

Seasonal Variation of Chemical Content and Heavy Metal Levels in Raw Milk

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Keywords

Raw milk, Seasonal variation,
Heavy metal, ICP-MS

Article information

Received: Jan 29, 2023

Revised: Jun 01, 2023

Accepted: Jun 03, 2023

Online: Jul 02, 2023

doi: [10.29002/asujse.1244032](https://doi.org/10.29002/asujse.1244032)

Abstract

Aksaray province is an important raw milk supplier for the dairy industry. In this respect, it is important to examine the milk quality of the region. In this case, milk was collected over a nine-month period from 413 cows at seven distinct locations in three different seasons. The percentages of fat, lactose, protein, dry matter, somatic cells, and the levels of heavy metals like arsenic, aluminum, cadmium, lead, and nickel were examined. All of these components and their levels of seasonal variations in milk were also researched.

Considering the average values of the parameters of the milk collected from all farms evaluated within the scope of the study, significant changes were detected. Significant changes were observed for fat, lactose, total solids, somatic cells, arsenic, aluminum, nickel, cadmium and lead. Only the protein value did not change depending on the seasons. The details of the changes on the basis of farms are also examined in the study.

1. Introduction

In terms of animal foods, milk and dairy products are among the most significant product types in Turkey and the rest of the world. Both for consumption directly and as ingredients in other food products, milk, and dairy products are significant in the food industry. Dairy products made from raw milk also play a significant role in both industrial food production and individual food consumption. In this context, raw milk quality and composition are seen as essential components for the food industry as well as consumer health.

The main criteria for determining milk quality are components such as dry matter, lactose, and fat [1]. It is also known that heavy metals are important in determining milk quality in industrialized regions [2]. These components directly affect the quality of the milk by changing the content. In addition, the quality of all dairy products is directly affected by the composition of the milk. The content of raw milk is affected by many factors such as animal age, breed, health, feeding status, season, geographical factors, etc. [3]. In this respect, the composition of milk due to different breeds and feeding regimes, different calving models, and breeding practices in different geographies also vary between countries.

A total of approximately 10 million tons of milk was produced in Turkey in 2021 [4]. This production value shows that dairy farming, production and consumption of milk and dairy products are at significant levels. From this point of view, the quality and composition of raw milk are very important for human health. On the other hand, as mentioned above, the composition of raw milk is not a fixed value and is highly variable. As shown in many studies, the composition of milk varies significantly with the seasons [5, 6].

Several researchers have found that the composition of cow's milk changes depending on the seasons, while somatic cell and total microbial load increases and fat, non-fat dry matter, protein values decrease in spring and summer. It is attributed to the fact that these seasons are the most suitable periods when fresh and green grasses are used as animal feed [7]. On the other hand, since the feed rations in modern farms are made more scientifically and technically than in traditional farms, the compositions (fat, protein, etc.) of the milk obtained from these farms have richer contents and a better economic value.

In addition to milk content, it is claimed that the levels of heavy metals in milk vary according to the seasons. In their study, Qin et al. observed that in addition to the seasonal variation of components such as protein, fat, and lactose in milk, the concentrations of all the elements determined such as copper, manganese, and zinc were also constantly varied within months [8]. Similarly, Kozhanova et al. also reported that the cadmium level in milk is lower in summer than in autumn

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[9]. The main hazard of heavy metals to human health is exposure to lead, cadmium, mercury and arsenic [10] and these heavy metals are mixed with biochemicals in normal body metabolic processes [11].

In light of these data, it is clear that the chemical structure and heavy metal content of raw milk might be at variable levels. As seen in the literature, seasonal changes directly affect the content of milk. From this point the detection of seasonal-based changes makes vital contributions to the literature. The fact that there is a significant amount of raw milk production in Aksaray makes this study important. For this purpose, fat, protein, lactose, dry matter, somatic cell values, and toxic heavy metal levels such as arsenic, aluminum, nickel, cadmium, and lead were investigated in raw milk in Aksaray province in seasonal cycles.

2. Materials and Methods

2.1. Materials

To evaluate the determined heavy metal contents and chemical composition, raw milk samples from dairy animals were collected from different regions of Aksaray province in accordance with the study's purpose. Raw milk samples were collected from the same point at 3 different times over a 6-month period. Milk samples representing a total of 413 cows were collected from the tanks of 7 farms in different locations. Thus, analysis was performed on 42 samples (n=42) with their parallels in 3 different periods from 7 different farms (7x3x2).

To prevent contamination from the transport containers, the samples were carried in polyethylene containers that had previously been cleaned with nitric acid. To represent all of the characteristics of the sample, 250 mL milk was taken from each sample point. The samples were rapidly taken into the sample preparation procedure after being brought to the laboratory while maintaining the cold chain (4 °C) via an in-car refrigerator.

2.2. Method

2.2.1. Sample Preparation and Microwave Incineration For ICP-MS

2 mL of milk samples were taken, 8 mL of 65 % (extrapur) nitric acid (E. Merck, Darmstadt, Germany) and 1 mL of 30 % hydrogen peroxide (E. Merck, Darmstadt, Germany) were added, and the mixture was held mouthed under a fume hood for 15-20 minutes. The samples were then burned using a microwave combustion device (Cem Corporation, Mars 5, U.S.A. -Maximum pressure 1600 psi, maximum temperature 310 °C-). After microwave digestion, samples were filtered through 0.45µm syringe filters and kept at +4 °C until ICP-MS analysis.

2.2.2. ICP-MS Analysis

The ICP-MS device was used to determine the heavy metal content of the samples, which were collected and microwave digested for analysis. Standards (Sigma Chem, St. Louis, ABD) with concentrations of 1, 5, 6, 10, 30, 50, 100, 150, and 200 g/kg were made from 10 mg/kg pure standards with equal concentrations of heavy metals for this purpose, and a calibration curve was drawn using them [12].

2.2.3. Chemical Analysis

Within the scope of the study, fat, protein, lactose and dry matter values were analyzed in the samples. These analyzes were performed with a Bentley 150 (Minnesota, USA) instrument. The device works with an optical infrared system and measures the energy absorption of milk components at certain wavelengths in the mid-infrared region. Somatic cell count was performed with Bentley Somacount FC (Minnesota, USA). It works on the principle of fluorescence-based flow cytometry. The devices meet the requirements of TSE (Turkish Standards Institute) standards [13, 14].

2.2.4. Statistical Analysis

Minitab 16.1 licensed program was used for statistical analysis of the data of all samples and the conformity of the data with One Way ANOVA to the normal distribution was analyzed. The contents of five different heavy metals (Al, As, Cd, Ni, Pb) and five chemical contents (fat, protein, lactose, dry matter and somatic cell) were evaluated in 42 (n=42) samples in a study with two replications.

3. Results and Discussion

After the samples were collected and properly prepared, chemical and ICP-MS analyzes were done. The data obtained were evaluated according to the season difference (summer, autumn, winter). Table 1. shows the mean values and statistical analysis of the samples. Table 2, Table 3 and Figure 1 show seasonal variation for each farm.

As can be seen in Tables, different levels of heavy metals and basic compound were found in raw milk throughout the seasons. Researchers from several fields have noted this issue in milk from various countries and regions [15-17]. It is known that there is a negative relationship between environmental temperature and milk fat and protein. Especially as the

environmental temperature increases, the amount of fat in the milk decreases [18]. In this study, it was determined that the amount of milk fat was at the lowest level in the summer months. Heck et al. reported that protein was at a lower level in the summer months and increased in the winter months [19]. This study, however, no significant relationship was found between protein levels and seasons. In addition, a significant relationship was found between lactose and total solid level and seasons in the study. Lactose reaches its lowest level in summer. Studies conducted in camel milk [20] and goat [21] milk have also reported that lactose level in milk decreases in summer months. Similarly, Zou et al. in their study on camel milk, reported that the levels of milk components change according to the seasons. Researchers reported that protein level was not affected by seasonal changes, but lactose and total solids content was affected by seasonal changes [22]. Celano et al. on the other hand, examined the changes in the components of milk in winter and summer seasons. According to the research, a significant relationship was found between seasonal variation and fat and lactose. However, no significant effect of seasonal changes on protein and total solids was detected [23].

Table 1. Average heavy metal and chemical contents of the samples and statistical analysis of data

Parameters	Season			P Value
	Summer	Autumn	Winter	
Overall Results				
Fat %	3.43 ^b ±0.22	3.52 ^b ±0.40	3.99 ^a ±0.36	0.000
Protein %	3.31 ±0.31	3.31 ±0.46	3.15 ±0.40	0.058
Lactose %	4.46 ^a ±0.09	4.69 ^b ±0.02	4.60 ^c ±0.08	0.000
Total Solid %	11,87 ^b ±0,41	11.75 ^b ±1.90	14.26 ^a ±0.38	0.000
Somatic Cell*	197 ^b ±91.80	164 ^b ±77.84	278 ^c ±97.49	0.000
As (µg/kg)	2.82 ^a ±0.23	0.02 ^b ±0.05	0.10 ^b ±0.24	0.000
Al (µg/kg)	142.91 ^a ±96.21	106.18 ^a ±124.46	39.31 ^b ±47.99	0.000
Ni (µg/kg)	181.34 ^a ±56.96	105.05 ^b ±15.65	9.09 ^c ±6.43	0.000
Cd (µg/kg)	nd	0.05 ±0.01	nd	
Pb (µg/kg)	2.15 ^a ±0.64	nd	0.49 ^b ±0.48	0.000

*cells/mL, nd: not detected, different letters in superscript on the same row indicate that the difference between seasons is significant at P<0.05.

According to these results, it is seen that especially the amount of dry matter is affected by seasonal changes. As another important data, it has been found that the fat, protein and lactose levels in milk are at relatively the same levels. However, it is possible to say that the level of somatic cells, which is another variable, reaches its highest level, especially in winter. The reason for this could be that hygiene is not adequately provided in barn conditions during the winter months and as a result of this situation, the animals could get more infections.

Table 2. Variation of heavy metal level according to farms

Dairy Farms	Heavy Metal (µg/kg)	Season			P Value
		Summer	Autumn	Winter	
Farm1	As	2.90 ^a ±0.00	nd	0.68 ^b ±0.01	0.000
	Al	54.72 ^a ±0.01	55.02 ^b ±0.04	137.25 ^c ±0.07	0.000
	Ni	95.02 ^a ±0.00	97.13 ^b ±0.00	10.53 ^b ±0.04	0.000
	Cd	nd	nd	nd	
	Pb	2.51 ^a ±0.01	nd	0.53 ^b ±0.01	0.000
Farm2	As	2.64 ^a ±0.01	0.15 ^b ±0.00	nd	0.000
	Al	223.74 ^a ±0.04	93.73 ^b ±0.11	83.10 ^c ±0.04	0.000
	Ni	265.60 ^a ±0.43	98.03 ^b ±0.03	9.50 ^c ±0.01	0.000
	Cd	nd	nd	nd	
	Pb	2.41 ^a ±0.01	nd	0.56 ^b ±0.02	0.000
Farm3	As	2.93 ±0.01	nd	nd	0.000
	Al	86.29 ^a ±0.18	19.56 ^b ±0.05	15.89 ^c ±0.07	0.000
	Ni	186.03 ^a ±0.04	136.05 ^b ±0.09	18.80 ^c ±0.09	0.000
	Cd	nd	nd	nd	
	Pb	1.17 ±0.01	nd	nd	0.000

Dairy Farms	Heavy Metal (µg/kg)	Season			P Value
		Summer	Autumn	Winter	
Farm4	As	2.78 ±0.01	nd	nd	0.000
	Al	84.42 ^a ±0.11	nd	9.01 ^b ±0.07	0.000
	Ni	160.78 ^a ±0.14	86.57 ^b ±0.09	16.26 ^c ±0.09	0.000
	Cd	nd	nd	nd	
	Pb	2.73 ^a ±0.01	nd	0.25 ^b ±0.01	0.000
Farm5	As	2.45 ±0.03	nd	nd	0.000
	Al	78.28 ^a ±0.18	229.28 ^b ±0.05	10.46 ^c ±0.02	0.000
	Ni	142.78 ^a ±0.05	104.77 ^b ±0.04	0.41 ^c ±0.01	0.000
	Cd	nd	0.26 ±0.01	nd	0.000
	Pb	2.47 ±0.02	nd	nd	0.000
Farm6	As	2.83 ±0.05	nd	nd	0.000
	Al	337.27 ^a ±0.15	nd	2.67 ^b ±0.03	0.000
	Ni	253.69 ^a ±0.14	95.32 ^b ±0.08	2.17 ^c ±0.10	0.000
	Cd	nd	0.11 ±0.01	nd	0.000
	Pb	1.14 ^a ±0.01	nd	0.57 ^b ±0.02	0.000
Farm7	As	3.21 ±0.05	nd	nd	0.000
	Al	135.66 ^a ±0.04	345.67 ^b ±0.42	16.80 ^c ±0.02	0.000
	Ni	163.51 ^a ±0.31	117.49 ^b ±0.33	5.96 ^c ±0.04	0.000
	Cd	nd	nd	nd	0.000
	Pb	2.61 ^a ±0.02	nd	1.50 ^b ±0.01	0.000

nd: not detected, different letters in superscript on the same row indicate that the difference between seasons is significant at P<0.05. As: arsenic, Al: aluminum, Ni: nickel, Cd: cadmium, Pb: lead

Table 3. Variation of basic compound level according to farms

Dairy Farms	Basic Compound (%)	Season			P Value
		Summer	Autumn	Winter	
Farm1	Fat	3.22 ^a ±0.02	3.15 ^b ±0.01	3.97 ^c ±0.01	0.000
	Protein	2.89 ^a ±0.01	2.98 ^b ±0.01	3.27 ^c ±0.01	0.000
	Lactose	4.43 ^b ±0.02	4.63 ^a ±0.02	4.44 ^b ±0.00	0.000
	Dry Matter	11.30 ^a ±0.03	11.42 ^b ±0.05	14.11 ^c ±0.03	0.000
Farm2	Fat	3.30 ^a ±0.01	3.58 ^b ±0.01	3.21 ^c ±0.01	0.000
	Protein	3.23 ^a ±0.01	3.27 ^b ±0.01	3.09 ^c ±0.01	0.000
	Lactose	4.42 ^a ±0.01	4.65 ^b ±0.02	4.67 ^c ±0.02	0.000
	Dry Matter	11.61 ^a ±0.02	11.90 ^b ±0.02	13.46 ^c ±0.01	0.000

Dairy Farms	Basic Compound (%)	Season			P Value
		Summer	Autumn	Winter	
Farm3	Fat	3.42 ^a ±0.01	3.02 ^b ±0.01	4.13 ^c ±0.01	0.000
	Protein	2.87 ^a ±0.01	2.49 ^b ±0.02	2.96 ^c ±0.06	0.000
	Lactose	4.34 ^a ±0.01	4.59 ^b ±0.01	4.63 ^c ±0.01	0.000
	Dry Matter	11.42 ^a ±0.02	11.03 ^b ±0.03	14.26 ^c ±0.03	0.000
Farm4	Fat	3.71 ^a ±0.01	3.64 ^b ±0.01	4.40 ^c ±0.01	0.000
	Protein	3.50 ^a ±0.01	3.22 ^b ±0.02	3.13 ^c ±0.06	0.000
	Lactose	4.50 ^a ±0.01	4.71 ^b ±0.02	4.65 ^c ±0.01	0.000
	Dry Matter	12.31 ^a ±0.03	12.43 ^b ±0.01	14.68 ^c ±0.01	0.000
Farm5	Fat	3.55 ^a ±0.01	3.80 ^b ±0.02	4.24 ^c ±0.01	0.000
	Protein	3.66 ^a ±0.01	3.84 ^b ±0.03	3.16 ^c ±0.02	0.000
	Lactose	4.52 ^b ±0.02	4.74 ^a ±0.02	4.51 ^b ±0.01	0.000
	Dry Matter	12.32 ^a ±0.02	12.58 ^b ±0.01	14.50 ^c ±0.02	0.000
Farm 6	Fat	3.68 ^a ±0.01	4.24 ^b ±0.01	4.06 ^c ±0.01	0.000
	Protein	3.45 ^a ±0.01	3.46 ^a ±0.01	3.24 ^b ±0.01	0.000
	Lactose	4.41 ^a ±0.01	4.76 ^b ±0.01	4.60 ^c ±0.00	0.000
	Dry Matter	12.24 ^a ±0.02	12.89 ^b ±0.02	14.45 ^c ±0.02	0.000
Farm7	Fat	3.11 ^a ±0.01	3.19 ^b ±0.01	3.90 ^c ±0.01	0.000
	Protein	3.59 ^a ±0.01	3.91 ^b ±0.02	3.18 ^c ±0.01	0.000
	Lactose	4.62 ^a ±0.02	4.78 ^b ±0.01	4.68 ^c ±0.01	0.000
	Dry Matter	11.87 ^a ±0.02	12.01 ^b ±0.02	14.38 ^c ±0.05	0.000

Different letters in superscript on the same row indicate that the difference between seasons is significant at $P < 0.05$.

It is suggested in many studies that especially the protein and fat content of milk decreases in the summer months compared to the winter months [9, 18, 24]. The researchers emphasized that the reason for this is that the cows are fed on more pasture during the summer months. In the study, the fat and protein levels of the milk varied, albeit at relatively low levels. Since the dairy animals within the scope of our research were fed completely with feed, it is considered that there is no significant difference. Similarly, Parmar et al. reported that the chemical content of milk changed within a 9-month period, and accordingly, there were differences in the amount of dry matter [25]. Researchers who obtained similar results in goat milk report that the content of milk changes depending on the seasons, and accordingly the content of dairy products is also affected [26]. In addition, significant relationship was found between somatic cell count and seasons in the study. Table 4 and Figure 2 show somatic cell change in detail.

Although it varies on farm basis, the average somatic cell count reached the highest level in winter. Similarly, Toghdory et al. reported that as the environmental temperature increased, the level of milk protein and fat and the number of somatic cells decreased significantly [27]. Researchers in different studies have shown an increase in somatic cell count during the summer months [28, 29]. It is estimated that this is due to the increased incidence of mastitis with temperature [29]. The different result in our study is thought to be due to mastitis, regardless of the season.

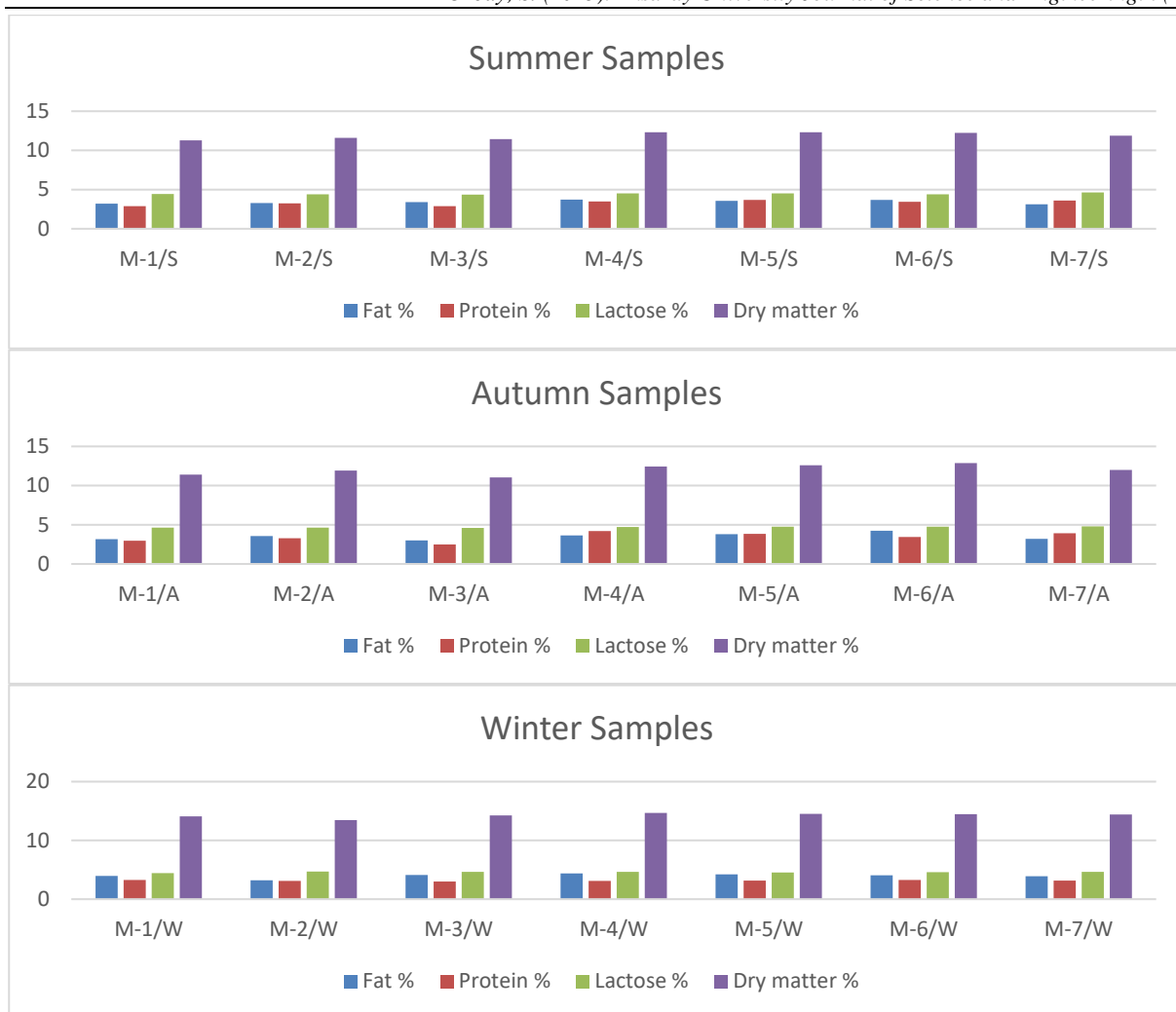


Figure 1. Variation of milk content according to seasons (M = Milk, Number = Farm (1 - Farm 1/ 2 - Farm 2/ 3 – Farm 3/ 4 - Farm 4/ 5 - Farm 5/ 6 -Farm 6/ 7 - Farm 7) , S=Summer, A=Autumn, W=Winter)

Table 4. Variation of somatic cells level according to farms

Dairy Farms	Season			P Value
	Summer	Autumn	Winter	
	Somatic Cell (cells / mL) × 1000			
Farm1	235 ^a ±7.91	195 ^b ±11.18	392 ^c ±9.28	0.000
Farm2	283 ^a ±2.45	261 ^b ±2.97	147 ^c ±1.41	0.000
Farm3	212 ^a ±6.07	158 ^b ±4.60	406 ^c ±4.66	0.000
Farm4	195 ^a ±3.56	205 ^b ±3.58	237 ^c ±1.95	0.000
Farm5	20 ^a ±1.52	35 ^b ±2.55	158 ^c ±1.48	0.000
Farm6	128 ^a ±2.51	71 ^b ±2.59	287 ^c ±4.62	0.000
Farm7	308 ^a ±5.63	225 ^b ±3.56	320 ^c ±4.92	0.000

Different letters in superscript on the same row indicate that the difference between seasons is significant at P<0.05.

In the study, a significant relationship was determined between the amount of all heavy metals and the seasons. Additionally in research, aluminum stands out as a significant pollutant due to its average concentration of heavy metals. In a survey that included nine countries, Turkey came out on top as the country with the highest aluminum content in milk [30]. In our study, cadmium was detected at low levels (0.02 µg/kg). It is known that foods contain cadmium at low levels (100 µg/kg) [31]. Usta and Güner reported that cadmium is detected at a maximum level of 1,162 mg/l in milk

[32]. In our study, the cadmium level is well below this level. In spite of the relatively low level of cadmium, a slight increase in lead level is also observed, especially in summer (2.14 µg/kg). Compared to the study, the Abou-Arab study found higher levels of lead in raw milk [33]. While the researcher detected 50 and 348 µg/kg lead and cadmium, respectively, Bilandzic et al. found lead in milk exceeding the maximum residue level in their study in Croatia [34]. According to studies on camel milk, the concentrations of cadmium, lead, arsenic, nickel, and aluminum were 0.4–1–0.2–3 and 51 µg/kg, respectively [12]. According to our study, nickel and aluminum specifically were found to be over these values, whereas cadmium, lead, and arsenic were found to be at lower or comparable amounts. It is also revealed that seasonal variations have an impact on the level of heavy metals in milk. Figure 3 shows heavy metal contents in milk on a seasonal basis.

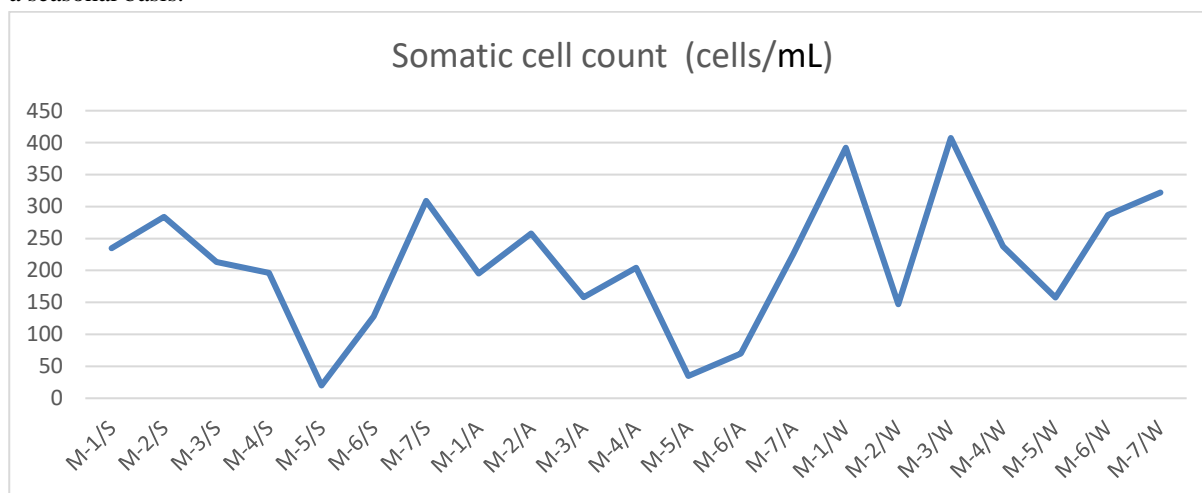


Figure 2. Changes in somatic cell level depending on the seasons (M = Milk, Number = Farm (1 - Farm 1/ 2 - Farm 2/ 3 - Farm 3/ 4 - Farm 4/ 5 - Farm 5/ 6 -Farm 6/ 7 - Farm 7), S=Summer, A=Autumn, W=Winter)

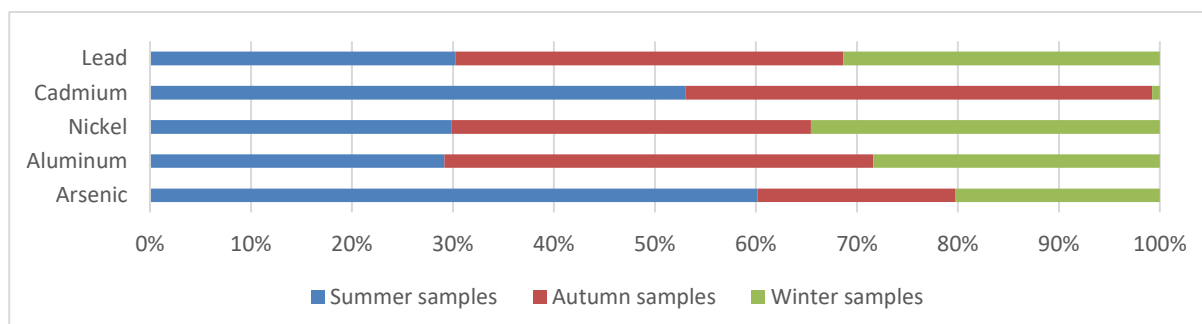


Figure 3. Heavy metal contents in milk on a seasonal basis.

It has been reported that heavy metals (Hg, Cr, Cd, Pb, and As) were detected in dairy products and milk in 20 provinces of China [35]. In a different study in which 64 milk samples were examined, it was reported that there was no heavy metal in milk samples (except copper) at a level that could pose a risk [36]. These different results are thought to be due to the regional and different variables of heavy metal pollution.

The level of heavy metals in raw milk can be affected by many factors. It has also been reported in different studies that the seasons change the heavy metal level in milk [37]. Additionally, both environmental and hygienic conditions affect this situation. In particular, the relationship between contaminated water and feed and the presence of heavy metals in milk is known [38]. This situation is specified as an indirect indicator of the hygienic status of milk [16, 39]. It is known that heavy metal contamination of milk can also occur from milk transport containers [40].

Arsenic, nickel, cadmium and lead values were found to be high in summer. Animals naturally drink more water in the natural summer months, so it is considered that the heavy metals that can be found in the water are more likely to pass into the milk. More research on this subject is needed.

In short, all components (fat, lactose, total solid, sometic cell, As, Al, Ni, Cd and Pb) differed significantly depending on the seasons, except for the protein amount in the study. In addition, seasonal changes were determined separately on the basis of farms in the sample. Similarly, it is emphasized that the content of sheep and goat milk changes significantly depending on the seasons [41]. In the study, heavy metal levels were generally higher in summer months. The ratio of milk components increased in the winter months and decreased in the summer months. Similar findings in milk

components values were obtained in Yörükoğlu's thesis study [42]. In addition, it is known that not only the milk components but also the average daily milk yield is affected significantly by the seasons [43].

According to the Turkish Food Codex Communiqué on Raw Milk and Heat Treated Drinking Milk, the analyzed raw milk can be considered suitable. Protein values are above the minimum 2.8 % value specified in the communiqué. The fat ratio of milk should be at least 3.5 % according to the communiqué. It has been observed that milk can remain below this level, albeit very slightly, in the summer months. In the somatic cell, maximum (500,000/mL) levels were not measured, even in the highest periods, according to the paper [44]. In the Turkish Food Codex Communiqué on Maximum Limits of Contaminants in Foodstuffs, there is only lead level limit for heavy metals. This limit is specified as 0.02 mg/kg wet weight. The lead value of all milk collected in the study for all seasons remained below the limit value [45].

As a result, a very substantial correlation between heavy metal levels and the season was discovered. It is possible to argue that somatic cell, lactose, fat, and therefore dry matter levels are impacted by seasonal fluctuations in terms of their chemical constituents. Seasons, milk protein were not shown to be correlated. The results supports the current studies on the subject in this respect. Both the heavy metal level and chemical structure of raw milk are affected by seasonal changes.

4. Conclusion

Milk and dairy products are important components of the diet of our society. In this context, it is critical to determine and examine the factors affecting milk quality. Research shows that the chemical composition and heavy metal contents of milk can change according to the seasons. In particular, these contents are directly related to the feed and water consumed by the animals. Depending on the seasons, it can cause significant variability in the feeding regime of animals and the amount of water drinking. In addition, the contaminants in the soil and water content of the regions where livestock is made appear as another important factor of variability. The change in the components of milk and the determination of the factors affecting it are important both for raw milk and for public health. As a result, it was seen that the seasons are an important factor affecting the milk components and quality and supported the previous studies. In terms of sustainability of quality, the seasonal variation factor should not be ignored by the producers.

Acknowledgment

I would like to thank Büşra ÖZBAY for the statistical analysis of all my studies.

Ethical Approval

This research does not need ethical approval.

Funding

Aksaray University Coordinating Office for Scientific Research Projects, Project Number: 2021-029.

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