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Near infrared reflectance spectroscopy of buffalo faeces to determine the nutritional characteristics of diets and digestibility: Preliminary study

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Abstract
The use of NIR technology in evaluating the nutritional qualities of buffalo diets is a promising and cost-effective method. The study conducted on 100 stool samples demonstrated that NIR could accurately predict the nutritional characteristics of diets and the digestibility of buffalo feed.
The study was conducted on a buffalo farm located in southern Italy. The animals from which the individual stool samples were collected were all in their third lactation and homogeneous in terms of milk production from the previous lactation.
The variability in stool samples obtained could be attributed to individual differences in animal nutrition and metabolism. However, despite this variability, the results obtained from the NIR analysis were promising and provided valuable insights into the nutritional quality of the diets fed to buffaloes.
The use of NIR technology can bring great benefits to buffalo farming by providing a quick and efficient way to evaluate the nutritional quality of diets. This information is essential to optimize feed composition and ensure animal health and productivity.
Overall, the study demonstrates the potential of NIR technology in evaluating the nutritional qualities of feces and predicting the nutritional characteristics of buffalo diets. Further research and validation of this method could significantly contribute to improving buffalo husbandry practices.

Keywords: NIRs, TMR, faeces, digestibility, buffalo

Introduction
Nutritional value plays a significant role in buffalo productivity. Food specialists have always emphasized the importance of valuating the nutritional value in Total Mixed Ration (TMR) provided to livestock. Meeting the recommended nutritional requirements described by nutritional systems helps ruminants produce as expected. The efficiency of metabolizable proteins (MP) is not only linked to milk or milk proteins production, but also affects overall animal production performance health status, reproduction, and profitability.
Chemical techniques to quantify quality parameters are costly and time-consuming. Fortunately, traditional methods of determining nutritional value have been replaced by Near-infrared spectroscopy (NIRs) due to the increased use of personal computers and advancements in mathematical procedures in chemometric studies (Pinotti *et al*., 2014) [16]. Collecting feaces is easy and does not harm or interfere with animals. Feaces contain chemical characteristics representative of the feed consumed and are linked to intake and digestibility. Fecal indicators have long been used to clarify dietary attributes (Ruiz-Saavedra *et al*., 2020) [17] are still use.
Increasing crude dietary protein as a strategy to meet the needs of essential amino acids has been used for a long time. However, this strategy leads to low efficiency of use, and unused nitrogen is excreted in urine, feaces, and milk (Al Rifai *et al*., 2020) [1].
The application of techniques to study the chemical and nutritional characteristics of faeces has been used to estimate the quality of the feed ration administered to cattle (Dixon *et al*., 2005; Jancewicz *et al*., 2017a and b; Johnson *et al*., 2017) [6, 8, 10].
The nitrogen content in feces, determined using NIRs, provides the ability to observe and

monitor health, welfare, and productive performance of the animals reared (Lyons *et al*., 2007) [13].

NIRS profiling can be used to predict diet composition and digestibility (Tolleson *et al*. 2015) [21].

Estimating the nutritional content of a sample can be done using NIRs, a nondestructive, rapid, accurate, and economical technique. A spectrum signature can be recorded by a spectrometer when electromagnetic radiations of given wavelengths are absorbed, reflected, or transmitted. Spectral signatures can be utilized to identify and predict certain features of the analysis by combining reference samples of known content and variable statistics (Næs *et al*., 2001) [14]. This method is known as NIRs (Cen *et al*., 2007) [4] when applied to the 700 to 2.500 nm region of the electromagnetic spectrum. NIRS has advantages over other laboratory analytical methods including the absence of reagents and the ability to determine multiple values in one analytical process (Stuth *et al*., 2003) [19].

The method of constructing a spectrochemical prediction model is called calibration (Shenk *et al*., 1996) [18].

Both physical and electronic steps are involved in calibration. To begin the process, you must acquire a sample set of the desired material. This is well distributed and represents the expected range of variation in the constituent of interest. Routine analysis strategies should be consistent with processing the NIR setting (Gamon *et al*., 2019) [7].

Drying and grinding procedures are crucial for NIRs analysis, as water is a strong NIR light absorber and particle size impact the spectrum (Pasikatan *et al*., 2001) [15].

The official AOAC recommends grinding the sample with a 1.1 mm grid for transmission penetration, to laboratory official techniques for determining the nutrient profile of feedstuffs.

Assessing the nutritive quality of the administered TMR is crucial for buffalo farming; success, as it affects animal performance and production efficiency.

NIR profiling can predict diet composition and digestibility, making it a valuable tool for assessing the nutritional characteristics of stool samples and predicting the nutritional characteristics and digestibility of buffalo diets.

Materials and Methods

The study was conducted on a buffalo farm located in southern Italy. The animals from which individual feaces samples were collected were all in their third lactation and homogeneous in milk production from the previous lactation.

The animals were raised according to standard farm procedures.

The animals (Mediterranean buffalo) had ad libitum access to a total mixed ration (60% maize silage, 17% hay, 24% concentrate).

The feaces were collected directly from the rectal ampoule and placed on steel trays. After mixing, a sample was taken and stored at -20 ºC, for future chemical analysis.

Total mixed ration (TMR) samples were dried at 65 °C in a controlled ventilation, while fecal samples were dried at 105 °C in an oven until the moisture content reached 5%. They were then stored dry in a closed container at room temperature until further processing. Prior to analysis, the samples were ground to a particle size of 1.1 mm to ensure sample homogeneity.

Digestibility (DMD %) was calculated using the following formula: DMD = (1-(Cd/Cf)) *100, where Cd is the concentration of acid insoluble ash in the TMR and CF in feaces.

The chemical characteristics of TMR and faecal samples were dry matter (DM), ash content, crude protein (CP), NDF and ether extract content, analyzed with NIRs.

The data are reported in table 1.

Table 1: Chemical characteristics of unifeed and fecal sample

		Unifeed	Feces
		μ ± ds	μ ± ds
DM	%	53.74 ± 1.38	13.95 ± 0.71
Ash	%	8.59 ± 0.08	15.24 ± 0.32
CP	%	19.84 ± 0.14	15.38 ± 0.21
EE	%	44.02 ± 0.31	42.73 ± 2.04
NDF	%	26.21 ± 0.26	36.04 ± 1.75
ADF	%	3.17 ± 0.10	3.30 ± 0.46
ADL	%	5.79 ± 0.13	1.93 ± 0.12

NIRs spectra were acquired using a Büchi Instruments Inc model NIRFlex N-500. The system utilizes the lasted version of the NIRWare Software Suite.

At 10 nm intervals, the diodes were arranged. The two spectral ranges of the instrument were merged at 950 nm, effectively spanning from 800 to 2500 nm.

The calibration and validation datasets were divided into 70 and 30 samples, with each third being assigned to the validation data set.

The repeatability of the reference method was measured for three NIRs calibration data sets.

Calibration is defined as the process of generating a spectrochemical prediction model (Williams, 1987). The performance of calibration was evaluated using the coefficient of determination (R^2) and standard error of calibration (SEC).

The selected standardization equation's analytical performance was evaluated through equation validation. The predictive ability of a calibration equation can be assessed by using the standard error of prediction (SEP): Terramoccia *et al*. (2005) [20] recommended that an equation is acceptable if SEC values are below 2 times SEP and R2 values are above 0.8%.

It is crucial to understand that NIR predictive equations are continually improved over time, and the reported results represent the best available now. Evaluating the slope and bias is also crucial in assessing equation performance (Li *et al*., 2007) [12], and it is important to minimize systematic overestimation or underestimation of values obtained from reference methods. Monitoring and quality control are essential to ensure both the instrument's accuracy and calibration.

Results and Discussion

Table 1's data is comparable to that reported by Boval *et al*. (2004) [3].

DMD in TMR ranged from 6% to 7; in feaces, it ranged approximately from 40% to 75. This variability in feaces is linked to individual animal effects.

Table 2 presents the calibration and validation statistics for the PLS (Coleman *et al*., 1989) model used to in predict protein content via NIRs.

Table 2: Calibration and validation statistics for PLS regression model

Calibration				Validation					
n	RMSEC	R^2	RMSECV	n	RMSEP	R^2	Bias	Slope	RPD
70	0.04	0.92	0.14	30	0.14	0.83	-0.04	2.39	10.50

RMSECV= root means square standard error of cross

validation.
RMSEP = root means square standard error of performance.
RPD= standard deviation of the reference method/RMSEP
The reported data are lower than those found by Boval *et al.* (2004) [3] and Keli *et al.* (2005) [12] in their work on cattle and sheep. R^2 values are significant for all parameters considered.
Figure 1 shows the observed values vs faecal DMD NIRs predicted values.

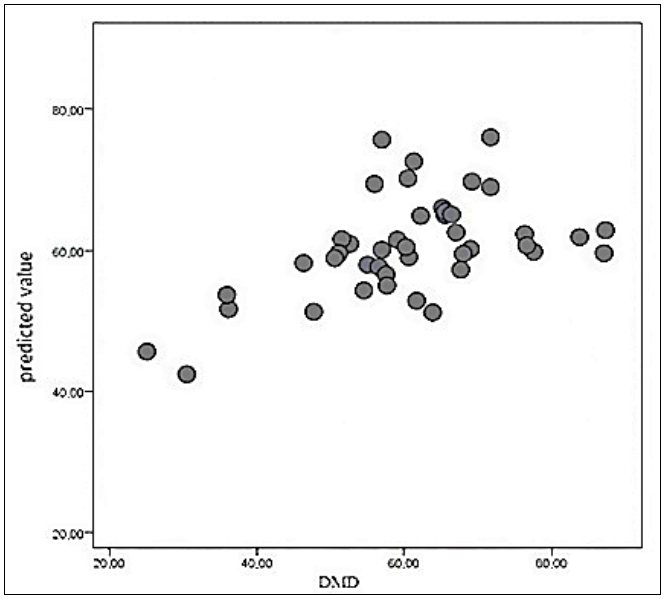

Fig 1: Observed values vs faecal DMD NIRs predicted values

Upon examining the figure, the indications obtained from studying the relationships between the parameters found in the feaces and prediction of diets of the digestibility may be limited and require further investigation.
Figure 2 reported the analysis of the coefficients that most influence the determination of DMD.
The normal distribution of data can be seen in the figure analysis, with most of the standardized residues falling within the ranges of -2 and 3 of the normal curves.
The significance of the coefficients' influence is greater starting from the top (EE) and less for the one indicated further down (DM).

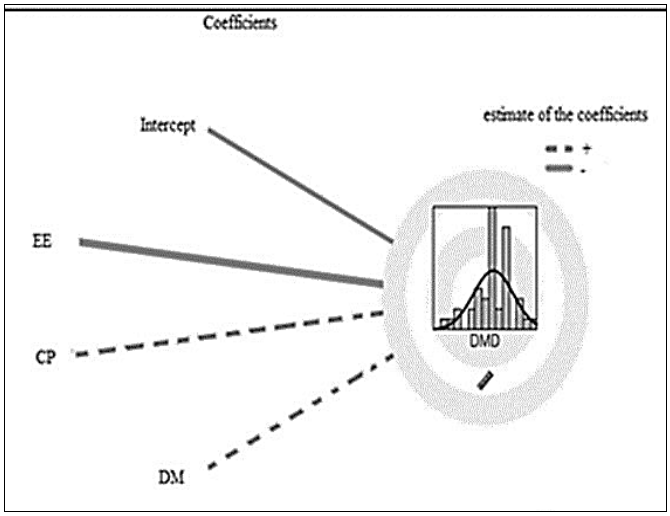

Fig 2: Analysis of the coefficients that most influence the determination of DMD

The greater impact of the ethereal extract on DMD can be justified by the fact that fatty acids in the intestine can combined with Ca salts to form soaps. Furthermore, in the faeces, there are bile pigments and ethereal extractable products of microbial origin.
Between 4000 and 6000 nm, there is a significant variation in the PLS model (Figure 3), with minor variations also present in the 6000 nm region.
Future research is necessary to determine if these recommendations are suitable for assessing faecal NIRs equations in predicting DMD.
Figure 3 shows that the residues are randomly scattered around the zero line, suggesting no clear pattern or tendency in the residues.

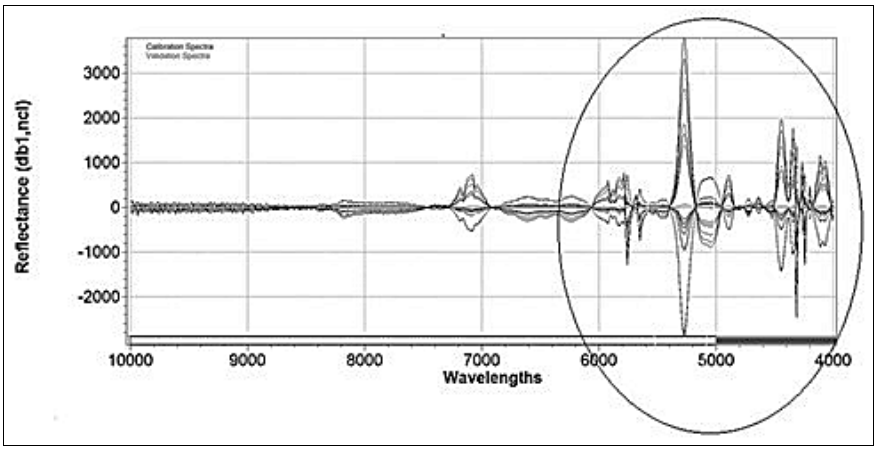

Fig 3: The residues are randomly scattered around the zero line

Additionally, the residue is relatively consistent across different values of the independent variable, indicating that outliers or influential data do not significantly affect the model. The model can predict results based on the independent variable because it meets the linear regression assumptions.

The reported results suggest that this technology has the potential to be used as a tool for predicting DMD in buffaloes fed diets.

Conclusion

Since these statistics, the method has been found to be suitable for screening purposes and useful for quickly monitoring the nutritional and digestibility characteristics using faeces without the need for sample preparation.

Recent advances in NIR_S technology have reduced the required amount of faeces for analysis, making it applicable in various animal management conditions.

Traditional protein measurements using AOAC methods are time-consuming and involve the use of solvents that must be disposed of as special waste.

In contrast, NIR chemical analysis is fast and does not produce special toxic industrial waste, making it a potential screening system for studying protein content.

Other results provide further evidence that faecal NIR profiling is a tool that may be used for predestining of digestibility of diets.

To simplify this, a rapid-scan series diode spectrometer was used to enable quick sample delivery for scanning.

Conflict of Interest

Not available

Financial Support

Not available

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