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Indigestible neutral detergent fiber evaluation with incubation in different species

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ADDITIONAL KEYWORDS
Digestibility.
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Polygastric.
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RESUMEN

We aimed to evaluate indigestible neutral detergent fiber (iNDF) feed concentration after cattle, goat, and sheep ruminal incubation, compare results with different estimate methods and connect with chemical analysis data. Four forages (corn silage, sugarcane, Brachiaria decumbens cv. Marandu, and Panicum maximum cv. Tanzânia), two concentrates (ground corn and soybean meal), a by-product (soybean hull), and three fecal samples (cattle, goat, and sheep) were incubated in Nellore steers, dry Saanen goats and Santa Inês sheep. Animals were previously adapted to the experimental diet for eight days, and incubation was performed for 240 hours. The concentration of iNDF was higher when samples were incubated in goat rumen compared with samples incubated in the rumen of cattle. Sheep ruminal incubation increased forages and tended to increase concentrate and by-product iNDF concentration, relative to cattle ruminal incubation. Moreover, sheep and goat ruminal incubation result in similar feed iNDF concentration. Besides, cattle feces had a higher level, and goat feces had lower iNDF levels than sheep feces. The CNCPS underestimated iNDF feed concentration. Estimates of uNDF from Conrad et al. (1984) were lower than iNDF level of sugarcane and higher than iNDF level of P. maximum and concentrates. In general, lignin concentration was the primary composition data related to the iNDF level. However, ADF was the best for forages ($R2 = 0.668$), and NDF was better for concentrates ($R2 = 0.454$). In conclusion, digestive process of different species affects iNDF feed concentration. The bias of models was considerable, and feed characteristics affect chemical composition and iNDF level.

Evaluación de fibra detergente neutra indigestible con incubación en diferentes especies

PALAVRAS CHAVE ADICIONAIS
Digestibilidade.
FDN.
Poligástricos.
Rúmen.

RESUMO

Objetivo do presente estudo foi avaliar a concentração da fibra em detergente neutro indigestível (FDNi) após a incubação ruminal em bovinos, caprinos e ovinos, e comparar os diferentes resultados com os métodos de estimativa e relacionar os dados com as análises químicas. Quatros forragens (silagem de milho, cana-de-açúcar, Brachiaria decumbens cv. Marandu e Panicum maximum cv. Tanzânia) dois concentrados (milho em grão e farelo de soja), um subproduto (casquinha de soja) e três amostras de fezes (bovino, caprino e ovino) foram incubadas em novilhos Nelore, cabras Saanen e ovinos Santa Inês. Os animais foram previamente adaptados a dieta experimental por 8 dias e incubação ocorreu por 240 horas. A concentração de FDNi foi mais alta nas amostras incubadas no rúmen de caprinos comparada com amostras incubadas no rúmen de bovinos. A incubação nos ovinos aumentou a concentração de FDNi nas forragens e tendeu a aumentar no concentrado e subproduto em relação a incubação no rúmen de bovinos. A concentração de FDNi foi similar para incubação em ovinos e caprinos. Em adição, as fezes bovinas tiveram alta concentração de FDNi, e as fezes de caprinos tiveram menor concentração de FDNi em relação às fezes de ovinos. O modelo CNCPS subestimou a concentração de FDNi nas amostras. Estimativas da FDN não degradável por Conrad et al. (1984) subestimou a concentração de FDNi na cana-de-açúcar e superestimou a concentração de FDNi no P.maximume concentrados. Em geral, a concentração de lignina foi ocomponente das amostrasmais relacionado com a concentração de FDNi. FDA foi a melhor para forragens ($R2 = 0.668$) e FDN foi melhor para concentrados ($R2 = 0.454$). Em conclusão, os processos digestivos das diferentes espécies afetam a concentração de FDNi do alimento. Erros nas estimativas dos modelos foram consideráveis e as características dos alimentos afetam a composição química e concentração de FDNi.

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INTRODUCTION

Ruminant animals have a developed and specialized digestion method that allows them to digest fibrous materials. This power is associated with pregastric retention and fermentation, which improves neutral detergent fiber (NDF) digestibility before the enzymatic digestion. Furthermore, carbohydrates are the primary source of energy available to the rumen microorganisms. After fermentation, these microorganisms provide volatile fatty acids for ruminant energy metabolism (Bergman, 1990).

Digestibility is one of the main feed attributes associated with a nutritive value (Colucci et al., 1989; Oba and Allen, 1999). However, *in vivo* evaluation by total fecal collection is an expensive and laborious process that requires the same animals, experimental control and may cause discomfort (France et al., 1988; Magalhães et al., 2018). Potentially digestible fiber (pdNDF) estimate could be obtained by the difference between NDF concentration and remaining undigested NDF (iNDF) after a period of ruminal incubation. Potentially digestible fiber (pdNDF) is an essential and easy evaluation that allows estimating *in situ* fiber digestibility (Nousiainen et al., 2004; Huhtanen et al., 2006). Indigestible NDF also plays a crucial role in dynamic rumen models, such as the Cornell Net Carbohydrate and Protein System (CNCPS) (Fox et al., 2004; Tylutki et al., 2008; Van Amburgh et al., 2015; Krizsan and Huhtanen, 2013) and NorFor (Volden, 2011) and can be used as a marker for fecal excretion or duodenal flow estimate (Waller et al., 1980).

Although there are known intrinsic factors that affect iNDF concentration, extrinsic factors are not known, such as diet composition (Krizsan and Huhtanen, 2013) and animal effect. According to Van Soest (1994), different feed behavior of ruminant species could affect the ruminal fermentation and ruminal digestive process, although, sheep have been extensively used as a model for research on ruminant digestion (Südekum et al., 1995).

Indigestible NDF is a principal preditor of feed nutritive value. However, nutritional models may not be correct, and animal models seem to affect iNDF estimates. We hypothesize that more selective animals (goats) have lower ruminal fiber digestibility, and this results in higher iNDF feed concentration than in less selective animals (especially cattle). We then evaluated the iNDF after cattle, sheep, and goat ruminal incubation and associated the results with chemical composition of feeds and current nutritional models.

MATERIAL AND METHODS

The present experiment was conducted at Jaboticabal, São Paulo, Brazil, on latitude South 21°15′22′′ and longitude West 48°18′58′′ and 595 meters a.s.l.

Feeds and chemical analysis

Samples of concentrates (ground corn and soybean meal), the by-product (soybean hulls), forages (corn silage, sugarcane, *Brachiariabrizantha* cv. Marandu and *Panicum maximum* cv. Tanzânia) and feces (cattle, sheep, and goats) were collected to evaluate iNDF concentration. Sugarcane was collected after the whole plant harvest. *Brachiaria brizantha* and *Panicum maximum* were sampled by simulated grazing, at native pasture and fertilized area, respectively. Feces were collected from the experimental animals on the 5th day of the trial. Wet samples of feed and feces were pre-dried in a forced-air oven at 55°C for 72 hours and subsequently processed in a Wiley knife mill with 1- and 2-mm pore screens, which were used for chemical analysis and ruminal incubation, respectively. Samples (1-mm screen) were analyzed for dry matter (method 930.15; AOAC, 2000), crude protein (N × 6.25; method 984.13; AOAC, 2000), ether extract (method 920.39; AOAC, 2000) and ash (method 942.05; AOAC, 2000). For neutral detergent fiber (NDF) and acid detergent fiber (ADF) evaluation, samples were placed in bags and digested in Van Soest et al. (1991) detergent for one hour at 90ºC. Acid detergent lignin (ADL) were analyzed using 72% sulfuric acid (Van Soest et al., 1991). The chemical composition of evaluated feed and feces are shown in **Table I**.

Animals and incubations

Three Nellore steers [300 kg of body weight (BW)], three Saanen dry goats (50 kg of BW) and three Santa Inês male sheep (50 kg of BW) were used. Animals were housed in individual pens and had free access to diet and water. Diet had 700 g/kg DM forage (corn silage). Animals were fed twice daily, at 700 and 1600 h, to provide 50 to 100 g/kg of the fresh matter daily orts. Experimental period lasted for 18 days, eight for diet adaptation, and ten for ruminal incubation.

Samples (2-mm screen) were placed in a 4 × 5 cm bag (non-woven tissue 100 g/m^2) and incubated in the rumen. Bags were previously washed in a neutral detergent solution (Robertson and Van Soest, 1981), dried and weighed. Less than 20 mg of sample per tissue cm^2 was used (Nocek, 1988). Incubation was performed for 240 hours, using three bags for each feed in each animal, with a nylon bag to pack TNT bags (Casali et al., 2008). At the end of incubation, bags were removed from the rumen and washed in cold water for 30 minutes. Bags were then washed in running water until whitening. Samples were evaluated for NDF concentration, as previously described.

Calculations and statistical analysis

Evaluated indigestible NDF concentrations were compared with uNDF estimate of Conrad et al. (1984) and iNDF estimate of Sniffen et al. (1992). Indigestible NDF from Sniffen et al. (1992) model were estimated as:

where ADL is the acid detergent lignin concentration of the feed.

Conrad et al. (1984) estimate were performed as:

where tdNDF is the truly digestible NDF concentration, which was estimated as:

where nNDF were estimated as NDF and neutral fiber indigestible protein concentration difference. Model bias was calculated as observed less estimated (predicted) values.

All data were separated by the feed classification (concentrate and by-product, forages, and feces). Indigestible NDF concentration of feeds was analyzed as split-plot design, using PROC MIXED of SAS (Statistical Analysis System, version 9.3) and considering the following statistical model:

$$Y_{ijkl} = \mu + S_i + \omega_{ij} + F_k + S{\times}F_{ik} + e_{ijk}$$

with and ; where: Y_{ijkl} is the observed value; μ is the overall mean; S_i is the fixed effect of species; ω_{ij} is the random effect of animal within species; $F_k$is the feed fixed effect; $S{\times}F_{ik}$ is the species × feed interaction effect; e_{ijk} is the random residual error; is the variance due to animal (parcel); MVN is the multivariate normal, and *R* is the variance-covariance matrix of residuals due to the multivariate analysis. Kenward and Roger's (1997) method was used for degree of freedom correction, while the Akaike method was used to choose one of the evaluated matrices of variance and covariance [CS, CSH, AR(1), ARH(1), TOEP, TOEPH, UN, FA(1), ANTE(1)]. The significant effect of feeds and species was studied by Fisher means test (LSD) and exact P values were shown.

Model accuracy evaluation was performed using a T-test (H_0: β_1= 0) for each feed. Additionally, the model bias in general and for each feed was evaluated using PROC MIXED of SAS 9.3 and considering each feed in each animal as an experimental unit (n=90). Similarly, feed average model errors were evaluated using Fisher means test (LSD). Simple linear regressions were conducted between iNDF concentration and sample chemical composition, for all feeds and each class, using PROC REG of SAS 9.3. Differences were considered significant at 0.05 level. Tendency was considered at $0.05 < P \leq 0.10$.

Table I. Chemical composition of evaluated feeds and feces (Composição química de rações e fezes avaliadas).

Item	Forages				Concentrates and by-product			Feces		
	CS*	SC†	BRA‡	PM§	GC¶	SM**	SH††	CF‡‡	SF§§	GF¶¶
Composition, g/kg										
Dry matter	346	291	249	233	901	910	905	210	399	410
Organic matter	968	958	904	918	976	927	944	875	887	851
Crude protein	72.0	42.3	118	212	84	513	273	182	161	140
Ash	31.6	42.1	96.3	81.7	23.9	62.8	56.1	125	113	149
Ecther Extract	25.2	12.0	17.4	15.6	40.1	19.7	16.2	29.7	27.5	25.4
Neutral detergent fiber	530	625	577	587	114	142	482	547	589	520
Acid detergent fiber	250	315	231	240	35.0	73.4	301	204	252	260
Acid detergent lignin	35.2	39.5	30.0	18.4	3.4	2.6	16.5	49.3	60.1	69.3

*CS: corn silage; †SC: sugarcane; ‡BRA: Brachiaria decumbens; §PM: Panicum maximum cv. Tanzânia; ¶GC: ground corn; **SM: soybean meal; ††SH: soybean hulls; ‡‡CF: cattle feces; §§SF: sheep feces; ¶¶GF: goat feces.

RESULTS

Indigestible neutral detergent fiber concentration

Species affected iNDF concentration after ruminal incubation in forages (P = 0.038; **Table II**) and tended to affect iNDF concentrations in feces and concentrates (P ≤ 0.097). Cattle ruminal incubation had lower iNDF concentration than goat ruminal incubation (P ≤ 0.026). Sheep incubation increased forage iNDF concentration (P = 0.023) and tended to increase concentrates iNDF concentration (P = 0.075) in relation to cattle incubation. Goat and sheep incubation showed similar iNDF concentrations (P ≥ 0.250).

There were species and feed interaction effects only for forage (P < 0.001; **Figure 1**). Corn silage and *P. maximum* had similar iNDF concentrations in different species of incubation (P ≥ 0.109). *B. decumbens* and sugarcane had lower iNDF in cattle than in sheep ruminal incubation (P < 0.05). *B. decumbens* had lower iNDF concentration in goat incubation than in sheep incubation (P < 0.05), and sugarcane incubation in goats had higher iNDF than cattle incubation (P < 0.05).

Indigestible NDF concentration was higher (P < 0.05) for feces and lower (P < 0.05) for concentrates, in relation to forage. Cattle feces had higher (P < 0.05; **Figure 2**) iNDF concentration than sheep feces, which had higher (P < 0.05) iNDF concentration than goat feces.

Model of indigestible NDF estimate

For all evaluated feed, Sniffen et al. (1992) equation estimated lower iNDF concentration than Conrad et al. (1984) equation (P < 0.001). Indigestible NDF to ADL ratio was 7.40 ± 0.34 (Mean ± SEM), significantly higher (P < 0.001; t-test) than the 2.4 considered for Sniffen et al. (1992). In general, Conrad et al. (1984) equation (10.6 g/kg) and Sniffen et al. (1992) equation (126 g/kg) underestimated (P < 0.001) iNDF concentration.

Between incubated forages, sugarcane showed the highest model error (P ≤ 0.05) (269 and 168 g/kg, for Sniffen et al. (1992) and Conrad et al. (1984) equations, respectively). *Panicum maximum* had the lowest model error (P ≤ 0.05). Sniffen et al. (1992) equation underestimated by 102 g/kg, and Conrad et al. (1984) equation overestimated by 103 g/kg the concentration of iNDF.

Table II. Average indigestible neutral detergent fiber after 240 hours of cattle, sheep and goat ruminal incubation (Fibra média de detergente neutro indidigível após 240 horas de incubação de bovinos, ovinos e caprinos).

Item	Species			SEM*	P†					
	Cattle	Goat	Sheep		Sp	Feed	Sp*Feed	C vs G	C vs S	G vs S
Indigestible neutral detergent fiber, g/kg										
Feces	315	366	342	7.78	0.097	0.001	0.272	0.038	0.216	0.250
Forages	194	236	238	5.91	0.038	<0.001	<0.001	0.026	0.023	0.940
CandB‡	35.4	43.2	41.7	1.19	0.077	<0.001	0.101	0.036	0.075	0.614

*SEM: Standard error of means; †Probabilities: Sp: Species effect; Feed: Feed effect; Sp*F: Specie and feed interaction effect; C vs G: Cattle vs Goat; C vs S: Cattle vs Sheep; G vs S: goat vs sheep effect. ‡Concentrates and by-product.

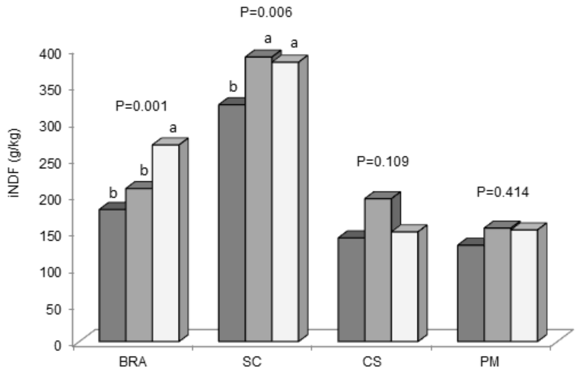

Figure 1. Average indigestible neutral detergent fiber (iNDF) concentration on evaluated forages after cattle, goat and sheep ruminal incubation (Concentração média de fibra de detergente neutro indidigesto (iNDF) em forragens avaliadas após bovinos, caprinos e ovinos incubação ruminal). BRA: Brachiaria decumbens; SC: sugar cane; CS: corn silage; PM: Panicum maximum cv. Tanzânia.

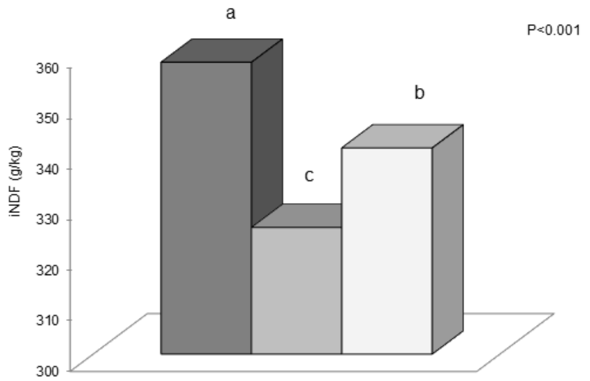

Figure 2. Average indigestible neutral detergent fiber (iNDF) on cattle, goat and sheep feces (Fibra média de detergente neutro indigesto (iNDF) em bovinos, cabras e ovinos fezes).

Moreover, corn silage (10.3 g/kg) had a lower ($P \leq 0.05$) estimate error than *B. decumbens* (77.6 g/kg). Furthermore, Conrad et al. (1984) equation was accurate ($P \geq 0.074$) for corn silage and *B. decumbens* evaluation.

Soybean meal and ground corn had similar model bias, while soybean hull had higher model estimate errors (85.8 g/kg). Conrad et al. (1984) equation overestimated the iNDF concentration of concentrates and byproduct ($P \leq 0.003$). Sniffen et al. (1992) overestimated iNDF concentration only for concentrates and was accurate for soybean hull ($P = 0.397$). In general, both evaluated systems underestimated fecal iNDF concentration ($P < 0.001$). Observation by estimated linear regression showed intercept of -5.23 and slope of 1.33 ($P < 0.001$ and $R^2 = 0.358$) for Conrad et al. (1984) model, and intercept of 2.8635 and slope of 2.246 ($P < 0.001$ and $R^2 = 0.767$) for Sniffen et al. (1992) model.

Indigestible NDF to composition linear regression

For forage, NDF, ADF, CP, ADL and CEL concentrations were used for iNDF estimate ($P < 0.001$). However, ADF showed a higher determination coefficient. For concentrates, NDF, ADF, ADL, CEL and HEM were used for iNDF estimate ($P < 0.001$), and NDF showed the highest correlation. The fecal composition is not associated with chemical composition ($P \geq 0.081$). In general, ADL showed the highest determination coefficient ($P < 0.001$ and $R^2 = 0.767$).

DISCUSSION

Indigestible NDF concentration in some feed and feces samples were studied after cattle, sheep, and goat ruminal incubation. Goats showed lower NDF ruminal degradation than cattle. According to Van Soest (1994), small ruminants are more selective than cattle, which could affect volatile fatty acids production, reduce ruminal pH and increase passage rate. Huston et al. (1986) highlight that other factors beyond diet composition, such as level of intake and ruminal retention, affect ruminal digestibility. Furthermore, these authors found a donor species of ruminal liquid effect on *in vitro* digestibility, which they related to microbial profile. Soto-Navarro et al. (2014) evaluated ruminal and total tract digestibility in cattle and sheep and found higher digestibility in cattle than sheep when using low-quality forage in the diet, which these authors associated with increased nutrient recycling. Moreover, Hofmann (1989) found lower cellulolytic ruminal activity for selector than other ruminants. In this study, goat incubation showed higher iNDF concentration than cattle, which suggests a more moderate ruminal cellulolytic activity. Lower iNDF level in goat feces than in sheep feces, and especially cattle, also confirms the lower fiber digestion capacity of goats.

Sheep and goat incubation had similar iNDF concentrations. However, sheep fecal iNDF concentration was higher than goat fecal level. We think that the selective behavior of these two small ruminant species allows the different fecal levels (Van Soest, 1994). In a practical aspect, using iNDF as a marker, incorrect assessment could affect estimates of intestinal flow and fecal excretion. Small ruminant incubation for estimating iNDF concentration in cattle studies results in partial recovery rate of the marker. On the other hand, lower iNDF level does not mean correct estimate and specific-species analysis seems to be the most recommended.

Nutritional models such as Cornell Net Carbohydrate and Protein System (CNCPS) (Sniffen et al. 1992) predicts iNDF from the ADL concentration. The iNDF fraction had been estimated ADL × 2.4 (Chandler et al., 1980). This relation was considered for overall forages. Huhtanen et al. (2006) described that this estimate had poor accuracy and precision and Palmonari et al. (2016) found iNDF:ADL ratio of 3.22 and 3.11 for grass hay and corn silage, respectively. In the current study, the iNDF:ADL ratio was in general 5.39 and 7.67 for forages, both higher than the 2.4 rates used in the Sniffen et al. (1992) equation.

We must highlight that Conrad et al., (1984) method focuses on estimated forage NDF ruminal digestibility in an animal at maintenance intake level, which is equivalent to nearly 48-hours ruminal digestion and not on iNDF. Weiss et al. (1992) already observed this difference and suggested that estimated uNDF should overestimate iNDF, once pdNDF ruminal digestibility is almost 0.80 at maintenance level. Conrad et al. (1984) model overestimated only *P. maximum*, concentrate, and by-product iNDF concentration was accurate for corn silage and *B. decumbems* and underestimated sugarcane iNDF concentration. Furthermore, between evaluated forages, *P. maximum* (146 g/kg) and corn silage (162 g/kg) had the lowest iNDF and sugarcane showed the highest iNDF concentration. Sugarcane unique features of low NDF content with low digestibility (Corrêa et al., 2003) underestimated model estimates.

Concentrates and by-products also had a small iNDF concentration. We could speculate that concentrates had little NDF (114.3 and 141.6g/kg for ground corn and soybean meal, respectively; **Table I**) and soybean hulls and *P. maximum* had small ADL concentration (16.5 and 18.4g/kg, respectively). Lignification of the cell wall is one of the main factors that affect fiber digestibility (Van Soest, 1994). Thus, lower ADL of these feeds is associated with overestimation. On the other hand, Conrad et al. (1984) equation also underestimated sugarcane and feces iNDF concentration.

In general, ADL showed the highest correlation with iNDF feed concentration (R^2=0.767). Palmonari et al. (2016) also found ADL as the single primary predictor of iNDF (R^2=0.67). These findings reflect a higher correlation between observed and estimated data in Sniffen et al. (1992) model than in the Conrad et al. (1984) model. Analyzing only forage, ADF showed the highest correlation with iNDF concentration, and NDF showed the highest correlation for concentrate and by-product. We think that feed composition could explain these effects. Concentrates had much lower ADL and ADF, which increased variation coefficient, and NDF became the main unique predictor. For forage, ADF concentration increased and became less variable. Additionally, low number of forages evaluated decreased the ADL and iNDF correlation. We also didn't see any chemical composition and iNDF fecal composition relationship. Fecal chemical composition is highly associated with digestion process. Increased nutrient digestibility decreases fecal concentration and increases the iNDF level, reducing their correlation.

CONCLUSIONS

Indigestible NDF estimate is dependent on ruminant species, and goats showed lower ruminal NDF degradability than cattle. Estimation models generally underestimate iNDF, and feed characteristics seem to affect its relationship with chemical composition.

CONFLICT OF INTEREST

The authors declare that are no conflicts of interest to the current manuscript.

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