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Influence of season and cows farming system on milk physical, chemical and hygienic traits

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Abstract

Raw cow milk quality and safety traits have been surveyed on samples collected from 67,703 dairy cows (breeds Friesian, Simmental, Brown and Pinzgau). Fat and proteins levels were higher during cold months than in summer ($p < 0.001$). The bacterial count (BC) indicated better milk hygiene in autumn and winter vs. warm seasons ($p < 0.001$). Annual mean reached 70.38±0.37 germs/ml x 10^3 (below the EU limit, 100 germs/ml x 10^3). The somatic cells count (SCC) followed the same trend, the annual level of 281.55±1.15 cells/ml x 10^3 was within the admitted range of 400 cells/ml x 10^3 (results confirmed by the enzymatic reductase tests: 1st class – Good quality milk). No antibiotics were detected. More germs were detected in cubicle accommodation system (bound cows; 75.83±0.48 germs/ml x 10^3) compared to the straw yard farms (unbound cows; 42.51±0.24 germs/ml x 10^3 ($p < 0.001$), as well as higher amount of somatic cells (308.74±1.52 vs. 172.65±1.06 cells/ml x 10^3). The milk complied with the safety standards. The state of facts could be improved in the surveyed territory, especially in small and medium sized farms, through the observation of good hygiene and management practices prior, during milking and along the dairy logistic chain.

Keywords: cow milk, bacterial count, somatic cells count, seasonality, farming system

1. Introduction

The quality of food products, primarily milk and dairy commodities, is a main concern in food safety and market economy. The rich biochemical and nutritional content of milk, which includes water, proteins, carbohydrates, lipids, macro and micro elements, as well as enzymes and vitamins, makes it a very favorable environment for the growth and development of contamination micro-organisms, some of which may have a harmful impact on consumers safety and on technological and commercial traits of the products, by producing multiple sensorial, chemical and microbiological depreciations or deviations (OLIVER et al. 2005 [24], HERVERT et al. 2016 [14]).

Usually, in dairy farming, the technological and physicochemical defects occurring in milk are quantitatively and qualitatively insignificant and easy to tackle, compared to the inner microbiological content of the milk, which is more difficult to manage (MURPHY & BOOR, 2000 [21]). The purpose of this study was to focus on the flaws that are technological or hygienic in nature, to draw attention on the difficulties existing in dairy farms and storage/processing facilities, with particular concern for the countryside households which provide a great amount of milk of uncertain quality and safety (ATASEVER et al.2012 [5], ALLORE et al., 1997 [2]).

The last few decades have seen an emergence of specialized studies that emphasize possible risk factors to consumers of milk and dairy products. Such reports focused on the persons who consume milk with a large content of germs and somatic cells and that use milk from

cows that were stimulated with hormones may develop certain diseases or even certain forms of cancer (Baer et al., 1989 [6], EPSTEIN, 1996 [8]). Most of the studies published over the last decades refer to the risks of drinking milk from cows given bovine somatotropin to stimulate milk production. In 1993, the Food and Drug Administration (FDA) of the U.S.A. approved for animal husbandry purposes the use of the recombinant bovine growth hormone (recombinant bovine somatotropin – rbST or rBGH) (USFDA, 2009 [31]) that could cause up to a 20% increase in milk production. This hormone was obtained through genetic engineering on Escherichia coli manipulations. Studies have shown that rBGH stimulates the hepatic production of IGF which is excreted through milk; its concentration being ten times higher than normal (AMERICAN CANCER SOCIETY, 2011 [3]). In addition, the bovine IGF is identical to human IGF and is not destroyed through pasteurization. Moreover, certain technological processes applied during milk transformation into cheese try to filtrate the bovine IGF in order to reduce its transfer to consumers (AKBACHE et al., 2009 [1]). However, studies from the same authority (USFDA) also states that the use or rBGH in dairy cows husbandry practice is not harmful for consumers (USFDA, 2009 [31]). In spite of this, a meta-analysis reviewing epidemiological studies incriminated the IGF1 originated from milk in the onset of severe dermatological conditions, especially throughout puberty in Western countries populations, whose nutritional habits include high intakes of animal proteins and among these, the dairy products contribute substantially (CLATICI et al., 2015 [7]). In some US dairy farms, this hormone is given 150 days after calving to prevent abortion. For 60 to 70% of the cattle herds, the use of BST starts on the 60th day after calving and is re-used every two weeks until dry-off. Through this procedure, farmers seek to increase the average production of milk by 5 to 7 kg per day per cow (Baer et al., 1989 [6], Epstein, 1996 [8]).

In the EU countries the use of hormones for the stimulation of milk and meat production (EUROPEAN COUNCIL, 1999 [9]), as well as of antibiotics as growth/production improvers (EUROPEAN PARLIAMENT & COUNCIL 2003 [10]), has been banned for several years, not only in cattle but in other species. Progress has been made since Romania became part of the EU in 2007, concerning the quality of milk but producers still require additional transition period, in order to improve the quality of raw milk and implicitly the quality of collection system and hygiene in the dairy farms. The extension provided to farmers, especially to the small size exploitation must be constructive in order to apply measures and implement programs designed to improve the quality and safety of raw cow milk, the collection system and its modernization as well as development of farms according to European requirements and regulations on animals' welfare, good practices and safety (MACIUC et al., 2003 [18], MACIUC et al. 2008 [19]).

We have undertaken this work to analyze the quality of milk in an important area for milk production in Romania in order to survey and avoid undesired risks and their consequences. Our analysis relies largely on observational – transversal acquired data, issued from certain laboratory seasonal tests, rather than experimental data collected due to special experimental designed protocol in which controlled factors have been introduced. Most of the data issued from assessments carried on the raw milk collected in bulk by a large milk processing company in Romania, throughout the whole 2014 year. Therefore, situation described in the paper is just a still "X-ray" picture of some of the milk traits from those very moments when samples were taken and from those particular farms and collecting itineraries, while the situation in the territory is continuously changing. Consequently, the facts and figures revealed here below must not be considering as an overview of the Romanian dairy farms status related to milk quality and safety.

2. Materials and Methods

The survey has been deployed across 7 Romanian counties, in North-East and South-East areas using adequate means of samples collection and storage (thermoregulated tanks, collecting points with refrigerating equipment and reception sites at producers). The milk was sampled from 17 collection centers, 5 associations of cattle breeders, 148 small farms and 174 large farms including agricultural companies. The genetic material examined from this region was represented by 67,703 dairy cows belonging to breeds Friesian, Simmental, Brown and Pinzgau. Other authors (SCHUTZ et al., 1994 [27]) reported on the certain genetic variability of the milk quality traits, so the variety of genotypes in our study is quite normal.

More than 8900 samples were analyzed and classified into centers and producers, months and seasons, together with the analysis of qualitative aspects regarding the content of fat, proteins and dry matter, temperature, density, acidity and the pH value, reductase microbial enzymatic occurrence test, bacterial count (BC thousand/ ml) and the somatic cells count (SCC thousand/ml), water addition and the presence of antibiotics in the samples. The values of the aforementioned traits were assessed in biochemical, enzymatic and food safety laboratories. The apparatus included the EkoMilk Bond Total, EkoTest, Bactocount 70 and Somacount testers.

The content of fat, proteins and dry matter, temperature, density, water addition, acidity and pH value were determined with the EkoMilk Bond Total device, previously calibrated using analytical potentiometric, titrimetric and densitometry standard methods in analyzing milk traits. The occurrence of antibiotics was tested by using the EkoTest device. Each sample meant 10 ml of raw milk, taken after proper homogenization of the bulk milk.

Bactocount 70 was used to assess the total bacterial count per milliliter of milk (BC/ml). Milk samples were stored in 10 ml sterilized plastic bottles, placed in a stand holding twenty samples at a time and introduced into the rail-support of the device. Three milliliter aliquots of milk are taken from each bottle and transported through a hose into an incubator with an analysis plate of 23 wells. One milliliter of milk and one milliliter of acridine-orange solution are mixed in each well. The incubation lasts for 7-8 minutes at 45°C and after which the sample is extracted with a syringe and diluted with a solution containing three drops of Triton X and 1 ml of ammonia / 1 l of distilled water. Then it is passed through a 0.6μ polyvinyl propylene filter membrane from which 1 ml is collected and sent to a laser-reading/counting device.

The Somacount uses an automatic microscope technique for counting somatic cells. The device uses the fluorescent dye ethidium bromide which colors the somatic cells, inducing a chemical-colorimetric complex with DNA inside the nucleus. When samples are subject to blue light, the cells become fluorescent and emit red light. The principle is called the counting of fluor-optoelectronic cells (FOE). An automatic pipette is used to introduce 0.5 ml of milk sample in the inlet chamber. Here the milk is mixed with 9.5 ml of warm dye. After 10 seconds of reaction time, 40 μl of colored cells suspension is dispersed as a thin film on the edge of a rotating disk - smear for the automatic microscope. The magnification is 500X. The cells are counted, and the total is posted. A discriminator eliminates the false pulses of the background noise, bacteria, and other foreign particles in the milk, ensuring the counting of only epithelial udder cells.

Conventional enzymatic colorimetric tests were applied on 10% of the samples processed for total number of germs assay, in order to identify and quantify reductase in milk samples and to indirectly appreciate the hygienic milk quality and the estimate microbial quantum. The blue methylene dye reduction test was performed using 1 ml Blue methylene 5% as reducible color witness and 10 ml of raw milk poured onto it and mixed. The velocity of color

degradation after sample incubation at 37ºC indicated the amount of microorganism load in the milk and its hygienic quality (1st class – Good – decoloring in > 5h30' = less than 500 germs/ml x 10^3; 2nd class – Satisfactory – decoloring in 2h-5h29' = 500 – 4000 germs/ml x 10^3; 3rd class – Poor – decoloring in 20'-1h59'= 4000 – 20000 germs/ml x 10^3; 4th class – Very poor – decoloring in < 20' = above 20000 germs/ml x 10^3) (SINGHAL et al., 1997 [29], OLIVER et al., 2005 [24]).
The second enzymatic method involves resazurin as witness reagent and its principle consist in the assessment of the color and tint of the prepared sample (1 ml resazurin + 10 ml raw fresh milk, mixed together in a sterile tube) after 1 h incubation at 37ºC. Classification: 1st class – Good – ultimate tint blue steel = less than 500 germs/ml x 10^3; 2nd class – Satisfactory – ultimate tint blue-violet to violet-red = 500 – 4000 germs/ml x 10^3; 3rd class – ultimate tint pink and light pink = 4000 – 20000 germs/ml x 10^3; 4th class – Very poor – no tint, totally white = above 20000 germs/ml x 10^3) (GRIMAUD et al. 2009 [12], USTUROI, 2012 [32]).
The acquired data were processed with the SPSS 22 software for WINDOWS, following the statistics methods presented by SCHUTZ et al., 1995 [28].

3. Results and discussion

In Romania, as far as quality is concerned, cow milk must have a minimum fat content of 3.5%, a minimum protein content of 3.2 %, a minimum density of 1.029 g/cm^3, a titratable acidity within the 16-19°T range. The temperature must not be higher than 14°C, the maximum bacterial count (BC) up to 100 germs/ml x 10^3 and the maximum Somatic Cells Count (SCC) less than 400 units/ml x10^3 (EUROPEAN PARLIAMENT & COUNCIL, 2004 [11]).
The milk yields displayed in table 1 represents the monthly average quantities of raw milk delivered to the processing company, by the surveyed farms.
According to the season, the highest amount of milk was collected in spring (26.9%) and the lowest in summer (22.49%). While in the other seasons the amounts were approximately equal.
The analysis of the milk quantity on a monthly basis shows that in March (9.29%) and April (9.03%) the greatest amounts of milk were collected and in the summer months (June-July-August) the lowest amounts of milk were yielded.
The dairy processing company we used in our study collects milk in a share of more than 60% from farms belonging to the mountainous area where calving usually takes place in early spring. In summer, the cows are grazed up in the mountains.
Most farmers' associations process their milk at this company. This is how we explain the high amounts of milk in spring.
The pH value was the most constant qualitative trait, the average monthly values varying between 6.78 and 6.80. Between the months or seasons of the year there were no significant differences for this quality trait. The variation coefficient was far below 5%, suggesting strong homogeneity for this trait.
The measured values indicated normal balanced milk, without contaminants or altered compounds, as well as low probability of udder pathology casuistic, knowing the average value of healthy milk pH should be of 6.7 (OKIGBO et al., 1985 [23]). However, the achieved values indicate a slightly migration above this threshold and this could indicate

possible udder distress. Such supposition should be cross-checked with other udder health parameters, such as somatic cells count (SCC), milk conductivity, lactose and citrate percentage (SLOTH et al. 2003 [30]). Among these supplementary parameters, we investigated the SCC and the results are discussed further in the article.

Table 1. Average milk yield and its physical-chemical traits in the surveyed area

Trait	Season	n	$\bar{x}$	$\pm s_{\bar{x}}$	S	V%
Yield (Kg)	Winter	1931	3845.19	39.41	1731.62	45.03
	Spring	2228	4031.60	38.09	1797.70	44.59
	Summer	2395	3371.75	42.18	2064.04	61.22
	Fall	2323	3738.54	41.45	1997.97	53.44
	YEAR	8877	3736.34	20.46	1927.83	51.60
pH (units)	Winter	1947	6.80	0.0007	0.03	0.44
	Spring	2235	6.79	0.0002	0.01	0.15
	Summer	2423	6.78	0.0002	0.01	0.15
	Fall	2385	6.79	0.0004	0.02	0.29
	YEAR	8990	6.79	0.0002	0.02	0.29
Acidity (°T)	Winter	1947	15.29	0.011	0.51	3.34
	Spring	2236	15.44	0.010	0.49	3.17
	Summer	2424	15.38	0.009	0.46	2.99
	Fall	2385	15.24	0.009	0.42	2.76
	YEAR	8992	15.34	0.005	0.48	3.13
Density (g/cm^3)	Winter	1946	1.0299	0.0002	0.01	0.97
	Spring	2234	1.0295	0.0004	0.02	1.94
	Summer	2417	1.0291	0.0006	0.03	2.92
	Fall	2385	1.0297	0.0006	0.03	2.91
	YEAR	8982	1.0296	0.0002	0.02	1.94
Fat (%)	Winter	1940	4.13^{a}	0.006	0.28	6.78
	Spring	2234	3.96^{b}	0.006	0.27	6.82
	Summer	2407	3.93^{c}	0.004	0.21	5.34
	Fall	2369	4.06^{b}	0.005	0.25	6.16
	YEAR	8950	4.02	0.003	0.26	6.47
Proteins (%)	Winter	1940	3.26^{b}	0.002	0.09	2.76
	Spring	2234	3.27^{b}	0.001	0.06	1.83
	Summer	2080	3.22^{a}	0.001	0.05	1.55
	Fall	2126	3.23^{a}	0.001	0.06	1.86
	YEAR	8380	3.25	0.001	0.06	1.85
Total Dry Matter (%)	Winter	1940	12.88^{a}	0.008	0.37	2.87
	Spring	2234	12.72^{c}	0.008	0.38	2.99
	Summer	2080	12.66^{c}	0.006	0.25	1.97
	Fall	2125	12.81^{a}	0.005	0.21	1.64
	YEAR	8379	12.77	0.004	0.32	2.51

ANOVA, within the same column, between the seasonal values of one trait:
$^{a \text{ vs. } b;\ b \text{ vs. } c}$ associated superscripts – distinguished significant differences, for $p < 0.01$
$^{a \text{ vs. } c}$ associated superscripts – highly significant differences, for $p < 0.001$

The temperature (°C) of the collected milk varied between+5.14°C in January and +10.27°C in August. This qualitative trait is in close co-relation with the months and seasons for collection, being strongly influenced by the temperature of the external environment, especially in small size farms.
The assessed values for the titratable acidity (°T) ranged within the technological limits, hence the average monthly values as well as the seasonal one did not pass above 16°T (yearly mean of 15.34±0.005°T), indicating thus a very good freshness status of all samples.
The milk density (g/cm^3) had slightly variable values above 1.029. The acquired data emphasized slight individual decreases of density in summer, below 1.029 and above 1.028 g/cm3, the differences being not relevant.
The fat content (%) in the milk varied according to the calendar month and the season in which milk was collected, while the annual average was 4.02%.
The data revealed values of more than 4% in January-March and September-December with a maximal value of 4.18% in January.
The lowest values of fat content, between 3.91% and 3.95% were measured in April-August.
The variability coefficient in the fat content of milk was between 5.34% and 6.82%. Despite these good homogeneity values, the ANOVA test revealed very significant differences between winter months and summer ones ($p < 0.001$), as well as distinguished significant differences ($p < 0.01$) for the other comparisons (winter vs. spring, spring vs. summer and summer vs. fall) (table 1).
The protein content (%) presented more constant values, with an annual mean of 3.25%. There was a slight increase during the cold months of the year, the maximum value being recorded in March (3.30%) and January (3.29%), while the minimal one in July 3.22%.
We notice that for this qualitative trait as well, in winter and spring, the milk had highest protein content, while the lowest one was measured in summer. This situation led to distinguished significant differences ($p < 0.01$) between the values recorded in the first semester (winter and spring) compared to those measured during the second one (summer and fall).
These two organic constituents of the milk, as main nutrients relevant for human consumers, have been found in a ratio of 1.24 parts Fat : 1 part Proteins, common for cow milk. In Romania, apart from dairy cows, there are also bred on small areas other species of large ruminants producing milk, such as buffalos, whose milk is richer both in proteins and lipids but the ratio we spoke above is far unbalanced, favoring the lipids content (1.77 parts Fat : 1 part Proteins) (VIDU et al. 2015 [34]). It would be interested to investigate the full quality traits of the milk issued from this species, focusing on hygiene level, hence there are small farms rearing both dairy cows and buffaloes.
The total dry matter (TDM %) of the collected milk had a relatively constant value in the calendar months in each season, but with significant differences between seasons. The milk collected in winter and fall had higher DM content (12.88 – 12.81%) than the milk collected in summer (12.66%) or in spring (12.72%), as very significant differences occurred between seasons ($p < 0.001$). Overall, basing on the data summarized in table 1, it could be stated that the collected milk was in accordance with the freshness and chemical-nutritional quality specified by the en-force regulations.
What about the hygienic quality and the safety of the analyzed samples? The total number of germs – bacterial count (BC - germs/ml x 10^3) in raw milk is presented in table 2 and emphasized microbial loads close to the maximal admitted limit (100 germs/ml x 10^3) especially during the summer period.

Throughout the studied year, the average value for BC was 70.38 germs/ml x 10^3. Analyzing this aspect with regard to the season, we found in summer the highest microbial contamination of the raw milk, i.e. 96.38 germs/ml x 10^3 and the lowest one cold months of fall (46.21 germs/ml x 10^3) and winter (54.87 germs/ml x 10^3), while the values detected throughout spring months led to an average bacterial count of 78.63 germs/ml x 10^3.

Table 2. Average values of milk hygiene, safety and adulteration parameters

Trait	Season	n	$\overline{X}$	$\pm s_{\bar{x}}$	s	V%
Total bacterial count (BC) (germs/ml x 10^3)	Winter	1835	54.87[a]	0.64	27.51	50.14
	Spring	2215	78.63[c]	0.73	34.34	43.67
	Summer	2358	96.38[c]	0.90	43.62	45.26
	Fall	2111	46.21[a]	0.30	13.83	29.93
	YEAR	8519	70.38	0.37	33.86	48.11
Reductase test methylene blue dye (degradation time: h ,min)	Winter	183	1st class–Good (degraded in 6h14'±3.5')			
	Spring	221	1st class–Good (degraded in 5h49'±2.4')			
	Summer	235	1st class–Good (degraded in 5h40'±1.9')			
	Fall	211	1st class–Good (degraded in 5h56'±3.1')			
	YEAR	850	1st class–Good (degraded in 5h54'±2.5')			
Reductase test resazurin method (ultimate developed tint)	Winter	183	1st class–Good (blue steel tint)			
	Spring	221	1st class–Good (blue steel tint)			
	Summer	235	1st class–Good (blue steel tint)			
	Fall	211	1st class–Good (blue steel tint)			
	YEAR	850	1st class–Good (blue steel tint)			
Somatic cells count (SCC) (cells/ml x 10^3)	Winter	1935	206.90[a]	1.77	77.88	37.64
	Spring	2204	278.07[c]	2.16	101.52	36.51
	Summer	2327	390.86[c]	3.68	177.37	45.38
	Fall	2322	237.51[a]	1.62	78.05	32.86
	YEAR	8788	281.55	1.15	107.38	38.14
Antibiotics occurrence	Winter	1940	ND*	-	-	-
	Spring	2234	ND	-	-	-
	Summer	2080	ND	-	-	-
	Fall	2323	ND	-	-	-
	YEAR	8577	ND	-	-	-
Water Addition (%)	Winter	1947	ND	-	-	-
	Spring	2234	1.73**	0.26	1.02	58.58
	Summer	2327	ND	ND	ND	ND
	Fall	2385	ND	ND	ND	ND
	YEAR	8893	1.73**	0.26	1.02	58.67

ANOVA, within the same column, between the seasonal values of one trait:
[a vs. c] associated superscripts – highly significant differences, for $p < 0.001$
*ND-not detected
** 1.73% – mean based on 15 frauds identified only, out of the whole sample pack

The ANOVA test emphasized very substantial differences for the bacterial count in milk, between the cold and the warm seasons ($p < 0.001$). The variability of this trait was within extreme limits and the dispersion indices confirm the extreme variability of BC/ml, the

standard deviation having a value of 33.86 germs/ml x 10^3 and the coefficient of variation, V%= 48.11. By analyzing the monthly evolution of the microbial load in milk, we have found that the milk with the lowest BC/ml was obtained in October-December. Our findings on the total bacterial count in milk were confirmed by the qualitative enzymatic test of reductase, whose results showed that all samples fall into the 1st hygienic quality class – Good. However, such a colorimetric test should be fine-tuned to increase its sensitivity and accuracy, in order to reveal microbial contamination at lower levels, hence the lowest detection threshold is 500 germs/ml x 10^3. Other studies (RADU-RUSU et al., 2013 [26]) run on samples collected from the farmers market (small size producers, even subsistence exploitations – backyard bard with 1-2 cows) revealed that 78% of the milk was of good quality, 19% of satisfactory quality and 2% of Poor quality, suggesting a real problem in milking practices, storage and management, especially in small size-familial dairy farms. Hence in the current study there were found values close to the maximal tolerable limit for the occurrence of germs in raw milk, this underlines an aspect which can be alarming, namely the hygienic quality of the collected milk. It also means that there were no antibiotics present in the collected milk that could destroy the unwilled microbiota, fact becoming true when antibiotic occurrence tests were carried on and the results proved to be negative (table 2).

The count of total number of somatic cells is the second qualitative trait concerning the sanitation of the milk collected for human consumption and for processing. All over the analyzed year (table 2), the SCC had an average value of 281.55 cells/ml x 10^3. The variability of this trait was extreme, the standard deviation having a value of stDev=107.38 cells/ml x 10^3 and the variation coefficient V%=38.14. These data suggest the precarious health condition of some cows' udders suffering from mastitis, especially when the animals are exposed to outer environment pathogens (LAMARCHE et al., 2000 [16]). When the SCC exceeds 400 cells/ml x 10^3 it raises a pertinent suspicion for at least subclinical mastitis in milked cows (ANDREI et al., 2011 [4]). The acquired values can indeed prove the fact that in the individual households and in the farms from where the milk was collected, mastitis is not detected and the milk coming from ill cows is neither milked separately nor removed from processing (VAN DEN BORNE et al. 2011 [33], JAMROZIK & SCHAEFFER, 2012 [15]).

According to the season of collection (table 2), the milk was always within the safety regulations of allowed SCC (400 cells/ml x 10^3); only in summer the measured level (390.86 cells/ml x 10^3) approached the upper tolerated limit, in other seasons the milk presented lower values (206.90 cells/ml x 10^3 in winter, 237.51 cells/ml x 10^3 during fall and278.07 cells/ml x 10^3 in springtime). There were found highly significant differences between winter and fall, on one side and spring-summer, on the other side ($p< 0.001$), suggesting a higher incidence of udder epithelial tissue distress in summer and even individual levels of somatic cells higher than the maximal threshold. This situation is not uncommon, hence another studies run abroad also revealed that in small dairy farms (herd size below 50 lactating cows) the incidence (19.1%) of the samples which exceeded the 400 cells/ml x 10^3 limit was significantly higher than in medium size or large size farms (1.1%) (NORMAN et al. 2011 [22]). Another research comparable to ours (HAND et al., 2012 [13]) identified also situations with more than 499 cells/ml x 10^3. Other studies (MOLLENHORST et al., 2011 [20], WILDMAN et al., 1982 [35]) emphasize the fact that the milking interval has a significant effect upon the number of somatic cells, although not a very strong one. The significance is much higher in the case of fluctuations in the milking interval which influence the number of somatic cells present in the milk. Our results lead us to the conclusion that on the whole, there were seasonal and annual average values which complied with the European quality standards. However, individual values in the database we built prior to data

processing, suggested a precarious cleanliness of milk obtained in small farms and individual households with 1-10 animals, both for BC and SCC.
Analysis of the milk quality indices also indicated adulteration of milk through water addition in just 15 samples - an average of 1.73% of water was added. This aspect confirms the necessity of a rigorous control in the collected milk and the enhancement of professional education in the case of dairy cows farmers.
One of the numerous factors influencing the quality of milk for processing is the accommodation system provided to dairy cows (LAMBERTZ et al., 2014 [17]). With this in mind, we also conducted investigations concerning the quality of the milk obtained according to the two systems of keeping cattle: straw yard-SY (free movement) and cubicle-CUB (bound). The acquired results are presented in table 3.

Table 3. The average values and the variability estimations for the quality and safety traits of the collected milk according to the cows' accommodation system

Trait	Cows' accommodation system	n	$\bar{x}$	$\pm s_{\bar{x}}$	S	V%
Yield (Kg)	SY*	1806	3392.82	50.54	2147.83	63.31
	CUB**	7071	3824.07	22.09	1857.49	48.57
pH (units)	SY*	1866	6.79	0.0002	0.01	0.15
	CUB**	7124	6.79	0.0002	0.02	0.29
Acidity (°T)	SY*	1866	15.24	0.009	0.41	2.69
	CUB**	7126	15.73	0.006	0.49	3.12
Density (g/cm^3)	SY*	1856	1.0298	0.0002	0.01	0.97
	CUB**	7126	1.0296	0.0002	0.02	1.94
Fat (%)	SY*	1824	4.06	0.006	0.26	6.40
	CUB**	7126	4.01	0.003	0.25	6.23
Proteins (%)	SY*	1675	3.28	0.001	0.06	1.83
	CUB**	6705	3.26	0.001	0.05	1.53
Total Dry Matter (%)	SY*	1675	12.81	0.009	0.38	2.97
	CUB**	6704	12.75	0.003	0.27	2.12
BC (germs/ml x 10^3)	SY*	1393	42.51^a	0.24	8.88	20.90
	CUB**	7126	75.83^c	0.48	40.52	53.43
Reductase test meth. blue dye	SY*	139	1^{st} class – Good (degraded in 6h09'±2.6')			
	CUB**	711	1^{st} class – Good (degraded in 5h48'±3.1')			
Reductase test resazurin method	SY*	139	1^{st} class – Good (blue steel tint)			
	CUB**	711	1^{st} class – Good (blue steel tint)			
SCC (cells/ml x 10^3)	SY*	1756	172.65^a	1.06	44.30	25.66
	CUB**	7032	308.74^c	1.52	127.39	41.26
Antibiotics occurrence	SY*	1675	ND	-	-	-
	CUB**	6902	ND	-	-	-
Water addition (%)	SY*	1767	4.50***	-	-	-
	CUB**	7126	1.54****	0.18	0.69	4.94

* SY – straw yard (cows not bound); ** CUB – Cubicle (cows bound)
ANOVA, within the same column, between the seasonal values of one trait:
a vs. c associated superscripts – highly significant differences, for $p < 0.001$
*** 4.50% absolute value, based on a single identified fraud

****1.54% mean value, based on 14 identified frauds

The evolution of the main physical and chemical parameters of the milk quality is almost similar within the two accommodation systems. Even though the milk yielded by the cows in the stray yard farming system is slightly richer in organic nutrients and total dry matter content than that issued from cubicle (bound) system, the differences were not found as statistically significant when the analysis of the variance testing has been applied. On the contrary, when the safety traits have been investigated, there have been found highly significant differences ($p < 0.001$) between the straw yard and cubicle accommodation systems, both for BC an SCC. Thus, the total bacterial count reached 42.51 germs/ml x 10^3 in the straw yard farms and 75.83 germs/ml x 10^3 in the cubicle farms, suggesting almost double bacterial contamination of the milk, in relation with the accommodation system. However, such levels were within the tolerable limits of the EU for the raw milk (max. 100 germs/ml x 10^3).

Results of the total number of germs are comparable to those recorded for the number of somatic cells. In the system of keeping cows bound (cubicle), the count of somatic cells reached 308.74±1.52 cells/ml x 10^3, which was within the safety limit for this trait (max. 400 cells/ml x 10^3). A better situation was obviously recorded for the milk collected from the unbound cows (straw yard system); the average value also was below the critical threshold, i.e. 172.65±1.06 cells/ml x 10^3. The ANOVA test revealed as highly significant the absolute difference of 136.09 cells/ml x 10^3 between the two accommodation systems ($p < 0.001$). In other studies carried out in Romania, the total amount of somatic cells reached 165.55 cells/ml x 10^3 (ONACIU et al., 2016 [25]) in the milk yielded from the cows maintained in grazing conditions, throughout the warm season of the year, suggesting that the accommodation system in stables could correlate with an increased amount of somatic cells excreted in milk.

A positive aspect regarding the milk quality is that for the analyzed situation no presence of antibiotics has been detected. The accommodation system for cows influenced in some little manner the other milk quality traits, such as the content of lipids, proteins, dry matter and density, hence they have high genetic determinism and less influence from exogenous factors. On the other hand, the acidity, pH, temperature, hygienic traits (microbial population and somatic cells in milk) are traits powerfully influenced by the exogenous environment factors. The calculation of average values close to the upper limits of bacterial count and somatic cells count indicated that certain individual values exceeded the safety threshold; therefore some measures have to be taken, in order to improve the udder health as well as the hygienic status and the safety level of the raw milk. Among these measures, may we recommend the usage of a better milking technique, the calibration of the milking equipment, better hygiene of the milking personnel, udder decontamination after milk ejection, better cleaning of the milking circuit, frequent survey for mammary gland issues and implementation of the proper veterinary treatments for the cows found with mastitis and their milking done separately. These milk safety traits must be controlled and improved through sanitary measures and good practices affecting the whole process of production-transport-processing which are easily manageable.

4. Conclusion

Milk issued from small individual cattle households had poor quality with values of BC and SCC close to or even above the threshold of actual sanitary regulations. This was due to the

fact that small size producers did not fully observe the good hygiene practices, both during milking or storage of the raw milk.
Besides this, there were identified issues of milk fraud, through dilution with water.
Within the individual households and larger farms from where the milk issued, the cases of mastitis were not surveyed or detected, hence the collected milk had no trace of antibiotics.
The season significantly affected the BC and the SCC, higher values being observed in summer ($p < 0.001$). Also, the accommodation system of the cows influenced the same safety traits of the raw milk (BC and SCC). Thus, the straw yard system proved to supply cleaner milk than the cubicle system ($p < 0.001$).
However, all the milk collected fully complied with the en-force regulations of the European Union concerning the physical, chemical quality traits and with the safety hygienic ones, including the bacterial count (BC) and the somatic cells count (SCC).

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