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ORIGINAL ARTICLE

Improving the nutritional value and rumen fermentation characteristics of sesame seed coats through bioconversion approach using exogenous fibrolytic enzymes produced by Trichoderma *longibrachiatum*

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Abstract
Sesame seed coat (SSC), agro-industrial waste of Halava, is characterized by high fiber content, moderate energy, low digestibility, and sugar. The aim of this study was to improve their nutritional value and rumen fermentation characteristics through a bioconversion approach using liquid exogenous fibrolytic enzymes (EFE) produced by Trichoderma *longibrachiatum*. SSC was pre-treated for 12 h with EFE (xylanases, endoglucanase, and exoglucanase) at concentrations of 0, 1, 2, and 4 µl g^{-1} dry matter (DM). The in vitro gas production technique with cow inoculum was used to determine rumen fermentation. At the end of fermentation, dry matter digestibility (DMD) as well as protozoa, ruminal fibrolytic enzyme activity, ruminal pH, and ammonia–nitrogen (NH_3-N) were determined, and net energy-lactation (NEL), total short-chain fatty acids (SCFA), and microbial crude protein synthesis (MCP) were estimated. Our results showed that the highest dose of EFE improved the amount and rate of rumen fermentation ($p < 0.05$) and decreased lag time in onset of fermentation ($p < 0.05$). In addition, it increased ($p < 0.05$) DMD, NEL, SCFA, and MCP, while NH_3-N decreased ($p < 0.05$) and ruminal pH was unaffected ($p > 0.05$). This result was due to hydrolysis ($p < 0.05$) of fiber content and release ($p < 0.05$) of total and reducing sugars, as well as the increase ($p < 0.05$) of ruminal fibrolytic enzyme activity and protozoa. The low and medium had no effect on the nutritional value of SSC. This eco-friendly bioprocess with the high dose of EFE can be a good strategy for bioconversion of these agro-industrial wastes into high-energy feeds.

Keywords Bioconversion · Sesame seed coat · Fibrolytic enzymes · Feedstuff livestock · Rumen fermentation

1 Introduction

Valorization of agro-industrial wastes has become one of the major challenges to reduce pollution [1]. The use of agro-industrial wastes as alternative livestock feed can be beneficial for feed safety, the livestock sector, and the environment [1–3]. However, much of this waste is burned or buried, rendering it unusable and polluting the environment [1]. The agro-industrial wastes of halva preparation (from dehulled, roasted, and grinded sesame seeds), commonly referred as sesame seed coat (SSC), are present in high quantities in North Africa, the Middle East, and Europe. These agro-industrial wastes, which are generated after dehulling of sesame seeds by water injection and sieving, consist of the testa, bran, and hull of the sesame seeds and possibly small or broken seeds that are lost during sieving. These agro-industrial wastes account for 14% of the sesame seeds used in halva preparation. It consists of a high proportion of dietary fiber, fat, and ash [4–6]. In Tunisia, the amount of this by-product is estimated to be about 1400 tonnes/year, it is usually discarded and rarely used as animal feed [4, 5]. Few studies have investigated their use as feed for livestock. In these studies, it was found that SSC can be added to the lambs' diet at 10% [7] and kids' diet at 20% [8]. At these proportions, SSC is considered safe and has no deleterious effects on growth performance [7, 8]. Although ruminants are capable of converting the gross energy of fibrous feeds into net energy, this bioconversion is very

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limited [9]. Several studies have shown that pre-treatment of fibrous ago-food wastes with exogenous fibrolytic enzymes (EFE) is a good nutritional strategy to improve ruminant performance through improved digestibility bioprocesses and reduce energy losses [10–14]. These feed additives can bioconvert dietary fiber to simple sugars during the pre-incubation period [13] and increase the microbial population in the rumen [15]. The extent of the bioprocess depended on the enzyme dose, enzyme complex, enzyme form, substrate, and dose-substrate interaction [12, 13]. In excessive doses, EFE has a depressant effect on digestion and fermentation [12, 13]. Excessive doses of EFE could hinder the attachment of microorganisms in the rumen to feed particles and produce antinutritional phenolic compounds during feed degradation [16]. Therefore, the effects of EFE need to be tested before widespread use in an area. To our knowledge, no study has investigated the effect of this additive on SSC. The hypothesis of this study is that EFE could be used as an ideal additive for the bioconversion of these wastes into high-energy feeds. This study was conducted to determine the optimal dose of EFE that would increase fermentation, digestibility, net energy lactation, total volatile fatty acids of SSC, and microbial protein synthesis.

2 Materials and methods

2.1 Collect of sesame seed coat, treated with enzymes fibrolytic exogen, and analyze their chemical composition

Samples of SSC were collected from two factories producing halva (Sfax, Tunisia). This by-product was minced to 1 mm size and treated with complexes of two commercial, liquid EFE mixtures of xylanase plus and cellulase plus (Dyadic International Inc. Jupiter, FL, USA) at a ratio of 1:1 (v/v). EFE was obtained from Trichoderma *longibrachiatum*. The treatment was performed according to the protocols of Abid et al. [13]. Briefly, the mixture was diluted in distilled water at 1 µl mixture preparation per 0.2 ml, 2 µl mixture preparation per 0.2 ml, or 4 µl mixture preparation per 0.2 ml and applied by spraying SSC with one of the enzyme solutions (or distilled water only in the control) at a rate of 0.2 ml of solution per gram of DM at 26 °C and 12 h before the start of in vitro incubation. Thus, the final concentration of this additive was 0 (control), 1 (low), 2 (medium), and 4 µl g^{-1} (high) DM weight. Xylanase, endoglucanase, and exoglycanase activities of EFE were measured under conditions corresponding to the rumen environment of pH 6.6 and temperature of 39 °C. Endoglucanase and exoglycanase activities were analyzed according to the protocols of Wood and Bhat [17]. Xylanase activity was analyzed according to the protocols of Baiely and Poutanen [18]. This feed additive has 2267 units of xylanases per ml, 1161 units of endoglucanase per ml, and 113 units of exoglucanase per ml.

Samples of SSC pre-treated with the appropriate doses were oven dried at 55 °C for 48 h and ground to 1 mm particle size. Crude protein (CP), ether extracts (EE), and ash were analyzed according to the Association of Official Chemists Analytical Chemists protocols [19]. Neutral detergent fiber (NDF), acid detergent fiber (ADF), and acid detergent lignin (ADL) were analyzed using a fiber analyzer (ANKOM220, Macedon, NY, USA) according to the protocol of Van Soest et al. [20]. Total phenols (TP) and total tannins (TT) were measured using the Folin-Ciocalteu colorimetric method with spectrophotometry (Shimadzu UV-1201 UV–Vis spectrophotometer) at 725 nm absorbance [21]. The condensed tannins were analyzed by acid-butanol-HCl-Fe method with spectrophotometry (Shimadzu UV-1201 UV–Vis spectrophotometer) at 550 nm absorbance [21]. Hydrolyzable tannins were determined by the difference between total and condensed tannins [21]. Reducing sugars were determined by the 3,5-dinitrosalicylic acid method with spectrophotometry (Shimadzu UV-1201 UV–Vis spectrophotometer) at 540 nm absorbance [22]. Total sugars were determined by phenol–sulfuric acid method with spectrophotometry (Shimadzu UV-1201 UV–Vis spectrophotometer) at 490 nm absorbance [23]. Calcium content was analyzed using a flame atomic absorption spectrophotometer (Varian AA140, Varian, Australia). Phosphorus content was analyzed using the colorimetric molybdovanadate method with spectrophotometry (Shimadzu UV-1201 UV–Vis spectrophotometer) at 690 nm absorbance [19].

2.2 In vitro rumen incubation

In vitro rumen incubation was used in this study to measure gas production according to the protocol of Theodorou et al. [24]. Rumen fluid was obtained from 2 slaughtered adult Holstein cows (body weight 650 kg) in a local slaughterhouse. Each cow was fed twice daily with a total ration consisting of 7 kg of oat hay and 3 kg of commercial concentrate. Rumen fluid was collected from different parts of the rumen. The rumen fluid was filtered through four layers of cheesecloth into a vacuum flask. The inoculum from these two cows was mixed in a 1:1 (v/v) ratio and immediately transported to the laboratory. The filtered rumen fluid was mixed with a 1:2 (v/v) buffered solution of Menke and Steingass [25] under rumen-like conditions (anaerobic and at a temperature of 39 °C). Samples of 0.2 g of ground SSC, untreated and treated with the appropriate dose of EFE, were incubated with 30 ml of the buffered rumen solution in serum bottles of 120 ml volume. In addition, negative control bottles (containing only 30 ml of the buffered rumen fluid) were prepared to correct for gas production from the buffered rumen fluid. Each treatment was performed in triplicate (3 replicates). These bottles were immediately flushed

with CO_2, capped, and incubated in a water bath at 39 °C. Gas pressure was measured with a pressure transducer and a data logger after 2, 4, 6, 8, 12, 24, 48, 72, and 96 h. This experiment was repeated three times (3 runs). The gas produced was corrected for the gas produced in the negative control bottles and converted to volume. Fermentation kinetic gas production was adjusted using a nonlinear option from SAS [26] according to Eq. 1 [27]:

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

where *GP* is the net gas production, t is the incubation time in h, *B* is the asymptotic gas in ml per g dry matter (DM), *C* is the constant gas rate in ml per h, and *Lag* is the delay in the onset of gas production in h.

The net energy lactation (NEL) was estimated according to Eq. 2, and total short-chain fatty acids (SCFA) were calculated according to Eq. 3: [25, 28]

$$NE_L = 0.101 \times GP24 + 0.051 \times CP + 0.112 \times EE \quad (2)$$

$$SCFA = -0.00425 + 0.0222 \times GP24 \quad (3)$$

Where *NEL* is net energy lactation in MJ per kg of DM; *GP*24 is net gas production (ml) from 200 mg of DM after 24 h of incubation; *CP* is crude protein in percentage of DM; and *EE* is ether extracts in percentage of DM.

At the end of incubation, the pH of the fermentation fluid was immediately measured using a pH meter (Jenway Ltd Felsted, model 3020, England). The contents of each serum bottle were filtered with filter paper (Whatman 541). The residues were collected and dried at 55 °C for 48 h to determine the DM digestibility (DMD) according to Eq. 4:

$$DMD = \frac{\text{initial DM} - \text{residual DM}}{\text{initial DM}} \times 100 \quad (4)$$

Microbial crude protein synthesis (MCP) was determined according to Eq. 5: [29]

$$MCP = DMD - 2.2 \times GP24 \quad (5)$$

where *MCP* is the microbial crude protein synthesis in mg per g of DM; *DMD* is the amount of digestible DM in mg per g at the end of incubation; and *GP*24 is the net gas production (ml) from 200 mg of DM of the substrate after 24 h of fermentation [29].

Xylanase, endoglucanase, and exoglycanase activities in the fermenter contents were determined according to the protocol of Patra et al. [30]. Briefly, 6 ml of filtrate was mixed with 1 ml of carbon tetrachloride and 1 ml of lysozyme. The mixture was incubated at 40 °C for 3 h and then sonicated at 4 °C. This was followed by centrifugation at 24,000 × g for 20 min at 4 °C, and the supernatant was used to determine enzymatic activities according to the protocol described above. For counting protozoa, 2 mL of the filtered liquid was reserved in 2 mL (1:1 v/v) methyl green formalin saline solution. Protozoa were counted by light microscopy using a Levy-Hausser counting chamber as described by Dehority [31].

For NH_3-N determination, 2 ml of the filtered liquid was preserved with 1 ml of 1 N H_2SO_4, and analysis was performed by the phenol-hypochlorite method with spectrophotometry (Shimadzu UV-1201 UV–Vis spectrophotometer) at 630 nm absorbance as described by Broderick and Kang [32].

2.3 Statistical analysis

All data were statically analyzed using the general linear model (GLM) of SAS [26]

$$Y_{ij} = \mu + Dose_i + \varepsilon_{ij}$$

where Y_{ij} is the observation, μ is the general mean, $Dose_i$ is the effect of the i^{th} dose, and ε_{ij} is the residual experimental residual error. Linear and quadratic orthogonal contrasts were tested using the contrast statement of SAS (26). The difference between doses was assessed using Tukey's multiple-range test. Differences are considered significant if p-value ≤ 0.05.

3 Results

Table 1 reveals that SSC was characterized by high NDF, fat, and ash compounds and low total and reducing sugar compounds. Table 1 shows that the pre-treated SSC with EFE at increasing the dose decreased (linear $p < 0.05$) NDF and ADF, and increased (linear $p < 0.05$) total and reducing sugar compounds of SSC. However, the other components of SSC remained unchanged. Compared with the control, only the highest dose had a significant effect on the chemical composition of SSC ($p < 0.05$). The cumulative gas production curve illustrates in Fig. 1 indicates that the highest dose of EFE increased ($p < 0.05$) the cumulative gas production after 24 h of fermentation until 96 h of fermentation (the end of fermentation) compared with untreated SSC. The other doses of EFE had no effect on cumulative gas production throughout the fermentation period compared with untreated SSC. The adjustment of gas production in Table 2 shows that SSC is a low and slow fermenting feed. From Table 2, it can be seen that the amount and rate of rumen fermentation were greater in SSC pre-treated SSC with the highest EFE dose than in untreated SSC, while their lag time at the onset of fermentation was less than in untreated SSC. However, other doses did not significantly affect ($p > 0.05$) the gas production kinetics of SSC. Table 3 summarized that DMD, NEL, SCFA, and MCP of the pre-treated SSC with the highest EFE were greater than untreated SSC while

Table 1 Effect of exogenous fibrolytic enzymes on chemical composition of sesame seed coats (g kg^{-1} dry matter)

Item	Control[1]	low	Medium	High	SEM	P-linear	P-quadratic
Crude protein	162	163	162	162	9	NS	NS
Ether extract	291	290	291	292	17	NS	NS
Neutral detergent fiber	307[a]	299[a]	290[ab]	271b	33	*	NS
Acid detergent fiber	153[a]	150[a]	142[ab]	135[b]	21	*	NS
Acid detergent lignin	21	21	20	21	4	NS	NS
Ash	119	120	121	122	9	NS	NS
Phosphore	2.3	2.2	2.4	2.3	0.7	NS	NS
Calcium	36.0	36.1	35.8	36.1	0.9	NS	NS
Total sugar	44[b]	44[b]	46[b]	72[a]	9	NS	NS
Reducing sugar	29[b]	32[b]	33[b]	59[a]	11	*	NS
Total polyphenol	8.1	8.0	8.2	7.9	0.6	NS	NS
Total tannin	4.8	4.9	4.7	4.9	0.5	NS	NS
Hydrolysable tannin	2.8	3.0	2.6	2.9	0.4	NS	NS
Condensed tannin	2.0	1.9	2.1	2.0	0.4	NS	NS

Control, untreated sesame seed coats; low, sesame seed coats pretreated with exogenous fibrolytic enzymes at 1 µL g^{-1} dry matter; medium, sesame seed coats pretreated with exogenous fibrolytic enzymes at 2 µL g^{-1} dry matter, High, sesame seed coats pretreated with exogenous fibrolytic enzymes at 2 µL g^{-1} dry matter

[a,b]Means in the same row with different superscripts letters differed at $p < 0.05$ (Tukey's test); *SEM* standard error of the means; *, $p < 0.05$; NS, $p > 0.05$

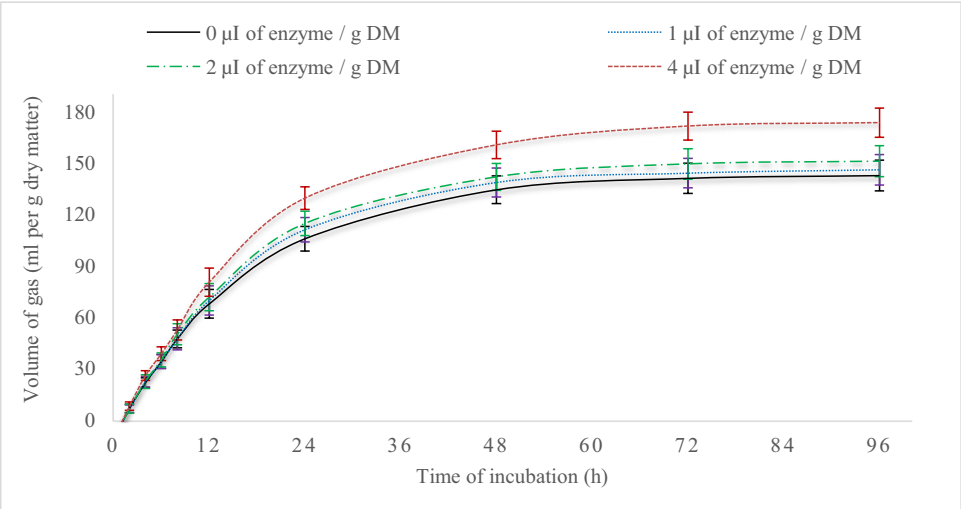

Fig. 1 Effect of exogenous fibrolytic enzymes on gas profile of sesame seed coats

Table 2 Effect of exogenous fibrolytic enzymes on gas production kinetics of sesame seed coats

Item	Control	Low	Medium	High	SEM	P-linear	P-quadratic
Asymptotic gas production (mL per g dry matter)	143.9[b]	145.1[b]	148.4[b]	177.1[a]	7.1	**	NS
Constant rate of gas production (mL per h)	0.060[b]	0.066[b]	0.065[b]	0.079[a]	0.011	*	NS
Delay at the start of gas production (h)	1.09[a]	1.02[a]	1.04[a]	0.88[b]	0.07	*	NS

Control, untreated sesame seed coats; low, sesame seed coats pretreated with exogenous fibrolytic enzymes at 1 µL g^{1} dry matter; medium, sesame seed coats pretreated with exogenous fibrolytic enzymes at 2 µL g^{1} dry matter, High, sesame seed coats pretreated with exogenous fibrolytic enzymes at 2 µL g^{1} dry matter

[a,b]Means in the same row with different superscripts letters differed at $p < 0.05$ (Tukey's test); *SEM* standard error of the means; **, $p < 0.01$; *, $p < 0.05$; NS, $p > 0.05$

Table 3 Effect of exogenous fibrolytic enzymes on rumen fermentation characteristic, dry matter digestibility, net energy-lactation and microbial crude protein synthesis of sesame seed coats

Item	Control	Low	Medium	High	SEM	P-linear	P-quadratic
Rumen fermentation characteristic							
Ph	6.78	6.72	6.75	6.70	0.13	NS	NS
Ammoniacal nitrogen (mg/100 ml)	268[a]	257[a]	246[ab]	231[b]	19	*	NS
Total short-chain fatty acids (mmol per 200 mg dry matter)	0.47[b]	0.48[b]	0.50[b]	0.59[a]	0.04	*	NS
Dry matter digestibility (%)	45.9[b]	46.8[b]	47.7[b]	52.2[a]	1.8	*	NS
Net energy-lactation (MJ per kg DM)	6.24[b]	6.27[b]	6.41[b]	6.78[a]	0.29	*	NS
Microbial crude protein synthesis (mg per g dry matter)	412.1[b]	420.4[b]	426.9[b]	463.6[a]	15.2	**	NS

Control, untreated sesame seed coats; low, sesame seed coats pretreated with exogenous fibrolytic enzymes at 1 µL g^{-1} dry matter; medium, sesame seed coats pretreated with exogenous fibrolytic enzymes at 2 µL g^{-1} dry matter, High, sesame seed coats pretreated with exogenous fibrolytic enzymes at 2 µL g^{-1} dry matter

[a,b]Means in the same row with different superscripts letters differed at $p < 0.05$ (Tukey's test); *SEM* standard error of the means; **, $p < 0.01$; *, $p < 0.05$; NS, $p > 0.05$

NH_3-N was lower than untreated SSC and ruminal pH was unaffected compared to untreated SSC. However, SSC pretreated with other doses had similar DMD, NEL, SCFA, MCP, NH_3-N, and ruminal pH compared with untreated SSC. Analysis of the ruminal fibrolytic enzyme activity and the number of rumen protozoa at the end of incubation in Table 4 shows that the high dose of EFE can increase the ruminal fibrolytic enzyme activity and the ruminal protozoa. All other doses have no significant effect ($p > 0.05$) on the activity of fibrolytic enzyme in the rumen and the number of protozoa in the rumen.

4 Discussion

The results of the present study indicate that SSC have a high NDF content (301 g kg^{-1} DM) with a low lignin content (21 g kg^{-1} DM), and a high CP content (162 g kg^{-1} DM), which is comparable to that of wheat and rice bran [33]. The SSC used in this study has higher CP contents than that found by Elleuch et al. [4]. This difference could be due to the industrial process and the variety of sesame seeds. In addition, this industrial waste can be used as a good source of minerals (119 g kg^{-1} DM), calcium (36 g kg^{-1} DM), and lipids (290 g kg^{-1} dry weight) for ruminant nutrition. This composition is higher than in classical ruminant feed [33]. Compared to other studies, the EE content of SSC is relatively high [4]. This controversial result may be attributed to industrial processing, which may remove more small and broken sesame seeds during the dehulling and sieving process, which are an important source of EE [4].

The total polyphenol content in SSC is moderate (8.1 g kg^{-1} DM), comparable to values reported by Elleuch et al. [5]. These secondary metabolites may improve oxidative stability and promote animal health [2]. The high levels of fiber, protein, and fat in SSC lead to ecological pollution when SSC is dumped in landfills, while the lack of recycling of these nutrients represents a waste of resources.

In vitro rumen fermentation is the most commonly used method to determine the nutritional value of feed [1, 10, 12–14, 33, 34] and shows that the DMD of SSC is very low compared to citrus pulp [35] and compared to DMD of untreated rice straw [34]. The low DMD of SSC is due to their high EE content (290 g kg^{-1} DM), which blocks the binding of the rumen microbiota to carbohydrates and their proliferation [36, 37]. The presence of total polyphenols,

Table 4 Effect of adding exogenous fibrolytic enzymes to sesame seed coats on rumen fibrolytic enzymes activities and protozoa population in rumen

	Control	Low	Medium	High	SEM	P-linear	P-quadratic
Xylanase activity (unites /ml)	1.07[b]	1.15[b]	1.12[b]	1.42[a]	0.21	*	NS
Endoglucanase activity (unites /ml)	4.20[b]	4.32[b]	4.29[b]	5.41[a]	0.36	*	NS
Exoglycanase activity (unites /ml)	25.1[b]	26.2[b]	26.5[b]	28.3[a]	1.23	*	NS
Total protozoa (10^3 cells/ml)	43.2[b]	47.2[b]	47.1[b]	57.2[a]	4.88	*	NS

Control, untreated sesame seed coats; low, sesame seed coats pretreated with exogenous fibrolytic enzymes at 1 µL g^{-1} dry matter; medium, sesame seed coats pretreated with exogenous fibrolytic enzymes at 2 µL g^{-1} dry matter, High, sesame seed coats pretreated with exogenous fibrolytic enzymes at 2 µL g^{-1} dry matter

[a,b]Means in the same row with different superscripts letters differed at $p < 0.05$ (Tukey's test); *SEM* standard error of the mean; *, $p < 0.05$; NS, $p > 0.05$

especially condensed tannins (2 g kg^{-1} DM) in this industrial waste forms indigestible complex mainly with proteins and to a lesser extent with polysaccharides and minerals at rumen pH condition (pH 5.5 to 7), prevents bacterial attachment to the feed, inhibits fiber-degrading bacteria populations, decreases rumen turnover rate, inhibits protease and carbohydrase enzymes, and reduces microbial digestibility of nutriment [38]. Changing the pH in the abomasum (pH 2.5 to 3.5) dissolved this complex, increasing the flow of undegradable dietary protein into the post-ruminal compartments without harming post-ruminal digestion [39, 40]. As a result, digestive utilization of dietary proteins is enhanced [40]. This increase in amino acid absorption contributes to fiber digestibility and energy balance, but not to protein synthesis [41]. This polyphenol compound indirectly reduces ammonia and nitrous oxide, which present an ecological problem, by shifting nitrogen extraction from urine (which is rapidly converted to ammonia and nitrous oxide) to nitrogen extraction from feces, which can be retained in soil [40, 42]. In addition, this compound can form a complex with salivary proteins, resulting in a lack of lubricity in the mouth, which leads to a reduction in the diet palpability and voluntary DM intake [43]. According to Makkar, [38] this polyphenolic compound is not degraded and not absorbed into the bloodstream, so it cannot damage organs. Hydrolyzable tannins were also detected in this industrial waste at 2.8 g kg^{-1} DM. This polyphenolic compound also has exhibit negative effects on feed digestion and rumen fermentation and can cause toxic effects (kidney and liver damage) when consumed in high amounts. In small amounts, this compound is efficiently detoxified in the liver [38]. The high NDF content in this waste acts as a barrier that reduces the access of rumen microbes and their hydrolytic enzymes to the nutriment compounds [44], and the cross-linking between the lignin component of the cell wall and the hemicellulose limits the digestion of NDF content of SSC [45].

Pretreatment of SSC with EFE hydrolyzed NDF from 307 to 271 g kg^{-1} DM and increased total sugars from 44 to 72 g kg^{-1} DM and reduced sugars from 29 to 59 g kg^{-1} DM, which likely improved palatability of SSC. In addition, the reduction in NDF fraction may lead to a reduction in rumen fill, which indirectly improves the intake of SSC. Comparable results were obtained for the chemical composition of olive leaves treated with EFE [13] and the feed intake of diets treated with EFE [15]. Increasing the sugar content in SSC increases the proliferation of protozoa in the rumen, science the sugar released from carbohydrates can be assimilated faster and more easily, providing additional energy for the activity of the rumen microflora [46]. In agreement with our results, Zhang et al. [15] demonstrated that the addition of an EFE preparation to the diets of bull improved protozoa and bacterial populations in the rumen. This improvement in rumen protozoa contributes to the increase in the activity of fibrolytic enzymes in the rumen. According to Takenaka et al. [47], rumen protozoa are responsible for up to 30% of fibrolytic enzyme activity in the rumen. In line with these findings, Zhang et al. [15] proved that EFE preparation increases microbial enzyme activity in vivo.

EFE significantly modulates the in vitro rumen fermentation of SSC. The curves of gas production showed significant differences between SSC pretreated with the high dose of EFE and untreated SSC after 24 h of fermentation until the end of fermentation. This result was comparable to that found in palm kernels treated with EFE (MAXFIBER – I®, SHAU-MANN GmbH, Wahlstedt, Germany) at 0.5 mg g^{-1} DM [48]. In contrast, Togtokhbayar et al. [49] showed that the addition of EFE (xylanase PLUS, Dyadic International, Inc., Jupiter, FL, USA) at concentrations 0.5, 1, 1.5, and 2 µL g^{-1} to wheat straw increased gas production at all incubation times. These contradictory results could be due to several factors related to the chemical composition of the feed and the type and dose of EFE. Fermentation adjustment showed that EFE increased volume and fermentation rate by 0.014 ml per hour due to the increase of easily and rapidly fermentable molecules such as soluble sugars in treated SSC and the increase of protozoa in the rumen. According to Chenost and Kayouli [50], faster fermentation of feeds promotes their breakdown into fine particles, increases the transit rate, and decreases rumen congestion. This result is consistent with an in vitro study on olive cake treated with an EFE preparation (50% cellulase Plus and 50% xylanase PLUS, Dyadic International, Inc., Jupiter, FL, USA) at a concentration of 4 µL g^{-1} [12]. Moreover, the lag phase is shortened, which could be due to the shortening of the adhesion time of the rumen microbiota to the SSC particles. This effect can be explained by the bioconversion of NDF to simple sugars, which acts as a chemoattractant for rumen microorganisms, thus shortening the lag time [46]. To our knowledge, this is the first study on the use of feed additives to modulate in vitro rumen fermentation of SSC. This improvement in in vitro fermentation of SSC by added EFE is similar to other agro-industrial wastes found in the literature [12, 13].

Only the high dose of EFE increased the DMD of SSC from 459 to 522 g kg^{-1}, stimulated the SCFA production from 0.47 to 0.59 mmol per 200 mg, and the NEL from 6.24 to 6.78 MJ per kg. It also improved rumen microbial protein synthesis, resulting in a reduction of NH_3-N in the rumen without altering rumen pH, which remained optimal for microbial metabolism [51]. Similarly, Abid et al. [13] demonstrated that the EFE preparation (50% cellulase Plus and 50% xylanase PLUS, Dyadic International, Inc., Jupiter, FL, USA) improved the nutritional value of olive cake only at high dose. Also, Sousa et al. [52] demonstrated that the application of an EFE preparation (Fibrozyme Alltech Inc.) on corn silage at high dosage was more effective

in improving DMD and reducing NH_3-N content in the rumen. An in vivo study also showed that feeding dairy cows with barley silage treated with an EFE preparation (AB Vista, Wiltshire, UK) at a dose of 0.75 µl g^{-1} reduced rumen NH_3-N and did not alter the rumen pH profile [53]. These results were attributed to the reduction of the NDF content of SSC, which hinders the digestibility and utilization of nutrients, as well as the increase in the activities of fibrolytic rumen enzymes and the proliferation of rumen protozoa, which are responsible for up to 20% of fiber digestion and 30% of microbial protein synthesis [54]. Consequently, pre-treatment of SSC with a high dose of EFE may be an eco-friendly strategy to improve nutritional value and reduce wastage of nutrients and pollution. In contrast to these results, a negative effect of a high dose of EFE preparation (50% cellulase Plus and 50% xylanase PLUS, Dyadic International, Inc., Jupiter, FL, USA) on the nutritional value of olive leaves was demonstrated [13]. The differences in the results may be related to the differences in the substrate and rumen ecosystem.

5 Conclusion

The results suggest that the efficacy of SSC pre-treated with EFE depends on the EFE dose. Only the highest dose of 4 µl g^{-1} DM allowed the partial bioconversion of NDF to simple sugars in the pre-incubation period, as well as the increase in the activity of fibrolytic rumen enzymes and rumen protozoa. Consequently, it improves DMD, bioconversion of NH3-N to rumen microbial protein, and conversion of gross energy to net energy of SSC. The use of treated SSC in animal feed represents a new source of revenue for the halva industry and replaces the cost of removing these residues; it is also a raw material with good nutritional value for the feed industry.

Abbreviations *ADF*: Acid detergent fiber; *ADL*: Acid detergent lignin; *B*: Asymptotic gas; *C*: Constant gas rate; *CP*: Crude protein; *DM*: Dry matter; *DMD*: Dry matter digestibility; *EE*: Ether extracts; *EFE*: Exogenous fibrolytic enzymes; *GP*: Net gas production; *GP24*: Net gas production after 24 h of incubation; *NDF*: Neutral detergent fiber; *NEL*: Net energy lactation; *MCP*: Microbial crude protein synthesis; *SCFA*: Short-chain fatty acids; *SEM*: Standard error of means; *SSC*: Sesame seed coat; *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

Ethics approval and consent to participate 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. All the authors of this article consented to participate.

References

1. Narimani Gharajeh S, Seifdavati J, Abdi-Benemar H, Salem AZM, Elghandour MM, Seyed Sharifi R (2021) Effects of nitrogen supplementation on bioconversion of potato waste by rumen fluid from slaughterhouses to produce eco-friendly products. Biomass Convers Biorefin., 1-10. https://doi.org/10.1007/s13399-021-02037-0
2. Correddu F, Lunesu MF, Buffa G, Atzori AS, Nudda A, Battacone G, Pulina G (2020) Can agro-industrial by-products rich in polyphenols be advantageously used in the feeding and nutrition of dairy small ruminants? Animals 10:131. https://doi.org/10.3390/ani10010131
3. Lucio VD, Susana B, Hernández-Domínguez EM, Villa-García M, Díaz-Godínez G, Mandujano-Gonzalez V, Mendoza-Mendoza B, Álvarez-Cervantes J (2021) Exogenous enzymes as zootechnical additives in animal feed: a review. Catalysts 11:851. https://doi.org/10.3390/catal11070851
4. Elleuch M, Besbes S, Roiseux O, Blecker C, Attia H (2007) Quality characteristics of sesame seeds and by-products. Food Chem 103:641–650. https://doi.org/10.1016/j.foodchem.2006.09.008
5. Elleuch M, Bedigian D, Besbes S, Blecker C, Attia H (2012) Dietary fibre characteristics and antioxidant activity of sesame seed coats (testae). Int J Food Prop 15:25–37. https://doi.org/10.1080/10942911003687231
6. Morsy MK, Sami R, Algarni E, Al-Mushhin AA, Benajiba N, Almasoudi AG, Mekawi E (2022) phytochemical profile and antioxidant activity of sesame seed (Sesamum indicum) by-products for stability and shelf life improvement of refined olive oil. Antioxidants 11:338. https://doi.org/10.3390/antiox11020338
7. Kaya I, Bölükbaş B, Aykut U, Uğurlu M, Muruz H, Salman M (2022) The effects of adding waste sesame seeds to diets on performance, carcass characteristics, and meat fatty acid composition of Karayaka lambs. Ankara Univ Vet Fak Derg 69:183–189. https://doi.org/10.33988/auvfd.843049
8. Awawdeh MS, Hananeh WM, Abdullah AY (2018) Histopathological effects of alternative feedstuffs (sesame hulls and Prosopis juliflora) on ruminal walls in black goat kids in Jordan. J Appl Anim Res 46(1):130–133. https://doi.org/10.1080/09712119.2016.1276020
9. Varga GA, Kolver ES (1997) Microbial and animal limitations to fiber digestion and utilization. J Nutr 127:819S-823S. https://doi.org/10.1093/jn/127.5.819S
10. Abid K, Jabri J, Beckers Y, Yaich H, Malek A, Rekhis J, Kamoun M (2019) Effects of exogenous fibrolytic enzymes on the ruminal fermentation of agro-industrial by-products. S Afr J Anim Sci 49(4):612–618. https://doi.org/10.4314/sajas.v49i4.2
11. Abid K, Jabri J, Ammar H, Ben Said S, Yaich H, Malek A, Rekhis J, Lopez S, Kamoun M (2020) Effect of treating olive cake with fibrolytic enzymes on feed intake, digestibility and performance in growing

lambs. Anim Feed Sci Tech 261:114405. https://doi.org/10.1016/j.anifeedsci.2020.114405
12. Abid K, Jabri J, Yaich H, Malek A, Rekhis J, Kamoun M (2022) Study of the influence of exogenous fibrolytic enzyme additive on chemical composition, fermentation characteristics, and nutritional value of brewer's spent grain. Food Sci Nutr 10(6):1707–1713. https://doi.org/10.1002/fsn3.2743
13. Abid K, Jabri J, Yaich H, Malek A, Rekhis J, Kamoun M (2022) In vitro study on the effects of exogenic fibrolytic enzymes produced from *Trichoderma longibrachiatum* on ruminal degradation of olive mill waste. Arch Anim Breed 65:79–88. https://doi.org/10.5194/aab-65-79-2022
14. Kholif AE, Gouda GA, Morsy TA, Matloup OH, Fahmy M, Gomaa AS, Patra AK (2022) Dietary date palm leaves ensiled with fibrolytic enzymes decreased methane production, and improved feed degradability and fermentation kinetics in a ruminal in vitro system. Waste Biomass Valor 13:3475–3488. https://doi.org/10.1007/s12649-022-01752-7
15. Zhang J, Wang C, Liu Q, Guo G, Huo W, Pei C, Jiang Q (2022) Influence of fibrolytic enzymes mixture on performance, nutrient digestion, rumen fermentation and microbiota in Holstein bulls. J Anim Feed Sci 31(1):46–54. https://doi.org/10.22358/jafs/147188/2022
16. Beauchemin KA, Colombatto D, Morgavi DP, Yang WZ (2003) Use of exogenous fibrolytic enzymes to improve feed utilization by ruminants. J Anim Sci 81(14_suppl_2):E37–E47. https://doi.org/10.2527/2003.8114_suppl_2E37x
17. Wood TM, Bhat KM (1988) Methods for measuring cellulase activities. Methods Enzymol 160(87):112. https://doi.org/10.1016/0076-6879(88)60109-1
18. Baiely P, Poutanen K (1992) Interlaboratory testing of methods for assay of xylanase activity. J Biotechnol 23:257–270. https://doi.org/10.1016/0168-1656(92)90074-J
19. Association of Official Chemists Analytical Chemists (2016) Official methodes of analysis of AOAC international, 20th Edn., AOAC International, Gaithersburg, MD, USA, ISBN 0–935584–87–0
20. Van Soest PV, Robertson JB, Lewis BA (1991) Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. J Dairy Sci 74:3583–3597. https://doi.org/10.3168/jds.S0022-0302(91)78551-2
21. Makkar HPS, Blummel M, Borowy NK, Becker K (1993) Gravimetric determination of tannins and their correlations with chemical and protein precipitation methods. J Sci Food Agr 61:161–165. https://doi.org/10.1002/jsfa.2740610205
22. Miller GL (1959) Use of dinitrosalicylic acid reagent for determination of reducing sugars. Anal Chem 31:426–428. https://doi.org/10.1021/ac60147a030
23. Dubois M, Gilles KA, Hamilton JK, Rebers PT, Smith F (1956) Colorimetric method for determination of sugars and related substances. Anal Chem 28:350–356. https://doi.org/10.1021/ac60111a017
24. Theodorou MK, Williams BA, Dhanoa MS, McAllan AB, France JA (1994) Simple gas production method using a pressure transducer to determine the fermentation kinetics of ruminant feeds. Anim Feed Sci Technol 48:185–197. https://doi.org/10.1016/0377-8401(94)90171-6
25. Menke KH, Steingass H (1988) Estimation of the energetic feed value obtained from chemical analysis and in vitro gas production using rumen fluid. Anim Res Dev 28:7–55
26. SAS Institute Inc (2011) SAS/STAT® 9.3, User's Guide, Cary, NC, USA. ISBN 978–1–60764–896–3
27. France J, Dijkstra J, Dhanoa MS, Lopez S, Bannink A (2000) Estimating the extent of degradation of ruminant feeds from a description of their gas production profiles observed in vitro: derivation of models and other mathematical considerations. Br J Nutr 83:143–150. https://doi.org/10.1017/S0007114500000180
28. Getachew G, Blummel M, Makkar HPS, Becker K (1998) In vitro gas measuring techniques for assessment of nutritional quality of feeds: a review. Anim Feed Sci Tech 72:261–281. https://doi.org/10.1016/S0377-8401(97)00189-2
29. Blümmel M, Makkar HPS, Becker K (1997) In vitro gas production: a technique revisited. J Anim Physiol An N 77:24–34. https://doi.org/10.1111/j.1439-0396.1997.tb00734.x
30. Patra AK, Kamra DN, Agarwal N (2006) Effect of plant extracts on in vitro methanogenesis, enzyme activities and fermentation of feed in rumen liquor of buffalo. Anim Feed Sci Technol 128(3–4):276–291. https://doi.org/10.1016/j.anifeedsci.2005.11.001
31. Dehority BA (1993) Laboratory manual for classification and morphology of rumen ciliate protozoa. CRC Press. https://doi.org/10.1201/9781351073912
32. Broderick GA, Kang JH (1980) Automated simultaneous determination of ammonia and total amino acids in ruminal fluid and in vitro media. J Dairy Sci 63:64–75. https://doi.org/10.3168/jds.S0022-0302(80)82888-8
33. Abaş I, Özpinar H, Kutay HC, Kahraman R, Eseceli H (2005) Determination of the metabolizable energy (ME) and net energy lactation (NEL) contents of some feeds in the Marmara region by in vitro gas technique. Turk J Vet Anim Sci 29:751–757
34. Datsomor O, Gou-Qi Z, Miao L (2022) Effect of ligninolytic axenic and coculture white-rot fungi on rice straw chemical composition and in vitro fermentation characteristics. Sci Rep 12:1–8. https://doi.org/10.1038/s41598-022-05107-z
35. Lashkari S, Taghizadeh A (2013) Nutrient digestibility and evaluation of protein and carbohydrate fractionation of citrus by-products. J Anim Physiol Anim Nutr (Berl) 97(4):701–709. https://doi.org/10.1111/j.1439-0396.2012.01312.x
36. Bionaz M, Vargas-Bello-Pérez E, Busato S (2020) Advances in fatty acids nutrition in dairy cows: from gut to cells and effects on performance. J Anim Sci Biotechnol 11:1–36. https://doi.org/10.1186/s40104-020-00512-8
37. Joy F, Johnson JA, Górka P, McKinnon JJ, Hendrick S, Penner GB (2021) Effect of dietary lipid inclusion from by-product-based pellets on dry matter intake, ruminal fermentation, and nutrient digestion in finishing beef heifers. Can J Anim Sci 101:481–492. https://doi.org/10.1139/cjas-2020-0133
38. Makkar HPS (2003) Effects and fate of tannins in ruminant animals, adaptation to tannins, and strategies to overcome detrimental effects of feeding tannin-rich feeds. Small Rumin Res 49(3):241–256. https://doi.org/10.1016/S0921-4488(03)00142-1
39. Min BR, Barry TN, Attwood GT, McNabb WC (2003) The effect of condensed tannins on the nutrition and health of ruminants fed fresh temperature forages. A review Anim Feed Sci Technol 106:3–19. https://doi.org/10.1016/S0377-8401(03)00041-5
40. Mueller-Harvey I (2006) Unravelling the conundrum of tannins in animal nutrition and health. J Sci Food Agric 86:2010–2037. https://doi.org/10.1002/jsfa.2577
41. Waghorn G (2008) Beneficial and detrimental effects of dietary condensed tannins for sustainable sheep and goat production-Progress and challenges. Anim Feed Sci Technol 147:116–139. https://doi.org/10.1016/j.anifeedsci.2007.09.013
42. Hristov AN, Oh J, Lee C, Meinen R, Montes F, Ott T, Firkins J, Rotz A, Dell C, Adesogan A, Yang W, Tricarico J, Kebreab E, Waghorn G, Dijkstra, J Oosting S (2013) Mitigation of greenhouse gas emissions in livestock production - a review of technical options for non-CO2 emissions, In: Gerber PJ, Henderson B, Makkar HPS (eds). FAO Animal Production and Health Paper No. 177. FAO, Rome, Italy. ISBN 978–92–5–107658–3
43. Lesschaeve I, Noble AC (2005) Polyphenols: factors influencing their sensory properties and their effects on food and beverage

preferences. Am J Clin Nutr 81:330S-335S. https://doi.org/10.1093/ajcn/81.1.330S
44. Rosser C, Terry SA, Badhan A, McAllister TA, Beauchemin KA (2022) Current knowledge and future opportunities for Ruminant Enzymes. In: Bedford MR, Partridge GC, Hruby M, Walk CL (ed) Enzymes in Farm Animal Nutrition. CABI Publishing, CAB International, London, UK, pp 153–169. https://doi.org/10.1079/9781789241563.0000.
45. Van Soest PJ (1994) Nutritional Ecology of the Ruminant, 2nd Edn., Comstock: Ithaca, NY, USA. ISBN-13: 978-0801427725
46. Lopez S (2005) In vitro and in situ techniques for estimating digestibility. In: Dijkstra J, Forbes JM, France J (ed) Quantitative aspects of ruminant digestion and metabolism. CABI Publishing, CAB International, Wallingford, UK, pp 87–122. https://doi.org/10.1079/9780851998145.0000
47 Takenaka A, Tajima K, Mitsumori M, Kajikawa H (2004) Fiber digestion by rumen ciliate protozoa. Microbes Environ 19:203–210. https://doi.org/10.1264/jsme2.19.203
48. Abid K, Jabri J, Beckers Y, Yaich H, Malek A, Rekhis J, Kamoun M (2019) Influence of adding fibrolytic enzymes on the ruminal fermentation of date palm by-products. Arch Anim Breed 62:1–8. https://doi.org/10.5194/aab-62-1-2019
49. Togtokhbayar N, Cerrillo MA, Rodriguez GB, Elghadour MMY, Salem AZM, Urankhaich C, Jigjidpurev S, Odongo NE, Kholif AD (2015) Effect of exogenous xylanase on rumen in vitro gas production and degradability of wheat straw. Anim Sci J 86:765–777. https://doi.org/10.1111/asj.12364
50. Chenost M, Kayouli C (1997) Utilisation des fourrages grossiers en regions chaudes. FAO production et santé animales. Paper No. 135. Rome, Italy. ISBN 9252039813–9789252039815
51. Dijkstra J, Ellis JL, Kebreab E, Strathe AB, López S, France J, Bannink A (2012) Ruminal pH regulation and nutritional consequences of low pH. Anim Feed Sci Tech 172(1–2):22–33. https://doi.org/10.1016/j.anifeedsci.2011.12.005
52. Souza JM, Souza JCSM, Sousa DO, Del Valle TA, Ghizzi LG, Alcântara AHD, Mesquita LG, Sousa RLM, Bueno ICS, Balieiro JCDC (2021) The effects of compound treatment of Aspergillus oryzae and fibrolytic enzyme on in vitro degradation, gas production and fermentative profile of maize silage and sugarcane silage. J Agric Sci 159(1–2):147–158. https://doi.org/10.1017/S002185962100037X
53. Refat B, Christensen DA, Ismael A, Feng X, Rodríguez-Espinosa ME, Guevara-Oquendo VH, Yang J, AlZahal O, Yu P (2021) Evaluating the effects of fibrolytic enzymes on rumen fermentation, omasal nutrient flow, and production performance in dairy cows during early lactation. Can J Anim Sci 102:1–11. https://doi.org/10.1139/cjas-2020-0062
54. Newbold CJ, De la Fuente G, Belanche A, Ramos-Morales E, McEwan NR (2015) The role of ciliate protozoa in the rumen. Front Microbiol 6:1313. https://doi.org/10.3389/fmicb.2015.01313