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Colostrum of current and rare cattle breeds: fatty acid pattern

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Abstract. Colostra in the early stage of lactation was obtained from cows belonging to different dairy breeds, but were kept under the same feeding and housing conditions. Crude fat content and the fatty acid composition of fat in the form of fatty acid methyl esters were determined.

Key words and phrases: fatty acid, colostrum, dairy cow.

There were not significant differences among breeds regarding the fat content of colostrum. The ratio of the saturated fatty acids within the sum of fatty acids was higher in the case of Jersey ($p < 0.05$) than that of Holstein-Friesian, Brown Swiss and Norwegian red. The fat of colostrum of Brown Swiss contained more monounsaturated fatty acids than that of the Jersey and Ayrshire. Holstein-Friesian proved to have higher ratios of polyunsaturated fatty acids within fat than Swedish red. The ratio of n6 fatty acids was higher in samples originated from Holstein-Friesian than that of Swedish red, Jersey, Norwegian red, and Ayrshire while the ratio of the n3 fatty acids did not differ among breeds. In consonance with the above results, the n6/n3 ratio was the highest in the colostrum of Holstein-Friesian cows.

1 Introduction

Fatty acid composition of colostrum can be affected by several factors. As in the case of normal milk, it depends partially on the lipid profile of the forage even in the case of ruminants. Some part of the long-chain n3-polyunsaturated fatty acids (n3-PUFA) originated from fish oil was transferred into the colostrum (*Cattaneo et al.*, 2006). Nevertheless, the organisms are willing to provide a certain level of long-chain PUFA in the colostrum, irrespective of the dietary sources and adipose tissue levels, as these fatty acids are important for the neonate (*Leiber et al.*, 2011). The fatty acid composition of normal milk is influenced by breed (*Soyeurt et al.*, 2006). There is scarce information on the genetic effect regarding the fatty acid profile of colostrum; therefore, the aim of our study was to compare the fatty acid composition of colostrum of different dairy cow breeds kept under the same feeding and housing conditions.

2 Material and methods

2.1 Milk sampling

Colostrum samples originated from the "Kőrös-Maros Biofarm" Ltd. from Gyulavári, Hungary. There are about 500 Holstein-Friesian cows in this plant. Individuals of further breeds were imported (Swedish red, Jersey, Brown Swiss, Norwegian red, Ayshire) and an experiment was conducted in order to compare their production. Experimental animals were loose-housed cows kept in small groups. The bedding of the cow-shed was deep litter and the house was provided with concreted open yard. The number of animals was 13–15 per part of the cow-shed. Feed and water were provided in the open yard. The

level of the water was fixed in the drinking trough and the feed was prepared with mixer-feeder wagon. Milking was obtained twice a day with herringbone milking parlour.

Feed met the requirements of bioproduction. The daily intake was 9 kg corn silage, 4 kg alfalfa silage, 2 kg alfalfa hay, and 9 kg triticale haylage. The amount of the provided concentrate (6–9 kg) depended on the milk production. The individual dry matter consumption was 19.1 kg, the milk production net energy 125 MJ, and the metabolizable protein content 2017 g.

Colostrum samples were taken on the first, third, and fifth days after calving. Three individuals from each breeds were milked and the lactations of the same animals were followed.

2.2 Chemical analysis

Chemical analysis was carried out at the Department of Chemistry-Biochemistry, Faculty of Animal Science, Kaposvár University. Crude fat content determination was carried out according to the MSZ ISO 8262-3 international standard. Fatty acid profile was determined in the form of fatty acid methyl esters. The homogenized sample was weighed into a flask, 8 cm^3 concentrated hydrochloric acid was added, and it was boiled for 60 minutes. After cooling down, 7 cm^3 ethanol and then 15 cm^3 diethylether was added, following a one-minute shaking. The next extraction was with 15 cm^3 petrolether (b.p.$<$ 60°C). After phase separation, organic phase, which contains about 150–200 mg fat, was separated and evaporated under vacuum on a rotadest. Then 4 cm^3 of 0.5 M sodium-hydroxide in methanol was added and boiled on a water-bath for 5 minutes. Then 4 cm^3 14% boron-trifluoride in methanol was added and boiled for 3 minutes, following the addition of 4 cm^3 n-hexane. It was boiled for one minute, then the level of the organic phase was brought to the neck of the flask with saturated sodium-chloride solution. When phases were separated, samples were taken for the analysis from the organic phase, and it was dry on sodium sulfate.

The fatty acid methyl esters (FAMEs) were separated on a 100 m $\times$ 0.25 mm wall-coated open-tubular (WCOT) column equipped with CP-SIL 88 (FAME) stationary phase. The quantitation of FAMEs was obtained with a flame ionization detector (FID) at 270 °C. The temperature of the splitter injector was 270 °C, the carrier gas was helium with the head pressure of 235 kPa. The oven was temperature-programmed from 140 °C (10 min.) with 10 °C/min increase up to 235 °C (26 min). The injected volume varied between 0.5 and 2 µl. The instrument was a Chrompack CP 9000 gas chromatograph.

The results were calculated as area % (the peak area of the given fatty acid methyl ester was divided by the sum of the peak areas and multiplied by 100) and expressed in fatty acid methyl ester % (w/w).

The influence of the breed and the time after calving on the fat content of colostrum and the fatty acid pattern was evaluated with analysis of variance.

3 Results

The crude fat content of colostrum samples did not differ by breeds ($p \geq 0.05$, Table 1). The variation within breeds was smaller than the variation in the function of time (Fig. 1). The average fat content measured on the first day (8.6%, $n = 18$) was significantly more than the one measured on the third day (3.9%, $n = 18$). In the case of samples milked on the fifth day of lactation, the average value of crude fat content was between the two above values (5.8% $n = 18$).

While the fat content of normal milk is affected by breed, in the case of colostrum, there were not significant differences detected by breeds within the examined time period.

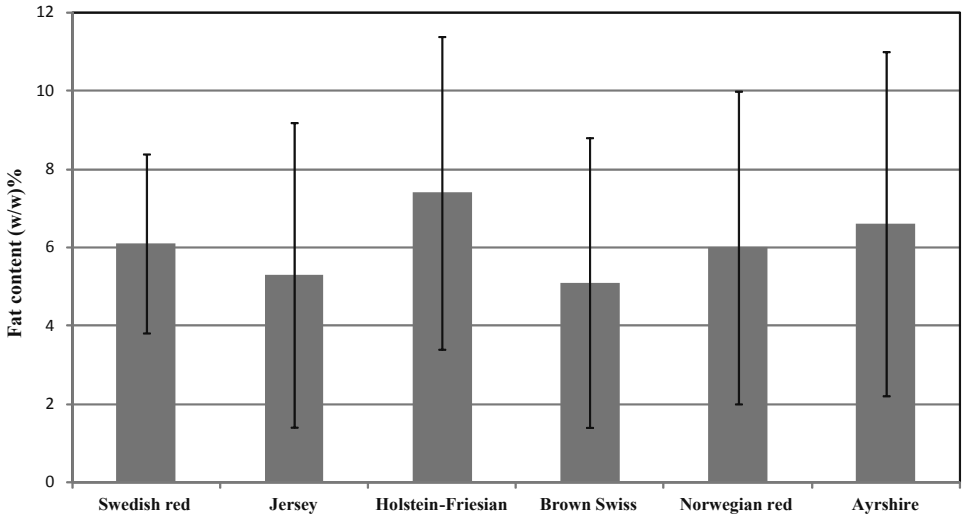

Figure 1: The fat content of colostra from different dairy cow breeds. Average and standard deviation of the samples milked on the first, third, and fifth days ($n = 9$)

Table 1: The characterization of fat content and fatty acid composition[2] of colostra of different breeds (n=9)[1]

	Swedish red		Jersey		Holstein-Friesian		Brown Swiss		Norwegian red		Ayrshire	
	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD
Crude fat content (%)	6.1	2.3	5.3	3.9	7.4	4.0	5.1	3.7	6.0	4.0	6.6	4.4
SFA	68.1^{abc}	6.4	72.7 c	3.5	64.5^{ab}	6.5	63.0^{a}	4.7	64.6^{ab}	4.2	69.7^{bc}	5.3
MUFA	27.7^{abc}	6.6	23.1^{a}	3.4	30.5^{bc}	6.9	32.5^{c}	5.1	31.1^{bc}	4.1	26.0^{ab}	4.9
PUFA	3.7^{a}	0.5	3.8^{ab}	0.6	4.7^{b}	0.5	4.1^{ab}	0.6	3.9^{ab}	0.6	3.9^{ab}	0.9
MUFA+PUFA	31.4^{abc}	6.4	27.0^{a}	3.5	35.2^{bc}	6.4	36.6^{c}	4.5	35.0^{bc}	4.1	29.9^{ab}	5.3
SFA:(MUFA+PUFA)	2.3^{abc}	0.6	2.8^{c}	0.5	1.9^{ab}	0.5	1.8^{a}	0.4	1.9^{ab}	0.4	2.4^{bc}	0.6
n3	1.1	0.2	0.9	0.2	1.0	0.2	1.0	0.1	0.9	0.4	1.3	0.5
n6	2.6^{a}	0.3	2.9^{a}	0.5	3.6^{b}	0.3	3.0^{ab}	0.5	3.0^{a}	0.4	2.6^{a}	0.8
n6:n3	2.5^{a}	0.4	3.2^{ab}	0.8	3.7^{b}	0.8	3.0^{ab}	0.3	3.8^{ab}	1.5	2.3^{a}	0.9

[1] Averages in one row with common superscript do not differ ($p \geq 0.05$).
The absence of superscripts in one row means that there are not any significant differences between the means.
SFA = the sum of the ratio of saturated fatty acids

[2] Expressed in fatty acid methyl ester %
MUFA = the sum of the ratio of monounsaturated fatty acids
PUFA = the sum of the ratio of polyunsaturated fatty acid

The ratio of the saturated fatty acids (SFA) in the colostrum fat of Jersey was higher ($p < 0.05$) than that of Holstein-Friesian, Brown Swiss, and Norwegian red (Table 1). The ratio of monounsaturated fatty acids (MUFA) was the highest in the case of Brown Swiss and the colostrum fat of this breed contained significantly more MUFA than the Jersey and Ayrshire.

The colostrum fat of Holstein-Friesian contained the highest amount of polyunsaturated fatty acids (PUFA). The sum of the unsaturated fatty acids (MUFA+PUFA) was significantly higher in the case of Brown Swiss than that of Jersey and Ayrshire.

The colostrum fat of Jersey had more saturated fatty acids related to the amount of unsaturated fatty acids SFA/(MUFA+PUFA) than the Holstein-Friesian, Brown Swiss, and Norwegian red (Fig. 2).

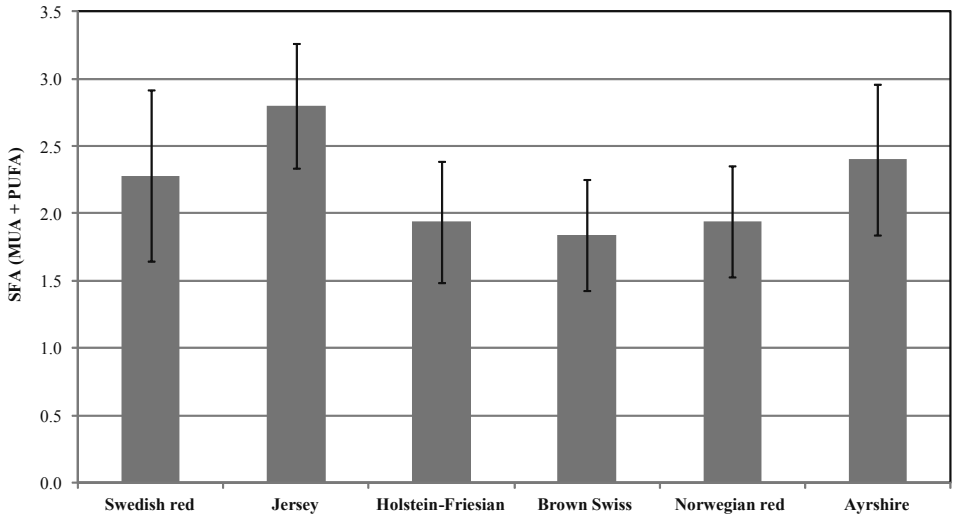

Figure 2: The ratio of the saturated and unsaturated fatty acids SFA/(MUFA+PUFA) in the colostra of different dairy breeds. Average and standard deviation of the samples milked on the first, third, and fifth days ($n = 9$)

The ratio of n3-fatty acids did not differ significantly in the colostrum fat of the breeds under investigation; however, some differences were detected in the case of n6-fatty acids. The colostrum fat of Holstein-Friesian contained more n6 fatty acids than that of Swedish red, Jersey, Norwegian red, and Ayrshire.

The n6/n3 ratio of fatty acids is shown on Fig. 3.

The average fatty acid composition of colostra can be seen in Table 2. In the group of short-chain saturated fatty acids, there was not significant difference in the ratio of butyric acid, caproic and caprylic acid among the breeds. The colostrum fat of Holstein Friesian contained less caprylic acid than the Ayrshire, less undecanoic acid than the Ayrshire, Swedish red, Norwegian red, and Brown Swiss. The ratio of lauric acid was less than the Jersey and the ratio of tridecanoic acid was also less than that of the Jersey, Norwegian red, and Ayrshire. In general, it can be stated that the colostrum fat of Holstein-Friesian contains less C10 – C13 saturated fatty acids than that of the other breeds under investigation.

There are not any obvious tendencies among breeds regarding the ratio of long-chain saturated fatty acids, being present in small amount. The colostrum fat of Holstein-Friesian contains less pentadecanoic acid but more margaric acid than the Jersey. Jersey contained significantly more arachidic acid than Swedish red and there were not significant differences among breeds in the ratios of behenic acid and lignoceric acid.

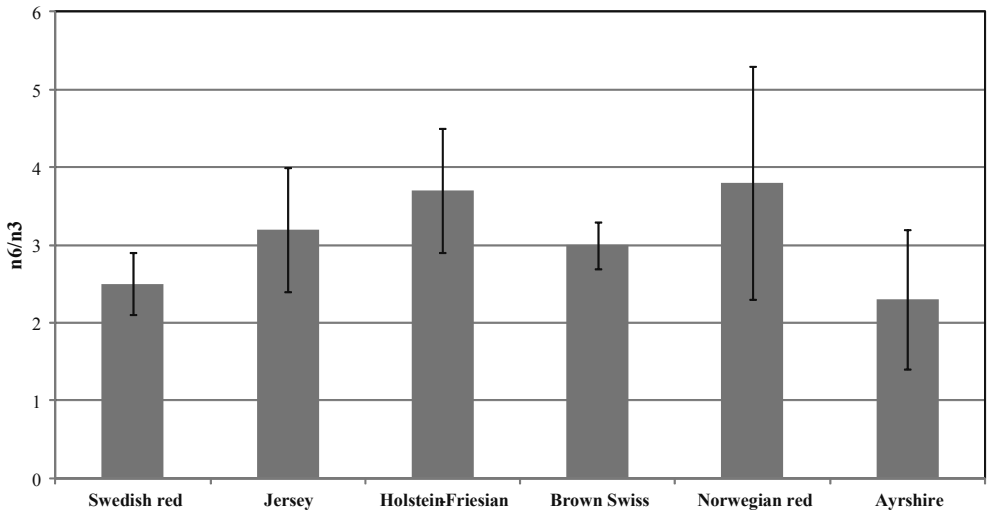

Figure 3: The ratio of n6/n3 fatty acids in the colostrum fat of different dairy breeds. Average and standard deviation of the samples milked on the first, third, and fifth days ($n = 9$)

Table 2: The average fatty acid content of colostrum (1st, 3rd, and 5th days) expressed in fatty acid methyl ester % (n=9)[1]
Part 1: C4-C17 fatty acids

Fatty acid		Swedish red		Jersey		Holstein-Friesian		Brown Swiss		Norwegian red		Ayrshire	
		mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD
Butyric acid	4:0	0.71	0.17	0.58	0.23	0.63	0.30	0.89	0.70	1.89	1.62	2.44	4.31
Caproic acid	6:0	0.85	0.32	0.80	0.26	0.62	0.13	0.86	0.25	0.88	0.31	0.75	0.22
Caprylic acid	8:0	0.72	0.24	0.66	0.24	0.43	0.09	0.64	0.15	0.65	0.20	0.60	0.16
Capric acid	10:0	1.97^{ab}	0.71	1.85^{ab}	0.67	1.03^{a}	0.28	1.47^{ab}	0.35	1.57^{ab}	0.45	1.60^{b}	0.36
Undecanoic acid	11:0	0.12^{b}	0.05	0.11^{ab}	0.06	0.04^{a}	0.01	0.07^{b}	0.02	0.11^{b}	0.05	0.10^{b}	0.04
Lauric acid	12:0	3.04^{ab}	0.90	2.94^{b}	0.62	1.88^{a}	0.66	2.22^{ab}	0.53	2.34^{ab}	0.51	2.72^{ab}	0.50
Tridecanoic acid	13:0	0.11^{ab}	0.04	0.12^{b}	0.04	0.06^{a}	0.02	0.08^{ab}	0.02	0.10^{b}	0.02	0.10^{b}	0.02
Myristic acid	14:0	12.39	2.97	12.75	1.90	10.13	3.76	10.35	3.07	10.23	2.43	12.65	2.70
Myristoleic acid	14:1	0.92^{c}	0.27	0.68^{abc}	0.20	0.46^{ab}	0.16	0.53^{ab}	0.20	0.62^{abc}	0.24	0.86^{abc}	0.32
Pentadecanoic acid	15:0	0.91^{ab}	0.07	1.01^{b}	0.15	0.75^{a}	0.13	0.85^{ab}	0.07	0.93^{ab}	0.09	0.88^{ab}	0.13
Palmitic acid	16:0	37.01^{ab}	5.07	40.78^{b}	5.16	35.54^{ab}	4.90	31.96^{a}	4.84	33.20^{ab}	4.79	37.75^{ab}	7.44
Palmitoleic acid	16:1	2.26	0.72	1.82	0.28	1.90	0.32	2.27	0.35	2.18	0.29	2.05	0.21
Margaric acid	17:0	0.93^{ab}	0.16	0.82^{a}	0.10	1.07^{c}	0.19	1.09^{c}	0.16	0.96^{b}	0.12	0.85^{ab}	0.13

[1] For superscripts, see Table 1.

Table 2: The average fatty acid content of colostrum (1st, 3rd, and 5th days) expressed in fatty acid methyl ester % (n=9)[1] Part 2: C18 fatty acids

Fatty acid		Swedish red		Jersey		Holstein-Friesian		Brown Swiss		Norwegian red		Ayrshire	
		mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD
Stearic acid	18:0	9.10	2.57	9.91	2.63	11.92	3.09	12.21	3.37	11.44	3.13	8.94	3.05
Elaidic acid	18:1n9t	1.14	0.47	1.05	0.32	1.12	0.37	1.53	0.35	1.20	0.46	0.96	0.45
Oleic acid	18:1n9c	22.41^{ab}	6.02	18.84^{a}	3.19	26.03^{ab}	6.32	26.98^{b}	4.58	25.94^{b}	3.98	21.28^{ab}	5.00
7-octadecenoic acid	18:1n7	0.90^{ab}	0.21	0.69^{a}	0.25	0.94^{ab}	0.17	1.07^{b}	0.20	1.04^{ab}	0.18	0.80^{ab}	0.21
Linoleic acid	18:2n6	2.08^{a}	0.16	2.19^{ab}	0.38	2.85^{c}	0.22	2.51^{bc}	0.31	2.47^{abc}	0.32	1.99^{abc}	0.73
γ-linolenic acid	18:3n6	0.03	0.01	0.03	0.01	0.03	0.01	0.03	0.01	0.02	0.01	0.03	0.01
α-linolenic acid	18:3n3	0.52^{ab}	0.15	0.40^{a}	0.11	0.43^{a}	0.07	0.59^{b}	0.06	0.44^{ab}	0.11	0.56^{ab}	0.22
c9, t11- conjugated linoleic acid c9,t11-18:2		0.40^{bc}	0.08	0.30^{a}	0.06	0.29^{a}	0.08	0.48^{c}	0.11	0.34^{ab}	0.09	0.35^{ab}	0.11

[1] For superscripts, see Table 1.

Table 3: The average fatty acid content of colostrum (1st, 3rd, and 5th days) expressed in fatty acid methyl ester % (n=9)[1] Part 3: C20-C24 fatty acids

Fatty acid		Swedish red		Jersey		Holstein-Friesian		Brown Swiss		Norwegian red		Ayrshire	
		mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD
Arachidic acid	20:0	0.14^{a}	0.02	0.19^{b}	0.04	0.18^{ab}	0.03	0.17^{ab}	0.04	0.17^{ab}	0.04	0.15^{ab}	0.03
Eicosenoic acid	20:1	0.07	0.02	0.07	0.02	0.08	0.01	0.08	0.01	0.09	0.02	0.08	0.03
Eicosadienoic acid	20:2	0.04	0.03	0.03	0.01	0.05	0.02	0.04	0.01	0.04	0.01	0.03	0.01
Behenic acid	22:0	0.11	0.05	0.12	0.04	0.11	0.03	0.10	0.03	0.10	0.04	0.10	0.04
Eicosatrienoic acid	20:3n6	0.17	0.07	0.23	0.08	0.28	0.18	0.20	0.10	0.17	0.08	0.20	0.09
Arachidonic acid	20:4n6	0.35^{ab}	0.09	0.41^{b}	0.08	0.49^{c}	0.15	0.30^{a}	0.11	0.32^{a}	0.12	0.35^{ab}	0.08
Lignoceric acid	24:0	0.04	0.01	0.09	0.05	0.07	0.03	0.07	0.03	0.06	0.01	0.07	0.04
Eicosapentanoic acid	20:5n3	0.15	0.05	0.13	0.05	0.10	0.04	0.10	0.04	0.12	0.07	0.22	0.11
Docosapentanoic acid	22:5n3	0.29	0.11	0.34	0.13	0.31	0.12	0.26	0.12	0.30	0.19	0.43	0.22
Docosahexanoic acid	22:6n3	0.11	0.05	0.07	0.04	0.17	0.17	0.06	0.03	0.06	0.04	0.08	0.05

[1] For superscripts, see Table 1.

The sum of SFA was mostly affected by the saturated fatty acids being present in high quantities. In the case of myristic acid, there were not significant differences among breeds, but the ratio of palmitic acid (dominating in the group of SFA) was not the same among breeds. The highest value was detected for the colostrum fat of the Jersey (40.8%), being significantly higher than what was measured for Brown Swiss (32.0%). The dominancy of palmitic acid within the group of SFA means that the differences in SFA ratio are mainly influenced by the variation of this fatty acid. The abundance of palmitic acid in the colostrum fat of Jersey made this breed having the most saturated character.

The colostrum fat of Holstein-Friesian contained a relatively small ratio of short-chain saturated fatty acids, but the ratio of palmitic acid (35.5%) was between the values of Jersey and Brown Swiss; therefore, instead of Holstein-Friesian, the colostrum fat of Brown Swiss proved to have the most unsaturated character.

There were not differences by breeds in the amount of some minor fatty acids with one double bond (palmitoleic acid, elaidic acid, eicosenoic acid). The colostrum fat of Swedish red contained almost twice as much myristoleic acid (0.92%) as Brown Swiss (0.53). The best source of 18 : 1n7 minor fatty acid was the colostrum fat of Brown Swiss.

Oleic acid is the main component of MUFA. The ratio of this fatty acid was significantly higher in the colostrum fat of Brown Swiss and Norwegian red (27.0% and 25.9%, respectively) than that of Jersey (18.8%). The oleic acid content of Holstein-Friesian colostrum fat was also high (26.0%).

In the case of PUFA occurring in smaller quantities, the ratio of 20 : 3n6, 20 : 5n3, 22 : 5n3, and 22 : 6n3 in the colostrum fat did not differ by breeds. The only exception was arachidonic acid. The colostrum fat of Norwegian red and Brown Swiss contained the lowest ratio of this fatty acid (0.32; 0.30%). Jersey had a little more (0.41%) and Holstein-Friesian had significantly highest ratio (0.49%) of arachidonic acid than that of the other breeds.

The ratio of c9, t11-conjugated linoleic acid (c9, t11-CLA) was significantly higher in the case of colostrum fat of Brown Swiss (0.48%) than that of Holstein-Friesian (0.29%) and Jersey (0.30). Similar tendency was observed in the case of linolenic acid (Table 2, Fig. 4). The colostrum fat of Holsten-Friesian contained significantly more linoleic acid (2.85%) than that of Swedish red (2.08%) and Jersey (2.19%) (Table 2).

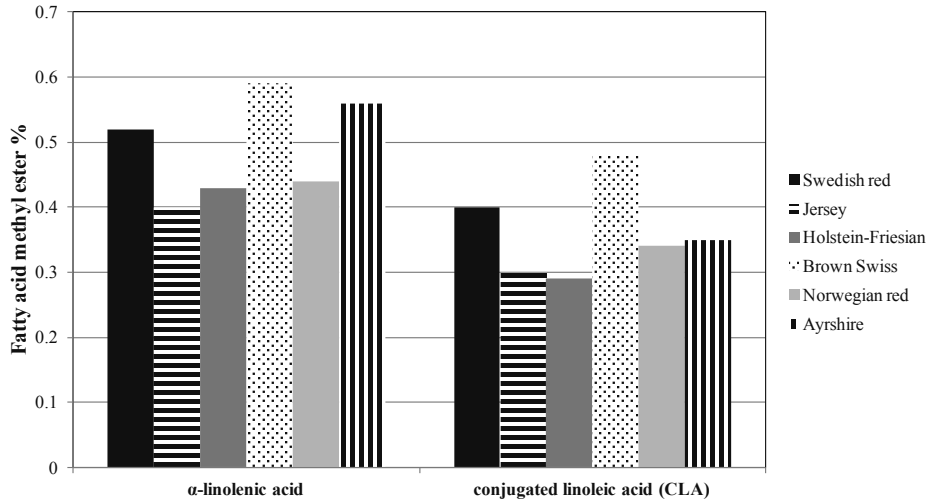

Figure 4: The ratio of c9, t11-conjugated linoleic acid (c9, t11-CLA) and α-linolenic acid in the colostrum fat of different dairy cow breeds ($n = 9$)

4 Summary

To summarize the results obtained for the fatty acid pattern of colostra, it can be concluded that a higher SFA ratio is characteristic to Jersey, a higher presence of MUFA is characteristic to Brown Swiss, and the colostrum of Holstein-Friesian is more abundant in PUFA than the other breeds. The colostrum fat of Jersey proved to have obviously more saturated character than that of Holstein-Friesian, Brown Swiss, and Norwegian red. The colostrum of Holstein-Friesian – with the exception of Brown Swiss – contained more n6 fatty acid than the other breeds under investigation. The pattern of c9, t11CLA and α-linoleic acid ratio in the fat is similar across the breeds. With the exception of the arachidonic acid, there were not any differences by breed with respect to the physiologically important long-chain n3-polyunsaturated fatty acids.

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Colostrum and milk of current and rare cattle breeds: protein content and amino acid composition

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Key words and phrases: amino acid, protein content, colostrum, dairy cow.

Abstract. The dry matter, crude protein, and amino acid content of colostrum and milk of different dairy cow breeds that were kept under the same conditions were determined. There were not notable differences between breeds regarding the dry matter, crude protein, and amino acid content of colostrum samples obtained on the first, third, and fifth days of lactation. The single exception was the third-day-colostrum from Holstein-Friesian that contained more of these compounds than the other breeds. The amino acid composition of protein from the colostrum of breeds under investigation was also very similar.

The milk samples of Jersey and Swedish red contained the highest amount of dry matter, crude protein, and that of amino acids in the examined lactation period (3-5 months after calving). The ratios of the individual essential amino acids within milk protein did not differ notably among breeds. Based on the Morup-Olesen biological value calculation, among the studied breeds, the amino acid composition of milk proteins from Holstein-Friesian was the most similar to the reference protein in the average of the 3-5 months of lactation.

1 Introduction

Colostrum is a transitional feed of the cow from the blood to the milk; therefore, its composition is similar to that of the blood. Its dry matter, crude protein, and mineral content are higher than that of the milk, but its lactose content is lower. The rapid change in the concentration of the given compounds is the most spectacular immediately after calving, the variability of compounds decreases from the third-fourth days of lactation. There are several other parameters affecting the composition of colostrum, e.g. breed, feeding, age (number of lactations), and health status (*Csapó*, 1984).

Contrary to colostrum, the composition of normal milk and the factors affecting it have been thoroughly studied. The dry matter and protein content of milk depends mostly on breed, lactation status, and age. Seasonal changes are associated with feeding (*Brunner*, 1976; *Cerbulis* and *Farrel*, 1974; *Csapó* and *Csapó-Kiss*, 1998).

Holstein-Friesian has become the dominating dairy cow breed in the most part of the world. The degree of utilization of the other breeds partially depends on their milk composition and their secondary properties. The main aim of our investigation was to compare the amino acid content and the amino acid composition of colostrum and milk of Holstein-Friesian and other dairy cow breeds kept under the same conditions.

2 Materials and methods

2.1 Milk sampling

Colostrum and milk samples originated from the "Kőrös-Maros Biofarm" Ltd. from Gyulavári, Hungary. There are about 500 Holstein-Friesian cows in this plant. Individuals of further breeds were imported (Swedish red, Jersey, Brown Swiss, Norwegian red, Ayshire) and an experiment was conducted in order to compare their production. Experimental animals were loose-housed cows kept in small groups. The bedding of the cow-shed was deep litter and the house was provided with concreted open yard. The number of animals was 13-15 per part of the cow-shed. Feed and water were provided in the open yard. The level of the water was fixed in the drinking trough and the feed was prepared with mixer-feeder wagon. Milking was obtained twice a day with herringbone milking parlour.

Feed met the requirements of bioproduction. The daily intake was 9 kg corn silage, 4 kg alfalfa silage, 2 kg alfalfa hay, 9 kg triticale haylage. The amount of the provided concentrate (6-9 kg) depended on the milk production. The individual dry matter consumption was 19.1 kg, the milk production net energy 125 MJ, and the metabolizable protein content 2,017 g.

Colostrum samples were taken on the first, third, and fifth days after calving. Three individuals from each breed were milked and the lactations of the same animals were followed.

The milk samples originated from three milking sessions of the trial flock. Milk samples originated from cows with similar days of lactation were sorted out (Table 1) and analysed.

Table 1: The average lactation time of the milked cows

Breed	**Days of lactation, average** ($n = 3$)		
	1st milking	**2nd milking**	**3rd milking**
Swedish red	99	118	157
Jersey	94	112	151
Holstein-Friesian	91	122	151
Brown Swiss	104	124	160
Norwegian red	89	110	149

2.2 Chemical analysis

Chemical analysis was carried out at the Department of Chemistry-Biochemistry, Faculty of Animal Science, Kaposvár University. The determination of dry matter content was based on the Hungarian standard MSZ 3744-81. The crude protein content was measured with a Kjeltec 2400 type automatic crude protein analyser and the related international standard (MSZ EN ISO 5983-2) was applied.

The amino acid content was quantified following acidic hydrolysis (6M hydrochloric acid solution, 110°C, 24 h) with an AAA 400 type amino acid analyser (Ingos).

The biological value was calculated on the basis of the Morup-Olesen index.

$$\text{Biological score} = 10^{2,15} \cdot q_{\text{Lys}}^{0,41} \cdot q_{\text{arom}}^{0,60} \cdot q_{\text{sulf}}^{0,77} \cdot q_{\text{Thr}}^{2,41} \cdot q_{\text{Trp}}^{0,41}$$

$q = a/a_{\text{ref}}$,
a = the ratio of the given essential amino acid/the ratio of the total essential amino acids in the sample protein,
a_{ref} = the ratio of the given essential amino acid/the ratio of the total essential amino acids in the reference protein,
arom = the sum of the ratio of aromatic amino acids (Tyr+Phe),
sulf = the sum of the ratio of sulfur containing amino acids (Cys+Met).

The reference protein was the 66:43 (w/w%) mixture of potato and egg because the highest nitrogen retention was observed at this ratio (*Morup and Olesen*, 1976).

3 Results

3.1 Colostrum

The dry matter content of the colostrum samples and the results of the crude protein content determination can be seen in Table 2. There was not any difference among breeds regarding these parameters in samples milked on the first and fifth days. The third-day-colostrum from Holstein-Friesians contained more dry matter and crude protein than that of the other breeds.

The dry matter and also the crude protein content of samples decreased significantly between the first and the third days. In the next time period (between the third and fifth days), there was no spectacular difference in the above-mentioned parameters with the exception of the colostrum of Holstein-Friesian and Ayrshire cows.

Table 2: The amount of some basic constituents of the colostrum of different dairy cow breeds

Days of lactation	Dry matter content (%)					
	Swedish red	Jersey	Holstein-Friesian	Brown Swiss	Norwegian red	Ayrshire
1	28.50	31.13	31.67	31.43	30.07	32.67
3	14.70	15.60	22.37	15.03	15.07	17.40
5	16.67	16.20	16.93	15.40	15.63	15.60

Days of lactation	Crude protein content % (N% × 6.38)					
	Swedish red	Jersey	Holstein-Friesian	Brown Swiss	Norwegian red	Ayrshire
1	17.31	19.16	19.75	17.66	18.71	19.50
3	5.97	6.59	11.57	5.23	4.59	8.26
5	4.95	5.80	5.04	4.51	4.75	5.36

The amino acid content of the colostra (Table 3) did not differ significantly by breeds on the first and the fifth days of lactation. However, there was some variation between the breeds on the third day. The colostrum of Holstein-Friesian cows contained more lysine, methionine, threonine, phenylalanine, valine, and isoleucine than that of the other breeds on the same day. There was a high decrease in the amino acid content between the first and the third days, following a moderate decrease or stagnation between days 3 and 5.

There were no spectacular differences in the amino acid composition of the colostrum proteins among the breeds. On the third and the fifth days, the colostrum proteins of Norwegian red and Brown Swiss contained a little more lysine and less threonine than that of the other breeds. Regarding the essential amino acids, the ratio of threonine showed the highest variation among breeds while the ratio of the other amino acids (phenylalanine, valine isoleucine, and leucine) was practically the same for the examined breeds.

In the function of lactation time, the composition of the colostrum proteins did not seem to differ notably between the first and the fifth days in the case of the most essential amino acids: the ratio of lysine, methionine, leucine, phenylalanine, and valine practically did not change. The ratio of threonine showed a decreasing tendency within the protein content. The ratio of non-essential glutamic acid and proline increased with time, similarly to the way it had been observed previously (*Csapó*, 1984).

Table 3: The essential and semi-essential amino acid content of colostrums originated from different cattle breeds (g amino acid/100 g sample)[1]

Amino acids	Day	Swedish red		Jersey		Holstein-Friesian		Brown Swiss		Norwegian red		Ayrshire	
		Mean	Std. deviation	Mean	Std. deviation	Mean	Std. deviation	Mean	Std. deviation	Mean	Std. deviation	Mean	Std. deviation
Thr	1st	1.047	0.093	1.263	0.270	1.237	0.071	1.100	0.030	1.143	0.249	1.160	0.044
	2nd	0.297	0.040	0.323	0.133	0.667	0.265	0.230	0.030	0.190	0.044	0.437	0.143
	3rd	0.207	0.035	0.290	0.096	0.230	0.070	0.173	0.021	0.190	0.046	0.230	0.062
Cys	1st	0.253	0.031	0.310	0.080	0.300	0.044	0.253	0.012	0.267	0.067	0.283	0.029
	2nd	0.077	0.015	0.080	0.053	0.180	0.062	0.050	0.010	0.037	0.012	0.110	0.035
	3rd	0.040	0.010	0.063	0.025	0.053	0.032	0.027	0.006	0.037	0.012	0.050	0.020
Val	1st	1.173	0.065	1.347	0.241	1.373	0.061	1.210	0.072	1.193	0.097	1.340	0.010
	2nd	0.360	0.040	0.393	0.137	0.757	0.248	0.307	0.035	0.263	0.051	0.527	0.150
	3rd	0.283	0.040	0.350	0.101	0.300	0.072	0.257	0.023	0.277	0.060	0.317	0.050
Met	1st	0.383	0.006	0.387	0.051	0.403	0.040	0.383	0.040	0.430	0.044	0.423	0.049
	2nd	0.140	0.010	0.157	0.050	0.247	0.067	0.127	0.006	0.117	0.021	0.187	0.055
	3rd	0.123	0.012	0.133	0.031	0.123	0.015	0.120	0.010	0.123	0.025	0.137	0.012
Ile	1st	0.663	0.015	0.690	0.098	0.730	0.092	0.677	0.064	0.727	0.067	0.747	0.055
	2nd	0.257	0.025	0.283	0.091	0.453	0.107	0.233	0.015	0.203	0.042	0.330	0.082
	3rd	0.223	0.021	0.247	0.065	0.227	0.040	0.210	0.017	0.217	0.045	0.237	0.021
Leu	1st	1.457	0.050	1.570	0.262	1.640	0.132	1.473	0.110	1.590	0.144	1.703	0.076
	2nd	0.507	0.051	0.557	0.181	0.953	0.261	0.443	0.035	0.400	0.079	0.717	0.180
	3rd	0.427	0.049	0.490	0.121	0.430	0.092	0.390	0.026	0.423	0.085	0.477	0.057
Tyr	1st	0.823	0.031	0.907	0.144	0.970	0.069	0.883	0.058	0.907	0.083	0.933	0.029
	2nd	0.277	0.025	0.307	0.116	0.557	0.159	0.247	0.025	0.210	0.044	0.393	0.106
	3rd	0.227	0.032	0.270	0.075	0.243	0.051	0.213	0.012	0.223	0.055	0.247	0.031
Phe	1st	0.723	0.025	0.777	0.127	0.817	0.076	0.730	0.052	0.777	0.065	0.813	0.040
	2nd	0.253	0.021	0.277	0.096	0.480	0.123	0.223	0.015	0.200	0.036	0.350	0.085
	3rd	0.213	0.021	0.250	0.066	0.217	0.040	0.197	0.015	0.210	0.046	0.233	0.025
Lys	1st	1.217	0.038	1.327	0.216	1.387	0.090	1.253	0.102	1.350	0.101	1.420	0.060
	2nd	0.443	0.047	0.477	0.156	0.833	0.227	0.403	0.031	0.357	0.074	0.620	0.160
	3rd	0.373	0.055	0.423	0.106	0.380	0.082	0.353	0.031	0.377	0.080	0.410	0.046

[1]n=3 for each time and breed

Having the protein fractions of colostrum analysed, it can be stated that the casein content already reached a stabile level after the third day while in the case of whey protein, there were some differences between values obtained on the third and the fifth days. The amount of non-protein nitrogen (NPN) decreased continuously during the observed time period.

3.2 Milk

The dry matter and protein content of the milk of the different breeds can be seen in Table 4. The concentrated milk breeds showed higher values in the case of these parameters. In the investigated time period (3-5 months), there are not any distinct tendencies regarding dry matter or crude protein content. This observation is contrary to the results of a previous investigation when an increase of these parameters from the second months of lactation was detected (*Csapó*, 1984).

Table 4: The amount of some basic constituents of the milk of different dairy cow breeds

Months of lactation	**Dry matter content** (%)					
	Swedish red	Jersey	Holstein-Friesian	Brown Swiss	Norwegian red	Ayrshire
3	12.00	14.06	13.18	13.28	12.07	12.02
4	12.83	16.29	13.71	12.98	12.40	12.65
5	12.48	15.12	13.74	12.29	13.28	12.88

Months of lactation	**Crude protein content %** (N% × 6.38)					
	Swedish red	Jersey	Holstein-Friesian	Brown Swiss	Norwegian red	Ayrshire
3	3.81	4.45	3.48	3.27	3.34	3.16
4	3.89	4.29	3.36	3.67	3.56	3.00
5	3.88	4.19	3.59	3.46	3.49	3.45

In the case of a given breed, the amino acid content of its milk did not seem to change in the function of time in the examined period, similarly to the protein content. In this experiment, the three-month - period was presumably too short to detect any changes if there were any. Analysing the differences between breeds regarding the amino acid content of their milk, it can be clearly seen that the amount of the essential amino acids (Table 5) is proportional to the protein content.

Table 5: The essential and semi-essential amino acid content of milks originated from different cattle breeds (g amino acid/100 g sample)[1]

Amino acid	Swedish red		Jersey		Holstein-Friesian		Brown Swiss		Norwegian red		Ayrshire	
	Mean	Std deviation	Mean	Std deviation	Mean	Std deviation	Mean	Std deviation	Mean	Std deviation	Mean	Std deviation
Thr	0.159	0.002	0.178	0.008	0.143	0.008	0.144	0.011	0.142	0.003	0.130	0.008
Cys	0.028	0.002	0.029	0.001	0.023	0.003	0.019	0.002	0.018	0.001	0.014	0.001
Val	0.218	0.003	0.237	0.011	0.214	0.009	0.216	0.016	0.213	0.006	0.195	0.004
Met	0.096	0.001	0.105	0.004	0.083	0.001	0.083	0.005	0.082	0.003	0.079	0.005
Ile	0.189	0.002	0.211	0.008	0.160	0.003	0.166	0.013	0.160	0.005	0.148	0.007
Leu	0.358	0.003	0.390	0.013	0.309	0.009	0.308	0.021	0.306	0.008	0.285	0.016
Tyr	0.179	0.001	0.201	0.009	0.151	0.006	0.154	0.010	0.151	0.004	0.140	0.006
Phe	0.177	0.002	0.196	0.007	0.153	0.006	0.155	0.011	0.152	0.006	0.143	0.008
Lys	0.312	0.002	0.345	0.011	0.258	0.011	0.261	0.017	0.262	0.008	0.242	0.014

[1]Average values of three milking sessions on the 3rd, 4th, and 5th month of lactation

The higher protein content in the case of Jersey and Swedish red was accompanied with higher essential amino acid concentration of their milk. The amino acid composition of milk protein showed only slight changes in the function of time. The amino acid pattern of the milk proteins also did not differ notably by breeds. The results of previous examinations led to the same conclusion regarding milk proteins of Jersey and Holstein-Friesian cows (*Csapó*, 1984).

When the biological value (Table 6) is calculated on the basis of the Morup-Olesen index, the deviation *in both directions* from the amino acid pattern of the reference protein lowers the score. Moreover, the small variance between the amino acid compositions of milk proteins of different breeds is magnified. Therefore, higher differences were detected in the biological value of milk protein originated from different breeds than that of the ratios of the individual amino acids.

Table 6: The biological value of milks originated from different cattle breeds[1]

	Swedish red	Jersey	Holstein-Friesian	Brown Swiss	Norwegian red	Ayrshire
Biological value	69	66	77	75	73	70

[1]Average values of three milking sessions on the 3rd, 4th, and 5th month of lactation

The biological value of the milk in the average of 3-5 months of lactation was 69 for Swedish red, 66 for Jersey, 77 for Holstein-Friesian, 75 for Brown Swiss, 73 for Norwegian red, and 70 for Ayrshire. All of the examined proteins contained higher ratio of lysine and aromatic amino acids (tyrosine and phenylalanine) than that of the reference protein, but the ratio of threonine and sulfur containing amino acids (cysteine + methionine) was lower. The essential amino acid composition of milk protein of Holstein-Friesian was the closest to the reference protein.

4 Summary

To summarize, it can be concluded that the dry matter, crude protein, and amino acid content of colostrums from the different breeds were similar. The only exception was the Holstein-Friesian, giving higher essential amino acid content colostrum on the third day. The colostrum from this breed also con-

tained more whey protein while the amount of casein was only a little less than that of the other breeds. Owing to the higher essential amino acid content of whey proteins, the colostrum of Holstein-Friesian was a more abundant source of these amino acids than that of the other breeds.

The crude protein content drop was different by breeds in the function of time. In most of the cases, low values were observed on the third day while in some cases, protein-fractions (e.g. whey protein, NPN) did not reach the levels of normal milk during this period.

Most of the essential amino acids changed proportionally to the crude protein content. The sum of the essential amino acids within colostrum protein decreased in the function of time. The essential amino acid content of milk was proportional to the crude protein content. Breeds having milk rich in protein gave milk with higher essential amino acid content because the amino acid composition of milk proteins was very similar.

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Analysis of the fatty acid pattern of milk from current and rare cattle breeds

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Abstract. Six different dairy breeds kept under the same farm and fed under the same conditions were evaluated with respect to the fat content and the fatty acid composition of their milk drawn on the 3-5th months of lactation.

Key words and phrases: fatty acid, milk, dairy cow.

The fat content of the milk of the breeds did not differ significantly ($p = 0.076$) owing to the high variance of samples in the examined time period. The mean value of the sum of saturated fatty acids was the highest in the case of Jersey (77.8%) while this value ranged from 71.8% to 74.8% for the other breeds. An inversely proportional tendency can be seen for the ratio of monounsaturated fatty acids: in this case, the mean value for Jersey was the lowest (18.9%) and the values of the other breeds ranged from 21.9 to 24.9%. The saturated fatty acid/unsaturated fatty acid ratio was significantly ($p < 0.05$) higher in the milk fat of Jersey than that of Swedish red, Holstein-Friesian, Norwegian red, and Brown Swiss. The $n6/n3$ ratio of fatty acids in the milk fat of the breeds under investigation did not differ significantly.

1 Introduction

The fatty acid composition of the consumed edible oils and fats is a matter of importance from nutritional point of view. The enhanced intake of some saturated fatty acids has been shown to be associated with the elevated risk of some diseases while other fatty acids proved to have health saving properties (*Chilliard et al.*, 2001). The unfavourable ratio of $n6$ and $n3$ fatty acids in the diet has been shown to enhance the level of health risks. In order to obtain better ratios, the intake of $n3$ fatty acids should be increased at the expense of $n6$ (*Riediger et al.*, 2009). Associated with the studies on the medical effect of the fatty acid pattern of food, efforts have been made to reveal the possibilities in order to change the fatty acid composition of foods. In the case of milk fat forage, feeding has been shown to be one of the most important factors. The effect of season and housing can also be originated in the influence of feeding. A moderate genetic variance regarding the fatty acid profile of milk was also verified (*Soyeurt et al.*, 2006). The aim of the present investigation was to compare the fat content and the fatty acid profile of the milk of different dairy cow breeds kept under the same feeding and housing conditions.

2 Materials and methods

2.1 Milk sampling

There were three farms involved in the investigations. The first part of the milk samples originated from the "Kőrös-Maros Biofarm" Ltd. from Gyulavári, Hungary. There are about 500 Holstein-Friesian cows in this plant. Individuals of further breeds were imported (Swedish red, Jersey, Brown Swiss,

Norwegian red, and Ayshire) and an experiment was conducted in order to compare their production. Experimental animals were loose-housed cows kept in small groups. The bedding of the cow-shed was deep litter and the house was provided with concreted open yard. The number of animals was 13-15 per part of the cow-shed. Feed and water were provided in the open yard. The level of the water was fixed in the drinking trough and the feed was prepared with mixer-feeder wagon. Milking was obtained twice a day with herringbone milking parlour.

Feed met the requirements of bioproduction. The daily intake was 9 kg corn silage, 4 kg alfalfa silage, 2 kg alfalfa hay, and 9 kg triticale haylage. The amount of the provided concentrate (6-9 kg) depended on the milk production. The individual dry matter consumption was 19.1 kg, the milk production net energy 125 MJ, and the metabolizable protein content 2017 g.

The milk samples originated from three milking sessions of the trial flock. Milk samples originated from cows with similar days of lactation were sorted out (Table 1) and analysed. Three individuals from each breed were milked and the lactations of the same animals were followed.

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Brown Swiss	104	124	160
Norwegian red	89	110	149

The next part of the study was conducted at "Gödrei Mezőgazdasági Zrt." in Gödre, Hungary and the Holstein-Friesian and Brown Swiss dairy herds of the farm were involved. Cows were kept in groups according to their milk yield. The number of animals in one group was 50-60. The bedding of the cow-shed was deep litter. Feed and water were provided in the open yard of the house with mixer-feeder wagon and fixed water level drinking trough. Milking was obtained with herringbone milking parlour. The animals involved in the experiment were selected based on their number of lactation and milk yield. The feeding ration for animals in the first part of lactation was 23 kg corn silage, 7 kg sugar beet pulp, and 4 kg alfalfa hay supplemented with

dairy concentrate. The dry matter content of the forage was 22.5 kg, the milk production net energy 142 MJ, and the metabolizable protein content was 2240 g.

The third part of the experiment was carried out at "Lukovics és Társa Ltd." in Magyarszék, Hungary. Individuals of Holstein-Friesian and Jersey milking herds were kept separately loose-housed in small groups. The feeding portion of the animals consisted of 20-23 kg corn silage, 3-5 kg haylage, and 3-4 kg hay that were completed with dairy concentrate depending on the milk production. The dry matter intake was 18 kg for Jersey and 21.5 kg for Holstein-Friesian. The net energy content of TMR was 135 MJ and it contained 2150 g metabolizable protein.

2.2 Chemical analysis

Chemical analysis was carried out at the Department of Chemistry-Biochemistry, Faculty of Animal Science, Kaposvár University. Crude fat content determination was carried out according to the MSZ ISO 8262-3 international standard. Fatty acid profile was determined in the form of fatty acid methyl esters. The homogenized sample was weighed into a flask, 8 cm^3 concentrated hydrochloric acid was added, and it was boiled for 60 minutes. After cooling down, 7 cm^3 ethanol and then 15 cm^3 diethylether was added, following a one-minute shaking. The next extraction was with 15 cm^3 petrolether (b.p.$<$ 60°C). After phase separation, the organic phase that contains about 150-200 mg fat was separated and evaporated under vacuum on a rotadest. Then 4 cm^3 of 0.5 M sodium-hydroxide in methanol was added, and boiled on a water bath for 5 minutes. Then 4 cm^3 of 14% boron-trifluoride in methanol was added and boiled for 3 minutes, following the addition of 4 cm^3 n-hexane. It was boiled for one minute, and then the level of the organic phase was brought to the neck of the flask with saturated sodium-chloride solution. When the phases were separated, samples were taken for the analysis from the organic phase, and it was dry on sodium sulfate.

The fatty acid methyl esters (FAMEs) were separated on a 100 m × 0.25 mm wall-coated open-tubular (WCOT) column equipped with CP-SIL 88 (FAME) stationary phase. The quantitation of FAMEs was obtained with a flame ionization detector (FID) at 270 °C. The temperature of the splitter injector was 270 °C; the carrier gas was helium with the head pressure of 235 kPa. The oven was temperature-programmed from 140 °C (10 min.) with 10 °C/min increase up to 235 °C (26 min). The injected volume varied between 0.5 and 2 µl. The instrument was a Chrompack CP 9000 gas chromatograph.

The results were calculated as area % (the peak area of the given fatty acid methyl ester was divided by the sum of the peak areas and multiplied by 100) and expressed in fatty acid methyl ester % (w/w).

In the case of the first farm, where six breeds were involved in the experiment, the influence of the breed on the fat content of milk and the fatty acid pattern was evaluated with one-way analysis of variance.

There were only two breeds to compare in the case of the second and third plants; therefore, the comparison of fat content and fatty acid profile of their milk was carried out with two sample T-tests.

3 Results

The milk of the six breeds kept on Biofarm did not differ in fat content at 95% level of significance ($p = 0.071$). Although the fat content (w/w%) of the milk of Jersey seemed to be the highest, the standard deviation was high (5.7 ± 1.3). The mean values of Swedish red, Norwegian red, Brown Swiss, and Ayrshire ranged between 3.8% and 4.1% (Table 2).

The ratio of saturated fatty acids (SFA) and that of monounsaturated fatty acids (MUFA) did not deviate by breeds at 95% level of probability ($p = 0.077$ and $p = 0.059$, respectively). The mean value of the SFA of Jersey was the highest (77.8%). The milk fat of the other breeds contained $71.8\% - 74.8\%$ SFA. An inverse tendency was observed for MUFA: the mean value for Jersey was the lowest (18.9%) while that of the other breeds were found between 21.9% and 24.9%.

The sum of the unsaturated fatty acids (MUFA+PUFA) also did not differ at 95% level of significance by breeds ($p = 0.076$), but the mean value of Jersey was the lowest among breeds (Table 2.)

The only acceptable significant difference appeared in the ratio of the saturated and unsaturated fatty acids SFA/(MUFA+PUFA). The milk fat of Jersey contained significantly ($p = 0.029$) more saturated fatty acids – related to unsaturated fatty acids – than Swedish red, Holstein-Friesian, Norwegian red, and Brown Swiss.

The milk fat of the breeds under investigation did not differ either in the amount or in the ratio of $n6$ and $n3$ fatty acids ($p > 0.154$).

Table 2: The characterization of fat content and fatty acid composition[1] of milk of different breeds (n=9)[2]

	Swedish red		Jersey		Holstein-Friesian		Brown Swiss		Norwegian red		Ayrshire	
	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD
Crude fat content	3.8	0.4	5.7	1.3	4.9	0.4	3.9	0.8	4.1	0.6	4.0	0.8
SFA	73.0	1.3	77.8	0.6	72.4	4.8	73.5	1.8	71.8	1.9	74.8	0.7
MUFA	23.6	1.1	18.9	0.5	24.4	4.6	22.9	1.5	24.9	1.9	21.9	0.6
PUFA	3.4	0.3	3.2	0.2	3.3	0.2	3.7	0.4	3.3	0.1	3.3	0.1
MUFA+PUFA	27.0	1.3	22.2	0.6	27.6	4.8	26.6	1.8	28.2	1.9	25.2	0.7
SFA/(MUFA+PUFA)	2.7^{a}	0.2	3.5^{b}	0.1	2.7^{a}	0.6	2.8^{a}	0.3	2.6^{a}	0.2	3.0^{ab}	0.1
n3	0.38	0.02	0.35	0.06	0.30	0.10	0.39	0.03	0.36	0.07	0.33	0.05
n6	2.7	0.2	2.6	0.1	2.6	0.1	2.9	0.3	2.5	0.1	2.6	0.1
n6:n3	7.1	0.7	7.6	1.2	9.5	3.9	7.5	0.3	7.2	1.6	7.8	0.9

[1] Expressed in fatty acid methyl ester %
[2] Averages in one row with common superscript do not differ ($p \geq 0.05$).
The absence of superscripts in one row means that there are not any significant differences between the means.
SFA = the sum of the ratio of saturated fatty acids.
MUFA = the sum of the ratio of monounsaturated fatty acids
PUFA= the sum of the ratio of polyunsaturated fatty acids

Table 3: The average fatty acid composition of milk of different breeds expressed in fatty acid methyl ester % (n=9)[1]
Part 1: C4-C17 fatty acids

Fatty acid		Swedish red		Jersey		Holstein-Friesian		Brown Swiss		Norwegian red		Ayrshire	
		mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD
Butyric acid	4:0	0.50	0.11	0.52	0.12	0.70	0.06	0.70	0.16	0.61	0.04	0.69	0.13
Caproic acid	6:0	1.12	0.06	1.12	0.05	1.19	0.06	1.20	0.12	1.12	0.14	1.18	0.03
Caprylic acid	8:0	1.11	0.08	1.07	0.06	1.07	0.09	1.13	0.09	1.03	0.18	1.06	0.01
Capric acid	10:0	3.46	0.42	3.60	0.53	3.13	0.65	3.39	0.26	3.09	0.77	3.10	0.09
Undecanoic acid	11:0	0.43	0.07	0.39	0.08	0.43	0.21	0.38	0.08	0.38	0.04	0.36	0.07
Lauric acid	12:0	4.61	0.63	5.09	1.11	4.10	0.96	4.45	0.31	4.27	1.03	4.10	0.16
Tridecanoic acid	13:0	0.31	0.09	0.33	0.08	0.34	0.23	0.27	0.08	0.36	0.14	0.25	0.04
Myristic acid	14:0	14.24	0.51	13.59	0.61	12.39	1.15	14.08	0.83	13.14	1.18	14.12	0.49
Myristoleic acid	14:1	1.07	0.04	0.93	0.10	1.10	0.31	1.07	0.15	0.97	0.05	1.09	0.35
Pentadecanoic acid	15:0	1.73	0.62	1.67	0.49	1.67	0.59	1.56	0.45	1.60	0.22	1.49	0.10
Palmitic acid	16:0	35.29	0.92	38.98	0.36	36.74	4.27	33.58	1.95	35.53	0.46	38.18	2.07
Palmitoleic acid	16:1	1.35	0.09	1.37	0.38	1.75	0.60	1.13	0.14	1.60	0.32	1.38	0.30
Margaric acid	17:0	0.87	0.07	0.85	0.06	0.98	0.15	0.89	0.02	0.87	0.06	0.83	0.05

[1] For superscripts, see Table 2.

Table 3: The average fatty acid composition of milk of different breeds expressed in fatty acid methyl ester % (n=9)[1]
Part 2: C18 fatty acids

Fatty acid		Swedish red		Jersey		Holstein-Friesian		Brown Swiss		Norwegian red		Ayrshire	
		mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD
Stearic acid	18:0	8.94	0.20	10.27	1.72	9.28	3.65	11.41	0.26	9.49	0.66	9.06	1.57
Elaidic acid	18:1n9t	1.01	0.03	0.98	0.34	1.13	0.15	1.00	0.41	0.82	0.14	0.87	0.30
Oleic acid	18:1n9c	19.29	1.04	14.89	0.77	19.36	5.33	18.82	1.42	20.55	1.67	17.77	0.94
7-octadecenoic acid	18:1n7	0.83^{ab}	0.05	0.69^{a}	0.06	0.98^{b}	0.13	0.80^{ab}	0.06	0.90^{ab}	0.16	0.77^{ab}	0.07
Linoleic acid	18:2n6	2.20	0.07	2.15	0.16	2.27	0.11	2.54	0.27	2.14	0.07	2.21	0.08
γ-linolenic acid	18:3n6	0.03	0.01	0.04	0.01	0.04	0.01	0.03	0.01	0.04	0.02	0.04	0.01
α-linolenic acid	18:3n3	0.30	0.02	0.27	0.05	0.23	0.10	0.32	0.02	0.29	0.06	0.26	0.04
c9, t11- conjugated linoleic acid	c9,t11-18:2	0.31	0.02	0.27	0.02	0.31	0.04	0.35	0.08	0.38	0.08	0.33	0.03

[1] For superscripts, see Table 2.

Table 3: The average fatty acid composition of milk of different breeds expressed in fatty acid methyl ester % (n=9)[1]
Part 3: C20 – C24 fatty acids

Fatty acid		Swedish red		Jersey		Holstein-Friesian		Brown Swiss		Norwegian red		Ayrshire	
		mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD
Arachidic acid	20:0	0.15	0.02	0.17	0.05	0.15	0.06	0.20	0.02	0.15	0.01	0.16	0.03
Eicosenoic acid	20:1	0.05	0.00	0.05	0.02	0.05	0.01	0.05	0.01	0.05	0.01	0.05	0.01
Heneicosanoic acid	21:0	0.03	0.01	0.04	0.01	0.04	0.03	0.05	0.01	0.05	0.01	0.04	0.02
Eicosadienoic acid	20:2	0.03	0.01	0.05	0.04	0.04	0.02	0.03	0.01	0.03	0.01	0.03	0.01
Behenic acid	22:0	0.11	0.01	0.08	0.02	0.09	0.02	0.11	0.02	0.10	0.01	0.10	0.03
Eicosatrienoic acid	20:3n6	0.16	0.07	0.14	0.02	0.10	0.02	0.12	0.02	0.10	0.01	0.11	0.00
Arachidonic acid	20:4n6	0.27	0.10	0.24	0.03	0.22	0.01	0.21	0.02	0.21	0.02	0.21	0.01
Lignoceric acid	24:0	0.13	0.10	0.07	0.03	0.05	0.02	0.07	0.02	0.06	0.01	0.08	0.03
Docosapentanoic acid	22:5n3	0.07	0.01	0.08	0.01	0.07	0.00	0.07	0.01	0.06	0.01	0.07	0.01

[1] For superscripts, see Table 2.

In the case of the individual fatty acids, there was only one detected significant difference: the milk fat of Holstein-Friesian contained more 7-octadecenoic acid (0.98%) than that of Jersey (0.69%; $p = 0.049$). The palmitic acid surplus of Jersey related to Norwegian red was not significant at 95% level ($p = 0.081$). The highest average value for linoleic acid was obtained with Brown Swiss, but the difference by breeds was not significant ($p = 0.050$).

The milk originated from the six breeds kept in Biofarm was examined in order to decide whether the time after calving exerts an effect on the crude fat content and the fatty acid composition, because some changes may occur in these parameters from the third to the fifth months of lactation. According to the results of the statistical calculations, there were not any significant differences ($p > 0.161$) either in the ratio of fatty acid groups {SFA, MUFA, PUFA, MUFA+PUFA, SFA/(MUFA+PUFA), n3, n6, n6/n3 ratio} or in the fat content of milk (Table 4).

Table 4: The crude fat content (w/w%) and the fatty acid composition[1] of milk obtained in different months after calving, expressed in the average of six breeds (n=6)

	3rd months		4th months		5th months	
	Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**
Crude fat	4.0	0.5	4.6	1.2	4.6	0.9
SFA	72.8	3.8	74.6	2.7	74.2	1.9
MUFA	23.7	3.7	22.2	2.6	22.4	1.8
PUFA	3.4	0.3	3.2	0.1	3.4	0.2
MUFA+PUFA	27.2	3.8	25.4	2.7	25.8	1.8
SFA:(MUFA+PUFA)	2.7	0.5	3.0	0.4	2.9	0.3
n3	0.4	0.0	0.4	0.0	0.3	0.1
n6	2.7	0.3	2.5	0.1	2.7	0.2
n6 : n3	7.4	0.8	7.1	0.9	8.8	2.7

[1] Expressed in fatty acid methyl ester %

The ratio of the individual fatty acids was also evaluated in the function of time after calving. The ratio of some long-carbon-chain saturated fatty acids being present in small amount changed between the third and the fifth months of lactation (Table 5). The odd number carbon containing undecanoic acid and pentadecanoic acid reached a maximum in the fourth month.

Table 5: The fatty acid composition[1] in the average of the six breeds (n=6)[2]

Fatty acid		3rd months		4th months		5th months	
		Mean	SD	Mean	SD	Mean	SD
Butyric acid	4:0	0.66	0.16	0.60	0.07	0.60	0.16
Caproic acid	6:0	1.14	0.12	1.14	0.04	1.19	0.06
Caprylic acid	8:0	1.08	0.12	1.05	0.06	1.12	0.07
Capric acid	10:0	3.30	0.65	3.14	0.40	3.44	0.35
Undecanoic acid	11:0	0.36[a]	0.06	0.48[b]	0.10	0.34[a]	0.05
Lauric acid	12:0	4.53	1.11	4.24	0.56	4.54	0.55
Tridecanoic acid	13:0	0.32	0.12	0.38	0.12	0.24	0.03
Myristic acid	14:0	13.37	1.31	13.27	0.61	14.14	0.79
Myristoleic acid	14:1	0.95	0.08	1.18	0.25	1.00	0.12
Pentadecanoic acid	15:0	1.50[a]	0.15	2.06[b]	0.34	1.30[a]	0.06
Palmitic acid	16:0	35.20	2.77	38.09	2.63	35.87	1.70
Palmitoleic acid	16:1	1.37	0.29	1.65	0.49	1.28	0.12
Margaric acid	17:0	0.89	0.05	0.94[b]	0.11	0.82[a]	0.03
Stearic acid	18:0	10.11	1.28	8.91	2.34	10.20	1.41
Elaidic acid	18:1n9t	0.96	0.27	0.87	0.30	1.08	0.11
Oleic acid	18:1n9c	19.50	3.61	17.61	2.82	18.23	1.62
7-octadecenoic acid	18:1n7	0.91	0.13	0.83	0.14	0.75	0.06
Linoleic acid	18:2n6	2.35	0.25	2.14	0.10	2.28	0.13
Arachidic acid	20:0	0.15[a]	0.03	0.14[a]	0.03	0.19[b]	0.02
γ-linolenic acid	18:3n6	0.04	0.01	0.04	0.01	0.04	0.01
Eicosenoic acid	20:1	0.05	0.01	0.04	0.01	0.05	0.01
α-linolenic acid	18:3n3	0.30	0.04	0.29	0.05	0.26	0.07
c9,t11- conjugated linoleic acid	c9,t11-18:2	0.34	0.06	0.32	0.06	0.32	0.04
Heneicosanoic acid	21:0	0.03[a]	0.01	0.05[b]	0.01	0.05[b]	0.01
Eicosadienoic acid	20:2	0.03	0.01	0.02	0.01	0.05	0.03
Behenic acid	22:0	0.10	0.02	0.09	0.01	0.11	0.01
Eicosatrienoic acid	20:3n6	0.12	0.02	0.12	0.02	0.13	0.06
Arachidonic acid	20:4n6	0.21	0.01	0.22	0.02	0.26	0.06
Lignoceric acid	24:0	0.09	0.08	0.05	0.01	0.08	0.01
Docosapentanoic acid	22:5n3	0.07	0.01	0.07	0.00	0.07	0.01

[1] Expressed in fatty acid methyl ester %

[2] For superscripts, see Table 2.

Milk fat samples originated from Holstein-Friesian and Brown Swiss cows in the farm in Gödre did not differ significantly in the ratio of saturated and

unsaturated fatty acids (see "SFA/(MUFA+PUFA)" row in Table 6). The ratio of the unsaturated fatty acids (PUFA+MUFA) did not differ in the milk fat, but significant differences were detected in the unsaturated fatty acid pool: the milk fat of Brown Swiss contained significantly ($p < 0.001$) more PUFA than that of Holstein-Friesian. The sum of PUFA and MUFA did not differ between the two breeds because the surplus of PUFA in the milk fat of Brown Swiss (0.5% more than that of Holstein-Friesian) was compensated with the relative deficiency of MUFA (1.7% less than that of Holstein-Friesian).

The milk fat of Brown Swiss contained more n6 and also more n3 fatty acids than Holstein-Friesian, but this excess existed with the same ratio of n6 and n3, that is, the n6/n3 ratio was the same in the milk fat of these two breeds (Table 6).

Table 6: The crude fat content (w/w%) and the fatty acid composition[1] of milk obtained from the two dairy herds in Gödre (n=20)[2]

	Holstein-Friesian		Brown Swiss	
	Average	**SD**	**Average**	**SD**
Crude fat	3.9	0.8	4.0	0.7
SFA	67.1	7.4	68.4	6.4
MUFA	29.4	4.7	27.7	3.3
PUFA	3.5^{a}	0.4	4.0^{b}	0.5
MUFA+PUFA	32.9	5.1	31.6	3.9
SFA/(MUFA+PUFA)	2.0	1.4	2.2	1.6
n3	0.26^{a}	0.02	0.30^{b}	0.05
n6	2.8^{a}	0.3	3.2^{b}	0.4
n6:n3	10.6	14.1	10.7	8.1

[1] Expressed in fatty acid methyl ester %

[2] For superscripts, see Table 2.

In the case of saturated fatty acids, the milk fat of Brown Swiss contained significantly more undecanoic acid and myristic acid than that of Holstein-Friesian (Table 7). Regarding the components of MUFA occurring in small amounts, Brown Swiss had more myristoleic acid and eicosenoic acid. There was not significant difference in the ratio of oleic acid giving the main part of MUFA (23.0% for Brown swiss and 25.0% for Holstein-Friesian). In the case of the fatty acids of PUFA, the milk fat of Brown Swiss was richer in linoleic acid ($p < 0.001$), α-linolenic acid, and conjugated linoleic acid ($p = 0.002$) than

that of Holstein-Friesian. The surplus of these three fatty acids is responsible for the excess of PUFA in the milk fat of Brown Swiss related to Holstein-Friesian.

Table 7: The fatty acid composition of the fat of milk from dairy herds in Gödre, expressed in fatty acid methyl ester % (n=20)[1]

Fatty acid		Holstein-Friesian		Brown Swiss	
		Average	SD	Average	SD
Butyric acid	4:0	0.77	0.13	0.77	0.08
Caproic acid	6:0	1.13	0.12	1.13	0.11
Caprylic acid	8:0	0.97	0.11	0.98	0.13
Capric acid	10:0	2.69	0.49	2.75	0.38
Undecanoic acid	11:0	0.28[a]	0.06	0.32[b]	0.06
Lauric acid	12:0	3.46	0.72	3.75	0.47
Tridecanoic acid	13:0	0.20	0.04	0.25	0.11
Myristic acid	14:0	10.97[a]	1.31	11.79[b]	0.77
Myristoleic acid	14:1	0.79[a]	0.13	0.96[b]	0.23
Pentadecanoic acid	15:0	1.17	0.17	1.19	0.18
Palmitic acid	16:0	33.71	2.94	34.43	2.32
Palmitoleic acid	16:1	1.73	0.36	1.64	0.35
Margaric acid	17:0	0.77	0.08	0.72	0.08
Stearic acid	18:0	10.88	1.18	10.11	1.64
Elaidic acid	18:1n9t	1.80	0.26	1.92	0.24
Oleic acid	18:1n9c	24.95	3.94	23.03	2.48
Linoleic acid	18:2n6	2.46[a]	0.17	2.83[b]	0.26
Arachidic acid	20:0	0.15	0.03	0.16	0.06
γ-linolenic acid	18:3n6	0.09[b]	0.04	0.05[a]	0.05
Eicosenoic acid	20:1	0.09[a]	0.04	0.15[b]	0.05
α-linolenic acid	18:3n3	0.26[a]	0.02	0.30[b]	0.05
c9,t11- conjugated linoleic acid	c9,t11-18:2	0.43[a]	0.07	0.51[b]	0.08
Eicosatrienoic acid	20:3n6	0.11	0.05	0.12	0.06
Arachidonic acid	20:4n6	0.15	0.03	0.16	0.04

[1] For superscripts, see Table 2.

In the case of milk samples originated from the farm situated in Magyarszék, the fat content of the milk of Jersey proved to be significantly higher ($p < 0.001$) than that of Holstein-Friesian (Table 8). The milk fat of Jersey contained more saturated fatty acid than that of Holstein-Friesian. The milk fat of Holstein-Friesian contained significantly more PUFA and unsaturated

fatty acid (PUFA+MUFA) but the MUFA-surplus was not significant at 95% level ($p = 0.052$). The milk fat of Jersey contained 4.2-fold more saturated fatty acid than unsaturated fatty acid SFA/(MUFA+PUFA); the value of this ratio (3.4) was significantly smaller for Holstein-Friesian. The milk fat of Holstein-Friesian contains a significantly higher ratio of n3 fatty acids than that of Jersey. Although the n6 fatty acids surplus in the milk fat of Holstein-Friesian did not prove to be significant, the p-value is on the limit value ($p = 0.05$). However, the n6/n3 ratio did not differ among breeds ($p = 0.340$).

Table 8: The crude fat content (w/w%) and the fatty acid composition[1] of milk obtained from the milking herds situated in Magyarszék (n=5)[2]

	Holstein-Friesian		Jersey	
	Average	**SD**	**Average**	**SD**
Crude fat	3.5[a]	0.8	7.0[b]	0.3
SFA	77.1[a]	2.3	80.6[b]	1.9
MUFA	19.6	2.2	16.7	1.7
PUFA	3.4[b]	0.4	2.7[a]	0.2
MUFA+PUFA	23.0[b]	2.3	19.4[a]	1.9
SFA/(MUFA+PUFA)	3.4[a]	0.4	4.2[b]	0.5
n3	0.71[b]	0.09	0.53[a]	0.06
n6	2.3	0.4	1.9	0.2
n6/n3	3.3	0.6	3.6	0.4

[1] Expressed in fatty acid methyl ester %

[2] For superscripts, see Table 2.

Saturated fatty acids, which are responsible for the saturated character of the milk fat of Jersey, appear from the evaluation of the individual fatty acids. In the group of saturated fatty acids, the excess of caproic acid and lauric acid is significant in Jersey (Table 9). Among unsaturated fatty acids, oleic acid – giving the bulk amount of MUFA – occurs in higher amount in the milk fat of Holstein-Friesian than that of Jersey. The same situation emerged in the case of the α-linolenic acid – being present in high ratio within PUFA – and docosapentaneoic acid.

Table 9: The fatty acid composition[1] of the fat of milk from dairy herds in Magyarszék, (n=5)[2]

		Holstein-Friesian		Jersey	
		Average	**SD**	**Average**	**SD**
Butyric acid	4:0	4.64	0.54	3.52	0,96
0.96 Caproic acid	6:0	1.16[a]	0.07	1.28[a]	0.07
Caprylic acid	8:0	1.02	0.09	1.10	0.09
Capric acid	10:0	2.90	0.44	3.38	0.35
Undecanoic acid	11:0	0.33	0.06	0.34	0.02
Lauric acid	12:0	3.78[a]	0.62	4.68[b]	0.44
Tridecanoic acid	13:0	0.24	0.09	0.28	0.03
Myristic acid	14:0	12.61	1.08	12.64	0.60
Myristoleic acid	14:1	0.92	0.26	0.89	0.13
Pentadecanoic acid	15:0	1.60	0.38	1.44	0.09
Palmitic acid	16:0	39.21	2.09	41.64	2.66
Palmitoleic acid	16:1	1.61	0.47	1.81	0.29
Margaric acid	17:0	0.88	0.12	0.83	0.04
Stearic acid	18:0	8.29	0.92	9.14	0.85
Elaidic acid	18:1n9t	1.05	0.31	0.85	0.24
Oleic acid	18:1n9c	15.98[b]	1.91	13.16[a]	1.47
Linoleic acid	18:2n6	1.96	0.38	1.60	0.14
Arachidic acid	20:0	0.13	0.01	0.14	0.02
γ-linolenic acid	18:3n6	0.02	0.01	0.02	0.00
Eicosenoic acid	20:1	0.04	0.01	0.04	0.01
α-linolenic acid	18:3n3	0.61[b]	0.08	0.46[a]	0.04
c9,t11- conjugated linoleic acid	c9,t11-18:2	0.29	0.05	0.24	0.04
Heneicosanoic acid	21:0	0.05	0.01	0.04	0.02
Eicosadienoic acid	20:2	0.04	0.02	0.03	0.01
Behenic acid	22:0	0.09	0.02	0.09	0.02
Eicosatrienoic acid	20:3n6	0.12	0.02	0.09	0.01
Arachidonic acid	20:4n6	0.21	0.03	0.17	0.04
Lignoceric acid	24:0	0.12[b]	0.04	0.03[a]	0.01
Docosapentanoic acid	22:5n3	0.10[b]	0.01	0.07[a]	0.02

[1] Expressed in fatty acid methyl ester %

[2] For superscripts, see Table 2.

4 Summary

Summarizing the results, it can be stated that the milk fat of Jersey possessed the most saturated character among the six breeds under investigation. The milk fat of Brown Swiss – owing to the abundance of linoleic acid, α-linolenic acid, and c9, t11-conjugated linoleic acid – was richer in polyunsaturated fatty acids than that of Holstein-Friesian (cows at the farm in Gödre). The milk fat of Brown Swiss contained more n6 and n3 than that of Holstein-Friesian (cows at the farm in Gödre); moreover, the milk fat of Holstein-Friesian was richer in n3 fatty acids than that of Jersey (animals at the plant in Magyarszék). Despite the differences in the absolute amount of n6 and n3 fatty acids, the n6/n3 ratio of milk fat did not differ significantly among breeds.

5 Acknowledgements

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Beneficial effects of probiotic microorganisms. A review

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Abstract. The aim of the present study was to describe the beneficial health effects of probiotic bacteria, manifested by inhibition of growth of pathogen microorganism strains and that of their colonization of the gastrointestinal tract of humans. The primordial mode of action by which a probiotic eradicates a pathogen can be facilitated by the production of antimicrobial substances such as bacteriocins, organic acids, and hydrogen-peroxide. Lactic acid bacteria and pathogens compete for receptor sites at the intestinal surface. Competition for these receptors will diminish the opportunity for pathogenic colonization and thus protect the host from infection. Many of the probiotic effects are mediated via immune regulation, in particular by the control of the balance of proinflammatory and anti-inflammatory cytokines.

Key words and phrases: probiotic microorganism, lactic acid bacteria, pathogen, competition, immune system.

1 General characterization

Probiotics are defined as viable microorganisms, which, in sufficient numbers, alter the microbiota of a host body compartment and thereby exert beneficial health effects (*Shida-Nanno*, 2008). The use of probiotics in enhancing intestinal health has been proposed for many years. As recently revisited by the Joint Food and Agriculture Organization/World Health Organization: probiotic strains are defined as "live microorganisms that, when consumed in an adequate amount as part of food, confer a health benefit on the host". Probiotic strains are considered non-pathogenic and safe (*Servin-Coccoiner*, 2003).

Lactic acid bacteria (LAB) are regarded as a major group of probiotic bacteria. LAB are usually described as Gram-positive microorganisms, devoid of cytochromes and preferring anaerobic conditions, but are aerotolerant, fastidious, acid-tolerant, and strictly fermentative, producing lactic acid as a main product. The most important genera are: *Lactobacillus*, *Lactococcus*, *Enterococcoccus*, *Streptococcus*, *Pediococcus*, *Leuconostoc*, and *Bifidobacterium*. Members of the LAB are usually subdivided into two groups based on their carbohydrate metabolism. The homofermentative group consisting of *Lactococcus*, *Pediococcus*, *Enterococcus*, *Streptococcus*, and some lactobacilli utilize the Embden-Meyerhof-Parnas (glycolytic) pathway to transform a carbon source chiefly into lactic acid. As opposed to homofermentors, heterofermentative bacteria produce equimolar amounts of lactate, CO_2, ethanol, or acetate from glucose exploiting phosphoketolase pathway. Members of this group include *Leuconostoc*, *Weissella*, and some lactobacilli (*Vasiljevic-Shah*, 2008).

2 Effect on lactose digestion

In humans, the most highly investigated aspect of probiotics in digestion is their compensation for lactase insufficiency. Numerous studies have shown that better lactose digestion occurs in lactose malabsorbers who consumed yoghurt rather than milk (*de Vrese et al.*, 2001).

Two hypotheses suggest that this effect does not correspond to a replacement of endogenous lactase by bacterial β-galactosidase. The gastric emptying of yoghurt has been found to be slower than that of milk, probably because of parameters such as viscosity or pH independent of the presence of bacteria. This delayed passage of lactose would give the residual endogenous lactase activity in the small intestine more time to hydrolyze the lactose. The second hypothesis is based on the fact that colonic microflora contribute to lactose

degradation in lactose maldigesters. Since LAB can stimulate colonic bacterial activity, it has been suggested that the beneficial effects of yoghurt in lactose malabsorbers result from an improved digestion of lactose in the colon. Even if these two hypotheses cannot be excluded, bacterial β-galactosidase probably cleaves lactose into galactose and glucose in the small intestine (*Fioramonti*, 2003).

3 Competition with pathogens

Many mechanisms have been postulated by which probiotics could enhance intestinal health, including competition for limited nutrients, inhibition of the epithelial and mucosal adherence of pathogens, inhibition of epithelial invasion by pathogens, the production of antimicrobial substances and/or the stimulation of mucosal immunity (*Servin-Coccoiner*, 2003).

Some probiotic bacteria with beneficial health effects have also been noted to adhere to the intestinal mucosa (Figure 1), suggesting that adhesive probiotics can prevent the subsequent attachment of pathogens, a phenomenon known as competitive exclusion.

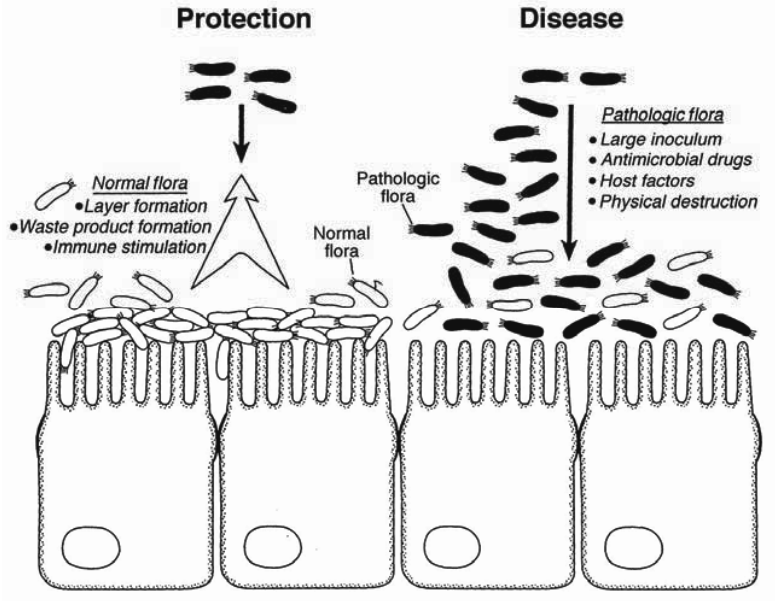

Figure 1: The mechanisms by which normal flora (probiotics) compete with gut pathogens (*Lu-Walker*, 2001)

The intestinal normal flora can enhance host defense by occupying the gut in large numbers and diversity, thus:

- preventing the colonization of the host by pathogens by competing for essential nutrients or epithelial attachment sites;

- producing antimicrobial compounds, volatile fatty acids, and modified bile acids that in turn create a luminal microenvironment unfavorable for the growth of pathogens;

- inducing recruitment of immune cells and activation of appropriate immune and inflammatory responses. Intestinal disease will occur when several factors that overcome these microenvironmental and immunologic responses disrupt the integrity of the epithelial defense by normal flora (*Lu-Walker*, 2001).

Two mechanisms of probiotic action have been identified to mediate the maintenance of the gastrointestinal microbial balance: production of antibacterial substances and competitive inhibition of pathogen and toxin adherence to the intestinal epithelium (*Vanderpool et al.*, 2008).

In vitro experimental studies have demonstrated that selected lactic acid strains are effective against diarrhoeagenic bacteria. By producing metabolites such as acetic and lactic acids, and thus lowering the pH, a large number of *Lactobacillus* strains inhibit the growth of bacterial pathogens. A strain of *L. lactis* selected for its ability to produce hydrogen peroxide, and *L. casei Shirota* or *L. acidophilus* YIT 0070, reduced the growth of *Escherichia coli* 0157:H7. The cell-free *L. casei* subsp. *rhamnosus* Lcr35 supernatant inhibited the growth of nine human pathogenic bacteria. Lactobacillus strains isolated from the human digestive tract have been found to inhibit the growth of four species known to be anaerobic bacterial aetiological agents of gastroenteric infections (*Servin-Coccoiner*, 2003).

Probiotic organisms are able to reduce the bacterial load and inflammation of *H. pylori* in animal and human studies. It has been suggested that the suppression effect is strain dependent. *L. casei Shirota* strain showed a significant reduction in the levels of *H. pylori* colonization. *L. johnsonii* La1 and *L. gasseri* OLL2716 were also found to reduce *H. pylori* colonization and inflammation. Similarly, *L. acidophilus* was able to inhibit the growth of *H. pylori*. Regular intake of yoghurt containing *Bifidobacterium animalis* Bb12 and *L. acidophilus* La5 may effectively suppress *Helicobacter pylori* infection

in humans. Several mechanisms regarding the effect of probiotics on *H. pylori* have been suggested including production of antimicrobial substances, enhanced gut barrier function, and competition for adhesion sites; however, the relative importance of these mechanisms is still unclear (*Vasiljevic-Shah*, 2008).

It was demonstrated that all bifidobacterial supernatants at pHs between 5.0 and 4.1 were able to produce strain-dependent growth inhibition of clostridia (*Trejo et al.*., 2006).

In the study of *Pan et al.*. (2008), there is characterized the potential probiotic *Lactobacillus acidophilus* NIT originally isolated in infant faeces. Results show that overnight culture of *L. acidophilus* was able to inhibit more pathogens than the supernatant and/or resuspended broth. The overnight culture had a strong inhibition to all the pathogens except *Clostridium histolyticum*. The reduction of *Escherichia coli* and *Salmonella typhimurium* adhesion to Caco-2 cells was more than 50% added with *Lactobacillus acidophilus* 10^8 CFU per well.

3.1 pH-lowering capacity

Probiotic bacteria, especially strains of *Lactobacilli*, produce acetic, lactic, and propionic acid that lower the local pH, leading to inhibiting the growth of a wide range of Gram negative pathogenic bacteria. Some *Lactobacillus* strains inhibit the growth of *Salmonella enterica* solely by the production of lactic acid. However, antibacterial effects of other strains of *Lactobacilli* may be the result of a combination of lactic acid and other unknown *Lactobacillus*-derived bactericidal substances by pH-dependent mechanism (*Vanderpool et al.*, 2008).

The analysis of organic acid composition of bifidobacterial supernatants shows that all strains under study produced lactic and acetic acid with the exception of two strains (539 and NCC 235) in whose supernatants acetic acid was not detected (*Trejo et al.*, 2006).

Short-chain fatty acids (acetic-, propionic-, and butyric acid), which are the fermentation products of saccharides, are repeatedly found in the colon of animals at various concentrations. Their presence in the human colon affects important biological processes, such as growth, metabolism, and differentiation of the intestinal epithelial cells; these processes are vital in maintaining the intestinal barrier integrity. Short-chain fatty acids, in particular butyric acid, affect the intestinal epithelial cell production of inflammatory cytokines that are pivotal to inflammation (*Koninkx-Malago*, 2008).

3.2 Bacteriocin production

Bacteriocins produced by LAB are classified into three main groups, lantibiotics being the most documented and industrially exploited. The groups are lantibiotics (Class I), nonlantibiotics, small heat-stable peptides (Class II), and large heat-labile protein (Class III) (*O'Sullivan et al.*, 2002).

Studies indicate that these probiotic-derived antibacterial substances exert their effects alone or synergistically to inhibit the growth of pathogens. The 2-component lantibiotics, produced by Gram-positive bacteria, such as *Lactococcus lactis*, are small antimicrobial peptides. These peptides have been found to be active at nanomolar concentrations to inhibit pathogens by targeting the lipid II component of the bacterial cell wall. Since lantibiotics are ribosomally synthesized and amenable to site-directed mutagenesis, they have the potential to serve as biological templates for the production of novel peptides with improved antibacterial functions. Other non-lanthionine containing bacteriocins are small antimicrobial peptides produced by *Lactobacilli* (*Vanderpool et al.*, 2008).

Analysis of the known genomic sequences of *Lactobacillus* strains including *L. plantarum*, *L. acidophilus* NCFM, *L. johnsonii* NCC 533, and *L. sakei* predicts a broad group of bacteriocins with divergent sequences. These peptides have a relatively narrow spectrum of activity and are mostly toxic to Gram-positive bacteria, including *Lactococcus*, *Streptococcus*, *Staphylococcus*, *Listeria*, and *Mycobacteria*. The primary mechanism of bacteriocin action is by forming pores in the cytoplasmic membrane of sensitive bacteria, but they can also interfere with essential enzyme activities in sensitive species. In addition, several strains of *Bifidobacteria* have been found to produce bacteriocin-like compounds toxic to both Gram-positive and Gram-negative bacteria (*Vanderpool et al.*, 2008).

The lantibiotic nisin naturally produced by *Lactococcus lactis* ssp. *lactis* is commercially available as food additive E234. The nisin variants A and Z, differing by one amino acid, are approved for use in foodstuffs by food additive legislating bodies in the US (Food and Drug Administration, FDA) and in the EU (*O'Sullivan et al.*, 2002).

Nisin binds to the carbohydrate moiety of the cell wall precursor lipid II, using it as a docking molecule prior to pore formation.

All forms of nisin are antimicrobially active against Gram-positive bacteria, such as LAB, *Listeria* sp., *Micrococcus* sp. and spore-forming bacteria like *Bacillus* sp. and *Clostridium* sp. The inhibiting mode of nisin towards vegetative cells consists of several phases. Nisin accumulates on the cell mem-

brane and penetrates into it, then aggregates within the membrane to form a water-filled pore (*O'Sullivan et al.*, 2002).

LAB capable of secreting antimicrobial peptides are used in a probiotic manner as food preservatives as well as health-promoting agents for humans and animals. Nisin applied as a food preservative extends the shelf life of a product. It is relatively stable in foodstuffs since 15-20% of nisin is lost in heat treatment. For probiotic purposes, bacteriocins are generally produced by a LAB strain in the product. The bacteriocin production is highest at the end of the exponential and early stationary phase. Some bacterial strains, such as *Clostridium botulinum* 169B and *Streptococcus bovis* JB1 are resistant to nisin. Resistance is assumed to be based on the enzymatic decomposition of nisin (*Breuer-Radler*, 1996).

3.3 Hydrogen peroxide production

The production of hydrogen peroxide (H_2O_2) is a widely accepted hypothesis to explain the inhibitory activity of LAB (*Charlier et al.*, 2009).

Servin-Coccoiner (2003) examined a strain of *Lactobacillus lactis*, selected for its ability to produce hydrogen peroxide. *L. casei Shirota* or *L. acidophilus* YIT 0070 reduced the growth of *Escherichia coli* 0157:H7. The production of hydrogen peroxide by LAB, particularly by lactobacilli, is antagonistic to *Staphilococcus aureus*. The production of hydrogen peroxide by LAB and its potential role in *S. aureus* inhibition is well-documented. Some lactobacilli strains are able to inhibit the growth of S. aureus by producing hydrogen peroxide at a concentration of 0.18 mmol/l. Hydrogen peroxide has a bacteriostatic effect at these concentrations and is bactericidal for concentrations up to 0.6 to 1.0 mmol/l. The authors concluded that hydrogen peroxide was involved in the capacity of these strains to inhibit *S. aureus* in mixed culture in laboratory media (*Vasiljevic-Shah*, 2008).

The use of *L. delbrueckii ssp. bulgaricus* in yoghurt may affect the survival of *L. acidophilus* and *Bifidobacterium* due to the acid and hydrogen peroxide produced during fermentation. The presence of oxygen (positive redox potential) in probiotic-containing products can have a detrimental effect on the viability of probiotics. Strains of *L. acidophilus* and *Bifidobacterium spp.* are microaerophilic and anaerobic, respectively. They lack an electron-transport chain, which results in the incomplete reduction of oxygen to hydrogen peroxide. Furthermore, they are devoid of catalase, thus incapable of converting hydrogen peroxide into water, the intracellular accumulation of hydrogen peroxide thus causing death of the cell was observed (*Vasiljevic-Shah*, 2008).

3.4 Competition at adhesion sites

Part of the beneficial effect of probiotics is reducing the establishment of pathogenic bacteria, such as *Salmonella*, a phenomenon called competitive exclusion. Probiotic bacteria interfere with the pathogen-receptor or toxin-receptor interactions. Preincubation of polarized monolayers of fully differentiated, villus-like Caco-2 cells with *Lactobacillus plantarum* MF1298 attenuated a decrease in transepithelial electrical resistance induced by *Listeria monocytogenes* (*Koninkx-Malago,* 2008).

Probiotics in the gastrointestinal tract decrease adhesion of both pathogens and their toxins to the intestinal epithelium. Several strains of *Lactobacilli* and *Bifidobacteria* are able to compete with pathogenic bacteria, including *Bacteroides vulgatus*, *Clostridium histolyticum*, *C. difficile*, *Enterobacter aerogenes*, *Listeria monocytogenes*, *Staphylococcus aureus*, *Salmonella enterica*, *Yersinia enterocolitica*, enterotoxigenic *E. coli*, and enteropathogenic *E. coli* for intestinal epithelial cell binding, and they can displace pathogenic bacteria even if the pathogens have attached to intestinal epithelial cells prior to probiotic treatment. However, specific probiotics or probiotic combinations should be selected based on their ability to inhibit or displace a specific pathogen (*Vanderpool et al.*, 2008).

Live and heat-killed *L. acidophilus* strain LB, which adheres to Caco-2 cells, inhibits adhesion to the brush border of diarrhoeagenic ETEC bearing colonization factor antigen CFA I or CFA II adhesive factors, in a concentration-dependent manner. Live and heat-killed *L. acidophilus* strain LB inhibits both cell association and the invasion of Caco-2 cells by enterovirulent *S. enterica* serovar *typhimurium*, EPEC, *Ersinia pseudotuberculosis* and *Listeria monocytogenes* in a concentration-dependent manner. Incubating Caco-2 cells with La1 was more effective before and during infection with enterovirulent *E. coli* than after infection by *E. coli*, indicating that La1 was able to prevent cell infection (*Servin-Coccoiner*, 2003).

Blockade of bacterial enterotoxin binding has also been demonstrated as a mechanism with therapeutic potential. The virulence factor of enterotoxigenic *E. coli* strains is a heat-labile enterotoxin that induces traveller's diarrhoea by binding to ganglioside GM1 on the surface of intestinal epithelial cells. By using a toxin-receptor blockade strategy, an engineered probiotic bacterium was generated to express glycosyltransferase genes from *Neisseria meningitides* or *Campylobacter jejuni* in a nonpathogenic *E. coli* strain (CWG308) (*Vanderpool et al.*, 2008).

Preincubation of *C. difficile* 43593 with neutralized supernatant of bifidobacterial strain CIDCA 5320 and CIDCA 5323 produced a decrease in the adhesion of clostridia to enterocytes (*Trejo et al.*, 2006).

The possible effect of *Lactobacillus gasseri* K7 strain to inhibit the adhesion of *Escherichia coli* O8:K88 to intestinal mucosa was studied by two models: on cultured Caco-2 cells and on small intestinal tissue of pigs. Lactobacilli were added simultaneously with *E. coli* (for competition assay), or the addition of lactobacilli alone was followed by the washing of unbound cells and the addition of *E. coli* cells (for exclusion assay). Preventive inoculation with K7 strain decreased the severity of infection and protected piglets against perishing, although it did not totally protect them against infection. Only the piglets inoculated with K7 strain did not show any infection symptoms and were in very good condition, which indicates the safe use of K7 strain. The applied lactobacilli probably prevented the adhesion of *E. coli* by competitive exclusion, which can include the occupation of specific *E. coli* binding sites or non-specific sterical hindrance of *E. coli* binding by lactobacilli (*Rogelj-Matijasic*, 2006).

In the study of *Candela et al.* (2008), strains belonging to *Bifidobacterium* and *Lactobacillus* were screened for their capability to compete with enteropathogens for enterocyte adhesion. The cell lines Caco-2 and HT29 were employed in adhesion experiments. It was demonstrated that *B. longum* Bar33, *B. lactis* Bar30, *L. acidophilus* Bar13, and *L. plantarum* Bar10 are effective in inhibiting the adhesion of *S. cholerasuis serovar typhimurium* and *E. coli* ETEC to Caco-2 cells. *L. acidophilus* Bar13 and *B. longum* Bar33 have the potential to protect intestinal cells from an acute inflammatory response.

Antagonistic effects of isolated lactic acid bacteria in our experiments were certificated using intestinal pathogen bacteria, which frequently cause food poisoning or intestinal diseases. Our isolated strains – exactly seven strains – produced metabolites, which have bacteriostatic effect on *Salmonella enteritidis* (*Both et al.*, 2009).

4 Stimulation of the immune system

The intestinal mucous membrane plays an important role in the exclusion and elimination of potentially harmful antigens and microorganisms and simultaneously provides the selective absorption of nutrients (*Herich-Levkut*, 2002).

The discovery that probiotics can stimulate an immune response provides a scientific basis for some of the observed probiotic effects. It was demon-

strated that administration of *L. casei* induced an increase in the production of circulating antibodies against *Pseudomonas aeruginosa*. Previous studies encouraged the use of certain lactobacilli as immunopotentiators. For example:

- it is important to know the type of immune cells that the LAB are able to stimulate, to know whether the induced immune response will be beneficial or not for the host (inflammatory or specific immune response);
- which is the most active strain;
- the dose required for maximum effect;
- when should it be administered;
- is it safe to use LAB or fermented milks in an immunosuppressed host (*Perdigon et al.*, 2001).

As mentioned above, probiotics serve not only to stabilize the gut microbiota but they can also potentially modulate the function of immune cells. Microorganisms in the gut lumen are recognized and processed by the immune system through several routes. a) Specialized epithelial cells called M (microfold) cells in the follicle-associated epithelium covering Peyer's patches or in the villi can take up probiotics directly by transcytosis. Macrophages (Mfs) or dendritic cells (DCs) are present immediately below M cells and then engulf probiotics and trigger immune responses. b) DCs in the intestinal lamina propria have been found to extend their dendrites between intestinal epithelial cells (IECs) and might directly sample and process probiotics in the gut lumen. c) Probiotics directly affect IECs to secrete an array of cytokines, which in turn modulate the immune functions of DCs, T cells, and B cells in the gut-associated lymphoid tissue (GALT) (Figure 2) (*Shida-Nanno*, 2008).

Macrophages and DCs exposed to probiotics can be observed to secrete a variety of cytokines. Chief among these are IL-12 and IL-10, which are key to controlling the balance of the immune response because the former augments cellular immunity whereas the latter suppresses inflammatory responses. Comparative analyses have revealed that the abilities of *Lactobacillus* strains to induce IL-12 production by macrophages are highly variable. Dietary supplementation with *L. rhamnosus* HN001 has been shown to increase NK cell number in humans and *Lactobacillus casei Shirota*-fermented milk enhances cytotoxic activity of NK cells (*Shida-Nanno*, 2008).

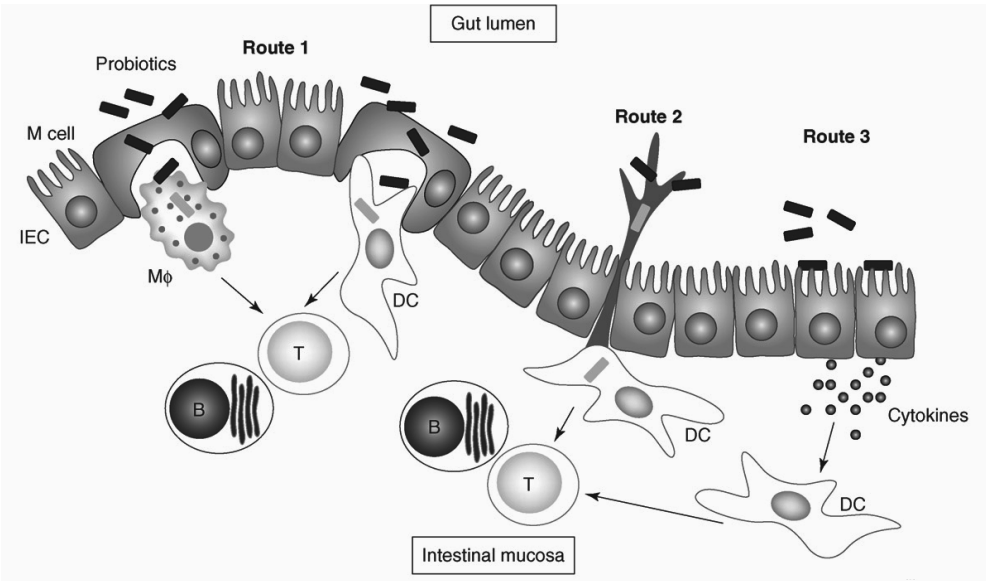

Figure 2: Three hypothetical pathways by which probiotics can trigger and modulate immune function in the intestine (*Shida-Nanno*, 2008)

There is accumulating evidence showing that dietary supplementation with probiotics augments innate immune functions including phagocytic activity of neutrophils and cytotoxic activity of NK cells. The activation of neutrophils and NK cells might be closely connected with the anti-infectious or anticancer abilities of probiotics. The abilities of lactobacilli to elicit IL-10 production from human DCs and PBMCs are weaker than those of bifidobacteria. That said, the addition of some *Lactobacillus* strains at high doses could induce high levels of IL-10, resulting in a decrease of IL-12 production in murine macrophage or DC cultures (*Shida-Nanno*, 2008).

Probiotics might also exert their anti-inflammatory activity by inhibiting the secretion of inflammatory cytokines such as IL-6 and IL-8. Furthermore, live but not heat-killed *L. casei* DN-114 001 cells could effectively downregulate spontaneous secretion of TNF-α by the inflamed mucosa of CD patients. Similarly, Caco-2 cells pretreated with live and heat-killed *L. rhamnosus* GG secreted much lower levels of IL-8 after stimulation with TNF-α (*Shida-Nanno*, 2008).

There are considerable differences in the ability of different probiotic bacteria to induce IL-12 and IFN-g. *Lactobacillus* and *Bifidobacterium* strains, which have previously been shown to stimulate IL-12 and IFN-g production in human PBMC, in the present study, were found to be relatively poor inducers of these cytokines. *S. thermophilus* and *Leuconostoc* strains used in the present study were extremely potent inducers of IL-12 and IFN-g, IL-10 was induced by *Bifidobacterium* and *Propionibacterium* strains whereas IL-10 production induced by *Streptococcus*, *Lactobacillus*, *Lactococcus*, and *Leuconostoc* strains remained at a low level (*Kekkonen et al.*, 2008).

There is a clear difference between Gram-negative non-pathogenic bacteria and LAB in their interaction with IEC. In direct interaction with IEC, both types of bacteria induce IFN-γ but the stimulating effect of LAB is restricted to the cellular surface molecule expression (*Herich-Levkut*, 2002).

Recently, IL-17-producing T helper cells (Th17 cells) have been regarded as crucial for the pathogenesis of several chronic inflammatory diseases. Th17 cells are abundant in the intestine; orally administered probiotics could affect the development of Th17 cells and ameliorate clinical symptoms. Regulation of Th17 cell functions might be the next target for probiotic modulation of the mucosal immune system (*Shida-Nanno*, 2008).

In sum, probiotics could potentially play a role in the control of the entire immune network by affecting diverse sets of immune-regulatory cells in the intestine.

The suppression of the formation of pro-inflammatory cytokines and chemokines in the presence of probiotics has been reported in several in vitro studies. The response of the immune system to a probiotic was weaker than in the presence of a Gram-positive pathogen. The cytokine response may vary greatly in the presence of different probiotics. The mixture of eight different probiotic and LAB strains including *L. acidophilus*, *L. delbrueckii ssp. bulgaricus, L. casei, L. plantarum, B. longum, B. infantis, B. breve*, and *S. thermophilus* upregulated the production of IL-10 (*Vasiljevic-Shah*, 2008).

In vitro immunomodulating capacity and mechanisms of LAB isolated from kefir grains and their individual supernatants by cytokine profiles were examined in the study of *Hong et al.* (2009). Time-dependent increases in cytokine levels were observed for both TNF-a and IL-6. There was no secretion of IL-12 and IL-1b for all treatments. The secretion of TNF-a, induced by isolated and reference strains – except for reference strain *Lb. kefiranofaciens* – was not changed compared to the kefir supernatant control whereas the production of IL-1b and IL-12 was significantly decreased. For IL-6, except for *Lb. kefiranofaciens* M1, low or no secretion was observed after co-cultured with isolated

strains. The secretion of IL-10, induced by isolated and reference strains – except for reference strain *Lb. kefiranofaciens* – was higher compared to the kefir supernatant control.

Bifidobacterium and *Lactobacillus* strains were assessed for their immunomodulating activity in the study of *Candela et al.* (2008). The cell lines Caco-2 and HT29 were employed in immune response experiments. The enterocyte-like Caco-2 cells have been extensively used to investigate the adhesion of probiotic bacteria to enterocyte. The enterocyte-like HT-29 cells represent a well-characterized model to study the enterocyte immune response to bacterial infection. Results show that neither probiotic strain induces the IL-8 secretion.

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Consumer attitudes to genetically modified foods – results of a nationwide study

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Abstract. Consumers today are becoming increasingly conscious and would like to make decisions in matters of shopping based on information they have collected. Secondary research has clearly shown that Hungarian customers reject GM foods. We used questionnaires in our primary research with 500 respondents. We analysed the first 13 from the 28 questions. More than 93% of the respondents had heard something about GM foods. They collect information mostly from television, newspapers and magazines are on the second place, Internet and radio on the third and fourth, respectively. When they hear the term 'genetically modified', they mainly think of an unnatural, unhealthy, and dangerous food product. Their impressions are definitely negative. A quarter of the respondents believe that GM technology has been used in human medicine for only a couple of years. They reject genetic modification entirely, especially in animals. It is crucially important to complement the missing facts, rectify incorrect information and publish scientific results so that consumers are well-informed, enough to be able to make a justified decision whether or not to buy GM food products.

1 Introduction

Research shows that the role of nutrition in human health is significant (*Berke*, 2004). Consumers are more and more aware of this fact; they are looking

Key words and phrases: genetically modified food, consumer attitudes.

for products which contribute to sustaining their health. Thus, we can say that today's consumers are becoming increasingly conscious and would like to make decisions in matters of shopping based on information they have collected. They are searching for manufacturers and products that they assume to be authentic; they are not willing to compromise in that (*Szakály*, 2008). This relates to genetically modified (GM) products, too. Secondary research has clearly shown that Hungarian customers reject transgenic foods. For example, according to the survey conducted by KÉKI (2002), 56% of the Hungarian consumers consider the general use of GM technology as harmful; among them, 30% consider it as very harmful (*Vajda*, 2005). According to results by Capital Research Institute, 62% of the consumers are afraid of the sale and consumption of GM foods. Today's conscious customers have the expectations connected to GM foods that they must not be harmful for their health but should have a positive influence on it. They feel it is important that scientists ease consumers' anxiety and that consumers can get enough information; for example, they would like to know about research results. More significantly, it is important for them to be able to make a choice between GM and conventional food products (*Bánáti*, 1999; *Szeitzné*, 2003).

Transgenic plants and food products with such ingredients are largely relevant today. It is a significant current issue nowadays characterized by a large amount of extreme and completely contradictory opinions and information. These products are available on the shelves of Hungarian shops (*Őri*, 2008; *Balogh et al.*, 2008); what is more, there have been food scandals connected to them, such as the GM rice found in Hungary. The important question is whether consumers have sufficient information in connection with these products. Besides, it is crucial whether they are able to make heads or tails of the large amount of contradictory views and information.

Therefore, our aim was to find out if consumers had heard anything about GM foods, and if they had, how much information they had, how authentic this information was, and what impression they had of these products.

2 Material and method

In our primary research, we used questionnaires with 500 respondents. Our research is nationally representative by age, gender, and region. The sample quota was taken on the basis of the 2001 census data of the Statistical Office. We selected the settlements randomly, but we were careful that at least one settlement per region was selected. The respondents were selected with

random-walk method based on the quota. The questionnaires were administered by students, PhD students, and teachers of universities and colleges in the selected towns. Respondents were anonymous and voluntary. The questionnaire listed closed-ended questions with one exception.

In the first half of the questionnaire, we enquired about the respondents' knowledge, information about and attitudes to GM foods. Then we asked about their willingness of consuming GM foods and their expectations in this respect.

This article publishes parts of our results. It analyses the first 13 questions of the total 28 found in the questionnaire.

The collected data were evaluated with SPSS 13. We calculated frequency distributions and means and carried out chi-squared tests. We examined the relationship between the different variables and background variables with the help of cross tables.

3 Result and evaluation

More than 93% of our respondents have heard something about GM foods. We will analyse their answers below. The number of respondents answering yes grew significantly with qualification. The same tendency was characteristic with monthly net average income per capita per household. Also, our results were significant by age; those who did not know anything about GM foods were primarily over 50 years of age.

Figure 1 shows where most respondents have heard about GM plants.

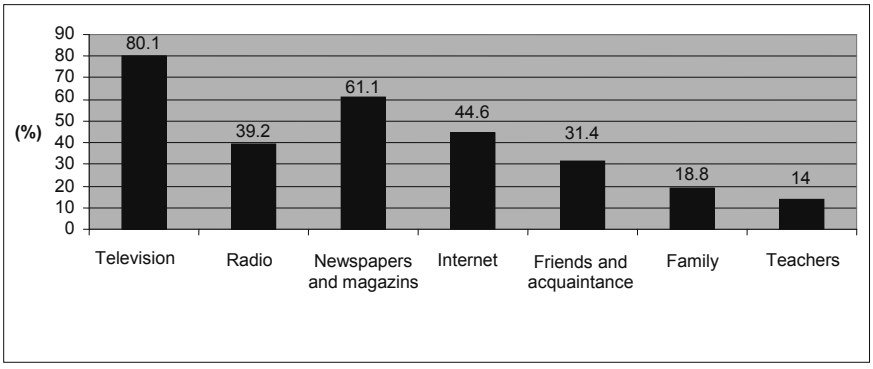

Figure 1: Consumers' source of information regarding GM foods (n=466) (%)

With regards to food products containing GM ingredients, most respondents collect information from television (80.1% mentioned it). With the development of communication technology, more and more channels are available for consumers. However, the primary source of information is still the television. This is shown by other researches, too, such as the study of Zoltán Szakály and his colleagues in 2008 (*Berke*, 2010), or the 2011 *Sonda Ipsos* research. Newspapers and magazines (61.1% mentioned them) are on the second place, the Internet on the third (44.6%), and the radio is on the fourth with slightly lower results: 39.2% (Figure 1). Women rather collect information from television (81.1% mentioned it), men collect information mostly from television (79.5% mentioned it), newspapers, and magazines (63.3% mentioned it), but many of them also indicated radio as a source of information. The higher their qualification was the more often they mentioned the radio, too. A lot of respondents collect information from the Internet, primarily those in their twenties and the middle-aged respondents. The number of respondents mentioning the Internet grows with higher qualification. Teachers' opinions might mean authentic information for students. Consequently, as television is the most preferred information source for consumers, it is this channel through which they should be provided with more information. For example, it would be important to broadcast professional debates and round-table discussions on this topic.

What comes to respondents' minds in connection with GM foods? Table 1 shows the most often mentioned associations.

Table 1: Impressions and associations in connection with GM foods (n=466).

What comes to your mind in connection with GM foods?

unnatural, artificial ♠
harmful (for health) ♠
corn, soy, potato, wheat . . . •
unhealthy ♠
dangerous ♠
interference with nature, against nature ♠
uncertainty, unpredictability ♠
money ♠
health ♦
science, development of technology ♦
biotechnology •

The following answers are on the first three places: transgenic food is unnatural and 'artificial' (10.7%), harmful (9.1%), unhealthy (6.3%), dangerous (6.3%), and against nature (4.4%). 8.8% of the respondents think of some GM plant when they hear the expression 'GM food', for example, corn, soy, potato, wheat. Corn and soy were mentioned primarily, which indeed belong to the most well-known and current GM plants. The reason for this is that – in addition to soy – we can hear mostly about corn in the news as it is the most widespread in the cultivation in the EU. Out of these corn types, MON810 is the most often mentioned, on which there has been a moratorium since 2005. Almost 100% of the soybeans sold in the market come from the American continent, where GM types are cultivated primarily. Other plants were also mentioned, such as tomato, which was the first GM plant (FalvrSavr tomato) on the market. Figure 2 shows that there are only negative associations with the exception of corn.

We wrote the words into the table in the order they were mentioned. Only the most often mentioned words were included in the table. The lines of the table were coloured on the basis of the colours used in Figure 2; namely, some of the words reflect positive (♦), negative (♠), or neutral (•) associations.

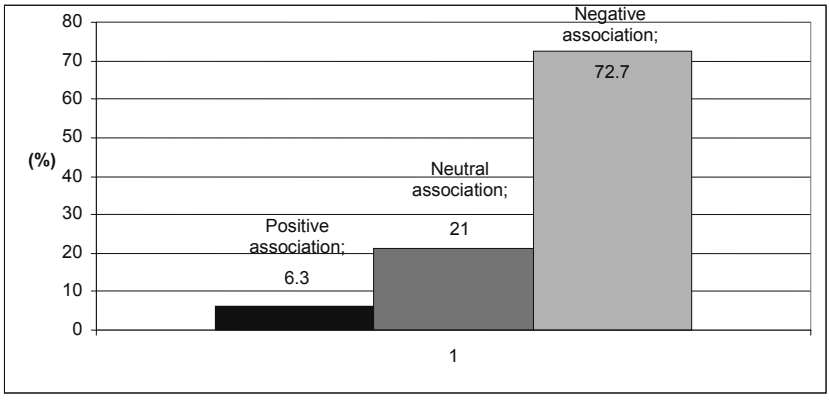

Figure 2: Associations in connection with GM foods (n=466)

The vast majority (72.7%) has negative associations in connection with GM foods; 21% has neutral associations; the latter mostly mentioned some plants or another word which was neither positive nor negative. 6.3% mentioned a positive word. With respect to the relationship between age and associations, we can say that respondents aged 18-29 had the most positive associations and respondents aged 50-59 had the most negative ones. By qualification, re-

spondents with primary school qualifications had the worst opinions. They are likely to have the smallest amount of information available about GM foods; this is why they reject them. Rejecting GM foods was more characteristic of families with children than young couples without children.

Respondents could choose several answers to the fourth question, namely, which GM plant they thought was cultivated on the largest area in the world (Figure 3).

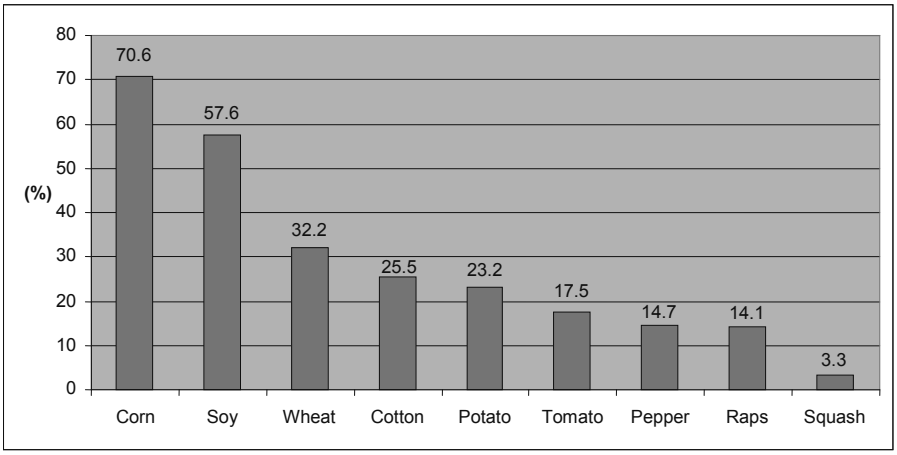

Figure 3: The GM plant cultivated on the largest area according to consumers (n=466) (%)

Most respondents chose corn (70.6%), soy is on the second place (57.6%), and wheat was often chosen, too (32.2%). Both men and women chose corn with more than 70%. The reason why it was so often mentioned is that this is the most often discussed topic in GMO news in Hungary. According to 2006 data, soy has the largest production area (58.6 million ha), corn has the second largest production area (25.2 million ha), cotton and rape are next with 13.4 million ha and 4.8 million ha, respectively (*Bánáti* and *Gelencsér*, 2007).

61.5% of the respondents said that GM foods are foods with ingredients that contain genes of another species, so they gave the correct answer. 21.9% of the respondents think that GM means a special manufacturing technology with which it is possible to keep the vitamin content of the food. The third answer, namely, that these foods contain a large amount of genes, was chosen by 7.2% of the respondents. The proportion of respondents giving wrong answers is still high (38.5%). With respect to the definition of the expression, the youngest

respondents are less informed and the respondents aged 50-59 are the most informed. Over 59 years of age, there is a decreasing tendency. The number of correct answers grows with qualification (except those with primary school qualification; this fact is probably due to the low number of respondents).

A significant proportion of the respondents (61.3%) suppose that genetic modification is used in human medicine, that is, in pharmaceutical industry. 25.3% of the consumers think that genetic modification has been used in pharmaceutical industry for only some years. Thus, in their opinion, this is an almost completely new method whereas it has been used in the pharmaceutical industry in addition to fermentation industry since the 1980s (*Bánáti* and *Gelencsér*, 2007). 25% of the respondents thought so (Figure 4).

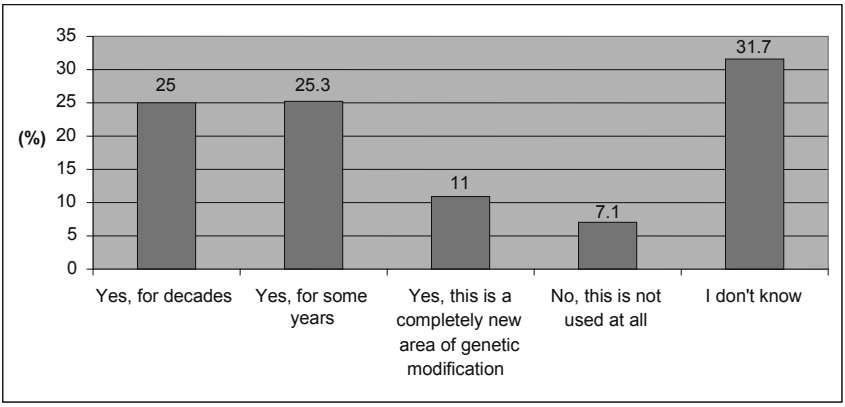

Figure 4: Consumers' opinion about the application of genetic modification in human medicine (n=465)

Table 2 shows that consumers cannot accept genetic modification at all. Most of all, they reject the genetic modification of animals.

Advocates of GM products often claim that such products might be a solution to the famine in the Third World. 51.4% of our respondents disagree with this argument; 81.9% would rather give them the unused cereals instead. They (56.6%) believe that only rich countries can profit from this; 22.3% think that also farmers can make a profit from the cultivation of such plants.

The majority (89.5%) agrees with the claim that until there is no sufficient scientific evidence regarding the safe consumption of such products, they should not be marketed. Considering, however, the opinion of respondents giving multiple answers (76.1%), the results show that they agree with the claim that research is still necessary in Hungary.

Table 2: The acceptability of genetic modification according to consumers (1 = strongly disagree, 5 = strongly agree)

	Mean	Standard deviation
The genetic modification of animals is unacceptable to me.	4.03	1.37
I cannot accept GM foods at all.	3.62	1.4
The genetic modification of plants is unacceptable to me.	3.74	1.36

According to 72.7% of the respondents, the spread of such plants endangers organic and conventional farming.

72.4% of the respondents think that the consumption of products containing GM ingredients might have unknown consequences.

4 Conclusions

The respondents are only partially informed about GM foods. Although some of them are trying to collect information, it is very difficult to digest this large amount of information as we tend to encounter a large number of contradictory views. Although conscious consumers are trying to make justified decisions, it is very difficult to stay realistic. Based on the information they have gained, and also emotionally, they have negative attitudes towards GM products. They consider them as artificial, unnatural, harmful for health, dangerous and risky. Consequently, consumers have no confidence in transgenic products.

There is a lot of distorted and incomplete information in different news items. It is the GM corn cultivated in the EU that is the best known. By contrast, soy is less often mentioned, although this is the GM plant that is most likely to be contained in foods in Hungary, too. The respondents cannot accept genetic modification, especially in animals. They trust scientific results; they would prefer to see scientific results as a preliminary condition of the distribution of such products. In addition, they fear that the spread of GM plants could endanger conventional and organic farming and they are also concerned about the environment. Nevertheless, they agree with the necessity of research.

It is a very important task to fill the information gaps, rectify the distorted information, and publish scientific results so that consumers are well-informed, enough to be able to make a justified decision whether or not to buy GM food products. Staff in shops should be knowledgeable about GM ingredients in particular products and consumers should be able to choose on this basis, too. Also, consumers should be able to understand the differences between the various products.

Thus, detailed and well-grounded marketing communication is essential in connection with genetic modification. Consumers' most important source of information is television; therefore, this channel should be exploited more for information; particularly, the scientific results should be published. In addition, it may be interesting to cover professional discussions and round-table talks. It would be worth editing a journal to be published twice a year which covers the most important news and results in this field. Schools (secondary and tertiary institutions) could get free copies while it would be available in other places, too. Thus, it could be a first step of informing and training teachers as they are an authentic source of information for their students.

There is a public revulsion against genetically modified products. A future study should examine consumers' expectations in this respect.

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Biofilm removal of *Pseudomonas* strains using hot water sanitation

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Abstract. Biofilm formation of *Pseudomonas aeruginosa* and *Pseudomonas stutzeri* and biofilm removal efficacy of hot water were examined. Both strains show different biofilm formation in BH broth and mineral water, but only a slight difference could be observed. Both *Pseudomonas* strain grew better in BH broth than in nutrient-poor medium. *Pseudomonas stutzeri* grow better in mineral water than *Pseudomonas aeruginosa*. There was no difference in attachment concerning the surface quality of the stainless steel coupons, irrespective of the growth medium. Hot water treatment reduced the number of planktonic cells with 4.5–6 log cycles, the number of adhered cells with 4.2–4.9 log cycles, and the number of biofilm cells with 2–3 log cycles, which was not sufficient to eliminate biofilms from the surface.

1 Introduction

The first report on biofilms was presented more then 60 years ago (*Zobell*, 1943). Biofilms are matrices of microorganisms embedded in their own microbial-originated extracellular polymeric substances (EPSs) attached to a solid surface or substratum. Biofilms are a concern in a broad range of areas, causing slime problems, reducing heat transfer efficiency in heat exchangers and condensers, great hygienic and financial concerns in the food, environmental

Key words and phrases: biofilm, hot water sanitation, *Pseudomonas*.

and biomedical fields (*Sihorkar* and *Vyas*, 2001; *Beczner*, 2001; *Maukonen et al.*, 2003). Biofilms are particularly problematic in food industry (*Frank et al.*, 2003; *Jessen and Lammert*, 2003). Biofilm bacteria are different from their planktonic counterparts (*Sauer et al.*, 2002). The attachment of bacteria to surfaces results in an increased (up to 1000 times higher) resistance to antimicrobial agents (*Stewart et al.*, 2000), and this makes their elimination from food processing facilities a big challenge (*Simoes et al.*, 2006).

Pathogenic bacteria are also able to form biofilms representing potential health risks (*Armon et al.*, 1997). *Pseudomonas aeruginosa* is a human opportunistic pathogen able to form biofilms on different biotic and abiotic surfaces, e.g. in water systems. It has been emerging as a primary source of nosocomial infections (*Kipnis et al.*, 2006), including infections of artificial implants, contact lenses, urinary cathetersacheal tubes (*Davey* and *O'Toole*, 2000). More than 60% of hospital-acquired infections are biofilm-related (*Ebrey et al.*, 2004). *Pseudomonas stutzeri* can be isolated from soil and surface water. Its presence in food and food environments is not regulated, but because of its good biofilm-producing ability it easily colonizes in pipes, heat-exchangers, air-conditioners, etc.; therefore, its growth should be controlled by sanitation procedures.

Bacteria associated with biofilms are much more difficult to kill and remove from surfaces than planktonic organisms. Biocides and disinfectants have been the principal weapons used to control unwanted biofilms. To kill and remove biofilm cells, cleaning procedures must break up or dissolve the EPS matrix of the biofilms to give the disinfectants access to the viable cells (*Simões et al.*, 2006). The removal of a mature biofilm most often requires extensive mechanical action, such as scrubbing or scraping in conjunction with the use of cleaning and sanitizing agents. However, care should be taken because some brushes and scrapers may be abrasive and leave scratches on surfaces, which promote biofilm formation. Disinfectants are less effective in the presence of organic material. Interfering organic substances, pH, temperature, water hardness, chemical inhibitors, concentration, and contact time generally control disinfectant efficacy (*Bremer et al.*, 2002). Chemicals must be rinsed off from surfaces, leaving no toxic residues. Hot water sanitation provides the green strategy for biofilm control. It has some advantages over chemicals: no chemical residues, no corrosion, and it can get to hard-to-reach areas. However, this practice is not advisable in some industries (e.g. dairy) because it aids in the formation of the conditioning layer by denaturing proteins and increasing the adhesion properties of the equipment.

The purpose of this study was to evaluate the removing and inactivating

effect of hot water sanitation on *Pseudomonas aerugonisa* and *Pseudomonas stutzeri* biofilms on stainless steel surface.

2 Materials and methods

2.1 Cultures

A culture collection strain of *Pseudomonas aeruginosa* (ATCC 9027) and a water source isolate of Pseudomonas stutzeri was used in the investigations. Strains were stored on agar slants at 4 °C. Strains were grown on BHI agar [brain extract, heart extract, and peptones 17.5 g; glucose 2.0 g; yeast extract 2.5 g; sodium chloride 5.0 g; di-sodium hydrogen phosphate 2.5 g; agar-agar 15.0 g; distilled water 1.0 l) (Merck 113825 0500)] at $30 \pm 2°C$ for 24–48 hrs.

2.2 Biofilm formation

Stainless steel coupons (30×9 mm) were used in the experiments. Stainless steel 75× was applied. Two types of stainless steel surfaces were used: stainless steel with smooth surface (SS) and stainless steel with matt surface (MS). A dilution of 106 cells ml^{-1} was prepared from each strain in 80 ml growth medium [BHI broth or still mineral water (MW)] in Petri-dishes (diam. 18 cm). Metal coupons were immersed and left in the growth medium containing the test microorganisms for biofilm formation. Twenty-four, 48, and 168 hours old biofilms of the strains were developed.

2.3 Hot water sanitation

After 24, 48, and 168 hours of biofilm formation, the coupons were rinsed with sterile water to remove unattached cells, and placed into hot water for 1 min, and rinsed with sterile water. The number of viable cells was counted as described in section 2.4. Other hot-water-treated coupons were then placed into sterile Petri dishes and poured with melted TGE agar (glucose 1.0 g; yeast extract 2.5 g; peptone 5.0 g; agar-agar 15.0 g; distilled water 1.0 l) (to detect any survival cells) as well as painted with acridine orange for microscopic investigations. Untreated coupons served as controls.

2.4 Detection of viable cell counts

Coupons were removed after 24, 48, and 168 hours from the Petri dishes containing BH or still mineral water, and rinsed with sterile water to remove

unattached cells. Coupons were placed into test tubes containing sterile glass beads and 10 ml sterile diluent. Tubes were vortexed for 2 minutes to remove attached cells from the surface of the coupons. Appropriate dilutions of these diluents were pour-plated and incubated at 30 °C for 24–48 hours. All measurements were carried out in duplicates.

2.5 Microscopic investigations

Coupons were investigated with epifluorescent microscope (Olympus BH-2, Olympus Optical Gmbh, Germany) after acridine orange (AO, 0.02 g/100 ml water) staining for 2 minutes.

2.6 Heat treatment of planktonic cells

A dilution of 10^6 cells ml^{-1} was prepared from each strain in 6 ml BH broth transferred into a 85 °C water bath for 1 minute (measuring the core temperature). The number of survival cells was determined with pour plating.

2.7 Heat treatment of cells on stainless steel surface

Volume of 0.1 ml of 106 cells ml^{-1} test cultures was spot-inoculated on the small stainless steel coupons. The surface was air-dried at room temperature. After drying, coupons were placed into Petri-dishes containing 85 °C water. Exposure time was 1 minute. Control coupons without heat treatment were also prepared as controls.

3 Results and discussions

3.1 Biofilm formation

Our studies were carried out under static conditions because they are as common as surfaces subjected to continuous liquid flow. Both strains showed biofilm formation on both stainless steel types although its quantity did not increase significantly with time (Figure 1).

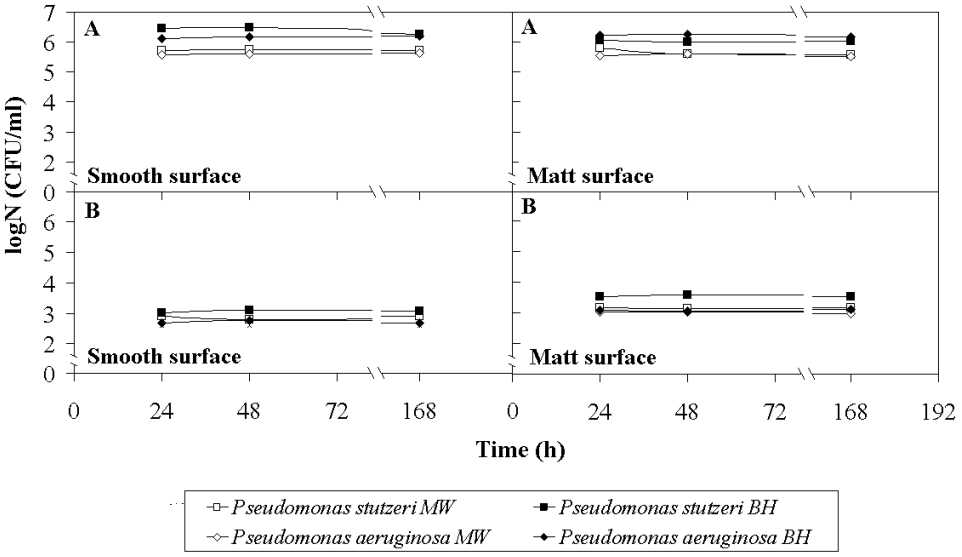
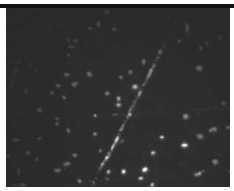
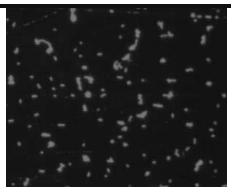
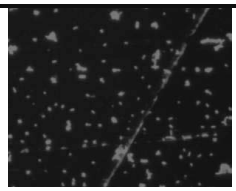
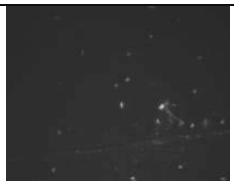
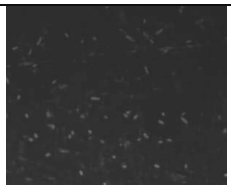
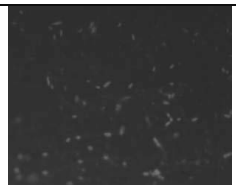
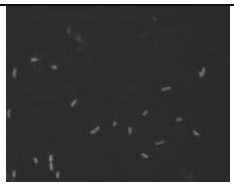
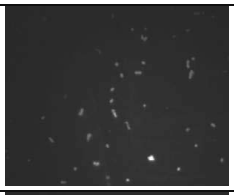
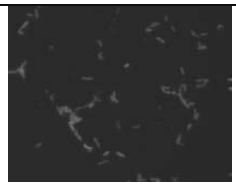
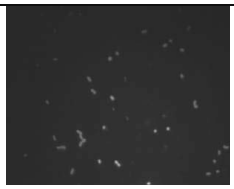
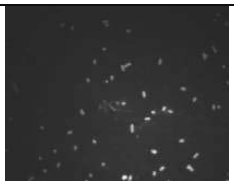
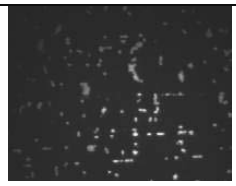


Figure 1: Viable number of irreversibly attached cells without (A) or with (B) hot sanitation treatment

Mays (2000) found similar degree of biofilm formation in bottled mineral water. Results showed that biofilm production was influenced by the growth medium used. Both strains grew better in BH broth compared to mineral water although many publications show that nutrient limitation enhances the attachment of cells to surfaces (*Poulsen*, 1999; *Ryu et al.*, 2004). On smooth surfaces, biofilm production of *P. stutzeri* in BH broth was higher compared to *P. aeruginosa* but the difference was not significant. *P. aeruginosa* produced higher amount of biofilm on matt surface in BH broth compared to mineral water while there was no significant difference in the case of *P. stutzeri*. The reason for that could be the water source origin of the *P. stutzeri* strain (Figure 1). This natural milieu provided good atmosphere for the adaptation of *P. stutzeri* cells to the harsh, low-nutrient-containing environment, and resulted in better biofilm formation ability.

The biofilm formation of the examined *Pseudomonas* strains was not affected by the surface quality of stainless steel coupons although sometimes significant attachments of bacteria to the cracks of stainless steel surfaces could also be seen. Some representative images obtained from microscopic observations of biofilm development during 6 days are shown in Table 1.

Table 1: Biofilm formation of Pseudomonas strains on stainless steel

	24 h	48 h	168 h
Ps. aeruginosa in BH			
Ps. aeruginosa in MW			
Ps. stutzeri in BH			
Ps. stutzeri in MW			

3.2 Hot water sanitation

Planctonic cells of *P. aeruginosa* showed a 6-log cycle and *P. stutzeri* a 4.5 reduction after hot water treatment. It was very effective against both strains in liquid medium. When *Pseudomonas aerugonisa* or *stutzeri* were adhered to stainless steel surface (cells attached to the surface reversible), the reduction was milder: 4.9 for *P. aeruginosa* and 4.2 for *P. stutzeri*, respectively. Biofilm cells showed the greatest resistance (3.2 log cycle reduction) against 85 °C water treatment, as it was expected (Figure 2).

It is well known that attached cells are much more resistant to antimicrobial treatments than their planktonic counterpart (*Norwood* and *Gilmour*, 2000; *González-Fandos et al.*, 2005; *Pap* and *Kiskó*, 2008). Reduction in mineral

water was more remarkable in the case of both *Pseudomonas* strains, which indicates a higher amount of glicocalix production in nutrient-poor medium (mineral water). In agreement with our work, other studies also showed that nutrient limitations result in higher glicocalix production, increasing the resistance of biofilm cells (*Brown* and *Hunter*, 1977; *Wrangstadh et al.*, 1986; *Kim* and *Frank* 1994; *Ryu et al.*, 2004).

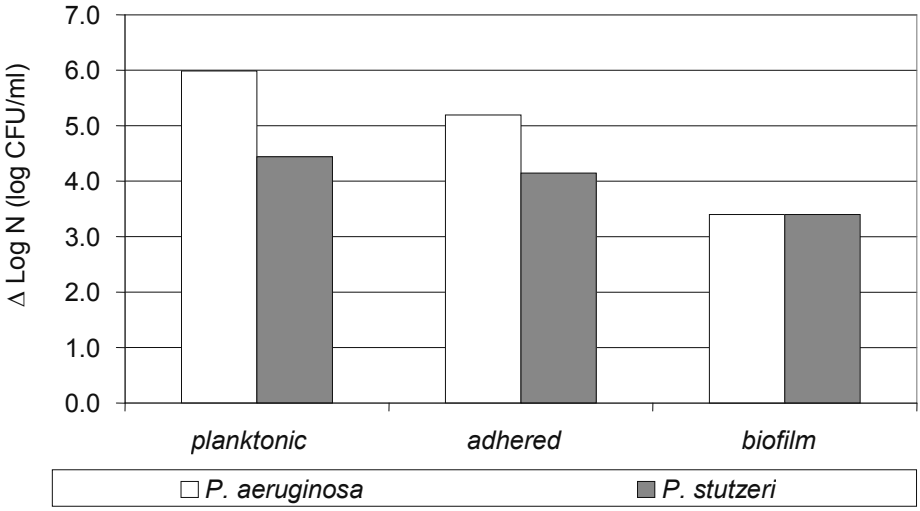


Figure 2: Heat inactivation effect of 85 °C hot water on planktonic, reversible adhered and irreversible attached (biofilm) cells

There was significant difference in heat resistance regarding stainless steel quality (Figure 3). Both *Pseudomonas* strains showed higher reduction on smooth surface. On matt surfaces, biofilm cells of *P. stutzeri* proved to be the most resistant.

Hot water treatment proved to be insufficient to destroy and remove *Pseudomonas* biofilms. A combination of heat and mechanical treatment or chemicals might be more successful. Results of *Jessen* and *Lammert* (2003) also suggested that individually applied disinfection treatments are not sufficient – they need to be used in combinations.

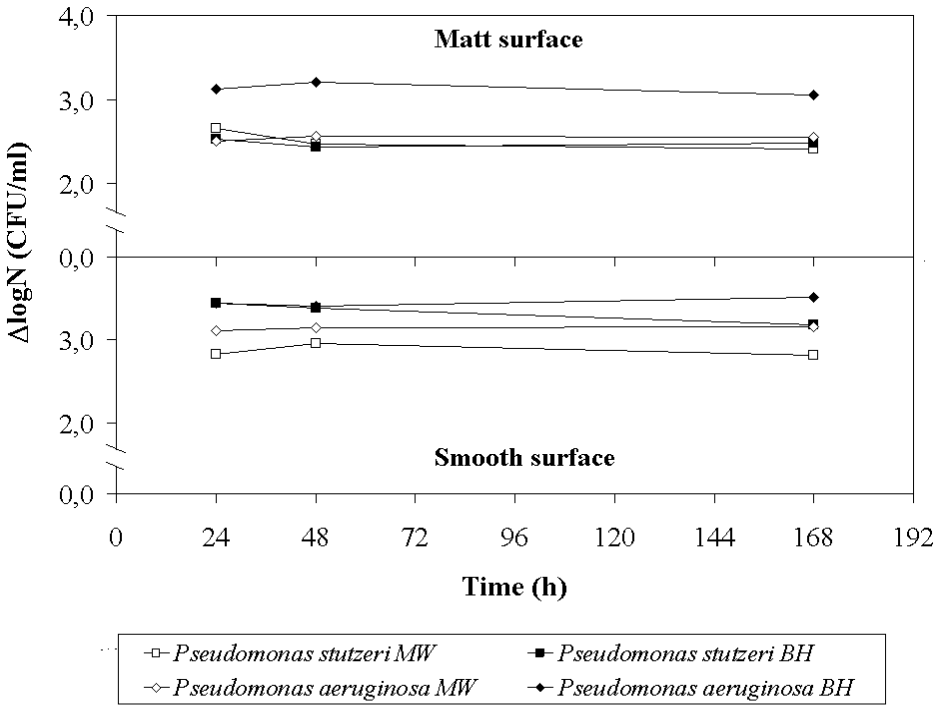


Figure 3: Heat inactivation effect of 85 °C hot water on biofilm cells on different quality stainless steel surfaces

4 Conclusions

Some equipment is designed to be sanitized by hot water or steam. It is successfully used in specific brewery, vinery, or dairy applications. Our results showed that although hot water sanitation reduced the number of *Pseudomonas* biofilm cells by about 3-log cycles, it left surviving cells, which enable further growth of biofilm. Therefore, it can not be considered an effective sanitation procedure unless its application is accompanied by further mechanical or chemical sanitation.

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The role of healthy nutrition in young peoples' life

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Abstract. In Hungary, nutrition plays a determining role in the development of the most serious chronic illnesses. Illnesses related/linked to nutrition/nourishment and lifestyle mean a serious problem. In Hungary, half of the total number of mortalities derives from cardiovascular diseases, a quarter of which comes from malignant tumour illnesses.

Besides these, childhood obesity means an increasingly bigger problem since the major part of obese children remain overweight also as an adult. According to some researches, 17.5 million children under 5 years are overweight in the world. There are twice as much obese children in the USA than they were in 1980; the number of obese adolescents has tripled since then.

The primary part of my research consists of a representative survey launched in the Southern Transdanubian Region. Eight hundred primary and secondary school students were involved in the survey in the county seats (Pécs, Kaposvár, Szekszárd) of the region.

Summarizing the results, it can be said that the parents' consciousness affects their child's values positively.

The most common sources of information are the parents, and they also occupy an outstanding place in terms of authenticity. They figure on the second place after the doctors and district nurses.

Key words and phrases: health, nutrition, young people, source of information.

1 Introduction

In Hungary, diet plays a key role in the development of the most serious diseases among the population. Diet- and lifestyle-related diseases cause an immense problem. In Hungary, coronary heart diseases account for half of all deaths and malignant tumour is responsible for 40% of deaths. As we know, all of these diseases causing death are related to nutrition. According to some researches, in the case of cardiovascular diseases, the effect of improper diet is over 30% while in the case of malignant tumours it is more than 35%. Besides these diseases, diet can also be brought into connection with obesity, diabetes, high blood pressure, osteoporosis, as well as, dental caries, food allergy, and intolerance [1]. As a result of this, every hour, 7 people die of obesity or of other obesity-related diseases in Hungary [2].

Childhood obesity is an extremely big problem since a significant number of children become obese when they grow up. According to the research of *Puska*, *Waxman* and *Porter* (2003) [3], 17.5 million children below 5 years of age are obese. In the United States, the number of obese children has doubled while among teenagers; this number has tripled since 1980 [4]. Childhood and teenage obesity has become a national economic problem of our days [5].

The main reason for obesity is the young peoples' improper health behaviour, a vital part of which is formed by nutrition habits. Children's nutrition habits start to develop at an early age, between 2 and 5 years of age; therefore, it is extremely important to take up proper eating habits since childhood nutrition habits can reduce the risk of adult age chronic diseases [6]. Teenage obesity has a 70% chance to result in adult age obesity. The chance increases to 80% if one or both of the parents are overweight or obese [7].

2 Materials and methods

During my secondary research, I studied mostly Hungarian and foreign specialized literature: special books, scientific journals, and I also analysed information available on the internet.

The primary part of my research consists of a representative survey launched in the Southern Transdanubian Region. Eight hundred primary and secondary school students were involved in the survey in the county seats (Pécs, Kaposvár, Szekszárd) of the region. The distribution of the questionnaires in the towns and within them, in the different school types (primary school, vocational school, technical school, secondary grammar school), according to

genders, was defined by the publication of the Central Statistical Office titled "The Main Data of Public Education in the Small Regions of Southern Transdanubia" [8]. The involved schools were selected arbitrarily, which was significantly influenced by the schools' willingness to take part in the survey. Within the schools, students from the 5th, 7th, 9th, and 11th grades were inquired. When choosing the age group, we followed the principles of the HBSC research to obtain comparable results. On the whole, we can say that the survey reflects the division rate of the school children attending different school types of the county seats of the Southern Transdanubian Region, and it is also representative concerning gender distribution.

The questionnaires were analysed with SPSS 13.0 programme. Significant corrections were counted (Pearson Chi^2 test) for the background variables (significance level: $p < 0.05$) using cross tables (Appendix 4), or in the case of the intervolume scales, variance analysis was applied using "ANOVA" tables.

3 Results and discussions

In the course of the representative survey, 800 primary and secondary school students were asked about their way of life and family patterns.

3.1 The importance of healthy nutrition

In the first group of questions, we asked the students to use school marks and judge how important healthy nutrition for them and for their father and mother is. The students who live in a one-parent family gave a zero mark in the case of the missing parent. We counted an average from the received results (Figure 1).

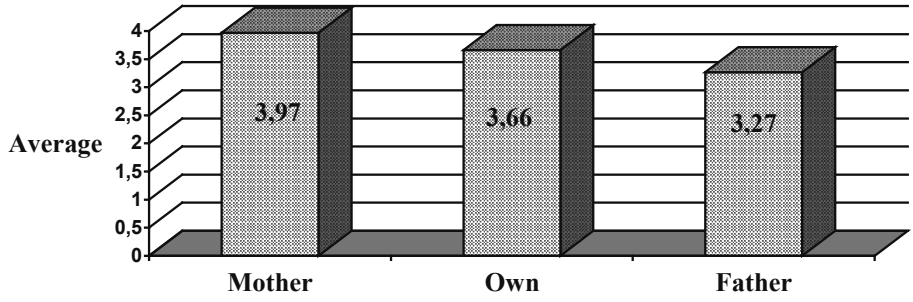

Figure 1: Importance of healthy nutrition

According to the results of the survey – within the family –, healthy eating is the most important for the mothers (3.97), they are followed by the children (3.66), and then the fathers (3.27).

3.2 Salubrity of nutrition and level of knowledge about healthy nutrition

In the next group of questions, the respondents had to estimate the wholesomeness of their own and their parents' nutrition. Similarly to the previous question, it can be seen that – within the family – the mother's nutrition is regarded to be the healthiest (3.57), they are followed by the children (3.23) and the fathers (3.02).

The respondent young people consider their state of health good (3.99), and they are followed by the mothers (3.72) and the fathers (3.40).

The existence of a link between healthy eating and the state of health is also supported by the results of our survey. The healthier people eat, the better their state of health becomes. It is due to the beneficial influence of nutrition on health, and is also due to a lot of diseases resulting from improper eating habits.

According to the surveyed young people, within their family it is their mother who knows most about healthy eating (4.04), they are followed by the children (3.78), and then the fathers (3.56).

The more the parents know about healthy eating, the higher the children's knowledge is.

Table 1: The importance of healthy nutrition, consciousness, knowledge, and state of health

Aspect	**Own judgement** (N=800)	**Mother's judgement** (N=793)	**Father's judgement** (N=771)
Importance of healthy eating	3.66	3.97	3.27
Healthiness of nutrition	3.23	3.57	3.02
State of health	3.99	3.72	3.40
Level of knowledge about healthy eating	3.78	4.04	3.57

It can be stated that the surveyed people consider healthy eating important (3.66); however, it can also be seen well that, compared to this, the healthiness of their diet (3.23) lags behind its importance. But the reason for this is not the lack of knowledge since – according to their judgement – their level of knowledge is higher (3.78) than both previous factors. The highest value was reached by the state of health: 3.99 average. A very similar tendency can be observed in the cases of both the mothers and the fathers; the difference is that here the state of health comes behind the level of knowledge.

Studying the unity of the families, we can say that – from the point of view of the importance of healthy eating, the healthiness of families and the level of knowledge – the mothers got the highest value; they are followed by the children, and then the fathers. The only exception from this is the state of health because, in this case, the children came first, and they are followed by the mothers, and then by the fathers. The possible reason for the fathers' worse judgement is that – within the families – it is mainly the mothers who are responsible for providing food, so the children have less information about their fathers' way of thinking. In every case, the results reflect the children's subjective judgement. Later on – in the next step of the research – we are planning to have the children and their parents fill in parallel evaluations in order to see how realistic the children's judgements are.

3.3 Perceived health index

Using the value obtained in the first four questions, we worked out a new index and called it perceived health index (PHI). The index is built on the following factors: the importance of healthy eating, the healthiness of eating, the sate of health, and the level of knowledge regarding healthy eating. An average was made of the four factors and the obtained results were defined in the following way: if the average value is between 1 and 2.50, then PHI is critical; if it is between 2.51 and 3.50, then it is mediocre; if it is between 3.51 and 4.50, then PHI is good and if the obtained average value is more than 4.51, then PHI is excellent. The value of PHI calculated for the whole family is 3.87, which means that the family's state of health is good. Counting the PHI for each member of the family, we obtained the results seen in Table 2.

Table 2: Perceived health index (PHI) in the family

Family member	N	PHI	
		Average	Standard Deviation
Child	800	3.80	0.78
Mother	793	4.06	0.77
Father	771	3.75	0.88

According to the applied index, the mother is the healthiest in the family. The PHI of the fathers and the children is almost the same; the difference between them is not significant. In our view, the mother's better result is due to that women are more interested in a healthy way of life, and in many cases, they do more to stay healthy.

3.4 The source of information related to healthy nutrition

We also wanted to find out information about young people's sources of information about healthy eating.

The three important sources of information are the parents (70.5%), the Internet (59.6%), and television (59.0%). It is a very good rate that seven out of ten young people turn to their parents for information about healthy eating. Doctors and nurses take the fourth place. Teachers occupy only the sixth place as only 39.7% of the children ask them for information about healthy eating.

When searching for detailed information on this issue, we were also looking for an answer to which of the listed sources of information is considered the most authentic by the surveyed children. They were allowed to choose only one of the already introduced answer categories. The obtained order can be seen in Table 3.

The order changed significantly. Doctors, nurses, parents, and the Internet proved to be the most authentic sources of information.

Table 3: Authenticity order of the sources of information (N=800)

Source of information	Head	%	Order	
			Authenticity	Frequency
Doctor/Nurse	317	39.7	1.	4.
Parents	140	17.5	2.	1.
Internet	115	14.4	3.	2.
Books	52	6.4	4.	7.
NK/NA	50	6.3	5.	13.
Trainers	36	4.5	6.	9.
Television	31	3.9	7.	3.
Teachers	21	2.6	8.	6.
Newspapers	15	1.9	9.	5.
Friends	7	0.9	10.	8.
Radio	6	0.7	11.	10.
Brother or sister	6	0.7	11.	11.
Other	4	0.5	13.	12.

4 Conclusions and recommendations

According to my results, it can be stated that the respondents consider healthy eating important and moderately important, but compared to this, the healthiness of their diet is behind its importance. The reason for this is not in the lack of their knowledge. According to the respondents, sometimes, the parents are also responsible for the children not being conscious enough (e.g. the children often get the sweets from their parents). The survey demonstrated that the importance of healthy diet is not significant enough in the families.

Although within the family, the mother has the greatest influence, we must not forget the fathers' role either since they also serve as a role model for their children. Their importance is supported by that 7 in 10 young people obtain information about healthy eating from their parents. The parents are given a serious task with this since they play an important part in providing their children with clear and relevant information.

According to the respondents, the most authentic sources of information are the doctors and nurses, but despite this, they only take the fourth place among the sources of information under consideration. Consequently, it becomes necessary to ensure the students the possibility to communicate with them more frequently.

Stressing the necessity of using the Internet is also a significant task since this is the second most frequently used source among the students, and it also takes a prominent place from the point of view of authenticity. However, it is important for the students to be able to select from the information available on the Internet since there are loads of articles and notes without any scientific basis.

Considering the obtained results, it would be important to forward the information not only to the children but it is also important to enhance the parents' consciousness, as well, since the results prove that the parents' consciousness has a great influence on their children's way of thinking – and the parents' consciousness highly depends on their education and knowledge.

In order to improve the parents' knowledge, brochures should be published, making use of the possibilities offered by collective marketing, or even lectures could be given at parents' meetings, or at other events where the emerging issues could be answered.

Children should be provided a lot more opportunities to communicate with doctors and nurses. They could also be supported by the schools so that the children are given the possibility to visit their doctors or nurses not only when they are ill but whenever they have any other problems too. Children would need more consulting hours with school doctors or nurses to be able to discuss healthy lifestyle, or if necessary, the different ways of body weight reduction or increase.

Since the Internet is the information source used by them most frequently, children could also be provided the chance to communicate with a school doctor or nurse in such a way. This way of communication could ensure anonymity for the children, which is very important for children of this age. Anonymity would make possible for them to get informed about topics that otherwise they would not dare bring forward, or would not want to ask, e.g. alcohol consumption, smoking, use of drugs, or sexuality.

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Potential impact of the climate change on the risk of mycotoxin contamination of agricultural products in Southeast Central Europe

A mini-review

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Abstract. Reports and modelling simulations by expert bodies, specialized institutions of the United Nations as well as everyday observations show that climate changes are occurring in our present epoch. Global warming and meteorological extremities, such as droughts, or even torrential rains, floods, and increasing frequency and duration of "heat waves" are in connection with this climate change, which is one of the greatest challenges of the 21st century. The climate change affects directly and significantly the agricultural production, diminishing thereby food security as well as risking public health, including the safety of our food and water sources. Drought stresses reduce the phytoimmunity of crop plants and extreme precipitations and heat waves increase the opportunity of growth of plant pathogenic microorganisms. Regarding food

Key words and phrases: climate change, Southeast Central Europe, food safety, mycotoxins, risk adaptation.

safety problems in connection with climate shifts, the mycological safety of foods and feeds is a subject of eminent importance because the factors mentioned previously could increase the contamination with toxigenic moulds and could increase the production of mycotoxins both prior to and after harvest. Species of the most important toxigenic moulds belong to the genera *Aspergillus*, *Fusarium*, and *Penicillium*. In the geographic region concerned in this review, various cereal grains, particularly maize and wheat, fodder plants, spice paprika as well as certain fruits such as apples and grapes plus their processed products are particularly important from this point of view. The growth and toxin production of such moulds are influenced very much by the eco-physiological factors, mainly the temperature and water activity of the attacked crops and products. Therefore, the relative risks of occurrence of specific moulds and their mycotoxins may change with the changing climatic conditions. Due to the so-called "mediterranization" of the geographic area concerned in this review, toxigenic moulds, formerly known as tropical and sub-tropical species may invade this region, which was a temperate climate in the past centuries. A project sponsored by the European Food Safety Authority investigated the possibility and modelling, predicting and mapping of the emergence of aflatoxin B1 in the European Union due to climate change. The main conclusion of the project consortium was that the risk of aflatoxin contamination is expected to increase in maize. In the case of the +2 °C temperature increase scenario as compared to the actual current temperatures, there is a clear increase in aflatoxin risks in the southern European countries, and low and medium risks at harvest in the four main maize producing countries (i.e. Romania, France, Hungary, and Northeast Italy). Several local investigations from countries in our region actually showed an increasing occurrence of *Aspergillus flavus* strains. Regarding *Fusarium* toxins, it can be expected that producers of fumonisins, such as *F. verticillioides* will also become more frequent. *F. graminearum* has become dominant in Europe instead of the previously dominant *F. culmorum*. Recently, so-called black aspergilli, known as ochratoxin and fumonisin producers, have been detected in Hungary also in onions and grapes.

1 Introduction

Reliable reports of expert bodies, specialized institutions of the United Nations as well as everyday observations show that climate changes are occurring in our present epoch. According to the Intergovernmental Panel on Climate Change (IPCC) and the World Meteorological Organization (WMO), a global warming is ongoing, which may be connected with the ever increasing human activities

of a tremendously growing world population since the commencement of the industrial revolution (*IPCC*, 2007; *WMO*, 2010). Meteorological extremities such as droughts, or even torrential rains, floods, and increasing frequency and duration of "heat waves" are in connection with this climate change. It is expected that such phenomena will increase during the coming decades (*Meehl and Tebaldi*, 2004; *Barriopedro et al.*, 2010).

The climate change affects directly and significantly the agricultural production, diminishing thereby the food security as well as creating public health risks, including the decreasing safety of our foods (*Páldy et al.*, 2004; *WHO*, 2008; *Miraglia et al.*, 2009). The present short review attempts to raise attention about a specific but important segment of these problems, with particular emphasis on the climate shift in Southeast Central Europe.

2 Climate change in Southeast Central Europe

The global warming tendency (*BEST*, 2011) and increasing contaminations and stress effects in relation to the meteorological extremities, the safety of foods and feeds as well as that of the water sources may increase infections and poisonings in this region (*Farkas and Beczner*, 2010). Drought stresses reduce the phytoimmunity of crop plants and extreme precipitations and heat waves increase the opportunity of growth of plant pathogenic microorganisms.

Ample evidence of these potential impacts are shown by documents and reports of the specialized agencies of the UN (e.g. *FAO*, 2008; *WHO*, 2008), the European Commission (e.g. *CEC*, 2009), and the relevant scientific literature (e.g. *Miraglia et al.*, 2009; *Tirado et al.*, 2010).

3 Mycological food safety

Regarding food safety problems in connection with climate shifts, the mycological safety of foods and feeds are a subject of eminent importance because the factors mentioned previously could increase their contamination with toxigenic moulds and could increase the production of mycotoxins both prior to and after harvest (*Cotty and Jaime-Garcia*, 2007; *Patterson and Lima*, 2010, 2011). Species of the most important moulds in this regard belong to the *Aspergillus*, *Fusarium*, and *Penicillium* genera. In the geographic region concerned in this review, various cereal grains, particularly maize and wheat, fodder plants, spice paprika as well as certain fruits, such as apples and grapes plus their processed products are particularly important from this point of

view (*Kovács*, 1998; *Fazekas et al.*, 2005; *Varga et al.*, 2005 a, b). The climate-stressed crops are increasingly sensitive to mould attacks and create increased mycotoxin risks thereby (*Guo et al.*, 2008). Consequently, moulds growing as plant pathogens and those that are mainly "post-harvest" mycotoxin producers in the food supply chain are of important hazards with special regard to aflatoxins, ochratoxin A, and fumonisins (*Farkas and Beczner*, 2009).

The growth and toxin production of such moulds are influenced very much by the eco-physiological factors, mainly the temperature and water activity (equilibrium relative humidity) of the attacked crops and products. Therefore, the relative risks of occurrence of specific moulds and their mycotoxins may change with the changing climatic conditions. It is an important consideration that the invasion by toxigenic moulds can be assisted also by those vectors, such as insect pests which migrate in relation to the climate shifts, too (*Patterson and Lima*, 2010). Due to the so-called "mediterranization" of the geographic area concerned in this review, toxigenic moulds, formerly known as tropical and sub-tropical species, may invade this region, which was of temperate climate in the past centuries. This is the reason why the Emerging Risks Unit of the European Food Safety Authority (EFSA) issued a call at the end of 2009 to perform a project to investigate the possibility and modelling, predicting and mapping of the emergence of aflatoxin B_1 in cereals in the EU due to climate change. The results of this project, performed by a consortium of Italian and Dutch experts, have appeared recently (*EFSA*, 2012). The main conclusion of the project consortium based on the predictive model developed for *A. flavus* growth and AFB_1 production linked to crop phenology data is that the risk of aflatoxin contamination is expected to increase in maize. In the +2 °C climate change scenario as compared to the actual current temperature, there is a clear increase in aflatoxin risk in areas such as the central and southern region of Spain, the south of Italy, Greece, North and Southeast Portugal, Bulgaria, Albania, Cyprus, and European Turkey. Besides high aflatoxin risk in these southern European countries, low and medium aflatoxin risk at harvest in the four main maize producing countries (Romania, France, Hungary, and Northeast Italy) were predicted. The aflatoxin risk indices above zero were defined as high when they were found between 141 and 180, medium between 101 and 140, and low between 41 and 100 AF risk index values. According to the risk maps for EU regions produced by the *A. flavus* AF model, wheat would present a negligible aflatoxin risk and rice no risk at all in the period between 2000 and 2010. The estimated AF risks cannot be quantitatively correlated with EU legal maximum levels of aflatoxin contamination. According to this important report and in agreement with a previous review (*Tirado et*

al., 2010) – in an agricultural context –, mycotoxin risk assessment should include a wider concept of risk evaluation. This is because "new mycotoxins could arise for new fungus and plant associations, making the occurrence of new risks or mycotoxins not yet considered as a new potential human and animal health threat".

Recently, several local investigations from countries in our region actually show an increasing occurrence of Aspergillus flavus strains capable of producing aflatoxins (*Torkar and Vengust*, 2008; *Tabuc et al.*, 2009, 2011; *Borbély et al.*, 2010; *Dobolyi et al.*, 2011).

Regarding *Fusarium* toxins, it can be expected that producers of fumonisins, such as *F. verticilloides* become more frequent related to rainfalls following dry periods. As a consequence of series of warm summers, *F. graminearum* has become dominant in Europe instead of the previously dominant *F. culmorum*. *Stroia and co-authors* (2010) found the presence of *Fusarium* species in all of the 56 samples of cereals (maize, wheat, barley, and oat) from Western Romania (different areas of Banat region), and the most frequent species were *F. graminearum* and *F. culmorum*. Besides DON and ZEA, *F. graminearum* produces NIV toxin (*Miller*, 2008). It is probable that formerly less known mycotoxins (e.g. moniliformin) may become important, too.

Recently, the so-called black aspergilli, known as ochratoxin and fumonisin producers, have been detected in Hungary also in onions and grapes (*Varga et al.*, 2007) while the appearance of *Pithomyces chartarum* as a pathogen of wheat was noted (*Tóth et al.*, 2007).

It can be concluded from this short review that due to the changing climate of the region concerned, one has to count continuously with the toxigenic species of *Penicillium* and *Fusarium* genera, and the species of both the widely spread aspergilli and those that were considered as associated with the warm areas may gain increasing importance. Preparing for an improved prevention of fungal infections and a better adaptation to the increasing mycotoxin risks, it is important to support investigations/researches for the better understanding of the eco-physiological connections of growth and the toxigenesis of moulds.

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