frontiers | Frontiers in Animal Science

TYPE Original Research
PUBLISHED 20 February 2025
DOI 10.3389/fanim.2025.1547776

OPEN ACCESS

EDITED BY
Sarita Bonagurio Gallo,
University of São Paulo, Brazil

REVIEWED BY
Ravikanth Reddy Poonooru,
University of Missouri, United States
Janaina Silveira Da Silva,
University of São Paulo, Brazil

*CORRESPONDENCE
Stefanie Alvarenga Santos
stefanie.alvarenga@ufba.br

RECEIVED 18 December 2024
ACCEPTED 04 February 2025
PUBLISHED 20 February 2025

CITATION
dos Santos ACS, dos Santos GR, da Silva MP, Mariz LDS, Alba HDR, Tosto MSL, Pereira ES, Pina DdS, de Azevedo JAG, de Carvalho GGP and Santos SA (2025) Estimation of indigestible NDF by *in situ* procedure in sheep: number of bags and fabric type. *Front. Anim. Sci.* 6:1547776.
doi: 10.3389/fanim.2025.1547776

Estimation of indigestible NDF by *in situ* procedure in sheep: number of bags and fabric type

Antônio Carneiro Santana dos Santos[1], Gisele Rocha dos Santos[1], Márcia Pereira da Silva[1], Lays Débora Silva Mariz[2], Henry Daniel Ruiz Alba[1], Manuela Silva Libânio Tosto[1], Elzania Sales Pereira[2], Douglas dos Santos Pina[1], José Augusto Gomes de Azevedo[3], Gleidson Giordano Pinto de Carvalho[1] and Stefanie Alvarenga Santos[1*]

[1]Escola de Medicina Veterinária e Zootecnia, Universidade Federal da Bahia, Salvador, Bahia, Brazil, [2]Departamento de Zootecnia, Universidade Federal do Ceará, Fortaleza, Ceara, Brazil, [3]Departamento de Ciências Agrárias e Ambientais, Universidade Estadual de Santa Cruz, Ilhéus, Bahia, Brazil

This study aimed to evaluate the effects of fabric type (F57 vs. non-woven textile [NWT]) and the number of bags incubated simultaneously in sheep on the determination of indigestible neutral detergent fiber (iNDF). Six fistulated sheep with an initial body weight of 46 $\pm$ 3 kg were used in the experiment, which followed a 6 × 6 Latin square design. The study consisted of six periods of 14 days each, allowing for a total incubation time of 336 hours. Two fabric types were used to incubate samples: F57 (Ankom, Fairport, NY) and NWT. Three sample types were analyzed to estimate and compare iNDF content: sorghum silage, ground corn, and sheep feces. For each sample type, bags of the two fabric types were incubated in sets of 4, 8, or 12 bags, totaling 12, 24, or 36 bags per animal per period, respectively. This resulted in a total of 144 bags incubated *in situ* per period. At the end of each incubation period, the bags were removed from the rumen, washed, oven-dried, and analyzed for NDF content. The results showed no significant differences (P > 0.05) in iNDF estimates between fabric types or across the different numbers of bags incubated. These findings indicate that both F57 and NWT fabrics are suitable for estimating iNDF in feed and feces samples from sheep. Moreover, using up to 36 bags did not compromise the results after 336 hours of incubation. However, further investigation is needed to determine whether incubating more than 36 bags could affect NDF degradation due to bag fill effects.

KEYWORDS
degradation, feces, F57, feeds, non-woven textile, small ruminant

1 Introduction

Internal markers are natural feed components that are neither digested nor absorbed in the gastrointestinal tract of animals. In ruminant nutrition trials, the primary internal markers used are indigestible neutral detergent fiber (iNDF) and indigestible acid detergent fiber (iADF), both of which can be quantified through *in situ* ruminal incubations. Several factors related to the *in situ* method can influence the accuracy and consistency of internal marker evaluation (Ørskov et al., 1980; Van der Koelen et al., 1992; Casali et al., 2008, 2009). Technical variables such as bag porosity and surface area, sample mass, post-incubation washing procedures, total incubation time, bag size, and the animal's basal diet have been shown to significantly affect the results (Lindberg, 1985; Nocek, 1988; Madsen and Hvelplund, 1994; Vanzant et al., 1998). To address these potential sources of variation, numerous standardization protocols have been developed over the years, aiming to enhance consistency and comparability across studies (Vanzant et al., 1998; Wang et al., 2021).

The use of F57 bags for analyzing indigestible fiber fractions is well-documented in both *in vitro* and *in situ* incubations (Adams et al., 2020; Coblentz et al., 2019; Defeo et al., 2020; Tassone et al., 2020; Valente et al., 2015). However, a significant limitation to the widespread adoption of F57 bags, particularly outside the United States, is their high cost, which limits their use in routine feed analysis. As alternatives, researchers have proposed non-woven textile (NWT) bags made from polypropylene as cost-effective alternatives for estimating iNDF and iADF (Casali et al., 2009; Valente et al., 2011a). These NWT bags provide a viable option for reducing costs while maintaining the accuracy and reliability of fiber analysis. Furthermore, the tensile strength of the F57 material was unaffected when tested with a dynamometer, whereas NWT was able to reduce its rupture strength after ruminal incubation without compromising the detergent extraction step. Besides, photomicrographs of F57 and NWT were similar, showing geometrically regular structures that did not obstruct the inflow of microorganisms or the outflow of degraded material.

Despite these advances, few studies have focused on standardizing *in situ* incubation methodologies for estimating iNDF in sheep. For example, Reis et al. (2017) compared *in situ* incubations between cattle and sheep, reporting that iNDF and iADF measured using NWT bags in cattle reached their asymptote in fewer hours compared to sheep. Interestingly, both species were incubated with the same number of bags, highlighting that methods validated for cattle may not be directly applicable to small ruminants. This discrepancy is likely due to differences in rumen volume and digestion dynamics between the two species, which can influence the digestion rate, and the time required to reach the indigestible fraction's asymptote.

Given the absence of a standardized methodology for determining the optimal number and type of bags for *in situ* incubation in sheep, this study aims to evaluate the effects of fabric type (F57 vs. NWT) and the number of bags incubated simultaneously on the estimation of iNDF in sorghum silage, ground corn, and sheep feces.

2 Material and methods

2.1 Location and ethical standards

The experiment was carried out at the Experimental Farm of São Gonçalo dos Campos – BA, in the research station of goats and sheep, belonging to the School of Veterinary Medicine and Animal Science of the Federal University of Bahia, Brazil (Universidade Federal da Bahia, Salvador, Bahia, Brasil).

All procedures were performed with authorization and strict accordance with the Ethics Committee for Animal Handling of the School of Veterinary Medicine and Animal Science of the Federal University of Bahia (Protocol number: 02/2019).

2.2 Animals and dietary handling

Six rumen-cannulated, non-castrated Santa Ines sheep, aged 9 to 15 months, with an average initial body weight (BW) of 46 ± 3 kg, were used. The animals were housed in covered individual pens of 1.0 m^2 (1.0 × 1.0m) with suspended floor made with wood slats, equipped with individual drinkers and feeders. Water and feed were supplied ad libitum. The animals were fed with a basal diet composed by 800 g/kg DM of sorghum silage as the forage source. The concentrate comprised 220 g/kg ground corn, 630 g/kg soybean meal, 100 g/kg corn germ, and 50 g/kg mineral mixture, on DM basis (Table 1).

Diets were offered daily twice daily (8:30 and 15:30 h), and were divided equally into two meals, to allow between 10 to 15% of

TABLE 1 Chemical composition of sorghum silage, concentrate and the mixed diet used to feed the sheep during the *in situ* incubation periods.

Nutritional fractions (g/kg DM)	Sorghum silage	Concentrate[5]	Basal diet[6]
Dry matter[1]	321	897	436
Organic matter	942	951	943
Crude protein	96	304	138
Ether extract	31	72	39
apNDF[2]	494	122	419
Non-fibrous carbohydrates[3]	320	453	348
iNDF[4]	259	24	212
Lignin	53	25	47

[1]g/kg fresh feed.
[2]Neutral detergent fiber corrected for ash and protein.
[3]Non-fibrous carbohydrates = Organic matter - (apNDF – crude protein – Ether extract).
[4] Indigestible neutral detergent fiber.
[5]Concentrate composed by ground corn, soybean meal, corn germ, urea, ammonium sulfate and mineral mixture, on DM basis. The warranty levels of mineral mixture were: 175 g/kg of sodium, 140 g/kg of calcium, 60 g/kg of phosphorus, 13 g/kg of sulfur, 3 g/kg of zinc, 1.5 g/kg of iron, 2 g/kg of manganese, 0.05 g/kg of molybdenum, 0.06 g/kg of iodine, 0.02 g/kg of cobalt, and 0.01 g/kg of selenium.
[6]Basal diet composed by 800 g sorghum silage and 200 g Concentrate per kg of dietary DM.

refusals. Daily, before offering the morning diet, the refusals were collected and weighed to proceed the feed adjustment.

2.3 Preparation of bags and samples

Bags from two types of fabric were used for the rumen incubations: Ankom F57 (Ankom Technology, Macedon, NY) and non-woven textile (NWT). The F57 bags were purchased directly from the manufacturer and the NWT (100 g/m^2) fabric was cut a (5 × 5 cm dimension. Both bags are made of synthetic fiber, which are stable in both acid and neutral mediums, with irregular geometrical fiber disposition (Casali et al., 2009).

Before receiving the samples, the NWT and F57 bags were prepared according to the Brazilian National Institute of Science and Technology in Animal Science (Detmann et al., 2021). Bags were washed in neutral detergent solution at 100°C for 15 minutes, then rinsed in hot water, due to the presence of small particles adhered to the original fabric (method number F-002/2). Later, to remove any residue, all bags were immersed in acetone and finally in distilled water. After washing, the bags were dried in an air-circulation oven at 55°C for 24 hours. Then, all the bags, F57 and NWT, were oven-dried at 105°C for 2 hours (method number G-003/1). After obtaining the bag's weight, the samples were added to suit the proportion of 20 mg of sample/cm^2 of surface (Nocek, 1988), resulting in approximately 500 mg of total sample. All the bags were thermally sealed after weighing and forwarded to the incubation.

The testing samples were 2 types of feeds (sorghum silage and ground corn grain) and 1 sample of feces from sheep fed with 50:50 forage concentrate ratio (Table 2). The sorghum silage and feces samples were dried in an air-circulation oven (55°C for 72 hours), and ground in a Wiley mill (Willey TE-650/1, Tecnal, São Paulo, Brazil). Samples were ground with a 2-mm mesh sieve for the *in situ* incubation (Valente et al., 2015; Reis et al., 2017) and with 1-mm to proceed laboratory analysis.

TABLE 2 Chemical composition of the testing samples added in bags of different fabric types to estimate indigestible neutral detergent fiber (iNDF) by *in situ* incubation procedure in the rumen of sheep.

Nutritional fractions (g/kg DM)	Sorghum silage	Ground corn	Sheep feces
Dry matter[1]	280	896	308
Organic matter	939	984	883
Crude Protein	107	84	192
Ether Extract	31	34	18
apNDF[2]	480	63	545
Non-fibrous carbohydrates[3]	321	803	128
Neutral detergent fiber	526	117	617
Acid detergent fiber	274	59	311
Lignin	54	19	74

[1]g/kg of fresh feed.
[2]Neutral detergent fiber corrected for ash and protein.
[3]Non-fibrous carbohydrates = Organic matter - (apNDF – crude protein – Ether extract).

2.4 Experimental design and incubation procedure

The experimental period lasted 94 days, with 10 days being designated to adaptation to diet, daily handling and facilities, and the remaining 84 days being used to perform the 6 experimental periods, which lasted 14 days each. For the incubation procedure, a 6 × 6 Latin square design was used (Figure 1). In each of the six periods, a round of incubation was performed on the animals, using 336 hours (14 days) as time limit to estimate the iNDF of the samples (Reis et al., 2017).

Bags from each fabric type were incubated in a total number of 4; 8 and 12 units per sample (Sorghum silage, ground corn and feces), totaling 12; 24 and 36 bags, per animal per period, respectively (Figure 1) for each fabric. Thus, six treatments were evaluated in each period of experimental design: 1) 12 units of NWT bags, 2) 24 units of NWT bags, 3) 36 units of NWT bags, 4) 12 units of F57 bags, 5) 24 units of F57 bags, 6) 36 units of F57 bags. These treatments were evaluated in six sheep using a Latin square design, in which each incubation procedure was repeated 6 times (periods). Thus, a total of 144 bags were *in situ* incubated per period. At the end of the experiment, a total of 864 bags were evaluated, with 144 bags x 6 periods.

Bags were tied together with a metallic chain equipped with a solid metallic weight with approximately 500g at its end, thus allowing the total immersion of the bags with samples in the ruminal content. The incubation time used to estimate the iNDF of the samples was 336 hours (Reis et al., 2017). After removing, all bags were washed in running water, until the water was completely cleared. After cleaning, bags were placed in the air-circulation oven with 55°C, where they were kept for 72 hours. Then, the bags were packed in plastic boxes, for further fiber analysis.

2.5 Chemical analysis

The feed and fecal samples were analyzed according to the protocols described by the Brazilian National Institute of Science and Technology in Animal Science (INCT-CA; Detmann et al., 2021). The following method numbers were used: DM (method G-003/1), ash (method M-001/2), ether extract (EE) (method G-005/2), neutral detergent fiber (NDF) (method F-002/2), acid detergent fiber (ADF) (method F-004/2), and lignin (method F-005/2). The total N content in samples of supplied ingredients, refusals, and feces were measured by the Kjeldahl method, and crude protein (CP) was calculated as N × 6.25 (INCT-CA; method N-001/2, Detmann et al., 2021). The neutral detergent fiber corrected for ash and protein (apNDF) was obtained after correction of the residues for the ash content (Mertens, 2002) and residual N (Licitra et al., 1996). The content of non-fibrous carbohydrates (NFC) was calculated according to Hall (2000): NFC =100 - (apNDF - CP - EE - ash).

The bags with residual sample after the incubation procedure were analyzed for NDF to quantify the iNDF, without the use of thermostable α-amylase and without the addition of sodium sulfite. After the washing and drying procedure, the bags were directed to the fiber analyzer (Ankom, 200 Fiber Analyzer, Ankom Technology), where they were boiled in neutral detergent solution at 100°C during 1h. Subsequently, the bags were dried in the air-circulation oven (55°C for 72 h), and then oven-dried at 105°C for 1 h. Afterward, they were stored in a desiccator in a proportion of (20 bags/desiccator) and weighed. The quantification of the indigestible fraction (iNDF) of each was measured gravimetrically.

2.6 Statistical analysis

The experimental design was 6 × 6 Latin square design, and statistical procedures were carried out using the SAS software (version 9.4). The data obtained were analyzed by the PROC MIXED (Statistical Analysis System - SAS Institute Inc., Cary, NC, USA) using the following statistical model:

$$Y_{ijkl} = \mu + N_i + F_j + (NF)_{ij} + a_k + p_l + e_{ijkl}$$

Where: μ = overall mean; N_i = fixed effect of the number of bags i; T_j = fixed effect of fabric type j; $(NF)_{ij}$ = fixed effect of the interaction between number and type of fabric, a_k = random effect of animal k; p_l = random effect of experimental period l; e_{ijkl} = random error between experimental units. The effects of the treatments were compared through analysis of variance adopting a significance level of 5%.

3 Results

Fabric types did not promote different estimates (P>0.05) on the iNDF content, regardless of the sample evaluated (Table 3). The iNDF in sorghum silage, ground corn and sheep feces (P>0.05) were similar when incubated in F57 or NWT bags, as well as for the number of 12, 24 or 36 bags placed inside rumen (P>0.05). In addition, the present study showed that the estimates of weighted standard-deviations for sorghum silage and ground corn obtained from 36 F57 bags incubated simultaneously were the smallest (Figure 2). Feces from sheep presented the smallest weighed standard deviation when 24 bags were also incubated in F57 bags (Figure 2). The weighed standard-deviations for ground corn were superior from the other ingredients in all treatments evaluated.

4 Discussion

In the current study, it was observed that both F57 and NWT bags could be incubated in the rumen of sheep to estimate the iNDF of feed and fecal samples. This result agreed

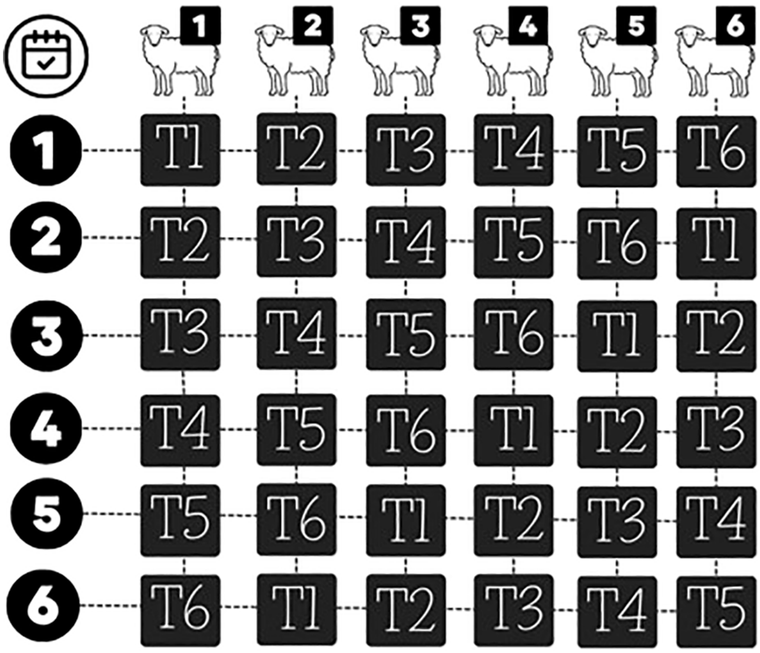

FIGURE 1
Schematic representation of the incubation procedure of three samples (sorghum silage, ground corn feces sheep) evaluated into six treatments (T) to estimate indigestible neutral detergent fiber (iNDF) in a Latin Square. Two types of fabric, Ankom F57 and non-woven textile (NWT), and three different number of bags were used for the rumen *in situ* incubations, as follow: T1 = 12 NWT bags (4 for Sorghum silage, for 4 ground corn and 4 for feces); T2 = 24 NWT bags (8 for Sorghum silage, for 8 ground corn and 8 for feces); T3 = 36 NWT bags (12 for Sorghum silage, for 12 ground corn and 12 for feces); T4 = 12 F57 bags (4 for Sorghum silage, for 4 ground corn and 4 for feces); T5 = 24 F57 bags (8 for Sorghum silage, for 8 ground corn and 8 for feces); T6 = 36 F57 bags (12 for Sorghum silage, for 12 ground corn and 12 for feces), totaling 144 bags per period and 864 bags in the complete experiment.

TABLE 3 Estimates of the indigestible neutral detergent fiber (iNDF) obtained from *in situ* incubation procedure in the rumen of sheep with different number of bags and fabric types.

Sample	Fabric type		Number of bags			SEM[2]	P-Value[3]		
	F57	NWT[1]	12	24	36		F	N	F × N
	iNDF[4] (g/kg DM)								
Sorghum silage	26.89	26.54	26.27	26.80	27.08	1.48	0.68	0.74	0.16
Ground corn	2.45	2.47	2.10	2.56	2.72	0.24	0.97	0.27	0.48
Sheep feces	41.52	41.37	41.01	42.55	40.78	1.31	0.91	0.51	0.57

[1]NWT, non-woven textile.
[2]SEM, standard-error of the mean.
[3]F × N, interaction between the factors: fabric type (F) and number of bags (N).
[4]iNDF, indigestible neutral detergent fiber (g/kg of dry matter).

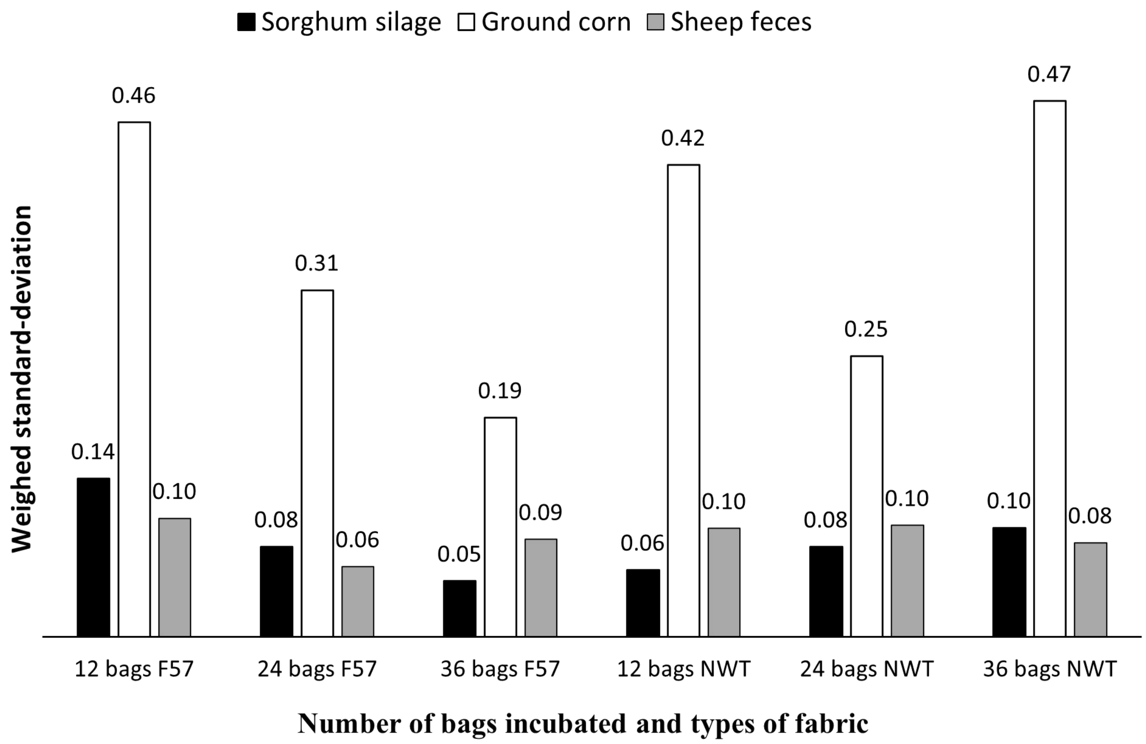

FIGURE 2
Weighed standard-deviation of indigestible neutral detergent fiber (iNDF) obtained from *in situ* incubation procedure in the rumen of sheep with different number of bags and fabric types.

with Valente et al. (2011a), who also reported that F57 can be replaced by NWT for the *in situ* procedures to estimate iNDF. According to these authors this result was based on the premise that these fabrics present similar physical structure and both F57 and NWT are produced by deposition of synthetic fiber without weaving.

Casali et al. (2009) evaluated these fabric types in cattle incubation and observed similar results for corn silage samples and other ingredients used in concentrates as wheat meal and soybean hulls. Pore size and texture of the fabric used to make the bags for *in situ* incubations can influence the turnover of the material inside the bags and their interaction with the rumen content (Kitessa et al., 1999; Valente et al., 2015). For Valente et al. (2011b) the structural surfaces of NWT and F57 are similar. These two types of fabrics present an irregular disposition of their textile fibers, resulting in similar porosities. Thus, Valente et al. (2011a) states that these types of fabrics promote similar input of microorganisms without particles loss and increasing degradation extension. However, the authors suggested that further studies are needed to accurately quantify the surface area available for microbial passage.

In general, NWT and F57 tissues showed similar behavior for the average iNDF for all samples. However, the weighted standard-deviation values were reduced when 36 bags were incubated simultaneously with F57 fabric. This treatment could be used when more accuracy is required in some situations. The weighed standard-deviation values were higher for ground corn when compared to the other samples. This can be explained by the reduced content of iNDF that remained inside the bag after 336 hours. Possibly, this reduced content limited the representation of the iNDF content and increasing the variation in the estimates. Besides, due to the similarity of the results obtained when comparing NWT and F57, we can infer that *in situ* incubation in sheep can be conducted with both fabric types and up to 36 bags. More studies are needed to evaluate the effect of a number bigger than 36 in sheep.

The total number of 36 bags incubated simultaneously in the rumen of sheep had no effect on the estimation of iNDF, regardless of the fabric type of bag. Chen et al. (2011) reported that there are few studies comparing the density of bags simultaneously incubated in the rumen. Valente et al. (2011a) reported that in long periods of *in situ* incubation, bags can lose resistance, leading to sample losses and errors in the iNDF estimates. Reis et al. (2017) stated that variations between results may be related to differences inherent in the particle size of the feed and the bags used in the incubation procedure. It would be expected that the increase in the number of bags incubated simultaneously in the rumen would influence the estimates of iNDF of the samples, however this phenomenon was not showed up to 36 bags after 336h.

In theory, it was expected that animals that underwent *in situ* incubations with the highest number of bags would generate overestimated estimates for iNDF. This effect would be related to the higher volume of bags, which in theory could impair the influx of fluids into the bags and, therefore, impairing the degradation of the samples by microorganisms. However, in this study, the similarity of the iNDF estimates regarding the number of bags incubated simultaneously in sheep may be associated with the length of time the bags remained incubated, which was probably enough to promote the target effective degradation.

5 Conclusion

The NWT and F57 bags were similar in the estimation iNDF from *in situ* rumen incubation procedure in sheep. The NWT bag can be an option to reduce costs, without compromising the iNDF estimates. The total number of 36 bags containing feeds or feces samples could be incubated simultaneously in the rumen of sheep without negative impact on estimating iNDF, regardless of the fabric type. If more accurate data is necessary, feces can be incubated up to 24 bags, but the incubation of 36 bags did not compromise the iNDF estimate. Further evaluations are recommended regarding a bigger number of incubated bags, as there are few studies evaluating *in situ* incubation procedures in sheep, especially concerning the iNDF estimation.

Author's note

This manuscript is part of the first author's Ph.D. thesis (A. C. S. Dos Santos). The thesis is entitled "Nutritional energy and protein requirements, improvement of *in situ* incubation methods in goats and sheep" and was a requirement for obtaining the Ph.D. degree in the Postgraduate Program in Animal Science at Universidade Federal da Bahia – Brazil.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: https://data.mendeley.com/datasets/3t2p7cjz2w/1.

Ethics statement

The animal study was approved by Ethics Committee for Animal Handling of the School of Veterinary Medicine and Animal Science of the Federal University of Bahia (Protocol number: 02/2019). The study was conducted in accordance with the local legislation and institutional requirements.

Author contributions

Ad: Data curation, Investigation, Methodology, Supervision, Validation, Writing – original draft. GdS: Methodology, Project administration, Resources, Writing – review & editing. Md: Supervision, Writing – review & editing, Methodology. LM: Conceptualization, Supervision, Validation, Visualization, Writing – review & editing. HA: Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. MT: Resources, Visualization, Writing – review & editing. EP: Funding acquisition, Visualization, Writing – review & editing. DP: Conceptualization, Data curation, Validation, Writing – review & editing. Jd: Conceptualization, Funding acquisition, Visualization, Writing – review & editing. GdC: Conceptualization, Data curation, Funding acquisition, Investigation, Validation, Visualization, Writing – review & editing. SS: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing.

Funding

The author(s) declare that financial support was received for the research, authorship, and/or publication of this article. The authors acknowledge the Foundation for research support of Bahia State – Brazil (Fundação de Amparo à Pesquisa do Estado da Bahia –

FAPESB), Brazilian National Council of Technological and Scientific Development (Conselho Nacional de Desenvolvimento Científico e Tecnológico- CNPq), Institutos Nacionais de Ciência e Tecnologia (INCT—Ciência Animal), and the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) for the financial support and scholarship.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Generative AI statement

The author(s) declare that no Generative AI was used in the creation of this manuscript.

Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

References

Adams, J. M., Norris, A. B., Batista, L. F., Rivera, M. E., and Tedeschi, L. O. (2020). Comparison of in *situ* techniques to evaluate the recovery of indigestible components and the accuracy of digestibility estimates. *J. Anim. Sci.* 98, 1–7. doi: 10.1093/jas/skaa296

Casali, A. O., Detmann, E., Valadares Filho, S. C., Pereira, J. C., Henrique, L. T., Freitas, S. G. D., et al. (2008). Influence of incubation time and particles size on indigestible compounds contents in cattle feeds and feces obtained by in *situ* procedures. *Rev. Bras. Zootec.* 37, 335–342. doi: 10.1590/S1516-35982008000200021

Casali, A. O., Detmann, E., Valadares Filho, S. C., Perreira, J. C., Cunha, M., Detmann, K. S. C., et al. (2009). Estimação de teores de componentes fibrosos em alimentos para ruminantes em sacos de diferentes tecidos. *Rev. Bras. Zootec.* 38, 130–138. doi: 10.1590/S1516-35982009000100017

Chen, Y., Penner, G. B., Li, M., Oba, M., and Guan, L. L. (2011). Changes in bacterial diversity associated with epithelial tissue in the beef cow rumen during the transition to a high-grain diet. *Appl. Environ. Microb.* 77, 5770–5781. doi: 10.1093/jas/skaa296

Coblentz, W. K., Akins, M. S., Ogden, R. K., Bauman, L. M., and Stammer, A. J. (2019). Effects of sample size on neutral detergent fiber digestibility of triticale forages using the Ankom DaisyII Incubator system. *J. Dairy Sci.* 102, 6987–6999. doi: 10.3168/jds.2019-16681

Defeo, M. E., Shampoe, K. V., Carvalho, P. H., Silva, F. A., and Felix, T. L. (2020). *In vitro* and in *situ* techniques yield different estimates of ruminal disappearance of barley. *Transl. Anim. Sci.* 4, 141–148. doi: 10.1093/tas/txz170

Detmann, E., Costa e Silva, L. F., Rocha, G. C., Palma, M. N. N., and Rodrigues, J. P. P. (2021). *Métodos para análise de alimentos* (Visconde do Rio Branco: Suprema), 214.

Hall, M. B. (2000). *Neutral detergent soluble carbohydrates nutritional relevance and analysis: A laboratory manual* (FL: Institute of Food and Agricultural Science, University of Florida).

Kitessa, S., Irish, G. G., and Flinn, P. C. (1999). Comparison of methods used to predict the *in vivo* digestibility of feeds in ruminants. *Aust. J. Agric. Res.* 50, 825–842. doi: 10.1071/AR98169

Licitra, G., Hernandez, T. M., and Van Soest, P. J. (1996). Standardization of procedures for nitrogen fractionation of ruminant feeds. *Anim. Feed Sci. Technol.* 57, 347–358. doi: 10.1016/0377-8401(95)00837-3

Lindberg, J. E. (1985). Estimation of rumen degradability of feed proteins with the in sacco technique and various *in vitro* methods: a review. *Acta Agric. Scand.* 25, 65–97.

Madsen, J., and Hvelplund, T. (1994). Prediction of in *situ* protein degradability in the rumen. Results of a European ringtest. *Livest. Prod. Sci.* 39, 201–212. doi: 10.1016/0301-6226(94)90185-6

Mertens, D. R. (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. doi: 10.1093/jaoac/85.6.1217

Nocek, J. (1988). *In situ* and other methods to estimate ruminal protein and energy digestibility: a review. *J. Dairy Sci.* 71, 2051–2069. doi: 10.3168/jds.S0022-0302(88)79781-7

Ørskov, E. R., Hovell, F. D., and Mould, F. T. A. P. (1980). The use of the nylon bag technique for the evaluation of feedstuffs. *Trop. Anim. Prod.* 5, 195–213.

Reis, M. J., Santos, S. A., Prates, L. L., Detmann, E., Carvalho, G. G. P., Santos, A. C. S., et al. (2017). Comparing sheep and cattle to quantify internal markers in tropical feeds using in *situ* ruminal incubation. *Anim. Feed Sci. Technol.* 232, 139–147. doi: 10.1016/j.anifeedsci.2017.08.013

Tassone, S., Fortina, R., and Peiretti, P. G. (2020). *In Vitro* techniques using the DaisyII incubator for the assessment of digestibility: A review. *Animals* 10, 1–24. doi: 10.3390/ani10050775

Valente, T. N. P., Detmann, E., Queiroz, A. C. D., Valadares Filho, S. D. C., Gomes, D. I., and Figueiras, J. F. (2011a). Evaluation of ruminal degradation profiles of forages using bags made from different textiles. *Rev. Bras. Zootec.* 40, 2565–2573. doi: 10.1590/S1516-35982011001100039

Valente, T. N. P., Detmann, E., and Sampaio, C. B. (2015). Review: recent advances in evaluation of bags made from different textiles used in *situ* ruminal degradation. *Can. J. Anim. Sci.* 95, 493–498. doi: 10.4141/cjas-2015-100

Valente, T. N. P., Detmann, E., Valadares Filho, S. C., Cunha, M., Queiroz, A. C., and Sampaio, C. B. (2011b). *In situ* estimation of indigestible compounds contents in cattle feed and feces using bags made from different textiles. *Rev. Bras. Zootec.* 40, 666–675. doi: 10.1590/S1516-35982011000300027

Van der Koelen, C. J., Goedhart, P. W., Van Vuuren, A. M., and Savoini, G. (1992). Sources of variation of the in *situ* nylon bag technique. *Anim. Feed Sci. Technol.* 38, 35–42. doi: 10.1016/0377-8401(92)90074-G

Vanzant, E. S., Cochran, R. C., and Titgemeyer, E. C. (1998). Standardization of in *situ* techniques for ruminant feedstuff evaluation. *J. Anim. Sci.* 76, 2717–2729. doi: 10.2527/1998.76102717x

Wang, W. K., Wang, Y. L., Li, W. J., Wu, Q. C., Yang, K. L., Li, S. L., et al. (2021). *In situ* rumen degradation characteristics and bacterial colonization of whole cottonseed, cottonseed hull and cottonseed meal with different gossypol content. *AMB Expr.* 11, 1–11. doi: 10.1186/s13568-021-01244-2