Chapter 4: Microplastics in Small Ruminants II

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Abstract

The purpose of this investigation was to evaluate the presence of microplastics in goats in one of the cantons, in the province of Manabí, Ecuador; the unit of analysis was goat feces *(Capra aegagrus hircus),* the type of research used was field, non-experimental, observational, transversal, descriptive and analytical. In this study, 100 fecal samples were collected for the purpose of processing them in the laboratory of the Facultad de Medicina Veterinaria y Zootecnia This was done using two types of techniques. The procedure to follow was to leave them in the incubator for 4 hours at a temperature of 37.5 °C with the help of a glass pipette, a drop of the sample was taken and placed on the slide and then covered with a glass plate coverslip and finally observe it under a microscope with a 10x objective. Obtaining as a result a prevalence of 93% with the supersaturated saline solution and 100% of potassium hydroxide solution to a 10% in the presence of microplastics in the analyzed samples. Regarding the sex variable, statistical significance was found in females corresponding to a free grazing system. It is important to keep in mind in future research the toxicity and its impact on the health that may affect goats.

Keywords: Feces, Goat, Microplastics, Prevalence, Supersaturated solution of salt

1 Introduction

It has taken 50 years for plastic to infiltrate every single facet of our existence. Through this time, world-wide plastic production has climbed annually, to an estimated 368 million tons in 2020. The continued use of this water will have great environmental impact, in so much as it functions as a pollutant carrier (Chusan & Cruz, 2023).

Plastic designates a material from polymer compounds that derived from organic material other than petrochemical derivatives. It has now turned into a major environmental polluter because it releases a large number of toxins when being produced or decomposed. One of its most disturbing characteristics is that it takes a long time to degrade, causing long-term damage to the environment (Posada, 2022).

Plastic has very versatile, excellent strength-to-weight ratio and its production thhe conventional materials in many applications. Nevertheless, its major disadvantage is the environmental load during the end-of-life, with the production of microplastic particles (Celi et al., 2023).

Even if there is no consensus about their exact size range, across studies MPs are usually referred as scraps of synthetic polymeric materials derived from petroleum with a size less than 5 mm (Zaragoza et al., 2023).

Microplastics are inert and recalcitrant materials and serve as vectors facilitating the attachment of heavy metals, pharmaceuticals, micro-organisms, viruses and toxins on their surfaces. This property allows them to remain viable in the environment maybe even longer (Sagot, 2022).

Regarding such anthropogenic pollutants, a recent study in Colombia evaluated microplastics in goat manure, and detected synthetic or semi-synthetic polymers of anthropogenic origin, ranging from less than 5 mm in size (2–5 mm). The highest abundance of MPs was in the Cusco region (35.2% = 647 MPs). Then Polonia-Villavieja with 29.2% (536 MPs), Xilópalos with 18.2% (334 MPs) and finally Los Hoyos with 17.3% (316 MPs), which is the one with the least amount of MPs (Serna, 2021).

Thus, the objective of this study is to evaluate the presence of microplastics in goat livestock in a canton of the province of Manabí, in the Ecuador. It's also aimed to determine MPs by the separation methods and compare the number of MPs in goat feces. Lastly, the findings will be integrated back with the study's independent variables.

II. Theoretical Framework

2.1 Domestic Goat (*Capra aegagrus hırcus*)

The goat is one of the oldest domesticated animals that people have. There is evidence that the he-goats were tamed in Asia about 7000-9000 years ago. It is known that the ancestor of a domestic animal is a wild animal. Thus, the most probable ancestor of a

domestic goat is a wild bezoar goat *(Capra aegagrus).* Multiple domestication events Looking at other animals that have become domesticated, it could be that several domestication events took place at the same time, with humans hunting these animals and letting baby animals get used to people. Hence, the goats became specialized in scavenging anthropogenic food sources, whether meat, milk or food remains (Rúa & Tarazona, 2023).

The oldest traces of the presence of these animals have been discovered in Ganj Dareh (Iran) dating from around 10,000 years BCE. Goats (*Capra hircus*) are domesticated ruminant mammals that stand about one meter tall and have a dainty body with short, rough hair systemically following a red color. They also possess large, hollow, twisted, and knobbed horns as well as a long, pendant tuft of hair from the lower jaw. They are typically white in color and have tender skin, although some animals may have black spots of the udders and ears.

This species is known for its resistance toward a stable environment and its sensitivity to radiation. Size depends upon selective breeding in each country, but is large, with a height of approximately 95 cm (38 inches), and a weight of 73 to 76 kg (160 to 170 pounds). Kids, fattened for meat production, have a good fattening performance as well as acceptance of the tender flesh. Due to their udder conformation, which enables easy manipulation of several animals in same herd, they are considered fit for mechanical milking. With respect to reproduction, they are capable of up to seven kids per litter (Zapata & Mellado, 2021).

Goats have historically had an important socio-economic impact on rural farming communities in some of the most remote areas in the world. The goat-breeding industry can exploit poor natural resources found in different areas to create a significant source of protein. This species is able to colonies habitats frequently inhospitable to other animals (Aguirre et al., 2021).

Ecuador regions: The central region of Ecuador, with its mountainous topography, is filled with steep ranges and valleys, offering a wide diversity of forage, climates and soils. Hence, animal and plant populations have become adapted to particular zones and have a long period of adaptation. This results in individuals in practice taking a number of generations to become the tool that they are in nature (Aguirre et al., 2021).

2.2 Production System

The integrated production system is in the tropical dry forest, land isn't good for sowing and planting. Utilization of large land areas is necessary for lack of other feed sources in

this system. The semi-intensive system means that animals are allowed to graze freely during the day and are housed in pens during the night, while they receive periodic supplements, like molasses, forage, salt, or concentrate. This system is frequently employed to monitor goats for when they are in a critical phase (e.g., during pregnancy and parturition) and to simplify management processes of treatment and milking (Chicaiza et al., 2023).

Intensive goat farming is increasing, and 250–1,000 head of goats in a barn is common. Confinement feeding can be internal – through the production of high nutirional value forage – or external, which is more expensive for the producer. In the intensive type, the most common forages are orchard grass (*Dactylis glomerata*), elephant grass (*Pennisetum purpureum*), alfalfa (*Medicago sativa*), clover (*Trifolium repens*), and in some cases, shrubs such as linden (*Tilia platyphyllos*), which are grown as live fences. These serve as feeders, from which the animals can eat, which give the system a semi-intensive nature (Pesántez & Sánchez, 2021).

2.3 Ruminant Nutrition

Ruminant animals are characterized by a four-chambered stomach, composing of the reticulum, rumen, omasum, and abomasum. The abomasum secretes digestive enzymes, just like the stomach in monogastric animals. Ruminants can eat grasses and forage because they can ferment structural polysaccharides, such as cellulose, hemicellulose and pectin — something that non ruminant species generally do not do.

This degradation occurs by fermentation in the rumen, where microorganisms live. Subsequently, the fermentation hydrolats are utilized to cover the animals' physiological requirements (Arias et al., 2020).

The rumen is an ecosystem that is populated with bacteria, protozoa, and fungi. This ecological context is dynamic and intricate, with an interplay among the microorganisms that compete for resources as it depends on the environmental conditions they faced. Inclusion of high amounts of concentrate in the diet causes damage to rumen balance. This is a disturbance in pH—changing from alkaline to acid— that affects rumen microbial activity, and consequently leads to competitive reduction in utilization efficiency of feed (Da Silva et al., 2022).

The quality and quantity of native and introduced pastures are closely related to livestock production. In the case of Manabí, a number of Fe materials are available, however, they are generally of low-yielding and poor quality when used as the sole source of protein in the diet for ruminants. This in turn has adverse effects on feed intake, digestibility and

the physiological responses of animals. As time goes by, goats keep grazing on grass and browse, mostly from the extensive breeding system (Roca et al., 2020).

2.4 Microplastics

Microplastics (MPs) are defined as plastic particles smaller than 5 mm in size, resulting from the degradation of synthetic polymers derived from petroleum. There is no single accepted definition, but microplastics were first coined by the National Oceanic and Atmospheric Administration in 2004, having been found earlier (dating back to the 1970s) by Thompson and colleagues in samples collected in the Atlantic Ocean and Bristol Channel. As a result, MPs have been found in all studied aquatic and terrestrial ecosystems, from the polar to the deep ocean (Zaragoza et al., 2023).

2.5 Classification of Microplastics

MPs can be categorized by color, size and type (e.g. foams, fibers, rigid and semi-rigid fragments, pellets and films). Pieces are plastic objects of not regular shape (rectangular, triangle) but whose structure is either rigid or semi-rigid. Color and size are very important as they affect the probability of ingestion. Whites, browns, and yellows of microplastics of commercial size have shown to be mistaken for prey by fish, several of which are consumed frequently. Furthermore, small microplastics are more biologically available, and can be ingested by a range of marine and terrestrial biota (Cruz et al., 2020).

Personal care products and machinery cleaning are the sources of primary microplastics. One of these additives, phthalates, is commonly employed as a pharmaceutical coating for capsules and tablets. Secondary microplastics form after microplastics themselves break down from exposure to elements such as sunlight, chemicals, organisms, and physical abrasion that break the polymer bonds and make smaller pieces. People can shed synthetic fibers from clothing while washing clothes, when they pick/wash plastic packaging or in marine environments where wind and waves help fibers escape the human grasp (Castañeta et al., 2020).

2.6 Microplastic Extraction Techniques

In recent studies a new enzymatic hydrolysis was developed to erase organic matter from microplastics without affecting the micro plastic polymer itself by immersing it in an enzyme solution. Enzymatic digestion also serves as an efficient alternative, for MPs degradation and released with their structure remaining. In addition, oxidation with 30% H_2O_2 has been reported to remove approximately 83% of organic matter from MPs, but

remaining efficacy may depend on sample size. These methods are crucial to MP research because of the variables at hand (Ortega & Solis, 2023; Sorolla, 2023).

Other researchers suggested tested some chemical solutions 20% HNO_3 and 10% HCl prepared at various temperatures with 24 hours exposure [16]. Other solutions including 30% H2O2 and 10.6% KOH were stored at room temperature for 7 days. These tests were utilized to determine the efficiency of biogenic organic material destruction and the tolerance of various synthetic polymers to the digestion protocols (Pfeiffer & Fischer, 2020).

2.7 Microplastic Identification

A few techniques are available for the detection of MPs. One is optical microscopy that employs stereoscopes and microscopes in order to manually differentiate and separate the particles according to their color, shape, size, and number. The method is cheap and practical. Another method, that of fluorescence microscopy, generates UV radiation with the help of Xe or Hg lamps and this radiation is subsequently reflected to the sample by a dichromatic mirror, allowing for the differentiation of MPs from other particles. Thirdly, SEM offers very high magnification, detailed images, thus enabling plastic particle size and shape analysis (Kadac et al., 2023).

2.8 Microplastics in the Land-Based Environment

In terrestrial systems the MP loading can be much higher than the marine environment. A high amount of MPs in soils could be found that is over marine ones. MPs can also be introduced into soil via different pathways, such as the addition of organic waste (e.g., compost and biosolids) that may harbor plastic, and plastic films that are commonly used in the agriculture (Zang et al., 2020) to enhance plant growth and water saving.

The organisms in terrestrial ecosystems are threatened by MP pollution that is transported there from different human activities. Gastrointestinal content studies constitute field evidence that freshwater and terrestrial species routinely consume MPs. For instance, a meta-analysis showed that freshwater fish accumulated, on average, 8.8 particles per individual, about four times more than marine ones (Ying et al., 2023).

2.8 Microplastic Contamination in Soil

Microplastics exist in the soils of different environments, including farmland, urban, industrial, forest, and highway soils. They have been recognized to be responsible on the soil structure deterioration and water-holding capacity mineral nutrients uptake

reduction. This reduces the biological activity of the microorganisms and crop development. Also, microplastics may attach with pollutants such as heavy metals, pesticides, or others, since they are becoming contaminants in the soil, they may have a toxic effect on the flora and fauna of the soil. Lastly, microplastics can be transported to the river, lake, and ocean through agricultural runoff (Sajjad et al., 2022).

2.9 Microplastic Toxicity

Impact of microplastics would be different with direct and indirect exposure. Acute toxicity (can be compared to immediate), violence is the toxicity and is the result of a direct exposure with the answer that involves instantaneously whereas chronic toxicity has the same roots of a direct answer but only over longer period of time. On the other hand, indirect exposure occurs due to uptake of microplastics and associated contaminants into the food chain that results in chronic toxicity of internal organs. The results are dependent on both magnitude and duration of exposure. Even in the predominating short-term exposure studies with microplastics <5 mm, findings indicate that the particles accumulate in the gut and result in physiological impacts even after short-term exposure (Qian et al., 2020).

2.10 Microplastics in Cattle

Taraconte Coscarelli et al. focused on ruminants regarding the existence of microplastics, particularly in the Chongón parish (Ecuador), where remarkable findings were observed. Microplastics were present in around 54% of the ruminal samples. Its importance derives from the variables used to find this output, breed, sex, diet, and grazing block. These are variables that will be considered as a key issues to investigate in the following studies of polygastrics (Chusan & Cruz, 2023).

III. Methods

3.3 Methodology

Unit of Analysis

Goat feces (*Capra aegagrus hircus*)

Type of Research

Cross-sectional, descriptive, analytical, observational, non-experimental and field-based.

Population and Sample

The number of goats was determined to be 597 heads in the Manabí province of Ecuador (INEC, 2020). Main breeds in coast: Anglo-Nubian and Criolla. A convenient sample of 100 goats was chosen for this study, considering economic constraints, to establish the presence of microplastics.

Statistical Analysis

Percentages, distribution tables, Pearson's Chi-square Test and Z-Test on SPSS version 26.

3.4 Variables

Dependent Variable
· Existence of microplastics in goat dung.
Independent Variables
· Age
· Sex
· Type of diet
Type of microplastic (MP)
· Color

3.5 Procedure

10% Potassium Hydroxide (KOH) Technique

Stool samples were collected and categorized based on an investigation report. The samples were packed and labelled with an adhesive label and trawled in a cooler with ice for transport. About 1-4 g of feces from individual animals were employed. Samples were weighed with an analytical balance in a watch glass.

To homogenize the content, 20 ml of 10% KOH was added in each test tube. The tubes with the samples were incubated at 37°C for 4 hours. After incubation, a 10 to 20 µl drop of supernatant was removed from each sample with a glass pipette. The supernatant was centrifuged onto slide, covered with a cover slip and examined under low (10x) and high (40x) microscope magnifications (Kadac et al., 2023).

Oversaturated Saline Solution Technique

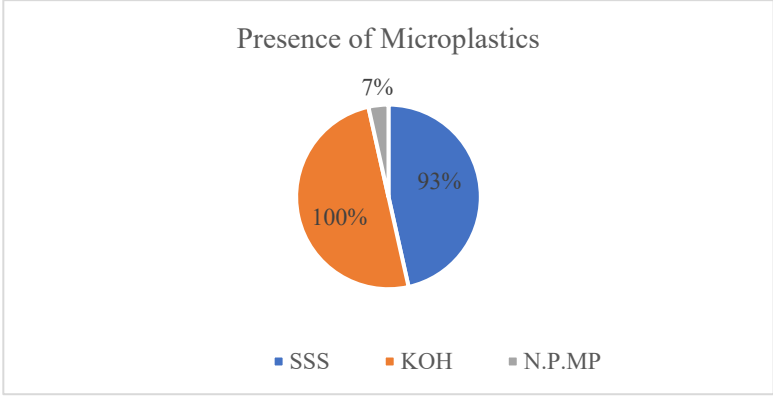
The mixture was prepared by mixing 75g of table salt in 250ml of distilled water, heated on an electric hob to achieve oversaturation in a beaker. The decanted solution was flavored through filter paper to remove any impurities that would be in table salt.

Then 2 g of feces were added to each test tube containing 28 ml of the oversaturated saline solution and homogenized.

The tubes were transferred to an incubator at 37°C and maintained for 4 hours at the fixed temperature. Following incubation, 10–20 µl of the supernatant of each sample were removed with a glass pipette. The supernatant was then transferred onto slides with cover slips and observed with the light microscope under magnifications of 10x and 40x (Hurtado and Torres, 2023).

Figure 1

Comparison of Oversaturated Saline Solution and 10% Potassium Hydroxide in the Detection of Microplastics



In the present study, a density separation method with oversaturated saline solution was employed, and 93% of MPs were detected in the goat feces samples. This is in contrast to the results obtained by Chusan & Cruz (2023), where 54% were positives from ruminant (bovine) faecal samples using the same SSS (Saline Solution Separation) procedure.

At the same time, 100% of the tested samples were found to be positive with KOH solution. Yan et al. (2020), potassium hydroxide (KOH) will dissolve proteins in feces in biological samples and facilitate fecal digestion.

Table 1

Cross-tabulation of the Variable "Presence of Microplastics in Oversaturated Saline Solution" with "Sex"

		Sex				Total	
		Female		Male			
		N	%	N	%	N	%
Presence of Microplastics	Yes	55a	93.2%	38a	92.7%	93	93.0%
	No	4a	6.8%	3a	7.3%	7	7.0%
Total		59	100.00%	41	100.00%	100	100.0%

Considering the sex, 93.2% of females and 92.7% of males had positive samples for microplastics using the oversaturated saline solution method. Conversely, with the potassium hydroxide solution, 59% of positive cases were females and 41 % were males.

In the research carried out by Priyanka & Dey (2018), the sex of animal is reported as an important determinant of foreign body ingestion. A higher incidence of rumen impaction due to plastics was recorded in females, than in males. Several physiological cues are responsible for this feeding response in females. Because of their larger reproductive lifespan, farmers usually retain the females longer than their male offspring, which increases their lifetime exposure to the risk of the ingestion and accumulation of foreign inside the rumen.

Results In the test oversaturated saline solution it was identified statistically significant ($p < 0.05$) by gender variable analyzed by Pearson's Chi-square. Pending the Z-test, the difference between the groups was not statistically significant.

Table 2

Cross-tabulation of the Variable *Color* in Oversaturated Saline Solution with the *Presence of Microplastics*

Presence of Microplastics (SSS)						
		Yes		No		Total
Color (SSS)		N	%	N	%	N / %
	Black	38a	40.9%	0b	0.0%	38 / 38.0%
	Green	24a	25.8%	0a	0.0%	24 / 24.0%
	Yellow	17a	18.3%	0a	0.0%	17 / 17.0%
	Blue	11a	11.8%	0a	0.0%	11 / 11.0%

	White	2a	2.2%	0a	0.0%	2 / 2.0%
	Red	1a	1.1%	0a	0.0%	1 / 1.0%
	0	0a	0.0%	0b	0.0%	0 / 0.0%
	Total	93	100.0%	7	100.0%	100 / 100.0%

For the oversaturated saline, the maximum percentage by weight were observed for black (40.9%) and green (25.8%). The smallest values of the medians were obtained for the white (2.2%) and the red (1.1%).

By comparing the variables color and microplastics the relationship between the two variables was found no to be statistically significant when using the statistical test Pearson´s Chi-square ($p > 0.05$). Similarly, using the Z- test, there was no significant difference between the groups.

Table 3

Cross-tabulation of the Variable *Color* in Potassium Hydroxide Solution with the *Presence of Microplastics*

	Presence of Microplastics (KOH)				
		Yes		Total	
Color (KOH)		N	%	N	%
	Black	41	41%	41	41%
	Yellow	29	29%	29	29%
	Blue	16	16%	16	16%
	Green	8	8%	8	8%
	White	5	5%	5	5%
	Red	1	1%	1	1%
	Total	100	100%	100	100%

-Highest concentrations in Black (41%) and Yellow (29%) were observed in the Potassium Hydroxide Solution. The lowest figures were recorded in white (5%) and red (1%).

A few research have reported that MPs were present in the manure samples from many different colored feed bags and common plastic products (as packaging materials like single-used plastic bags, cups and bottles). Furthermore, coloured MPs could be produced from short-life packaging, as well as from long-life plastic-wrapped products (Wu et al., 2021; Zhang et al., 2018).

Table 4

Cross-tabulation of the Variable *Presence of Microplastics in Oversaturated Saline Solution* with the Variable *Fibers*

		Febres					
		Yes		No		Total	
		N	%	N	%	N	%
Presence of Microplastics (SSS)	Yes	93_a	100.0%	0_b	0.0%	7	7.0%
	No	0_a	0.0%	7_b	100.0%	93	93.0%
Total		93	100.0%	7	100.0%	100	100.0%

According to the results in Table 1, 93% of the samples presented microplastics in the form of fibers in the OSS. In the Potassium Hydroxide Solution, MPs with a typical fiber shape were detected in 100% of the samples. These findings are in discrepancy with the study performed by baderi et al. (2023) found, that within the different microplastic shape categories fibers were the highest occurring type, representing 39%.

In evaluating MPs presence in fiber form, it indicates that there were no significant differences ($p > 0.05$) among the categories evaluated according to Pearson Chi-square. But when the Z test was used, a discrepancy existed between the groups.

Table 5

Cross-tabulation of the Variable *Presence of Microplastics in Oversaturated Saline Solution* with the Variable *Fragments*

		Fragmentes				Total	
		Yes		No			
		N	%	N	%	N	%
Presence of Microplastics	Yes	31_a	100%	62_a	89,9%	93	93%
	No	0_a	0%	7_a	10,1%	7	7%
Total		31	100%	69	100%	100	100%

From the information provided in the table, it is evident that the MPs with the fragmented form accounted for 31% of the samples in the OverSat Saline Solution. In Potassium Hydroxide Solution, 29% of the samples exhibited MPs that were in fragmented form.

When evaluating MPs in fragment with Over Saturated Saline Solution, it was found no difference ($p > 0.05$) between the analyzed categories, according to the Pearson Chi-square Test. So as the Z-test showed no significant difference between the groups.

In the Naderi et al. (2023) did on microplastic morphology, fibers (39%) and fragments (44%) were the most abundant in runoff. This indicates that in the present study, streams that goats are known to drink water from on an opportunistic basis (a source of unknown quality) can spread microorganisms and microplastics, all sourced from human waste in the vicinity.

Eriksen et al. (2021) reported samples structures and content only by applying simple description techniques and concluded plastic films were the most abundant Sample category, but without excluding the presence of not only fibers and fragments, but also clear occurrence of plastic bags and ropes.

In the feeding variable, 100% of the animals were raised under free grazing system, in an extensive production system feeding on shrub and wild grass and taking potable water during the dry season.

V. Conclusions

In goats, from all feces collected in a canton of the Manabí province, Ecuador, 98.5% were positive for microplastics (MPs), with supersaturated saline solution and potassium hydroxide methodologies.

When the potassium hydroxide solution was applied, 100% of MPs in same feces were detected, whereas 93% of MPs were found in the feces extract by the density separation method with oversaturated saline solution.

If the sex variable was concerned, the girls (female) appeared to have statistically significant ($p < 0.05$). In addition, black color at other count (40.9% in SSS, 41% in KOH) was recorded.

Further research and experiments should be performed to explore the occurrence of MPs in goat livestock organs and to elucidate their negative health effects. In addition,

comparative studies between feed and water sources are recommended to determine water sources with the greatest MP levels.

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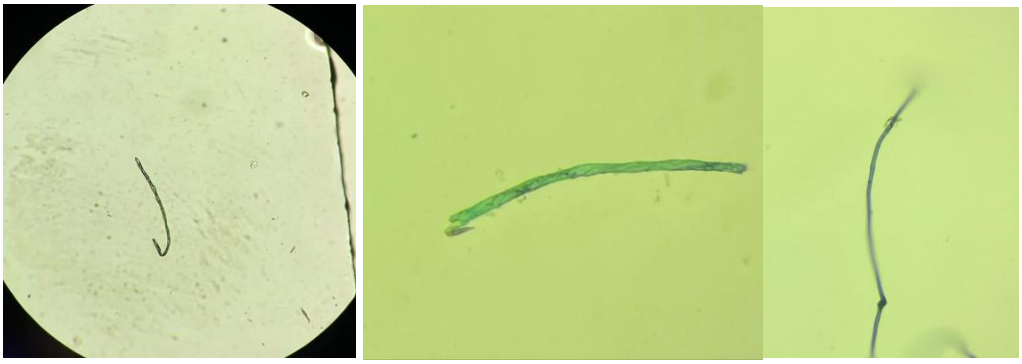
VII. Annexes

Annex 1
Microplastics found in the collected samples



Microplástics (fibers) in feces sample of *Capra aegagrus*. 10X