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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 reaches humans as the particulates could pass the intestinal barriers contaminating blood and animal products. Until now, there is not a simple, quick, sustainable, and reliable method to detect microplastics in feeds. The objective of this work was to investigate the ability of Near-Infrared Spectroscopy to detect microplastics in ruminant feeds. Two types of instruments were tested using five feedstuffs (corn silage, mixed hay, rye grass silage, soy grain and total mixed ration). Two types of crumbled contaminants (low-density polyethylene and polystyrene) were accurately mixed at 0, 1, 3 and 5 mass mg g-1. The pool of the five matrices examined by the benchmark instrument (714-3333 nm) allowed an accuracy around 0.8 mg g-1 and a detection limit around 1 mg g-1 however, in separate calibrations, the errors could be halved. A short range of wavelength (714-1070 nm) or a smart NIRS instrument have prevented an acceptable discrimination of the concentrations. Following these preliminary results, any validation on other samples and with different and powerful NIRS tools is encouraged.**

Keywords— NIRS; SCÏO; feeds; microplastics; Low Density Polyethylene; Polystyrene.

I. INTRODUCTION

Animal welfare is 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].

One problem that is poorly investigated in ruminant welfare is plastic pollution in livestock farms and particularly in feedstuffs. In fact, a huge amount of plastic materials are used to store ruminant feeds, especially dairy cows' ones. For example, low-density polyethylene (LDPE) films are widely used to cover horizontal silos and to wrap and tie bales [3]. In addition, it is also necessary to consider plastics coming from outside the farms that may pollute the feeds through wind, rain, etc. Plastics undergo to a systematic fragmentation driven mainly by ultraviolet radiation and mechanical abrasion, thus generating small plastic fragments (< 5 mm), called microplastics (MPs) [4]. MPs can be divided into two main categories: primary MPs that are directly immitted in the environmental and secondary MPs originating from the fragmentation of large plastic litter [5].

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 proved their presence in cow and pig feeds. Moreover, it also demonstrated the MPs presence in meat and milk showing that they can pass into animal products and could also be a problem for human health [8].

Until now, most MPs detection methods have been time consuming, requiring many steps and the use of reagents or enzymes. In addition, they are extremely heterogenous depending on the nature of the matrix 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 were detected after different steps: 1) sampling (collection and preparation of the samples); 2) extraction of MPs from the samples; 3) quantification and identification of MPs [11, 12, 13]. Moreover, for feeds, as they are considered complex samples, because they have constituents that are difficult to remove and are composed of multiple elements, the MPs extraction could be even more complex and could require different steps of organic matter degradation [13]. There are currently no specific analytical methods to detect MPs in feeds. They were extracted from feeds just by van der Veen et al. [8], but this protocol is not specific for feeds, and involves the use of ungreen solvents like methanol and tetrahydrofuran [14].

To overcome these problems, one possible solution 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 common in analytic process, including detection of contaminants and analysis of feeds and dairy products. The new generation of portable and handheld devices allow to perform the analyses during the production to evaluate results [14]. NIR spectroscopy works 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 combination vibrations for a limited number of molecular vibrations (C–H, O–H, N–H). It can be used to quickly determine properties of materials without altering the sample. Therefore, NIRS used in combination with chemometric methods represent an important instrument for classification tasks and quantitative analyses. As it has the 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 microplastics 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 NIR 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 soil [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 some ruminant feeds.

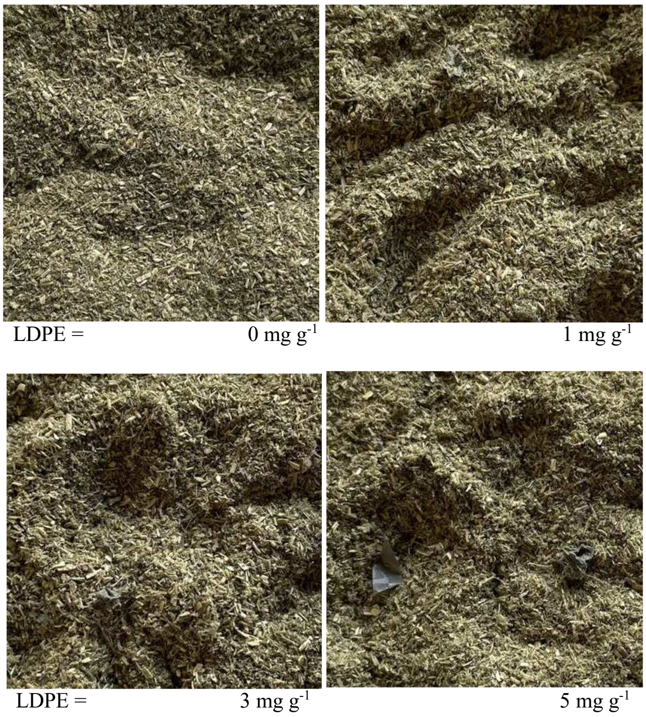
II. MATERIALS AND METHOD

Four feeds and a total mixed ration (TMR) were used to test the NIRS' ability to identify the different concentration of two MPs polymers. The feeds were chosen among the most commonly used in the farm which were: corn silage, rye grass silage, total mixed ration, soy grain mixed hay and TMR. They were dried at 60 °C and grinded with a 4 mm sieve (mixed hay and soy grain with a 2 mm sieve).

The two chosen MPs polymers were the LDPE and the Polystyrene (PSE). They were reduced under 5 mm and added to the feeds in the following percentages: 0.0, 0.1, 0.3 and 0.5 % (equivalent to 5, 3, 1 and 0 mass mg g^{-1}, respectively) (Figure 1).

The samples were examined in reflectance mode by a benchmark instrument IdentiCheckTM FT-NIR-IR system (PE, Perkin-Elmer, Beaconsfield, England) in the 714-3333 nm range, with 2751 absorbance points, as described in [21]. Each absorbance *spectrum* was the average of 26 scans and each sample was mixed up and replicated 10 times. The absorbance *spectra* were imported by GRAMS/AITM 7.02 in the Win-ISI III (Infrasoft International, Port Matilda, PA, USA) software and processed, after standardization and first derivation, by means of the modified Partial Least Squares method. The stability of the models was assessed by a cross-validation, allowing elimination of outliers exceeding a critical 'T-Student' = 2.5 and 'Global H' = 10 values [22].

FIGURE 1. Grinded mixed hay polluted at different concentrations of LDPE: 0, 1, 3, 5 mg g^{-1}.



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

The percentages of pollution were fitted as raw values, disregarding the MPs polymers in the five feeds and grouping them all together as a total and within each of them. Otherwise, the relevance of the MPs polymers in the NIR *spectra* was assessed by directly fitting three dummy variables (1 = none, 2 = LDPE, 3 = PSE) in the total feeds model. The relative percentage difference parameter (RPD = SD/SECV) was retained as indicator of performance. Moreover, some spectral features of the SCÏO^{TM} were studied in each feed differentiating the MPs polluted from the feeds by the ratio ln(R_MPs/R_Feed) where R is the reflectance. Moreover, two simple regression models were built, selecting the best parameters for discriminating the type and the quantity of MPs, for each feed.

III. RESULTS AND DISCUSSION

The NIR *spectrum* of MPs at 1 ÷ 5 mg g^{-1} concentration is clearly identifiable in the NIR-IR *spectrum* (714-3333 nm, PE tool) of the fodder (mixed hay and silages), soy grain and total mixed ration.

In a total dataset, the feed source, the two types of MPs polymers and the concentrations are perceived by the NIR *spectra* at a similar level that is higher for the PE than PE\ (Table 1).

In the separate datasets of the five feeds the cross-validate standard error (SECV) from the full *spectra* (PE), for the MPs concentration descends from 0.63 mg g^{-1} to a half average value (0.35 mg g^{-1}), that increased to 1.13 mg g^{-1} and 0.64 mg g^{-1}) when using the reduced *spectra* (PE\).

The Fig. 2 reports the calibration plot corresponding to the 0.92 R^2 for the total concentration of Tab. 1 with a Standard Error in Calibration (SEC) of 0.50 mg g^{-1}, and the R^2 cross-validate (1-VR) reduced to 0.80.

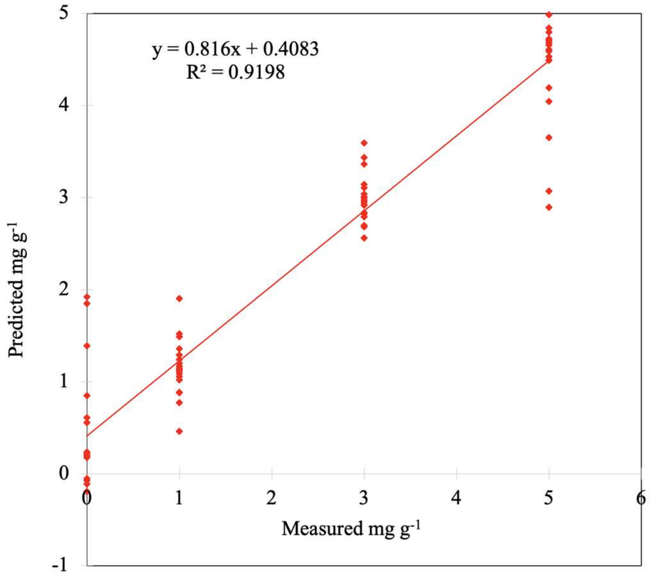
The analysis of the full *spectra* (PE) in the total of the samples gives a RPD for the concentration of 2.2, a value however apt to "approximate" quantitative prediction [22]. Otherwise, with the cut *spectra* the RPD falls to 1.4, a value not able to a prediction, due to the excessive heterogeneity of the matrices used for calibration. As can be seen in Tab. 1, a calibration for each matrix improves performance for both used ranges (PE, PE\).

TABLE 1. Results of the calibration and cross validation of the theses on the NIR *spectra* of the devices (PE = Perkin Elmer, 714-3333 nm; PE\ = PE, 714-1070 nm).

Feed	**Theses / Pollution**	**Instr.**	**N**	**Mean** mg g^{-1}	**SD** mg g^{-1}	**SEC** mg g^{-1}	**R^2**	**SECV** mg g^{-1}	**1-VR**	**RPD**
Total	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
	MPs Type	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
	Concentration	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
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
Soy grain	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

SD = Standard Deviation; SEC = Standard Error in Calibration mode; R^2 in calibration mode; SECV = Standard Error in Cross Validation mode; 1–VR = R^2 in cross validation mode; RPD = SD / SECV Relative Prediction Deviation [22].

FIGURE 2. Plot of the measured *vs* predicted microplastic concentration from the total dataset using the benchmark instrument with the full *spectra* 714-3333 nm. The Standard Error in Calibration was 0.50 mg g^{-1}.



The SCÏO^{TM} performances seems fairly good, provided that the sample was triplicated, the prediction averaged and paired to the assigned data. In fact (Tab. 2) the average R^2 is 0.69 but the error (SEC) is, on average, limited to 0.79 mg g^{-1} value that approaches the average value of 0.84 SECV obtained from the PE\ of the 5 feeds. In the Fig. 3 is shown the plot of the measured *vs* predicted microplastic concentration from the hay dataset using the SCÏO^{TM} instrument. The Standard Error in Calibration is 1.16 mg g^{-1}.

The present results are resumed in an accuracy, around 0.8 mg g^{-1}, as shown by the SECV for the total concentration. The detection limit at the zero concentration is calculated to be around 0.4 mg g^{-1} as highlighted in Fig. 1.

These values are better than the results of Paul et al. [10] on the MPs in the soil that showed a 10 mg g^{-1} accuracy and a detection limit of ~15 mg g^{-1}. Similar results were obtained by Huang et al. [11] with a spectroscopic low-cost ultra-gravimetric method for quantifying MPs in soils and compost that showed accuracy from 0.6 to 0.9 mg g^{-1} also using limited low-cost instruments as observed in the present work about the SCiO^{TM} instrument.

A systematic feature of the spectra obtained from polluted samples is a different reflectance pattern. As shown in the Fig 4, the ratio ln(R_MPs/R_Feeds) for the 3 mg g-1 contaminated feeds is higher than the 1 mg g-1, equivalent in all the five tested feeds.

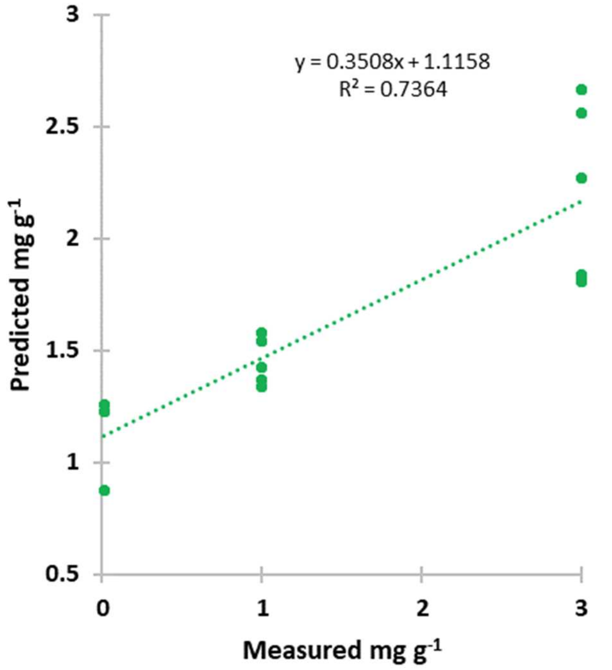
This result derives from a higher reflectance of the blue light flashed by the SCÏOTM all along the spectral range, when the feed is more contaminated. As expected, thanks to its complex organic composition, the raw feeds are prone to be more

TABLE 2. Results of the calibration of the MPs concentration (in mg g^{-1}) on the NIR *spectra* of the device SCÏO^{TM} (740-1070 nm) with average prediction per repeated sample.

Feed	**N**	**Mean** mg g^{-1}	**SD** mg g^{-1}	**SEC** mg g^{-1}	**R^2**
Corn silage	43	1.49	1.18	0.39	0.93
Mixed hay	41	1.64	1.16	0.90	0.65
Rye grass silage	40	1.63	1.19	1.07	0.38
Soy grain	41	1.51	1.14	0.86	0.65
Total mixed ration	43	1.56	1.18	0.75	0.84
Average		1.57	1.17	0.79	0.69

SD = Standard Deviation; R^2 in calibration mode SEC = Standard Error in Calibration mode

FIGURE 3. Plot of the measured *vs* predicted microplastic concentration from the hay dataset using the SCIÖ^{TM} (740-1070 nm). The Standard Error in Calibration was 1.16 mg g^{-1}.



absorbing the radiation than the pure plastic material, more reflective.

Considering the set of wavelengths in the SCÏO^{TM} *spectrum* selected first by a stepwise regression (Fig. 5) for the five feeds no special spectral range is concerned. The type of plastic is effectively represented in the NIR *spectra*, but it fits less than the percentage of pollution, as shown by an average of R^2 0.40 and 0.53, respectively. Note that this pattern is inversed for the rye grass (Rye).

IV. CONCLUSION

The direct scan of raw samples of common animal feeds and TMR prepared by standard methods for a NIRS examination can detect a MPs pollution at least up to 1 mg g^{-1} (~1 g kg^{-1}) within the range of 0 – 0.5 mg g^{-1}. A specific calibration for a single feed would greatly improve the ability of NIRS to discriminate polluted samples.

The real difficulty in creating a database to be used for the identification of microplastics in feeds consists in finding certainly free-plastic feeds. Currently, the diffusion of microplastics is such which seems to be almost impossible to find free-plastic feeds in farms. Following these preliminary results, any validation on other samples/feeds and with different and powerful NIRS tools is encouraged.

FIGURE 4. Ratio ln(R_MPs/R_Feed) for 1 and 3 mg g^{-1}.

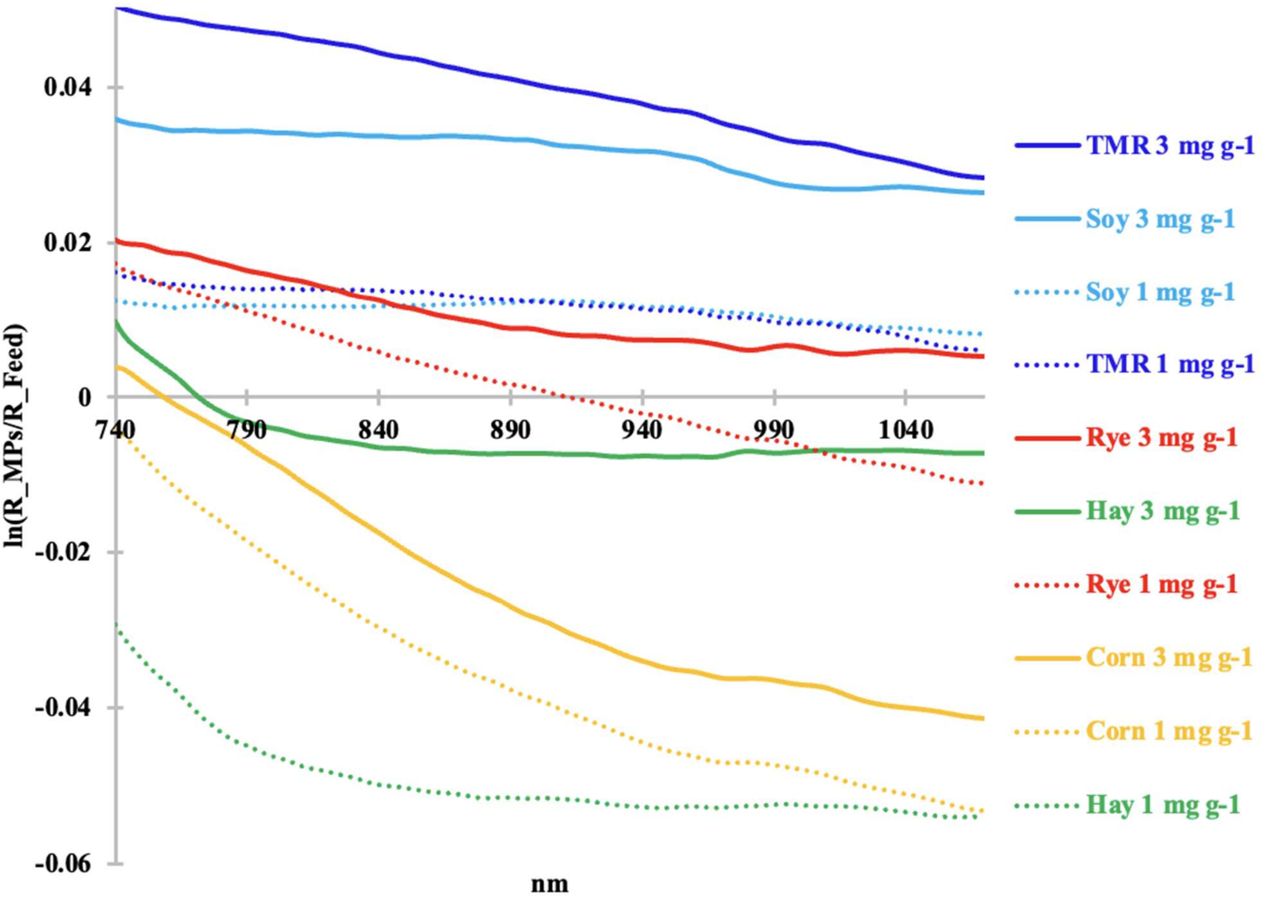
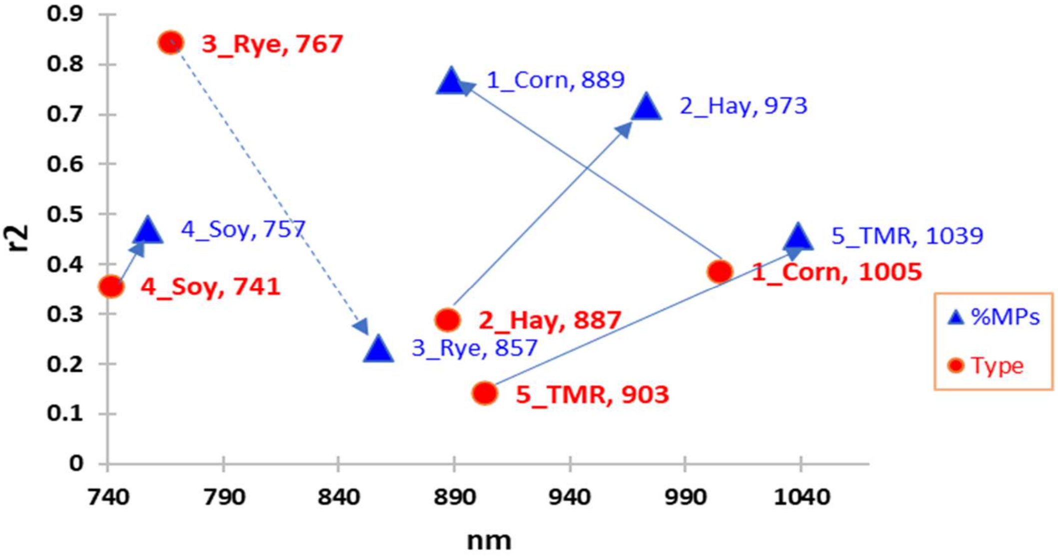


FIGURE 5. Plot of the wavelength in the SCIO *spectrum* (740-1070) nm selected first by a stepwise regression for % MPs and Type *vs.* the r^2, separately for the five feeds.



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