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Determination of the fatty acid composition and fatty acids trans-isomers in the horse, stall horse, mutton, beef and pork meat

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ABSTRACT

In this study, we have focussed on the fatty acid composition of the meat of various animals raised in the Republic of Kazakhstan. We have analyzed pasture horse meat, stall horse meat, lamb, beef, and pork meat. Samples from four carcass muscles (back, hip, rib, and neck) were tested. Comparative analysis of the content of trans isomers of fatty acids (TFA) was performed. The analysis of the obtained samples showed that the TFA content is significantly (p <0.05) different in different parts of the carcasses of all animals. Their highest content was observed in the mutton sample, which reached 79.56-82.04%. The beef was next after mutton (6.20-9.64%). Less than in mutton and beef, but more than in pork and TFAs were contained in stall horse meat (2.75-5.52%). Of the two types of horse meat, there was less TFA in horse meat of pasture content (1.85-3.46%). Compared to all studied samples, the lowest level of trans fatty acids was in pork (0.91-1.39%). In horse meat of both types, TFAs were present in trans-9-C16: 1. More types of TFA were found in the meat of other animals: in mutton (trans-9-C14: 1; trans-9-C16: 1; trans-9-C18: 1; trans-11 C18: 1; trans-9-trans- 12-C18: 2), in beef (trans-9-C16: 1; trans-9-C18: 1; trans-11-C18: 1; trans-9-trans-12-C18: 2), in pork (trans-9-C16: 1). In addition to TFA, an analysis was made of the ratio of omega-6 and omega-3 (ω-6: ω-3). Considering that the lower the ratio of ω-6: ω-3 in fat, the healthier it is for the human body, the most optimal among the studied samples in terms of the ratio of ω-6: ω-3 fatty acids was mutton (1.83-2.35) and horse meat of stall keeping (1.76-6.53). The most unfavourable ratios were in the pork samples (17.46-35.69). The ratio ω-6: ω-3 in other animals was within the following limits: beef (5.35-9.13), horse meat of pasture content (7.08-10.50).

Keywords: meat, lipids, trans fatty acids, analysis, chromatography

INTRODUCTION

In the food industry, along with valuable products worldwide, there are types of food products that harm the human body. Foods with high TFAs are among the most unhealthy foods. TFA consumption is associated with an increased risk of developing coronary heart disease (CHD) - the main harm to the human body **[1]**, **[2]**, **[3]**.
In recent decades, partially hydrogenated vegetable oils have become food's main source of TFA **[4]**. The composition and content of TFAs formed during the hydrogenation of vegetable oils are influenced not only by the initial fatty acid composition of the oils but also by the process conditions: catalyst, temperature, and duration. The main part of TFA is represented by isomers of octadecenoic acid (from trans-4 to trans-16-C18: 1) with a predominance of isomers of trans-9-C18: 1 (elaidic acid); trans-10-C18: 1, as well as trans-11-C18: 1 (vaccenic acid) **[5]**, **[6]**. They are naturally produced by biohydrogenation in the stomach of ruminants. TFAs formed in the stomach of ruminants are contained in all the fats of these animals **[7]**.

Biohydrogenation of unsaturated fatty acids in the rumen of ruminants aims to protect bacteria from their toxicity. As a result of this process, unsaturated fatty acids are converted into saturated ones through isomerization into intermediate compounds of trans isomers of fatty acids with subsequent hydrogenation of double bonds. The number of microorganisms capable of hydrolyzing lipids is small. Indeed, lipolytic bacteria have been isolated from the rumen, including various strains of *Anaerovibrio lipolytica* and other bacteria belonging to the genera

Butyrivibrio, *Clostridium*, and *Propionibacterium*. The main path of biohydrogenation is the initial isomerization of unsaturated C18: 2n-6 (linoleic) fatty acid into conjugated cis-9-trans-11-C18: 2 (rumenic), followed by hydrogenation of the resulting isomer to trans-11-C18: 1 (vaccene) **[8]**. The final product of biohydrogenation is a trans-11-C18: 1 fatty acid additionally hydrogenated to C18: 0 (stearic) **[8]**.

Along with an increased risk of CHD, high TFA consumption is associated with sudden death, diabetes mellitus, and increased markers for systemic inflammation **[9]**, **[10]**. The earliest and most significant effect on increasing the level of "bad" cholesterol - low-density lipoprotein (LDL) and reducing the level of "good" cholesterol - high-density lipoprotein (HDL) **[11]**. HDL transports cholesterol to the liver for further removal from the human body. In contrast, LDL delivers cholesterol to the arteries, which in excess accumulates on the walls of the arteries, leading to atherosclerosis, heart attack, or stroke **[7]**.

TFAs by changing the structure of phospholipids and cholesterol in the membrane lead to a decrease in the activity of the model receptor. As fatty acids in the trans configuration take on a more linear form, they promote stronger intermolecular interactions, leading to a higher melting point. This affects protein metabolism and the interaction of phospholipids containing TFAs with the membrane of cholesterol **[11]**.

There is a lot of evidence that fatty acids of both partially hydrogenated vegetable oils and fats of ruminants are effectively absorbed into chylomicrons, except for fatty acids having double bonds in positions from 2 to 7. The residues of chylomicron triacylglycerol reaching the liver are absorbed and repackaged and then enter into the blood as LDL. Fractions of triacylglycerol transported to peripheral tissues are hydrolyzed by lipase of the enzyme lipoprotein and enter the cells. As a result, an increased LDL cholesterol level leads to the development of coronary heart disease. According to the American Nutrition Association the Heart Foundation, the TFA content in ground beef is 1 g, and in animal fat from 0 to 5 g. The content of TFA in food products may vary from country to country. For example, foods in the U.S. and some Middle Eastern and South Asian countries may contain up to 7% TFA **[12]**.

A comparison of TFAs of hydrogenated vegetable and animal fats on low-density lipoproteins (LDL) and high-density lipoproteins (HDL) plasma showed the ratio of LDL: HDL with the consumption of animal fats also increased. According to this comparison, the authors believe there is no threshold at which TFAs do not worsen the lipoprotein profile. Analysis of various clinical studies showed that high consumption of TFA in ruminants led to undesirable changes in plasma lipoproteins **[13]**.

The researchers compared the amount of TFA in raw and cooked meat, adipose tissue, and offal from New Zealand mutton and beef. As a result of the comparison, the following data were obtained: the TFA content of the total amount of fatty acids was 5.61% in beef adipose tissue, 3.09% in beef meat and offal, 9.60% in mutton adipose tissue, 6.10% in mutton meat and offal. In the adipose tissue of beef and mutton, the TFA content was slightly higher than in meat and offal **[14]**.

According to a study in France, the content of trans C18: 1 acid in the total chromatogram of the methyl ester of fatty acid in a sample of French mutton was 4.8% of the total amount of fatty acids. This chromatogram did not detect the trans-C16: 1 acid content **[15]**.

In a study of the fatty acids of lamb meat sold in northern Spain, the content of trans isomers of fatty acids in its meat was determined. The total content of TFA in different periods of the year reached high rates. The average minimum and maximum average values correspond to: 1.97% and 16.5% **[16]**.

The content of TFA in mutton and its fat is higher than in other products: bovine muscle 3.6%, bovine fat 4.9%, mutton muscle 8.2%, mutton fat 9.7%, pork muscle 0.5%, pork fat 0.7%, eggs 1.3%, chicken light meat 0.8%, chicken dark meat 0.8% **[17]**.

Depending on the diet, the total composition of the trans isomers of two and three double bonds of fatty acids in ruminants varies from 0.8-4.5 g per 100 g of muscle fat **[18]**.

As a result of evaluating the fatty acid content of horse meat available in retail sale in Spain, it was found that the horse steak *Longissimus thoracis et lumborum* from butcher shops in different regions and at different times of the year contained a low level of TFA among fatty acids (0.180-0.212%). In addition, polyunsaturated fatty acids n – 3 useful for humans were found in an amount of 1.52% in muscle fat and 27.9% in the fat of back meat **[19]**.

So far, many countries have adopted measures to regulate food products containing TFA. In our country, following the technical regulations of the countries of the Customs Union for fat and oil products, since 2018, the permissible level of TFA is 2% of the total fat content in the product **[20]**. The requirement to enter the TFA content during product labelling applies only to fat and oil products. Until now, no other mechanisms have been envisaged to reduce the TFA content in other food products. Therefore, controlling and reducing trans fats in meat products are very relevant for Kazakhstan.

In this study, the fatty acid composition of the meat of various types of animals (horse meat, lamb, beef, and pork) was determined, which made it possible to carry out a comparative analysis of the content of trans isomers of fatty acids in the meat of these animals, which is used in a wide range of products.

Scientific Hypothesis

The quantitative content of fatty acid trans-isomers varies in different animal species and also depends on the animal keeping (stall or pasture).

MATERIAL AND METHODOLOGY

Samples

For the analysis, pasture horse meat (Kazakh horse breed), stall horse meat (Kazakh horse breed), mutton (North Kazakh merino breed), beef (Auliekol breed), and pork (White breed) were purchased from meat markets and supermarkets in Almaty, Kazakhstan. Samples were obtained from each animal's four carcass muscles (back muscles, thigh muscles, rib muscles, neck muscles), an average of 500 grams of sample. The samples obtained were stored at a temperature of -18 °C. Horse meat for pasture and stall keeping was supplied from farms engaged in horse breeding and inappropriate feeding.

Chemicals

Hexane (Labor Farma Limited Liability Partnership, Kazakhstan).
Ethyl alcohol (90%, Pharmacy 2010 Limited Liability Partnership, Kazakhstan).
Potassium hydroxide (Labor Farma Limited Liability Partnership, Kazakhstan).
Methanol (Labor Farma Limited Liability Partnership, Kazakhstan).

Animals and Biological Material

The biological materials used in this work were horse meat, beef, and pork purchased from supermarkets.

Instruments

Shaker (S-3L, producer (ELMI) Limited trade development, Latvia).
Gas chromatograph (Agilent 6890N, producer (Agilent Technologies) Incorporated, United States of America).
Gas chromatograph Agilent 6890N (Agilent Technologies, USA) equipped with a flame ionization detector (Agilent Technologies, USA). The column was CPTM-Sil 88 (Chrompack, The Netherlands) 100 m in length, 0.25 mm i.d., 0.2 μm in film thickness. The temperature of the injector and detector was set at 250 ℃. The carrier gas is hydrogen at a flow rate of 1 ml/min.
Water bath (TW-2.03, producer (ELMI) Limited trade development, Latvia).
Evaporative porcelain cup (123/50-250, producer (Altey Group) Limited liability company, Russia).
Measuring flask (500 ml, producer (Altey Group) Limited liability company, Russia).
Desktop High-speed heavy-duty centrifuge with cooling (AWTech H2100R, producer (Analytics and High Technology) Autonomous non-profit organization, Russia).

Description of the Experiment

Sample preparation:

Lipid extraction was carried out using a solution of hexane and ethyl alcohol (2: 1). Homogenization of 50 grams of meat from each sample is carried out by grinding in a porcelain cup with a porcelain mortar. After placing a homogeneous homogenized sample of meat in a 500 ml flask, the following solvents were added: 200 ml of hexane and 100 ml of ethanol to precipitate proteins and extract fatty acids and TFA. After adding the solvents, the flask with the sample was placed on a shaker for thorough mixing at 180 rpm for 3 to 4 hours, during which extraction occurred. The resulting supernatant was evaporated in a water bath at 70 ±2 ºC. After evaporation, the sample is poured into an Eppendorf and placed in the freezer to check the sample.

To prepare methyl esters of fatty acids (FAME), a mixture of 1 g of potassium hydroxide (KOH) and 20 ml of methanol (CH_3OH) was added to 1 μl of the sample in a ratio of 1:20. Added 2 ml of hexane. The resulting aliquot was mixed on a mixer and centrifuged at 10,000 rpm for 5 min, t = 38 °C.

Number of samples analyzed: Meat from four different places of the carcasses of five animals were used in the study of samples

Number of repeated analyses: Each study was carried out five times, with the number of samples being twenty, which amounted to hundred repeated analyses.

Number of experiment replication: The study was repeated five times, with the experimental data processed using mathematical statistics methods.

Laboratory Methods

Extraction of lipids

The extraction of lipids in meat was carried out by the Folch method. This method allows you to isolate 90-95% of all cellular lipids. Lipid extraction was carried out using a solution of hexane and ethyl alcohol (2:1). Homogenization of 50 grams of meat from each sample is carried out by grinding in a porcelain cup with a porcelain mortar. After placing a homogeneous homogenized sample of meat in a 500 ml flask, the following solvents were added: 200 ml of hexane and 100 ml of ethanol to precipitate proteins and extract fatty acids and TFA. After adding the solvents, the flask with the sample was placed on a shaker for thorough mixing at 180 rpm for 3 to 4 hours, during which extraction occurs. The resulting supernatant was evaporated in a water bath at 70 ±2 °C. After evaporation, the sample is poured into an Eppendorf and placed in the freezer to check the sample.

Preparation of methyl esters of fatty acids (FAME)

To prepare methyl esters of fatty acids (FAME), a mixture of 1 g of potassium hydroxide (KOH) and 20 ml of methanol (CH_3OH) was added to 1 µl of the sample in a ratio of 1:20. Added 2 ml of hexane. The resulting aliquot was mixed on a mixer and centrifuged at 10,000 rpm for 5 min, t = 38 °C.

Analysis

The analysis of methyl esters of fatty acids were analysed on an Agilent 6890N gas chromatograph (Agilent Technologies) with a plasma ionization detector on a capillary column (length 100 m, inner diameter 0.25 mm, layer thickness 0.2 µm). Detector temperature was set to 250 °C, division ratio, division ratio 50:1, injection volume 1 µl, head 2 ml/min, oven temperature 120 °C, 1 min, 10 °C/min to 175 °C, 10 min, 5 °C/min to 210 °C, 5 min 5 °C/min to 230 °C, 5 min **[21]**.

Quantitative analysis was carried out on the assumption that the total area of the peaks of all components of the analyzed sample is 100% (GOST 31754-2012). The mass fraction of the methyl ester of each fatty acid X_i, %, was calculated by the formula (1):

$$X_i = \frac{A_i}{\sum A_i} \times 100 \quad (1)$$

Where: A_i – is the peak area of the methyl ester of a single fatty acid; $\sum A_i$ – the sum of the areas of all peaks of fatty acid methyl esters.

The final measurement result was the arithmetic mean of two parallel measurements of the fatty acid mass fraction, determined from the automatic calculation of chromatograms obtained under repeatability conditions when the acceptability condition is met. The boundary of the interval in which the relative error was found with a confidence level of p = 0.95 followed GOST 31754-2012 **[22]**.

Statistical Analysis

The Statistica 12.0 software (StatSoft Inc., Tulsa, OK, USA) was used for processing experimental data using mathematical statistics methods. The accuracy of obtained experimental data was determined using the Student's t-test with a confidence coefficient ≤0.05 with many parallel definitions of a least 5 (confidence probability p = 0.95). Linear programming problems were solved using the MS Excel table processor's 'Search for a solution setting (Excel Solver).

RESULTS AND DISCUSSION

The results obtained showed a significant difference in the fatty acid composition of the muscle tissues of various animal species: horses, sheep, cattle, and pigs. Due to the high content of SFAs, the fattest among the studied samples was mutton (79.56-82.04%). The fat content in pork was less than in mutton but more than in beef and horse meat, in which saturated (50.42-64.39%) and polyunsaturated (22.93-42.99%) fatty acids occupied a significant part.

In horsemeat of pasture content, compared to horsemeat in stalls, less saturated (37.01-37.53% < 40.32-59.45%), polyunsaturated (11.43-19.76% < 15.85-26.97%), and more monounsaturated (43.23-51.04% > 17.05-40.36%) fatty acids.

Different TFA content was observed in different parts of all animals' carcasses. Their highest content was observed in the mutton sample, which was 7.98-10.92%. The beef was next after mutton (6.20-9.64%). Less than in mutton and beef, but more than in pork and TFAs were contained in stall horse meat (2.75-5.52%). Of the two types of horse meat, there was less TFA in horse meat of pasture content (1.85-3.46%). Compared to all studied samples, the lowest level of trans isomers of fatty acids was in pork (0.91-1.39%).

In horse meat of both types, TFAs were present in trans-9-C16: 1. More types of TFA were found in the meat of other animals: mutton (trans-9-C14: 1; trans-9-C16: 1; trans-9-C18: 1; trans-11 C18: 1; trans-9-trans-12 -C18: 2), beef (trans-9-C16: 1; trans-9 C18: 1; trans-11-C18: 1; trans-9-trans-12-C18: 2), pork (trans-9 C16: 1).

The optimal ratio of omega-6 and omega-3 (ω-6: ω-3) is in the range of 5-10, and for medical nutrition, in the range of 1-5 **[23]**, **[24]**. The most optimal among the studied samples in terms of the ratio ω-6: ω-3 fatty acids was mutton (1.83-2.35) and horse meat of stall content (1.76-6.53). The most unfavourable ratios were in the pork samples (17.46-35.69). The ratio ω-6: ω-3 in other animals was within the following limits: beef (5.35-9.13), horse meat of pasture content (7.08-10.50).

Table 1 Fatty acid content in the muscle lipid fraction of grass-fed horse meat: % of total fatty acids, g/100 g of meat.

Fatty acids	Loin	Round	Rib	Neck
	% of total fatty acids			
∑ SFA	37.43 ±0.62^{a}	37.53 ±0.40^{a}	37.34 ±0.64^{a}	37.01 ±0.41^{a}
∑ UFA	62.57 ±0.67^{a}	62.47 ±0.82^{a}	62.66 ±1.10^{a}	62.99 ±0.58^{a}
∑ MUFA	48.12 ±0.54^{b}	51.04 ±0.93^{b}	44.05 ±0.82^{a}	43.23 ±0.67^{a}
∑ PUFA	14.44 ±0.23^{b}	11.43 ±0.12^{a}	18.61 ±0.32^{c}	19.76 ±0.41^{c}
PUFA/SFA	0.38	0.30	0.50	0.53
UFA/SFA	1.67	1.66	1.68	1.70
∑ TFA	1.85 ±0.02^{a}	3.46 ±0.06^{c}	2.13 ±0.04^{b}	2.64 ±0.05^{b}
∑ ω-3 PUFA	1.79 ±0.02^{c}	1.01 ±0.01^{a}	1.68 ±0.02^{b}	1.72 ±0.03^{c}
∑ ω-6 PUFA	12.66 ±0.25^{b}	10.42 ±0.17^{a}	16.93 ±0.26^{c}	18.04 ±0.20^{d}
ω-6/ω-3	7.08	10.29	10.09	10.50

Note: $^{a-d}$ means within the same row with different uppercase letters differing significantly among different meat samples (p <0.05). SFA – Saturated Fatty Acids; UFA – Unsaturated Fatty Acids; MUFA – Monounsaturated Fatty Acids; PUFA – Polyunsaturated Fatty Acids. All values are expressed as the mean + SD (standard deviation).

Table 2 The fatty acid content in the muscle lipid fraction of grain-fed horse meat: % of total fatty acids (TFA), g/100 g of meat.

Fatty acids	Loin	Round	Rib	Neck
	% of total fatty acids			
∑ SFA	40.32 ±0.81^{a}	49.80 ±0.55^{b}	55.98 ±1.08^{c}	59.45 ±0.63^{d}
∑ UFA	59.68 ±0.48^{d}	50.20 ±1.03^{c}	44.02 ±0.67^{b}	40.55 ±0.87^{a}
∑ MUFA	40.36 ±0.44^{c}	23.54 ±0.42^{b}	17.05 ±0.16^{a}	24.70 ±0.38^{b}
∑ PUFA	19.31 ±0.31^{b}	26.65 ±0.39^{c}	26.97 ±0.45^{c}	15.85 ±0.36^{a}
PUFA/SFA	0.48	0.54	0.48	0.27
UFA/SFA	1.48	1.01	0.79	0.68
∑ TFA	2.75 ±0.04^{a}	5.54 ±0.08^{c}	3.56 ±0.04^{b}	5.52 ±0.09^{c}
∑ ω-3 PUFA	5.99 ±0.15^{b}	9.65 ±0.13^{d}	7.78 ±0.05^{c}	2.11 ±0.04^{a}
∑ ω-6 PUFA	13.33 ±0.13^{a}	17.01 ±0.26^{b}	18.37 ±0.24^{c}	13.74 ±0.23^{a}
ω-6/ω-3	2.23	1.76	2.36	6.53

Note: $^{a-d}$ means within the same row with different uppercase letters differing significantly among different samples of meat (p <0.05). SFA – Saturated Fatty Acids; UFA – Unsaturated Fatty Acids; MUFA – Monounsaturated Fatty Acids; PUFA – Polyunsaturated Fatty Acids. All values are expressed as the mean + SD (standard deviation).

Table 3 Fatty acid content in the muscle lipid fraction of mutton meat: % of total fatty acids (TFA), g/100 g of meat.

Fatty acids	Loin	Round	Rib	Neck
	% of total fatty acids			
∑ SFA	82.04 ±0.94[a]	80.36 ±0.99[a]	81.72 ±1.02[a]	79.56 ±1.03[a]
∑ UFA	17.96 ±0.33[a]	19.64 ±0.24[b]	18.28 ±0.35[a]	20.44 ±0.41[b]
∑ MUFA	14.11 ±0.19[a]	13.91 ±0.21[a]	14.71 ±0.22[a]	14.08 ±0.20[a]
∑ PUFA	3.85 ±0.07[b]	5.72 ±0.09[c]	3.57 ±0.06[a]	6.36 ±0.09[d]
PUFA/SFA	0.05	0.07	0.04	0.08
UFA/SFA	0.22	0.24	0.22	0.26
∑ TFA	9.95 ±0.14[c]	7.98 ±0.13[a]	10.92 ±0.14[d]	8.89 ±0.09[b]
∑ ω-3 PUFA	1.36 ±0.03[b]	1.71 ±0.03[c]	1.26 ±0.01[a]	2.03 ±0.02[d]
∑ ω-6 PUFA	2.49 ±0.04[a]	4.01 ±0.06[b]	2.31 ±0.02[a]	4.33 ±0.08[c]
ω-6/ω-3	1.83	2.35	1.84	2.13

Note: [a-d] means within the same row with different uppercase letters differing significantly among different meat samples (p <0.05). SFA – Saturated Fatty Acids; UFA – Unsaturated Fatty Acids; MUFA – Monounsaturated Fatty Acids; PUFA – Polyunsaturated Fatty Acids. All values are expressed as the mean + SD (standard deviation).

Table 4 Fatty acid content in the muscle lipid fraction of beef meat: % of total fatty acids (TFA), g/100 g of meat.

Fatty acids	Loin	Round	Rib	Neck
	% of total fatty acids			
∑ SFA	72.95 ±1.12[b]	76.18 ±1.22[c]	77.67 ±1.21[c]	67.68 ±1.35[a]
∑ UFA	27.05 ±0.35[b]	23.82 ±0.35[a]	22.33 ±0.32[a]	32.32 ±0.57[c]
∑ MUFA	23.85 ±0.38[b]	18.63 ±0.29[a]	19.35 ±0.31[a]	19.04 ±0.27[a]
∑ PUFA	3.19 ±0.04[a]	5.19 ±0.07[b]	2.99 ±0.04[a]	13.29 ±0.20[c]
PUFA/SFA	0.04	0.07	0.04	0.20
UFA/SFA	0.37	0.31	0.29	0.48
∑ TFA	6.72 ±0.13[a]	9.64 ±0.17[c]	6.20 ±0.09[a]	8.64 ±0.16[b]
∑ ω-3 PUFA	ND*	0.51 ±0.01[b]	0.47 ±0.01[a]	ND*
∑ ω-6 PUFA	3.19 ±0.05[b]	4.67 ±0.09[c]	2.52 ±0.03[a]	13.29 ±0.21[d]
ω-6/ω-3	–	9.13	5.35	–

Note: [a-d] means within the same row with different uppercase letters differing significantly among different meat samples (p <0.05). SFA – Saturated Fatty Acids; UFA – Unsaturated Fatty Acids; MUFA – Monounsaturated Fatty Acids; PUFA – Polyunsaturated Fatty Acids. All values are expressed as the mean + SD (standard deviation).

Table 5 Fatty acid content in the muscle lipid fraction of pork meat: % of total fatty acids (TFA), g/100 g of meat.

Fatty acids	Loin	Round	Rib	Neck
	% of total fatty acids			
∑ SFA	52.79 ±0.56[b]	64.39 ±0.58[d]	58.31 ±1.28[c]	50.42 ±0.55[a]
∑ UFA	47.21 ±0.46[c]	35.61 ±0.49[a]	41.69 ±0.24[b]	49.58 ±0.71[d]
∑ MUFA	7.99 ±0.13[b]	12.69 ±0.16[d]	9.95 ±0.14[c]	6.58 ±0.09[a]
∑ PUFA	39.23 ±0.38[c]	22.93 ±0.34[a]	31.74 ±0.30[b]	42.99 ±0.58[d]
PUFA/SFA	0.74	0.36	0.54	0.85
UFA/SFA	0.89	0.55	0.71	0.98
∑ TFA	0.91 ±0.01[b]	1.47 ±0.02[c]	1.39 ±0.02[c]	0.72 ±0.01[a]
∑ ω-3 PUFA	1.91 ±0.003[c]	0.62 ±0.01[a]	1.72 ±0.02[b]	2.10 ±0.03[d]
∑ ω-6 PUFA	37.31 ±0.49[c]	22.30 ±0.42[a]	30.02 ±0.43[b]	40.90 ±0.32[d]
ω-6/ω-3	19.49	35.69	17.46	19.51

Note: [a-d] means within the same row with different uppercase letters differing significantly among different meat samples (p <0.05). SFA – Saturated Fatty Acids; UFA – Unsaturated Fatty Acids; MUFA – Monounsaturated Fatty Acids; PUFA – Polyunsaturated Fatty Acids. All values are expressed as the mean + SD (standard deviation).

The digestive system of non-ruminants like horses is different from ruminants. The difference lies in the low biohydrogenation activity of fatty acids since they are absorbed before they are microbially attacked in the hindgut (cecum and colon) **[25]**, **[26]**. The digestive physiology of horses is characterized by high levels of polyunsaturated fatty acids and low levels of trans fats, which makes horse meat healthier. This is confirmed by one of the studies to assess the composition of horse meat fatty acids carried out by scientists in Spain. According to these studies, the total fatty acid content was 1970 mg/100 g of meat. Of these, NLC – 47.8 34.2%; MUFA – 32.3-35.4% (cis-MUFA – 99.4% and trans-MUFA – 0.180-0.212%); CLA – 0.0768-0.0941%; PUFA – 23.4-26.6%. The main trans-MUFAs were elaidic (9 t-18: 1; 0.0776%) and vaccenic (11 t-18: 1; 0.0273%) acids **[27]**, **[28]**.

Since animals with a single stomach, unlike ruminants, do not have a significant effect on fatty acids during the digestion of food, the fatty acid composition of meat of non-ruminant animals, such as horse meat and pork, may differ mainly due to the composition of fatty acids of the products of their diet **[29]**, **[30]**.

According to the WHO recommendation, the total intake of TFA should not exceed 1% of the total caloric intake, which corresponds to less than 2.2 g per day on a diet of 2000 calories **[31]**, **[32]**. Investigating the problem of the content of trans isomers of fatty acids in meat products, scientists have concluded that it is necessary to control them. The reasons for this are an increase in the share of TFAs during the storage of natural raw materials, heat treatment, and the addition of various enzymes. Also, using various stimulants and metabolic regulators of the growth and development of animals can lead to an increase in TFA **[33]**, **[34]**.

In Kazakhstan, under the technical regulations of the countries of the Customs Union for fat and oil products, since 2018, the permissible level of trans isomers of fatty acids is 2% of the total fat content in the product. The requirement to enter the TFA content during product labelling applies only to fat and oil products. Until now, no other mechanisms have been envisaged to reduce the TFA content in other food products. Therefore, the control and reduction of trans fatty acids in meat products are very relevant for Kazakhstan.

CONCLUSION

Analysis of the obtained samples showed that, in comparison with other animal species, pork had the lowest content of trans fatty acids. A comparison of horse meat of various types of feeding showed that horse meat raised on pasture contains less TFA than horse meat of stall content. Mutton and beef, in comparison with other animals, contained high levels of trans fats, which were presented in several species: mutton (trans-9-C14: 1; trans-9-C16: 1; trans-9-C18: 1; trans-11- C18: 1; trans-9-trans-12 C18: 2), beef (trans-9-C16: 1; trans-9-C18: 1; trans-11-C18: 1; trans-9-trans-12- C18: 2), pork (trans-9-C16: 1). One type of trans fatty acids (trans-9-C16: 1) was found in horse and pork meat. Based on the results, we can conclude that pork is the most favourable for health in terms of TFA content. However, in terms of the ω-6: ω-3 ratio, pork has the largest deviations from the norm compared to other types of meat. The ω-6: ω-3 ratio of beef and mutton is favourable, but at the same time, these samples have the highest TFA content. The TFA values were close to the recommended ones in horse meat samples, and the ratio of ω-6: ω-3 fatty acids was within the optimal range. However, compared with the samples of stall-keeping horse meat, the TFA level is lower in the samples of pasture-containing horse meat. Therefore, the choice of pasture-containing horses can contribute to a more favourable composition of fatty acids in horse meat. Mutton, beef, and pork were less favourable in terms of fatty acid composition. However, mutton, beef, and pork are some of the main and traditional raw meat sources worldwide. Therefore, producing meat products with a low content of trans fatty acids should be carried out by developing and applying technologies, regardless of the type of raw meat used.

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