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RESEARCH PAPER

Improvement in nutritional quality of fibrous food via *in vitro* digestion by *Aspergillus niger*

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Abstract

A. Velásquez, P. Marnet, and R. Arias. 2015. Improvement in nutritional quality of fibrous food via *in vitro* digestion by *Aspergillus niger*. Cien. Inv. Agr. 42(1): 45-55. In a first experiment, the effect of *in vitro* incubation with *Aspergillus niger* (*An*) on the chemical composition of different fibrous substrates was studied. In a second experiment, the effect of incubation time (0, 72 and 144 h) on *in vitro* digestion with *An* of dry matter (IVDMD), neutral detergent fiber (DVNDF) and acid detergent fiber (DVADF) was evaluated for different substrates. Wheat straw, WS; barley straw, BS; oat hulls, OH; dehydrated alfalfa, DA; and dehydrated ryegrass, DB substrates were evaluated. In both experiments, incubations were performed in 250 mL Erlenmeyer flasks; 2 g of substrate was added to a culture medium (pH = 6), and incubated at 28 °C with constant ventilation. The *An* dose consisted of 3 mL of a solution of 5.3×10^6 spores mL^{-1} per flask. Incubation time for the first experiment was 144 h. After incubation, the contents of the flasks were homogenized in a blender for one minute. Subsequently, bromatological analysis was conducted without separating the *An* biomass. No interaction effects among any studied variables were observed. *An* incubation effected cell wall (NDF) and lignocellulose (ADF) content. An increase in true protein (TP) and soluble nitrogen (SN) was observed for all substrates tested, but responses differed depending on the type of substrate. The largest increases in TP were observed in DA and DB substrates (0.55 and 0.63% DM, respectively). IVDMD, DVNDF and DVADF were affected by the type of substrate and incubation time. The highest value of $IVDMD_{144}$ was observed in the DAAn treatment (49.47% DM), followed by the DBAn treatment, with a value of 45.51% DM. OHAn showed the lowest value of $IVDMD_{144}$ (29.38% DM). The results suggest that *An* possesses fibrolytic and metabolic potential for improving the nutritional value of fibrous foods through of the digestion of structural carbohydrates and the liberation of nitrogenous fractions embedded in cell walls, producing a change in the chemical composition and the potential digestibility of treated foods.

Key words: *Aspergillus niger,* fibrous substrates, i*n vitro* digestion, nutritional quality.

Introduction

High cellulose, hemicellulose and lignin content of fibrous foods are among the major factors responsible for the low nutritional values observed in livestock feed. This is not only because of the chemical composition of fibrous foods but also because of their low digestive potential in the animal's gastrointestinal system. These foods, under a biotechnology program

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using saprophytic fungi, could be modified to improve their nutritional quality by decreasing the cell wall content via digestion, and also by increasing nutrients obtained from the biomass of the microorganism growing on the incubated substrate (Kuhad *et al*., 1997; Shrivastava *et al*., 2012). Wheat straw (WS) and barley straw (BS) are important agricultural residues in the La Araucanía region and are usually burned or discarded. Furthermore, oat hulls (OH), an agro-industrial by-product, are also generated in significant quantities in this region of Chile. These fibrous foods have the potential to be used as animal feed, preferably in ruminants. However, the nutritional value of these foods is low, even for ruminants, due to their high lignocellulosic fractions and low crude protein (CP), thus limiting their use as dietary ingredients (Nagarajan, 2005; Arora *et al*., 2011). Indeed, bromatological measurements on WS and OH performed in our laboratory yielded values of 86.9% and 76% neutral detergent fiber (NDF), 55.6% and 42% acid detergent fiber (FDA), and 4.1% and 4.6% CP, respectively (Velásquez and Arias, 2013). Sarkar *et al*. (2012), in Pensupa *et al*. (2013), found WS to contain 35–45% cellulose, 20–30% hemicellulose, 8–15% lignin, 3.1% protein and 10.1% ash. The low digestibility of lignocellulosic fractions is due to lignin-covered polysaccharide microfibers, structured in a compact, crystalline, heterogeneous complex, where the lignin protects the polysaccharides against action by hydrolytic enzymes and other external factors, in addition to stabilizing the complex (Leonowicz *et al*., 1999; Villas-Boas *et al*., 2002). The high lignocellulosic content present in these agricultural residues and agro-industrial by-products inhibits the enzymatic action of the microbial community in the rumen, resulting in minimal degradation of organic matter, and consequently reduced availability of energy and nitrogen for the ruminal ecosystem (Sahoo *et al*., 2002; Shrivastava *et al*., 2012). Various techniques have been applied to improve the nutritional value of these fibrous foods, including physical, chemical and biological treatments (Liu *et al*., 1999; Tuyen *et al*., 2012). The biological methods include those based on the use of hydrolytic enzymes and microorganisms, particularly saprophytic fungi with fibrolytic potential (Zafar *et al*., 1996; Kuhad *et al*., 1997; Rodrigues *et al*., 2008; Gaitán-Hernández *et al*., 2011). These biological methods can improve the nutritional quality of fibrous foods by increasing true protein (TP) content (Velásquez *et al*., 2012), and improve digestibility by decreasing cell wall content (Kuhad *et al*., 1997; Villas-Boas *et al*., 2002). The advantage of biological methods for improving the nutritional quality of these foods over physical and chemical techniques is their low environmental impact, which generate little pollution and require little energy for their application (Basu *et al*., 2002; Shrivastava *et al*., 2012). *Aspergillus niger* (*An*) is a saprophytic fungus with potent fibrolytic activity, belonging to the phylum Ascomycota, class Ascomycetes, order Eurotiales and family Trichocomaceae. This enzymatic ability to digest cell walls, along with starches, simple sugars and proteins, make it a suitable fungus to grow on fibrous substrates (Hernández-Díaz *et al.,* 2010; Rajesh *et al*., 2010). These filamentous fungi are naturally adapted for growth on plant surfaces, which they use as support for the development of aerial mycelia and reproductive structures. This close contact with the substrate stimulates secretion of the hydrolytic enzymes responsible for extracellular digestion (Jones, 1994). Among the reported hydrolytic enzymes that can be synthesized by *An* are cellulases, exo-β-1,4-mannosidase, endo-α-1.5-arabinase, α-l-arabinofuranosidase, endo-galactanase, feruloyl esterase (Park *et al*., 2002; Howard *et al*., 2003; Sánchez, 2009), glucoamylase, xylanases, endo-β-1.4-glucanase and exo-β-1.4-glucanase (Kang *et al*., 2004; Pensupa *et al*., 2013). Considering this powerful pool of fibrolytic enzymes, *An* could degrade most fibrous substrates, using the carbon skeletons and energy generation to fuel reproduction and growth. Consequently, the first objective of this study was to evaluate the effect of *in vitro* incubation of different substrates with *An*

on the chemical composition of fibrous foods. The second objective was to study the effect of substrate type and incubation time on *in vitro* digestion of dry matter, neutral detergent fiber and acid detergent fiber by *An*.

Materials and methods

Experiment 1: Effect of in vitro incubation with Aspergillus niger on the chemical composition of fibrous substrates

An strains (ATCC-1015) were provided by the veterinary medicine school's microbiology laboratory (Catholic University of Temuco). *An* strains were seeded in Petri dishes with a potato dextrose agar culture medium, then dried in a forced air oven (Arquimed WTB Binder) at 26 °C for 4 days, and cooled and held at 4 °C until incubation. Incubations were performed in 250 mL Erlenmeyer flasks fitted with a glass and rubber stopper to ensure aerobic conditions during incubation (Robinson and Nigam, 2003; Velásquez and Arias, 2013). The flasks were sterilized with alcohol (98%) and UV radiation (Laminar Flow Cabinet: Streamline SHC-4A1) for 30 min. Two grams of substrate were added to a culture medium consisting of 8 mL of 50 mM Tris buffer (pH = 6), 2 mL of streptomycin antibiotic (0.1% w/v) and 2 mL of urea 1% (w/v). Urea was added to ensure that nitrogen was not a limiting factor for *An* metabolism. The dose of *An* (inoculum) per flask consisted of 3 mL of a solution with 5.3×10^6 spores mL^{-1} (determined by hemocytometer). This dose established a substrate-limiting kinetic condition, which was determined by preliminary experiments in our laboratory. Basal input of *An* to the culture medium was corrected by inoculum incubation in inert material, without organic substrate (Velásquez *et al*., 2012). The incubation time was 144 h under continuous ventilation (28 °C). After incubation, we homogenized the total contents of the flask in a blender for one minute. Subsequently, bromatological analysis was conducted on the total mass incubated, without separating the *An* biomass.

Collection and preparation of fibrous substrates

Triticum aestivum (wheat straw, WS), *Hordeum vulgare* (barley straw, BS), *Avena sativa* (oat hulls, OH), *Medicago sativa* (dehydrated alfalfa, DA) and *Lollium perenne* (dehydrated ryegrass, DB) were evaluated. Ten subsamples (0.5 kg) of each substrate were collected and were subsequently mixed. WS and BS were obtained from crop stubble randomly selected from within La Araucanía Region. OH was a by-product of the industrial processing of oats for human and animal consumption; samples were also randomly selected from La Araucanía Region agro-industries. DA and DB were cut before flowering from pastures established within the IX region (first cut), and then were processed in the laboratory. During collection, all samples were visually inspected for structural damage and contaminants; any impurities in vegetable materials were removed. In addition, substrates were checked for the presence of phytopathogenic agents by visual inspection, and also by microscopic examination in the laboratory. Similarly, OH was inspected for contaminating microbial agents and purity (visually and microscopically). All substrates (subsamples combined) were washed with distilled water for 20 minutes, then autoclaved in 250 mL flasks at 121 °C for 15 min, and finally dried in an oven at 60 °C for 48 h. Subsequently, samples were chopped (size range 0.2–0.5 cm). Later, all substrates were subjected to chemical composition analysis.

Chemical composition of fibrous substrates and generated biomass

Bromatological analysis was performed on fibrous substrates (Table 1) and post-incubation biomass (experiments 1 and 2). AOAC (1990) methods were used to determine dry matter (DM, official method 934.01), ash (official method 942.05) and N (official method 984.13) content. Neutral detergent fiber (NDF) and acid detergent fiber (ADF) were determined by the method of Van

Table 1. Chemical composition of fibrous substrates.

Substrate[1]	DM[2] %	Ash % DM	CP % DM	NDF[3] % DM	ADF % DM	TP[4] % CP	SN % CP	NDIN[5] % CP	ADIN % CP
WS	96.3[6]	8.1	4.0	86.2	54.6	85.0	22.8	48.5	26.3
BS	96.8	7.5	4.4	84.7	55.6	89.4	24.1	47.2	25.9
OH	97.1	7.4	4.5	86.3	65.4	86.5	18.3	41.3	26.8
DA	93.5	11.4	18.3	41.7	28,3	89.6	21.2	23.1	13.4
DB	94.2	12.3	11.2	57.8	34.5	90.4	14.3	32.1	15.9

[1]**WS: wheat** straw, BS: barley straw, OH: oats hull, DA: dehydrated alfalfa, DB: dehydrated ballica.
[2]**DM: dry** matter, CP: crude protein.
[3]**NDF: neutral** detergent fiber, ADF: acid detergent fiber.
[4]**TP: true** protein, SN: N soluble fraction.
[5]**NDIN: insoluble** N in neutral detergent, ADIN: insoluble N in acid detergent.
[6]**Each value** represents the chemical assay of three samples.

Soest *et al*. (1991). Fibrous fractions were determined with alpha amylase; these were expressed exclusive of residual ash. True protein (TP) was determined from buffer-insoluble N, and the fraction of trichloroacetic acid (TCA)-insoluble N to buffer-soluble N. Soluble N (SN) was measured by vacuum filtration (Whatman paper N°41) using the Kjeldahl method (AOAC, 1990). Insoluble N in neutral detergent fiber (NDIN) and insoluble N in acid detergent fiber (ADIN) were determined by measuring Kjeldahl N in the fiber residues.

Experiment 2: Effect of incubation time on the dry matter, neutral detergent fiber and acid detergent fiber digestion of different substrates digested with Aspergillus niger (An)

Using the same incubation protocol as the first experiment, a second trial measured the effect of substrate type and incubation time (0, 72 and 144 h) on the dry matter (IVDMD), NDF (DVNDF) and ADF (DVADF) digestion of fibrous foods digested with *An* (*in vitro*). Preliminary experiments in our laboratory (unpublished) showed an asymptotic time of DM digestion of approximately 120 h; however, the levels of digestion for shorter periods of time are unknown. Separate experimental setups were used for each incubation time. The inoculum dose ensured a substrate-limiting condition, sufficient to evaluate the susceptibility of the substrate to enzymatic digestion. After 72 h and 144 h, the flasks were withdrawn from the stove and 10 mL of cold water (7 °C) was added to each flask to stop *An* enzymatic activity. The contents of the flasks were subsequently filtered using Whatman filter paper (N° 41) in a vacuum funnel; mass balance was performed after bromatological analysis of the undigested residue to determine IVDMD, DVNDF and DVADF. Values from each treatment were corrected using the respective blanks, which consisted of substrates incubated without *An*.

Statistical analysis

For both tests, the experimental design was completely randomized, with factorial arrangement, 2×5 and 5×3, for experiments 1 and 2, respectively. For experiment 1, the first factor corresponded to incubation with and without *An*, and the second corresponded to the 5 types of fibrous substrates. For experiment 2, the first factor corresponded to the 5 types of substrate, and the second factor corresponded to the three incubation times (0, 72 and 144 h). The general statistical model for both experiments was $Y_{ijk}=\mu+\alpha_i+\beta_j+(\alpha\beta)_{ij}+e_{ijk}$, where Y_{ijk} = the observed value (chemical composition, IVDMD, DVNDF and DVADF); μ = the generalized mean; α_i = the effect of the i^{th} level of factor α; β_j= the effect of the j^{th} level of factor β; $(\alpha\beta)_{ij}$ = the interaction between the i^{th} level of factor α and the j^{th} level of factor β; e_{ijk} = the experimental

error. Each experiment was repeated three times in duplicate. The normality of the data was verified using the Shapiro-Wilk procedure (P>0.05). Levene's statistic was used to test for homogeneity of variance (P>0.05). In both experiments, the experimental unit was a matrass (250 mL) with its respective incubation. The results were subjected to an ANOVA with a significance level of 5%, with an analysis of interaction and main effects. Treatment means were compared using Tukey's method (5% significance). Statistical analysis was performed with JMP® software (version 5.0.1.2, SAS Inc., Cary, NC, 2003).

Results

Experiment 1: Effect of in vitro incubation with Aspergillus niger on the chemical composition of fibrous substrates

No interaction effect was observed among any of the studied variables (P=0.28–P=0.37). Incubation with *An* affected the amount of NDF (P≤0.001), ADF (P≤0.001), TP (P≤0.01), SN (P≤0.01), NDIN (P≤0.05) and ADIN (P≤0.05). Additionally, chemical composition was affected by the type of substrate (P≤0.0001–P≤0.01) (Table 2). The greatest reduction in NDF (P≤0.05) was observed with the substrate BS (24.68% DM), and the smallest reduction was observed (P≤0.05) with the substrate DA (15.56% DM). The average decrease in NDF across all substrates was 20.86% DM. The greatest decrease in ADF (P≤0.05) was observed in OH (8.53% DM), and the smallest decrease was observed (P≤0.05) in DA (4.73% DM). The average decrease in ADF was 6.71% DM for all substrates. However, the greatest increases in TP (P≤0.05) were observed in the substrates DA and DB (0.55% and 0.63% DM, respectively). WS, BS and OH substrates showed an average increase of 0.32% DM and were not different from one another (P>0.05). These changes suggest a possible increase in *An* biomass as a by-product of digestion and growth on the fibrous substrates, with a consequent increase in protein synthesis. Furthermore, DA showed the greatest increase in solubility of nitrogenous compounds, increasing SN 12.11% CP, whereas DB showed the smallest increase in SN (6.84% CP). On average, across all substrates, SN increased by 9.69% CP. For N associated with cell walls (NDIN), WS showed the greatest decrease (P≤0.05), with a value of 4.57% CP; there was no difference in NDIN among the rest of the substrates, which exhibited an average decrease of 4.04% CP. For N insoluble in acid detergent fiber (ADIN), BS substrate showed the greatest decrease (P≤0.05), yielding a value of 3.19% CP. The smallest decrease in ADIN (P≤0.05) was in DB (1.46% CP). Although proteolytic activity of *An* was not measured directly in this study, the decreased the levels of NDIN and ADIN, and increased solubility of nitrogen compounds (SN), suggest some proteolytic activity by *An*, corroborating the proteolytic potential observed in other assays developed in our laboratory, and in the laboratories of others.

Experiment 2: Effect of incubation time on the dry matter, neutral detergent fiber and acid detergent fiber digestion of different substrates digested with Aspergillus niger (An)

The results of the experiment on time of incubation and *in vitro* digestion of DM (IVDMD), NDF (DVNDF) and ADF (DVADF) in different substrates by *An* are presented in Table 3. First, the statistical analysis indicated that there was no interaction effect among any of the parameters studied (P=0.28-P=0.46). The results revealed that $IVDMD_{72}$ and $IVDMD_{144}$ were affected by the type of substrate (P≤0.001 and P≤0.001, respectively). The greatest value of $IVDMD_{144}$ (P≤0.05) was observed in the treatment DAAn (49.47% DM), followed by the treatment DBAn (45.51% DM). $IVDMD_{144}$ did not differ between WSAn and BSAn (P>0.05), and averaged 33.77% DM. OHAn had the lowest level (P≤0.05) of $IVDMD_{144}$ (29.38% DM). $DVNDF_{72}$ and $DVNDF_{144}$ were both affected by the type of substrate (P≤0.001 and P≤0.001), and there were differences between them in all

Table 2. Effect of *in vitro* incubation with *Aspergillus niger* (*An*) and type of substrate on the chemical composition of fibrous foods (WS: wheat straw, BS: barley straw, OH: oats hull, DA: dehydrated alfalfa and DB: dehydrated ballica). Experiment 1.

Treatment[1]	NDF[2] % DM	ADF % DM	CP[3] % DM	TP % DM	SN % CP	NDIN[4] % CP	ADIN % CP
WSAn	61.74 b[5]	46.24 b	4.12	3.78 a	32.34 a	43.96 b	23.67 b
WSs	85.55 a	53.63 a	4.09	3.47 b	23.01 b	48.53 a	26.34 a
BSAn	59.54 b	46.94 b	4.47	4.23 a	34.72 a	43.45 b	22.85 b
BSs	84.22 a	54.31 a	4.42	3.91 b	23.41 b	47.33 a	26.04 a
OHAn	64.32 b	54.35 b	4.62	4.24 a	27.89 a	36.12 b	24.66 b
OHs	85.77 a	62.88 a	4.56	3.92 b	19.02 b	40.28 a	26.74 a
DAAn	26.77 b	23.12 b	18.42	16.86 a	34.56 a	19.81 b	12.11 b
DAs	42.33 a	27.85 a	18.32	16.31 b	22.45 b	23.76 a	13.67 a
DBAn	38.54 b	29.68 b	11.34	10.76 a	21.38 a	29.03 b	14.67 b
DBs	57.35 a	35.23 a	11.21	10.13 b	14.54 b	33.21 a	16.13 a
P(subs)	<0.0001	<0.0001	<0.001	<0.001	<0.001	<0.01	<0.01
P(An)	<0.001	<0.001	0.35	<0.01	<0.01	<0.05	<0.05
P(subsxAn)	0.28	0.33	0.31	0.37	0.29	0.36	0.35
SEM	1.83	1.77	0.084	0.075	0.61	1.32	0.63

Different letters within a column indicate significant differences between treatments with/without An (P≤0.05).
[1]WSAn: wheat straw with Aspergillus niger (An), WSs: wheat straw without An, BSAn: barley straw with An, BSs: barley straw without An, OHAn: oats hull with An, OHs: oats hull without An, DAAn: dehydrated alfalfa with An, DAs: dehydrated alfalfa without An, DBAn: dehydrated ballica with An, DBs: dehydrated ballica without An.
[2]NDF: neutral detergent fiber, ADF: acid detergent fiber.
[3]CP: crude protein, TP: true protein, SN: N soluble fraction.
[4]NDIN: insoluble N in neutral detergent, ADIN: insoluble N in acid detergent.
[5]Each value represents incubation assays for three repetitions with duplication.

Table 3. Effect of substrate type and incubation time on *in vitro* digestion with *Aspergillus niger* (*An*) of dry matter, neutral detergent fiber and acid detergent fiber[1]. Experiment 2.

Treatment[2]	$IVDMD_{72}$ % DM	$IVDMD_{144}$ % DM	$DVNDF_{72}$ % NDF	$DVNDF_{144}$ % NDF	$DVADF_{72}$ % ADF	$DVADF_{144}$ % ADF
WSAn	25.72 c[3]	33.12 c	16.33 c	27.83 c	8.74 b	13.78 b
BSAn	26.97 c	34.41 c	17.68 c	29.30 c	7.95 b	13.57 b
OHAn	20.65 d	29.38 d	13.45 d	25.00 d	7.73 b	13.56 b
DAAn	37.78 a	49.47 a	24.76 a	36.76 a	11.52 a	16.98 a
DBAn	34.31 b	45.51 b	21.28 b	32.80 b	10.33 a	15.75 a
P(subs)	<0.001	<0.001	<0.001	<0.001	<0.01	<0.01
P(time)	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
P(subsxtime)	0.41	0.36	0.33	0.42	0.46	0.28
SEM	0.053	0.042	0.023	0.017	0.011	0.008

Different letters within a column indicate significant differences (P≤0.05).
[1]$IVDMD_{72}$: in vitro dry matter digestion at 72 h, $IVDMD_{144}$: in vitro dry matter digestion at 144 h, $DVNDF_{72}$: in vitro neutral detergent fiber digestion at 72 h, $DVNDF_{144}$: in vitro neutral detergent fiber digestion at 144 h, $DVADF_{72}$: in vitro acid detergent fiber digestion at 72 h, $DVADF_{144}$: in vitro acid detergent fiber digestion at 144 h.
[2]WSAn: wheat straw with Aspergillus niger (An), BSAn: barley straw with An, OHAn: oats hull with An, DAAn: dehydrated alfalfa with An, DBAn: dehydrated ballica with An.
[3]Each value represents incubation assays for three repetitions with duplication.

substrates tested (P≤0.01), showing the influence of the incubation time; there was no interaction between incubation time and substrate type. The highest value for $DVNDF_{144}$ (P≤0.05) was observed in the DAAn treatment (36.76% NDF), followed by the DBAn treatment (32.8% NDF). WSAn and BSAn did not differ (P>0.05) in $DVNDF_{144}$, with an average of 28.57% NDF. The lowest value for $DVNDF_{144}$ (P≤0.05) was observed in the OHAn treatment (25% NDF). Moreover, $DVADF_{72}$ and $DVADF_{144}$ were affected by substrate type (P≤0.01 and P≤0.01, respectively). Similarly, incubation time affected the amount of the lignocellulosic fraction digested in all substrates studied (P≤0.01). DAAn and DBAn treatments showed the highest values of $DVADF_{144}$ (P≤0.05), with no differences between them (P>0.05); these treatments averaged 16.37% ADF. The $DVADF_{144}$ of WSAn, BSAn and OHAn treatments were similar (P>0.05), with an average of 13.64% ADF. As seen in Table 3, in all substrates and parameters evaluated, the level of digestion was lower at 72 h of incubation than at 144 h incubation (P≤0.05). In general, these results suggest that the hydrolytic activity of *An* continues after 72 h incubation, peaking at 144 h.

Discussion

One of the primary findings of this research is the decrease in NDF and ADF content of the substrates tested, as a result of digestion by *An* under *in vitro* conditions. In effect, the decrease in NDF and ADF content, observed in all substrates studied (Table 2), would allow improved digestive potential, especially for WS, BS and OH substrates, because these could be included as ingredients in ruminant diets. This is possible due to the decrease in cell wall content, and in particular, the lignocellulosic fractions, which are the most resistant to the enzymatic action of microorganisms in the rumen (Sniffen *et al*., 1992; Colombatto *et al*., 2007). However, digestibility is the most appropriate parameter to describe substrate susceptibility to the enzymatic degradation system. Reviewing the levels of digestion (Table 3), it can be seen that degradation was generally higher with the DA and DB substrates (forage). The average value of those substrates was 34.78% for $DVNDF_{144}$ and 16.37% for $DVADF_{144}$ compared with WS, BS and OH substrates, which averaged 27.38% and 13.64%, respectively. This relationship was clearly associated with $IVDMD_{144}$ values, given the original magnitude of fibrous fractions in the foods assessed (the correlation coefficient (r) between $IVDMD_{144}$ and $DVNDF_{144}$ was 0.979; and between $IVDMD_{144}$ and $DVADF_{144}$ was 0.972, across all substrates). In the case of treatments DAAn and DBAn, $IVDMD_{144}$ values averaged 47.49%, whereas the WSAn, BSAn and OHAn treatments averaged 32.3%, suggesting that the DM of forage foods was more responsive to *An* hydrolytic enzymes (under *in vitro* conditions), resulting in a greater degree of degradation. This difference could be due to variation in the chemical composition of the substrates. The DA and DB forages had a lower initial level of NDF and ADF relative to WS, BS and OH substrates (Table 1). Most likely, the chemical structures in the WS, BS and OH cell walls were more lignified, and therefore possessed a more complex integration of lignin in the cellulosic matrix, making it more resistant to fibrolytic enzyme action (Shrivastava *et al*. 2012). Generally, the complexity of the lignocellulosic matrix and its interaction with proteins increases in more advanced growth stages of the plant. It is noted that WS and BS corresponded to post-harvest waste, and OH corresponded to a more aged plant biomass, whereas DA and DB came from first cut forage plants with a phenological state prior to flowering. To improve access of fungal enzymes to the organic matter in the substrates, it is necessary to break the lignocellulosic and hemicellulosic structures located in the cell walls (Kuhad *et al*., 1997; Colombatto *et al*., 2007); when this occurs, the digestion of substrates is accelerated and higher levels of degradation can be achieved. It should be considered that during the process of enzymatic digestion of organic matter, a diverse pool of polysaccharidases and proteases act synergistically, and can liberate

nitrogen compounds and carbon skeletons highly resistant to degradation. Indeed, *An* has a powerful pool of fibrolytic and proteolytic enzymes (Villas-Boas *et al*., 2002; Howard *et al*., 2003; Pensupa *et al*., 2013), which would explain the levels of degradation of DM and fibrous structures found in this investigation. With respect to incubation time, the results showed differences in the magnitude of *An* digestion achieved between 72 h and 144 h, a phenomenon observed for all substrates and for all parameters measured (Table 3). Although a kinetic study of the degradation of DM and fibrous fractions was not performed in this study, these results show that digestion had not begun to asymptote at 72 h; that occurred later in incubation. In previous studies, our laboratory found asymptotic values of *An* digestion of fibrous substrates with kinetic times approaching 100–120 h. Therefore, in this study, 144 h was chosen as end of incubation. It should be noted that the average level of digestion at 72 h of incubation, for all substrates, was 29.09% DM, 18.7% NDF and 9.25% ADF to $IVDMD_{72}$, $DVNDF_{72}$ and $DVADF_{72}$, respectively. These values represent 75.79%, 61.64% and 62.83%, respectively, of the average digestion achieved at 144 h of incubation (across all substrates). These observations allow us to state that a significant fraction (> 60%) of the hydrolytic activity of *An* took place during the first 72 h. However, *An* requires additional incubation time to reach its full digestive potential. Furthermore, the observed increase of SN in all treatments (Table 2) reveals probable proteolytic activity, although it was not directly measured in this investigation. Normally, solubilization of nitrogenous compounds is the result of protein degradation. Moreover, the decrease in NDIN and ADIN content in all treatments ($P \leq 0.05$) is directly related to increased SN, indicating possible proteolytic activity of *An* during incubation. Additionally, this situation can be connected to the increase in TP content in all treatments, which could be attributed to the increase in *An* cell biomass growing on the substrates. Fiber digestion of the substrates tested (NDF and ADF) provided *An* with energy and carbon skeletons for cell reproduction and growth, implying, although it was not measured directly, a likely increase in microbial protein synthesis. In this context, it is noted that the largest increases in TP ($P \leq 0.05$), the nutritional parameter that can be directly linked to protein synthesis, were observed in the DAAn and DBAn treatments, which averaged a 0.59% increase in TP. In comparison, WSAn, BSAn and OHAn treatments averaged a TP increase of 0.32%. A possible explanation for these differences is that forage foods (DA and DB) showed the greatest digestibility of DM, NDF and ADF, which allowed access to more energy, carbonaceous skeletons and nitrogen sources for *An* metabolism during incubation. Additionally, there may have been some variability in the efficiency of TP synthesis performed by *An*, as a result of digesting and metabolizing substrates and nutrients of different chemical nature (Velasquez *et al*., 2012).

In general, it can be stated that the results of this investigation confirm the fibrolytic potential of *An*, which could improve the nutritive value of fibrous foods. This would be achieved by digestion of structural carbohydrates, together with release nitrogenous fractions located in cell walls, producing a change in the potential digestibility of treated foods. Additionally, it should be considered that *An* cell biomass (growing on these substrates) forms part of the final mass of the bioprocess, providing protein of high biological value, carbohydrates and vitamins (Rajesh *et al*., 2010), along with other nutrients contained in the fungal protoplasm.

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Resumen

A. Velásquez, P. Marnet y R. Arias. 2015. Mejoramiento de la calidad nutritiva de alimentos fibrosos a través de la digestión *in vitro* con *Aspergillus niger.* Cien. Inv. Agr: 42(1): 45-55. En un primer ensayo se estudió el efecto de la incubación *in vitro* con *Aspergillus niger (An) y el* tipo de sustrato sobre la composición química de alimentos fibrosos. En un segundo ensayo se evaluó el efecto del tipo de sustrato y el tiempo de incubación (0, 72 y 144 h) sobre la digestión *in vitro* con *An* de la materia seca (IVDMD), fibra detergente neutro (DVNDF) y fibra detergente ácida (DVADF). Se evaluaron los sustratos paja de trigo: WS, paja de cebada: BS, cáscara de avena: OH, alfalfa deshidratada: DA y ballica deshidratada: DB. Para ambos experimentos las incubaciones se realizaron en matraces Erlenmeyer de 250 mL; la masa de sustrato a incubar fue de 2 g, adicionados a un medio de cultivo (pH=6), incubado con sistema de ventilación constante a temperatura de 28 ºC. La dosis de *An* consistió en 3 mL frasco^{-1} de una solución de 5,3 × 10^6 esporas mL^{-1}. El tiempo de incubación para el primer ensayo fue de 144 h. Transcurrido este tiempo, se procedió a homogenizar (Blender) el contenido total de los matraces por un minuto. Posteriormente, se realizó un análisis bromatológico a la masa total incubada, sin separar la biomasa de *An*. No se observó efecto de la interacción en ninguna de las variables estudiadas. La incubación con *An* mostró un efecto sobre el contenido de paredes celulares (FDN) y las fracciones lignocelulósicas (FDA). Se observó un incremento en la Proteína Verdadera (TP) y en el Nitrógeno Soluble (SN) en todos los sustratos evaluados, y su respuesta fue diferente según el tipo de sustrato. Los mayores incrementos en TP se observaron en los sustratos DA y DB (0,55 y 0,63% DM, respectivamente). IVDMD, DVNDF y DVADF se vieron afectadas por el tipo de sustrato y el tiempo de incubación. El valor más alto de $IVDMD_{144}$ se observó en el tratamiento DAAn (49,47% DM), seguido de DBAn, con un valor de 45,51% DM. OHAn mostró el valor más bajo de $IVDMD_{144}$ (29,38% DM). Los resultados permitieron inferir que *An* posee el potencial fibrolítico y metabólico para mejorar el valor nutritivo de alimentos fibrosos, a través de la digestión de los carbohidratos estructurales y liberación de fracciones nitrogenadas incrustadas en las paredes celulares, implicando un cambio en la composición química y en la digestibilidad potencial de los alimentos tratados.

Palabras clave: *Aspergillus niger*, calidad nutricional, digestión *in vitro*, sustratos fibrosos.

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