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ORIGINAL ARTICLE

Nutritional value assessments of peanut hulls and valorization with exogenous fibrolytic enzymes extracted from a mixture culture of *Aspergillus* strains and *Neurospora intermedia*

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Abstract
Peanut hulls are abundant waste with high bioactive compounds, antioxidant activity, and cell wall polysaccharides but low nutritional value. The aim of this study was to valorize this agricultural waste into alternative ruminant feed with exogenous fibrolytic enzymes (EFE) produced by fermentation of mixture culture of *Aspergillus* strains (*A. niger*, *A. tubingensis*, *A. oryzae*, and *A. sojae*) and *Neurospora intermedia*. Peanut hulls were pretreated for 24 h with increasing EFE levels 0, 1, 2, and 4 mg/g dry matter. The results showed that the effectiveness of this additive depended on EFE level. The low EFE level did not affect their nutritional value. The moderate and high EFE levels converted part of their cell wall polysaccharide compound into non-fiber carbohydrates and solubilize their organic matter without altering their bioactive compounds and their antioxidant activity. Consequently, these two levels of EFE accelerate rumen fermentation process, reduce the time of onset of rumen fermentation, and improve cell wall polysaccharide digestibility, net energy lactation, and short-chain fatty acid production. However, only the high EFE level promoted the proliferation of rumen protozoa and the amount of fermentation and dry matter digestibility and reduced rumen ammonia nitrogen by conversion into microbial crude protein. In conclusion, this practical bioprocess with the highest EFE level can be used as an effective tool for bioconversion of these wastes into energy feeds with high bioactive compounds and antioxidant activity to substitute expensive ruminant feeds. This strategy can provide a new source of revenue for the peanut shelling and peanut oil industries and protect the environment from the pollution.

Keywords Bioconversion · Peanut hulls · Exogenous fibrolytic enzymes · Valorization · Energy feed · Antioxidant activity

1 Introduction

The use of agricultural and agro-industrial wastes as unconventional feed for ruminants has become an interesting strategy to reduce feed costs, consumption between animal feed and human food, and environmental pollution [1–3]. Peanut (*Arachis hypogaea* L.) is an annual Leguminosae cultivated in Asia, Africa, Oceania, and America mainly for its seed and oil production. When peanut seeds are shelled from the pod, a large amount of outer shell is produced, estimated at about 14 million tons annually worldwide, known as peanut hulls. These abundant agricultural wastes are often discarded and slowly degrade under natural conditions, resulting in a potential source of environmental pollution [4–6]. Therefore, the recycling of these wastes represents a major environmental challenge. Various studies have shown that these agricultural wastes can be used as cheap roughage for rams up to 40% [7] and for cattle and dairy cows up to 20% [8]. Using them as an alternative food has several health benefits, such as adsorbing intestinal enterotoxins, improving intestinal health, lowering blood pressure, reducing inflammation and urinary excretion, relieving cough, and clearing mucus from the lungs [5, 9]. However, the high proportion of lignocellulose compounds of over 750 g/kg dry matter limits the proportion of nutrients that can be effectively degraded in the rumen [10]. Various strategies with chemical additives such as pretreatment with urea or lime can improve their

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nutritional value [7, 10]. However, few studies have investigated the effect of biological pretreatment on the nutritional value of peanut hulls. Abo-Donia et al. [10] reported that pretreated with *Trichoderma viride* can be used as a practical tool to modify their chemical composition and improve their palatability, pansal fermentation process, and nutrient digestibility due to the extracellular enzymes produced by these fungi. Reducing the cost of enzyme production has drawn the attention of nutritionists to its potential use as a biological pretreatment to improve feed efficiency in ruminants [11]. In this regard, several studies have proved that pretreated fiber feeds with exogenous fibrolytic enzymes (EFE) break down its cell wall contents into simple sugars during the pre-incubation period [12, 13]. Therefore, they stimulate the rumen fermentation process [12, 13] and fiber digestibility both in situ [14, 15] and in vivo [15–19] while improving growth performance [15, 17, 18] and lactation performance of ruminants [16, 19]. However, the effectiveness of EFE is variable and depends on several factors including ruminant species, animal production, feed composition, enzyme activity, and application methods [20, 21]. It is important to note that excessive levels of EFE can reduce rumen microbiota attachment to feed particles [22], fiber digestibility [23], and lactation performance in dairy cows [23]. Despite increasing research on this topic and the abundance of peanut hulls worldwide, there is no report in the literature on the effect of EFE on the nutritional value of peanut hulls. With this in mind, our study was conducted to determine the optimal levels of EFE extracted from a mixed culture of *Aspergillus* strains (*A. niger*, *A. tubingensis*, *A. oryzae*, and *A. sojae*) and *Neurospora intermedia* in order to convert this waste into an alternative energy feed for ruminants. We hypothesize that the pretreated peanut hull with EFE would improve its nutritional value without altering its bioactive compounds and antioxidant activity.

2 Materials and methods

2.1 Collection of peanut hulls and pretreatment with exogenous fibrolytic enzymes

Samples of fresh peanut hulls (*Chounfakhi* variety) were harvested from different fields in the Cap Bon region of northeastern Tunisia with a subhumid bioclimate and brought to the laboratory. These agricultural wastes were dried at 55 °C for 48 h and ground through a 1-mm sieve using a Retsch mill (Retsch ZM200, Retsch GmbH, Haan, Germany).

Peanut shells were pretreated with commercial EFE preparations in lyophilized form (MAXFIBER-I®, SHAUMANN GmbH, Wahlstedt, Germany) prepared by fermentation of a mixture of *Aspergillus* strains (*A. niger*, *A. tubingensis*, *A. oryzae*, and *A. sojae*) and *Neurospora intermedia*. Four EFE levels (0, 1, 2, and 4 mg) were diluted with 200 mL of distilled water and sprayed onto 200 g of substrate at 26 °C room temperature for 24 h. The experimental EFE values were 0 (control, without EFE), 1 (low EFE level), 2 (medium EFE level), and 4 (high EFE level) mg EFE/g dry matter peanut hulls. The low EFE level used in this study was chosen based on the results of a previous in vitro study by Jabri et al. [24], which showed that this EFE preparation at this level improved rumen fermentation of wheat straw. The medium EFE level and high EFE level used in this study consisted of multiplying this dose by × 2 and × 4, respectively, to investigate the effects of increasing the EFE level on the nutritional value of peanut hulls.

The enzyme activity of the commercial EFE preparation was analyzed for its xylanase, endoglucanase, and exoglucanase activities using spelt xylan, carboxymethylcellulose sodium salt, and cellulose, respectively, at pH of 6.6 and temperature 39 °C, which reflects the normal condition of the rumen, according to the protocol of Baiely and Poutanen [25] and Wood and Bhat [26]. The enzyme activity of the commercial EFE preparation was 1180 UI xylanases, 750 UI endoglucanase, and 740 UI exoglucanase/g.

2.2 Chemical composition and antioxidant activity

Chemical composition and antioxidant activity were examined after pretreatment with the appropriate level of EFE. Crude protein (CP, N × 6.25; method 968.06), ether extract (EE, method 920.30), and ash (method 923.03) were analyzed according to the standard method proposed by the Association of Official Chemists Analytical Chemists [27]. Neutral detergent fiber (NDF), acid detergent fiber (ADF), and acid detergent lignin (ADL) were determined according to the method described by Van Soest et al. [28] using an ANKOM220 fiber analyzer (ANKOM technology, Macedon, NY, USA). The soluble organic matter was determined following the protocol described by Abid et al. [29]:

Non-fiber carbohydrate (NFC) content was calculated according to Eq. 1 [30]:

$$\mathrm{NFC} = 100 - (\mathrm{NDF} + \mathrm{CP} + \mathrm{EE} + \mathrm{Ash}) \quad (1)$$

Peanut hull extract was prepared according to the method described by Adhikari et al. [5]. Briefly, samples of pretreated peanut hulls were mixed with absolute methanol (1:10 w/v) incubated in an incubator at a temperature of 25° C and an oscillation speed of 150 rpm for 6 h. Then the mixture was centrifuged at 13,000 g at ambient temperature for 10 min. The supernatant was filtered and used to determine antioxidant activity and bioactive molecule activity. Total polyphenols were analyzed by the Folin–Ciocalteu colorimetric method at an absorbance of 750 nm wavelength. Gallic acid was used as a reference standard, and the result was expressed on µg

gallic acid equivalent/g sample [31]. Condensed tannins were analyzed by the vanillin method at an absorbance of 500 nm wavelength. Catechin was used as reference standard, and result expressed on µg catechin equivalent/g sample [32]. Flavonoids were determined by the colorimetric aluminum chloride method at an absorbance of 500 nm wavelength. Quercetin was used as a reference standard, and the result was expressed on µg quercetin equivalent/g sample [33].

Antioxidant activity was measured by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scaring method at an absorbance of 517 nm wavelength flowing to the method described by Xu and Chang [34]. Results of radical scavenging activity were expressed as a percentage of the DPPH and calculated according the Eq. 2:

$$\text{DPPH radical scavenging (\%)} = \left[1 - \frac{\text{Absorbance of DPPH and sample} - \text{absorbance of methnol and sample}}{\text{Absorbance of DPPH and methanol} - \text{asorbance of methanol}}\right] \times 100 \quad (2)$$

2.3 In vitro ruminal incubation

The in vitro rumen fermentation test was performed using gas production following the methodology described by Theodorou et al. [35] to evaluate in vitro rumen incubation. Rumen fluid content was obtained from 2 slaughtered adult Holstein cows (~6 years old and 650 kg body weight, fed the same diets 7 kg oat hay, and 7 kg commercial concentrate) at different locations in the rumen. The rumen liquor was filtered through four layers of cheesecloth, mixed in equal volume ratio, and rapidly transported to the laboratory in thermos flasks preheated to 39 °C and purged with CO_2. In the laboratory, rumen inoculum was mixed with an artificial buffer solution (1:2 ratio) prepared according to the method of Menke and Steingass [36] at 39 °C with continued flushing with CO_2 at 39 °C. Samples of 200 mg of dry matter peanut hulls pretreated with 0, 1, 2, and 4 mg EFE/g dry matter were incubated with 30 mL of the buffered rumen inoculum in pre-warmed (39 °C) sterile amber glass serum bottles of 120 mL. Amber glass serum bottles containing only 30 mL of the rumen buffered inoculum were used as negative controls to correct the gas production from the rumen buffered inoculum. All bottles were immediately sealed with a rubber cap and an aluminum crimp cap and incubated in a shaking water bath at a constant temperature of 39 °C and an oscillating speed of 125 rpm. The gas pressure in each incubated bottle was measured after 2, 4, 6, 8, 12, 24, 48, 72, and 96 h of incubation using a pressure transducer connected to a data logger and converted to volume using Eq. 3 described by Mauricio et al. [37]:

$$\text{Gas volume(mL)} = \left[\text{Gas pressure(psi)} \times 4.8843\right] + 3.1296 \quad (3)$$

Data of the cumulative volume of biogas production generated were fitted with an exponential mathematical model according to Eq. 4 [38] using the nonlinear package from SAS Institute Inc [39]:

$$GP_{(t)} = B\left(1 - e^{-C(t-\text{Lag})}\right) \quad (4)$$

where GP is the net gas production (mL/g dry matter); t is the incubation time (hours); B is the asymptotic gas production (mL/g dry matter); C is the constant gas production rate (mL/h); and Lag is the time of onset of rumen fermentation (hours).

At the end of fermentation, the rumen pH was immediately measured using a pH meter (Jenway Ltd Felsted, model 3020, England). The contents of each serum bottle were filtered using filter paper disks (Whatman 541). Residues were collected, and their dry matter and NDF were determined according to protocols published by the Association of Official Chemists Analytical Chemists [27] (method 934.01) and Van Soest et al. [28], respectively. In vitro dry matter digestibility and in vitro NDF digestibility were determined according to Eq. 5 and Eq. 6, respectively:

$$\text{In vitro dry matter digestibility (\%)} = \frac{\text{initial dry matter} - \text{residual dry matter}}{\text{initial dry matter}} \times 100 \quad (5)$$

$$\text{In vitro NDF digestibility (\%)} = \frac{\text{initial NDF} - \text{residual NDF}}{\text{initial NDF}} \times 100 \quad (6)$$

Samples of 1 mL of the filtered rumen liquor from each bottle were mixed with 1 mL of physiological methyl green formalin solution. The total number of protozoa was determined by light microscopy and Levy-Hausser counting chamber according to the protocol of Dehority [40].

In addition, 4 mL of the filtered rumen liquor from each bottle was preserved by adding 2 mL of 1 N H_2SO_4. Samples were analyzed for ammonia nitrogen by the phenol-hypochlorite method with an absorbance of 630 nm as described by Broderick and Kang [41].

Net energy of lactation, short-chain fatty acids, and microbial crude protein were estimated from the gases produced after 24 h of incubation according to the regression equations (Eq. 7, Eq. 8, and Eq. 9) proposed by Menke and Steingass [36], Getachew et al. [42], and Blümmel et al. [43], respectively:

$$\text{Net energy of lactation} = 0.101 \times \text{GP24} + 0.051 \times \text{CP} + 0.112 \times \text{EE} \quad (7)$$

$$\text{Short} - \text{chain fatty acids} = -0.00425 + 0.0222 \times \text{GP24} \quad (8)$$

$$\text{Microbial crude protein} = \text{amount of digestible dry matter} - 2.2 \times \text{GP24} \quad (9)$$

where the net energy of lactation is in MJ per kg dry matter; short-chain fatty acids in mmol/200 mg dry matter; and microbial crude protein in mg/g dry matter; GP24 is net gas production after 24 h of incubation in mL/200 mg dry matter; CP is crude protein in percent of dry matter; EE is ether extract in percent of dry matter; and amount of digestible dry matter is in mg/g at the end of incubation.

2.4 Statistical analysis

This experiment was repeated three times (3 runs). For each run, three replicates were performed for each EFE level. All collected data was statistically analyzed using the GLM model (Proc GLM) using SAS Institute Inc. [39], with the static model flowing:

$$Y_{ij} = \mu + \text{level}_i + \varepsilon_{ij}$$

where Y_{ij} is the observation of the dependent variable, μ is the overall mean, level $_i$ is the effect of the ith EFE level ($i = 0$, 1, 2 and 4), and ε_{ij} is the residual experimental error associated with the observation.

The linear and quadratic effect of increasing the EFE level was tested using the polynomial contrast statement of SAS Institute Inc. [39], and the orthogonal polynomial contrast coefficients were determined using Proc IML of SAS Institute Inc. [39]. The difference between the means of the treatments was compared using Tukey's multiple range test. Mean differences were considered significant when the p value was less than 0.05%.

3 Results

Table 1 reveals that peanut hulls are a good source of fiber (NDF and ADF), total polyphenols, and flavonoids, a moderate source of protein and ash, and a low source of EE and NFC. These agricultural wastes have high antioxidant activity. Pretreatment of these agricultural wastes with moderate or high EFE level significantly reduced the NDF and ADF compound and increased the concentration of NFC and soluble organic matter compound, while the other compounds and their antioxidant activity were not affected compared to the untreated peanut hulls. The low EFE level has no significant effect on the chemical compounds and antioxidant activity of peanut hulls compared to untreated peanut hulls. The effects of increasing the EFE levels on the kinetics of rumen fermentation (B, C, and lag), in vitro dry matter digestibility, in vitro NDF digestibility, net energy of lactation, short-chain fatty acids, microbial crude protein, ammonia nitrogen, rumen protozoa, and rumen pH are shown in Table 2.The low EFE level has no significant impact on the overall parameter study in Table 2. Moderate and high EFE level increases rumen fermentation rate, fiber digestibility, net energy lactation, and short-chain fatty acids and decreases delay time in starting fermentation. The highest EFE level also improved the proliferation of rumen protozoal, amount of fermentation, dry matter digestibility, and microbial crude protein and decreased rumen ammonia nitrogen and rumen pH compared to untreated peanut hulls.

Table 1 Effect of exogenous fibrolytic enzymes on chemical composition, secondary compounds, and antioxidant activity of peanut hulls

Item	EFE level (mg EFE g/dry matter)				SEM	Polynomial contrast	
	0	1	2	4		Linear	Quadratic
Crude protein (g/kg dry matter)	67	69	70	70	7	NS	NS
Ether extract (g/kg dry matter)	17	16	18	17	4	NS	NS
Neutral detergent fiber (g/kg dry matter)	680 [a]	666 [a]	609 [b]	580 [c]	20	**	NS
Acid detergent fiber (g/kg dry matter)	582 [a]	573 [a]	528 [b]	510 [b]	16	*	NS
Acid detergent lignin (g/kg dry matter)	92	93	91	90	8	NS	NS
Ash (g/kg dry matter)	43	45	44	45	7	NS	NS
Non-fiber carbohydrate (g/kg dry matter)	193 [c]	204 [c]	259 [b]	287 [a]	18	**	NS
Soluble organic matter (g/kg dry matter)	13.2 [b]	13.7 [b]	17.2 [a]	20.8 [a]	1.7	**	NS
Total polyphenols (μg gallic acid equivalent/ g)	799	791	794	790	9	NS	NS
Condensed tannins (μg catechin equivalent/ g)	313	307	308	309	8	NS	NS
Flavonoids (μg quercetin equivalent/ g)	607	606	609	570	10	NS	NS
DPPH radical scavenging activity (%)	89.4	89.2	88.9	89.1	1.4	NS	NS

PPH, 2,2-diphenyl-1-picrylhydrazyl; *SEM*, standard error of the means; [a,b,c], means value flowed by different superscript letters in the same line differed at $p < 0.05$; **, $p < 0.01$;*, $p < 0.05$; *NS*, $p > 0.05$

Table 2 Effect of exogenous fibrolytic enzymes on nutritional value of peanut hulls

Item	EFE level (mg EFE/ g dry matter)				SEM	Polynomial contrast	
	0	1	2	4		Linear	Quadratic
Asymptotic gas production (mL/g dry matter)	251.6 ^{b}	256.5 ^{b}	260.9 ^{b}	292.3 ^{a}	12.1	**	NS
Constant rate of gas production (mL/h)	0.031 ^{b}	0.032 ^{b}	0.040 ^{a}	0.043 ^{a}	0.005	**	NS
Delay at the start of gas production (h)	1.23 ^{c}	1.20 ^{c}	0.89 ^{b}	0.58 ^{a}	0.09	***	NS
Dry matter digestibility (mg/g)	488 ^{b}	491 ^{b}	516 ^{ab}	552 ^{a}	16	*	NS
Natural detergent fiber digestibility (mg/g)	369 ^{c}	376 ^{c}	395 ^{b}	421^{a}	13	*	NS
Microbial crude protein (mg/g dry matter)	431.5 ^{b}	432.4 ^{b}	440.5 ^{b}	471.0 ^{a}	18.3	**	NS
Ammonia nitrogen (mg/100 mL)	183 ^{a}	182 ^{a}	159 ^{ab}	141^{b}	17	*	NS
Net energy lactation (MJ/kg dry matter)	3.12 ^{c}	3.22 ^{c}	3.75 ^{b}	4.27 ^{a}	0.37	**	NS
Short-chain fatty acids (mmol/200 mg dry matter)	0.57 ^{c}	0.59 ^{c}	0.70 ^{b}	0.81 ^{a}	0.05	**	NS
pH	6.73 ^{a}	6.73 ^{a}	6.72 ^{a}	6.61 ^{b}	0.04	*	NS
Total protozoa (10^5 cells/ mL)	6.21 ^{b}	6.30 ^{b}	6.49 ^{b}	6.96 ^{a}	0.21	*	NS

^{a,b,c}, means value flowed by different superscript letters in the same line differed at $p < 0.05$; *SEM*, standard error of the means; ***, $p < 0.001$; **, $p < 0.01$; *, $p < 0.05$; *NS*, $p > 0.05$

4 Discussion

The *Chounfakhi* peanut hulls are a potential source of secondary metabolites, particularly flavonoids content, due to their self-defense mechanisms against soil microbial attack and soil oxidation [5]. The use of biomass with high flavonoid content in animal feed can reduce diseases related to oxidative stress, risk of acidosis, bacterial pathogenicity, rumen methanogen microbiota, and postpartum inflammation and improve energy metabolism [44]. The condensed tannins detected in these agricultural wastes are beneficial to improving protein flux and absorption in the small intestine, reducing methane emissions and toxic effects of high rumen ammonia nitrogen [45, 46]. The excellent antioxidant activity detected on peanut hulls allows it to be used in food and pharmaceutical applications. Comparable with peanut hulls collected from Korea [5], *Chounfakhi* peanut hulls collected from Tunisia have better bioactive compound and antioxidant activity. This difference could be due to the diversity of peanut varieties, soil types, climatic conditions, and maturity stage.

It is relevant to point out that these agricultural wastes collected in Tunisia are a potential source of fiber (NDF, ADF). Comparable results have been reported from peanut hulls collected from Nigeria [7, 47], China [6, 9], and Egypt [10]. The ADF content of peanut hulls is well above the minimum recommended level for dry cow diet (21% dry matter) to ensure proper rumen function [30] and higher than that of oat hay, alfalfa hay, and common vetch hay [48]. In this context, these agricultural wastes can be used as a valuable biomass alternative to hay in ruminant diets. However, the relatively high lignin content of peanut hulls can reduce their nutritional value. Our results are comparable to those found in peanut hulls collected in Egypt [10]. The CP content of these agricultural wastes is comparable to that of oat hay [48] and is considered sufficient to meet the minimum requirements for optimal growth, function, and maintenance of adequate fibrolytic activity of the rumen microflora [49]. According to Adhikari et al. [5], peanut hulls contain eight essential amino acids (histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, and valine) that must be included in the diet. It is important to note that these agricultural wastes can be used as a moderate source of mineral nutrients. In this same context, Akinfemi et al. [47] reported that potassium, calcium, magnesium, and phosphorus are the main minerals of peanut hulls. However, these agriculture wastes have a low EE and NFC concentrations. The EE and NFC compounds detected on peanut hulls collected in Tunisia are comparable to those on peanut hulls collected from Nigeria [7] and China [9], and they are also comparable to EE on oat hay, alfalfa hay, and common vetch hay [48].

In vitro rumen fermentation is one of the most widely used methods for rapid and efficient prediction of the nutritional value of feed value under simulated rumen conditions [14]. Our results indicate that peanut hulls have low fermentation, rumen digestibility, and low energy value compared to sugar beet pulp and citrus pulp [1]. This result is consistent with in vitro rumen fermentation kinetics and in vivo dry matter digestibility of peanut hulls collected from Egypt [10]. The low nutritional value of peanut hulls is probably due to their high content of cell wall tissue, which is difficult for rumen microbes to metabolize and their relatively high lignin tissue, which is not degraded in the rumen environment and presents a physical barrier for the rumen microbiota and digestive enzymes to penetrate to the other components [49]. In addition, the condensed tannins detected on these wastes may be associated with carbohydrates and proteins in the rumen, reducing the adhesion of rumen

microbiota to the feed particles and impair their metabolism [45, 46].

Pretreatment of peanut hulls with EFE tended to linearly increase (P linear < 0.10) their crude protein content with increasing the EFE level. This result is consistent with previous studies that reported that crude protein concentration of alfalfa hay treated with various EFE preparations tended to be greater than untreated [50]. In addition, recent studies had shown that ensiling various fibrous forages such as date palm leaves, rice straw, wheat straw, corn stalks, and sugarcane bagasse with a preparation of fibrolytic enzymes increased their crude protein content compared to ensiling without an enzyme preparation [19, 51, 52]. This positive effect is due to the source of the enzyme, which is a protein with catalytic properties. This improvement in crude protein may improve the growth and function of the rumen microflora [49]. Furthermore, the moderate and high EFE levels have greater ability to bio-convert NDF and ADF polymers into NFC compounds and to solubilize organic matter compounds of peanut hulls. This bioprocess improved linearly with increasing EFE level. Consistent with our results, Abid et al. [13] reported a linear effect of increasing the levels of EFE (50% cellulase plus and 50% xylanase plus, Dyadic International, Inc., Jupiter, FL, USA) on cell wall bioconversion of pretreated sesame seed coats into sugar components. However, other studies reported that the same EFE preparation had no effect on the soluble organic matter of palm byproducts [29]. This discrepancy can be explained by the difference in the cell wall structure of the substrate and the short duration of the enzyme–substrate interaction (12 h) in the study by Abid et al. [29] compared to our study (24 h), which limits the enzymatic hydrolysis of the cell walls of the substrate. This positive effect of pretreating of peanut hulls, particularly at high EFE levels, can lead to a reduction in rumen filling and potentially improve their palatability as well as their intake. These results agree with the in vivo study by Zhang et al. [15] who showed that increasing the dose of EFE (Shanxi Dayu Biotechnology Co., Ltd., Ruicheng, China) improved feed intake in Holstein bulls. The linear increase in NFC with increased EFE levels provides an additional energy source for rumen protozoa proliferation, especially at high doses. This increase in rumen protozoa improves the rumen fermentation environment by scavenging oxygen to maintain anaerobiosis and indirectly improves the development of bacteria and fungi [53]. This finding is consistent with previous in vitro and in vivo studies showing a positive response of EFE preparation on rumen microbiota and rumen microbial enzyme activity [13, 15]. This positive bioconversion of peanut hulls and proliferation of rumen protozoa provide rapidly fermentable substrates in the rumen and shorten the delay phase of the onset of rumen formation, especially at high EFE levels. This result is in agreement with a previous study showing a linear improvement in fermentation with increased levels of EFE preparations (50% cellulase plus and 50% xylanase plus, Dyadic International, Inc., Jupiter, FL, USA) from pretreated olive cake [12] and sesame seed coats [13]. Consequently, the linear increase in EFE levels linear promotes digestibility of cell wall polysaccharides, net energy lactation of peanut hulls, and short-chain fatty acids production. According to Oba and Allen [54], this improvement in in vitro NDF digestibility can increase feed intake and 4% fat corrected milk yield of dairy cows by 0.17 kg and 0.25 kg per unit improvement in NDF digestibility, respectively. Consistent with our results, Zhang et al. [15] reported that increasing the levels of EFE preparation (Shanxi Dayu Biotechnology Co., Ltd., Ruicheng, China) linearly improved the effective NDF degradability in situ and NDF digestibility in vivo. However, Refat et al. [16] found that increasing the level of EFE preparation (AB Vista, Wiltshire, UK) had a cubic effect on total tract NDF digestibility in vivo and lactation performance of dairy cows. This difference between the cited study and the current work can be explained by the differences in EFE preparation and substrate used. Only the high EFE level stimulated the fermentation amount, in vitro dry matter degradation, and bioconversion of rumen ammonia nitrogen to microbial crude protein. The latter results are consistent with previous in situ experiments that proved that pretreatment of whole plant faba bean silages with EFE (AB Vista, UK) improved both NDF degradability and dry matter degradability [14] and with the in vivo study that proved that barley silage pretreated with an EFE preparation (AB Vista, Wiltshire, UK) reduced rumen ammonia nitrogen of dairy cows [55]. It is important to highlight that the highest EFE level used in this study lowered rumen pH. This result is consistent with previous in vivo studies that reported higher levels of EFE (Shanxi Dayu Biotechnology Co., Ltd., Ruicheng, China) decrease rumen pH in Holstein bulls [15]. This effect could be produced by the improvement of the rumen fermentation process, the increase of NFC compound, and the increased production of short-chain fatty acids. However, with the high EFE level, the rumen pH value is still in the optimal pH range (6.4 to 6.8), which favors the proliferation of rumen microbiota and feed digestion [56].

5 Conclusions

Peanut hulls are characterized by high bioactive compounds, antioxidant activity, and fiber but low nutritional value. Pretreatment of these agricultural waste with EFE extracted from a mixture culture of *Aspergillus* strains (*A. niger*, *A. tubingensis*, *A. oryzae*, and *A. sojae*) and Neurospora intermedia converted into energy feed for ruminants without altering their bioactive compounds and their antioxidant activity. However, the effectiveness of this bioprocess

depends on the level of EFE. The maximum improvement was obtained with the highest level of EFE, which caused bioconversion of NDF and ANDF compounds to NFC and solubilization of organic matter during the pre-incubation period. In vitro fermentation tests showed that the highest level of EFE improved rumen protozoa proliferation, rumen fermentation process, digestibility of NDF and dry matter compounds, net energy lactation, production of short-chain fatty acids, and bioconversion ammonia nitrogen to microbial crude protein. This green strategy creates new market opportunities for these agricultural wastes, which can be used by the feed industry to replace expensive feed, and provides a new source of revenue for the peanut shelling and peanut oil industries.

Abbreviations *ADF*: Acid detergent fiber; *ADL*: Acid detergent lignin; *B*: Asymptotic gas production; *C*: Constant gas production rate; *CP*: Crude protein; *DPPH*: 2,2-Diphenyl-1-picrylhydrazyl; *EE*: Ether extract; *EFE*: Exogenous fibrolytic enzymes; *GP*: Net gas production; *GP24*: Net gas production after 24 h of incubation; *Lag*: Time of onset of rumen fermentation; *NDF*: Neutral detergent fiber; *NFC*: Non-fiber carbohydrate; *SEM*: Standard error of means; t: Incubation time

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Data availability The datasets and materials used during the current study are available from the corresponding author upon reasonable request.

Declarations

Ethical approval The article does not contain any studies with human participants. It also does not perform experiments directly on animals. So, this experience does not need ethics statement.

Consent to participate All the authors of this article are consented to participate.

Competing interests The authors declare no competing interests.

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