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Estimates of *in situ* digestibility and fibrous compounds in feeds for ruminants

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ABSTRACT. Current paper assesses the relationship between *in situ* incubation time, particle size and types of materials on estimates of indigestible fractions of dry matter (iDM) and neutral detergent fiber (iNDF) in cattle feed and feces. Samples of soybean meal, alfalfa and feces of cattle fed on high concentrated diets were analyzed. The samples were processed in a Wiley mill, with 0.5 and 1.5 mm porosity for food and 1.5 mm for feces, which were packed in 4 × 5 cm F57 bags (Ankon®) and nonwoven fabric (100 g m^{-2}) of two brands. The material was divided into two groups, with and without treatment with acetone, and five replications. Samples of each group were incubated in the rumen of a multiparous cow adapted to a 70:30 diet, forage:concentrate diet, for 240 hours. The iDM and iNDF levels were evaluated sequentially to interpret the percentages of undigested material. Data underwent analysis of variance (ANOVA, $p < 0.05$) and means were compared by Tukey's test ($p < 0.05$). Material, size of particles and incubated material affected iDM and iNDF estimates.

Keywords: digestion, neutral detergent fiber, nonwoven fabric, F57.

Estimativa de digestibilidade *in situ* e componentes fibrosos em alimentos para ruminantes

RESUMO. A premissa é avaliar a relação do tempo de incubação *in situ*, tamanho de partículas e tipos de materiais sobre as estimativas das frações indigestíveis da matéria seca (MSi) e da fibra em detergente neutro (FDNi) em alimentos e fezes bovinas. Estudaram-se amostras de farelo de soja, alfafa e fezes de bovinos alimentados com dietas com alto concentrado. As amostras foram processadas em moinho de facas com peneiras de porosidade 0,5 e 1,5 mm para alimentos e 1,5 para fezes, acondicionadas em sacos de F57 (Ankon®) e tecido não tecido (100 g m^{-2}) de duas marcas na dimensão 4 × 5 cm. Os materiais foram divididos em dois grupos, com e sem tratamento com acetona e cinco repetições, incubadas no rúmen de uma vaca multípara adaptada à dieta 70:30, volumoso:concentrado, respectivamente, por 240h. 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 tipo de material utilizado, tamanho de partículas e material incubado sobre as estimativas de MSi e FDNi.

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

Introduction

The use of intake internal indexes, such as the indigestible fraction of food, is a highly promising tool in studies on animal nutrition (Van Soest, 1994), since it estimates such parameters as fecal production, digestibility coefficients, food ingestion and nutrient flow in the animals' gastrointestinal tract (Berchielli et al., 2000; Zeoula et al., 2002). Among the intake internal markers, the insoluble and indigestible fibrous fraction of food, specifically indigestible neutral detergent fiber (iNDF) has been widely employed as an internal index due to its greater precision (Piaggio et al., 1991; Zeoula et al., 2000).

In spite of several studies, there are many divergences on the incubation period required to represent the indigestible fraction in the rumen. The literature gives 96h (Ruiz et al., 2001), 120h (E. Detmann et al., 2004), 144h (Berchielli et al., 2000), 192h (Zeoula et al., 2002) and 288h (Huhtanen et al., 1994). According to Berchielli et al. (2000), food digestibility decreases as the sample's exposure time to the rumen micro-biota is reduced, providing

an unreal reproduction of the samples' indigestible fraction. On the other hand, a longer period may damage the material due to rumen mobility, and bag pores may be obstructed due to the disposal of degraded matter or rumen microorganisms.

However, results by Huhtanen et al. (1994) that *in vivo* dry matter digestibility may be predicted with great precision when the fiber's indigestible wastes from *in situ* procedures are evaluated and compared to estimates by *in vitro* procedures. *In vitro* incubations may have the disadvantage of particles adhering to the tube walls, preventing any contact with the rumen *inoculum* and increasing misleadingly post-incubation wastes (Freitas et al., 2002). Consequently, *in situ* procedures seem to be able to give more correct results for internal index concentrations in feed and feces. On the other hand, there are contradictions with regard to the implementation, fitness and performance in *in situ* methods. Further, the material used, grinding of ingredients, incubation time and type of food have not been standardized or validated for the constitution of protocols.

The relevance of studies on protocol standardization to estimate the rates of indigestible compounds in *in situ* procedures should be enhanced. Current analysis evaluates the type of materials, the post-incubation and post-extraction physical wholeness with neutral detergent, particle size at a fixed incubation time on the estimates of indigestible compounds in Dry Matter (iDM) and indigestible Neutral Detergent Fiber (iNDF) in the cattle feed and feces.

Material and methods

The experiment was performed at the Laboratory of Animal Nutrition of the Empresa Brasileira de Pesquisa Agropecuária (EMBRAPA) Pecuária Sudeste, São Carlos, São Paulo State, Brazil. Samples of soybean meal, alfafa (*Medicago sativa* L.) and cattle feces were used. Feed samples were collected on the cattle ranch and feces were harvested from high production crossbreed cattle (Holstein x Jersey) during lactation, fed on a roughage:concentrate (50:50) diet. The samples were pre-dried in a forced air buffer (60ºC for 72 hours) and processed in a Wiley mill with 0.5 and 1.5 mm-pore sieves. Milling with 0.5 mm pores was employed only for soybean meal and alfafa for better precision in the laboratory feed analysis.

Samples' dry matter (DM), crude protein (CP) and mineral matter (MM) were evaluated following Silva and Queiroz (2002); neutral detergent fiber (NDF) and acid detergent fiber (ADF) were assessed according to Van Soest et al. (1991). Further, 0.5 g of the sample were conditioned in previously dried and weighed nonwoven fabric bags to determine NDF and ADF in the samples of soybean meal and alfafa by fiber analyzer Ankon®. Table 1 shows results of the analysis.

Table 1. Chemical composition of cattle feed and feces.

Ingredients	DM[1]	CP[2]	NDF[3]	ADF[4]	Lignin[5]
Soybean meal	93.48	48.35	15.34	9.20	3.12
Alfafa	95.67	25.87	37.84	30.63	7.44
Cattle feces	91.78	12.22	65.22	34.78	2.40

[1]DM = Dry Matter - 105ºC (%); [2]CP = Crude Protein (%); [3]NDF = Neutral Detergent Fiber (%); [4]ADF = Acid Detergent Fiber; [5]Lignin (%).

Feed samples were weighed and conditioned in fabric F57 (Ankom®); nonwoven fabric (100 g m^{-2}) 'A'; nonwoven fabric (100 g m^{-2}) 'B' to evaluated rates of indigestible Dry Matter (iDM) and indigestible Neutral Detergent Fiber (iNDF). Treatments with and without acetone were performed for each material prior to weighing of samples and incubation of materials. Tissues were immersed in acetone for 5 minutes and treatments consisted of NWF 'A'; NWF 'B'; NWF 'A' + Acetone; NWF 'B' + Acetone; F 57; F 57 + Acetone, with five replications for each feed and for each treatment. NWF bags were sized 4 × 5 cm, with correction rates for each step and densities, as given in Table 2. Samples were conditioned in all the bags, at 20 mg of DM per square centimeter (Nocek, 1988).

Table 2. Correction and density rates of Nonwoven Fabric (NWF).

Material[1]	DM*i*[2]	CV	NDF*i*[3]	CV[4]	Dens.[5]	CV
NWF 'A'	0.0477	6.70	0.0240	6.62	100.79	5.14
NWF 'B'	0.0941	8.44	0.0523	10.25	106.41	5.40
F 57	0.0448	7.24	0.0292	7.80	-	-
NWF 'A' + Acetone	0.0639	6.56	0.0322	8.43	103.25	7.64
NWF 'B' + Acetone	0.0997	17.61	0.0533	12.85	104.51	5.75
F 57 + Acetone	0.0599	6.56	0.0375	15.17	-	-

[1]Materials for the two treatments, with and without acetone; [2]DM*i* = correction rates of control (CRC) after the incubation period (mg); [3]FDN*i* = correction rates of control after the extraction process of NDF (mg); [4]CV = Coefficient of Variation (%); [5]Dens. = Density of materials (g m^{-2}). Rates were calculated based on total size of bags after manufactured. Correction rates for control were obtained after each process, dried in non-ventilated buffer (105ºC for 3 hours) and conditioned in a desiccator for 1 hour.

The bags were incubated for 240h (E. Detmann et al., 2001) in the rumen of a Holstein x Jersey crossbreed cow, fed on a roughage:concentrate (70:30) diet. After incubation, the bags were removed, washed in running water till their complete clarification, dried and weighed to determine the indigestible dry matter; extraction with ND solution was performed (Mertens, 2002) by fiber analyzer equipments Ankom$^{200®}$. After extraction, the bags were washed in hot water and acetone, and dried at room temperature for 15 min. and afterwards in a buffer at 105ºC for 3 hours.

The bags were then conditioned in a desiccator and weighed on an analytic scale after cooling.

Fragments from the tissues of all the bags were fixed with carbon glue in metal holders and gold-covered (20 nm) with a Sputter Coater SCD050 / LEICA for electronic microscopy analysis. They were evaluated at 150 X magnification by Scanning Electron Microscope (SEM) (JSM-6510 / JEOL) by secondary electrons with acceleration tension of 5 KV.

Data underwent analysis of variance (ANOVA, $p < 0.05$) and means were compared by Tukey's test ($p < 0.05$), with Statistical Analysis System (SAS, 2004).

Results and discussion

iDM and iNDF rates for soybean meal, alfafa and cattle feces (Table 3) were different ($p > 0.05$) and higher rates were reported for feces samples followed by alfafa and soybean meal. As a rule, results obtained with F57 were higher ($p > 0.05$) when compared with other materials with or without acetone treatment.

Table 3. Indigestible Dry matter and Indigestible Neutral Detergent Fiber.

Ingredients[1]	Materials	iDM[2]	SD[3]	iNDF[4]	SD
	NWF 'A'	-0.99^{Aa}	1.52	-0.01^{Aa}	0.69
	NWF 'B'	-3.00^{ABa}	3.66	-3.52^{Ba}	1.23
Soybean meal	F 57	-0.44^{Aa}	1.15	-3.41^{Ba}	0.43
(1.5 mm)	NWF 'A' + Acetone	-5.54^{Ba}	1.68	-2.69^{Ba}	1.01
	NWF 'B' + Acetone	-3.83^{ABa}	1.90	-3.52^{Ba}	0.88
	F 57 + Acetone	-2.55^{ABa}	1.85	-4.47^{Ba}	0.76
	NWF 'A'	19.48^{Bb}	1.67	14.61^{Cb}	0.96
	NWF 'B'	19.69^{Bb}	2.09	13.38^{BCb}	1.30
Alfafa	F 57	24.42^{Ab}	0.96	12.97^{BCb}	0.56
(1.5 mm)	NWF 'A' + Acetone	17.77^{Bb}	1.92	13.34^{BCb}	1.03
	NWF 'B' + Acetone	17.47^{Bb}	1.59	11.76^{ABb}	1.39
	F 57 + Acetone	19.54^{Bb}	1.18	10.39^{Ab}	0.56
	NWF 'A'	52.23^{BCd}	2.03	35.54^{Cd}	1.96
	NWF 'B'	48.92^{Cd}	3.04	31.99^{ABd}	1.84
Cattle feces	F 57	58.26^{Ad}	1.29	33.68^{BCd}	0.47
(1.5 mm)	NWF 'A' + Acetone	44.45^{Dd}	4.56	30.55^{Ad}	3.03
	NWF 'B' + Acetone	44.44^{Dd}	3.84	29.92^{Ad}	1.80
	F 57 + Acetone	53.68^{Bd}	2.99	31.93^{ABd}	1.42
	NWF 'A'	0.23^{Aa}	2.78	-0.05^{Ba}	1.03
	NWF 'B'	-1.88^{Aa}	0.74	-3.28^{Aa}	0.08
Soybean meal	F 57	-0.17^{Aa}	0.42	-3.42^{Aa}	0.21
(0,5 mm)	NWF 'A' + Acetone	-2.78^{Aa}	1.60	-1.36^{ABa}	0.62
	NWF 'B' + Acetone	-2.95^{Aa}	2.64	-3.01^{Aa}	1.18
	F 57 + Acetone	0.40^{Aa}	0.74	-3.37^{Aa}	0.28
	NWF 'A'	26.35^{ABc}	1.18	21.49^{Cc}	0.50
	NWF 'B'	26.31^{ABc}	2.36	19.27^{BCc}	0.94
Alfafa	F 57	28.48^{ABc}	0.15	18.28^{ABc}	0.02
(0.5 mm)	NWF 'A' + Acetone	21.85^{Cc}	0.11	18.66^{Bc}	0.25
	NWF 'B' + Acetone	29.91^{Ac}	1.71	21.05^{Cc}	0.25
	F 57 + Acetone	25.84^{Bc}	3.10	16.13^{Ac}	2.70

[1]Feed after two grindings and cattle feces; [2]iDM = indigestible Dry Matter (%); [3]iNDF = indigestible Neutral Detergent Fiber (%); [4]SD = Standard Deviation. Capital letters mean significant differences between materials; small letters mean significant differences between the ingredients; means are compared by Tukey's test ($p < 0.05$).

Results are very similar to those by Casali et al. (2009) when they compared NDF and ADF in food samples obtained from NWF and F57 bags. Since these authors did not report any significant difference between the tissues, the use of NWF as alternative material becomes possible. Although response to current analysis was similar, iDM and iNDF had overestimated rates for soybean meal in all types of material. Rates may actually been directly related to incubation time. According to Detmann et al. (2007), different materials may require different protocols to estimate the indigestible fraction of DM, NDF and ADF. Casali et al. (2008) later confirmed the great variability of critical periods between indexes and food, from 87.8h (iDM in wheat meal) to 268.6h (iNDF for corn silage with a 3 mm-size particle). Different incubation times should be adopted for different food types for a higher precision of results with fibrous compounds.

The period of rumen incubation is one of the variables with the greatest influences on the representation of non-digested wastes in *in situ* incubation procedures. Casali et al. (2008) registered a 43.74% rate for iDM and 52.56 for iNDF in cattle feces from high concentrated diets. The above rate was similar for iDM, with the exception of material F57 and NWF 'A', with rates above 52.23%. Moreover, iNDF rates were lower, averaging 32.26%. However, Casali et al. (2008) used critical times 167.8h for iDM and 186.9h for iNDF, whereas a fixed time of 240h was used in current study. This period may have also overestimated iDM rates for soybean meal, as Berchielli et al. (2000) underscored. Above result goes against the theory by Mertens (1993) in which the indigestible fraction represents the fraction which is incapable of being used by microbial and animal enzymatic systems when there is no limit in exposure time. Casali et al. (2008) obtained 4.54% at a critical time of 88.4h for all materials and millings. However, other factors such as milling, NDF and ADF of food may be related, besides incubation time.

Casali et al. (2008) did not report any significant difference ($p > 0.05$) between the particle sizes (1, 2 and 3 mm) in some materials. Different rates for iDM occurred in the case of sugar cane due to the size of the particle, greater for 3 mm milling. The occurrence of contaminants reported by Huhtanen et al. (1994) and Casali et al. (2009) showed that low fiber quality enhanced a more intense microbial activity. Indigestible fiber rates for alfafa for 0.5 mm milling were greater when compared to 1.5 mm milling ($p < 0.05$) which was probably related to the smallest particle size providing a greater surface for microbial degradation, as Figure 2 and Table 3 show. In fact, iDM rates for F57 were greater and they suggested that, in spite of the fact that milling

provided a greater specific surface, F57 restricted microbial activity during the incubation period analyzed. The contamination of the material evidenced this fact by a more intense activity of the microorganism (Figure 2A), missing in NWF and F57 + Acetone (Figure 2).

The electronic photomicrographs of the tissues in Figures 1 and 2 evidenced the physical wholeness of all the material co-related to blank materials for correction (Figure 1) after the process with ND. Further, iNDF rates did not have the same response standard of materials for iDM for the different matters, due to F57 at the specific time period, and indicates a slower bacterial activity in the degradation of NDF and a limitation to the discharge of degraded matter. The tissue's pore size may compromise the removal of gases derived from fermentation for F57 and NWF, respectively, according to Uden et al. (1974) and Nocek (1988).

After the incubation process, non-desirable materials (Figure 2A) may be eliminated in the fiber extraction process, at overestimated rates. In the case of F57+Acetone, the iDM and iNDF rates were higher than the material without acetone. Treatment with acetone (Figure 2B) may enhance a great fluidity with the medium when compared with the respective materials (BCO) without the food sample (Figure 2A and B). In the same materials where no sample existed for the activity of microorganisms, there was a smaller concentration of residue adherent to the material (Figure 1A and B), also reported by Casali et al. (2009).

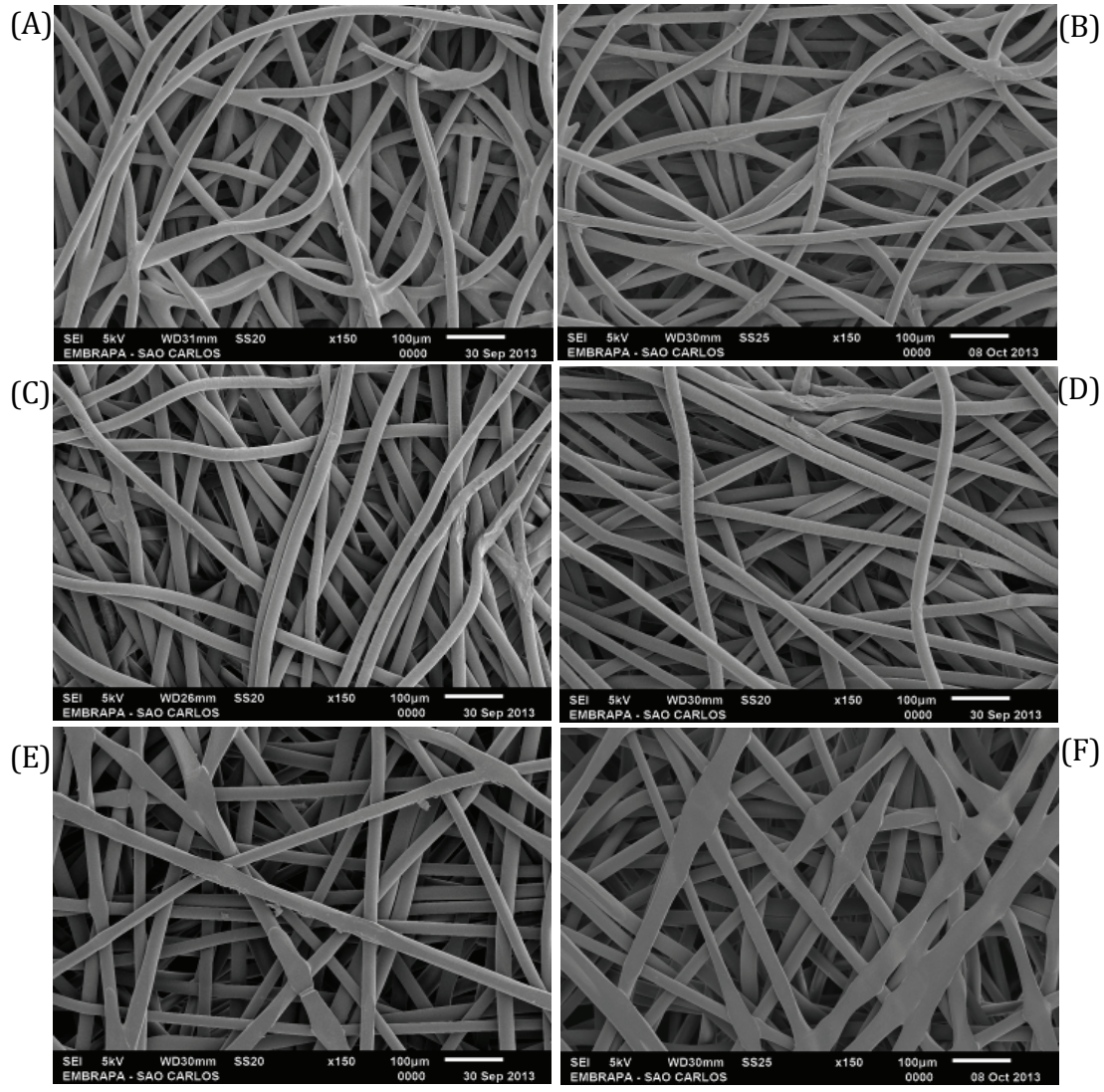

Figure 1. Scanning electron photomicrographs of blanks (BCO), (A) – F57; (B) – F57+ACETONE; (C) – NWF 'A'; (D) – NWF 'A'+ACETONE; (D) – NWF 'B'; (F) NWF 'B'+ACETONE. Images were taken with 150 x resolution after processes (*in situ* incubation and NDF extraction).

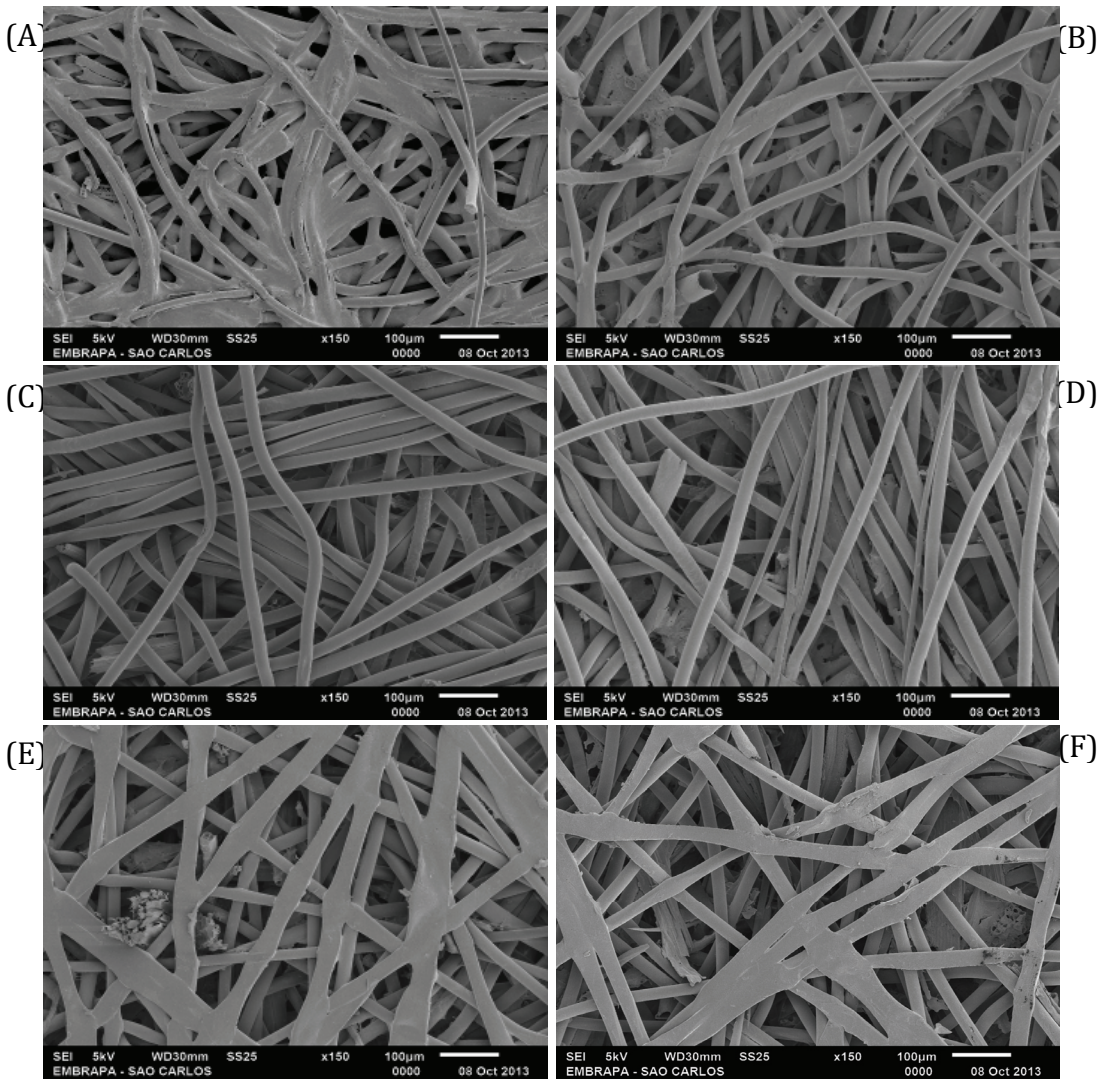

Figure 2. Scanning electron photomicrographs of materials incubated with alfafa, (A) – F57; (B) – F57+ACETONE; (C) – NWF 'A'; (D) – NWF 'A'+ACETONE; (D) – NWF 'B'; (F) NWF 'B'+ACETONE. Images were taken with 150 x resolution after processes (*in situ* incubation and NDF extraction).

Further studies on the exact quantification of the surface actually available for the microbial passage and discharge of degradation products with NWF have been suggested so that standards for *in situ* procedures and internal consumption markers could be obtained. Since NWF may be easily obtained in Brazil at a low price, one should focus on the quality of the product even though results obtained did not reveal any difference between the two types of NWF used. Caution should be taken for the general use of NWF in *in situ* procedures since rates of relative standard deviation for the material were higher when compared with those by F57 ($p > 0.05$).

Conclusion

Due to its preciseness of results, tissue F57 (Ankom®) is recommended to determine NDF rates by the *in situ* technique. The nonwoven fabric (NWF) (100 g m-2) may be a promising replacement for F57 for the quantification of indigestible fibrous compounds in food. It is not only a low-cost product but provides estimates at the same level as F57. Due to slight differences only between the two products, further research work on this material should be undertaken for routine analysis in laboratories for the standardization of results.

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