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# Comparing sheep and cattle to quantify internal markers in tropical feeds using in situ ruminal incubation

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A R T I C L E I N F O

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A B S T R A C T

The main focus of this work was to verify the hypothesis that there are differences between cattle and sheep to obtain internal markers in tropical forages using in situ ruminal incubation. A presupposition that the indigestible fraction is exclusively inherent to feed was considered, and its ruminal incubation in different animal species should not change the estimate of this fraction; but should change the minimum time required to obtain this value, or the critical-time (Tc). The Tc to obtain indigestible fractions were compared between species for neutral detergent fiber (INDF) and acid detergent fiber (IADF) in feeds and feces. A total of 16 samples were divided into two groups of forages; one group of concentrates and another one of feces. These samples were placed inside bags and incubated in the rumens of 4 sheep and 4 cattle, at following time-points: 0, 12, 24, 48, 96, 144, 192, 240, 288, and 336 h, using two 4 × 4 Latin squares. There was no effect on the species with regard to the degradation rate (kd) of both the aNDF and ADF in alfalfa hay ($P = 0.36$; $P = 0.14$). All other forages, which were tropical types, were affected by animal specie ($P < 0.05$). Cattle was associated with lesser Tc when compared to sheep, both for INDF and IADF. All concentrate feeds were affected by the species ($P < 0.05$), with sheep providing greater Tc for both undegradable fractions. Feces from cattle and sheep fed with low concentrate required higher Tc when incubated in sheep ($P < 0.05$), while feces from cattle and sheep fed with low concentrate required the same Tc to obtain IADF in cattle or sheep ($P = 0.19$; $P = 0.11$). Sheep is not a practical recommendation to obtain internal markers based on in situ trials, due to the high incubation time length to obtain the non-degraded fraction of feeds and feces. Internal markers IDNF and IADF from sheep trials can be obtained from 216 h of in situ incubation in cattle.

## 1. Introduction

The ruminant species suitable for in situ incubation of feeds and fecal samples obtained in digestibility trials evokes divergent opinions among researchers. Internal markers obtained with in situ degradation assays in the bovine rumen, count with the

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advantage of a larger number of bags incubated per unit of time due to the higher ruminal volume (Bulo et al., 1992); additionally, well-defined protocols on the method and incubation period for indigestible feed fractions are already available for the bovine species (Valente et al., 2015). In theory, the undegradable content is inherent on feeds (Ørskov, 2000), regardless of the species that will receive it. However, it is known that the ruminant species may experience interference in the rate of degradation (Huntington and Givens, 1997).

Once the undegraded residue of in situ ruminal incubation shows asymptotic value, or, the undegraded fraction can only be truly obtained in procedures considering mathematical functions that whose result tend to infinity (Detmann et al., 2007), ruminal incubation time applied in sheep and cattle could differ, and, consequently different degradation rates (kd) could be verified. Thus, the minimum time required to reach the asymptote could diverge between ruminant species, which may affect the in situ methods to obtain indigestible fractions. However, in the absence of valid protocols, doubts have been raised regarding possible differences in the microbial population that lead to differences in sample exposure time to estimate the asymptotic values.

Authors such as Cochran et al. (1986) and Valente et al. (2011) evaluated ruminal in situ incubation of temperate and tropical climate forages, and they recommended incubation times of 144 and 288 h to estimate indigestible fibrous fractions, respectively. However, these trials were performed exclusively with cattle. Thus, it is necessary to investigate and recommend protocols to obtain indigestible fibrous fractions, which also could be used as internal markers for digestibility trials with sheep. Additionally, it was hypothesized that sheep fecal samples from digestibility trials probably can be incubated in the bovine rumen, which would represent optimization of the time spent in incubating process.

Thus, the objective of this work was to verify the hypothesis that both cattle and sheep can be used to obtain internal markers in tropical forages using in situ ruminal incubation, as well as to compare the critical times required to obtain indigestible fibrous fractions of forages, concentrates, and feces from cattle and sheep digestibility trials.

## 2. Material and methods

### 2.1. Animals, local and diet

All procedures with the animals were approved by the Committee on Ethics in the Use of Animals of the School of Veterinary Medicine and Animal Sciences of the Federal University of Bahia, with protocol number 22/2015. The experiment was conducted at the Experimental Farm of the same institution, located in the municipality of São Gonçalo dos Campos, BA. The chemical analyses were performed at the Laboratory of Animal Nutrition.

In situ degradation kinetics of neutral detergent fiber (aNDF) and acid detergent fiber (ADF) were evaluated in four castrated crossbred cattle, male, with a mean body weight (BW) of 400 ± 25 kg and four uncastrated crossbred sheep, male, with a mean BW of 45 kg ± 3 kg, all cannulated in the rumen. The animals were kept in individual stalls, equipped with feeders and drinkers with free access to water. Sheep were dairy handled in the individual pens where they were kept during the incubation procedures, and cattle were contained using cages.

All animals were fed with sorghum silage and concentrate at an 80:20 proportion based on dry matter (DM), following the recommendation of Huntington and Givens (1997). The same diet with 120 g of crude protein (CP) per kg of DM was used for both species. The concentrate was composed of 379 g/kg ground corn; 586 g/kg of soybean meal and 34 g/kg of commercial mineral mixture for growing sheep or cattle, depending on the species to be supplemented, based on dietary DM. Diets were provided at 9:00 a.m. and 4:00 p.m. in similar proportions. Total DM intake has been adjusted to keep leftovers at a maximum of 5–10% (Moya et al., 2011) of the quantity supplied for cattle and 10–15% (Bickell et al., 2014) of the quantity offered for sheep, in order to allow *ad libitum* intake.

### 2.2. Forages, concentrates and feces samples evaluated

A total of 12 feeds samples were evaluated (Table 1): Brachiaria grass (*Braquiária Brizanta* Stapf), Sugarcane (*Saccharum officinarum*), Elephant grass (*Pennisetum purpureum*), Tifton-85 hay (*Cynodon* spp.), Alfalfa hay, soybean meal, wheat bran, ground corn, cottonseed cake, millet silage (*Pennisetum glaucum*), corn silage, and sorghum silage, and four feces samples (Table 1): feces from cattle fed with high concentrate (cattle-HC), feces from cattle fed with low concentrate (cattle-LC), feces from sheep fed with high concentrate (sheep-HC), feces from sheep fed with low concentrate (sheep-LC). Fecal samples were obtained from two different experimental digestibility trials, one for each species, with different animals of the present study. The LC diet fed to cattle was composed of 70:30 fresh sugarcane and concentrate, while the HC diet consisted of 40:60 fresh sugarcane and concentrate, on DM basis. The LC diet fed to sheep was composed of 60:40 Tifton-85 hay and concentrate, while the HC diet consisted of 100:0 concentrate.

Feed samples were subjected to partial drying in a forced ventilation oven (55° C) for 72 h. After drying, the samples were ground in a Willey mill (Willey TE-650/1, TECNAL, São Paulo, Brazil) at 2 mm for in situ incubation (Valente et al., 2015) and at 1 mm for subsequent laboratory analysis. The samples of feeds and feces were analyzed for DM and mineral matter (MM) according to official methods 934.01, 105 °C for 16 h and 942.05, 600 °C for 4 h (AOAC, 2005), respectively. The total N was quantified using a three step micro Kjeldhal analyses (sulphuric acid digestion, basic distillation and chloride acid titration), according to the official method 968.06 (AOAC, 2005), and this value was multiplied by 6.25 to obtain the crude protein (CP).

The aNDF content in feeds and feces were evaluated by placing the samples in 100 ml autoclavable flasks, following the proportion of 1 g of sample per 100 ml of detergent (Mertens, 2002) with thermostable α-amylase (Ankom Technology, Tecnoglobo

**Table 1**
Chemical composition[a] of feeds and feces used on in situ rumen incubations in sheep and cattle.

| Items | DM | OM | CP | aNDF | ADF | Lignin |
|---|---|---|---|---|---|---|
| Brachiaria grass[b] | 253 | 937 | 94 | 666 | 292 | 31 |
| Elephant grass[b] | 215 | 942 | 152 | 676 | 283 | 20 |
| Tifton-85 hay | 822 | 932 | 121 | 716 | 320 | 50 |
| Sugarcane | 317 | 976 | 29 | 373 | 219 | 41 |
| Corn silage | 331 | 966 | 69 | 522 | 235 | 36 |
| Sorghum silage | 271 | 957 | 99 | 613 | 315 | 65 |
| Millet silage | 255 | 926 | 91 | 638 | 391 | 100 |
| Alfalfa hay | 884 | 920 | 162 | 495 | 320 | 91 |
| Soybean meal | 893 | 936 | 503 | 90 | 68 | 2 |
| Wheat bran | 897 | 946 | 254 | 353 | 116 | 39 |
| Ground corn | 911 | 985 | 109 | 93 | 08 | 1 |
| Cottonseed cake | 918 | 960 | 291 | 527 | 273 | 70 |
| Sheep feces HC[c] | 386 | 906 | 205 | 327 | 170 | 56 |
| Sheep feces LC[d] | 411 | 900 | 144 | 528 | 276 | 73 |
| Cattle feces HC[e] | 324 | 932 | 141 | 266 | 127 | 37 |
| Cattle feces LC[f] | 324 | 882 | 145 | 386 | 235 | 55 |

[a] DM = dry matter (g/kg fresh sample); OM = organic matter (g/kg DM), CP = crude protein (g/kg DM); aNDF = Neutral detergent fiber assayed with thermostable amylase (g/kg DM); ADF = Acid detergent fiber (g/kg DM).
[b] Grasses harvested at 60 days of regrowth.
[c] HC: high concentrate (0:100).
[d] LC: low concentrate (50:50).
[e] HC: high concentrate (30:70).
[f] LC: low concentrate (60:40).

Equipamentos, Curitiba, Brazil) and without the addition of sodium sulphite (aNDF). The samples were autoclaved at 110 °C for 60 min in 4.3–5.7 psi (Barbosa et al., 2015). The ADF was sequentially analyzed following the same procedures for aNDF, but without the addition of thermostable α-amylase. Washing and filtration procedures for aNDF and ADF followed the protocol described by Barbosa et al. (2015). Lignin(sa) was measured according to the official method 973.18 (AOAC, 2005) using 72% sulfuric acid.

### *2.3. Experimental design and in situ incubation procedures*

The samples of feeds and feces were divided into two groups of forages; one a group of concentrates and another one of feces of cattle and sheep previously fed with different diets. The first group (G1) comprised the forages: Brachiaria grass, Sugarcane, Elephant grass and Tifton-85 hay; the second group (G2) also comprised forages: Alfalfa hay, Millet silage, Corn silage and Sorghum silage; the third group (G3) comprised only concentrate ingredients: ground corn, Soybean meal, Wheat bran and cottonseed cake; in the fourth group (G4) only fecal samples were allocated: feces cattle-HC, cattle-LC, sheep-HC and sheep-LC.

The experiment lasted 66 days, comprising 10 days of adaptation to the facilities and diets, and four incubation periods of 14 days each. The incubation procedure was performed using two 4 × 4 Latin squares, one for each species. Thus, in each incubation period, each group of sample (G1, G2, G3 and G4) was incubated in each animal of each species, and therefore, the same feed was incubated four times in each animal until the end of the procedure in both species (Fig. 1). Latin square design was only used to assist and organize the information obtained in the field, allowing measurements of different feeds without the confounding effect on the animal, which is common in such experiments. Thus, this design was not a tool to guide statistical evaluations, i.e., not to estimate variability. The objective was to control the sources of variation and avoid bias.

Non-woven textile (NWT) bags (Valente et al., 2015) with a dimension of 4 × 5 cm were used to pack the samples, with 50 µm pore size. The NWT bags were washed in neutral detergent solution (Mertens, 2002) at 100 °C for 15 min, and then washed in hot water and acetone (Detmann et al., 2012). The bags were subjected to partial drying in a forced ventilation oven (55 °C) for 72 h.

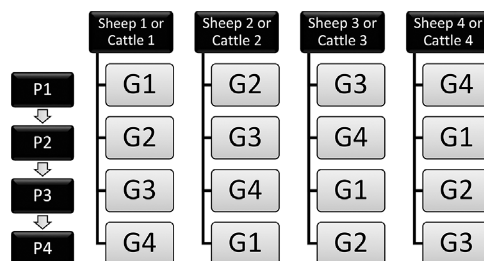


**Fig. 1.** Schematic presentation of incubation procedure with 12 feeds and 4 feces samples divided into 4 different groups. Each one of the 16 samples was incubated 4 times in the rumen of a cattle and 4 times in sheep, totalizing two 4 × 4 Latin square design. Each of the 32 experimental units received a pool of 40 bags, which were ruminally incubated over time, with exception for 0-time.

Subsequently, the bags were oven-dried at 105 °C for 1 h (method 934.01; AOAC, 2005). After obtaining the bags' weight, the feeds and fecal samples were weighed inside the bags in the proportion of 20 mg of sample per $cm^2$ of surface, and then the bags were sealed. Incubation times were 0, 12, 24, 48, 96, 144, 192, 240, 288, and 336 h. In the present study, considering the limited capacity of the sheep rumen, schedules 2, 4 and 6 h, although important in the investigation of latency times, were not evaluated, as it was decided to extend the time of the investigation to reach the asymptotic values for the degradation curves.

The proposed scheme allowed the evaluation of 40 bags per animal per period, comprising 10 evaluation times for each of the four groups of feeds or feces. A total of 36 of the 40 bags were incubated in reverse order of the time-points in the rumen of each ovine or bovine, so as to be withdrawn simultaneously at the end of each period. The bags were attached to a metal chain with a heavy end to allow for the total immersion of the samples in the rumen fluid. The remaining 4 bags of each group at time 0 were not submitted to ruminal incubation. These bags were washed out only under tap water. Thus, a total of 320 bags were evaluated per incubation period (8 animals × 40 bags), and at the end of the trial, 1280 bags (320 bags × 4 periods) were evaluated in total.

After removal of the rumen, all bags (including time-0 bags) were washed with tap water to remove any remnant soluble material. The bags were manually washed up to total whitening. The bags were then subjected to partial drying at 55 °C for 72 h for subsequent fiber analysis. Later, the bags were treated with a neutral detergent, following the proportion of 1 g of sample per 100 ml of detergent (Mertens, 2002) for 1 h in an autoclave at 110 °C for 60 min in 4.3–5.7 psi (Barbosa et al., 2015), and washed in boiling water and acetone. Thereafter, they were dried in a forced-air oven at 55 °C for 72 h, and then in a non-ventilated oven at 105 °C for 45 min, stored in a desiccator (20 bags/desiccator) and weighed for quantification of the non-degraded aNDF. The procedure described for quantification of the non-degraded aNDF was again performed, while replacing the neutral detergent with an acid detergent in order to estimate the non-degraded ADF.

### 2.4. Statistical analysis

The aNDF and ADF residue profiles were studied using the asymptotic model of Mertens and Loften (1980), which was used to compare the degradation rates (kd) obtained in the ruminal incubation with cattle and sheep. The following model (1) was used to estimate parameters of the degradation curves:

$$Rt = D1 \times ((B \times e^{-kd1 \times t}) + \mathrm{I}) + D2 \times ((B \times e^{-kd2 \times t}) + I)$$

Where: Rt = non-degraded residue of aNDF or ADF (%) at incubation time t; D1 and D2 are dummy variables corresponding to sheep and cattle species: D1 = 0 and D2 = 1 corresponding to the degradation residues obtained in cattle, and D1 = 1 and D2 = 0 corresponding to the degradation residues obtained in sheep; B = potentially degradable insoluble fraction of aNDF or ADF (%); I = undegradable fraction (%), which represents the contents of INDF or IADF; kd1 and kd2 correspond to the ruminal degradation rates of B fraction of feeds or feces incubated in sheep (kd1) or cattle (kd2) species; T = residence time in the rumen.

The model was adapted in order to allow the comparison between the degradation rates obtained in the two species. The parameter I was used to estimate the internal markers, as well as the fraction B, that were not decomposed by the species. This proposed model assumed that the non-degradable content of a feed is in itself an inherent characteristic, regardless of the species that will receive it for in situ incubation (Detmann et al., 2008). Thus, the information sought was the incubation time required in each species to estimate the parameter I.

Statistical comparisons were made by the model identity test (Regazzi, 2003). In this case, two different adjustments were performed for each sample. In the first adjustment, it was assumed that the dimensions of the degradation rates (kd1 and kd2) between cattle and sheep were similar; hence, the "restricted model" was adjusted, regarding the dummy variable described in the model. In the second adjustment, those degradation rates (kd1 and kd2) were supposed to be different between cattle and sheep, thus being called a "complete model", in which the dummy variable described in the model was significant. From this information, the statistical comparison was performed using the $\chi^2$ distribution as follows:

$$\chi^2_{calc.} = -n \times 1\mathrm{n}(\frac{RSSc}{RSSr})$$

$$d.f. = p(c) - p(r)$$

where $\chi^2_{calc}$ is the calculated value for $\chi^2$, n is the number of observations used in each fit for the samples, RSSc is the sum of squares of the residue of the complete model, RSSr is the sum of squares of the residue of the restricted model, d.f. is the number of degrees of freedom used to perform the test, p(c) is the number of parameters considered to fit the complete model, and p(r) is the number of parameters considered to fit the restricted model. For all samples evaluated p(c) = 4, p(r) = 3, then d.f. = 1. The non-linear fittings were obtained with the Marquardt algorithm, and t statistics were used to construct the confidence intervals of the parameters ($1 - \alpha = 0.95$). The procedures were performed using PROC NLIN of SAS (version 9.2).

The quantification of the required time (critical time – Tc) to estimate the non-degradable residues was performed based on the properties of the asymptotic confidence intervals for the parameter I of the equation (1). The description of the Tc was conditioned to the previous result obtained by the model identity test; hence, there were two different models by species or a single model for both species. The estimates of Tc by feed or feces, and by species (if there is significance) were obtained by the iterative procedure as suggested by Casali et al. (2008), using the adjusted equation in each situation. The Tc were established as the time in which the estimate of the non-degraded residue became numerically identical to the upper limit of the asymptotic confidence interval

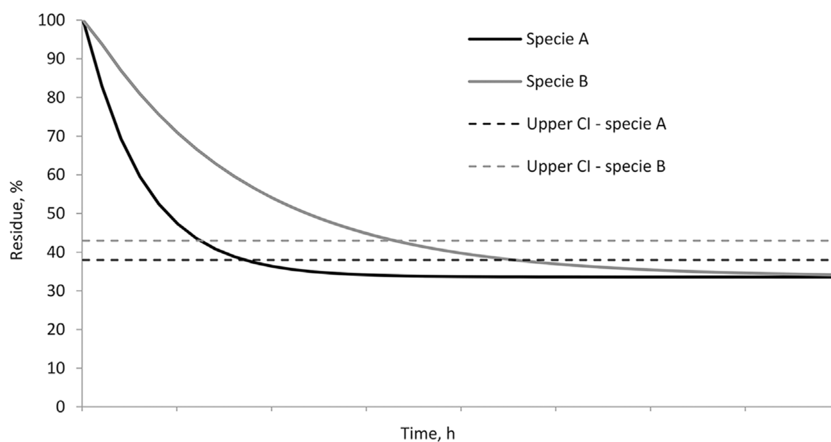


**Fig. 2.** Schematic representations of the expected profiles of non-degraded residue of neutral detergent fiber or acid detergent fiber, which were exponentially fitted over time for sheep and cattle. The dashed lines represent the upper limits of the asymptotic confidence interval at 95% of the parameter I estimated for each specie. The critical time (Tc) of each indigestible fraction was estimated when the dashed line crossed its respective continuous line.

$(1 - \alpha = 0.95)$ of parameter I (Fig. 2).

## 3. Results

The values of kd for NDF (Table 2) were different among species ($P < 0.01$) in most forages, with highest values obtained in cattle in relation to sheep. The same result was observed (Table 3) in the kd for ADF ($P < 0.05$). Alfalfa hay was an exception in forages because there was no difference in the kd between the species for both NDF ($P = 0.36$) and for ADF ($P = 0.14$). The concentrate feeds had higher kd for NDF ($P < 0.01$) when incubated in cattle compared to sheep. The kd for the ADF of concentrates were also higher for cattle ($P < 0.05$), with ground corn showing a trend ($P = 0.05$) for this pattern. Estimates of non-degraded fractions of ADF in concentrates presented greater asymptotic standard-error associated with them (Fig. 3), indicating low reproducibility of ADF as a marker for concentrates.

The value of kd for NDF in the feces sample from cattle-HC was similar when incubated in sheep or cattle ($P = 0.51$). In addition, there was a noticeable effect of the species in the kd for NDF of the other fecal samples ($P < 0.05$), in which the kd was higher when

**Table 2**
Estimates of potentially degradable fraction of neutral detergent fiber (pdNDF), fractional rate of the dynamics of ruminal degradation of B fraction (kd) obtained from cattle and sheep, indigestible fraction of NDF (INDF), upper limit of the asymptotic confidence interval at 95% (UL) for INDF estimate, and critical time (Tc) to reach INDF estimate in both species.

| | | kd ($h^{-1}$) | | | | | Tc (h) | |
|---|---|---|---|---|---|---|---|---|
| | pdNDF | Cattle | Sheep | P-value | INDF | UL | Cattle | Sheep |
| Forage 1 | | | | | | | | |
| *Brachiaria* grass | 67.90 | 0.032 | 0.012 | < 0.01 | 33.56 | 36.96 | 96.2 | 256.0 |
| *Elephant* grass | 68.18 | 0025 | 0.013 | < 0.01 | 33.89 | 36.59 | 129.1 | 279.8 |
| Sugarcane | 33.77 | 0.023 | 0.007 | < 0.01 | 64.24 | 67.67 | 98.6 | 307.7 |
| Tifton-85 hay | 57,50 | 0.023 | 0.010 | < 0.01 | 39.95 | 42.87 | 129.0 | 304.9 |
| Forage 2 | | | | | | | | |
| Alfalfa hay | 33.35 | 0.031 | 0.031 | 0.36 | 67.21 | 69.93 | 80.1 | 80.1 |
| Corn silage | 70.58 | 0.018 | 0.013 | < 0.01 | 31.05 | 35.35 | 155.5 | 215.2 |
| Millet silage | 47,25 | 0.008 | 0.006 | < 0.01 | 48.17 | 61.66 | 156.7 | 208.9 |
| Sorghum silage | 56.52 | 0,018 | 0.008 | < 0.01 | 43.24 | 47.02 | 150.3 | 337.9 |
| *Concentrate* | | | | | | | | |
| Ground corn | 61.32 | 0.019 | 0.011 | < 0.01 | 25.54 | 33.27 | 109.0 | 188.1 |
| Cottonseed cake | 60.97 | 0.018 | 0.009 | < 0.01 | 33.32 | 37.18 | 153.3 | 306.5 |
| Soybean meal | 85,61 | 0.035 | 0.016 | < 0,01 | 19.25 | 24.02 | 82.5 | 180.5 |
| Wheat bran | 48.01 | 0.035 | 0.011 | < 0.01 | 38.59 | 42.03 | 75.3 | 239.5 |
| *Feces*[a] | | | | | | | | |
| Cattle HC | 37.29 | 0.013 | 0.013 | 0.51 | 58.67 | 67.01 | 115.2 | 115.2 |
| Cattle LC | 34.79 | 0.021 | 0.006 | < 0.01 | 65.16 | 70.78 | 86.8 | 303.7 |
| Sheep HC | 55.08 | 0.021 | 0.012 | 0.02 | 45.77 | 50.14 | 115.2 | 211.1 |
| Sheep LC | 27.50 | 0.016 | 0.004 | < 0.01 | 70.03 | 76.33 | 92.1 | 360.7 |

[a] HC = high concentrate and LC = low concentrate.

**Table 3**
Estimates of potentially degradable fraction of acid detergent fiber (pdADF), relative rate of the dynamics of ruminal degradation of B fraction (kd) obtained from cattle and sheep, indigestible fraction of ADF (IADF), upper limit of the asymptotic confidence interval at 95% (UL) for IADF estimate, and critical time (Tc) to reach IADF estimate in both species.

| | | kd ($h^{-1}$) | | | | | Tc (h) | |
|---|---|---|---|---|---|---|---|---|
| | pdADF | Cattle | Sheep | P-value | IADF | UL | Cattle | Sheep |
| Forage 1 | | | | | | | | |
| *Brachiaria* grass | 69.08 | 0.022 | 0.008 | < 0.01 | 28.80 | 32.41 | 131.8 | 354.2 |
| *Elephant* grass | 65.33 | 0.027 | 0.013 | < 0.01 | 34.61 | 38.22 | 107.2 | 222.7 |
| Sugarcane | 40.05 | 0.021 | 0.009 | < 0.01 | 60.07 | 62.77 | 129.7 | 310.9 |
| Tifton-85 hay | 60.97 | 0.016 | 0.009 | < 0.01 | 41.16 | 45.70 | 163.4 | 285.9 |
| Forage 2 | | | | | | | | |
| Alfalfa hay | 33.53 | 0.022 | 0.022 | 0.14 | 61.29 | 65.28 | 96.7 | 96.7 |
| Corn silage | 71.90 | 0.018 | 0.012 | 0.02 | 29.03 | 34.21 | 146.1 | 219.2 |
| Millet silage | 47.71 | 0.009 | 0.005 | < 0.01 | 51.70 | 60.12 | 180.7 | 338.7 |
| Sorghum silage | 58.32 | 0.017 | 0.009 | < 0.01 | 44.86 | 48.80 | 161.4 | 301.3 |
| *Concentrate* | | | | | | | | |
| Ground corn | 57.27 | 0.009 | 0.005 | 0.05 | 36.20 | 57.22 | 111.3 | 200.5 |
| Cottonseed cake | 36.96 | 0.035 | 0.007 | < 0.01 | 45.85 | 49.41 | 66.8 | 381.2 |
| Soybean meal | 60.20 | 0.098 | 0.041 | 0.03 | 18.15 | 23.28 | 25.1 | 60.1 |
| Wheat bran | 42.09 | 0.014 | 0.006 | < 0.01 | 49.03 | 46.11 | 131.1 | 287.0 |
| *Feces*[a] | | | | | | | | |
| Cattle HC | 31.79 | 0.013 | 0.006 | < 0.01 | 66.51 | 73.51 | 116.4 | 252.1 |
| Cattle LC | 29.27 | 0.021 | 0.021 | 0.19 | 72.08 | 76.76 | 87.3 | 87.3 |
| Sheep HC | 35.57 | 0.050 | 0.010 | < 0.01 | 66.48 | 71.33 | 39.8 | 199.2 |
| Sheep LC | 24.19 | 0.011 | 0.011 | 0.11 | 68.14 | 77.77 | 83.7 | 83.7 |

[a] HC = high concentrate and LC = low concentrate.

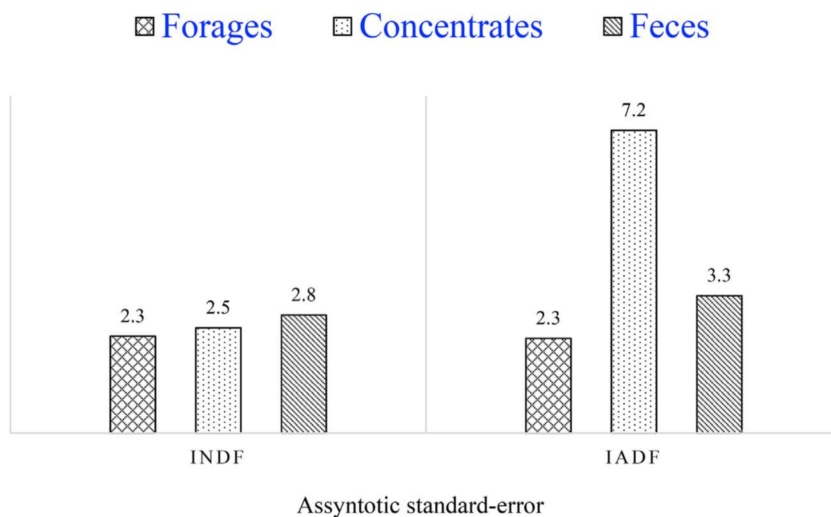


**Fig. 3.** Average values for asymptotic standard-errors of indigestible fractions obtained from cattle and sheep for each evaluated group of samples.

incubated in cattle. The values of kd for ADF in fecal samples from cattle-LC (P = 0.19) and sheep-LC (P = 0.11) were similar when incubated in sheep or cattle. The kd for ADF of fecal samples obtained in sheep-HC and cattle-HC were higher when incubated in cattle (P < 0.01).

Critical-time values greater than 150 h were required to estimate INDF in forages when using cattle; for example, sorghum silage with Tc 150.3 h, and corn silage with Tc 155.5 h. When the same forages were incubated in sheep, these values were 337.9 and 215.2 h, respectively. The sorghum silage incubated in sheep required Tc superior than 336 h, which was the maximum time-point of evaluation in this study; the same occurred with feces from sheep-LC. A Tc of 98.6 h was required to estimate the INDF of sugarcane in cattle, while for sheep this value was 307.7 h. Among all forages, alfalfa hay presented the lowest Tc to estimate INDF, 80.1 h, which was similar for incubation in both species. Concentrates feeds as cottonseed cake and wheat bran required greater Tc when incubated in sheep, requiring 306.5 and 239.5 h, respectively, for quantification of INDF. However, when incubated in cattle, these Tc were 153.3 and 75.3 h. Additionally, in the concentrate group, the lowest Tc to obtain INDF was for soybean meal, being 82.5 h for cattle and 180.5 h for sheep. Feces samples from animals fed with low concentrate diets required higher Tc to obtain estimates of

INDF in sheep; but the same behavior was not observed for cattle, which provided lower Tc for these samples within the feces group.

Critical-time values greater than 180 h were required for the quantification of forage IADF when using cattle; for example, millet silage with Tc of 180.7 h, and Tifton-85 hay, with Tc of 163.4 h. When the same forages were incubated in sheep, these values were 338.7 and 285.9 h, respectively. Millet silage and Brachiaria grass incubated in sheep required values higher than 336 h, the maximum time-point of evaluation in this experiment; so did the cottonseed cake sample. The Tc of 129.7 h was required to estimate the IADF of sugarcane in cattle, while for sheep this value was 310.9 h. Among the forages, alfalfa hay presented the lowest Tc to estimate IADF, 96.7 h, which was similar for incubation in both species. Cottonseed cake and wheat bran presented higher Tc for incubation in sheep, requiring 381.2 and 287.0 h, respectively, for IADF estimation. However, when incubated in cattle, these concentrates required 66.8 and 131.1 h of incubation. In the concentrate group, the lowest Tc to obtain ADF was obtained for soybean meal, 25.1 h for cattle and 60.1 h for sheep. Feces from cattle-HC showed higher Tc to obtain estimates of IADF when incubated in sheep. The Tc required to obtain IADF of feces samples from animals fed low concentrate diets were less than 100 h when incubated in both cattle or sheep.

## 4. Discussion

When adopting the following assumptions: that the indigestible fraction is an intrinsic characteristic of the substrate (Ørskov, 2000), and it is not influenced by physical characteristics or other components of the feed and feces incubated, it is possible to infer that differences in the kd and the Tc between the sheep and the cattle obtained in this study may be associated to the anatomical and physiological differences of the rumen in the evaluated species.

Huntington and Givens (1997), did not observe differences in the kd for NDF in alfalfa hay when they evaluated temperate forages incubated in Holstein cows and Suffolk sheep. The research on the different animal species used in degradation tests is probably more relevant when evaluating forages from tropical climate. In this work, samples of feed and feces incubated in cattle rumen showed higher ruminal degradation rates. This disparity between species can be attributed to their ruminal environment. These species present distinct ruminal volume, thus providing differences in the dilution of the bags immersed, in the quantity, and in the profile of microorganisms present in the degradation site.

Legumes from temperate climate, such as alfalfa hay, presented higher values than those obtained for tropical climate forages, such as sorghum silage, Tifton-85 hay, sugarcane, Elephant grass and Brachiaria grass. This difference can be due to the fact that C4 forages have lower content of core lignin than C3 grasses and legumes (Jung 1989). The core lignin is usually bound to hemicellulose by covalent attachment, and the same does not occur with cellulose. Thus, forages with lower content of hemicellulose in the cell wall, such as alfalfa hay, tend to be less inhibited by lignification. According to Van Soest (1996), legumes show differentiation in cell wall composition, which gives them higher cellulose and pectin contents (Van Soest et al., 1991), and consequently a higher rate of degradation of potentially degradable NDF. However, few legumes are adapted to forage cropping in tropical regions.

The results of this work are compatible with those presented by Soto-Navarro et al. (2014). These authors reported a higher Tc required to estimate NDF disappearance in sheep rumen when compared to cattle, and lower kd values. The authors explained this result by a possible lower efficiency of ruminal microorganisms in sheep when degrading less digestible fractions of the feeds. However, studies comparing the density and diversity of ruminal microbiota among ruminants are still limited (Chen et al., 2011). It is recognized that factors such as the passage rate and intake can affect the population of microorganisms and consequently the degradation rate of feed (Foster et al., 2007). It can thus be inferred that, in addition to the shorter retention time of sheep in relation to cattle (Soto-Navarro et al., 2014), the limited ruminal volume could reflect a lower relative population of microorganisms in sheep ruminal environment when compared to cattle. These facts could justify the reduced degradation rates in a majority of the evaluated feeds in sheep.

The ruminant species represented a source of variation on kd for NDF and ADF, even for tropical forages with higher nutritional quality. The only exception was for alfalfa hay. Thus, even the best tropical forage options receive the adverse effects of lignification. Soto-Navarro et al. (2014) also worked with C3 and C4 forages incubated in both species and inferred that sheep can be used as models to evaluate the degradability of high and medium quality forages for cattle. However, for low quality forages (C4), the same cannot be stated. The authors confirmed their hypothesis that cattle digest low quality forages more efficiently than sheep, and did not recommend the replacement of these species in tests for the construction of degradation curves or even for digestibility studies. In contrast, in the present work, the objective was to determine the Tc used in each species to reach the indigestible fraction by estimating the degradation extent of a given feed, which required lengthy time and shorter evaluation intervals. In this context, the sheep rumen possibly restricted the evaluation of both, the rate and the extent of degradation, which are dependent on a greater amount of time points for evaluation and major time length for tropical forages incubation. In this context, cattle could replace sheep as the animal model for ruminal fermentation.

Lignin is usually recognized for its activity on inhibition of microbial access to the feed cell wall, and for the reduction in fiber digestion rate (Krizsan and Huhtanen, 2013; Van Soest, 1994). The greater lignification of the cell wall in the evaluated silages is an intrinsic characteristic of the tropical climate forages. In addition, high temperatures raise the rate of the enzymatic processes associated with lignin biosynthesis. Tropical plants are subjected to long nocturnal periods, when soluble carbohydrates are used for cellular respiration, which reduces the non-fibrous carbohydrates' content and the quality of these forages (Van Soest, 1996).

Lopes et al. (2015) evaluated two corn silages that differed in fiber digestibility for dairy cows. The authors have reported that CNCPS model calculates the INDF fraction as 2.4 × lignin for corn silage, but they observed that the pool of INDF is smaller when calculated as 2.4 × lignin than when determined by long incubation time either 240 (in vitro) or 288 h (in situ) incubations. Higgs et al. (2015) also recommended 240 h of in vitro incubation to estimate INDF when updating the CNCPS System feed library, which is

in accordance with that reported by Lopes et al. (2015) concerning to the requirement for long time incubation system for INDF analysis. Krizsan et al. (2010) also recommended 288 h of in situ incubation to obtain INDF. Colombini et al. (2012) considered questionable the assumption that indigestible NDF is equal to 2.4 times lignin(sa) for any feed, once the ratio between lignin and INDF is not constant, and particularly it is lower in most corn silages and grasses.

In the present study, the reduced ADF content in ground corn and soybean meal samples, and the higher asymptotic standard-errors associated with non-degraded fractions of ADF, indicated a limitation for IADF as a marker for this ingredient type. According to Detmann et al. (2007) ADF is more sensitive to systematic errors arising from weighing procedures, which reduces its accuracy as a marker; especially in concentrate feeds, where it is present in lower levels.

Recent studies comparing sheep and cattle on internal markers evaluation are scarce. Kozloski et al. (2014) compared ADF and lignin(sa) to estimate digesta flow and ruminal digestibility in sheep and cattle, and concluded that ADF was more precise for estimating DM digestibility in both species. However, the authors did not evaluate IADF, which could be more suitable as internal marker, because INDF is an internal marker intimately associated with dietary fiber, and its fecal recovery works as an ideal marker (Huhtanen et al., 2010). Lee and Hristov (2013) have compared acid-insoluble ash (AIA) and IADF for dairy cows and observed that INDF was more reliable than AIA to estimate fecal digestibility and total tract digestibility. Sampaio et al. (2011) stated that internal markers indigestible dry matter, INDF and IADF are suitable to estimate fecal excretion without bias inherent to marker fecal recovering, besides presenting higher precision of the estimates when compared with other external marker as titanium dioxide and chromic oxide.

Variations between the Tc of feeds and feces to estimate non-degraded fractions were verified. The high values obtained in sheep, when compared to cattle, may indicate that there was probably insufficient incubation time length to reach the asymptotic value of the adjusted curve for this species. It must be emphasized that the temporal variability among the two species can be attributed to the lower effectiveness of substrate degradation by the microbial population in sheep in order to reach the indigestible fraction. However, we suggest that further research could be conducted with tropical forages incubated in sheep, using longer incubation time length and shorter evaluation intervals. Data are still required to infer about the in situ degradation curve in sheep using as few bags as possible.

In relation to feces, in spite of the significant lignification, these constitute relapsed material to the rumen environment, which is evident in characteristics that differ from the other fibrous feeds (Casali et al., 2008). The kd for ADF of cattle-LC and sheep-LC did not differ between the two species available for incubation, in addition to presenting reduced Tc. This can be explained by the content of ADF being lower in the concentrates and higher in forages. This association may indicate that reduced passage rate of diets composed by low concentrate (higher contents of ADF) could reduce potentially degradable fractions of feces generated from these diets.

Critical-times between 144 and 192 h in cattle were observed for both, the INDF and the IADF evaluation, and by adopting a multiple of 24 h for an analytical recommendation (Valente et al., 2015), an incubation protocol from 216 h in cattle could be suggested to evaluate feeds and feces obtained in digestibility trials of tropical forages in sheep. These results, when obtained for cattle, are compatible with other in situ studies involving different feeds. Casali et al. (2008) found a Tc of 196.5 and 248.8 h to estimate INDF and IADF, respectively, in tropical forages. Valente et al. (2015) found a Tc of 274.5 h INDF in tropical grasses. Krizsan and Huhtanen (2013) suggested 288 h of in situ incubation to determine INDF for any feed incubated in cattle. Digestion of NDF continues even after long incubation periods in situ, suggesting that extended incubations are required to precisely estimate the INDF concentration of a feed.

The variation between experiments may also occur due to differences inherent to bags used in each incubation procedure and with the particle size of the incubated feed. According to Kramer et al. (2013) particle loss during in situ rumen incubation cannot be excluded, and is likely to vary among feedstuffs. These authors highlighted that particle size area decreased linearly with increasing incubation, and hence recommended 288 h of in situ feed incubation. All these studies were totally carried out using cattle. The present work demonstrated that the same feeds incubated both in sheep and cattle receiving the same diet, required greater incubation time when evaluated in sheep. In addition, incubation time-points higher than 336 h were obtained, which demonstrates how important is to know the time required to obtain degradation extension of feed, using a time length as short as possible.

## 5. Conclusion

After analyzing all data, we do not recommend sheep to obtain internal markers based on in situ trials, due to the high incubation time length to obtain the non-degraded fraction of feeds and feces. Otherwise, the internal markers IDNF and IADF from sheep trials can be obtained from 216 h of in situ incubation in cattle. Nevertheless, as this study presented a comparison between species, we suggest to maintain the previous literature recommendation of 288 h as Tc for in situ incubation in cattle, which demonstrated that the longer the incubation time applied to the in situ tests in cattle, the greater the accuracy of the estimates. We do not recommend IADF as an internal marker when diet is mainly based on concentrates. It is necessary to emphasize that when trials are carried out to estimate degradability parameters (B, kd and I) and, consequently, to estimate effective degradability, sheep and cattle present different degradation curves, which may restrict the species to determine the degradable fractions of feed by itself.

## Conflict of interest

There is no conflict of interest.

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