Original Article

Quantitative and Qualitative Assessment of Microplastics in Drinking Water, Raw Materials, and Animal Feed Additives

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ABSTRACT

Background: Numerous studies have shown that exposing livestock to environmental and nutritional pollutants can endanger their health, leading to decreased production and product quality. Additionally, it can potentially transfer pollutants to higher levels of the food chain through contaminated products. Plastic materials have recently garnered significant attention as pollutants. Despite the crucial role of livestock and poultry products in human nutrition, providing a significant portion of the required protein, limited research has been conducted in this area.

Objectives: This study was done to estimate the extent of microplastics in drinking water, complete diet, and certain feedstuffs in two dairy farms located in Tehran and Babol.

Methods: Water and feed samples were collected in accordance with the standards of the National Organization of Iran. Microscopical examination of samples was done after digesting their organic matter contents with potassium hydroxide (KOH), and their size, color, and quantity were determined. The ingested number of microplastics by each cow was estimated based on the diet consumption.

Results: The findings revealed the presence of a large number of microplastics in feedstuffs, varying in size and color. A significant difference in particle sizes of microplastic samples was observed between the two farms. It was estimated that each cow is ingesting over 5,000 microplastic particles daily through their feed.

Conclusion: It can be concluded that dairy cows are exposed to microplastics through their diet, and corn grain was the most contaminated diet ingredient. Particle sizes range from 100 to 700 µm.

Keywords: Dairy farm, Feedstuffs, Food chain, Microplastics, Pollutants

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Introduction

The health and well-being of dairy cows are crucial for producing high-quality products. Exposure to environmental pollutants and nutritional factors may impact animal health, reduce milk and/or meat production and quality, and potentially transfer pollutants to the human food chain (Glatz et al., 2020). Plastic is one of the most significant pollutants that has recently received attention. The massive production, perceived lack of danger, and absence of cost-effective recycling options have resulted in plastics becoming the largest volume of waste in a growing trend. Plastics consist of various materials and chemical compounds with different shapes, colors, sizes, degradability, weights, and densities. The combination of these characteristics makes it challenging to track and identify their origin and the source of their dispersal in the environment (Ziani et al., 2023).

In the process of degradation and decomposition of plastics, particles that reach a size smaller than 5 mm to approximately 1 µm are referred to as microplastics. Microplastics can be formed through the fragmentation of larger plastic pieces or the breakdown of fibers and threads from synthetic polymers (secondary microplastics), or they can be produced and released through various means, often accompanied by wastewater or environmental accidents (primary microplastics) (Zhang et al., 2020; Lei et al., 2017). The threats posed by the increasing production and consumption of these synthetic materials have been recognized by the World Health Organization as "emerging pollutants" (Yu et al., 2022).

The most likely route of exposure to microplastics in animals is through ingestion and consumption (Shi et al., 2022), although other entry pathways, such as respiratory or dermal routes, are also possible. In recent years, the presence of microplastics in soil, water, and agricultural products has become a concern due to the potential entry of microplastics into animal bodies from a food safety perspective. Despite the significant role of various animal products in human nutrition by providing a substantial portion of protein requirements through meat, dairy products, and other related animal-sourced products, limited research has been conducted in this area. Based on our studies, it is evident that there is a lack of quantitative and qualitative assessment of microplastics in various types of animal food, especially in Iran. Therefore, the aim of this study was to identify and estimate the quantity of microplastics in drinking water, complete diet, and some raw materials fed to dairy cows in two industrial dairy farms located in the Tehran and Babol regions.

Materials and Methods

Samples collection

Samples were collected from two dairy farms with significant geographical differences. The first farm (farm A) was located in Qarchak-Varamin, Tehran Province, and the second farm (farm B) was located in Babol, Mazandaran Province. Sampling was conducted using standard methods to collect primary sources of animal food from dairy farms. The samples included water (2 liters), raw materials (corn grain, barley grain, rapeseed meal, soybean meal, alfalfa, and wheat bran), mineral and vitamin supplements, salt, and total mixed ration (TMR), each about 500 g.

Sample preparation

In the laboratory, 5 g of each feed sample and 100 mL of water were used for the initial digestion process. The organic liquid was then removed using potassium hydroxide (KOH 10%), and the remaining liquid was filtered using a nitrocellulose filter (2.0 µm). The filtered samples were placed in a 6-mm plate in a jar and kept in a horizontal position away from dust for further observations and analysis. The samples were then observed under a stereomicroscope in a clean laboratory environment. Subsequently, 5 g of each sample was repeatedly mixed with six times its volume of water (approximately 30 mL) using a magnet stirrer. The mixture was then washed with a 500-µm sieve, and the bottom liquid was collected.

Observation and examination of microplastics

To determine the length, width, color, and shape of each suspected plastic particle, each particle was observed using a TCA-3.0 Mega camera connected to a stereomicroscope with different magnifications and using additional white light. Each photo was analyzed using the using the software package Image-J, and the type of microplastics, including fragments (irregular fragments and foam particles), microbeads, microfilaments, film, as well as the color of microplastics, were measured and recorded.

Accuracy of the test

To separate organic and environmental particles, microplastic particles were examined using a loop with different magnifications and the hot needle method.

Smooth papers were placed in specialized plates with a diameter of 1 cm and were kept in foil containers to prevent environmental contamination. During the test process, a water container was placed as a control sample in the test location to identify any environmental contaminants (microplastics) that may have accidentally entered the samples during the testing process. To prevent any potential contamination from the technician's clothing, a laboratory white coat was worn (Gallagher et al., 2016).

Estimation of daily exposure to microplastic particles

To investigate exposure to microplastic particles in animals, the diet composition and daily feed intake of cows weighing approximately 600 kg were examined. Based on the obtained data, the number of ingested microplastic particles from each diet ingredient by a cow was calculated using the Equation 1:

1. Number of daily ingested particles = (Particle counts in 5g feed × daily intake of feed (kg)×200)

Statistical analysis

Statistical analysis was performed using quantitative or qualitative non-parametric tests with SPSS statistics software, version 20 after determining the normal distribution of data. The number, shape, color, and size of the observed microplastics in each sample type were compared between the two farms. A t-test was also used to compare the mean sizes of the particles between the samples from the two dairy farms. Significance was declared at $P<0.05$.

Results

In the sampling of water and various animal feeds from two dairy farms, different types of microplastics were observed and photographed using a microscope. These particles varied in size, number, shape, and color.

Size examination of microplastic particles

The Mean±SD of particle size in the samples of both farms is shown in Table 1. In farm A, the mean size of the microplastic particles (in µm) in different feed samples were as follows: Complete diet (662), barley grain (566), mineral supplement (562), corn grain (384), wheat bran (352), vitamin supplement (326), soybean meal (291), water (277), sodium salt (206), and alfalfa hay (121). In Farm B, the mean size of the microplastic particles in feedstuff samples were as follows: Alfalfa hay (497), corn grain (437), vitamin supplement (432), sodium salt (344), complete feed (323), mineral supplement (312), water (214), soybean meal (183), and wheat bran (179). In the samples of wheat bran, soybean meal, mineral supplement, and complete diet, there was a significant difference in the size of microplastics between the two farms. As shown in Table 1, the particle sizes of the samples from farm A were significantly larger than those from farm B, except for the corn grain, vitamin supplement, sodium salt, and alfalfa hay samples.

The complete diet in dairy farm A had the largest size, while the particles found in alfalfa hay had the smallest size. In dairy farm B, the largest particles were found in alfalfa hay, and the smallest particles were found in soybean meal samples.

Examination of the shape and number of microplastic particles

Figure 1 compares the number of particles in both dairy farms. Higher contamination with microplastics was observed in corn grain samples compared to other feedstuff samples in both dairy farms. In general, the number of particles in the samples of dairy farm B was higher than that of farm A, except for mineral and vitamin samples (Table 2). Figures 2 and 3 show the diversity of particle shapes in water and feedstuff samples of dairy farms A and B, respectively. The shape of particles was divided into four Groups: fiber, fragment, microbead, and film. In both farms A and B, almost all types of samples exhibited the most common form of fiber-like particles, except in the corn grain samples of farm B, where the predominant forms were film-like particles, and in wheat bran and mineral supplements, which had more fragment-like particles.

Examination of the color diversity of particles

Figure 4 shows the various colors of microplastics found in some feedstuff samples. The color diversity in each sample type was as follows:

Water: Water from dairy farm A contained more black particles than other colors, while in dairy farm B, more white particles were observed. The color diversity of dairy farm A was lower than that of dairy farm B.

Corn grain: In dairy farm A, the most abundant microplastics were red, followed by irregular shapes, small beads, and film. In dairy farm B, the majority of microplastics were pink-colored, primarily consisting of film and string.

Table 1. Mean particle sizes (µm) in Varamin (A) and Babol (B) dairy farms

Sample Type	Mean±SD Particle Size (µm) Varamin Farm	Mean±SD Particle Size (µm) Babol Farm	P (t-test)
Alfalfa hay	121±42.1	497±65.9	0.037
Barley grain	566±36.1	314±61.3	0.012
Corn grain	384±61.4	437±60.6	0.15
Mineral supplement	562±51.7	312±68.4	0.037
Sodium salt	205±45.4	344±22.6	0.062
Soybean meal	291±29.3	183±84.3	0.001
Vitamin supplement	326±52.3	432±60.4	0.110
Wheat bran	352±73.4	179±90.4	0.005
Complete diet	662±43.8	323±19.4	0.002
Drinking water	277±87.7	214±34.6	0.30

Barley grain: In dairy farm A, black microplastics were more prevalent, primarily consisting of string-like particles. In dairy farm B, the majority of microplastics were brown-black, often appearing in string-like forms.

Wheat bran: In dairy farm A, yellow-colored microplastics were more abundant, followed by other colors. In dairy farm B, most of the microplastic samples were beige-colored, with pink-colored particles coming in second.

Soybean meal: In dairy farm A, red and black microplastics were more prevalent. In dairy farm B, red and beige-colored particles were more common.

Table 2. Estimation of daily ingested particle numbers through each type of feed

Feed Type	Number of Particles (in 5 g Sample) in Each Farm		Daily Intake (kg)	Daily Ingested Particle Numbers	
	A	B		A	B
Alfalfa hay	114	119	6	136800	142800
Barley grain	89	153	4	71200	122400
Corn grain	142	278	6	170400	333600
Mineral supplement	41	31	0.26	2132	1612
Sodium salt	54	56	0.14	1512	1568
Soybean meal	127	168	5	127000	168000
Vitamin supplement	88	32	0.21	3696	2352
Wheat bran	51	112	2	20400	44800
Total	706	949	23.61	533140	816124

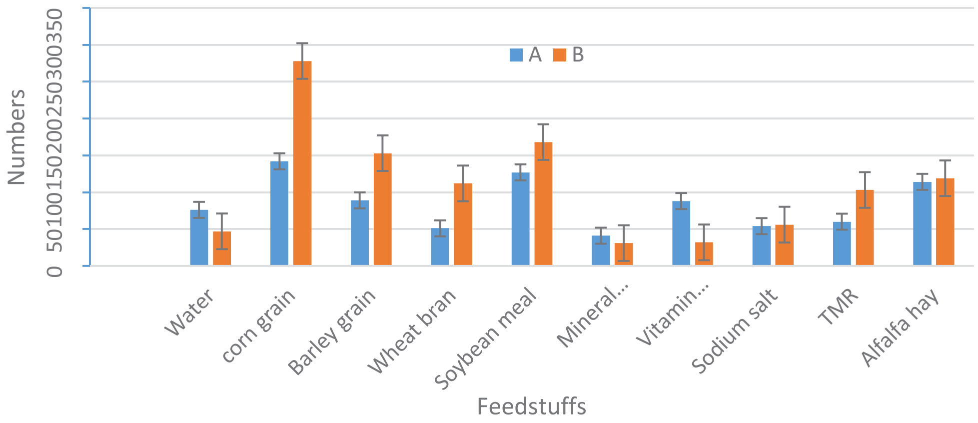

Figure 1. Comparison of the number of microplastic particles in 5-gram feedstuff samples from dairy farms A and B

Mineral supplement: In dairy farm A, red microplastics dominated, followed by colorless particles. In dairy farm B, blue-colored microplastics were more abundant, with colorless particles coming in second. Microplastics in dairy farm B exhibited a greater diversity of colors than those in dairy farm A.

Vitamin supplement: In dairy farm A, colorless particles were more abundant, followed by green particles. In dairy farm B, the majority of microplastics were colorless, followed by brown particles. Both dairy farms exhibited a high level of color diversity.

Sodium salt: In both dairy farms A and B, black-colored microplastics were more abundant, followed by colorless particles.

Complete diet: In dairy farm A, numerous colorless particles were observed, with some exhibiting a shiny appearance. In dairy farm B, colorless and beige particles dominated.

Alfalfa hay: In both dairy farms A and B, colorless particles were more abundant. The particle samples of dairy farm A exhibited a lower level of color diversity than those of dairy farm B.

Conclusion on color diversity in samples

The greatest color diversity was observed in the vitamin supplement samples from dairy farm A, with 12 different colors present. In dairy farm B, the barley grain samples exhibited the highest level of color diversity, with 13 different colors observed. The mean number of colors observed in both dairy farms was 8 different colors. In dairy farm A, the majority of microplastics were colorless, followed by red and black particles. In dairy farm B, the most abundant colors were beige, colorless, and red.

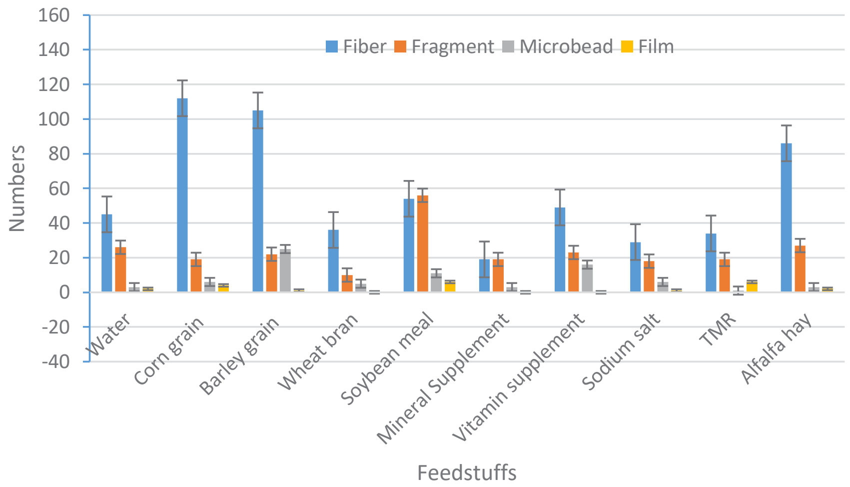

Figure 2. Number of different types of microplastic particles in 5-gram feedstuff samples from dairy farm A

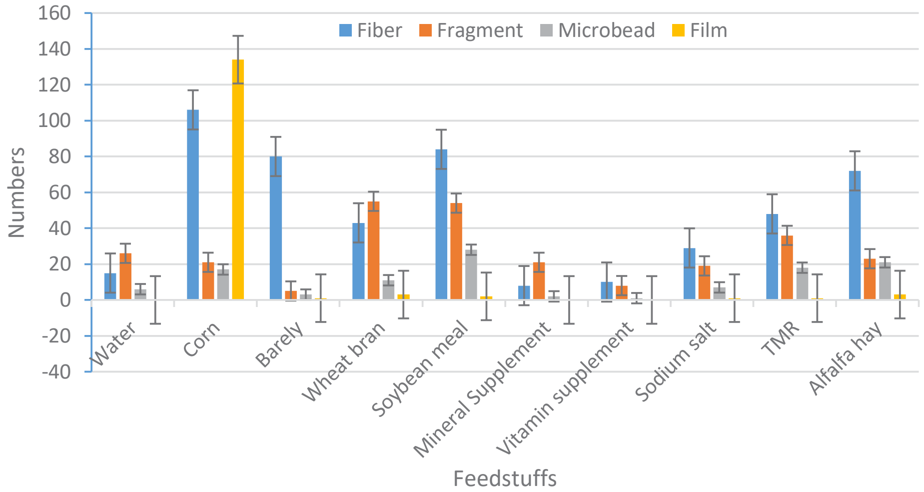

Figure 3. Number of different types of microplastic particles in 5-gram feedstuff samples from dairy farm B

Daily exposure to microplastics through feedstuffs

Table 2 presents the calculated total number of daily ingested microplastic particles by a cow in both dairy farms A and B. The estimated daily intake rate of microplastic particles in dairy farm B (816124) was higher than that of farm A (533140). The higher number of daily ingested particles in both farms came from corn grain (32% to 40.8%), alfalfa hay (17.5% to 25.7%), soybean meal (20.6% to 23.8%), and barley grain (13.4% to 15%), respectively. The lower number of ingested par-

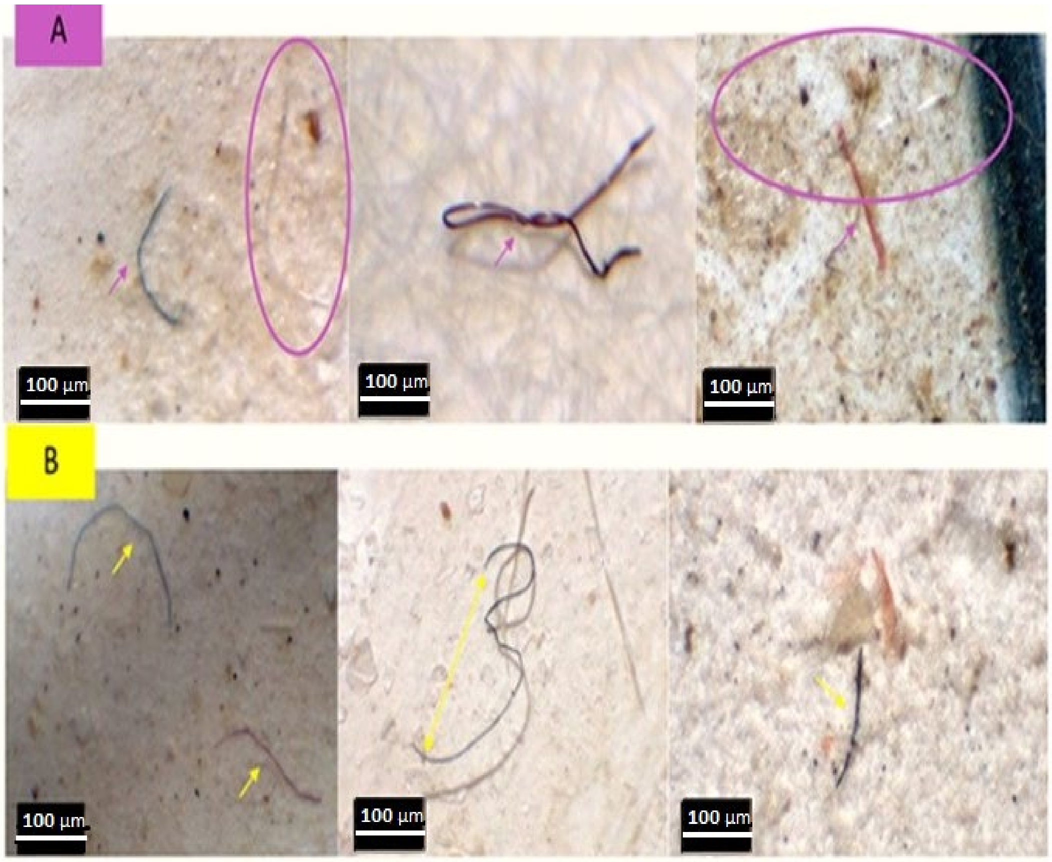

Figure 4. Color diversity of some fiber microplastics that were found in feedstuff samples of farm A and farm B

Note: Pictures were captured by microscope (×2.5 magnification).

ticles also came from sodium salt, mineral and vitamin supplements, and wheat bran, which accounted for about 5.1 to 6.1% of the total numbers in both farms.

Discussion

Microplastics are small plastic particles with a size of less than 5 mm that can come from various sources, including the decomposition of large plastic items, such as bottles and bags, in the environment. In agriculture, microplastics can enter the food chain through agricultural activities, water, and contaminated feed. Marine and plant-based ingredients used in livestock feed, such as soybean meal, cottonseed meal, and sugarcane molasses, may be contaminated with microplastics present in agricultural runoff or wastewater.

In this study, microplastic particles in water and raw materials of livestock feed, including corn grain, wheat bran, soybean meal, alfalfa, and mineral supplements, were quantitatively and qualitatively analyzed. Samples were collected from two regions that differed in their geographical location. The mean size of the particles was found to be between 100 and 700 µm. In region A, which is closer to an industrial city in Tehran, larger particles were found. Among the ten sample types collected, six samples from dairy farm B had more particles than those from dairy farm A. The most contaminated sample in both farms was corn grain, while the least contaminated sample was mineral supplements. The higher contamination of corn grain in dairy farm A may be due to the presence of a large number of microplastic fibers in the farm's water supply, and the presence of microplastic particles in the form of fibers and films in dairy farm B.

In general, the diversity of colors indicates different sources of microplastic release into the environment (Gallagher et al., 2016). In this study, the most common colors observed in both farms were colorless, gray, and red. Based on the observations made during the sampling process and previous studies, it can be concluded that the source of the observed microplastics can be attributed to packaging materials. The colorless particles may originate from plastics that have separated from packaging materials and entered the feed. The red color is associated with the ropes used to package the feed.

In general, samples with an internal source had less contamination than imported feedstuffs. Among the internal samples, those from northern regions of the country had higher contamination. Regarding drinking water, a very small number of particles observed were microplastics, leading to the conclusion that underground waters still have little pollution.

The presence of microplastics in water and feedstuffs is a new concern due to its potential negative effects on animal health and food safety. Microplastics can enter the agricultural cycle through industrial runoff and urban wastewater used as fertilizer (Piehl et al. 2018). These plastic particles can enter the food chain when animals graze on contaminated fields or consume processed feed containing significant amounts of microplastics. Microplastics can then accumulate in the bodies and tissues of animals when they ingest contaminated feed or water. Several studies have shown that microplastics may have various adverse effects on livestock, including gastrointestinal problems, oxidative stress, reproductive disorders, and immune toxicity (Urli S., 2023). For example, a study found that exposure of poultry to microplastics led to reduced growth rates and increased mortality in broiler chickens (Sharma et al., 2024). Another study reported that ingested microplastics cause inflammation and damage to organs such as the liver, spleen, kidneys, heart, and lungs in goats (Omidi, 2012). Moreover, microplastics are associated with hormonal disorders that can affect reproduction and growth processes in livestock. Furthermore, the transfer of microplastics from livestock to consumers is a significant concern. Concerns about human health related to microplastics primarily stem from their potential entry into the food chain through water, air, and contaminated food sources such as meat, dairy products, and processed foods (European Commission, 2023). After consumption, these tiny particles can accumulate in various tissues and organs throughout the body, leading to several adverse effects on human health. It has been shown that microplastics can potentially contribute to chronic inflammation and various diseases, such as obesity, diabetes, cardiovascular diseases, and cancer (Liu et al., 2022), hormonal disorders, and effects on the nervous system, cognitive abilities, and behavior (Wu et al., 2019). However, more research is needed to fully evaluate this risk and develop strategies to minimize it (Queilon, 2023; Aardema 2024).

In this study, for the first time, the daily exposure of livestock to microplastics was evaluated. Livestock in farm A are exposed to a daily total of about 533140 microplastic particles through their feed, while those in farm B are exposed to 816124 particles. The consequences of this pollution on animal health and subsequently human health through consuming contaminated meat and dairy products are a new area of research. Adverse effects include decreased feed efficiency and changes in nutrient absorption that negatively impact growth rates (Rochman et al., 2015). Reproductive disorders, such as changes in reproductive physiology that include decreased testosterone levels and poor sperm quality (Cole et al., 2018), growth problems such as structural changes in bones, liver toxicity, and neurodevelopmental disorders, particularly during early growth stages (Sazakli et al., 2019), and suppression of the immune system with increased susceptibility to infections (Détrée et al., 2018), are also reported among other adverse effects.

Currently, there is no definitive answer regarding which specific compounds or components in animal feed are more prone to microplastic contamination, and research on this topic is a new and necessary area of study. To fully understand the scope and sources of microplastic contamination in animal feed and to find ways to reduce or eliminate it, more research is needed. Meanwhile, focusing on using high-quality materials and local agricultural products when possible, as well as employing suitable filtration systems, may help reduce microplastic contaminants in the production process.

While there is a lack of information on which specific foods contain the highest levels of microplastics, several studies have pointed to various pathways through which these contaminants can enter animal feed. Some research suggests that industrial wastewater and urban runoff, as well as agricultural practices, can introduce microplastics into livestock water sources (Piehl et al., 2018). Soils can also absorb and retain microplastics produced by industrial activities, urban wastewater, or atmospheric deposition (Koelmans et al., 2016). Plants grown in contaminated soils may accumulate microplastics and ultimately enter an animal's diet through grains or forage (Schwabl et al., 2020, Piehl et al., 2020). Concentrate may also be contaminated from raw materials obtained from sources known to be contaminated with microplastics, such as grains or plant-based proteins that were not processed under controlled conditions before reaching the final product (Rochman et al., 2015).

To date, there is no comprehensive research that specifically addresses the challenge of identifying which components of animal diet, including wheat, corn, barley grain, and other concentrated feed or silage, contain the highest levels of microplastic contamination. However, numerous studies have shown that multiple factors play a role in the presence of microplastics in various stages of the agricultural supply chain and may potentially affect these materials.

Researchers and experts in the food and animal feed industry are actively exploring ways to reduce microplastic contamination in animal feed. Some possible approaches include improving farm management practices, implementing good agricultural practices, such as maintaining suitable irrigation systems, reducing synthetic fertilizer and pesticide use, minimizing soil contamination, and effective waste management for livestock farms (European Commission, 2023). Installing filtration systems in water sources used for animal consumption is also an effective way to remove microplastics (European Parliament and Council of the European Union, 2023). Using higher-quality raw materials and adopting stricter quality control measures during raw material preparation for animal food production can also ensure that fewer microplastics enter the final product. Additionally, exploiting advanced technologies for identifying and classifying contaminated grains before combining them into the final animal food product can significantly improve its quality (FAO, 2023). Other techniques for achieving lower microplastic levels in animal feed include processes such as milling, sieving, and cleaning (EPA, 2021). Each strategy targets various aspects of the supply chain to minimize the entry of microplastics into the animal diet and ultimately protect animal and human health. However, it is essential to remember that ongoing research and collaboration between feed producers, farmers, and other relevant stakeholders are necessary to assess the practicality and effectiveness of these strategies.

The limitations of this study can be mentioned as being unable to measure particles at the nanoscale. The analysis of observed microplastic particles can help to determine the type of polymer and ultimately identify the main sources of contamination in animal food.

Conclusion

Microplastic particles were observed in all of the tested feedstuff samples, with the corn grain samples being the most contaminated in both farms. The number of particles in the corn samples in farm B was higher than that of farm A. The mean particle size in samples from regions A and B ranged from 121 to 661 µm and 179 to 497 µm, respectively. Microplastics exhibited a wide variety of shapes and colors, but the most common shape observed was fibrous, and colorless (transparent) particles were more prevalent than other colors. Based on the estimates performed, dairy cattle are exposed to between 500,000 and 800,000 microplastic particles daily just through their feed. It is recommended that the approximate rate of excretion of these particles be investigated by studying cow feces samples to facilitate the calculation of their probable absorption rate by the digestive system. Examining milk from dairy cows in terms of microplastic excretion is also important because milk is consumed by both calves and humans and is part of the human food chain.

Ethical Considerations

Compliance with ethical guidelines

There were no ethical considerations to be considered in this research.

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Authors' contributions

All authors contributed equally to the conception and design of the study, data collection and analysis, interception of the results and drafting of the manuscript. Each author approved the final version of the manuscript for submission.

Conflict of interest

The authors declared no conflict of interest.

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