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Microplásticos en materia fecal de rumiantes en Ecuador

Microplastics in ruminant feces in Ecuador

Microplásticos em fezes de ruminantes no Equador

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Resumen

El presente estudio proporciona evidencia de la presencia de microplásticos en la materia fecal de bovinos, ovinos y caprinos de producción en el Ecuador como un indicador relevante en la comprensión de los procesos de fragmentación, mecanismos de biodistribución y posibles efectos de la contaminación en la cadena agroalimentaria. Un total de 300 muestras de heces de rumiantes se analizaron a través de una investigación de campo, observacional y transversal. Utilizando la técnica de flotación de solución salina saturada, se encontró que el 75,67% de las muestras estaban contaminadas con microplásticos. Los análisis estadísticos, que incluyeron tablas de distribución de frecuencia, prueba de Chi Cuadrado de Pearson, prueba Z y Odds Ratio utilizando el software SPSS versión 26, demostraron que el tipo de alimentación es un factor significativo en la presencia de microplásticos en las heces de los rumiantes. Además, se observó un aumento del 87% en la reducción de microplásticos en la materia fecal de rumiantes en sistemas de pastoreo en relación con aquellos que recibieron alimentación suplementaria balanceada. Finalmente, se encontró un incremento del 85,3% en la presencia de microplásticos en heces de bovinos que pastaban cerca de vías. Estos resultados señalan la importancia de considerar el tipo de alimentación, el entorno de pastoreo al abordar la contaminación por microplásticos en la cadena agroalimentaria y sus posibles implicaciones en sistemas de una salud.

Palabras clave: Heces, Partículas de Plástico, Flotación, Contaminación, Fauna.

Summary

The current study provides evidence of the presence of microplastics in the feces of cattle, sheep, and goats under production in Ecuador as a relevant indicator in the understanding of fragmentation processes, biodistribution mechanisms, and possible effects of contamination in the agri-food chain. A total of 300 samples of ruminant feces were analyzed in a field, observational, and cross-sectional study. Using the saturated salt solution flotation technique, 75.67% of the samples were found to be contaminated with microplastics. Statistical analyses, which included frequency distribution tables, Pearson's Chi-Square test, Z-test, and Odds Ratio using SPSS version 26 software, showed that the type of feeding is a significant factor in the presence of microplastics in ruminant feces. In addition, an 87% increase in the reduction of microplastics in ruminant feces was observed in grazing systems compared to those receiving balanced supplemental feeding. Finally, there was an 85.3% increase in microplastics found in the feces of cattle grazing near roads. These results suggest the importance of considering the type of feed and the grazing environment when addressing microplastic contamination in the agri-food chain and its potential impact on one health systems.

Key words: Feces, Plastic Particles, Flotation, Pollution, Fauna.

Resumo

O objetivo do presente estudo foi fornecer evidências da presença de microplásticos nas fezes de bovinos, ovinos e caprinos em produção no Equador como um indicador relevante para a compreensão dos processos de fragmentação, mecanismos de biodistribuição e possíveis efeitos da contaminação na cadeia agroalimentar. Um total de 300 amostras de fezes de ruminantes foram analisadas num estudo de campo, observacional e transversal. Utilizando a técnica de flotação em solução salina saturada, verificou-se que 75,67% das amostras estavam contaminadas com microplásticos. As análises estatísticas, que incluíram tabelas de distribuição de frequências, teste Qui-Quadrado de Pearson, teste Z e Odds Ratio utilizando o software SPSS versão 26, mostraram que o tipo de alimentação foi um fator significativo na presença de microplásticos nas fezes de ruminantes. Além disso, foi observado um aumento de 87% na redução de microplásticos nas fezes de ruminantes em sistemas de pastagem em comparação com aqueles que receberam alimentação suplementar balanceada. Por fim, houve um aumento de 85,3% na presença de microplásticos nas fezes de bovinos que pastam perto de estradas. Estes resultados sugerem a importância de considerar o tipo de alimentação e o ambiente de pastagem ao abordar a contaminação por microplásticos na cadeia agroalimentar e o seu potencial impacto nos sistemas de saúde.

Palavras chave: Fezes, Partículas de Plástico, Flutuação, Poluição, Fauna

Introduction

In 2020, 337 million tons were the amount reached in the annual production of plastics; in fact, there will be a nearly estimation of 11 billion tons of plastic waste in the environment by 2025 [(1)], with the highest amount of microplastics (MP) found in densely populated urbanized regions [(2)]. The new historical era: Plasticine, requires an extensive search for scientific indicators on the levels of exposure to microplastics, their additives and compounds that are disposed of in the environment, and their consequent effects on human health [(3).]

Nowadays, MP contamination in the agri-food chain is considered an emerging problem as MP has been detected in freshwater [(4)]. The One Health approach, which provides a modern perspective focused on a multidisciplinary approach to health, enables the formulation of research on the presence, interaction, and possible effects on humans, animal health, and the environment [(5)] to monitor the environment and food for plastic contamination [(6).]

The worldwide expansion of plastics is due to their physico-chemical, mechanical, and technological properties; they offer material with hardness, tensile and impact strength, machinability, elasticity, and low production costs. The main compounds used in the plastics industry are polyethylene (PE), polystyrene (PS), polypropylene (PP), polyethylene terephthalate (PET) and polyvinyl chloride (PVC) [(7)].

There are additives associated with MPs to improve their physico-chemical properties, such as flame inhibitors, plasticizers, stabilizers, colorants, lubricants, antistatic agents, slip agents, curing agents, foaming agents, and biocides [(8)].

The association of various types of plastics with additives such as phthalates and bisphenol A (BPA) has been found to lead to increased risk in physiological functions. Phthalates are used in the industry to impart softness and flexibility to polyvinyl chloride (PVC), and BPA is used to impart translucency, in addition to improving mechanical and thermal properties [(9) .]

MPs are classified into primary and secondary particles; primary particles are intentionally produced by industry to produce various plastic products, while secondary particles are the product of the disintegration, friction of materials or waste released into the environment [(10)] .

The physical structure of plastic breaks down into fragments over time until it is no longer visible to the human eye, leading to microplastics. In 2004, the term MP was first coined to refer to small plastic particles < 5 mm [(11)]. Once plastics end up in the environment, they are fragmented into microplastics by physical, chemical, biological, and mechanical processes [(12),] fragmentation, weathering, hydrolysis, UV radiation and biodegradation [(7)]. These can alter the abiotic properties of matrices and interfere with essential ecosystem functions that affect ecosystem services (e.g., biogeochemical processes), which, in turn, can affect human health [(13)]; this particular pollutant represents a major threat to organisms in various ecosystems, including human health [(14)].

MP is a ubiquitous contaminant that can be found in air, water, and soil. Based on several studies, it can be considered that the main exposure to MP is through the gastrointestinal tract by ingesting food. Indeed, the most common plastic particles found in food are blue and have a fiber shape[(15)].

MPs and nanoplastics (NPs) can permeate and accumulate in the soil, thereby affecting plant growth and development [16]. Several foods are contaminated with MPs such as apples, carrots, and lettuce [17], radish and broccoli [18], rapeseed [19], and they can be found in seeds, roots, stems, and leaves [20]. In tissues such as liver, meat and intestines of cattle and sheep, between 0.14 and 0.13 MP/g, respectively, were detected, beef showed the highest concentration of MP (0.19 MP/g) [21] are particles because of contact with containers [12].

There is evidence of a small increase in MP contamination in milk processing and packaging conditions according to several investigations [22]. In the food chain, contamination with polyethersulfone (PES) and polysulfone (PSU) fibers, and membrane materials in dairy processes has been registered, indicating contamination in technological processes [23]; however, exposure to microplastics resulting from milk consumption may also differ according to people's consumption habits [24]. It is also possible to consider the transfer of MP from the environment to the body of cattle through their feed and contamination during meat processing and the consequent health risks to consumers [21].

In vitro studies in human cell lines have shown controversial results regarding the toxicity of MPs in the intestinal system. On the contrary, studies in mammals suggest that MPs may have adverse effects in terms of toxicity, immunotoxicity and dysbiosis of intestinal cells [25].

The main factor in addressing the plastic threat is to reduce the use of plastic as much as possible and, if used, to give more preference to choosing biodegradable plastic than its recalcitrant counterparts [26], as the use of alternative non-plastic materials ensures microbiological safety [13]. Furthermore, mandatory standardization of the determination of MP in food products, including raw materials, is considered vital to ensure confidence among authorities, the food industry, and consumers [22].

This research is intended to establish a baseline with information obtained by studying the presence of microplastics in fecal samples, with the objective of conducting a series of studies on production animals, food of animal origin and their traceability.

Materials and Methods

The following research was conducted in several locations in the provinces of Guayas and Manabi in Ecuador, with a tropical savanna climate. In the present study, field, observational, cross-sectional, non-experimental, correlational, quantitative, and analytical research was conducted.

It was carried out the analysis of MP in 300 samples of feces (100 for each species) of ruminants: cattle, sheep, and goats of the tropical region.

Statistical analysis

For the statistical analysis, frequency distribution tables, Pearson's Chi-square test, Z test, and Odds Ratio were used with SPSS version 26.

Sampling procedure

Animals in this research were randomly selected, in different establishments for each species. The fecal analysis does not require refrigeration or freezing as long as the sample arrives at the laboratory immediately, using fresh samples collected directly from the rectum before the first evacuation in the morning.

Procedure

The processing of the samples was carried out in the multifunctional laboratory of the Faculty of Veterinary Medicine and Zootechnics of the University of Guayaquil, using the modified Willis Molloy technique.

1. Collect the feces samples in a container with their corresponding data and place them in a cooler.
2. Once all the samples were taken, we went to the laboratory of the faculty, so we proceeded to use the biosafety norms.
3. The samples were weighed in a digital balance with the test tube establishing a unit of measurement of 2.5 g per sample.
4. With the help of a wash bottle, we place the supersaturated solution in each 50 ml test tube, and with a tongue depressor, we stir it to homogenize it, and then we place a piece of aluminum foil over the test tubes.
5. Then we put the test tubes in the incubator at a temperature of 37.5°C for four hours.
6. At the end, with the help of the micropipette (regulated to 50 µl), we place on the microscope slide a drop of the supernatant of the sample processed for four hours, then we cover it with the coverslip, seal it with gloss enamel and observe under the 10x and 40x microscope.

The modified Willis-Molloy technique is a qualitative test that has been modified to identify microplastics by separating them from fecal matter with the appropriate specific gravity to determine the type of microplastic involved [18].

Results

Regarding the determination of the presence of microplastics, there was evidence of 75.67% in the study of 300 ruminants, including cattle, sheep, and goats, with 54% of positive results in cattle, 80% in sheep, and 93% in goats, in samples analyzed using the flotation technique with supersaturated saline solution (Table 1).

Table 1. Presence of MP in ruminants, flotation study in feces samples.

Species	Frequency	Percentage

Cattle	Yes	54	54
	No	46	46
	Total	100	100
Sheep	Yes	80	80
	No	20	20
	Total	100	100
Goats	Yes	93	93
	No	7	7
	Total	100	100

For the breed variable, since the animals were of mixed breed and different species, it was not possible to perform statistical calculations. We found 72.7% of the cases in sheep of the Pelibuey breed (n:22).

The current research showed an association between the gender variable and the presence of MP in ruminants, indicating different groups between the presence of microplastics using the Z test, and establishing that male ruminants are 35% less likely to present MP, according to the risk estimation (*P*-value Chi^2: 0.043, OR:0.542, 95% CI 0.29 - 0.98; *P*-value: 0.044), Table 2.

There was no association in the sex variable for the goat species. While in cattle a statistical association was established: males 40.5% (15/37) and females 61.9% (39/63), where 58% of males have a lower probability of presenting MP than females (*P*-value Chi^2: 0.038, OR:0.42, 95% CI 0.18 - 0.96; *P*-value: 0.040).

Table 2. Cross-tabulation of the presence of MP by sex in ruminants.

			Presence of Microplastics		**Total**
			Yes	**No**	
Sex	**Male**	Count	82 $_a$	34 $_b$	116
		% within Sex	70,69%	29,31%	100%
	Female	Count	145 $_a$	39 $_b$	184
		% within Sex	78,80%	21,20%	100%
Total		Count	227	73	300
		% within Sex	75,67%	24,33%	100%

Similarly, a high statistical association is reported between the variable age and the presence of MP in ruminants, mentioning a difference between the groups that present MP in feces according to the Z-test, and it can also be determined through the estimation of risk with 72% less likely to present MP in young animals when they have not yet entered a reproductive stage (*P*-value Chi^2: < 0.001, OR: 0.285, 95% CI 0.15 - 0.52; *P*-value: 0.0001), Table 3. No association was reported between the variable age and the presence of PM in cattle, goats, and sheep.

Table 3. Cross-tabulation of the presence of MP by age variable in ruminants.

			Presence of Microplastics		**Total**
			Yes	**No**	
Age	**Growth**	Count	46 $_a$	35$_b$	81

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group		% within age group	56,79%	43,21%	100%
	Adult	Count	181 a	38 b	219
		% within age group	82,65%	17,35%	100%
Total		Count	227	73	300
		% within age group	75,67%	24,33%	100%

In the variable feeding type, a statistical association was found between grazing and mixed feeding (grazing and concentrates) with the presence of MP in ruminants, with a difference between the groups presenting MP in feces according to the Z-test. Finally, by risk estimation, it can be determined that ruminants consuming only pasture are 87% less likely to present MP compared to animals fed both concentrates and pasture (P-value Chi2: 0.01, OR: 0.041, 95% CI 0.002 - 0.68) P-value: 0.026), Table 4.

Table 4. Analysis of the presence of PM by feeding variables and **concentrate** supplementation.

			Presence of Microplastics		**Total**
			Yes	**No**	
Type of power supply	**Grazing**	Count	192 a	63 b	255
		% within type of power supply	75,29%	24,71%	100%
	Mixed	Count	35 a	10 b	45
		% within type of power supply	77,78%	22,22%	100%
Total		Count	227 a	73	300
		% within type of power supply	75,67%	24,33%	100%

In this study, it is not possible to calculate the independent variables of water source and breed, since all the animals had a drinking water source, and they were categorized as mixed-breed.

Discussion

Due to their ability to digest fiber and produce high-quality animal products from non-edible foods, ruminants can play an important role in the human diet. Dairy cows, sheep, and goats consume mainly foods that are inedible for humans such as grasslands and wild pasture areas, which means that 89%, 89%, and 86% respectively of the proteins in the average diet, do not compete with human nutrition. In the case of energy, these figures are 86%, 88%, and 83%, respectively. Finally, the more forages and grasses in the diet, the more efficient the production systems will be in converting energy and protein [27].

Sewage, wells, groundwater, or drinking water can be one of the main sources of contamination of pasture or forage. It has been established that the spread of MP targets sewage treatment plants as a common source from which they can access natural waters, groundwater, or terrestrial ecosystems [28], often present in fresh, drinking [4] and domestic water.

Additionally, it is of scientific interest to carry out more in-depth studies to assess the interaction between water sources, their distribution, and use, as these are a possible source of contamination of aquatic and terrestrial ecosystems, biota, and human health. In addition, the study of MP in wastewater treatment plants in large cities, the continuous monitoring of coastal pollution, and the establishment of environmental and food safety regulations are key [29].

Further studies have demonstrated the incorporation of MPs into the soil. It is believed that the long-term application of manure as organic fertilizer contributes 43 - 75.9% of the MPs in soils [30]. In addition, microplastics are excreted in animal feces and pose an indirect threat when applied as organic fertilizers to agricultural fields, contaminating plants with microplastics or reducing their productivity [13]; another way in which MPs enter soils is through diffuse aeolian transport [31].

In the current study, fecal or manure samples were found to be contaminated with 75.67% of MP in the target species. Other studies show the presence of 40% MP in manure samples, with higher levels of polyethylene and polypropylene found in a sheep farm in the northern province of Shaanxi in China [32]. With all this evidence, it could be concluded that animal manure is a source of microplastics entering the environment due to its widespread use as a fertilizer on agricultural lands around the world. Furthermore, studies of manure treatment to remove microplastics have shown that each of the microplastics studied exhibits unique behavior during composting [33]. It is also very likely that the digestive systems of different farm animals affect the excretion of microplastics. For instance, a recent study found no microplastics in cow manure fertilizers and suggested that the long digestive process in cattle may have resulted in microplastics being completely digested in the cows' intestines [34].

Fecal material is a key determinant of microplastic exposure in farm animals [33], with grazing cattle more likely to ingest pasture-aged fibrous and lamellar MPs [35], and then release a large amount of microplastics to the field in their feces [36]. Moreover, most particles are expected to be eliminated in feces after biliary excretion and macrophage migration. Therefore, microplastics are often found in the feces of production animals [37], considering cattle as the carrier and vector of contamination [38].

Studies have shown that MP/NPs enter the body of animals through water, food, breath, and even skin, which later have access to the blood circulation through the lungs and digestive tract and these can be eventually accumulated in various tissues (1). The most common route of exposure is considered to be the gastrointestinal tract [12] which can be considered the main route of internalization of MP as the respiratory tract can prevent larger particles (>10 µm) from reaching the alveoli [37]. Translocation studies have even been reported in animal tissue, especially with smaller microplastics (<150 µm) [13].

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Microplastics can be transported from plant roots to leaves [39] and affect plant growth, development, and function, including germination, inhibition of root and leaf growth, and tissue composition, [40,7]Previous research showed the accumulation of MPs in vascular systems of plant tissues, particularly in vascular bundles and veins of leaves, and in cell walls and intercellular spaces [41].

After degradation and fragmentation, MP contaminates fields by transferring these fragments or fibers from the soil to plants, providing a source of contaminated plant food for both humans and animals [39]; grazing livestock are more likely to ingest MP in contaminated soils [35], especially in intensive agricultural systems [32].

Our results demonstrated that the supplementation of concentrates in ruminant diets can be identified as a possible factor to increase the amount of MP in feces. It is fundamental to establish that ruminants can have a specialized diet through the administration of highly edible concentrates with less than 30% crude protein (CP) rich in cereals and poorly edible concentrates with more than 30% CP, where the main component is the Alfalfa legume. Finally, agricultural producers often use levels of 15% and 30% to allow the distribution of concentrates to animals in a range from high-energy/low-protein to low-energy/high-protein [27]

These findings are in line with Priyanka & Dey (2018) who conducted a study that claims that there is a statistical association between the female sex variable and the presence of MP in ruminant feces. Females have a higher risk of ingestion and accumulation of foreign bodies in the rumen throughout their life; due to several physiological factors such as increased nutritional requirements during reproduction, pregnancy, and lactation, negative energy balance, and mineral deficiencies.

MP residues are a major source of contamination in soils, which can then spread to various ecosystems and, due to their small size, are easily ingested by organisms and subsequently transported through the food chain [42]. Additionally, it has been shown that processing and packaging can also increase the number of microplastics in animal products, possibly even exceeding the concentrations originally present in the product [13], gradually increasing with the complexity of the processing and packaging processes [1].

The management of research work to establish a baseline, standardization of techniques, and validation of tests to improve the sensitivity and specificity in assessing the presence of MP in human food sources, such as the findings of the study with the marine snail Thaisella chocolate, made it possible to determine the potential bioindicator for monitoring MP [43]. Other studies have demonstrated the presence of MPs in takin (*Budorcas taxicolor*) and leopard cat (*Prionailurus bengalensis*), providing the first report on exotic fauna. However, data on microplastic exposure in wildlife are still limited [44].

The significant contributions of new studies allow us to evaluate the use of MP filtration, identification, and characterization techniques to broaden the analysis of solid or liquid samples by determining the best test in red blood cells in sheep [45], small intestine samples [46] and MP bioaccumulation processes in animal tissues [1].

One option to reduce the MP recontamination process in agricultural areas may be the incorporation of biochar in composting processes to promote the degradation of microplastics [47]; energy-efficient fuel production from the bioconversion of microplastics to polyhydroxyalkanoate (PHA) shows great potential and sustainable alternatives to petroleum-derived plastics [48].

The few and limited studies on the determination of microplastics in biological samples of production animals show great methodological challenges in the detection and characterization of MP. The urgent need to assess contamination levels in the agri-food chain requires the identification of reliable methods to detect, sample, and measure MP in different animal products [38].

Conclusions

On this basis, we conclude that 75.67% of MPs were detected in stool samples processed by density separation techniques in saturated saline solution. Samples of feces in cattle showed 54%, in sheep 80%, and in goats 93%.

One of the independent variables to be highlighted is sex in ruminants, where the group of females presented a higher degree of contamination with 78.8%, compared to males with 70.69%, showing a significant difference (p<0.05) in favor of females.

To sum up, we have shown that the type of feeding is one of the main factors to look at since it is reflected that any grazing system can be a variable to account for the reduction of microplastics in ruminant feces, with 87%, compared to the group that has a mixed feeding by supplementing concentrates. Also, an increase in the presence of microplastics in the feces of cattle is established at 85.3% when they are fed in grazing systems near roads.

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