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Effect of Exogenous Fibrolytic Enzymes Supplementation or Functional Feed Additives on In Vitro Ruminal Fermentation of Chemically Pre-Treated Sunflower Heads

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Citation: Jabri, J.; Ammar, H.; Abid, K.; Beckers, Y.; Yaich, H.; Malek, A.; Rekhis, J.; Morsy, A.S.; Soltan, Y.A.; Soufan, W.; et al. Effect of Exogenous Fibrolytic Enzymes Supplementation or Functional Feed Additives on In Vitro Ruminal Fermentation of Chemically Pre-Treated Sunflower Heads. *Agriculture* **2022**, *12*, 696. https://doi.org/10.3390/agriculture12050696

Academic Editor: Dongyang Liu

Received: 17 March 2022
Accepted: 10 May 2022
Published: 14 May 2022

Abstract: This study aims to provide possible utilization of sunflower head byproduct (SFH) as a feedstuff by implementing chemical pretreatments (4% sodium hydroxide (SFH_{NaOH}) or 4% urea (SFH_{urea}) and supplementation with either exogenous fibrolytic enzymes (EFE) or functional feed additive (FFA). The experimental EFE was a complex (1:1, *v*/*v*) of two enzyme products with high activity of β-1,3-1,4-glucanase and endo-1,4-β-D-xylanase and applied at 0 (SFH_{out}), 1, 2, 5, and 10 µL/ gdry matter, while FFA was a fermentation byproduct rich in cellulase and xylanase activities, applied at 0 (SFH_{out}), 0.5, 1, 2, and 4 mg/g DM. SFH_{urea} had the highest ($p < 0.05$) crude protein (CP) content compared to other SFH substrates. Linear enhancements ($p < 0.05$) in kinetics of gas production (GP), metabolizable energy (ME), organic matter digestibility (OMD) and total short-chain fatty acids (SCFAs) concentrations were observed for all SFH substrates supplemented with EFE. The SFH_{out} had the highest ($p < 0.05$) potential GP, maximum rate (Rmax) of GP, ME, OMD and SCFAs. Supplementation of EFE was more pronounced than FFA in affecting the kinetic parameters of in vitro GP for all SFH substrates. SFH_{out} supplemented with EFE seems to be the most promising substrate to enhance microbial fermentation in vitro.

Keywords: sunflower head; chemical composition; digestibility; in vitro; chemical pretreatments; exogeneous enzymes; feed additive

1. Introduction

Sunflower (*Helianthus annuus*) (SF) includes 67 annual and perennial species that differ in location and growth mode [1]. Although it is widely used for seed and oil production, the plant itself and its crop residues (heads and leftover stalks) have become an increasingly important roughage for livestock production and have been used for this purpose since the early 20th century [2,3]. After harvesting SF for silage, livestock grazed on the leftover stalks and heads that were very palatable, although they are characterized by low protein and fibrous materials contents [4,5]. Regarding the nutritional characteristics of SFH, Aregheore [6] indicated relatively high cell wall contents of NDF (480 g/kg DM), ADF (299 g/kg DM), and ADL (100 g/kg DM). In the case of the whole plant residues, Lardy and Anderson [7] reported that SF silage can be 80 to 85% as valuable as corn silage. However, there is very little evidence on the dietary inclusion of SF residues in ruminant nutrition.

SF byproducts are characterized by high variability in nutrient contents. Rasool et al. [8] reported that SFH inclusion at the ration of 20% in small ruminant diets led to a significant increase in feed intake and weight gain, compared to sunflower stalk, wheat straw, rice straw, and rape straw. Nagalakshmi et al. [9] found that incorporating SFH up to 500 g/kg along with 100 g/kg sorghum straw in a complete diet (roughage concentrate ratio, 60:40) in growing lambs has improved average daily weight gain by 12.9%, consequently reducing meat production costs compared to a conventional diet consisting of concentrate mixture and chopped maize. Similarly, Amini-Jabalkandi et al. [10] have investigated the effects of the substitution of alfalfa hay with SF residue silage on the fattening efficiency of male buffaloes and have indicated that 50% of substitution can be considered as an optimal point without negative effects on performance. Given their high richness in cell wall contents, and for a better valorisation in animal nutrition, different SFH treatments were proposed to modify the rumen microbial fermentation towards more fiber degradation. Pretreatment methods based on chemical, thermo-chemical, mechanical, and biological treatments are currently used to improve the utilization of the lignocellulosic byproducts [11]. Among these methods, alkaline chemical pretreatments are classified as the most common low cost and effective processes used for SF residues [12]. Alkaline pretreatment, such as urea or sodium hydroxide (NaOH), can affect the chemical composition and utilization of the SFH (especially with high-oil varieties) [7]. In this context, Soltan et al. [12] reported that feed supplements containing primarily cellulolytic and xylanolytic activities enhance the utilization of forage with high lignocellulose contents in ruminant diets. In addition to a relatively high content of fibrolytic enzymes and essential nutrient contents (e.g., crude protein, minerals, vitamins, etc.), it is believed that functional feed additives (FFAs), which are issued from fiber microbial fermentation, may provide benefits beyond satisfying traditional nutrient supplies for ruminants. Ammoniation of SF-cob residues increased the crude protein (CP) content without affecting NDF concentration or the DM degradability [13]. However, a study conducted by Sariçiçek and Gripoglu [14] demonstrated that chemical pretreatments have no positive impact on the nutritional value of SFH. Against this background, this study aims to assess the effect of chemical pretreatments (urea or NaOH) of SFH on in vitro ruminal fermentation of cows. The use of fibrolytic enzymes on different forms of SFH (chemically treated or untreated) was also assessed in vitro.

2. Materials and Methods

2.1. Plant Material

After the seeds' harvest, in September in the mature–ripe stage, SFH were collected from different private fields located in the northwest region of Tunisia. They were separated from the stalks and left in the fields for sun-drying (30–35 °C) for 7 days. About 30 kg of dried SFH samples were randomly collected and transported to the laboratory, chopped into small stands (5 cm) using a cutting mill (Pulverisette, Fritsch, Benelux Scientific, Gent, Belgium) to ensure better homogenization. They were divided after well-mixing into six sub-samples of 5 kg in each, which were then used directly for further treatments.

2.2. Pre-Treatment of SFHs

Three pretreatments were applied on the samples, with two subsamples for each treatment. The first treatment was allocated to alkaline pretreatments with 4% NaOH (SFH_{NaOH}) and 4% urea solutions (SFH_{urea}) as described by [15,16], respectively. The two SFH_{NaOH} samples were kept at room temperature for 48 h before the in vitro assay. The SFH_{urea} samples were hermetically sealed in plastic bags for two months before they were exposed to air for 48 h prior to the in vitro assay. The last two sub-samples were used as the control without any pretreatment (SFHout).

After pretreatments, 500 g subsamples were collected randomly from each SFH preparation (SFH_{out}, SFH_{NaOH} and SFH_{urea}), dried overnight at 55 °C and milled in a hammer mill (Retch SK 100 standard, Gubeisen, Germany) using a 1 mm sieve for subsequent analysis.

2.3. Chemical Analysis

Dry matter (DM, method ID 934.01), organic matter (OM, method ID 942.05), ether extract (EE, method ID 920.30), CP (method ID 984.13), and crude fiber (CF, ID 962.09) contents were determined following the methods of the Association of Official Analytical Chemists [17]. Neutral detergent fiber (NDF), acid detergent fibre (ADF), and acid detergent lignin (ADL) were determined in sequence using the ANKOM fibre analyzer (ANKOM, A2001, New York, NY, USA) in a fiber filter bag (F57-ANKOM Technology Corporation, Macedon, NY, USA) [18] applying the reagents described by [19]. The NDF was determined using sodium sulfite addition, instead of amylase. Calcium content (Ca) was measured using an atomic absorption spectrophotometer (Varian AA140, Varian, Australia) (method 968.08; [20]). Total phosphorus (P) contents were analyzed by the molybdovanadate colorimetric method (method 965.17; [21]) using a spectrophotometer (Shimadzu UV-1201 UV-Vis). All chemical analysis of SFH have been done in nine replicates.

2.4. Feed Additives and Determination of Their Enzymatic Activities

Two complexes (considered as feed additives (FA) of exogeneous fibrolytic enzymes (EFE) were supplemented to SFH preparations (SFH_{out}, SFH_{urea}, SFH_{NaOH}) at four different doses. The first complex was a mixture of two liquid commercial enzymatic preparations (1:1, *v*/*v*), which are the Cellulase PLUS (E.C. 3.2.1.4) with a high level of cellulase and beta-1,3-1,4-glucanase and the Xylanase PLUS (E.C. 3.2.1.8) with high levels of xylanase. Both commercial enzyme products contained additional side activities like pectinase, mannanase, β-glucosidase, β-xylosidase, α-L-arabinofuranosidase, amylase and protease (Dyadic International, Jupiter, FL, USA) and were produced by the fermentation of non-GMO Trichoderma longibrachiatum. This enzymatic complex was applied to the three SFH preparations at four experimental levels: (0, 1, 2, 5, and 10 µL /g DM). The second FA was a functional feed additive product (FFA; Max Fiber, Provita Supplements SCHAUMANN GmbH, Bad Laasphe, Germany). It is a powder by-product of solid-state fermentation of five different fungi species: Aspergillus niger, Aspergillus tubingensis, Aspergillus orzyae, Aspergillus sojae, and Neurospora intermedia incubated on four defined substrates: rapeseed meal, sugar beet molasses, corn gluten, and corn powder and containing 315 g kg^{-1} CP. The FA products possessed xylanase, endoglucanase, and exoglucanase activities and were applied at four experimental doses (0, 0.5, 1, 2, and 4 mg/g DM) to the basal feed substrate (SFH_{out}, SFH_{urea}, SFH_{NaOH}).

To measure the enzymatic activities of the experimental FA, an enzyme extraction step was done as described by [22] before its application to the SFH preparations. 1 g of FA product was incubated in 10 mL of citrate buffer (pH = 6.6) followed by homogenization. The supernatant was separated from the solid biomass by centrifugation and then was used as a source of liquid enzyme preparation.

The experimental FA (EFE and FA extracts) were assayed for endoglucanase, exoglucanase, and xylanase activities (Table 1) at normal ruminal conditions (pH = 6.6; 39 °C) following the procedure described by [22,23]. The activities of enzymes were de-

termined in triplicate; one international unit (IU) was defined as the amount of enzyme required to release 1 µmol of released reducing sugar (glucose or xylose) per one minute from the corresponding substrate at pH 6.6 and 39 °C [24]. The substrates were 1% (*v*/*v*) medium-viscosity carboxymethylcellulose sodium salt (CMC, Catalog no. C-4888, Sigma Aldrich, Darmstadt, Germany), cellulose (Sigmacell Cellulose–Type 20, 20 µm, Cotton linters/Cellulose/Cellulose powder, Catalog no. S-3504, Sigma Aldrich, Germany) and oat spelt xylan (1% (wt/vol), catalog no. X-0627, Sigma Aldrich, Germany). The absorbance was read at 540 nm against glucose and xylose standard curves. In order to ensure the creation of a stable enzyme-feed complex, FA was supplemented at room temperature (20–23 °C) for 20 h before the in vitro ruminal incubation [25]. Samples of 200 mg SFH preparations were weighed in 160 mL glass serum incubation bottles.

Table 1. Xylanase, endoglucanase, and exoglucanase activities of the experimental feed additives.

Enzyme Product	Enzymatic Activity (IU) [1]		
	Xylanase	**Endoglucanase**	**Exoglucanase**
Exogenous fibrolytic enzymes	22.760 ± 29	1568 ± 110	164 ± 3.9
Functional feed additive	118 ± 6.0	92 ± 1.0	91 ± 0.3

[1] Xylanase activity was expressed as µmoles of xylose released per minute per milliliter of the enzyme (IU: International Unit). Endoglucanase and exoglucanase activities were expressed as µmoles of glucose released per minute per milliliter of the enzyme (IU). Mean ± StD, $n = 3$ replicates.

2.5. In Vitro Gas Production

The in vitro gas production (GP) technique as described by [26] was used to evaluate the experimental treatments. Two red Holstein cows (bodyweight 490.4 ± 4.23 kg) fitted with a permanent ruminal cannula were used as ruminal inoculum donors to carry out the in vitro assay. The animals were fed a diet consisting of 8 kg of lucerne hay (*Medicago sativa*) and 8 kg of commercial concentrate distributed twice a day with free access to water and mineral/vitamin licks.

The technique of in vitro batch culture using gas-tight fermentation bottles (117 mL) was carried out as described by [26]. Samples of 200 ± 10 mg DM grounded SFH_{out}, SFH_{NaOH}, and SFH_{urea} were weighed into fermentation bottles with six replications each. Thereafter, the corresponding enzymatic dose was added to each sample.

Twenty hours later, a sample of rumen contents was withdrawn via an electric pump, immediately transferred to the laboratory, then mixed and strained through 4 layers of cheesecloth at 39 °C and under CO_2 flow. The fermentation inoculum was prepared by mixing the fresh ruminal fluid to an anaerobic buffer medium (pH) prepared as described by [26] in a ratio of 1:2 (ruminal fluid: medium buffer. Each incubation bottle was filled with 30 mL fermentation inoculum under continuous CO_2 flow, then sealed by butyl rubber stopper and aluminum crimp cap, and immediately incubated at 39 °C for 96 h. Six negative blanks bottles (fermentation inoculum without substrate) were used to correct the GP produced by residual fine feed particles in ruminal fluid. Similarly, six serum bottles were incubated with 200 mg of inulin as standard to ensure the effectiveness of the used inoculum.

The headspace GP was recorded from each incubated bottle after 2, 4, 6, 8, 12, 24, 48, 72, and 96 h of incubation. The GP was measured by inserting a 23-gauge (0.6 mm) needle attached to a pressure transducer connected to a visual display [27]. Incubations were carried out in three replicated runs at different weeks and arranged as a factorial design 4 × 2 × 3 (4 enzymatic doses × 2 feed supplementations × 3 SF preparations).

2.6. Calculations

The recorded gas pressure was corrected for the blanks and then converted to the volume of gas produced at each measuring time (2, 4, 6, 8, 12, 24, 48, 72, and 96 h) using the following equation:

$$GP\ (\text{mL/g}\ DM) = Pr \times \frac{Vf - Vi}{P_{atm}}$$

Pr: recorded gas pressure (bar)
Vf: total volume of the incubation bottle (=117.39 mL)
Vi: volume of inoculum added to each bottle
P_{atm}: atmospheric pressure (=1.01325 bar)

In order to estimate the kinetic parameters of *GP*, *GP* data were fitted using the monophasic model proposed by [28]:

$$GPt = \frac{A}{\left[1 + \left(\frac{B}{t}\right)^{c}\right]}$$

where *GPt* (mL/g DM) denotes the cumulative gas production at time *t* (h); *A* (mL/g DM) is the potential gas production; *B* is the time of incubation at which half of *A* has been produced (h), *C* is the sharpness of the curve, and *t* is the total in vitro incubation time (96 h).

The maximum rate of *GP* (*Rmax*) and the time (*Tmax*) at which the maximum rate of *GP* was attained were calculated according to the equations proposed by [29]:

$$Rmax\ (\text{mL/h}) = AB^{C}C\left[\frac{Tmax^{(-C-1)}}{[1 + B^{C}\ \times Tmax^{-C}]^{2}}\right]$$

$$Tmax(\text{h}) = B\left[\frac{C-1}{C+1}\right]^{1/C}$$

The different fitted parameters *A*, *B*, and *C* were calculated using the residual least-squares procedure using the reduced generalized gradient algorithm from the solver function in the Microsoft Excel Software (2013).

The organic matter degradability (OMD) and the metabolizable energy (ME) were estimated based on the *in vitro GP* after 24 h of incubation (*GP24h*; mL/200 mg DM) according to the regression equations proposed by [26] as following:

$$\text{OMD (\%)} = 14.88 + (0.889 \times \text{GP}_{24\text{h}}) + (0.45 \times \text{CP}) + (0.0651 \times \text{ash})$$

$$\text{ME (MJ/kg DM)} = 2.2 + (0.136 \times \text{GP}_{24\text{h}}) + (0.057 \times \text{CP}) + (0.00286 \times \text{EE}^{2})$$

The total short-chain fatty acids (SCFAs) concentration was estimated according to [30] as:

$$\text{SCFA}_{\text{S}}\ (\text{mmol/200 mg DM}) = -0.00425 + (0.0222 \times \text{PG}_{24\text{h}})$$

where *CP* is the crude protein content (%*DM*), *EE* is the ether extract (%*DM*), ash is the ash content (%*DM*).

2.7. Statistical Analysis

The in vitro GP data were analyzed as a completely randomized design with three replicates per treatment per run based on the GLM procedure from the SAS® Studio software 3.6 (SAS Institute Inc., Cary, NC, USA). The incubation bottle was the experimental unit. Results of chemical composition were tested using a one-way analysis of variance (ANOVA). The cubic effects of FA doses were not analyzed for their inexplicability in biology [31]. For all SFH substrates, a statistical model considered the SFH preparations

(substrates), the feed supplement doses, and interaction (substrates × feed supplementation dose) as fixed factors using the following model:

$$Y_{ij} = \mu + SFH_i + D_j + (SFH_i \times D_j) + e_{ij}$$

where (*Yij*) is the measured parameter when μ is the overall mean, *SFH* substrates (i = 1–4) is the *SFH* preparations supplemented by *FA* (j = 1–5) including the control, and e is the experimental error, and $(SFH_i \times D_j)$ is the interaction between chemical pretreatments and the *FA* doses. The Duncan test (p = 0.05) was used to compare the means [32]. Means were declared significantly different when the p-value was less than 5%.

3. Results

3.1. Effect of Alkaline Pretreatments of SFH on the Chemical Composition

The chemical composition of the SFH substrates is presented in Table 2. The treatment of SFH with urea (SFH_{urea}) gave rise to the highest CP, CF and ADF ($p < 0.05$) contents. P content, however, was the lowest in SFH_{urea}. SFH_{NaOH} had lower ($p < 0.05$) DM and ash contents compared to other SFH treatments. The SFH_{out} exhibited the highest ($p < 0.05$) DM and Ca and the lowest ($p < 0.05$) ADF contents compared with other SFH preparations.

Table 2. Effect of alkaline pretreatments (NaOH or urea) on chemical composition (g/kg DM) of SFH.

Items	SFH_{out}	SFH_{NaOH}	SFH_{urea}	SEM	*p*-Value
Dry matter	881 ^{a}	314 ^{c}	696 ^{b}	3.2	*
Ash	109 ^{ab}	99 ^{b}	113 ^{a}	1.0	**
Crude protein	94 ^{b}	82 ^{b}	220 ^{a}	3.4	***
Crude fiber	140 ^{b}	159 ^{ab}	185 ^{a}	8.4	*
Neutral detergent fiber	242 ^{a}	182 ^{b}	250 ^{a}	7.4	**
Acid detergent fiber	108 ^{c}	128 ^{b}	208 ^{a}	17.4	***
Acid detergent lignin	18	19	17	3.4	NS
Phosphorus	1.4 ^{a}	1.4 ^{a}	1.3 ^{b}	0.4	*
Calcium	7.3 ^{a}	5.2 ^{b}	5.0 ^{b}	0.3	*

^{a,b,c} means within a row with different superscripts differ significantly ($p < 0.05$). SEM: standard error of the mean. * p-value < 0.05, ** p-value < 0.01, *** p-value < 0.001, NS: not significant.

3.2. Effect of FA Supplementation on Kinetics of In Vitro Gas Production

Effects of FA supplemented to SFH_{out}, SFH_{NaOH}, and SFH_{urea} on the volume of in vitro GP and ruminal GP kinetics are presented in Figure 1 and Table 3, respectively. The FA supplemented to SFH_{out} did not affect ($p > 0.05$) the GP volume produced from 2 h till 12 h (Figure 1A). However, linear increases ($p < 0.05$) were observed from 24 h to the end of the incubation period by the increasing levels of FA supplementations. This resulted in a linear increase ($p < 0.05$) in the estimated potential of GP (A) of the SFH_{out} with increasing doses of supplemented FA (Table 3), while no effects ($p > 0.05$) were observed in the other parameters of kinetics of GP (B, C, $R_{max,}$ and T_{max}) values for SFH_{out} samples supplemented with FA. The contrast test was observed with SFH_{NaOH} samples where neither the GP volumes (Figure 1B) nor the GP kinetics (Table 3) were affected by FA supplementation. Linear increases ($p < 0.05$) were observed by the FA supplemented to SFH_{urea} in GP (Figure 1C) produced in all incubation times (except 2 and 4 h), values of A, B, and Rmax (Table 3).

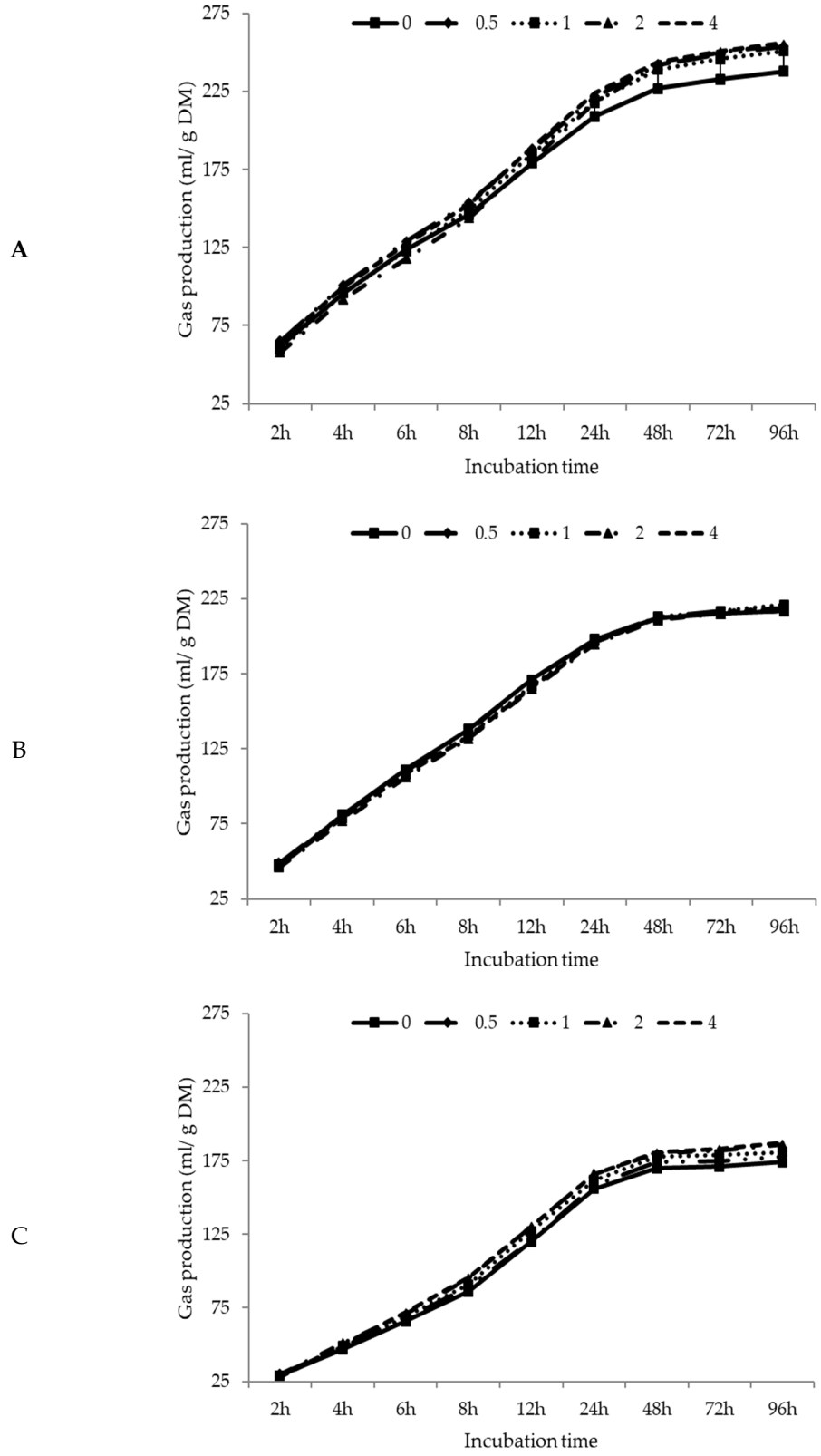

Figure 1. Effects of FFA supplemented to pretreated SFH ((**A**): SFH_{out}, (**B**): SFH_{NaOH}, (**C**): SFH_{urea}) on the volume of in vitro gas production.

Table 3. Effects of FFA supplemented to pretreated SFH (SFH_{out}, SFH_{NaOH}, SFH_{urea}) on the kinetics of the in vitro gas production.

Substrate	FFA (µL/g DM)	Gas Production Kinetics				
		A (mL/g DM)	B (h)	C (h)	R_{max} (mL/h)	T_{max} (h)
SFH_{out}	0	238	5.3	1.28	29.1	1.03
	0.5	254	5.4	1.28	30.2	1.04
	1	251	5.8	1.32	28.4	1.34
	2	255	6.2	1.31	26.9	1.37
	4	256	5.6	1.28	29.5	1.09
	p linear	*	NS	NS	NS	NS
SFH_{NaOH}	0	218	5.5	1.53	24.4	1.98
	0.5	220	5.6	1.47	24.2	1.83
	1	220	5.8	1.46	23.6	1.83
	2	217	5.6	1.49	23.1	1.98
	4	219	5.9	1.47	23.5	1.89
	p linear	NS	NS	NS	NS	NS
SFH_{urea}	0	174	7.66	1.69	14.3	3.37
	0.5	178	7.62	1.64	14.6	3.13
	1	182	7.52	1.67	15.0	3.23
	2	186	7.04	1.65	16.5	3.15
	4	187	7.54	1.67	15.5	3.24
	p linear	*	*	NS	**	NS
SEM		17.1	0.53	0.09	3.38	0.53
FFA		***	NS	NS	NS	NS
Substrate		***	***	***	***	***
FFA × Substrate		*	**	NS	NS	NS

A = estimated potential gas production; B = time of incubation at which the half of A has been produced; C = sharpness of the curve; R_{max} = the maximum rate of gas production; T_{max} = time at which the maximum rate of gas production is attained. SEM: standard error of the mean. * *p*-value < 0.05, ** *p*-value < 0.01, *** *p*-value < 0.001, NS: not significant.

The volume of gas produced from 24 h to 96 h and the total GP values (A) were affected ($p < 0.05$) by FA, and substrate × FA interaction, while the SFH substrate significantly ($p < 0.05$) affected the volume of GP in all incubation times and all GP kinetic parameters. Generally, the highest ($p < 0.001$) values of volume of gas produced at the different incubation times and the GP rate were detected for SFH_{out}, while the lowest values were for SFH_{urea}.

3.3. Effect of EFE Supplementation on Kinetics of In Vitro Gas Production

Results of EFE supplemented to SFH_{out}, SFH_{NaOH}, and SFH_{urea} on the volume *of in vitro* GP and ruminal GP kinetics are presented in Figure 2 and Table 4, respectively. Generally, EFE supplementations affected the GP and GP kinetics more than FFA. The contrast analysis revealed significant linear increases ($p < 0.05$) in EFE on the volume of gas produced in all incubation times (from 2 till 96 h) as well as the total GP values (A) within each SFH substrate. Values of B were affected ($p < 0.05$) by the EFE supplementation in SFH_{out} and SFH_{urea} while remaining unchanged in the SFH_{NaOH} form. Values of C were not affected by any of the SFH preparations, while increasing linear ($p < 0.05$) trends were observed in values of R_{max} and T_{max} (except for SFH_{urea}).

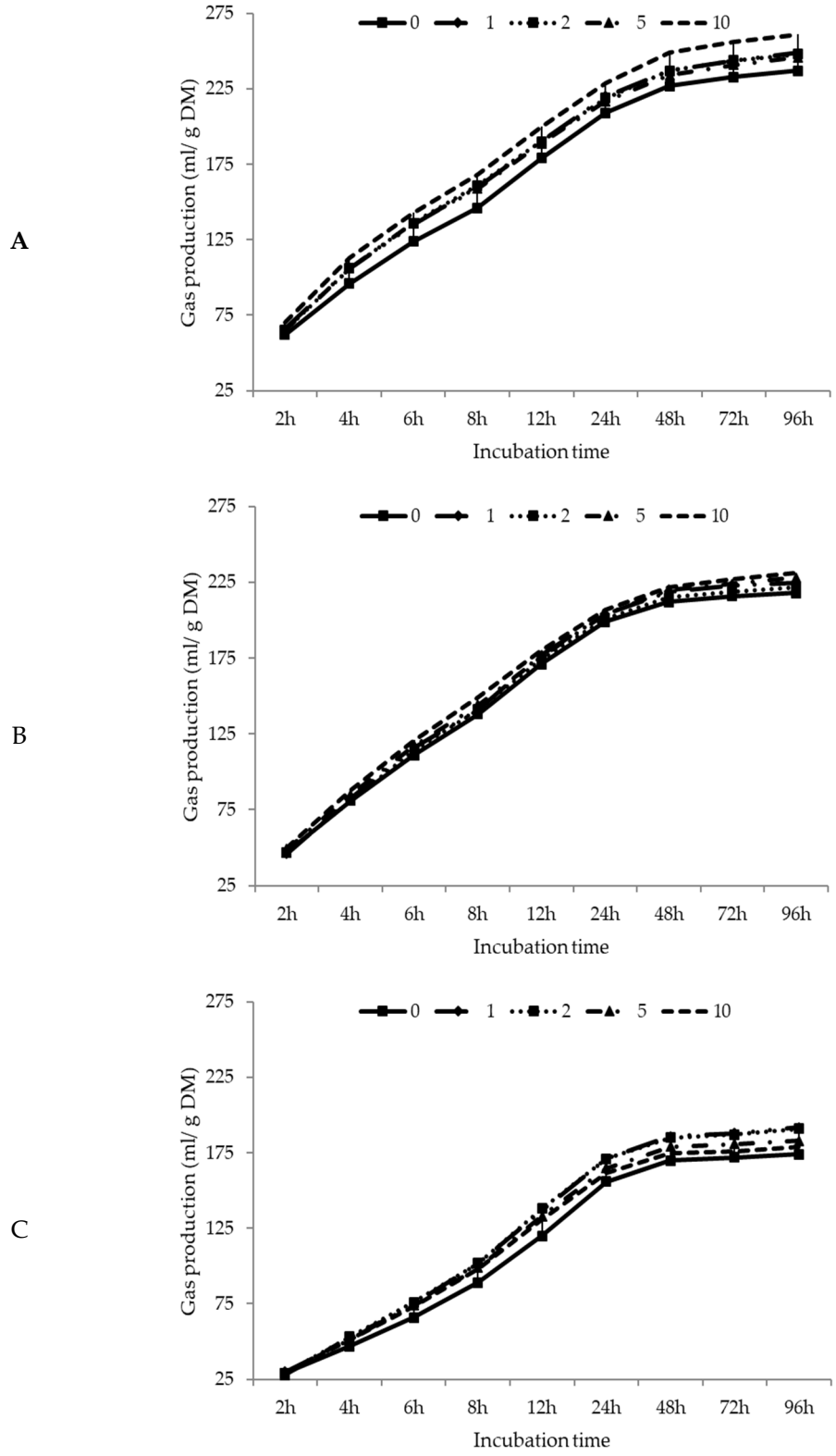

Figure 2. Effects of EFE supplemented to pretreated SFH ((**A**): SFH_{out}, (**B**): SFH_{NaOH}, (**C**): SFH_{urea}) on the volume of in vitro gas production.

Table 4. Effects of EFE supplemented to pretreated SFH (SFH_{out}, SFH_{NaOH}, SFH_{urea}) on the kinetics of in vitro gas production.

Substrate	EFE (μL/g DM)	Gas Production Kinetics				
		A (mL/g DM)	B (h)	C (h)	R_{max} (mL/h)	T_{max} (h)
SFH_{out}	0	238	5.3	1.28	29	1.03
	1	249	4.9	1.28	32	0.95
	2	248	5.0	1.29	32	0.98
	5	246	4.9	1.28	33	0.93
	10	261	4.9	1.26	35	0.88
	p linear	*	*	NS	**	**
SFH_{NaOH}	0	218	5.5	1.53	24	1.98
	1	224	5.7	1.53	24	2.04
	2	222	5.6	1.53	25	1.97
	5	228	5.6	1.48	25	1.83
	10	231	5.4	1.48	26	1.77
	p linear	***	NS	NS	**	*
SFH_{urea}	0	174	7.7	1.69	14	3.37
	1	192	7.3	1.70	16	3.31
	2	191	7.2	1.68	16	3.12
	5	183	7.2	1.70	16	3.11
	10	179	7.0	1.72	16	3.16
	p linear	*	**	NS	**	NS
SEM		16.3	0.57	0.1	3.99	0.54
p-Value						
EFE		**	***	NS	***	***
Substrate		***	***	***	***	***
EFE × Substrate		NS	*	NS	**	NS

A = estimated potential gas production; B = time of incubation at which half of A has been produced; C = sharpness of the curve; R_{max} = the maximum rate of gas production; T_{max} = time at which the maximum rate of gas production is attained. SEM: standard error of the mean. * *p*-value < 0.05, ** *p*-value < 0.01, *** *p*-value < 0.001, NS: not significant.

The volume of gas produced during all incubation times and all parameters of the GP kinetics (except C values) were affected ($p < 0.05$) by EFE and substrate, while no EFE × substrate interaction effects were observed on all GP and GP kinetic parameters except the volume of GP produced at 2 h and 4 h, as well as the values of B, and R_{max}.

3.4. Effect of FFA Supplementation on ME, OMD, and SCFAs

Results of the effects of FFA supplemented to SFH_{out}, SFH_{NaOH}, and SFH_{urea} on the ME, OMD and SCFAs are presented in Table 5. The dose–response analysis showed that the FFA supplementation enhanced the ME, OMD, and SCFAs for SFH_{out} (linearly, $p < 0.05$) and SFH_{urea} (Quadratiecly, $p < 0.05$), while no effects ($p > 0.05$) were observed for the SFH_{NaOH} diets. For all SFH diets, values of ME, OMD, and SCFAs were significantly affected ($p < 0.001$) by the substrate, while neither FFA nor FFA × substrate interaction had significant effects. Generally, the greatest ($p < 0.001$) increases in ME, OMD, and ruminal SCFAs were detected for $SFH_{out,}$ while the lowest values were for SFH_{urea}.

Table 5. Effects of FFA supplemented to pretreated SFH (SFH_{out}, SFH_{NaOH}, SFH_{urea}) on metabolizable energy, organic matter digestibility and ruminal total short chain fatty acids concentrations (SCFAs).

Substrate	FFA (µL/g DM)	Metabolizable Energy (MJ/kg DM)	Organic Matter Digestibility (g/kg DM)	Total SCFAs (mmol/200 mg DM)
SFH_{out}	0	8.45	570	0.82
	0.5	8.74	589	0.97
	1	8.71	588	0.96
	2	8.65	583	0.96
	4	9.00	606	1.01
	p linear	*	*	*
	p quadratic	NS	NS	NS
SFH_{NaOH}	0	8.10	545	0.87
	0.5	8.20	552	0.89
	1	8.16	549	0.88
	2	8.25	555	0.90
	4	8.33	560	0.91
	p linear	NS	NS	NS
	p quadratic	NS	NS	NS
SFH_{urea}	0	7.72	535	0.68
	0.5	8.14	562	0.75
	1	8.14	562	0.76
	2	7.99	552	0.73
	4	7.90	546	0.72
	p linear	NS	NS	NS
	p quadratic	*	*	*
SEM		0.09	0.61	0.01
FFA		NS	NS	NS
Substrate		***	***	***
FFA × Substrate		NS	NS	NS

SEM: standard error of the mean. * *p*-value < 0.05, *** *p*-value < 0.001, NS: not significant.

3.5. Effect of EFE Supplementation on ME, OMD, and SCFAs

Table 6 presents the effects of EFE supplemented to SFH diets on ME, OMD, and SCFAs. The dose–response analysis revealed that EFE supplemented to SFH_{out} linearly enhanced ($p < 0.05$) the values of ME, OMD, and ruminal SCFAs, while no effects were observed by SFH_{NaOH} and SFH_{urea} diets. For all SFH diets, values of ME, OMD, and SCFAs were significantly affected ($p < 0.01$) by the EFE substrate, and their interaction. The highest ($p < 0.001$) increases in ME, OMD, and ruminal SCFAs were detected for SFH_{out} compared to other substrate diets. EFE supplemented at 10 µL/g DM was the most efficient dose to enhance the fermentation and nutrient digestibility compared to the other doses.

Table 6. Effects of EFE supplemented to pretreated SFH (SFH_{out}, SFH_{NaOH}, SFH_{urea}) on metabolizable energy, organic matter digestibility and ruminal total short chain fatty acids concentrations (SCFAs).

Substrate	EFE (µL/g DM)	Metabolizable Energy (MJ/kg DM)	Organic Matter Digestibility (g/kg DM)	Total SCFAs (mmol/200 mg DM)
SFH_{out}	0	8.45	570	0.82
	1	8.80	593	0.98
	2	8.70	587	0.97
	5	8.70	587	0.97
	10	8.84	596	0.99
	p linear	*	*	*
	p quadratic	NS	NS	NS

Table 6. *Cont.*

Substrate	EFE (µL/g DM)	Metabolizable Energy (MJ/kg DM)	Organic Matter Digestibility (g/kg DM)	Total SCFAs (mmol/200 mg DM)
SFH_{NaOH}	0	8.10	545	0.87
	1	8.10	545	0.88
	2	8.06	542	0.87
	5	8.00	539	0.86
	10	8.03	541	0.86
	p linear	NS	NS	NS
	p quadratic	NS	NS	NS
SFH_{urea}	0	7.72	535	0.68
	1	7.80	539	0.70
	2	7.90	546	0.71
	5	7.98	552	0.73
	10	7.99	552	0.73
	p linear	NS	NS	NS
	p quadratic	NS	NS	NS
SEM		0.13	0.80	0.04
FFA		***	***	***
Substrate		***	***	***
FFA × Substrate		**	***	***

SEM: standard error of the mean. * *p*-value < 0.05, ** *p*-value < 0.01, *** *p*-value < 0.001, NS: not significant.

4. Discussion

In the present study, CP content (90 g/kg DM) of SFH_{out} was in the range of values (65–110 g/kg DM) reported by Özelçam et al. [33] on different Turkish SFH varieties. Gholami-Yangije et al. [34] reported lower CP content (28 g/kg DM) in dried Iranian SFH. They indicated higher cell wall contents in terms of NDF (553 g/kg DM), ADF (457 g/kg DM), and ADL (24 g/kg DM) in SFH, compared to our study (248, 108 and 18 g/kg DM, for NDF, ADF, and ADL, respectively). However, NDF and CF of the studied samples are somewhat similar to those reported by Özelçam et al. [33] in different SFH varieties (NDF: 255–302 g/kg DM; CF: 154–182 g/kg DM). Such variations can be attributed to diversity in varieties, climate and soil conditions, maturity stage, and the proportions of residues (stalks, heads without seeds, and mixtures of stalks and heads). On the other hand, it is pertinent to mention that ADF contents in all SFH substrates (108 g/kg DM) were far from the minimum level recommended in dairy diets (210 g/kg DM) as suggested by National Research Council (NRC) [35]. In this context, it was proposed that SFH substrates can be a valuable substitute for conventional roughages in a complete diet where roughage is included at up to 60% [36].

Based on their CP and NDF contents, the investigated SFH substrates are comparable to ground corn at 95 g/kg DM CP and 210 g/kg DM NDF [37]. However, compared to wheat straw, bean straw, and SF stalks, which are commonly used in the ruminant diet in Tunisia, all SFH preparations showed higher CP, ash, and EE, and lower fiber contents. Consequently, according to Özelçam et al. [33], the experimental SFH preparations are considered to have relatively better nutritional value than dry forages, used in ruminant diets, and could significantly contribute to ruminant livestock feeds [8].

The increases in ADF values caused by the NaOH and urea pre-treatments can be explained by the solubilization of hemicellulose and increases in cellulose fractions. Rezende et al. [38] reported that the pretreatment of cellulosic biomass by increasing the level of NaOH could solubilize up to 58% of lignin and 86% of hemicellulose. The same observation was reported by Moradi et al. [39] on pistachio byproducts. Xu et al. [40] proved that the solubilization of hemicellulose by NaOH or H_2SO_4 treatments can improve the cellulose crystallinity, which could negatively affect the digestibility of lignocellulosic biomass and prevent the effective penetration of digestive enzymes. This finding could explain the decrease in the extent and the rate of in vitro ruminal fermentation, ME, OMD

and SCFAs concentrations of SFH_{NaOH} and SFH_{urea} as compared to SFH_{out}. Comparatively, Moradi et al. [39] revealed that the alkali pretreatment of the pistachio byproducts decreased significantly the in vitro recorded GP. Therefore, the untreated form of SFH (SFH_{out}) seems to be more effective for ruminant feeding.

The experimental FFA presented lower efficiency than EFE regarding GP values, most GP kinetic parameters, the estimated OMD, ME and total SCFAs concentrations in all SFH substrates. These results can be attributed to the higher activity of the xylanase, endoglucanase, and exoglucanase enzymes found in EFE compared to FFA. The fibrolytic enzymes detected in EFE may act through hydrolyzing the cell wall polymers, providing an additional energy source for the ruminal microorganisms' growth and preparing the cell wall surface for microbial attachment [41]. It was proposed that exogenous enzymes hydrolyze the digestible carbohydrates at the plant cell wall surface [42]. This reduces the accumulation of the lignin-carbohydrate complexes and alters the structure of the feed, making it more susceptible to ruminal degradation and microbial colonization [43], which supports the growth and activity of rumen microorganisms.

The most significant improvement effect of EFE was mainly detected for SFH_{urea} on rumen fermentation and nutrient digestibility; this was probably due to higher CP content, which could deteriorate the active sites of fibrolytic enzymes on the substrate. These findings align with a previous study conducted by Eun et al. [44]. They applied a synergistic effect between ammonia pretreatment (NH_3) and EFE supplementation to increase the in vitro degradability of rice straw. Thus, we can conclude that the additional source of nitrogen delivered by ammonia or urea stimulates the EFE effect. The exact mechanism by which the synergy between urea pretreatment and EFE was created remains unexplained. However, urea pretreatment increases rumen N-NH_3 content [45], as well as the total number of ruminal bacteria [46] including ruminal fibrolytic microbes [47]. Moreover, the EFE provides an energy source for ruminal microorganisms by hydrolyzing the cell wall polysaccharides [48].

Wang et al. [49] proved that the pretreatment of wheat straw with NaOH enhanced the efficacy of exogenous enzymes on ruminal fermentation in addition to the rate, and the extent of dry matter digestibility. However, these effects were not observed in our study, since SFH_{out} had the most promising fermentation values compared to SFH_{NaOH}. The variability and inconsistencies in the results can be attributed to the enzyme type, activity level and to the kind of substrate since the chemical composition of wheat straw and SFH differs widely [50].

Both the experimental additives in this experiment had almost the same effect trends on in vitro ruminal fermentation, especially for SFH_{urea} and SFH_{out}. However, there remains some difference in optimal dose, sensitivity to the chemical agent, and efficiency level to improve the ruminal utilization of SFH, which could be due to the amounts of fibrolytic activities present in the enzymatic product. In this connection, Vahjen and Simon [51], who investigated 18 commercial enzymes, indicated that commercial enzyme preparations display different modes of action, depending on enzyme concentrations, the molecular weight of fibrolytic components, substrate specificity and proteolytic stability within the digestive tract.

5. Conclusions

The pretreatments of SFH with exogenous cellulase and xylanase has the potential to improve the in vitro ruminal fermentation and the digestive use parameters. Using two different types of EFE showed some differences in optimal dose, sensitivity to other chemical treatments, and efficiency to improve ruminal fermentation, which could be due to the specificity enzyme-substrate and to the amounts of added fibrolytic activities from each commercial enzymatic product. Moreover, the EFE could create a synergy effect with urea pretreatment for in vitro ruminal fermentation. However, as compared to the untreated form, the observed synergy was less effective, which could be due to the presence of antinutritional factors released after chemical treatment. We suggest that there is no

major beneficial effect of the use of the chemical treated form of SFH. The study may stimulate new avenues of further research into the investigation of other combinations of fibrolytics such as esterase-cellulase and xylanase on the fermentation parameters of SFH.

Author Contributions: Conceptualization, M.K. and J.J.; methodology, M.K., Y.B., J.J. and H.Y.; software, Y.B. and J.J.; validation, H.A., M.I.A., Y.A.S., W.S., A.M. and M.K.; formal analysis, H.A., A.S.M., Y.A.S., J.J. and M.K.; investigation, J.J., K.A., H.A., Y.A.S.; resources, H.A., J.J., Y.A.S., W.S., A.M., Y.B.; data curation, J.J., Y.B., K.A., H.A., Y.A.S. and M.K.; writing—original draft preparation, J.J., K.A. and M.K.; writing—review and editing, J.J., H.A., Y.A.S., M.C., M.E.d.H.M., M.I.A., W.S., A.S.M.; visualization, H.A., M.I.A., M.C., M.E.d.H.M., W.S., M.K.O. and J.J.; supervision, M.K., Y.B. and A.M.; project administration, M.K., H.Y. and J.R.; funding acquisition, M.K.O. and W.S. All authors have read and agreed to the published version of the manuscript.

Funding: This research was supported by the laboratory of Animal nutrition: Management of the health and quality of animal production [LR14AGR03] (Ministry of Higher Education and Scientific Research, Tunisia), the precision livestock and nutrition unit, Gembloux Agro-Bio Tech, University of Liège and the Researchers Supporting Project number (RSP-2021/390), King Saud University, Riyadh, Saudi Arabia.

Institutional Review Board Statement: This study was carried out in an approved establishment for animal experimentation under the "Institutional Review Board" of the University of Gembloux, Belgium. The protocol was designed and all practices were performed according to the Directives of the European Parliament and Committee 2010/63/EU regulation about animal welfare.

Informed Consent Statement: Not applicable.

Data Availability Statement: The data presented in this study are available on request from the corresponding author.

Acknowledgments: The authors want to thank the Researchers Supporting Project number (RSP-2021/390), King Saud University, Riyadh, Saudi Arabia.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. FAO. *Sunflower Crude and Refined Oils*; Agribusiness Handbook; Investment Centre Division; FAO: Rome, Italy, 2010. Available online: https://cupdf.com/document/sunflower-crude-and-refined-oils.html (accessed on 5 June 2013).
2. Gaines, W.L.; Nevens, W.B. *The Sunflower as a Silage Crop: Composition and Yield at Different Stages of Maturity*; Agricultural Experiment Station Bulletin No. 268; University of Illinois: Urbana, IL, USA, 1925.
3. Ray, G.S. *Sunflowers for Silage*; Extension Bulletin Series 1. No. 158·A; Colorado Agricultural College Extension Service: Fort Collins, CO, USA, 1919.
4. Lardy, G. *Sunflower Silage may be an Option for Drought-Stressed Sunflower Crop*; North Dakota State University Extension Service: Fargo, ND, USA, 2009.
5. Heuzé, T.; Trang, G.; Hassoun, P.; Lebas, F. Sunflower Forage and Crop Residues, Feedpiedia, a programme by INRAE, CIRAD, AFZ and FAO. 2015. Available online: https://www.feedipedia.org/node/143 (accessed on 13 July 2019).
6. Aregheore, E.M. Growth rate, apparent nutrient digestibility and some blood metabolites of Gwembe Valley Goats on rations based on crop residues in the hot dry season in Zambia. *Trop. Anim. Health. Prod.* **2001**, *33*, 331–340. [CrossRef] [PubMed]
7. Lardy, G.; Anderson, V. *Alternative Feeds for Ruminants*; North Dakota State University; Department of Animal Sciences: Fargo, ND, USA, 2015. Available online: https://www.ag.ndsu.edu/publications/livestock/alternative-feeds-for-ruminants/as1182.pdf (accessed on 8 March 2019).
8. Rasool, E.; Khan, M.F.; Nawaz, M.; Rafiq, M. Utilization of sunflower crop residues as feed in small ruminants. *Asian-Australas. J. Anim. Sci.* **1998**, *11*, 272–276. [CrossRef]
9. Nagalakshmi, D.; Reddy, D.N.; Prasad, M.R. Effect of feeding expander extruder processed sunflower heads based complete diets on performance, nutrient digestibility and carcass characteristics of lambs. *Indian J. Anim. Res.* **2017**, *51*, 280–285. [CrossRef]
10. Amini-Jabalkandi, J.; Pirmohammadi, R.; Razzagzadeh, S. Effects of different levels of sunflower residue silage replacement with alfalfa hay on Azeri male buffalo calves fattening performance. *Italian J. Anim. Sci.* **2007**, *6*, 495–498. [CrossRef]
11. Zhurka, M.; Spyridonidis, A.; Vasiliadou, I.A.; Stamatelatou, K. Biogas Production from Sunflower Head and Stalk Residues: Effect of Alkaline Pretreatment. *Molecules* **2020**, *25*, 164. [CrossRef] [PubMed]
12. Soltan, Y.A.; Abdalla, A.L.; Silva, L.R.F.; Natel, A.S.; Morsy, A.S.; Louvandini, H. Response of different tropical pasture grass species to treatment with fibrolytic enzymes in terms of in vitro ruminal nutrient degradation and methanogenesis. *Anim. Nutr. Feed Technol.* **2013**, *13*, 551–568.

13. Snyaman, L.D.; Joubert, H.W. The chemical composition and in vitro dry matter digestibility of untreated and ammoniated crop residues. *S. Afr. J. Anim. Sci.* **2002**, *32*, 83–87. [CrossRef]
14. Sariçiçek, B.Z.; Gripoglu, A.V. Feed Values of Sunflower Heads and Stovers Treated with Different Methods and Possibilities of Using as Roughage. *Tarim Bilimleri Derg.* **1997**, *3*, 52–56.
15. Dulphy, J.P.; Breton, J.; Bienaime, A.; Louyot, J.M. Etude de la valeur alimentaire des pailles de céréales traitées ou non à la soude: I- Influence du traitement à la soude. *Ann. Zootech.* **1982**, *31*, 195–214. [CrossRef]
16. Chermiti, A.; Nefzaoui, A.; Cordesse, R.; Amri, T.; Laajili, M. Paramètres d'uréolyse et digestibilité de la paille traitée à l'urée. *Ann. Zootech.* **1989**, *38*, 63–72. [CrossRef]
17. Helrich, K.; Association of Official Analytical Chemists. *Official Methods of Analysis of the Association of Official Analytical Chemists*, 15th ed.; The Association of Official Analytical Chemists: Arlington, TX, USA, 1990.
18. Ammar, H.; López, S.; Bochi, O.; García, R.; Ranilla, M.J. Composition and in vitro digestibility of leaves and stems of grasses and legumes harvested from permanent meadows at different stages of maturity. *J. Anim. Feed Sci.* **1999**, *8*, 599–610. [CrossRef]
19. Van Soest, P.J.; Robertson, J.B.; Lewis, B. Methods for Dietry Fiber, Neutral Detergent Fiber, and Nonstarch Polysaccharides in Relation to Animal Nutrition. *J. Dairy Sci.* **1991**, *74*, 3583–3597. [CrossRef]
20. AOAC. *Official Methods of Analysis*, 17th ed.; The Association of Official Analytical Chemists: Gaithersburg, MD, USA, 2000.
21. Norsalwani, T.L.; NikNorulaini, N.A.R.; NurulJannah, H.; MoftahMassaud, B.N.; Hideyuki, N.; Mohd Omar, A.K. Cellulase activity in solid-state fermentation of palm kernel cake with Trichodermareesei. *Malays. J. Microbiol.* **2012**, *8*, 235–241. [CrossRef]
22. Bailey, M.J.; Biely, P.; Kaisa, P. Interlaboratory testing of methods for assay of xylanase activity. *J. Biotechnol.* **1992**, *23*, 257–270. [CrossRef]
23. Wood, T.M.; Bhat, K.M. Biomass, Cellulose and Hemicellulose. *Methods Enzymol.* **1988**, *160*, 632–634.
24. Giraldo, L.A.; Tejido, M.L.; Ranilla, M.J.; Ramos, S.; Carro, M.D. Influence of direct- fed fibrolityc enzymes on diet digestibility and ruminal activity in sheep fed a grass hay-based diet. *J. Anim. Sci.* **2008**, *86*, 1617–1623. [CrossRef]
25. Beauchemin, K.A.; Rode, L.; Sewalt, V.J. Enzyme Additives for Ruminant Feeds. U.S. Patent 5,720,971, 24 February 1998. Available online: https://patentimages.storage.googleapis.com/aa/c9/5a/b9add11c22817e/US5720971.pdf (accessed on 15 November 2019).
26. Menke, K.H.; Steingass, H. Estimation of the energetic feed value obtained from chemical analysis and in vitro gas production using rumen fluid. *Anim. Res. Dev.* **1988**, *28*, 7–55.
27. Theodorou, M.K.; Williams, B.A.; Dhanoa, M.S.; McAllan, A.B.; France, J. A simple gas production method using a pressure transducer to determine the fermentation kinetics of ruminant feeds. *Anim. Feed. Sci. Techn.* **1994**, *48*, 185–197. [CrossRef]
28. Groot, J.C.; Cone, J.W.; Williams, B.A.; Debersaques, F.M.; Lantinga, E.A. Multiphasic analysis of gas production kinetics for in vitro fermentation of ruminant feeds. *Anim. Feed Sci. Technol.* **1996**, *64*, 77–89. [CrossRef]
29. Yang, H.J.; Tamminga, S.; Williams, B.A.; Dijkstra, J.; Boer, H. *In vitro* gas and volatile fatty acids production, profiles of barley and maize and their soluble and washout fractions after feed processing. *Anim. Feed Sci. Technol.* **2005**, *120*, 125–140. [CrossRef]
30. Getachew, G.; Blummel, M.; Makkar, H.P.S.; Becker, K. In vitro gas measuring techniques for Assessment of nutritional quality of feeds: A review. *Anim. Feed Sci. Technol.* **1998**, *72*, 261–281. [CrossRef]
31. Tang, S.X.; Zou, Y.; Wang, M.; Salem, A.Z.M.; Odongo, N.E.; Zhou, C.S.; Han, X.F.; Tan, Z.L.; Zhang, M.; Fu, Y.F.; et al. Effects of exogenous cellulase source on in vitro fermentation characteristics and methane production of crop straws and grasses. *Anim. Nutr. Feed Technol.* **2013**, *13*, 489–505.
32. Duncan, D.B. Multiple Range and Multiple F Tests. *Biometrics* **1955**, *11*, 1–42. [CrossRef]
33. Özelçam, H.; İpçak, H.H.; Özüretmen, S. Feed value of sunflower heads in different varieties. *Int. J. Agric. Environ. Biores.* **2017**, *2*, 58–63.
34. Gholami-Yangije, A.; Pirmohammadi, R.; Khalilvandi-Behroozyar, H. The potential of sunflower (Helianthus annuus) residues silage as a forage source in Mohabadi dairy goats. *Vet. Res. Forum* **2019**, *10*, 59–65. [CrossRef]
35. National Research Council. *Nutrient Requirements of Dairy Cattle*, 7th ed.; National Academies Press: Washington, DC, USA, 2001.
36. Anandan, S.; Kumar, G.K.A.; Rudraswamy, M.S. *In vitro* evaluation of sunflower heads based complete feeds. *Indian J. Anim. Nutr.* **2002**, *19*, 374–377. [CrossRef]
37. Maia, M.O.; Susin, I.; Ferreira, E.M.; Nolli, C.P.; Gentil, R.S.; Pires, A.V.; Mourao, G.B. Intake, nutrient apparent digestibility and ruminal constituents of sheep fed diets with canola, sunflower or castor oils. *Rev. Bras. Zootec.* **2012**, *41*, 2350–2356. [CrossRef]
38. Rezende, C.A.; de Lima, M.A.; Maziero, P.; Deazevedo, E.R.; Garcia, W.; Polikarpov, I. Chemical and morphological characterization of sugarcane bagasse submitted to a delignification process for enhanced enzymatic digestibility. *Biotechnol. Biofuels* **2011**, *4*, 54. [CrossRef]
39. Moradi, M.; Afzalzadeh, A.; Behgar, M.; Norouzian, M.A. Effects of electron beam, NaOH and urea on chemical composition, phenolic compounds, in situ ruminal degradability and in vitro gas production kinetics of pistachio byproducts. *Vet. Res. Forum.* **2015**, *6*, 111–117.
40. Xu, N.; Zhang, W.; Ren, S.; Liu, F.; Zhao, C.; Liao, H.; Xu, Z.; Huang, J.; Li, Q.; Tu, Y.; et al. Hemicelluloses negatively affect lignocellulose crystallinity for high biomass digestibility under NaOH and H2SO4 pretreatments in Miscanthus. *Biotech. Biofuels* **2012**, *5*, 58. [CrossRef]
41. Morgavi, D.P.; Beauchemin, K.A.; Nsereko, V.L.; Rode, L.M.; Iwaasa, A.D.; Yang, W.; Mcallister, T.A.; Wang, Y. Synergy between the ruminal fibrolytic enzymes and enzymes from T. longibra-chiatum. *J. Dairy Sci.* **2000**, *83*, 1310–1321. [CrossRef]

42. Thammiah, V.; Samanta, A.K.; Senani, S.; Sridhar, M. Scope of exogenous enzymes in enhancing ruminant productivity. *J. Adv. Vet. Anim. Res.* **2017**, *5*, 00137. [CrossRef]
43. Dean, D.B.; Staples, C.R.; Littell, R.C.; Kim, S.; Adesogan, A.T. Effect of method of adding a fibrolytic enzyme to dairy cow diets on feed intake digestibility, milk production, ruminal fermentation, and blood metabolites. *Anim. Nutr. Feed Technol.* **2013**, *13*, 337–353.
44. Eun, J.-S.; Beauchemin, K.A.; Hong, S.-H.; Bauer, M.W. Exogenous enzymes added to untreated or ammoniated rice straw: Effects on in vitro fermentation characteristics and degradability. *Anim. Feed Sci. Technol.* **2006**, *131*, 87–102. [CrossRef]
45. Van Soest, P.J. *Nutritional Ecology of the Ruminant*, 2nd ed.; Cornell University Press: New York, NY, USA, 1994.
46. Paengkoum, S.; Wanapat, M.; Wachirapakorn, C.; Nontaso, N. The effect of roughage and urea solution infusion levels on ruminal NH3-N concentration and nutrient digestibility in beef cattle and swamp buffaloes. *Sci. Eng. Health Stud.* **2010**, *4*, 47–55. [CrossRef]
47. Vinh, N.T.; Wanapat, M.; Khejornsart, P.; Kongmun, P. Studies of diversity of rumen microorganisms and fermentation in swamp buffalo fed different diets. *J. Anim. Vet. Adv.* **2011**, *10*, 406–414. [CrossRef]
48. Wang, Y.; McAllister, T.A. Rumen microbes, enzymes and feed digestion-A review. *Asian-Australas. J. Anim. Sci.* **2002**, *15*, 1659–1676. [CrossRef]
49. Wang, Y.; Spratling, B.M.; Zobell, D.R.; Wiedmeier, R.D.; McAllister, T.A. Effect of alkali pretreatment of wheat straw on the efficacy of exogenous fibrolytic enzymes. *J. Anim. Sci.* **2004**, *82*, 198–208. [CrossRef]
50. Krueger, N.A. Effect of Fibrolytic Enzymes on the Nutritive Value of Tropical Forages and Performance of Beef Steers. Ph.D. Thesis, Department of Animal Science, University of Florida, Gainesville, FL, USA, 2006.
51. Vahjen, W.; Simon, O. Biochemical characteristics of non-starch polysaccharide hydrolyzing enzyme preparations designed as feed additives for poultry and piglet nutrition. *Arch. Anim. Nutr.* **1999**, *52*, 1–14. [CrossRef]