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Effectiveness of near-infrared spectroscopy to predict the chemical composition of feces and total-tract apparent nutrients digestibility estimated with undigestible neutral detergent fiber or acid-insoluble ash in lactating buffaloes' feces

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ABSTRACT

Following a comparison of nutrient total-tract digestibility estimates in lactating buffaloes using single-point undigestible NDF (uNDF) or acid-insoluble ash (AIA) as internal markers, the potential of fecal near-infrared spectroscopy (NIRS) to provide calibration equations for the assessment of the chemical composition of feces and nutrient total-tract digestibility estimated with internal markers was explored. Chemical analyses were performed on 147 fecal samples from lactating buffaloes reared on 5 farms in central Italy (Naples). Each farm fed a silage-based TMR to the buffaloes, and the TMR was sampled in the 2 d before the fecal collection. The TMR and individual fecal samples were collected and analyzed for DM, OM, ash, AIA, ether extract (EE), starch, fiber fractions (amylase-treated NDF without residual ash [aNDFom], amylase-treated NDF inclusive of residual ash [aNDF], ADF without residual ash [ADFom], ADF, hemicellulose, cellulose, ADL, uNDF), N, CP and CP bound to aNDF (NDICP) and to ADF (ADICP). The uNDF content was determined through a 240-h in vitro fermentation and employed, together with AIA as markers, to estimate the total-tract apparent digestibility and total-tract digestibility of DM, OM, ash, N, CP, EE, aNDFom, aNDF, NDIP, ADFom, and ADF, ADIN, ADL, hemicellulose, cellulose, starch, NFC, and the B3 fraction of N (NB3). No correlation was found between DM and OM digestibility estimated with AIA and uNDF as internal markers. Weak correlations were detected for all the other nutients digestibilities, and strong correlations were observed for EE, ADFom, hemicellulose, NDIN, ADIN, NB3, NFC, and starch. The sample set (n = 147) was divided in a calibration set (n = 111) and a validation set (n = 36) to "train" and "validate" the fecal NIRS curve through an external validation process. An estimation usable for preliminary or initial evaluation was obtained for N, CP, and aNDF fecal content. An excellent prediction was obtained for total tract digestibility of ADIN (R^2 = 0.90) when estimated with uNDF as the internal marker. The NIRS technology was not able to accurately predict all the other traits and the estimated nutrient digestibility of lactating buffalo diets from fecal spectra.
Key words: fecal near-infrared spectroscopy, buffaloes, undigested neutral detergent fiber, markers

INTRODUCTION

The interest in Mediterranean buffalo farming is increasing worldwide (mainly in Asia, followed by Africa and Europe; FAO, 2023) and, over the past 20 years, much effort has been focused on increasing the milk yield in Italy (+132 kg of milk per buffalo per lactation, http://bollettino.aia.it) and all over the world (+68 million tons from 2001 to 2021, AIA, 2023). This species is particularly interesting for its unique milk quality, and most of the scientific literature focuses on milk and cheese production and characteristics (Manuelian et al., 2017; Pasquini et al., 2018), while nutrient digestibility is less studied, even though it is important in determining diet and livestock efficiency.

Efficiency can be enhanced through precision feeding techniques, including the optimal combination of feeds (forages, concentrates, and additives) followed by accurate output measurements (milk, urine, and feces) as a tool to further fine tune the diet. This, in turn, improves digestion and overall nutrient availability (van Empel et al., 2016). Nutrient total-tract digestibility is one of the traits that allows for the assessment of energy value of feed and feed efficiency. Given a specific diet, the evalu-

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ation of digestibility is a phenotypical trait that can be used to improve feed efficiency (Babar et al., 2013). The gold standard method to evaluate nutrient digestibility is total fecal collection, which is expensive, labor intensive, and not applicable under on-farm conditions (e. g. grazing animals; Battelli et al., 2020). Thus, several internal and external markers have been tested in comparison to total fecal collection in ruminants (Fondevila et al., 1995; de Carvalho et al., 2013; Wang et al., 2020). According to Huhtanen et al. (1994), acid-insoluble ash (**AIA**) and undigestible NDF (**uNDF**) appear to be the most promising markers to predict digestibility in cattle. Generally, an internal marker must be undigestible and unabsorbable, and thus 100% recoverable (Huhtanen et al., 1994). In buffalo, Cr_2O_3 (chromic oxide), AIA, and lignin have been compared with total fecal collection (Wang et al., 2020). In the latter study, AIA was demonstrated to be the best option in this species, showing a 97% of recovery, and other authors haver reported 105% recovery in buffaloes (Sriwattanasombat and Wanapat, 1983). Indeed, this marker showed good performance in other species with a recovery averaging 100% (84%–151%; Sales and Janssens, 2003). However, this marker could be overestimated in a dry hay-based diet, when forages are contaminated by dust or soil or when bedding material is ingested (Huhtanen et al., 1994). Alternatively, uNDF proposed for cattle (Cotanch et al., 2014; Fustini et al., 2017; Raffrenato et al., 2018) demonstrated a higher recovery when animals were fed with a silage-based diet (Huhtanen et al., 1994), and showed a complete recovery in sheep and goat studies (de Carvalho et al., 2013).

The study of animal fecal matter has long been recognized as a valuable means to assess diet utilization and animal health (Şeker et al., 2010). Despite the accuracy and precision, nutritional analyses like digestibility are expensive and time consuming. Moreover, the in vitro evaluation of digestibility involves the use of biological inocula, which potentially reduces the repeatability of analysis (Simoni et al., 2021b). However, technological progress has introduced a powerful and efficient tool, near-infrared spectroscopy (**NIRS**), which offers a promising alternative for rapid, cost-effective, and noninvasive analysis (Dixon and Coates, 2009). This technology can be considered as a potential option, despite some inconsistent results for specific chemical and nutritional characteristics (Righi et al., 2017; Brogna et al., 2018; Simoni et al., 2021a). Several NIRS calibrations have demonstrated good ability of NIRS in assessing uNDF in forages (Nousiainen et al., 2004; Refat and Yu., 2022) and cattle feces (Brogna et al., 2018). Based on Kho et al. (2023), fecal NIRS calibrations should be considered species specific, and to the best of our knowledge no calibration has been tested and validated in buffalo.

Following a comparison of nutrient total-tract digestibility estimates in lactating buffaloes using single-point uNDF or AIA as internal markers, the potential of fecal NIRS to provide calibration equations for the assessment of the chemical composition and nutrient total-tract digestibility estimated with internal markers was explored.

MATERIALS AND METHODS

According to Italian law on animal experimentation and ethics (DL 04/03/2014 n. 26), this study does not require ethical approval. The trial has been conducted in a responsible manner without affecting animal health and behavior.

Sample Collection and Chemical Analysis

A total of 147 individual, fresh fecal samples were obtained from lactating buffaloes (155.8 ± 57.4 DIM, 3.1 ± 2.2 parity, and 10.5 ± 2.6 kg milk yield) from 5 different farms located in the area of Mozzarella di Bufala Campana PDO. More specifically, the number of collected samples was 28 from 2 farms, and 29, 30, and 32 from the remaining 3 farms. The selected farms provided a silage-based TMR to buffaloes to reduce the feed sorting risk. Moreover, farms were chosen based on the variability in their dietary formulations, having a forage-to-concentrate ratio ranging from 25:75 to 52:48. All diets, fed once a day in the morning, were based on corn silage, alfalfa hay and haylage, mixed hay, concentrates, and a mineral–vitamin supplement (Table 1). Furthermore, all diets showed similar chemical and nutritional composition. The TMR was sampled over 2 d before the fecal collection in each farm during feed delivery. After collection, the samples were stored at −20°C in the Department of Veterinary Medicine and Animal Production of the University Federico II (Napoli, Italy), shipped and chemically analyzed at the Laboratory of Feed Analysis of the Department of Veterinary Science of the University of Parma (Parma, Italy). A single fecal sample, weighing approximatively 2 kg, was collected from the rectum of the animals approximatively 3 h after the feed delivery.

Both TMR and fecal samples were oven dried at 55°C to constant weight and then ground in a Retch SK mill (Bauknecht, Stuttgart, Germany) to pass a 1-mm screen. An aliquot of each fecal sample was chemically analyzed, and another aliquot was subjected to spectrophotometric analysis. The chemical composition of the diets and of the fecal samples was determined as described in Simoni et al., 2021b. Briefly, DM, ash, and ether extract (**EE**) content were determined following European Commission Regulation No. 152/2009 (European Commission, 2009) recommendations. The amylase-treated NDF with-

out residual ash (**aNDFom**), ADF without residual ash (**ADFom**), and ADL were analyzed according to Mertens et al. (2002) with the use of heat-stable amylase, without sodium sulfite, and corrected for ash. For the boiling and filtering phase, a semi-automated system (FIWE Raw Fiber Extractor, VELP Scientifica, Usmate Velate, Italy) was employed. All samples were run in duplicate and the second repetition was performed to collect the amylase-treated NDF inclusive of residual ash (**aNDF**) and ADF residues for fiber-bound N determination. A further repetition of the fiber fractions analysis was performed sequentially for hemicellulose (**HC**) and cellulose (**CEL**) determination (Robertson and Van Soest, 1981). The HC content was calculated as the difference between aNDF and ADF obtained by sequential analysis type, whereas CEL content was calculated as the difference between ADF and ADL. The N content, NDIN, and ADIN were determined by the combustion digestion of the sample at 900°C in an excess of oxygen by Dumatherm (Gerhardt GmbH &Co, Königswinter, Germany) as described by Mihaljev et al. (2015). The starch content was analyzed by enzymatic method (method 2014.10; AOAC International, 2014). The NFC were calculated as a difference between 100% and the sum of ash, CP, EE, and aNDFom percentages. The AIA was determined on 5 g of sample, burned on a Bunsen burner and boiled on a hotplate with 2*N* HCl for 15 min (Van Keulen and Young, 1977), then filtered (Whatman no. 41), transferred to a porcelain crucible, according to the European Commission Regulation no. 152/2009 (European Commission, 2009) and ashed by ignition at 550°C. The uNDF content was determined through 240-h fermentation, according to Raffrenato et al. (2018), in an in vitro batch system using rumen fluid collected at the slaughterhouse from 4 cows and processed as described by Simoni et al. (2021c) as inoculum. A total of 3 consecutive runs were needed to analyze all the samples. The rumen fluid was kept at 39°C under anaerobic conditions, was blended and filtered through 4 layers of cheesecloth, and was inoculated at the ratio of 1:4 in the buffer solution in a flask containing 0.5 g of sample. The whole mixture was incubated in an in vitro batch fermentation system (Goering and Van Soest, 1970). The chemical composition of the diets fed to the buffaloes are reported in Table 1. The estimated apparent total-tract (**tta**) digestibility (**De**) of DM (**ttaDMDe**), OM (**ttaOMDe**), ash (**ttaAshDe**), EE (**ttaEEDe**), CP (**ttaCPDe**), NFC (**ttaNFCDe**), starch (**ttaStarchDe**) and total-tract (**tt**) De of aNDFom (**ttaNDFomDe**), aNDF (**ttaNDFDe**), ADFom (**ttADFomDe**), ADF (**ttADFDe**), HC (**ttHCDe**), and CEL (**ttCELDe**), NDIN (**ttNDINDe**), ADIN (**ttADINDe**), and NB3 (**ttNB3De**) were calculated using the uNDF or the AIA of the TMR and of the related fecal samples as internal markers as described by Fustini et al. (2017), Righi et al. (2017), and Simoni et al.(2021a,b). The values obtained were then associated with the spectra collected scanning the fecal samples during the calibration and validation process. The relationships between nutrient digestibility estimated using the 2 internal markers were assessed by Pearson correlation and linear regression using the software SPSS (IBM SPSS Statistics for Windows, Version 28.0; IBM Corp., Armonk, NY).

Table 1. Average chemical composition of the diets fed to the lactating buffaloes

Item[1]	Mean	SD	Min.[2]	Max.[2]	CV, %
Ingredient					
Corn silage	20.3	8.9	10.2	32.0	43.9
Wheat silage	11.0	—	11.0	11.0	—
Mixed hay	11.2	1.3	10.3	12.1	11.1
Alfalfa hay	14.4	2.4	12.7	16.1	16.9
Straw	10.1	7.0	5.1	15.1	69.6
Rye grass	15.3	—	15.3	15.3	—
Alfalfa haylage	8.3	1.8	7.1	9.5	21.1
Brewers grain	50.3	2.5	47.5	52.2	4.9
Earlage	14.5	—	14.5	14.5	—
Corn meal	14.4	—	14.4	14.4	—
Flaked corn	4.9	1.9	3.5	6.2	38.2
Soybean meal	5.6	4.3	2.0	10.4	78.0
Feedstuff	14.1	12.9	6.2	36.8	91.4
Hydrogenated fat	1.2	0.3	0.9	1.3	21.6
Sucrose	0.6	0.1	0.5	0.8	21.0
Minerals and vitamins	0.6	0.2	0.4	0.9	33.9
Chemical composition					
DM, % as fed	44.3	7.7	39.0	57.1	17.4
OM	92.4	1.2	90.5	93.6	1.3
Ash	8.0	1.2	6.7	9.4	15.1
EE	5.2	2.0	2.8	7.0	39.1
aNDFom	42.9	3.0	38.1	45.9	6.9
aNDF	44.7	4.3	39.5	50.5	9.5
ADFom	25.6	3.6	23.4	32.0	14.1
ADF	26.2	1.2	24.5	27.7	4.6
ADL	4.8	0.6	4.0	5.7	13.3
HC	16.7	3.4	12.3	21.5	20.3
CEL	21.4	1.2	19.9	23.0	5.6
N	2.3	0.1	2.2	2.6	6.2
CP	14.5	1.0	13.4	16.0	7.0
NDIN	0.5	0.2	0.3	0.8	34.7
ADIN	0.2	0.1	0.1	0.3	28.2
NB3	0.3	0.1	0.2	0.5	14.3
NFC	28.9	4.3	23.2	35.2	14.8
Starch	21.7	3.1	16.9	25.8	14.1
AIA	1.8	0.3	1.4	2.4	18.1
uNDF	14.7	1.5	12.4	16.5	10.4

[1]Given as percent of DM, unless otherwise noted.
[2]Min. = minimum; max. = maximum.

NIRS Spectra Collection and Calibration Development

Visible/near-infrared spectra were collected scanning the 147 dried and ground buffalo fecal samples in the wavelength range of 400–2,500 nm with a spectral resolution of 0.5 nm using a NIRS DS2500 instrument (FOSS Electric A/S, Hillerød, Denmark). An aliquot of 30 g was placed and analyzed in a large glass FOSS cup (diameter

105 mm, depth 35 mm) and the spectrum obtained was the average of 32 subspectra collected at different points during the automatic rotation of the cup and recorded as log(1/reflectance).

A chemometric analysis was carried out using WinISI 4 software (Infrasoft International, Port Matilda, PA) through modified partial least squares (**mPLS**) regression analysis (Osborne et al., 1993) to correlate spectral information to reference values using the complete dataset. Before data modeling, raw spectra underwent several scatter corrective methods, including detrending, standard normal variate (**SNV**), SNV+detrending, and multiplicative scatter correction to reduce noise and remove imperfections from the data matrix (De Marchi et al., 2017). Spectral derivation was applied in combination using various traits (Shenk et al.1989). To increase calibration accuracy, spectral outliers were eliminated using the Mahalanobis distance (Global H > 3.0), followed by 3 rounds of chemical outliers' elimination using the t statistic (>3.0). Samples whose predicted value differed more than 3 standard errors from the respective reference value were removed (Goi et al., 2019, 2022) before building the final infrared model. The prediction equations obtained were validated using a 5-fold cross validation and an external validation procedure, which required in both cases the selection of 5 random representative subsets from the entire data. Of 5 groups 4 were intended as a training set used to develop the model, which was then validated on the validation set that excluded from the calibration. This procedure was performed until all the subsets had been used once as the validation set.

The validation of the predictive ability of the infrared models was done using an independent data validation approach. The external validation process involved the creation of a calibration set selecting 75% of samples from the complete dataset and its use to generate a final prediction equation, which was then tested on the remaining 25% of samples. The creation of the dataset into the 2 subsets was done using a random selection procedure, which resulted in a comparable mean and standard deviation for each trait for the 2 subsets (calibration and validation). The optimal calibration models were identified based on the number of latent factors selected to minimize the root mean square error of cross validation, the standard error of cross validation ($\mathbf{SE_{CrV}}$) and of external validation ($\mathbf{SE_{ExV}}$), the coefficient of determination of cross validation ($\mathbf{R^2_{CrV}}$) and of external validation ($\mathbf{R^2_{ExV}}$), and the residual predictive deviation (**RPD**) of external validation ($\mathbf{RPD_{ExV}}$).

Table 2. Average chemical composition of the feces collected from lactating buffaloes (n = 147)

Chemical composition[1]	Mean	SD	Min.[2]	Max.[2]	CV, %
DM, % of predried samples[3]	93.4	0.7	91.5	96.4	0.7
OM	84.5	2.9	75.3	91.7	3.4
Ash	15.4	2.7	10.4	24.7	17.8
EE	1.9	0.5	0.9	3.8	24.4
aNDFom	57.5	4.7	40.4	68.7	8.2
aNDF	62.1	4.7	45.2	71.5	7.6
ADFom	36.1	3.3	27.2	43.6	9.2
ADF	39.9	3.4	27.2	47.0	8.5
ADL	9.8	1.5	3.1	15.5	15.0
HC	24.2	3.7	10.7	38.6	15.1
CEL	27.9	3.8	15.2	37.6	13.7
N	2.3	0.2	1.8	2.8	9.1
CP	14.2	1.3	11.1	17.5	9.1
NDIN	0.8	0.1	0.5	1.3	14.3
ADIN	0.5	0.1	0.3	0.8	18.0
NB3	0.3	0.1	0.2	0.8	29.3
NFC	10.9	2.6	1.8	18.0	23.5
Starch	1.7	0.6	0.3	5.6	37.4
AIA	6.1	1.0	3.9	8.1	17.0
uNDF	41.4	5.7	27.6	60.1	13.7

[1]Percent of DM, unless otherwise indicated.

[2]Min. = minimum; max. = maximum.

[3]DM of samples as they were used for NIR spectra acquisition; samples were predried at 55°C to constant weight.

RESULTS

Chemical Composition of Lactating Buffaloes' Feces

The fecal samples were characterized by an average DM, OM, and ash content of 93.4%, 84.5%, and 15.4% of DM respectively (Table 2). It should be highlighted here that only the DM of predried samples is reported in Table 2, whereas the DM expressed as a percentage of the sample as-is amounted to an average of 15.4% ± 3.8%, which is typical of buffaloes' feces. Fecal EE content ranged from 0.9% to 3.7% of DM. The amount of aNDFom in feces ranged between 40.4% and 68.7% DM, and the aNDF content ranged from 45.1% to 71.5% of DM. Approximately 60% of aNDFom was represented by ADFom, and approximately 17% was ADL (57.4%, 36.1%, and 9.79% of DM, respectively). The predominant subcomponent of the aNDFom is CEL (45%), and, on average, HC accounted for 39% of this fraction. The average fecal N content was 2.3% of DM, with NDIN and ADIN accounting for 34% and 20% of the total N, respectively; thus 14% of the total fecal N could be considered potentially digestible fiber-bound nitrogen, being an analog to the B3 fraction of the dietary proteins. The NFC were on average 10.9% of DM, whereas fecal starch ranged between 0.01% and 7.21% of DM. The fecal marker content was on average 6.08% and 41.3% of DM for AIA and uNDF, respectively. Overall, the variability of each component was greater than 7.61%, except for the DM of predried feces (0.71%) and OM (3.38%), which showed substantially lower variability.

Total-Tract Apparent Nutrients Digestibility Estimated with uNDF or AIA

The tta and tt nutrients De using uNDF or AIA are reported in Tables 3 and 4, respectively. The variability of all analyzed digestibility traits estimated from uNDF or AIA was greater than 6%, except for ttaDMDe (5.55% and 5.96% for uNDF and AIA, respectively), ttaOMDe (5.06% and 5.63% for uNDF and AIA, respectively), ttaStarchDe (1.07% and 0.92% for uNDF and AIA, respectively), and ttaNFCDe (5.10% and 3.59% for uNDF and AIA, respectively).

The ttaDMDe ranged between 55.0% and 74.2% (Table 4), whereas ttaOMDe ranged between 58.0% and 77.4%. The total-tract digestibility of ash (**ttAshDe**) and ttaEEDe averaged 32.4% and 84.7%, respectively. The digestibility of aNDFom, aNDF, ADFom, and ADF were on average 52.9%, 50.2%, 50.1%, and 46.2%, respectively. The ttHCDe ranged between 16.4% and 74.9%, whereas cellulose digestibility varied from 25.1% to 74.1%. The ttaCPDe, ttNDINDe, ttADINDe, and ttNB3De were on average 65.5%, 38.5%, 37.1%, and 55.9% respectively. The ttaNFCDe ranged from 73.9% to 97.4% and almost all of the starch was digested in buffaloes (97.3% on average).

On average all the traits were lower when using uNDF as internal marker compared with AIA, with the exception of ADIN, which was about 10% higher when calculated using uNDF. The digestibility of DM, OM, N, CP, EE, NDIN, starch, NFC, and NB3 were 5 percentage points lower when using uNDF. Among the different components, the average starch digestibility was comparable, showing just 0.4% difference; EE and NFC estimated with the 2 markers were also close, with 1.6% and 2.2% difference, respectively. All the traits estimated from fecal AIA fell into a wider range than when estimated from uNDF, except for ttADFDe, ttADINDe, ttHCDe, ttCELDe, ttaStarchDe, and ttaNFCDe.

Table 3. Descriptive statistics of average estimated total-tract apparent (tta) and true (tt) nutrients digestibility (De) using fecal uNDF as a marker in feces of lactating buffaloes (n = 147)[1]

Trait	Mean	SD	Min.	Max.	CV, %
ttaDMDe	64.7	3.6	55.0	74.2	5.6
ttaOMDe	67.6	3.4	58.0	77.4	5.1
ttAshDe	32.4	13.4	3.3	58.5	41.4
ttaEEDe	84.7	8.6	60.3	95.2	10.1
ttaNDFomDe	52.9	5.0	41.4	64.3	9.4
ttaNDFDe	50.2	5.6	35.4	66.7	11.2
ttADFomDe	50.1	8.7	31.3	69.7	17.3
ttADFDe	46.2	7.2	22.9	62.4	15.6
ttHCDe	48.4	11.0	16.4	74.9	22.6
ttCELDe	53.9	8.4	25.1	74.1	15.6
ttaNDe	65.5	5.3	49.2	76.0	8.1
ttaCPDe	65.5	5.3	49.2	76.0	8.1
ttNDINDe	38.5	17.7	4.3	69.6	45.9
ttADIND	37.1	27.0	1.0	85.3	72.9
ttNB3De	55.9	19.9	6.8	88.5	35.5
ttaNFCDe	86.4	4.4	73.9	97.4	5.1
ttaStarchDe	97.3	1.0	92.2	99.5	1.1

[1]Feces were sampled one time per day from each buffalo per farm. Min. = minimum; max. = maximum.

Table 4. Descriptive statistics of average estimated total-tract apparent (tta) and true (tt) nutrients digestibility (De) using fecal AIA as a marker in feces of lactating buffaloes (n = 147)

Trait	Mean	SD	Min.[1]	Max.[1]	CV, %
ttaDMDe	69.6	4.1	57.4	79.3	6.0
ttaOMDe	72.0	4.1	60.0	81.2	5.6
ttAshDe	39.7	12.0	3.4	67.1	30.1
ttaEEDe	86.3	8.8	55.1	96.4	10.2
ttaNDFomDe	59.0	7.3	41.3	74.4	12.5
ttaNDFDe	56.6	7.8	36.2	70.6	13.9
ttADFomDe	56.3	10.2	31.0	74.8	18.2
ttADFDe	53.5	6.6	30.5	66.9	12.3
ttHCDe	54.2	13.3	19.7	80.1	24.6
ttCELDe	60.2	7.3	38.0	76.1	12.1
ttaNDe	70.1	5.4	54.1	82.4	7.7
ttaCPDe	70.1	5.4	54.1	82.4	7.7
ttNDINDe	43.4	21.4	0.7	75.5	49.2
ttADINDe	29.1	19.2	0.5	63.8	66.0
ttNB3De	59.1	20.9	2.3	91.6	35.4
ttaNFCDe	88.6	3.2	80.8	98.1	3.6
ttaStarchDe	97.7	0.9	93.5	99.6	0.9

[1]Min. = minimum; max. = maximum.

Nutrient Total-Tract Digestibility in Lactating Buffaloes Estimated from uNDF or AIA

Except for ttaDMDe and ttaOMDe, all correlations and regressions between nutrients digestibility estimated from uNDF or AIA, shown in the Table 5, were significant. The strongest correlations ($r > 0.7$) were obtained for ttaEEDe, ttHCDe, ttNDINDe, ttADINDe, ttNB3De, ttaNFCDe, and ttaStarchDe. Nonsignificant regressions were obtained for ttaDMDe and ttaOMDe, but all the other analyzed traits were found to be significant. The strongest regressions were found for ttaEEDe, ttNDINDe, ttNB3De, ttaNFCDe, and ttaStarchDe; apart from ttNDINDe, all showed negative intercepts.

NIRS Assessment of Fecal Chemical Composition

The data in Figure 1 depict the NIRS average spectral profiles of the fecal and ration samples examined in this study. In general, the spectral patterns of the 2 matrixes followed the same trend regarding absorbance peaks. However, fecal samples exhibited higher absorbance than TMR within the wavelength range up to 1,500 nm.

Predictive performance of NIRS models developed for buffalo fecal compositional traits in external validation are shown in Table 6. The percentage of outliers detected

Table 5. Relationship between digestibility traits estimated with AIA or uNDF as internal markers

	Linear regression				Pearson correlation	
Item	Intercept	Slope	R^2	*P*-value	r	*P*-value
ttaDMDe	62.50	0.03	0.002	0.640	0.039	0.320
ttaOMDe	60.76	0.10	0.013	0.173	0.113	0.086
ttAshDe	2.45	0.74	0.412	<0.001	0.642	<0.001
ttaEEDe	5.71	0.92	0.885	<0.001	0.957	<0.001
ttaNDFomDe	37.85	0.26	0.144	<0.001	0.38	<0.001
ttaNDFDe	34.12	0.29	0.156	<0.001	0.395	<0.001
ttADFomDe	18.54	0.56	0.436	<0.001	0.660	<0.001
ttADFDe	34.04	0.23	0.043	0.012	0.177	0.019
ttHCDe	7.79	0.74	0.693	<0.001	0.833	<0.001
ttCELDe	17.31	0.61	0.278	<0.001	0.527	<0.001
ttaNDe	28.87	0.52	0.286	<0.001	0.545	<0.001
ttNDINDe	6.46	0.74	0.787	<0.001	0.913	<0.001
ttADINDe	28.27	0.17	0.024	0.061	0.757	<0.001
ttNB3De	−3.12	0.97	0.880	<0.001	0.930	<0.001
ttaNFCDe	−19.17	1.20	0.739	<0.001	0.859	<0.001
ttaStarchDe	−2.53	1.00	0.782	<0.001	0.905	<0.001

in fecal samples was <10%, except for starch (18.0%), which had the worst prediction performance. The number of latent factors (**LF**) was found to have wide variability ranging from 3 (ADL, % of DM) to 11 (ADF, % of DM). The best prediction models with greater R^2_{ExV} and RPD values were observed for EE (R^2_{ExV} = 0.72; RPD_{ExV} = 1.89), aNDFom (R^2_{ExV} = 0.72; RPD_{ExV} = 1.89), aNDF (R^2_{ExV} = 0.77; RPD_{ExV} = 2.12), N (R^2_{ExV} = 0.76; RPD_{ExV} = 2.06), and CP (R^2_{ExV} = 0.83; RPD_{ExV} = 2.32). Average prediction accuracy was observed for DM (R^2_{ExV} = 0.63; RPD_{ExV} = 1.65), ash (R^2_{ExV} = 0.70; RPD_{ExV} = 1.66), ADF (R^2_{ExV} = 0.66; RPD_{ExV} = 1.43), and AIA (R^2_{ExV} = 0.60; RPD_{ExV} = 1.60). The lowest accuracy was obtained for predicting fecal starch.

NIRS Evaluation of Nutrient Digestibility

The goodness-of-fit statistics of mPLS regression model's external validation for tta or tt nutrients De using fecal uNDF and AIA as markers of buffalo feces are shown in Table 7 and Table 8. All calibration models developed using both fecal markers had a percentage of outliers below 10% except for ttADLDe (19.3% for uNDF and 10.8% for AIA) and ttNB3D (10.8% for AIA only). The number of LF ranged from 3 (ttADINDe) to 10 (ttaCPDe) for nutrient digestibility estimated using the fecal uNDF marker and from 1 (ttaStarchDe and ttaNFCD) to 12 (ttNB3D) for nutrient digestibility estimated using the AIA marker. The scatter correction

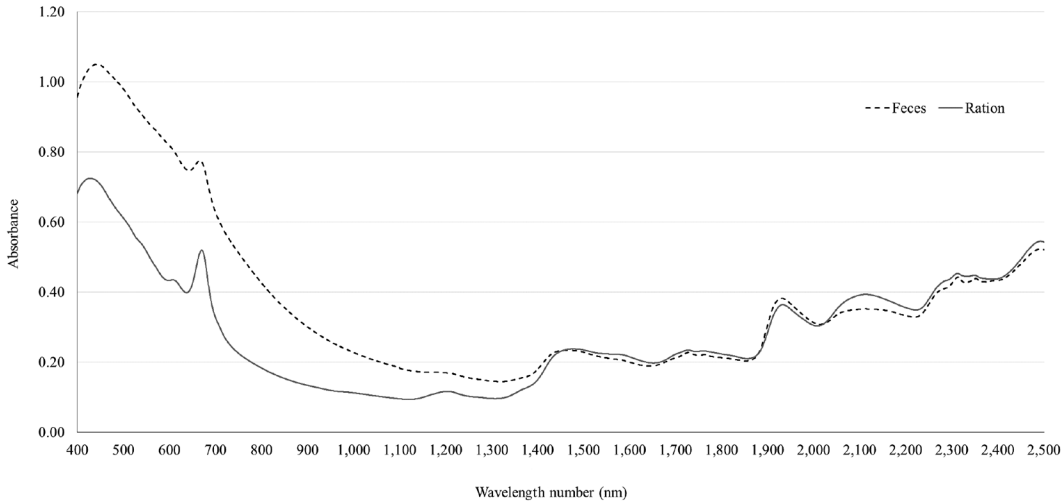

Figure 1. Near-infrared average spectra of ration and fecal samples.

Table 6. Fitting statistics of modified partial least square regression models in external validation for chemical composition of buffalo feces, developed using visible/NIR spectroscopy

	Calibration set (n = 111)					Validation set (n = 36)				
Trait[1]	n_{CrV}[2]	% outliers	LF	SE_{CrV}	R^2_{CrV}	SE_{ExV}	R^2_{ExV}	Bias	Slope	RPD_{ExV}
DM, % of predried sample[3]	103	7.21	7	0.26	0.75	0.34	0.63	0.04	1.01	1.65
OM	107	3.60	8	1.29	0.77	1.62	0.63	−0.39	0.80	1.55
Ash	105	5.41	4	0.89	0.88	1.40	0.70	0.04	0.76	1.66
EE	103	7.21	5	0.18	0.80	0.27	0.72	−0.05	1.02	1.89
aNDFom	103	7.21	8	2.10	0.78	2.31	0.72	0.41	1.05	1.89
aNDF	103	7.21	6	1.95	0.84	1.86	0.77	0.18	1.03	2.12
ADFom	104	6.31	10	1.50	0.79	2.20	0.57	0.21	0.75	1.44
ADF	106	4.50	11	1.90	0.67	2.19	0.66	2.19	0.68	1.43
ADL	109	1.80	3	0.89	0.42	1.84	0.14	0.05	1.01	1.09
HC	102	8.11	5	2.38	0.36	2.24	0.49	0.42	0.94	1.39
CEL	107	3.60	9	2.23	0.57	2.98	0.44	0.63	1.04	1.32
N	101	9.01	8	0.09	0.80	0.10	0.76	0.01	1.06	2.06
CP	101	9.01	7	0.48	0.86	0.56	0.83	0.08	1.15	2.32
NDIN	107	3.60	4	0.07	0.47	0.10	0.45	0.02	1.03	1.41
ADIN	105	5.41	4	0.04	0.55	0.08	0.21	0.03	0.71	1.04
NB3	109	1.80	7	0.07	0.33	0.10	0.17	0.00	0.63	1.08
NFC	104	6.31	4	1.96	0.30	2.19	0.16	0.21	0.75	1.09
Starch	91	18.02	4	0.45	−0.02	0.63	0.06	−0.01	−1.66	0.97
AIA	103	7.21	7	0.59	0.64	0.72	0.60	−0.02	1.08	1.60
uNDF	105	5.41	8	3.44	0.61	4.01	0.48	0.67	0.94	1.39

[1]Given as percent of DM, unless otherwise noted.

[2]nCrV = number of actual cross validation samples used from the calibration set.

[3]DM of samples as they were used for NIR spectra acquisition, samples were predried at 55°C to constant weight.

methods that garnered the highest selection were the absence of correction and the SNV method with "None" and "SNV" with spectral derivation parameters set to 0, 0, 1, 1. The initial digit represents the derivative order, the second denotes the interval for derivative computation, the third indicates the smoothing segment, and the final digit corresponds to the secondary smoothing segment (Catunda et al., 2022). Using fecal uNDF as a marker of predried feces, only 3 traits were predicted with a calibration model exhibiting $R^2_{ExV} > 0.66$, namely ttaEEDe (0.89), ttNDINDe (0.74), and ttADINDe (0.90). The prediction models based on AIA as a marker were found to have an $R^2_{ExV} > 0.66$ only for ttaEEDe, with a value of 0.86. Similarly, the largest RPD values were found in the same traits with values between 2 and 2.4 for ttaEEDe based on the AIA marker (2.22), ttNDINDe based on uNDF marker (1.96), between 2.4 and 2.9 for ttaEEDe (2.78), and greater than 2.9 for ttADINDe estimated based on AIA (3.16 estimated from uNDF). All the remaining prediction models had a $R^2_{ExV} < 0.66$ and achieved RPD values between 0.88 and 1.65. Based on R^2_{ExV},10 out of a total of 18 prediction models were found to be better predicted using uNDF as a marker rather than AIA, and only one trait (ttADFomDe) had equal predictive performance. The worst prediction models were calculated for ttaNDFomDe ($RPD_{ExV} = 0.88$) and ttaStarchDe ($R^2_{ExV} = 0.08$) using uNDF as marker.

DISCUSSION

The results presented are subject to a few limitations: animals were sampled only once, and this could have reduced the overall accuracy of diet digestibility estimation, because uNDF may not be uniformly excreted throughout the day (Morris et al., 2018). However, each fecal spectrum was obtained from an individual fecal sample whose marker content, determined through wet chemistry, was employed to calculate the digestibility coefficient to be associated with the spectrum itself. Thus, each spectrum is associated with a specific real extent of digestion, related to the sample. In this direction the use of single-point sampling increases the variability of the data for the NIRS calibration. Moreover, single sampling is the most employed protocol in the field by nutritionists because of its practicality. Higher sampling frequency, duplication, and replication of fecal sampling might have improved the accuracy of digestibility estimations, with possible improvement of NIRS calibration. Moreover, TMR samples were from the whole pen and were not necessarily fully representative of the diets consumed by the cows used for fecal sampling (i.e., some variation in refusals could have occurred). Thus, the average intake of indigestible markers from the pen might not have been representative of the intake of the sampled cows. The second limitation is related to the methodology applied to determine the fecal EE content, which did not include

Table 7. Fitting statistics of modified partial least square regression models in external validation for estimated total-tract apparent (tta) or true (tt) nutrients digestibility (De) using fecal uNDF as a marker in feces of lactating buffaloes, developed using visible/NIR spectroscopy

	Calibration set (n = 111)						Validation set (n = 36)			
Trait	n_{CrV}[1]	% outliers	LF	SE_{CrV}	R^2_{CrV}	SE_{ExV}	R^2_{ExV}	Bias	Slope	RPD_{ExV}
ttaDMDe	108	2.70	7	2.90	0.31	3.94	0.10	−0.24	0.42	0.97
ttaOMDe	104	6.31	8	2.72	0.31	3.46	0.21	0.12	0.63	1.09
ttAshDe	106	4.50	6	9.36	0.52	8.58	0.58	0.05	0.93	1.53
ttaEEDe	103	7.21	7	2.21	0.92	3.34	0.89	−0.93	1.14	2.78
ttaNDFomDe	105	5.71	7	3.94	0.36	5.72	0.15	−0.14	0.36	0.88
ttaNDFDe	107	3.60	7	4.46	0.43	4.73	0.28	0.23	0.54	1.05
ttADFomDe	106	4.50	8	5.66	0.58	5.73	0.55	−0.33	0.83	1.47
ttADFDe	105	5.71	9	4.90	0.46	6.86	0.29	−1.87	0.68	1.10
ttHCDe	104	6.31	7	7.47	0.50	10.32	0.32	0.19	0.68	1.17
ttCELDe	106	4.50	9	5.69	0.48	7.90	0.31	−2.43	0.67	1.11
ttaNDe	106	4.50	6	3.67	0.55	4.10	0.36	0.72	0.76	1.22
ttaCPDe	106	4.50	10	3.73	0.51	4.40	0.31	−0.88	0.66	1.13
ttNDINDe	101	9.01	7	8.84	0.73	9.13	0.74	0.19	0.94	1.96
ttADINDe	94	7.45	3	7.22	0.93	8.64	0.90	0.31	0.91	3.16
ttNB3De	100	5.66	9	11.01	0.69	12.52	0.61	0.90	0.78	1.53
ttaNFCDe	103	7.21	7	3.09	0.47	2.99	0.58	−0.12	0.93	1.56
ttaStarchDe	105	5.71	7	0.82	0.10	1.08	0.08	0.01	0.55	1.03

[1]n_{CrV} = number of actual cross validation samples used from the calibration set.

acidification, potentially leading to an overestimation of the EE digestibility (Palmquist et al., 2017). Last, considering the specific area of interest where the sampling was conducted, our results may be considered to be specific to the conditions of the present trial, including diets and nutrient composition.

Chemical Evaluation and Characteristics of Feces

The choice to use data from multiple farms is rooted in the recognition that building models based on a single farm can lead to overly precise results (overfitting, Hawkins, 2004; Mota et al., 2021). Such models may only be applicable in the specific context of that farm and may not be applicable in other contexts.

The composition of the diets considered in the present study is typical of rations fed to lactating Mediterranean buffaloes (Neglia et al., 2014; Albano et al., 2020; Evangelista et al., 2022). Although buffalo feces have been analyzed in several studies to estimate diet digestibility (Khattab et al., 2010; Hassaan et al., 2022; Yadav et al., 2022), the fecal composition has been reported only in a few cases, and mostly without describing the composition of the diet (Al-Asfoor et al., 2012; Paula et al., 2020;

Table 8. Fitting statistics of modified partial least square regression models in external validation for estimated total-tract apparent (tta) or true (tt) nutrients digestibility (De) using fecal AIA as a marker in feces of lactating buffaloes, developed using visible/NIR spectroscopy

	Calibration set (n = 111)						Validation set (n = 36)			
Trait	n_{CrV}[1]	% outliers	LF	SE_{CrV}	R^2_{CrV}	SE_{ExV}	R^2_{ExV}	Bias	Slope	RPD_{ExV}
ttaDMDe	104	6.31	9	3.62	0.21	4.62	0.18	−0.56	0.43	0.95
ttaOMDe	101	9.01	3	3.21	0.35	3.97	0.27	−0.73	0.67	1.12
ttAshDe	108	2.70	3	9.17	0.51	8.12	0.45	0.74	0.49	0.99
ttaEEDe	101	9.01	3	2.28	0.91	4.68	0.86	−1.13	1.33	2.22
ttaNDFomDe	104	6.31	3	5.56	0.40	7.58	0.18	−0.58	0.53	1.03
ttaNDFDe	104	6.31	3	6.33	0.36	7.40	0.19	−0.42	0.56	1.05
ttADFomDe	104	6.31	10	6.30	0.63	7.93	0.54	−0.19	0.56	1.35
ttADFDe	104	6.31	4	5.60	0.21	5.96	0.11	−0.22	0.58	1.04
ttHCDe	106	4.50	10	10.37	0.50	9.77	0.50	−0.75	0.70	1.31
ttCELDe	106	4.50	5	6.26	0.26	6.85	0.22	−2.57	0.61	1.00
ttaNDe	104	6.31	3	3.79	0.50	4.42	0.33	−0.94	0.61	1.10
ttaCPDe	104	6.31	3	3.79	0.50	4.86	0.33	−0.94	0.61	1.00
ttNDINDe	105	5.41	2	11.80	0.69	13.15	0.62	−0.11	0.99	1.65
ttADINDe	84	5.62	8	11.15	0.67	15.28	0.46	−4.77	0.75	1.25
ttNB3D	99	10.81	12	9.60	0.75	13.72	0.59	−0.16	0.93	1.58
ttaNFCD	107	3.60	1	2.38	0.40	2.77	0.28	−0.46	0.70	1.14
ttaStarchDe	93	16.22	1	0.73	0.004	1.15	0.13	−0.34	−1.29	0.89

[1]n_{CrV} = number of actual cross validation samples used from the calibration set.

Lisanti et al., 2021). Besides the lack of diet composition details, the comparison with published data might be challenging due to differences in breed, stage of lactation, and fecal sampling protocol. Bovera et al. (2007) showed the chemical composition of Mediterranean buffalo feces without differentiating between lactating and dry animals. Lisanti et al. (2021) reported the fibrous fraction content of domestic and wild buffalo feces. Furthermore, Al-Asfoor et al. (2012) mentioned the fecal composition of water Nili-Ravi buffalo heifers fed with different carbon/nitrogen and NDF/soluble carbohydrates ratios. Moreover, in the present study feces were collected as a single time point, which is the common practice adopted in the field, but may not represent accurately the daily fecal composition. The DM content of the feces (15.4% ± 3.8%, as-is), as well as ash, EE, NDF and ADF percentages, are consistent with values reported by Bovera et al. (2007). Our results related to NDF, ADF, and ADL are close to those reported for low carbon/nitrogen and NDF/soluble carbohydrates ratio by Al-Asfoor et al. (2012), and ADF, CEL, and ADL content are similar to those of domestic buffalo reported by Lisanti et al. (2021). Only the maximum NDF and HC values found in the present study are comparable with those found in domestic buffalo feces in the last cited study; this may be due either to a higher dietary fiber digestibility fed in the present study or to a lower digestibility of neutral detergent solubles. The aNDFom and ADFom values are lower compared with aNDF and ADF, respectively, as consequence of ash correction. On average, ash bound to NDF and ADF is 30% and 39.5% of fecal ash, respectively. According to Van Soest (1994) the former contains soil mineral silica, insoluble in neutral detergent, and various minerals and some naturally occurring rare earth. The lower values observed for ash bound to NDF, as compared with those of ADF, may be due to the solubilization of biogenic silica, which is quantitatively recovered in the ADF residue as opposed to neutral-detergent extraction.

In our study, the N content of buffalo feces ranged from 1.78% to 2.80% DM, which is lower than the values observed for buffalo heifers' feces by Al-Asfoor et al. (2012) as a consequence of the higher N requirement of lactating animals and to the relative supply. The NDIN and ADIN values in buffalo feces are close to, and lower than, the values found in beef cattle (Simoni et al., 2021b). The observed lower fecal ADIN content in buffalo, compared with beef cattle, may be a result of the lower ADIN levels of the diets consumed (averaging 0.19% of DM).

The fecal starch content in our study (average 1.66% of DM) is lower than the values observed in male (Paula et al., 2020), lactating, and dry Mediterranean buffaloes (Bovera et al., 2007), and the minimum value observed (0.25% of DM) is close to the average value found in heifers' feces (Al-Asfoor et al., 2012). This may be due to the higher efficiency in starch digestion of lactating animals or to different dietary starch sources (Grant and Ferraretto, 2018). The first hypothesis is supported by data from Righi et al. (2007) on lactating cows. In the latter study, starting from an average dietary starch content of 18.1% of DM, consistent with those received by the buffalo enrolled in the present study, the authors found fecal starch proportions ranging from 0.11% to 2.75% of DM, determined with the polarimetric method, which could have led to an underestimation of its content.

The literature generally lacks comprehensive data on the marker content in buffalo diets and feces, making comparisons challenging. A back calculation from the data of Hart and Wanapat (1992) on swamp buffalo (9.8% dietary AIA; marker recovery 94%) indicated a higher fecal AIA content (22% of DM) than what we observed in our study (6.08% of DM). In line with other results, the fecal AIA of steers fed a corn silage-based diet were 3.3 and 3.9 times higher than dietary AIA (Thonney et al., 1985). Fecal uNDF, starting from a dietary concentration of 14.6% of DM, averaged 41.3% of DM, whereas a study on Murrah buffaloes reported a dietary uNDF content of about 36% of DM, which lead to an estimated fecal uNDF content of 61% of DM (Soares et al., 2011), which is consistent with the highest values found in our study. The uNDF:ADL ratio ranged from 2.71 to 6.20, indicating a lower digestibility of dietary fiber when compared with dairy cows (1.58–4.10; Righi et al., 2017) and beef cattle (2.19 on average; Simoni et al., 2021b).

Total-Tract Apparent and True Digestibility

The most widely used internal marker in digestibility studies is AIA, which, along with short execution times, has many advantages, including the simplicity of analysis and of the required equipment (Wang et al., 2020), and the high or even complete recovery rate found in several species, including dairy and beef cattle, buffaloes, sheep and goast, pigs, horses, dogs, ostriches, and humans (Sales and Janssens, 2003; Wang et al., 2020). Thonney et al. (1985) demonstrated that AIA can be used to predict digestibility in cattle when diets contain AIA at more than 0.75% of DM, and diurnal and daily variations in fecal AIA content were insignificant. Similar indications were given for other species (Sales and Janssens, 2003). High accuracy ($R^2 > 0.75$) was reported when AIA and indigestible NDF (**iNDF**) were used to estimate nutrient digestibility in cattle (Pepeta et al., 2022). However, Lund et al. (2007) found a highly variable fecal marker recovery as a result of the type of forage fed to the animals, especially when iNDF intake was low (<750 g/d). Additionally, the sampling protocol adopted affected uNDF recovery, being multiple daily samples required for this

marker (Morris et al., 2018). Based on the amount of feed offered, in the present study, the minimum required intake values for both markers were exceeded. However, adhering to common field practices, we collected a single sample per cow per day, potentially altering the uNDF results, which may not accurately represent the relative average fecal content. Based on Thonney et al. (1985), this risk could be lower in the case of AIA

Our results for ttaDMDe, ttaOMDe, ttaEEDe, ttaNDFDe, ttaADFDe, ttHCDe, ttCPDe, ttaStarchDe, and ttaNFCDe, estimated using AIA as a marker, are consistent with the literature on Mediterranean buffaloes fed similar diets (Campanile et al., 2008; Serrapica et al., 2022) and lactating river buffalo worldwide (Azzaz et al., 2015; Qamar et al., 2016; de Moura Lima et al., 2021). Thus, exhibiting a wide range of outcomes, our database can be considered representative for lactating buffaloes. The lower digestibility of the fibrous fractions inclusive of their ash content compared with the corrected ones (aNDF vs. aNDFom, and ADF vs. ADFom) is related to the ash correction itself, which always increases digestibility. The ranges of ttAshDe, ttCELDe, and ttADLDe are similar to those found in nonpregnant, nonlactating swamp buffalo by Wang et al. (2020), even though their results showed less variability as a consequence of the specific physiological phase, which is less subject to variation.

The digestibility estimated from AIA or from uNDF did not differ by more than 7 percentage points on average, with exceptions for ttAshDe, ttADFDe, ttADINDe, and ttADLDe, which exhibited differences between 7 and 9 percentage points. None of the previously cited studies reported the digestibility of N bound to fibrous fractions. However, a study conducted on beef cattle estimating nutrient digestibility, using uNDF as an internal marker, showed higher digestibility of NDIN (52.0% of DM on average) and ADIN (47.0% on DM on average; Simoni et al., 2021b). This can be related to the higher intake of lactating buffaloes compared with beef animals, which affects the passage rate, reducing diet utilization, or to a different forages' digestibility.

Nutrient Total-Tract Digestibility in Lactating Buffaloes Estimated from uNDF or AIA

The uNDF has been shown to be more accurate than AIA when compared with total fecal collection in predicting fecal output and nutrient digestibility of lactating cows, but as observed by Morris et al. (2018) the accuracy of the marker seems to depend on diet typology, marker digestibility, analytical method and errors, sampling protocol and species of interest. In fact, the cited authors highlight inconsistent results regarding the markers' performance in predicting digestibility in dairy cows. Recently, a study conducted on lactating dairy cattle reported a good relationship ($r = 0.93$) between the iNDF intake and the fecal flow of iNDF (Lund et al., 2007). However, the study evaluated the recovery of iNDF by feeding different forages ad libitum (grass hay, early-cut grass silage, late-cut grass silage, whole crop barley silage, lucerne hay, maize silage, and pea silage) as the only feed or supplemented either with soybean or wheat meal. An average iNDF recovery of 1.01 g/g, ranging from 0.64 and 0.84 g/g in early-cut grass silage supplemented and unsupplemented respectively, and 1.20 g/g in maize silage was found. The highest recovery of iNDF in maize silage-based diet (as those fed in the present trial) led to an underestimation of nutrients digestibility. Consistently, in the present study, digestibility estimated using uNDF as a marker was lower compared with those obtained using AIA. It was demonstrated in buffalo that uNDF, compared with total collection, underestimated digestibility (Maeda et al., 2011; Soares et al., 2011). Because AIA demonstrated around 100% of recovery in the same species (Sales and Janssens, 2003; Wang et al., 2020) we can speculate that our results are in line with the previous cited studies. On the other hand, it has been demonstrated that AIA, unlike uNDF, are unaffected by daily variation (Thonney et al., 1985). Hence, AIA is more suitable for use when sampling is conducted, as in our case, only once a day. In-depth studies are needed to define whether the marker should be chosen on the basis of the forage base in the diet (Van Soest, 1994), if a glucogenic diet can have an effect on feeding behavior (Oba and Allen, 2003), and thus if multiple-step point sampling is needed to avoid the potential variation in the flux of markers via the digestive tract (Morris et al., 2018).

When estimated with uNDF, the ttaDMDe, ttaOMDe, and ttADFDe were not comparable with those estimated with AIA. All the other variables where moderately ($0.3 < r < 0.7$) or strongly ($r > 0.7$; digestibility of EE, NDIN, ADIN, HC, starch, NFC, and NB3) correlated when estimated with the 2 different markers. Specifically, the values can be considered comparable in the cases of EE, NDIN, starch, and NB3, having a Pearson correlation higher than 0.9, a coefficient of determination higher than 0.8, an intercept close to 0, and slope of approximately 1. When estimated with the AIA, the digestibility values are similar to those obtained through total fecal collection in horses (Bergero et al., 2004; Bergero et al., 2005), reaching correlation coefficients higher than 0.80 (Miraglia et al., 1999), except for crude protein digestibility (Bergero et al., 2004). These results are explained by the better recovery rate of AIA, which was around 100% in several species (Sales and Janssens., 2003),

and, particularly in buffalo, was demonstrated to be 97% (Wang et al., 2020).

Our results are partially in line with those obtained on dairy heifers (Mota et al., 2013) and on castrated buffalo (Soares et al., 2011), in which digestibility values obtained using uNDF were underestimated if compared with total fecal collection. In fact, Soares et al. (2011) found that using indigestible ADF at 144 h, iNDF (at 144 h and 288 h), indigestible DM at 144 h, or chromium oxide as markers, the estimated fecal DM production was higher than the real excretion measured through total fecal collection. This led to an underestimation of the nutrient digestibility, as observed in the present study. As deduced by Berchielli et al. (2005), the markers can have a different behavior based on the type of forage fed to the animal, which in turn may affect their recovery. Moreover, a recent study from Del Valle et al. (2022) showed that the fecal recovery of markers was affected by a 2-way interactions effect between the fat supplementation and the internal marker and also by sampling time.

NIRS Prediction of Fecal Composition

As previously observed in other studies on dairy and beef cattle, the traits ash and ADF exhibited R^2_{ExV} values of 0.70 and 0.66, respectively, that suggest an approximate quantification. Their RPD_{ExV} values (1.66 and 1.43, respectively) showed that they can be useful only to detect extreme values as reported by Grelet et al. (2021). The RPD is a highly stringent metric that is particularly used to compare models with one another because it is dimensionless, providing a standardized assessment of performance. A good prediction of CP was achieved, with an accuracy of estimate from fecal samples comparable to the one reported by Decruyenaere et al. (2009) using a cross-calibration approach on 78 samples of bovine feces.

Comparable accuracies were observed for aNDF, N, and ash, aligning with the findings of Simoni et al. (2021b), who assessed the NIRS predictive performance on cattle feces. However, a lower accuracy (0.66) was observed specifically for ADF, compared with the 0.82 of the other traits. Similarly to Simoni et al. (2021b), results reported nonusable predictive performance for uNDF. It is crucial to highlight that Simoni et al. (2021b) used the R^2_{CrV} as the reference parameter, whereas in this study the value obtained from external validation was employed as the reference parameter.

Moreover, based on the recommendations of Williams (2014), and given the R^2_{ExV} and RPD_{ExV} values, the prediction models of the buffaloes' feces composition traits were not usable in 13 out of 20 cases. Seven models have achieved an R^2_{ExV} value exceeding approximately 0.45, indicating potential for further enhancements. This improvement could be accomplished by expanding the sample size under investigation, thereby increasing its variability. However, the poorer outcomes observed can be attributed to the potential inadequacy of NIRS in accurately predicting these traits within the selected wavelengths.

NIRS Prediction Models of Total-Tract Apparent and True Nutritional Digestibility

The present study investigates the use of uNDF or AIA as markers to develop and examine several NIRS predictive models for tta nutrients digestibility from buffaloes' feces. When performing the external validation, the tta digestibility of ash exhibited the highest predictive accuracy, as evidenced by the R^2_{ExV} value of 0.58 and the RPD_{ExV} of 1.53, among the various traits evaluated. Similarly, a previous study conducted by Simoni et al. (2021b) has also explored and developed prediction models using uNDF as a marker, but specifically on fecal samples of beef cattle, yielding comparable outcomes in terms of accuracy. Conversely, the other variables investigated in these 2 studies yielded comparatively lower outcomes in terms of NIRS predictive models.

Among the range of predictive models developed for estimating tta or true nutrients digestibility using fecal uNDF as a marker in predried feces of lactating buffaloes, ttNDINDe and ttADINDe showed R^2_{ExV} values that can be considered suitable for an approximate quantification. When focusing on prediction patterns formulated using fecal AIA as a marker in buffalo feces, only ttaEEDe exhibited excellent performance with an R^2_{ExV} value of 0.86 and an RPD_{ExV} of 2.22. However, despite the good R^2_{ExV} found for ttaEEDe when both reference markers were considered in this study, due to methodological issues, it cannot be representative of the NIR ability in predicting EE digestibility.

CONCLUSIONS

The NIRS technology efficiently estimated N, and consequently CP, EE, aNDFom, and aNDF content of Mediterranean buffalo feces, but failed to estimate other compositional characteristics. Moreover, NIRS can predict ttNDIDe and ttADINe when estimated using uNDF. However, it is unable to predict all the other nutrients digestibilities when estimated using uNDF or AIA as markers. The EE, NDIN, ADIN, HC, starch, NFC, and NB3 digestibility values obtained using uNDF and AIA are strongly correlated, whereas the DM and OM digestibility values obtained with the 2 markers are not correlated.

NOTES

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Nonstandard abbreviations used: ADFom = ADF without residual ash; ADICP = CP bound to ADF; AIA = acid-insoluble ash; aNDF = amylase-treated NDF inclusive of residual ash; aNDFom = amylase-treated NDF without residual ash; CEL = cellulose; De = digestibility; EE = ether extract; HC = hemicellulose; iNDF = indigestible NDF; LF = latent factors; max. = maximum; min. = minimum; mPLS = modified partial least squares; NB3 = B3 fraction of N; n_{CrV} = number of actual cross validation samples used from the calibration set; NDICP = CP bound to aNDF; NIR = near infrared; NIRS = near-infrared spectroscopy; OM = organic matter; R^2_{CrV} = R^2 of cross validation; R^2_{ExV} = R^2 of external validation; RPD = residual predictive deviation; RPD_{ExV} = RPD of external validation; SE_{CrV} = SE of cross validation; SE_{ExV} = SE of external validation; SNV = standard normal variate; tt = total tract; tta = apparent total tract; ttaCPDe = apparent total-tract digestibility of CP; ttADFDe = total-tract digestibility of ADF; ttADFomDe = total-tract digestibility of ADFom; ttADINDe = total-tract digestibility of ADIN; ttADLDe = total-tract digestibility of ADL; ttaDMDe = apparent total-tract digestibility of DM; ttaEEDe = apparent total-tract digestibility of EE; ttAshDe = total-tract digestibility of ash; ttCELDe = total-tract digestibility of CEL; ttHCDe = total-tract digestibility of HC; ttaNDFDe = total-tract digestibility of aNDF; ttaNDFomDe = total-tract digestibility of aNDFom; ttNB3 = total-tract digestibility of NB3; ttNDINDe = total-tract digestibility of NDIN; ttaNFCDe = apparent total-tract digestibility of NFC; ttaOMDe = apparent total-tract digestibility of OM; ttaStarchDe = apparent total-tract digestibility of starch; uNDF = undigestible NDF.

REFERENCES

AIA. 2023. Produzione media per Bufala. Accessed Mar, 29, 2023. http://bollettino.aia.it/Contenuti.aspx?CD_GruppoStampe=RS&CD_Specie=C4.

Al-Asfoor, H., E. Schlecht, A. Sundrum, and A. Schiborra. 2012. Varying the dietary supply of C and N to manipulate the manure composition of water buffalo heifers in Oman. J. Agric. Rural Dev. Trop. Subtrop. 113:125–136.

Albano, L., N. Ciriello, G. Auriemma, R. Palomba, G. Grazioli, and F. Sarubbi. 2020. Nitrous oxide emissions of the Italian Mediterranean buffalo breeding: Influence of management. Asian J. Res. Biosci. 2:68–76.

AOAC International. 2014. Dietary starch in animal feeds and pet food (AOAC 2014.10). AOAC International, Rockville, MD.

Azzaz, H. H., H. M. Ebeid, T. A. Morsy, and S. M. Kholif. 2015. Impact of feeding yeast culture or yeast culture and propionibacteria 169 on the productive performance of lactating buffaloes. Int. J. Dairy. Sci. 10:107–116. https://doi.org/10.3923/ijds.2015.107.116.

Babar, M. E., T. Hussain, and A. Wajid. 2013. Latest biotechnological approaches for efficient buffalo production. Buffalo Bull. 32:142–150.

Battelli, M., L. Rapetti, A. Rota Graziosi, S. Colombini, G. M. Crovetto, and G. Galassi. 2020. Use of undigested NDF for estimation of diet digestibility in growing pigs. Animals (Basel) 10:2007. https://doi.org/10.3390/ani10112007.

Berchielli, T. T., S. G. de Oliveira, E. N. V. M. Carrilho, J. V. Feitosa, and A. D. Lopes. 2005. Comparação de marcadores para estimativas de produção fecal e de fluxo de digesta em bovinos. R. Bras. Zootec. 34:987–996. https://doi.org/10.1590/S1516-35982005000300032.

Bergero, D., G. Meineri, N. Miraglia, and P. G. Peiretti. 2005. Apparent digestibility of hays in horses determined by total collection of faeces and using internal marker methods. J. Food Agric. Environ. 3:199–202.

Bergero, D., N. Miraglia, C. Abba, and M. Polidori. 2004. Apparent digestibility of Mediterranean forages determined by total collection of faeces and acid-insoluble ash as internal marker. Livest. Prod. Sci. 85:235–238. https://doi.org/10.1016/S0301-6226(03)00137-4.

Bovera, F., R. Tudisco, S. Marono, M. I. Cutignelli, S. Calabrò, F. Infascelli, and V. Piccolo. 2007. Relationship between fecal scoring and in vitro digestibility of the diet in buffalo cows. Ital. J. Anim. Sci. 6(Suppl. 2):555–558. https://doi.org/10.4081/ijas.2007.s2.555.

Brogna, N., A. Palmonari, G. Canestrari, L. Mammi, A. Dal Prà, and A. Formigoni. 2018. Technical note: Near infrared reflectance spectroscopy to predict fecal indigestible neutral detergent fiber for dairy cows. J. Dairy Sci. 101:1234–1239. https://doi.org/10.3168/jds.2017-13319.

Campanile, G., F. Zicarelli, D. Vecchio, C. Pacelli, G. Neglia, A. Balestrieri, R. Di Palo, and F. Infascelli. 2008. Effects of *Saccharomyces cerevisiae* on in vivo organic matter digestibility and milk yield in buffalo cows. Livest. Sci. 114:358–361. https://doi.org/10.1016/j.livsci.2007.11.002.

Catunda, K. L. M., A. C. Churchill, S. A. Power, and B. D. Moore. 2022. Near-infrared spectroscopy calibration strategies to predict multiple nutritional parameters of pasture species from different functional groups. J. Near Infrared Spectrosc. 30:254–263. https://doi.org/10.1177/09670335221114746.

Cotanch, K. W., R. J. Grant, M. E. Van Amburgh, A. Zontini, M. Fustini, A. Palmonari, and A. Formigoni. 2014. Applications of uNDF in ration modeling and formulation. Pages 114–131 in Cornell Nutr. Conf. Feed Manuf. Cornell University.

de Carvalho, G. G. P., R. Garcia, A. J. V. Pires, R. R. Silva, E. Detmann, R. L. Oliveira, and L. S. O. Ribeiro. 2013. Long-term bias of internal

markers in sheep and goat digestion trials. Asian-Australas. J. Anim. Sci. 26:65–71. 10.5713/ajas.2011.11408.

De Marchi, M., C. L. Manuelian, S. Ton, D. Manfrin, M. Meneghesso, M. Cassandro, and M. Penasa. 2017. Prediction of sodium content in commercial processed meat products using near infrared spectroscopy. Meat Sci. 125:61–65. https://doi.org/10.1016/j.meatsci.2016.11.014.

de Moura Lima, E., J. A. C. Vargas, D. I. Gomes, R. P. Maciel, K. S. Alves, W. F. Oliveira, G. L. Aguiar, G. de Carvalho Reis, L. R. S. Oliveira, and R. Mezzomo. 2021. Intake, digestibility, and milk yield response in dairy buffaloes fed *Panicum maximum* cv. Mombasa supplemented with seeds of tropical açai palm. Trop. Anim. Health Prod. 53:178. https://doi.org/10.1007/s11250-021-02626-1.

Decruyenaere, V., Ph. Lecomte, C. Demarquilly, J. Aufrere, P. Dardenne, D. Stilmant, and A. Buldgen. 2009. Evaluation of green forage intake and digestibility in ruminants using near infrared reflectance spectroscopy (NIRS): Developing a global calibration. Anim. Feed Sci. Technol. 148:138–156. https://doi.org/10.1016/j.anifeedsci.2008.03.007.

Del Valle, T. A., L. Y. Sakamoto, E. M. C. Zilio, L. G. Ghizzi, J. A. Marques, J. M. Souza, M. S. S. Dias, and F. P. Rennó. 2022. Effects of sampling time on internal markers concentration and fecal recovery in dairy cows. Livest. Sci. 258:104897. https://doi.org/10.1016/j.livsci.2022.104897.

Dixon, R., and D. Coates. 2009. Review: Near infrared spectroscopy of faeces to evaluate the nutrition and physiology of herbivores. J. Near Infrared Spectrosc. 17:1–31. https://doi.org/10.1255/jnirs.822.

European Commission. 2009. Commission regulation (EC) no. 152/2009 of 27 January 2009. Laying down the methods of sampling and analysis for the official control of feed. Off. J. Eur. Union. 54:1–130.

Evangelista, C., U. Bernabucci, and L. Basiricò. 2022. Effect of antioxidant supplementation on milk yield and quality in Italian Mediterranean lactating buffaloes. Animals (Basel) 12:1903. https://doi.org/10.3390/ani12151903.

FAO. 2023. Crops and livestock products. Accessed Mar. 29, 2023. https://www.fao.org/faostat/en/?#data/QCL/visualize.

Fondevila, M., C. Castrillo, J. Gasa, and J. A. Guada. 1995. Rumen-undegradable dry matter and neutral detergent fiber as ratio indicators of digestibility in sheep given cereal straw-based diets. J. Agric. Sci. 125:145–151. https://doi.org/10.1017/S0021859600074591.

Fustini, M., A. Palmonari, G. Canestrari, E. Bonfante, L. Mammi, M. T. Pacchioli, G. C. J. Sniffen, R. J. Grant, K. W. Cotanch, and A. Formigoni. 2017. Effect of undigested neutral detergent fiber content of alfalfa hay on lactating dairy cows: Feeding behavior, fiber digestibility, and lactation performance. J. Dairy Sci. 100:4475–4483. https://doi.org/10.3168/jds.2016-12266.

Goering, H. K., and P. J. Van Soest. 1970. Forage fiber analyses (apparatus, reagent, procedures and some applications). Agriculture Handbook no. 379. Page 20. USDA-ARS, Washington, DC.

Goi, A., J. F. Hocquette, E. Pellattiero, and M. De Marchi. 2022. Handheld near-infrared spectrometer allows on-line prediction of beef quality traits. Meat Sci. 184:108694. https://doi.org/10.1016/j.meatsci.2021.108694.

Goi, A., C. L. Manuelian, S. Currò, and M. De Marchi. 2019. Prediction of mineral composition in commercial extruded dry dog food by near-infrared reflectance spectroscopy. Animals (Basel) 9:640. https://doi.org/10.3390/ani9090640.

Grant, R. J., and L. F. Ferraretto. 2018. Silage review: Silage feeding management: Silage characteristics and dairy cow feeding behavior. J. Dairy Sci. 101:4111–4121. https://doi.org/10.3168/jds.2017-13729.

Grelet, C., P. Dardenne, H. Soyeurt, J. A. Fernandez, A. Vanlierde, F. Stevens, N. Gengler, and F. Dehareng. 2021. Large-scale phenotyping in dairy sector using milk MIR spectra: Key factors affecting the quality of predictions. Methods 186:97–111. https://doi.org/10.1016/j.ymeth.2020.07.012.

Hart, F. J., and M. Wanapat. 1992. Physiology of digestion of urea-treated rice straw in Swamp buffalo. Asian-Australas. J. Anim. Sci. 5:617–622. https://doi.org/10.5713/ajas.1992.617.

Hassaan, N. A., M. Khorshed, M. Shoukry, A. Abedo, A. El-Nasser Khattab, and N. E. El-Bordeny. 2022. Effect of phytase supplementation on blood chemistry and milk composition of lactating buffaloes. Egypt. J. Chem. 65:133–142. https://doi.org/10.21608/ejchem.2021.96005.4501.

Hawkins, D. M. 2004. The problem of overfitting. J. Chem. Inf. Comput. Sci. 44:1–12. https://doi.org/10.1021/ci0342472.

Huhtanen, P., K. Kaustell, and S. Jaakkola. 1994. The use of internal markers to predict total digestibility and duodenal flow of nutrients in cattle given six different diets. Anim. Feed Sci. Technol. 48:211–227. https://doi.org/10.1016/0377-8401(94)90173-2.

Khattab, H. M., S. A. El-Nor, S. M. Kholif, H. M. El-Sayed, O. H. Abd El-Shaffy, and M. Saada. 2010. Effect of different additive sources on milk yield and composition of lactating buffaloes. Livest. Sci. 131:8–14. https://doi.org/10.1016/j.livsci.2010.02.016.

Kho, E. A., J. N. Fernandes, A. J. Tilbrook, G. P. Fox, M. T. Sikulu-Lord, A. C. Kotze, A. M. Beasley, P. J. James, D. R. Tolleson, and D. Cozzolino. 2023. State of the art and the future of fecal analysis using infrared spectroscopy. Appl. Spectrosc. Rev. 58:755–785. https://doi.org/10.1080/05704928.2022.2143795.

Lisanti, E., R. D. Wulaningsih, and A. Arwin. 2021. Van soest and mineral analysis of wild buffaloes feces (*Bubalus bubalis* L.) in Savana Baluran National Park, East Java Indonesia. IOP Conf. Ser. Mater. Sci. Eng. 1098:052053. https://doi.org/10.1088/1757-899X/1098/5/052053

Maeda, E. M., L. M. Zeoula, H. C. C. Gomes, G. Jacobi, F. L. Simioni, and R. A. de Oliviera. 2011. Evaluation of markers used in the studies of intake and digestibility in cattle and buffalo. Arch. Zootec. 60:123–131. https://doi.org/10.21071/az.v60i229.4696.

Manuelian, C. L., G. Visentin, C. Boselli, G. Giangolini, M. Cassandro, and M. De Marchi. 2017. Short communication: Prediction of milk coagulation and acidity traits in Mediterranean buffalo milk using Fourier-transform mid-infrared spectroscopy. J. Dairy Sci. 100:7083–7087. https://doi.org/10.3168/jds.2017-12707.

Mertens, D. R., M. Allen, J. Carmany, J. Clegg, A. Davidowicz, M. Drouches, K. Frank, D. Gambin, M. Garkie, B. Gildemeister, D. Jeffress, and C. S. Jeon. 2002. Gravimetric determination of amylase-treated neutral detergent fiber in feeds with refluxing in beakers or crucibles: Collaborative study. J. AOAC Int. 85:1217–1240. https://doi.org/10.1093/jaoac/85.6.1217.

Mihaljev, Ž., S. Jaksic, and M. Ž. Baloš. 2015. Comparison of the Kjeldahl method, Dumas method and NIR method for total nitrogen determination in meat and meat products. J. Agroaliment. Processes Technol. 21:365–370.

Miraglia, M., D. Bergero, B. Bassano, M. Tarantola, and G. Ladetto. 1999. Studies of apparent digestibility in horses and the use of internal markers. Livest. Prod. Sci. 60:21–25. https://doi.org/10.1016/S0301-6226(99)00043-3.

Morris, D. L., L. R. Rebelo, P. A. Dieter, and C. Lee. 2018. Validating intrinsic markers and optimizing spot sampling frequency to estimate fecal outputs. J. Dairy Sci. 101:7980–7989. https://doi.org/10.3168/jds.2018-14717.

Mota, D. A., T. T. Berchielli, R. C. Canesin, A. P. O. Sader, J. D. Mesana, and M. V. Biehl. 2013. Estimation of fecal production, digesta flow and digestibility in dairy heifers with different indicator. Acta Sci. Anim. Sci. 35:17338. https://doi.org/10.4025/actascianimsci.v35i2.17338.

Mota, L. F., S. Pegolo, T. Baba, F. Peñagaricano, G. Morota, G. Bittante, and A. Cecchinato. 2021. Evaluating the performance of machine learning methods and variable selection methods for predicting difficult-to-measure traits in Holstein dairy cattle using milk infrared spectral data. J. Dairy Sci. 104:8107–8121. https://doi.org/10.3168/jds.2020-19861.

Neglia, G., A. Balestrieri, B. Gasparrini, M. I. Cutrignelli, G. Bifulco, A. Salzano, R. Cimmino, E. Varricchio, M. J. D'Occhio, and G. Campanile. 2014. Nitrogen and phosphorus utilisation and excretion in dairy buffalo intensive breeding. Ital. J. Anim. Sci. 13:3362. https://doi.org/10.4081/ijas.2014.3362.

Nousiainen, J., S. Ahvenjärvi, M. Rinne, M. Hellämäki, and P. Huhtanen. 2004. Prediction of indigestible cell wall fraction of grass silage by near infrared reflectance spectroscopy. Anim. Feed Sci. Technol. 115:295–311. https://doi.org/10.1016/j.anifeedsci.2004.03.004.

Oba, M., and M. S. Allen. 2003. Effects of corn grain conservation method on feeding behavior and productivity of lactating dairy cows at two dietary starch concentrations. J. Dairy Sci. 86:174–183. https://doi.org/10.3168/jds.S0022-0302(03)73598-X.

Osborne, B. G., T. Fearn, and P. H. Hindle. 1993. Practical NIR Spectroscopy with Application in Food and Beverage Analysis. Page 227. Addison-Wesley Longman.

Palmquist, D. L., and T. C. Jenkins. 2017. A 100-Year Review: Fat feeding of dairy cows. J. Dairy Sci. 100:10061–10077. https://doi.org/10.3168/jds.2017-12924.

Pasquini, M., A. Osimani, S. Tavoletti, I. Moreno, F. Clementi, and M. F. Trombetta. 2018. Trends in the quality and hygiene parameters of bulk Italian Mediterranean buffalo (*Bubalus bubalis*) milk: A three year study. Anim. Sci. J. 89:176–185. https://doi.org/10.1111/asj.12916.

Paula, T. V., C. L. Francisco, A. M. Castilhos, P. R. L. Meirelles, A. G. Jacaùna, H. L. Correa, C. S. Oliveira, F. de Barros, V. R. M. Jacob, and A. M. Jorge. 2020. Fecal parameters as indicators of starch digestibility and feedlot performance of water buffaloes. J. Anim. Sci. 98(Suppl. 4):299–300. https://doi.org/10.1093/jas/skaa278.539.

Qamar, M. S., Mahr-Un-Nisa, M. Sarwar, Zia-Ul-Rahman, and T. Huma. 2016. Substituting corn steep liquor with urea as nitrogen equivalence on nutrients intake, digestibility, blood biochemistry and milk composition in early lactating Nili-Ravi buffaloes. J. Anim. Plant Sci. 26:1212–1217. https://thejaps.org.pk/docs/v-26-05/03.pdf.

Raffrenato, E., D. A. Ross, and M. E. Van Amburgh. 2018. Development of an in vitro method to determine rumen undigested aNDFom for use in feed evaluation. J. Dairy Sci. 101:9888–9900. https://doi.org/10.3168/jds.2018-15101.

Refat, B., and P. Yu. 2022. Evaluation of prediction of indigestible fiber fraction (iNDF) of whole-crop barley silage by using non-destructive spectroscopic techniques as a fast-screening method: Comparison between FTIR vs. NIR. Can. J. Plant Sci. 102:1130–1138. https://doi.org/10.1139/cjps-2022-0054.

Righi, F., A. Quarantelli, L. Tonelli, M. Renzi, and B. Gandolfi. 2007. Use of Penn State Particle Separator for the evaluation of total mixed rations typical of Parmigiano Reggiano cheese production area. Ital. J. Anim. Sci. 6(Suppl. 1):347–349. https://doi.org/10.4081/ijas.2007.1s.347.

Righi, F., M. Simoni, G. Visentin, C. L. Manuelian, S. Currò, A. Quarantelli, and M. De Marchi. 2017. The use of near infrared spectroscopy to predict fecal indigestible and digestible fiber fractions in lactating dairy cattle. Livest. Sci. 206:105–108. https://doi.org/10.1016/j.livsci.2017.10.006.

Robertson, J. B., and P. J. Van Soest. 1981. The detergent system of analysis and its application to human foods. Pages 123–158 in The Analysis of Dietary Fiber in Food. W. P. T. James and O. Theander, ed. Marcel Dekker. New York, NY.

Sales, J., and G. P. J. Janssens. 2003. Acid-insoluble ash as a marker in digestibility studies: A review. J. Anim. Feed Sci. 12:383–401. https://doi.org/10.22358/jafs/67718/2003.

Şeker, E., Y. Kuyucuoğlu, B. Sareyyüpoğlu, and H. Yardımcı. 2010. PCR detection of shiga toxins, enterohaemolysin and intimin virulence genes of *Escherichia coli* O157:H7 strains isolated from faeces of Anatolian water buffaloes in Turkey. Zoonoses Public Health 57:e33–e37. https://doi.org/10.1111/j.1863-2378.2009.01285.x.

Serrapica, F., F. Masucci, G. De Rosa, A. Braghieri, F. Sarubbi, F. Garofalo, F. Grasso, and A. Di Francia. 2022. Moving buffalo farming beyond traditional areas: Performances of animals, and quality of mozzarella and forages. Agriculture 12:1219. https://doi.org/10.3390/agriculture12081219.

Shenk, J. S., M. O. Westerhaus, and S. Abrams. 1989. Protocol for NIR calibrations: Monitoring analysis results and recalibration. Pages 104–110 in Near Infrared Spectroscopy (NIRS): Analysis of Forage Quality. G. C. Marten, J. S. Shenk, and F.E. Barton II, ed. USDA-ARS, US Government Printing Office, Washington, DC.

Simoni, M., A. Canepa, R. G. Pitino, A. Quarantelli, and F. Righi. 2021a. Digestibility and sorting of hay-based total mixed rations employed in the Parmigiano-Reggiano area as affected by dietary particle size distribution. Large Anim. Rev. 27:123–132.

Simoni, M., A. Goi, M. De Marchi, and F. Righi. 2021b. The use of visible/near-infrared spectroscopy to predict fiber fractions, fiber-bound nitrogen and total-tract apparent nutrients digestibility in beef cattle diets and faeces. Ital. J. Anim. Sci. 20:814–825. https://doi.org/10.1080/1828051X.2021.1924884.

Simoni, M., E. Tsiplakou, R. Pitino, A. Quarantelli, and F. Righi. 2021c. Determination of the optimal priming interval of rumen fluids as inocula for the in vitro digestibility trials through radial enzyme diffusion method. Anim. Prod. Sci. 61:525–531. https://doi.org/10.1071/AN20197.

Soares, L. F. P., A. Guim, M. D. A. Ferreira, C. Modesto, Â. Maria, V. Batista, P. De Barros, and S. Monteiro. 2011. Assessment of indicators and collection methodology to estimate nutrient digestibility in buffaloes. R. Bras. Zootec. 40:2005–2010. https://doi.org/10.1590/S1516-35982011000900023.

Sriwattanasombat, P., and M. Wanapat. 1983. Supplementation of urea-treated straw with dried leucaena (*Leucaena leucocephalia*) and water hyacinth (*Eichhornia crassipes*) leaf meals. Pages 170–179 in The National Buffalo Research and Development Center Project. Department of Livestock Development. Bangkok, Thailand.

Thonney, M. L., B. A. Palhof, M. R. De Carlo, D. A. Ross, N. L. Firth, R. L. Quaas, D. J. Perosio, D. J. Duhaime, S. R. Rollins, and A. Y. M. Nour. 1985. Sources of variation of dry matter digestibility measured by the acid insoluble ash marker. J. Dairy Sci. 68:661–668. https://doi.org/10.3168/jds.S0022-0302(85)80872-9.

van Empel, M. J., H. P. S. Makkar, J. Dijkstra, and P. Lund. 2016. Nutritional, technological and managerial parameters for precision feeding to enhance feed nutrient utilization and productivity in different dairy cattle production systems. Perspect. Agric. Vet. Sci. Nutr. Nat. Resour. 11:1–27. https://doi.org/10.1079/PAVSNNR201611037.

Van Keulen, J., and B. A. Young. 1977. Evaluation of acid-insoluble ash as a natural marker in ruminant digestibility studies. J. Anim. Sci. 44:282–287. https://doi.org/10.2527/jas1977.442282x.

Van Soest, P. J. 1994. Nutritional Ecology of the Ruminant. 2nd ed. Page 476. Comstock Publishing.

Wang, Z., Y. Wu, L. Shi, L. Cui, X. Li, C. He, L. Yang, and X. Li. 2020. Evaluation and mining the applicable methods of roughage digestibility determination for buffalo (*Bubalus bubalis*). Trop. Anim. Health Prod. 52:2639–2646. https://doi.org/10.1007/s11250-020-02292-9.

Williams, P. 2014. The RPD statistic: A tutorial note. NIR News 25:22–26.

Yadav, B., P. Yadav, M. Kumar, S. Vasvani, M. Anand, A. Kumar, D. K. Swain, S. Yadav, and A. K. Madan. 2022. Effect of heat stress on rumen microbial diversity and fermentation pattern in buffalo. Advanced Gut & Microbiome Research 2022:1–14. https://doi.org/10.1155/2022/1248398.

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