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Evaluation *in situ* digestibility of alfalfa in different grinds and textiles

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ABSTRACT. Indigestible fractions of dry matter (iDM) and neutral detergent fiber (iNDF) in the feed of ruminants are mainly estimated by *in situ* incubation time with regard to particle size and textile types. Samples of alfafa, ground into three particle sizes, were analyzed. Samples, processed in a Willey mill with 1.0; 2.0 and 3.0 mm sieve pores, were conditioned in F57 (Ankon®), nonwoven (100 g m^{-2}) and polyester textile bags measuring 4 x 5 cm. Material was divided into 13 incubation periods and 8 replications, and incubated in the rumen of two multiparous cows adapted to a 70:30 diet, roughage:concentrate, respectively, for 288h. iDM and iNDF rates were evaluated sequentially for non-digested percentage and data underwent analysis of variance (ANOVA $p < 0.05$); means were compared by tukey's test ($p < 0.05$). Particle size and material employed for incubation affected iDM and iFDN at the initial periods of incubation ($p < 0.05$).

Keywords: digestion, neutral detergent fiber, F57, nonwoven textile, polyester textile.

Avaliação de digestibilidade *in situ* de alfafa em diferentes moagens e tecidos

RESUMO. A determinação do tempo de incubação *in situ*, em relação ao tamanho de partículas e tipos de tecidos, é o aspecto principal sobre as estimativas das frações indigestíveis da matéria seca (MSi) e da fibra em detergente neutro (FDNi) em alimentos para ruminantes. Estudou-se amostra de alfafa, moídas em três tamanhos de partículas. As amostras foram processadas em moinho, com facas e porosidades das peneiras ajustadas a 1,0; 2,0 e 3,0 mm, as amostras foram acondicionadas em sacos F57 (Ankon®), tecido não tecido (100 g m^{-2}) e tecido de poliéster, confeccionados na dimensão 4 x 5 cm. Os materiais foram divididos em 13 tempos de incubação e oito repetições, incubadas no rúmen de duas vacas multíparas adaptadas à dieta 70:30, volumoso:concentrado, respectivamente, por tempo total de 288h. Os teores de MSi e FDNi foram avaliados sequencialmente para interpretação das porcentagens de material não digerido, e os dados foram submetidos à análise de variância (ANOVA, $p < 0,05$), e as médias comparadas posteriormente pelo teste de tukey ($p < 0,05$). Houve efeito do tamanho de partículas e material utilizado para incubação sobre as estimativas de MSi e FDNi, nos tempos iniciais de incubação ($p < 0,05$).

Palavras-chave: digestão, fibra em detergente neutro, F57, tecido não tecido, poliéster.

Introduction

High quality forage is a highly important factor for animal production on pasture. Feed quality, however, is related to management, soil fertility and mainly the type of forage grass. Since alfafa is a high quality forage grass, albeit characterized by high digestibility and fast ruminal degradation (Rodrigues, Comeron, & Vilela, 2008), it should be focused upon in assessments for the recuperation of indigestible fractions such as internal indicators, inconsistencies of results for incubation time and material used in *in situ* assays, as already analyzed by Detmann et al. (2007) and Kuwahara Souza, Soares, Costa, and Meirelles (2015).

The insoluble and indigestible fibrous fraction of feed, specifically indigestible neutral detergent fiber (iNDF), is extensively used due to preciseness in results (Piaggio, Prates, Pires, & Ospina, 1991). According to Van Soest (1994), the indigestible fraction of feed for internal intake indexes is a highly promising tool in studies on animal nutrition. Berchielli, Andrade and Furlan (2000) report that iNDF is usually employed in estimates such as fecal production, coefficients of digestibility, food ingestion and flow of nutrients through the gastrointestinal tract of the animals.

Further, incubation time and material used to condition the samples in the rumen are basic factors

to standardize methodology for the *in situ* determination of the indigestible fraction. According to Mertens (1993), the indigestible fraction is an asymptotic concept, or rather, it is truly measured in procedures performed in an infinite scale of time. Contrastingly, in their work on the dynamics of ruminal degradation of feed produced in tropical and non-tropical situations, Detmann et al. (2008) insist that the definition is inefficient for tropical conditions. In fact, *in situ* procedures are based on finite scales of times, assuming a relatively high temporal rate to materialize the asymptotic concept.

Nylon, the standard material for *in situ* incubation procedures (Nocek, 1997), showed ambiguous rates in estimates of *in situ* indigestible residues for 240h, due to particle loss through the pores of nylon bags (Piaggio et al., 1991) and to the integrity of the material after procedures to determine iDM and iNDF (Valente et al., 2011). The Ankom® system, synthetic fiber bags insoluble in acid and neutral medium, has been used for more precise and favorable results (Berchielli, Sader, Tonani, Paziani, & Andrade, 2001). However, high costs in routine feed analysis in the system shifted usage towards nonwoven textile (NWT), weight 100 g m^{-2}, as an alternative (Casali et al., 2009).

However, there is a decrease in the integrity of the material in long incubation periods, even though no difference exists when compared to the Ankom® system (Valente et al., 2011). Since preciseness of results is required, Kuwahara et al. (2015) enhance the existence of variability in the quality of the material on the Brazilian market.

Results by Casali et al. (2008) showed different critical times (hours) of incubation for feeds with regard to indigestible dry matter (iDM): 170.8 (elephant grass - 73.13% NDF); 139.1 (Brachiaria hay - 77.31% NDF); 137.5 (sugarcane - 50.70% NDF); 207.8 (corn silage - 3.0 mm - 51.07% NDF); 217.2 (corn straw - 89.84% NDF). In fact, results reveal that rates of fibrous components of feed may be directly related to incubation time. Although Casali et al. (2008) reported difference in incubation time for sugarcane only, with different grindings, Kuwahara et al. (2015) registered that alfafa ground at 0.5 mm (37.84% NDF) overestimated iDM and iMDF rates. According to Casali et al. (2008), 3.0 mm grindings may cause lack of precision in results, attributing the smallest specific surface for microbial activities (Nocek, 1997). At the same time, it should be underscored that a greater particle size may favor the sample's non-homogeneity with regard to the amount of incubated material (20 mg cm^{-2}), especially in roughage, corroborating the lack of precision in results. The standardization of the sample preparation with 2 mm sieves is recommended for *in situ* protocols (Hvelplund & Weisbjerg, 2000; National Research Council [NRC], 2001)

Current assay therefore evaluates the types of material for the conditioning of samples in the rumen of animals, particle size and incubation time on the estimates of indigestible compounds in Dry Matter (iDM) and indigestible Neutral Detergent Fiber (iNDF) in alfafa.

Material and methods

Current experiment was conducted at the Laboratory of Animal Nutrition of Embrapa Pecuária Sudeste, São Carlos, São Paulo State, Brazil. Samples of alfafa (*Medicago sativa* L.), harvested at the Embrapa unit during the summer, at the phenological stage in which 10% of the plants featured floral buds, were used. Samples, divided into three sub-samples, were pre-dried in a forced air buffer at 60ºC for 72 hours, following Silva & Queiroz (2002), and ground in a Wiley mill. Treatments consisted of three grinding processes, with adjustment of knives and sieve porosity (1.0, 2.0, 3.0 mm), three types of materials to condition the samples in the animal's rumen (Ankom® F57, NWT and nylon) and 13 incubation times at 24-h intervals. A sub-sample of approximately 300 g was removed and the fractioning of particle sizes was quantified in each grinding treatment. A vibration sieve (Retsch® AS 200), with a set of 9 sieves, was employed at a 20-min. sieving period.

Samples were analyzed for dry matter (DM), mineral matter (MM) and crude protein (CP) rates, following Silva & Queiroz (2002), given in Table 1. The chemical composition of alfafa comprised 37.8% neutral detergent fiber (NDF), 30.6% acid detergent fiber (ADF) and 7.4% lignin, following methodology by Van Soest and Robertson (1985).

Table 1. Chemical composition of alfafa in different grindings.

Ingredient	DM[1]	MM[2]	CP[3]	
Alfafa – 1.0 mm	94.68	9.38	22.17	cv 1.21
Alfafa – 2.0 mm	94.24	9.62	22.58	cv 1.92
Alfafa – 3.0 mm	94.82	9.62	20.81	cv 5.18

[1]DM = Dry Matter - 105ºC (%); [2]MM = Mineral Matter (%); [3]CP = Crude Protein (%); [4]cv = Coefficient of variation (%).

Alfafa samples were weighed and conditioned in F57 (Ankom®), nonwoven textile (NWT 100 g m^{-2}) and nylon textile (50 μm) bags to evaluate rates of indigestible dry matter (iDM) and indigestible neutral detergent fiber (iNDF). NWT and nylon bags measuring 4 x 5 cm were made. Samples were conditioned in all bags with 20 mg of DM per cm^2 (Nocek, 1997).

Experimental design consisted of randomized blocks with a 3 x 3 x 13 factorial design (three types of grinding; three types of material; 13 incubation intervals, with eight replications, involving two animals. Bags were incubated in the rumen of two cross-breed Holstein x Jersey cows fed on a 70:30 (roughage: concentrate) diet. Incubation period totaled 288 hours, with 24-h intervals, for the composition of each incubation time, with 13 periods: 0, 24, 48, 72, 96, 120, 144, 168, 192, 216, 240, 264, and 288 hours. Samples were analyzed directly for NDF rates at zero time for the respective materials and grindings. After each incubation time, the bags were removed, washed in running water till total whitening, dried at 105ºC for three hours, conditioned in a dryer, cooled and weighed on an analytic scale to determine non-digestible dry matter. Further, extraction on the same material and textile used for incubation was undertaken with neutral detergent solution (Mertens, 2002) by Ankom® fiber analyzer equipment (American Oil Chemists' Society [AOCS], 2009).

Data were submitted to analysis of variance (Anova, $p < 0.05$) and averages were compared by tukey's test ($p < 0.05$) with Statistical Analysis System (SAS, 2003).

Results and discussion

Figure 1 shows size particles of each grinding. All treatments provided particle size less than expected. Most sieves and knives adjusted to 3.0 mm had particle sizes ranging between 0.5 and 1.5 mm, with significant dispersion when compared to 1.0 and 2.0 mm grinding. Greater homogeneity in particle size was better for the two treatments. Greater preciseness should be expected for laboratory analyses, as Table 1 shows, where CP rates were more exact with more intense grinding, also reported by Casali et al. (2008). These authors added that grinding with 2.0-mm-pore sieve may be suggested for the standardization of samples in *in situ* analyses (NRC, 2001).

According to Ørskov (2000), the indigestible fraction is the exclusive characteristic of the substrate and not of the fermentation medium or particularity of the experimental material. Casali et al. (2008) did not report possible loss of the particle related to textile porosity, with the exception of sugarcane where the indigestible fraction was higher in 3 mm (32.07%) when compared to 2mm (28.63%) and 1 mm (28.51%). The authors also registered that if real loss of particle occurred, iNDF and iADF rates must also be affected, which was not observed by the authors. They also reported that iDM fraction is made up of essentially insoluble components, similar to the iDF and iADF fractions. Although data in current experiment corroborate the theory by Casali et al. (2008), Kuwahara et al. (2015) reported that more intense grindings may overestimate iDM and iNDF rates at an average of 6.57% for alfafa when grinding shifted from 1.5 mm to 0.5 mm for a fixed period of 240 hours.

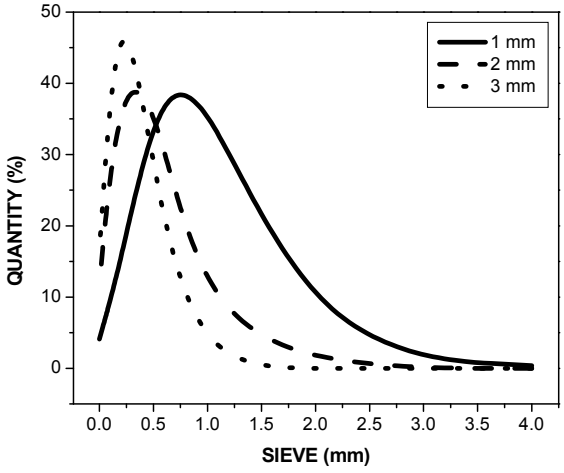

Figure 1. Quantity of sample (%) per sieve (mm) in different grindings for alfafa. Amount of fractioning of particle sizes per vibratory sieve Retsch® AS 200.

When the concept by Mertens (1993), previously discussed, is taken into consideration, coupled to the description by Detmann et al. (2008) as a slightly precise concept, results of current analysis (Figure 2 and Tables 2 and 3) reveal that the nylon textile in the three grindings had lower iDM and iNDF rates when compared to other treatments ($p < 0.05$) in the initial period of incubation. After 96 hours of incubation, all materials and grindings are likely to have similar rates where the differences between material, times and grinding may be due to the contamination of F57 and TNT textiles, as reported by Casali et al. (2009) and Kuwahara et al. (2015).

At zero time, direct extraction in neutral detergent solution in Ankon® fiber analyzer, nylon textile in 1.0 and 2.0 mm grindings overestimated NDF rates (Table 3) where, according to Huhtanen Kaustell, and Jaakkola (1994), the exactness of results and possible loss of particle are compromised, as reported by Casali et al. (2009). Results in current assay corroborate these theories where, despite less intense grindings in treatments with 3.0 mm particles, also demonstrated particles lower than 0.5 mm (Figure 1) which, besides triggering the loss of particles, also affects the instability of results. Although textile integrity was not evaluated, Valente et al. (2011) report the

decrease of resistance to NWT and Nylon tension when compared to F57, after the determination of iDM and iNDF where the possible influence of results in reuse is related.

When Casali et al. (2009) compared the integrity of the materials nylon, Ankom® and nonwoven textile by electronic photomicrographs, they failed to report any difference in the integrity of pre- and post-incubation nylon. According to these authors, NWT and F57 provided structures that might limit the introduction of microorganisms and the exit of non-degraded fibrous particles. According to Udén, Parra and Van Soest (1974), pore reduction may decrease the elimination of gases from fermentation, with a lower degradation of NDF which may explain greater iNDF estimates (Casali et al., 2009). However, Lindberg and Knutsson (1981), quoted by Udén and Van Soest (1984), evaluated the removal of particles in *in situ* incubation procedures in textile with different porosities (5, 10 and 20 μm) and verified less fibrous residues for the reduction of porosity in the initial incubation periods. However, after 24 hours of incubation, the authors reported that degradation curves converged, while residues were considered similar for all types of porosity after 72 hours. Results corroborate a behavior similar to current analysis (Tables 2 and 3), contrastingly to Huhtanen et al. (1994) and Casali et al. (2009).

Casali et al. (2009), Valente et al. (2011) and Kuwahara et al. (2015) did not report significant differences between the textiles Ankom® and NWT, with the later coming up as an alternative. According to Detmann et al. (2008), different materials may require distinct protocols to estimate the indigestible fraction of DM, NDF and ADF, confirmed by Casali et al. (2008) in the critical periods between indexes and feed.

Corrêa, Magalhães and Siqueira (2012) evaluated the degradability of dry matter and the fibrous fraction of the leguminous *Stylozanthes guianensis* using 5.0 mm grindings and nylon bags (50 μm) and reported that degradability rates remained constant after 48 hours of incubation, with a 75.70% effective degradability for DM, 59.01% for NDF and 76.81% for ADF. Stability after this period was also reported by Santos et al. (2012) when they used silage with elephant grass (*Pennisetum purpureum* Schum.), nylon textile (50 μm) and 2.0 mm grinding. Figure 2 demonstrates similarity in results in current analysis when nylon was employed.

Similarity in iDM and iNDF rates at the last period of incubation suggests that differences in initial times for nylon may be related to high rates of ruminal degradation of alfafa plus a greater fluidity of the material when compared with F57 and TNT. In fact, they provide a slower rate in bacterial activity in the degradation of NDF as they limit the exit of degraded material. Undesired materials after the incubation process may be eliminated in the fiber extraction process, with inconsistent rates.

Table 2. Non-digestible dry matter in different grindings, materials and incubation times[1].

Time	1.0 mm			2.0 mm			3.0 mm		
	NWT	F57	Nylon	NWT	F57	Nylon	NWT	F57	Nylon
0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
CV [2]	-	-	-	-	-	-	-	-	-
1	43.79^{Ba}	47.41^{Aa}	27.88^{Da}	40.92^{Ba}	47.52^{Aa}	27.66^{CDa}	43.45^{Ba}	49.62^{Aa}	31.17^{Ca}
CV	10.98	6.85	32.14	13.89	9.13	18.01	12.10	8.82	9.52
2	27.75^{BCb}	35.71^{Ab}	18.79^{Cb}	29.77^{BCb}	35.45^{Ab}	20.85^{Cb}	33.73^{Bb}	38.58^{Ab}	22.96^{Cb}
CV	16.82	5.30	10.70	8.80	9.25	4.68	4.28	7.53	8.15
3	23.49^{Dbc}	27.58^{Bc}	20.34^{Db}	25.85^{BCbc}	31.76^{Abc}	23.45^{CDb}	26.89^{BCc}	30.01^{Abc}	25.12^{Cb}
CV	6.39	13.46	9.22	11.70	14.39	10.83	11.50	7.77	8.05
4	23.22^{CDbc}	25.86^{BCc}	20.24^{Db}	23.96^{Cc}	28.73^{ABcd}	23.11^{Cb}	24.64^{Cc}	29.22^{Ac}	26.16^{ABCb}
CV	11.74	7.11	4.53	10.42	5.24	8.58	7.48	4.75	15.40
5	23.09^{BCbc}	24.94^{BCcd}	20.19^{Cb}	24.72^{BCc}	27.68^{ABcd}	22.11^{BCb}	27.07^{ABc}	28.06^{Ac}	24.05^{Bb}
CV	9.46	12.84	10.58	11.44	14.82	5.56	7.79	7.77	10.72
6	22.12^{Cc}	24.34^{BCcd}	22.15^{Cb}	24.15^{ABCc}	27.74^{Acd}	23.98^{BCb}	26.28^{ABc}	26.32^{ABcd}	23.84^{BCb}
CV	5.85	6.84	21.89	4.31	5.06	13.42	11.62	9.82	12.31
7	19.53^{Cc}	23.54^{BCcd}	23.07^{BCb}	21.43^{BCc}	22.35^{BCd}	24.44^{Bab}	23.07^{BCc}	27.55^{Ac}	25.49^{Bb}
CV	4.83	11.32	13.96	10.69	9.69	7.75	4.95	7.33	7.27
8	20.75^{Cc}	24.34^{BCcd}	26.11^{ABab}	22.44^{Cc}	27.42^{ABcd}	27.16^{Aa}	24.39^{BCc}	28.92^{Ac}	25.53^{Bb}
CV	7.47	5.93	15.97	5.65	6.00	10.43	4.73	2.98	25.77
9	21.80^{Cc}	24.92^{BCc}	23.50^{ABCab}	22.69^{BCc}	23.83^{BCd}	26.00^{Aab}	25.62^{ABc}	26.56^{ABCcd}	26.29^{Ab}
CV	10.34	9.65	12.45	8.26	5.76	13.92	12.28	18.50	6.46
10	20.72^{Bc}	25.34^{ABc}	21.71^{Bb}	23.41^{Bc}	25.39^{ABd}	25.26^{ABab}	23.28^{Bc}	26.69^{Acd}	26.15^{ABb}
CV	9.72	6.83	19.42	5.72	12.76	10.28	10.69	12.30	19.33
11	22.11^{Dc}	26.17^{BCc}	23.25^{CDab}	22.52^{Dc}	28.31^{ABcd}	23.56^{CDb}	23.76^{CDc}	30.82^{Ac}	25.01^{BCb}
CV	12.07	8.03	20.00	9.04	13.29	10.23	8.34	13.29	11.20
12	19.60^{Bc}	20.39^{Bd}	21.35^{Bb}	21.82^{ABc}	22.66^{ABd}	21.21^{Bb}	25.21^{Ac}	22.82^{ABd}	24.42^{ABb}
CV	3.37	13.24	20.26	2.71	12.63	14.62	2.44	22.26	4.51

[1]Rates of indigestible dry matter in alfafa in three grindings, materials and incubation times (%); [2]CV = Coefficient of Variation (%). Capital letters denote significant differences among materials of each grinding; small letters denote significant differences between incubation times; means are compared by tukey's test ($p < 0.05$).

Table 3. Indigestible neutral detergent fiber in different grindings, materials and incubation times[1].

Time	1.0 mm			2.0 mm			3.0 mm		
	NWT	F57	Nylon	NWT	F57	Nylon	NWT	F57	Nylon
0	38.40^{Ba}	42.85^{Aa}	34.22^{Ca}	36.80^{Ba}	44.17^{Aa}	35.73^{BCa}	39.08^{ABa}	40.34^{ABa}	37.58^{Ba}
CV [2]	10.52	2.36	1.64	6.91	1.70	2.63	5.53	2.85	5.93
1	31.77^{Ab}	30.35^{Ab}	18.20^{Cbc}	30.17^{Ab}	31.11^{Ab}	18.79^{Cb}	32.50^{Ab}	32.97^{Ab}	22.39^{Bb}
CV	10.35	6.87	27.33	12.88	7.42	15.10	8.94	10.71	12.75
2	20.17^{Bc}	20.91^{Bc}	12.92^{Cd}	21.78^{ABcd}	21.96^{ABc}	14.28^{Cc}	25.19^{Ac}	24.43^{Ac}	16.62^{Cc}
CV	11.36	7.54	11.59	6.77	5.97	4.62	5.70	6.18	9.88
3	17.94^{Bd}	16.79^{Bd}	13.70^{Cd}	19.25^{Abd}	19.39^{Acd}	15.74^{Bc}	21.99^{Acd}	18.17^{Bd}	18.23^{Bc}
CV	4.69	11.26	7.09	8.23	13.59	13.01	7.84	9.05	11.83
4	17.38^{Bd}	15.04^{Cd}	12.19^{Dd}	18.17^{ABd}	16.68^{Bd}	14.23^{Cc}	19.19^{Ad}	18.15^{ABd}	17.15^{Bc}
CV	4.78	4.75	6.22	6.47	5.26	13.94	7.65	4.23	10.60
5	17.66^{Bd}	14.95^{CDd}	12.94^{Dd}	18.97^{Bbd}	17.33^{BCd}	15.10^{Cc}	22.50^{Acd}	17.47^{Bd}	16.78^{Bc}
CV	5.69	12.62	10.04	6.52	15.10	9.03	8.82	8.41	13.96
6	16.89^{Bd}	15.56^{BCd}	14.21^{Cd}	17.99^{ABd}	17.68^{ABd}	16.13^{Bc}	19.70^{Ad}	16.61^{Bd}	15.70^{BCc}
CV	4.73	3.20	18.30	3.51	4.71	10.37	11.91	12.03	17.95
7	15.41^{Bd}	14.98^{Bd}	15.55^{Bcd}	16.98^{Bbd}	15.29^{Bd}	16.61^{Bc}	18.92^{Ad}	18.13^{Ad}	17.10^{ABc}
CV	2.86	8.42	13.57	6.58	4.42	5.27	6.00	8.77	15.25
8	16.42^{Bd}	13.68^{Cd}	16.06^{Bcd}	17.48^{ABd}	15.28^{Bd}	18.22^{ABbc}	19.42^{Ad}	16.94^{Bd}	17.14^{ABc}
CV	2.89	4.19	13.58	4.02	2.29	11.85	6.13	10.84	22.56
9	16.92^{Bd}	14.89^{Cd}	14.47^{Cd}	17.86^{Bd}	15.61^{Cd}	16.37^{BCc}	19.95^{Ad}	17.03^{Bd}	17.16^{Bc}
CV	5.25	5.13	10.27	4.99	4.19	11.97	9.35	13.89	7.91
10	16.11^{ABd}	13.65^{Cd}	14.61^{BCd}	17.40^{Ad}	14.64^{BCd}	17.84^{Abc}	18.83^{Ad}	15.35^{Bd}	17.97^{Ac}
CV	5.05	7.34	10.10	5.67	8.35	5.96	9.41	14.87	10.29
11	17.46^{Bd}	13.66^{Dd}	14.11^{CDd}	18.18^{Ad}	15.40^{Cd}	15.89^{Cc}	18.78^{Ad}	16.97^{BCd}	18.15^{ABc}
CV	7.43	4.76	11.69	6.01	12.63	5.68	8.65	12.63	9.85
12	16.02^{Cd}	17.99^{BCd}	12.99^{Dd}	18.02^{BCd}	19.70^{Bcd}	13.64^{Dc}	21.00^{Ac}	19.29^{Bd}	16.17^{Cc}
CV	3.19	4.41	13.13	4.08	5.74	11.28	2.85	20.52	5.05

[1]Indigestible Neutral Detergent Fiber rates in alfafa in three grindings, materials and incubation times (%); [2]CV = Coefficient of Variation (%).Capital letters denote significant differences among materials of each grinding; small letters denote significant differences between incubation times; means are compared by tukey's test ($p < 0.05$).

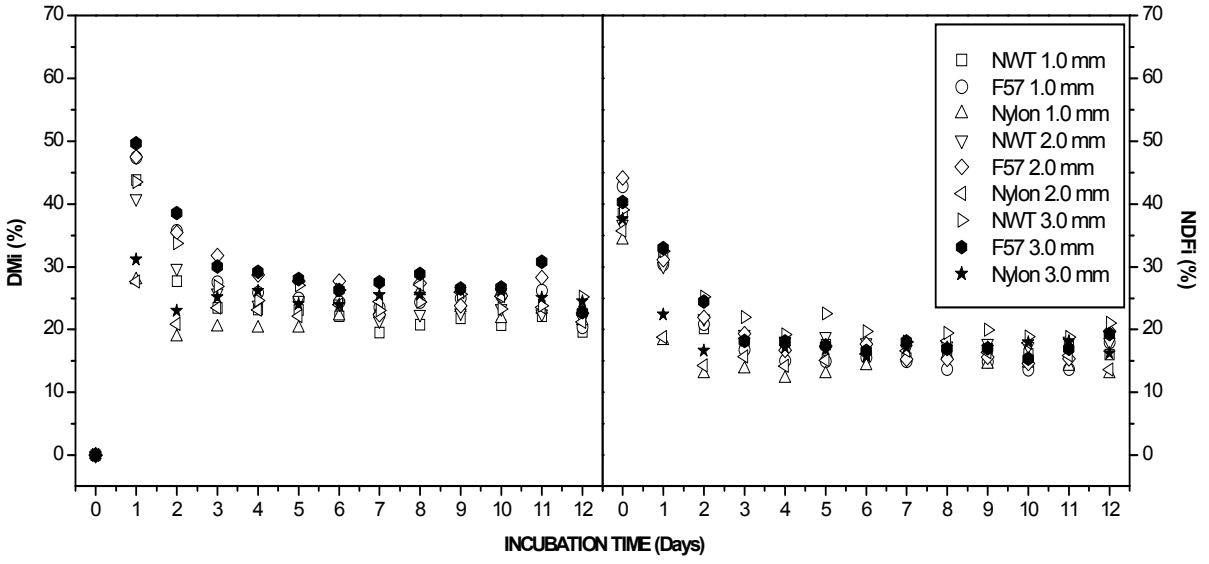

Figure 2. Mean rates of indigestible dry matter – iDM (%) and indigestible neutral detergent fiber – iNDF (%) in different grindings, materials and incubation times.

Inconsistency of data in the literature for the standardization for *in situ* procedures and internal consumption markers suggests further research for incubation time, material and textile to condition material in the rumen. Since nylon requires different grindings and F57 has high costs for routine lab tests, NWT may be an alternative due to its availability on the Brazilian market and to the quality of the material for preciseness in results. However, caution should be taken in the use of NWT in *in situ* procedures.

Conclusion

Nylon inconsistencies to determine dry matter and indigestible fibrous fraction of food recommends F57 textile (Ankom®) to determine indigestible fiber rates in neutral detergent by the *in situ* technique due to exactness of results. Since F57 textile is expensive for routine lab tests, the nonwoven textile (100 g m^{-2}) may be its substitute due to estimates similar to those of F57. Since

several divergences are extant in comparative studies, further research work on the material must be accomplished for its use so that standardization of results may be obtained.

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