

# Rapid detection of microplastics in feeds using near-infrared spectroscopy

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## ABSTRACT

The presence of microplastics in the forage and feedstuffs of domestic animals represents an imminent threat to the entire food chain that may reach humans since the particles could be transferred into the intestinal barriers and contaminate blood and animal products. Until now, there is no simple, rapid, sustainable, and reliable method to detect microplastics in animal feed. The objective of this study was to investigate the ability of near-infrared spectroscopy (NIRS) to detect microplastics in ruminant feeds. Two types of instruments were tested using four feeds (corn silage, mixed hay, rye grass silage, soybean meal) and a total mixed ration. Two types of crumbled contaminants, low-density polyethylene and polystyrene, were accurately mixed at ratios of 0, 1, 3, and 5 mg g<sup>-1</sup>. The pool of the five matrices examined by the benchmark instrument (714-3333 nm) yielded an accuracy of approximately 0.8 mg g<sup>-1</sup> and a detection limit of about 1 mg g<sup>-1</sup>, however, the errors could be halved in separate calibrations. A short wavelength range (714-1070 nm) or a smart NIRS instrument proved an acceptable discrimination of the concentrations. Following these preliminary results, any validation on other samples with different and powerful NIRS tools is encouraged.

Section: RESEARCH PAPER

**Keywords:** Feeds; low density polyethylene; microplastics; near-infrared spectroscopy; polystyrene; SCiO™

**Citation:** G. Masoero, S. Barbera, H. Kaihara, S. Mabrouki, S. Glorio Patrucco, Kh. Abid, S. Tassone, Rapid detection of microplastics in feed using near-infrared spectroscopy, Acta IMEKO, vol. 13 (2024) no. 2, pp. 1-6. DOI: [10.21014/acta\\_imeko.v13i2.1663](https://doi.org/10.21014/acta_imeko.v13i2.1663)

**Section Editor:** Leopoldo Angrisani, Università degli Studi di Napoli Federico II, Naples, Italy

**Received** August 29, 2023; **In final form** December 6, 2023; **Published** June 2024

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## 1. INTRODUCTION

Animal welfare is becoming increasingly important to society [1]. Ruminant welfare must be of crucial interest as the number of herds is constantly growing to meet the demand for meat and milk [2].

A problem that is poorly investigated in ruminant welfare is plastic pollution in livestock farms and particularly in feedstuffs. In fact, a substantial amount of plastic material is used to store ruminant feed, especially for dairy cattle. For example, low density polyethylene (LDPE) films are widely used to cover horizontal silos and to wrap and tie bales [3]. Plastics undergo a systematic fragmentation, driven mainly by ultraviolet radiation and mechanical abrasion, generating small plastic fragments (< 5 mm) called microplastics (MPs) [4]. MPs can be divided into two main categories: primary MPs that are directly released in the environment and secondary MPs originating from the fragmentation of large plastic litter [5]. It is crucial to acknowledge the environmental impact of MPs pollution

because they can contaminate feeds through various channels like wind, rain, and other environmental elements.

Recent studies have demonstrated the presence of MPs in cow blood and sheep feces, showing that ruminants ingest MPs [6], [7]. In fact, a pilot study has detected their presence in cow and pig feeds. Moreover, the presence of MPs was also confirmed in both meat and milk, indicating that they can be transferred into animal products and potentially pose health concerns for humans [8].

Until now, most MPs detection methods have been time-consuming, requiring many steps and the use of reagents or enzymes. They are also extremely heterogeneous depending on the nature of the matrix being analyzed [9]. The actual analysis of MPs includes primarily microscopic and micro-spectroscopic techniques (micro-Raman; micro-FTIR spectroscopy), which can only be used in absence of contaminating organic and inorganic materials [10]. With these techniques, MPs are detected after different steps: 1) sampling (collection and preparation of samples); 2) extraction of MPs from the samples; 3)

quantification and identification of MPs [11], [12], [13]. Furthermore, the MPs extraction from feeds could be even more complicated and might require different steps for organic matter degradation, because feeds are considered complex samples due to their constituents which are difficult to remove and are composed of multiple elements [13]. Currently, there is no specific analytical method to detect MPs in feeds. They were extracted from feeds solely by van der Veen et al. [8], however, this protocol is not specific for feeds and involves the use of ungreen solvents such as methanol and tetrahydrofuran [14].

A possible solution to overcome these problems could be the use of the near-infrared spectroscopy (NIRS). NIRS has been used for decades as an innovative technique in agriculture, becoming the most widely used analytic process, including detection of contaminants and analysis of feeds and dairy products. The new generation of portable and handheld devices allows to perform the analyses during the production to evaluate results [14]. NIRS operates in the electromagnetic spectrum from 667 to 2500 nm by measuring light scattered off and through a sample. NIR spectra are characterized by the broad overlapping bands of overtone and the combination of vibrations for a limited number of molecular vibrations (C–H, O–H, N–H). It can quickly determine material properties without altering the sample. Therefore, NIRS, when used in combination with chemometric methods, represent an important instrument for classification tasks and quantitative analyses. Due to its lower sensitivity to water and the possibility to work with quartz materials for fibers and optical elements, it can be used for the classification of most common synthetic polymers. Paul et al. [9] verified that NIRS is an ideal technology to detect MPs in soils; it can be applied for the automated sorting of plastic waste [15], often in combination with hyperspectral imaging [16]. Karlsson et al. [17] demonstrated the potential of three different hyperspectral imaging devices working in the NIRS in combination with multivariate data analysis for the mapping of MPs on filters. The NIRS for MPs has also been studied by other authors in soils [18], [19] and in waters [20]. However, up to now no study has investigated its use to detect MPs in feeds.

The aim of this study was to investigate the ability of NIRS to detect MPs at different concentrations in ruminant feeds.

## 2. MATERIALS AND METHODS

Four common ruminant feeds (corn silage, rye grass silage, soybean meal, and mixed hay) and a total mixed ration (TMR) were used to assess the NIRS' ability to identify the different concentrations of two types of MPs: LDPE and Polystyrene (PS). The feed samples were dried at 60 °C and ground with a 4 mm sieve (mixed hay and soybean meal with a 2 mm sieve). The LDPE and PS polymers were ground under 5 mm and added to the feeds in the following proportions: 0.0, 0.1, 0.3 and 0.5 % (equivalent to 0, 1, 3 and 5 mg g<sup>-1</sup> on a dry weight basis, respectively) (Figure 1). The samples were examined by a benchmark instrument, IdentiCheck™ FT-NIR-IR system (PE, Perkin-Elmer, Beaconsfield, England), in reflectance mode, spanning the range of 714-3333 nm and consisting of 2751 absorbance points, as described in Schymanski et al. [21]. Each absorbance spectrum was the average of 26 scans and each sample was mixed and replicated 10 times. The absorbance spectra were imported by GRAMS/AITM 7.02 in the WinISI III software (Infrasoft International, Port Matilda, PA, USA) and processed, after standardization and first derivation, by means of the modified Partial Least Squares method. The performance of the

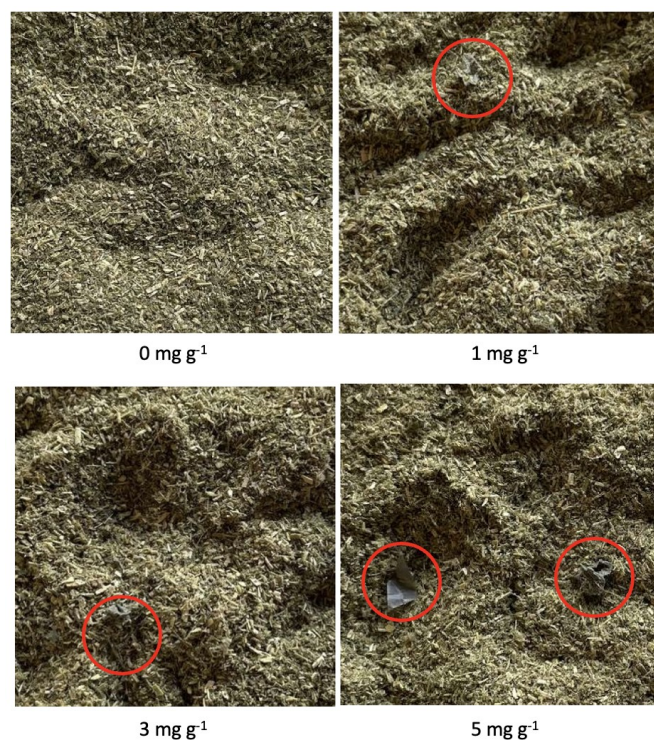


Figure 1. Ground mixed hay contaminated with different concentrations of LDPE (0, 1, 3, 5 mg g<sup>-1</sup>). LDPE particles are highlighted with red circles.

models was assessed by a cross-validation, allowing elimination of outliers exceeding a critical 't di Student' > 2.5 and 'Global H (Mahalanobis distance on PLS scores)' > 10 [22].

Another smart NIRS instrument, the SCiO™ v 1.2 molecular sensor (Consumer Physics Inc., Tel Aviv, Israel), was used for scanning the samples using a blue light over the 740-1070 nm NIR range. The 331 reflectance points of the spectra were processed by using the WinISI III software within each feed type but the R<sup>2</sup> in calibration mode was set up averaging the prediction per each repeated sample. The full-range NIR-IR spectra (PE: 714-3333 nm) of the benchmark instrument was also cut at the 714-1070 nm (PE\ ) to compare the quality of the smart NIRS instrument.

The concentrations of MPs were calculated based on raw values for each feed, or without considering the polymer types, or feed groups, or for concentrations. Otherwise, the relevance of the MP polymers in the NIR spectra was assessed by directly fitting three dummy variables (1 = none, 2 = LDPE, 3 = PS) in the total feeds model. The Ratio of Prediction to Deviation parameter (RPD) was calculated as the standard deviation (SD) divided by the standard error in cross-validation (SECV) and served as a performance indicator. Moreover, some spectral features of the SCiO™ were studied individually in each feed differentiating the whole class of contaminated MPs from the non-contaminated samples by the ratio  $\ln(R_{MPs}/R_{Feed})$ , where  $R_{MPs}$  is the reflectance of feed contaminated by MPs, and  $R_{Feed}$  is the reflectance of non-contaminated feed. Furthermore, a stepwise regression was applied to select the most prominent wavelengths to identify the type of plastic and to predict the quantities of MPs in each feed.

Table 1. Results of the calibration and cross-validation statistics (without outliers) for microplastic contamination determinations in various items on the NIR spectra of the devices (PE = Perkin Elmer, 714-3333 nm; PE\ = PE selected, 714-1070 nm; *SD* = standard deviation; *SEC* = standard error in calibration; *R*<sup>2</sup> in calibration; *SECV* = standard error in cross-validation;  $1-VR = R^2$  in cross-validation; *RPD* = ratio of prediction to deviation calculated as *SD* / *SECV* [22]).

Items	Theses	Instrument	<i>n</i>	Mean mg g <sup>-1</sup>	<i>SD</i> mg g <sup>-1</sup>	<i>SEC</i> mg g <sup>-1</sup>	<i>R</i> <sup>2</sup>	<i>SECV</i> mg g <sup>-1</sup>	1- <i>VR</i>	<i>RPD</i>
Corn silage	Concentration	PE	65	2.63	1.86	0.17	0.99	0.49	0.93	3.8
		PE\	62	2.48	1.84	0.66	0.87	1.13	0.62	1.6
Mixed hay	Concentration	PE	61	2.52	1.76	0.10	1.00	0.39	0.95	4.5
		PE\	61	2.67	1.80	0.46	0.93	0.75	0.83	2.4
Rye grass silage	Concentration	PE	61	2.48	1.81	0.11	1.00	0.46	0.94	3.9
		PE\	60	2.42	1.79	0.56	0.90	0.87	0.76	2.1
Soybean meal	Concentration	PE	60	2.50	1.79	0.14	0.99	0.35	0.96	5.1
		PE\	60	2.47	1.80	0.47	0.93	0.64	0.87	2.8
Total mixed ration	Concentration	PE	87	1.95	1.92	0.31	0.97	0.63	0.89	3.1
		PE\	84	1.93	1.91	0.57	0.91	0.81	0.82	2.3
Overall	Feeds	PE	325	3.15	1.38	0.33	0.94	0.56	0.84	2.5
		PE\	314	3.16	1.35	0.78	0.66	0.87	0.59	1.6
	MP Types	PE	325	1.34	0.63	0.18	0.92	0.28	0.81	2.3
		PE\	329	1.36	0.62	0.38	0.63	0.42	0.54	1.5
	Concentrations	PE	323	2.36	1.81	0.50	0.92	0.81	0.80	2.2
		PE\	326	2.39	1.81	1.23	0.54	1.29	0.49	1.4

### 3. RESULTS AND DISCUSSION

The NIR spectra of MPs at 1 to 5 mg g<sup>-1</sup> concentration were clearly identifiable in the PE of the fodder (mixed hay and silages), soybean meal, and TMR (Table 1, *R*<sup>2</sup>). In the PE\, the ability to identify MPs concentrations decreased with the *SECV* ranging from 0.64 to 1.13 mg g<sup>-1</sup>, while the *SECV* in the PE yielded smaller values ranging from 0.35 to 0.63 mg g<sup>-1</sup>.

The overall NIR ability to differentiate a group (feeds, MP types, or concentrations) in PE was reliable (*R*<sup>2</sup>: 0.94, 0.92, 0.92 respectively, Table 1) with a slight decrease compared to the ability when calibrated in individual feeds. However, the overall ability in the PE\ notably decreased (*R*<sup>2</sup>: 0.66, 0.63, 0.54 respectively).

Figure 2 reports a calibration plot corresponding to the *R*<sup>2</sup> of 0.92 for the total concentration of Table 1 with a standard error

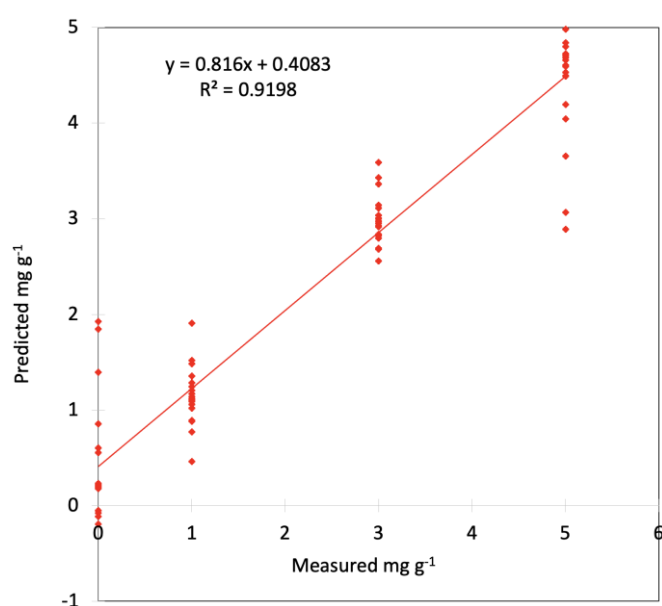


Figure 2. Plot of the measured vs predicted microplastic concentrations from the total dataset using the benchmark instrument with the full spectra (PE: 714-3333 nm). The standard error in calibration was 0.50 mg g<sup>-1</sup>.

in calibration (*SEC*) of 0.50 mg g<sup>-1</sup>, and the *R*<sup>2</sup> in cross-validation ( $1 - VR$ ) reduced to 0.80.

As it can be seen in Table 1, the analysis of PE in the overall concentrations resulted in the *RPD* of 2.2 which is apt to “approximate” quantitative prediction [22]. However, in the PE\, the *RPD* dropped to 1.4, which is not suitable for prediction. On the other hand, a single calibration for each feed improved the *RPD* compared to the overall calibration for both the spectral ranges, with a more noticeable enhancement for PE than for PE\. The decrease of the *RPD* in the overall calibration could be due to the excessive heterogeneity of the matrices used for calibration.

The present results were summarized in an accuracy of about 0.81 or 1.29 mg g<sup>-1</sup>, as shown by the *SECV* for the overall concentration from the PE or PE\ spectral ranges obtained from the instrument. The detection limit at the zero concentration was calculated at 0.4 mg g<sup>-1</sup> as an intercept of the linear equation in Figure 2.

The SCİO™ performances appeared satisfactory only in separate calibrations when the predictions from triplicated samples were averaged and then paired with the assigned data. In fact, in Table 2, the average *R*<sup>2</sup> was 0.69 and the average *SEC* was limited to 0.79 mg g<sup>-1</sup> which was apparently better than the *SEC* of 1.23 mg g<sup>-1</sup> obtained from the PE\ of the overall pooled feeds (Table 1). Figure 3 shows a plot of the measured vs predicted MP concentrations from the mixed hay dataset, as an example, by using the SCİO™ instrument. The *SEC* was 0.86 mg g<sup>-1</sup>.

Table 2. Results of the calibration of the microplastics concentrations on the NIR spectra of the device SCİO™ (740-1070 nm) with average prediction per repeated sample (*SD* = standard deviation; *R*<sup>2</sup> in calibration; *SEC* = standard error in calibration).

Feed	<i>n</i>	Mean mg g <sup>-1</sup>	<i>SD</i> mg g <sup>-1</sup>	<i>SEC</i> mg g <sup>-1</sup>	<i>R</i> <sup>2</sup>
Corn silage	43	1.49	1.18	0.35	0.93
Mixed hay	41	1.64	1.16	0.86	0.74
Rye grass silage	40	1.63	1.19	0.96	0.38
Soybean meal	41	1.51	1.14	0.77	0.65
Total mixed ration	43	1.56	1.18	0.67	0.84
Average		1.57	1.17	0.79	0.69

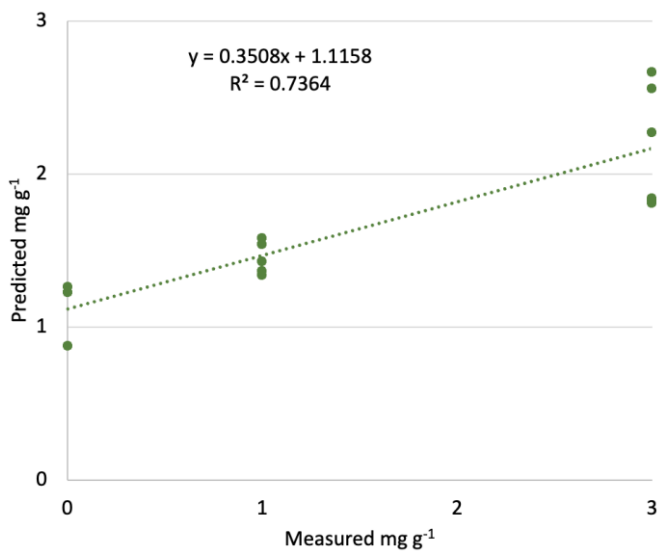


Figure 3. Plot of the measured vs predicted microplastics concentrations from the mixed hay dataset using the SCİÖ™ (740-1070 nm). The standard error in calibration was 0.86 mg g<sup>-1</sup>.

A systematic feature of the SCİÖ™ *spectra* obtained from contaminated samples is a different reflectance pattern. As shown in Figure 4, the ratio  $\ln(R_{MPs}/R_{Feeds})$  for the 3 mg g<sup>-1</sup> contaminated feeds was higher than the ones at 1 mg g<sup>-1</sup>, which

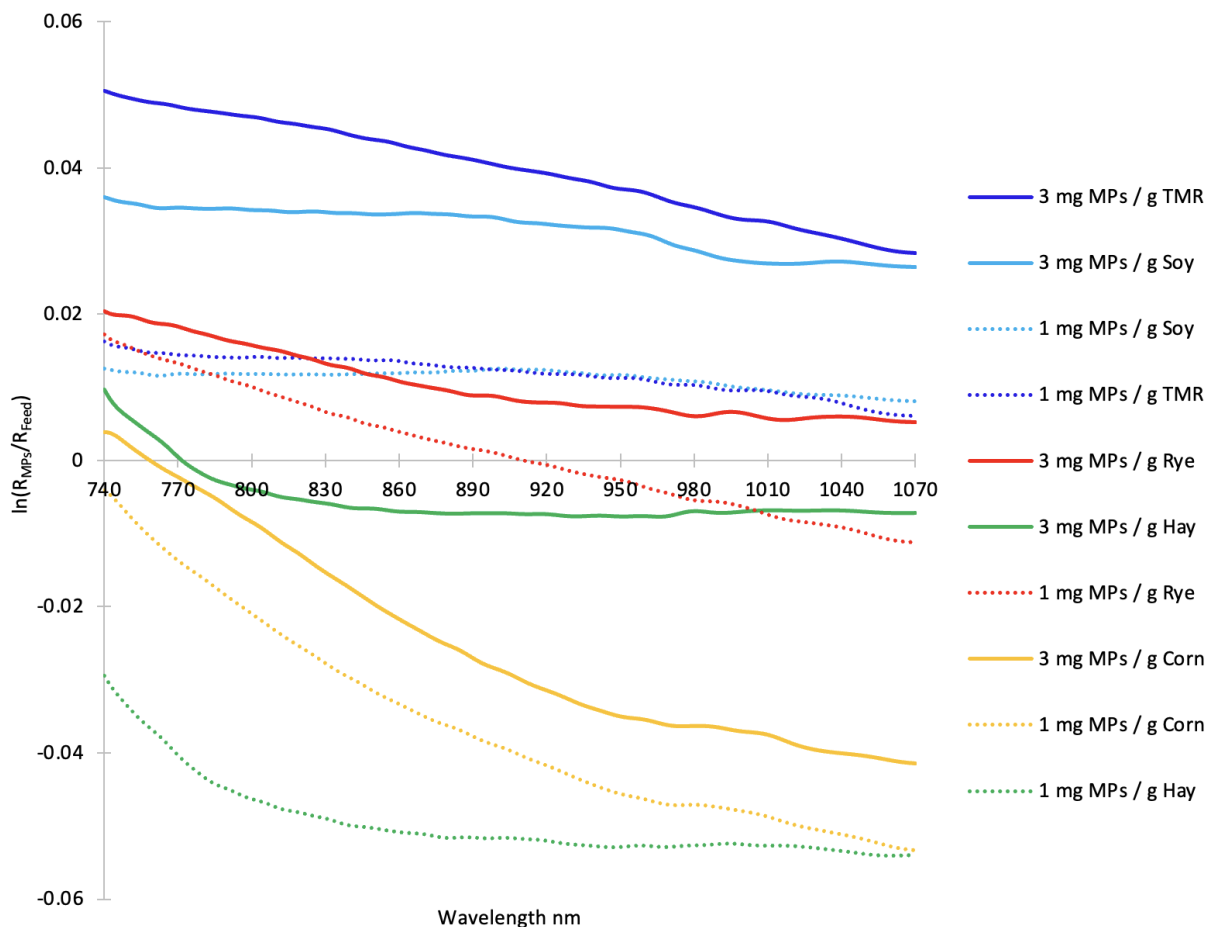


Figure 4. Different reflectance pattern expressed in ratio  $\ln(R_{MPs}/R_{Feed})$ , where  $R_{MPs}$  = the reflectance of feed contaminated by microplastics and  $R_{Feed}$  = the reflectance of non-contaminated feed, for 1 and 3 mg g<sup>-1</sup> of microplastics across the four feeds (Corn = corn silage, Hay = mixed hay, Rye = rye grass silage, Soy = soybean meal) and total mixed ration (TMR) in the SCİÖ™ *spectra* (740-1070 nm).

was equivalent in all the tested feeds and TMR. This result could be derived from a higher reflectance of the blue light flashed by the SCİÖ™ across the entire spectral range, when the feed was more contaminated. As expected, due to its complex organic composition, the raw feeds were more prone to absorbing the radiation than the pure plastic materials, making them more reflective.

In the SCİÖ™ *spectra* for the four feeds and TMR, it is interesting to consider the first representative wavelengths selected by the stepwise regression for two theses (the percentage of MPs and type of MPs), and their univariate coefficients of determination ( $r^2$ ). Initially, it is evident that no special spectral range is highlighted as all the predominant wavelengths vary (Figure 5). Additionally, the percentage of MPs is tendentially better predicted compared to identifying the type of MPs except for rye grass silage, as indicated by an average  $r^2$  of 0.53 and 0.40 respectively.

In summary, NIRS is more accurate in predicting the quantity of MPs rather than identifying the type of MPs.

#### 4. CONCLUSIONS

The direct scan of raw samples of common animal feeds and TMR prepared by standard methods for NIRS examination performed by the benchmark NIR-IR -not from a smart NIRS instrument - was able to detect MPs pollution at least up to 1 mg g<sup>-1</sup> (~1 g kg<sup>-1</sup>) within the range of 0 to 0.5 mg g<sup>-1</sup>. A specific

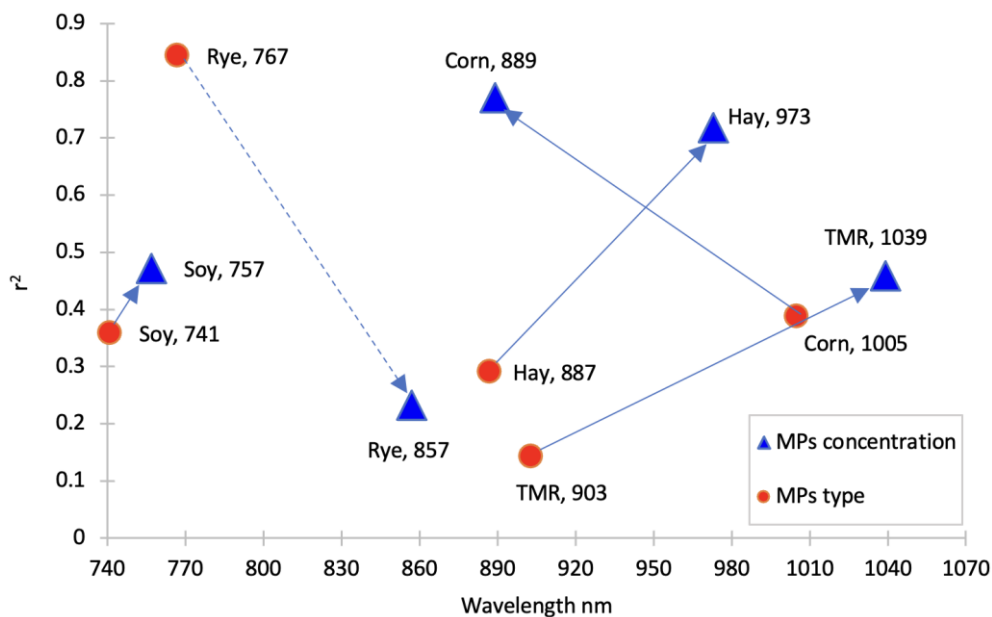


Figure 5. Univariate coefficient of determination ( $r^2$ ) for the first predominant wavelengths selected by a stepwise regression for concentrations of microplastics (1, 3 and 5 mg g<sup>-1</sup>) and type of microplastics, separately for the four feeds (Corn = corn silage, Hay = mixed hay, Rye = rye grass silage, Soy = soybean meal) and total mixed ration (TMR) in the SCiO™ spectra (740-1070 nm).

calibration for a single feed enhanced the ability of NIRS to discriminate polluted samples.

The real challenge in creating a database for the identification of MPs in feeds lies in finding feeds which are definitely free of MPs. Currently, the diffusion of MPs is such which seems to be nearly impossible to find plastic-free feeds in farms. Following these preliminary results, any validation on other samples/feeds with different and powerful NIRS tools is encouraged.

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