na sedmodnevnoj pretežno kiseloj prehrani. Ispitanici su samostalno, prateći upute, mjerili i bilježili (u ponedjeljak, četvrtak i nedjelju) pH pljuvačke i prvog jutarnjeg urina, natašte i prije pranja zuba. Pratili smo linearni trend promjena pH kroz vrijeme eksperimenta. Bilježi se pad vrijednosti pH pljuvačke u periodu konzumacije acidnih namirnica, dok je pad vrijednosti pH urina bio izraženiji. Također, bilježi se blagi porast pH pljuvačke u periodu alkalne prehrane, a pH urina se ne mijenja. Ova pilot studija sa malim brojem uzoraka, ukazuje na postojanje veze između konzumacije alkalne, odnosno acidne hrane i vrijednosti pH pljuvačke i urina. Potrebno je provesti istraživanje sa većim uzorkom.

*Ključne riječi: hrana, pH, pljuvačka, urin*

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