



## The potentially anticancer effect of Busha cattle milk

Vesna Gantner<sup>1\*</sup>, Krešimir Kuterovac<sup>2</sup>, Marko Samardžija<sup>3</sup>, Zvonimir Steiner<sup>1</sup>,  
Ranko Gantner<sup>1</sup> and Klemen Potočnik<sup>4</sup>

<sup>1</sup> Josip Juraj Strossmayer University of Osijek, Faculty of Agrobiotechnical Sciences, Osijek, Croatia

<sup>2</sup> Agroporc Ltd., Petrinja, Croatia

<sup>3</sup> University of Zagreb, Faculty of Veterinary Medicine, Zagreb, Croatia

<sup>4</sup> University of Ljubljana, Department of Animal Science, Biotechnical faculty, Domžale, Slovenia

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### ABSTRACT

This study analysed the fatty acid profiles of milk from two breeds of cattle, Simmental and Busha, to investigate their potentially anticancer effect. Milk samples were obtained during the summer season while the animals were grazing on pasture. The study determined the amounts of linoleic acid, conjugated linoleic acid,  $\alpha$ -linolenic acid, arachidonic acid, eicosapentaenoic acid, and docosahexaenoic acid, as well as the total amounts of saturated fatty acids (SFA), mono-unsaturated fatty acids (MUFA), polyunsaturated fatty acids PUFA, n-3 PUFA and n-6 PUFA. According to this research, milk obtained from the Busha breed, which is an indigenous cattle breed, was found to have higher quantities of essential fatty acids, including linoleic, arachidonic, eicosapentaenoic, and docosahexaenoic acids, compared to milk obtained from Simmental cows. The study also revealed that Busha milk contains higher quantities of total MUFA, PUFA, n-3 PUFA, and n-6 PUFA. The presence of these fatty acids in higher concentrations indicates that Busha milk is highly nutritious and may have possible anticancer potential. Therefore, it is crucial to consider this finding when formulating strategies for the preservation of the breed. This involves facilitating sustainable breeding to ensure the longevity of this breed and the production of high-quality dairy products.

**Key words:** milk; fatty acids; anticancer potential; Simmental; Busha

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### Introduction

Cancer represents a complex set of diseases caused by the abnormal growth and spread of cells in the body that are not controlled ([DESAI et al., 2023](#)). These cells can reproduce quickly, leading to the development of tumors or malignant masses that can interfere with the regular functioning of organs and tissues. The tumors can invade surrounding tissues and organs, and in some instances, they

can spread to other parts of the body via the bloodstream or lymphatic system and establish new tumors. Cancer can impact any part of the body, including the lungs, liver, colon, breasts, skin, and others.

Cancer, as a complex and multifactorial disease, may appear due to a variety of factors. It is primarily caused by genetic mutations that occur

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\* Corresponding author:

within cells, which can be triggered by a range of environmental factors and lifestyle choices ([LIU et al., 2022](#)). In addition to genetic mutations, several other risk factors can contribute to the development of cancer, including exposure to certain chemicals and substances, radiation exposure, a family history of cancer, certain infections, and the natural process of ageing ([MEIJERS and DE BOER, 2019](#)).

Recognizing the symptoms of cancer is crucial in identifying the disease at an early stage. Common symptoms include persistent fatigue, unexplained weight loss, pain, skin changes, and respiratory issues. Early detection increases the chances of successful treatment. The process of diagnosing cancer involves various stages of evaluation that start with a thorough assessment of the patient's medical history, including an analysis of any risk factors and symptoms they may have ([NOORELDEEN and BACH, 2021](#)). Medical professionals then conduct a physical examination to check for any signs of cancer, such as lumps or abnormal growths. Imaging tests, such as CT scans, MRIs, and PET scans, are used to visualize the internal organs and detect any abnormalities. Finally, laboratory tests, including biopsies and blood tests, are performed to identify cancer cells, and determine their type and stage.

There are a variety of treatment options that can be employed to combat cancer. Surgery is frequently the initial choice for extracting tumors, primarily if the cancer is localized. This process involves the removal of the diseased tissue or organ. According to [BORTOT et al. \(2023\)](#), surgical resection forms the foundation of solid tumor treatment. Chemotherapy is another prevalent treatment option that employs drugs to eliminate cancer cells. These drugs are typically administered through the veins or orally, and can reach cancer cells throughout the body. Radiation therapy, on the other hand, utilizes high-energy radiation to annihilate cancer cells. This therapy can be administered externally or internally, depending on the cancer type and its location. Immunotherapy and targeted therapy are novel approaches that have demonstrated promise in treating cancer. Immunotherapy operates by stimulating the patient's immune system to identify and obliterate cancer cells. Targeted therapy,

conversely, employs drugs that exclusively target cancer cells, thus sparing healthy cells. Hormone therapy is another type of cancer treatment that is applied to treat specific types of cancer that are hormone-sensitive. It operates by obstructing the production or activity of hormones that promote the growth of cancer cells. The selection of cancer treatment is dependent on various factors, including the cancer type and stage, the patient's overall health, and their personal preferences.

In the realm of cancer prevention, there are numerous precautionary measures one can undertake to significantly reduce the risk of developing malignant tumors. One of the most efficacious methods of preventing cancer is to adopt a healthy lifestyle that includes a balanced diet and consistent physical activity. Healthy diet can bolster the body's defences against a range of cancers, while regular exercise can help maintain a healthy weight and reduce the likelihood of developing certain malignancies. In addition to a healthy lifestyle, abstaining from tobacco use and curtailing excessive alcohol consumption can significantly diminish the chances of developing cancer. Tobacco usage is the primary cause of preventable cancer-related deaths worldwide, and quitting smoking is the most crucial step one can take to guard against malignancy. Likewise, excessive alcohol consumption has been correlated with an escalated risk of several types of cancer, including liver, breast, and colon cancer. Preventing certain infections is also vital in curbing the likelihood of developing cancer. For instance, the human papillomavirus (HPV) is a prevalent sexually transmitted infection that can cause cervical cancer, while hepatitis B and C are viral infections that can lead to liver cancer. Vaccines are obtainable to shield against these infections, and it is imperative to consult with a healthcare professional about which vaccines are appropriate for one's needs. Lastly, it is paramount to stay abreast of recommended cancer screening programs based on one's age and risk factors. Regular cancer screenings, such as mammograms and colonoscopies, can aid in the early detection of cancer, which is when it is most treatable.

There is no one food that can guarantee the prevention of cancer, but consuming a healthy and

balanced diet that is rich in nutrients may contribute to overall health, and potentially decrease the risk of developing specific types of cancer. Therefore, following a diet that emphasizes the consumption of nutrient-dense foods may be helpful in promoting optimal health and reducing the possibility of developing cancer. The correlation between milk and its potential role in cancer prevention has been investigated in several studies ([BADAWY et al., 2018](#); [EL-GAWAD et al., 2021](#); [EL-MAKSOUUD et al., 2021](#); [MAHMOUDI et al., 2023](#)). Milk and other dairy products are a great source of essential nutrients, including calcium, vitamin D, proteins, and milk fat, which are all vital for maintaining good health and well-being. However, it is important to note that the relationship between milk and cancer risk is intricate and can vary depending on the type of cancer. Some research studies have suggested that certain elements present in milk, such as calcium and vitamin D, may have protective effects against colorectal cancer. Furthermore, the anticancer effect of fatty acids was analyzed in numerous scientific papers ([KUMARI et al., 2012](#); [KAPLAN et al., 2013](#); [NARAYANAN et al., 2015](#); [HASI et al., 2019](#); [VENN-WATSON et al., 2020](#); [DAY et al., 2022](#); [NATH et al., 2022](#); [ZHANG et al., 2022](#)).

Fatty acids are carboxylic acids that consist of an aliphatic chain, either saturated or unsaturated. They are usually present in the form of triglycerides, phospholipids, and cholesteryl esters. Fatty acids have crucial roles in multiple functions, such as: signal-transduction pathways, cellular fuel sources, protein modification, energy storage, and they form essential structural components of cells. The classification of fatty acids is based on length and saturation. They can be categorized into short-chain fatty acids (SCFAs), medium-chain fatty acids (MCFAs), long-chain fatty acids (LCFAs), and very long-chain fatty acids (VLCFAs). Saturated fatty acids do not contain C=C double bonds, whereas unsaturated fatty acids have one or more C=C double bonds. Furthermore, several studies have indicated that the overall composition, as well as the fatty acid profile of milk, is affected by the species, breed, parity, lactation stage, season, feeding and individual animal effect ([ŠKRTIĆ](#)

et al., 2008; [POTOČNIK et al., 2011](#); [KOMPAN and KOMPREJ, 2012](#); [MOLLICA et al., 2012](#); [GANTNER et al., 2015](#); [JASTRZEBSKA et al., 2017](#); [RODRÍGUEZ-ALCALÁ et al., 2017](#)).

Milk fat is considered to be the most complex of all natural fats, as it contains nearly 400 different types of fatty acids, according to [MÅNSSON \(2008\)](#). The origin of milk fatty acids is almost equally divided between two sources: the feed, and the microbial activity in the rumen of the cow. The lipids in bovine milk are primarily present in globules, forming an emulsion. According to [MÅNSSON \(2008\)](#), almost 70% of the total fat content in milk is saturated fat. Out of this 70%, about 11% of it is made up of short-chain fatty acids, of which almost half is butyric acid. It was also found that approximately 25% of the fatty acids in milk are mono-unsaturated, while 2.3% are poly-unsaturated, with an omega-6/omega-3 ratio of approximately 2.3. Furthermore, it was found that roughly 2.7% of the total fatty acid composition in milk is made up of trans fatty acids. According to [GÓMEZ-CORTÉS et al. \(2018\)](#), lipids constitute a significant component of milk, playing a crucial role in the nutritional and economic value of this dairy product. These lipids provide a valuable source of energy and contribute to the unique sensory and physical characteristics of dairy products. Additionally, milk fat serves as a vehicle for naturally occurring fat-soluble vitamins (A, D, E, and K) and  $\beta$ -carotene, a pro-vitamin A carotenoid. Recent research has challenged the notion that dairy products are unhealthy due to their high content of saturated fatty acids (SFA) [GÓMEZ-CORTÉS et al. \(2018\)](#). While it was once believed that consuming certain SFAs in excess could lead to health problems, it turns out that the presence of various components in the dairy matrix - such as calcium, peptides, phosphorus, and the milk fat globule membrane - can modify blood lipid responses to SFA intake ([THORNING et al., 2017](#)). Additionally, milk fat contains bioactive fatty acids that are not found in significant amounts elsewhere in our diets, such as butyric acid and conjugated linoleic acid, which have potential health benefits ([KRATZ et al., 2013](#)).

Polyunsaturated fatty acids (PUFAs) are a group of essential fatty acids that play a crucial role in human nutrition, and are found in various foods, including milk. These fatty acids are vital for maintaining good health and are associated with numerous benefits, such as improving heart health, brain function, and overall well-being. A study conducted by [ŠREDNICKA-TOBER et al. \(2016\)](#), based on a systematic literature review and meta-analysis comparing organic and conventional cattle milk, found that organic milk has significantly higher concentrations of total PUFA and n-3 PUFA (omega-3 fatty acids) compared to conventional milk. Specifically, organic milk was found to have an estimated 7% higher total PUFA and a substantial 56% higher n-3 PUFA content. The beneficial fatty acids found in organic milk, such as eicosapentaenoic acid (EPA), arachidonic acid (AA), and docosahexaenoic acid (DHA), have specific roles in maintaining good health. EPA is important for cardiovascular health, AA is essential for the development of organs, tissues, and the nervous system, while DHA is crucial for brain development and vision. Furthermore, PUFAs are essential for maintaining cell membranes, supporting brain function, and reducing inflammation.

Linoleic acid (LA) is a type of polyunsaturated fatty acid (PUFA) that can be found in different foods, including milk. Conjugated Linoleic Acid (CLA) is a unique fatty acid present in milk. CLA is a mixture of positional and geometric isomers of linoleic acid (C18:2). These isomers have two conjugated double bonds at different carbon positions in the fatty acid chain. Among natural dietary sources, milk fat is the richest in CLA, containing an average of 4.5 mg CLA per gram of fat. According to [CHINNADURAI and TYAGI \(2011\)](#), the CLA content in milk can be enhanced by dietary manipulation, especially the addition of plant oils rich in unsaturated fatty acids. Linoleic acid, a type of omega-6 fatty acid, has been associated with numerous health benefits.

This study aimed to investigate the potential anticancer effect of milk by analyzing the amount of some polyunsaturated fatty acids, as well as the total amount of saturated, monounsaturated and

polyunsaturated fatty acids, in the milk of two different breeds of cattle, namely Simmental and Busha.

### Materials and methods

During this research, milk samples were obtained in the summer season (July) from two distinct cattle breeds, namely Simmental (12 animals) and Busha (10 animals). The cows were divided into groups on the basis of two criteria: their lactation stage, which was divided into three stages (I. calving - 100 days of lactation; II. 100 - 200 days of lactation; III. 200 - 300 days of lactation), and their parity, which was divided into two groups (I. primiparous cows; II. cows in second and higher lactation). The animals involved in the study were healthy at the time of sample collection, and were grazing on pasture at an altitude of 150 m, with no additional supplementation. The animals were in their first to third parity. Milk samples were taken from each cow during milking, and transported to the laboratory. The milk samples were subjected to analysis to determine their fatty acid profiles. Specifically, the amounts of linoleic acid, conjugated linoleic acid,  $\alpha$ -linolenic acid, arachidonic acid, eicosapentaenoic acid, and docosahexaenoic acid were determined. Additionally, the total amount of saturated (SFA), monounsaturated (MUFA), and polyunsaturated (PUFA) fatty acids, as well as the total amounts of n-3 PUFA and n-6 PUFA, were calculated.

In the laboratory, a gas chromatograph (Agilent 8860; Agilent Technologies. Inc., Santa Clara, CA, USA) was used for the analysis of fatty acid methyl esters. The gas chromatograph was equipped with a flame ionization detector (FID) and an automatic liquid sampler (ALS) for the analysis. The injector temperature was set at 200°C and the detector temperature at 240°C. A capillary column DB-23 (Agilent Technologies, Santa Clara, CA, USA) was used for chromatography, which had a length of 60m, a column inner diameter of 0.25mm, and an active layer thickness of 0.25 $\mu$ m. The gas chromatography process was initiated at an initial column temperature of 150°C for 2 minutes, after which the temperature was gradually increased to

230°C by heating at 5°C/min, and held steady at that temperature for 20 minutes. Hydrogen was utilized as the carrier gas, with a flow rate of 1 mL/

min. Results were collected and processed using the computer program OpenLAB CDS ChemStation Workstation VL. Identification of fatty acids was completed through a comparison of retention times with methyl standards (Sigma Aldrich Chemie, GmbH and Supelco, St. Louis, MI, USA). Nonadecanoic acid methyl ester (C19:0) was used for quantification. The percentage of each fatty acid relative to the total fatty acids was calculated to determine the fatty acid composition.

The statistical software SAS ([SAS Institute Inc., 2019](#)) was used to prepare the data for analysis and perform statistical calculations. In order to evaluate the effect of an animal's breed (Simmental and Busha) on the values of fatty acids in milk, a GLM (Generalized Linear Model) procedure in SAS was applied, using the least-square means (LSMeans). The statistical model (1) applied in the analysis was as follows:

$$y_{ijkl} = \mu + L_i + P_j + B_k + e_{ijkl}, \quad (1)$$

where:

$Y_{ijklmn}$  = estimated fatty acid (FA)

$\mu$  = intercept;

$L_i$  = fixed effect of lactation stage  $i$ ;

$P_j$  = fixed effect of parity  $j$ ;

$B_k$  = fixed effect of animal's breed  $k$ ;

$e_{ijkl}$  = residual.

To determine the significance of differences between the estimated LSMMeans, the t test was applied in the SAS GLM procedure.

## Results

The essential information regarding daily milk production, the content of fat, protein, and lactose, and the somatic cell count of the cows of the analyzed breeds are presented in Table 1. The data show that Simmental cows produced significantly more milk per day than Busha cows. However, the milk of the Simmental cows had a lower content

Table 1. Least squares means of daily milk production traits in relation to breed

Trait	Simmental breed	Busha breed
Daily milk yield, kg	6.64 <sup>A</sup> ± 0.42	3.35 <sup>B</sup> ± 0.38
Fat content, %	4.63 ± 0.20	4.75 ± 0.18
Protein content, %	3.18 ± 0.05	3.27 ± 0.05
Lactose content, %	4.72 ± 0.05	4.64 ± 0.04
Somatic cell count (000)	1151.27 ± 361.36	795.16 ± 325.39

\* LSMMeans in the same row marked with different letters differ statistically highly significant (P<0.01)

Table 2. Least squares means of polyunsaturated fatty acids (PFAs) in relation to breed (g/100g of total FA)

PUFA	Simmental breed	Busha breed
Linoleic acid (LA), C18:2(n-6)	1.51a ± 0.23	2.44b ± 0.38
Conjugated linoleic acid (CLA), (C18:2, cis-9, cis-12)	1.25a ± 0.59	1.02b ± 0.37
$\alpha$ -linolenic acid (ALA), C18:3(n-3)	1.18a ± 0.23	1.13a ± 0.24
Arachidonic acid (AA), C20:4(n-6)	0.10a ± 0.02	0.32b ± 0.22
Eicosapentaenoic acid (EPA), C20:5(n-3)	0.11a ± 0.03	0.13a ± 0.05
Docosahexaenoic acid (DHA), C22:6(n-3)	0.02a ± 0.01	0.05b ± 0.02

\* LSMMeans in the same row marked with different letters differ statistically significant (P<0.05)

of fat and protein, but a higher content of lactose compared to that of the Busha cows. Moreover, the Busha cows had a lower somatic cell count than the Simmental cows, indicating better udder health. According to [IVANKOVIĆ et al. \(2016\)](#), Busha cows yielded between 6.45 and 11.91 kg of milk per day in the second month of lactation, with an average milk fat content of 4.15% and an average milk protein content of 3.45%.

The amount of polyunsaturated fatty acids (PCFAs) in the milk of the Simmental and Busha breeds is presented in Table 1. Statistical analysis showed significant differences between the amount of linoleic, conjugated linoleic, arachidonic, and docosahexaenoic acid in the milk of the Simmental and Busha breeds. A statistically significant ( $P < 0.05$ ) higher value of linoleic acid (LA) was determined in the milk of Busha cattle, amounting to 2.44 g/100g of the total FA. On the other hand, a statistically significant ( $P < 0.05$ ) higher amount of conjugated linoleic acid (CLA) was found in the milk of Simmental cows (1.25 g/100g of total FA). Although the amount of  $\alpha$ -linolenic acid (ALA) did not differ statistically significantly ( $P > 0.05$ ), a higher amount was determined in the Simmental breed (1.18 g/100g of total FA). The amounts of arachidonic (AA),

0.32 g/100g of total FA, eicosapentaenoic (EPA), 0.13 g/100g of total FA, and docosahexaenoic acid (DHA), 0.05 g/100g of total FA, were higher in the Busha cattle with statistically significant ( $P > 0.05$ ) differences observed for AA and DHA. The observed difference in EPA was not statistically significant ( $P > 0.05$ ).

The total amounts of saturated (SFA), monounsaturated (MUFA), and polyunsaturated (PUFA) fatty acids in the milk of Simmental and Busha cows are presented in Figure 1. Also, the total amounts of n-3 PUFA and n-6 PUFA in relation to the animal's breed are presented in Figure 1. Milk from the Simmental cows contained higher amounts of saturated fatty acids (SFA) amounting to 63.7 g/100g of the total FA, while the milk of the Busha breed contained higher amounts of monounsaturated (MUFA) and polyunsaturated (PUFA) fatty acids, amounting to 35.3 g/100g of the total FA of SFA, and 5.6 g/100g of the total FA of PUFA. Also, the milk of the Busha was shown to have a higher amount of n – 6 PUFA (2.9 g/100g of total FA), while the amount of n-3 PUFA was similar in both breeds, with slightly higher value observed in the Busha cows.

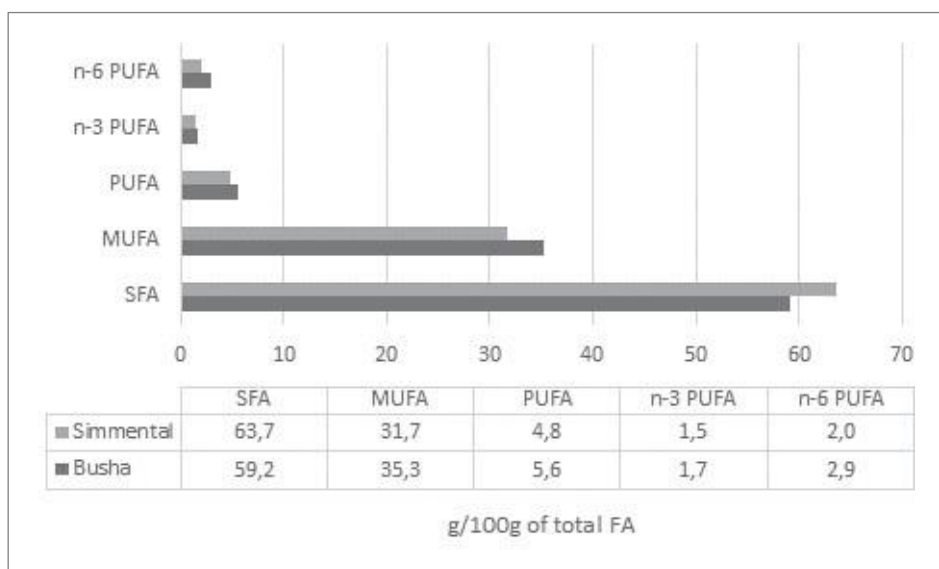


Fig. 1. The total amount of saturated (SFA), monounsaturated (MUFA), polyunsaturated (PUFA), n-3 PUFA and n-6 PUFA in relation to the animal's breed (g/100g of total FA)

## Discussion

One of the most promising benefits of polyunsaturated fatty acids (PUFA) is their potential anti-cancer effects. Studies have shown that conjugated linoleic acid (CLA), which is produced from linoleic acid, may have anticarcinogenic effects, particularly in mammary, stomach, skin, and prostate cancers ([PARODI, 1999](#)). In addition to its potential anti-cancer properties, linoleic acid may also contribute to heart health by exhibiting antiatherogenic properties. It can help lower cholesterol levels, reduce inflammation, and improve blood vessel function, all of which are key factors in maintaining a healthy heart. Linoleic acid has also been found to have antidiabetic effects, as it can improve insulin sensitivity in the body. This means that it can help regulate blood sugar levels, which is particularly beneficial for individuals who are at risk of developing type 2 diabetes. Furthermore, linoleic acid can influence the immune system by exhibiting immunomodulatory effects. It plays a key role in the production of certain immune cells, and can help regulate the body's response to infection and inflammation. To increase the concentration of CLA in milk, feeding strategies that increase linoleic acid (C18:2) and linolenic acid (C18:3) in animal diets can be implemented. These fatty acids serve as the main precursors of CLA, and increasing their intake in animal diets can lead to higher levels of CLA in the milk that is produced ([CHINNADURAI and TYAGI, 2011](#)). According to a study conducted by [DACHEV et al. \(2021\)](#), CLA has exhibited remarkable anti-carcinogenic activity. It has been found that the different isomers of CLA show anti-tumor effects by influencing the proliferation and apoptosis of tumor cells. Additionally, CLA has been associated with reducing mutagenic processes, which may help safeguard against genetic damage that could potentially lead to cancer. Moreover, CLA acts as an antioxidant, helping to counteract oxidative stress and protect cells from damage. This is particularly significant as antioxidants play a crucial role in preventing cancer development. In a study conducted by [TANAKA et al. \(2011\)](#), the effect of CLA in preventing cancer development was emphasized. The high amount of linoleic acid

in Busha milk (2.44 g/100g of total FA), as well as the high amount of conjugated linoleic acid in the milk of Simmental cows (1.25 g/100g of total FA), indicates the high nutritional and potentially anti-cancer properties of the milk of both breeds.

$\alpha$ -Linolenic acid (ALA) is a type of n-3 polyunsaturated fatty acid (n-3 PUFA) found in various seeds, oils and fats (also in milk fat). ALA has recently become a focus of research due to its potential role in preventing cancer. According to [YAN et al. \(2024\)](#), ALA is effective in inhibiting cancer cell growth by slowing down the process of cell division, thereby helping to control tumor growth. In addition, ALA has been found to promote apoptosis, which is the programmed death of cells, and this process helps to eliminate abnormal or damaged cells, preventing their uncontrolled growth. Moreover, ALA plays a crucial role in suppressing tumor metastasis, which is the spread of cancer cells to other parts of the body, as well as angiogenesis, which is the formation of new blood vessels that nourish tumors. By interfering with these processes, ALA helps to limit cancer progression. Another benefit of ALA is that it acts as an antioxidant, which helps to protect cells from oxidative damage, and reduce oxidative stress. This is particularly important in the prevention of cancer development, as oxidative stress is known to contribute to the development of cancer. In summary, ALA has been found to have a range of potentially anticancer effects, including slowing down cell division, promoting apoptosis, suppressing tumor metastasis and angiogenesis, and acting as an antioxidant ([SELVARAJ, 2017](#); [YAN et al., 2024](#)). Our results revealed that both breeds produce milk with a similar amount of ALA. The results showed that the concentration of ALA in the milk of Simmental and Busha cows was 1.18 and 1.13 g/100g of total fatty acids, respectively. This indicates that both breeds of cows have similar potential for providing health benefits through the consumption of their milk.

Arachidonic acid (AA) is a polyunsaturated omega-6 fatty acid. AA is present in the phospholipids of cell membranes, particularly in phosphatidylethanolamine, phosphatidylcholine,

and phosphatidylinositides. It is widely distributed in the body, with high concentrations found in the brain, muscles, and liver. AA is a crucial player in cellular signalling as it acts as a lipid second messenger, regulating signalling enzymes, such as phospholipase C (PLC)- $\gamma$ , PLC- $\delta$ , and protein kinase C (PKC)- $\alpha$ , - $\beta$ , and - $\gamma$  isoforms. AA is also known to act as a vasodilator, which helps to increase blood flow in the body. In terms of dietary sources, AA is naturally present in various foods, including meat, eggs, and dairy products. Additionally, the body can synthesize AA from linoleic acid, another essential fatty acid, through enzymatic processes. One of the essential roles of AA is its involvement in the normal inflammatory process. During injury or irritation, AA is activated and plays a vital role in initiating and resolving the inflammatory response. AA is converted into various bioactive lipid mediators, including prostaglandins, leukotrienes and thromboxane, which help to mediate inflammation, and contribute to the healing process. In conclusion, AA is a crucial polyunsaturated fatty acid that plays a vital role in cellular signalling, inflammation, and blood flow regulation. It can be obtained from dietary sources or synthesized in the body, and is essential for maintaining optimal health. [SALEM and VAN DAEL \(2020\)](#) stated that the lipid composition of human milk is unique, and provides long-chain polyunsaturated fatty acids (LC-PUFAs), particularly arachidonic acid (AA) and docosahexaenoic acid (DHA) in a specific manner. Although AA is present in human milk at lower concentrations compared to other fatty acids, it is the most predominant long-chain polyunsaturated fatty acid. AA plays an important role in physiological development and its related functions during early life nutrition, making it a crucial nutrient during infancy and childhood. Therefore, it is important to pay appropriate attention to its nutritional status and presence in the infant diet. If breastfeeding is not feasible and formula feeding is used, experts recommend adding both AA and DHA at the levels found in human milk. Furthermore, AA is a significant topic of research in the context of cancer. Recent studies have focused on its impact on breast cancer ([LI et al., 2022](#)), and it has been discovered that high

levels of AA metabolism can enhance the prognosis for patients with breast cancer. This is intriguing because earlier studies suggested that AA and its metabolites may contribute to the development of cancer. Researchers speculate that the positive effects of high AA metabolism could be due to immune-related pathways that are more active in patients with high AA metabolism. A new AA metabolic prognostic signature for breast cancer prognosis has even been created, which includes genes such as SPINK8, KLRB1, APOD, and PIGR. The COX-2 pathway is another area of interest in cancer studies. This pathway has been linked to reduced survival rates in various types of cancer, including colon cancer, colorectal cancer, breast cancer, gliomas, prostate cancer, oesophageal carcinoma, pancreatic cancer, and lung carcinoma ([BORIN et al., 2017](#)). The effects on cancer cells of AA exposure vary depending on the type of cancer. For example, in some types of cancer, such as hepatic and lung cell carcinomas, AA exposure has been shown to increase nSMase activity, which could be useful for cancer treatment. However, in breast cancer cells (MCF-7), AA exposure has not been shown to significantly affect nSMase activity ([TALLIMA and EI RIDI, 2023](#)). In this study, the milk of Busha cattle was found to contain a high amount (0.32 g/100g of total FA) of AA. This finding underscores the potential health benefits of consuming this type of milk, especially for infants and in the prevention of cancer. Therefore, it is recommended to consider the inclusion of Busha cattle milk in the diets of individuals seeking to improve their overall health and well-being.

Eicosapentaenoic acid (EPA) is a type of omega-3 polyunsaturated fatty acid. Studies have shown that EPA can effectively fight cancer cells ([OGO et al., 2018](#)). When combined with certain anticancer drugs, even low concentrations of EPA can have a synergistic effect, suppressing the growth of cancer cells. This could be particularly beneficial for elderly or high-risk patients, as it can reduce the side effects of treatment. EPA also affects the activity of cytokines, which play a role in inflammation. By inhibiting the activation of NF- $\kappa$ B and the production of IL-6 in oesophageal cancer cells, EPA may improve the outcome of cancer

surgery ([ANDO et al., 2019](#)). Additionally, EPA suppresses angiogenesis, which is the formation of new blood vessels that cancer cells need to grow. By reducing the secretion of IL-6 and VEGF from colon cancer-associated fibroblasts, EPA can help prevent the growth of cancer cells. In summary, EPA is a promising candidate for cancer therapy due to its many beneficial effects. Furthermore, EPA is a bioactive omega-3 fatty acid that plays a crucial role in human physiologic functions. EPA acts as a precursor to metabolites such as prostaglandin-3, thromboxane-3, and leukotriene-5, which have been associated with the prevention of platelet aggregation, the regulation of blood pressure, and the reduction of inflammation. Recent studies suggest that EPA intake may reduce the risk of cardio-vascular diseases, alleviate depressive symptoms, and potentially benefit conditions such as menopausal symptoms and rheumatoid arthritis. The primary dietary sources of EPA are oily fish, such as cod liver, herring, mackerel, salmon, menhaden, and sardine, and supplements derived from fish oil or algae oil. EPA is also present in human breast milk, and is crucial for normal retinal and brain development during fetal growth and the first two years of life. This study found that Busha cows' milk contains higher levels of EPA compared to Simmental cows' milk, suggesting that Busha milk may be a highly recommended food source due to its rich EPA content.

Docosahexaenoic acid (DHA), an omega-3 polyunsaturated fatty acid, is found in varying amounts in human breast milk and plays essential roles in normal retinal and brain development during fetal growth and the first two years of life. DHA also exhibits potential in mitigating colorectal cancer. Current studies have demonstrated that administering DHA alongside standard chemotherapy regimens can lead to a synergistic anticancer effect against colorectal cancer cells ([JIN et al., 2021](#)). A recent investigation evaluated the effect of DHA in combination with isoliquiritigenin (ISL) on CRC cells. The results illustrated that the combined treatment induced apoptosis, confirmed by Annexin V/PI staining and apoptosis-associated protein expression. This effect was achieved by inducing apoptosis

caspase-dependently, augmenting reactive oxygen species (ROS) generation, increasing Fas ligand mRNA expression, and promoting cytochrome c release, with the involvement of ERK and JNK phosphorylation. Furthermore, DHA has been shown to induce oxidative stress and oxidative DNA adduct formation in certain human cancer cells, by depleting intracellular glutathione (GSH) and decreasing mitochondrial function in cancer cells. Additionally, DHA has been observed to potentiate the anticancer effect of the menadione/ascorbate redox couple by increasing mitochondrial superoxide and accelerating ATP depletion ([IVANOVA et al., 2023](#)). In this research, a significantly ( $P < 0.05$ ) higher amount of DHA was found in the milk of the Busha breed (0.05 g/100g of total FA) than from Simmental cows, indicating the high nutritional and health value of this milk. Furthermore, higher amounts of total MUFA, PUFA, n-3 PUFA, and n-6 PUFA were determined in the milk of Busha cows in comparison to the Simmental breed. A study conducted by MANSON (2008) revealed that the Swedish cattle population had a higher level of SFA compared to the two breeds analyzed in this research. The SFA content in the Swedish cattle population accounted for approximately 70% of the total fatty acids, which was a noteworthy difference. Furthermore, MANSON (2008) reported that 25% of the MUFA and 2.3% of PUFA were present in the total fatty acids. In contrast, the present study found that both Busha and Simmental breeds had higher levels of MUFA, with 35.5% in Busha and 31.7% in Simmental. Similarly, the levels of PUFA were also higher in both breeds, with 5.6% in Busha and 4.8% in Simmental. These results provide valuable insights into the composition of the fatty acids in different cattle populations, and the possible nutritional and health potential of their milk and dairy products.

### Conclusions

The present research determined that the milk of the Busha breed, an indigenous cattle breed, contained higher quantities of essential fatty acids, such as linoleic, arachidonic, eicosapentaenoic, and

docosaehaenoic acids, compared to Simmental cows. Furthermore, Busha milk contains higher amounts of total monounsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA), as well as n-3 PUFA and n-6 PUFA. These higher concentrations of the analyzed fatty acids indicate that Busha milk has high nutritional value and possible anticancer potential. Therefore, it is crucial to integrate this finding into development strategies for animal production, which would enable the sustainable breeding and production of this highly valuable breed, and its milk and dairy products. This step would also contribute to the conservation of the endangered Busha breed.

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### Declaration of competing interests

No potential conflict of interest was reported by the authors

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**GANTNER, V., K. KUTEROVAC, M. SAMARDŽIJA, Z. STEINER, R. GANTNER, K. POTOČNIK:**  
**Potencijalni antikancerogeni učinak mlijeka autohtone hrvatske pasmine krava buša. Vet. arhiv 95, 173-186, 2025.**

#### **SAŽETAK**

U istraživanju su analizirani profili masnih kiselina u mlijeku dviju pasmina goveda, simentalske i buše, s ciljem utvrđivanja njihova potencijalnog učinka protiv raka. Uzorci mlijeka uzimani su tijekom ljetne sezone dok su životinje bile na ispaši. Istraživanjem su utvrđene količine linoleinske kiseline, konjugirane linoleinske kiseline,  $\alpha$ -linoleinske kiseline, arahidonske kiseline, eikozapentaenske kiseline i dokozaheksaenske kiseline, kao i ukupne količine zasićenih masnih kiselina (SFA), mononezasićenih masnih kiselina (MUFA), polinezasićene masne kiseline PUFA, te n-3 PUFA i n-6 PUFA. Rezultati istraživanja pokazali su da mlijeko dobiveno od krava autohtone pasmine buša ima, u usporedbi s mlijekom dobivenim od krava simentalske pasmine, veće količine esencijalnih masnih kiselina, uključujući linolnu, arahidonsku, eikozapentaensku i dokozaheksaensku kiselinu. Istraživanjem je također utvrđeno da mlijeko buše sadržava veće količine ukupnih masnih kiselina MUFA, PUFA, n-3 PUFA i n-6 PUFA. Prisutnost ovih masnih kiselina u većim koncentracijama upućuje na to da je mlijeko buše nutritivno visokovrijedno i da može imati antikancerogeni potencijal. Stoga je pri formuliranju strategija za očuvanje pasmine ključno uzeti u obzir ovaj nalaz. To uključuje omogućivanje održivog uzgoja kako bi se osigurala dugovječnost ove pasmine i proizvodnja visokokvalitetnih mliječnih proizvoda.

**Ključne riječi:** mlijeko; masne kiseline; antikancerogeni potencijal; simentalac; buša

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