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ORIGINAL ARTICLE

Fermentative profile and nutritional value of untreated and alkali-treated faba bean (*Vicia faba* L.) straw supplemented with exogenous fibrolytic enzymes derived from *Trichoderma longibrachiatum*, *Aspergillus strains*, and *Neurospora intermedia*

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Abstract
The faba bean straw (FBS) is a faba bean plant by-product characterized by high fiber and crude protein content, and low digestibility. This study aimed to improve the nutritional value and ruminal fermentation of FBS by combining chemical and biotechnological treatments. The FBS was subject of two alkali treatments: 4% NaOH (NFBS) and 4% urea (UFBS), and exogenous fibrolytic enzyme (EFE) supplementation using two enzymatic complexes: *Trichoderma longibrachiatum* EFE (DCX) at 0, 1, 2, 5, and 10 µL/gDM and *Aspergillus strains* and *Neurospora intermedia* EFE (MaxFiber) at 0, 0.5, 1, 2, and 4 mg/gDM of untreated FBS (CFBS), NFBS, and UFBS. All supplemented FBS preparations were incubated with buffer solution, and fresh cows' ruminal fluid. At the end of incubation period (96h), the in vitro ruminal fermentation parameters as the extent (A), the rate of GP (Rmax), and the digestive use parameters: organic matter digestibility (OMD), metabolizable energy (ME), and volatile fatty acids (VFA) were determined. Our results proved that EFE's effect depended on the enzymatic dose and the alkali treatment. The DCX supplementation effect was more pronounced than the MaxFiber. The highest improvements were recorded for CFBS supplemented by DCX (5µL/gDM), by 43.6%, 60.2%, 27%, 25.9%, and 43.5% for A, Rmax, ME, OMD, and VFA, respectively, as compared to the control. However, the association between EFE and alkali decreased the efficiency of EFE. Therefore, using EFE supplementation to the CFBS could generally provide an energy-protein-rich bio-converted by-product as compared to commonly used cereal straw in ruminant nutrition.

Keywords Agricultural by-product · Faba bean straw · In vitro ruminal fermentation · Microbial fibrolytic enzymes · Ruminant

1 Introduction

Faba bean straw is a by-product of the faba bean plant, which is a type of legume commonly grown for its seeds. Faba bean straw is high in fiber and is often used as a feed ingredient for livestock [1], particularly for ruminants such as cattle, sheep, and goats, especially during shortage periods. Ruminants can digest the straw cellulose, which provides a source of energy and fiber. This can help to improve the health of their digestive system and can also help to prevent digestive disorders such as bloating [2]. In addition to providing energy, faba bean straw can also provide a source of protein for ruminants, making it a valuable feed ingredient [1]. Overall, the use of faba bean straw in ruminant feeding can help to improve the health and performance of these animals. However, agricultural by-products such as the faba bean straw are composed of lignin, cellulose, and hemicellulose, which are structural polymers that give plants their strength and rigidity. These polymers are difficult to digest, which makes lignocellulosic biomass less digestible than other types of feed [1, 3]. To improve the digestive use of such agricultural by-product, various treatments have been developed such as physical, chemical, and biological treatments [4–6]. Despite some improvements in digestibility, the

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level of digestive use of straw remains insufficient and continues to restrict the amount of available digestible energy to ruminants [7].

The exogenous fibrolytic enzymes (EFE) produced by microorganisms are being increasingly used to improve fiber digestibility, feed efficiency, and animal performance as they overcome the limitations of other methods that have been used for this purpose [8]. These enzymes are believed to work by breaking down complex plant cell wall polysaccharides, such as cellulose and hemicellulose, into simpler sugars that can be fermented by rumen microorganisms. So, the main purpose of EFE supplementation is to provide additional nutrients from the indigestible, potentially digestible, and digestible portions of the cell wall [9]. However, even though positive effects were obtained, up-to-date inconsistent results have been recorded among studies for animal responses [10] due to many reasons such as fibrolytic activity, dosage, method of enzyme addition, diet constituents, and supplemented substrate [11, 12]. The efficiency of these enzymes may differ between the microorganisms according to their genetic makeup [13, 14]. The *Trichoderma longibrachiatum* produces a wide range of fibrolytic enzymes such as cellulases, hemicellulases, and ligninases, which are capable of breaking down complex plant cell walls with significant improvement of ruminal digestion [13, 15]. In contrast, *Aspergillus* strains produce mainly cellulases, while *Neurospora intermedia* produces mostly xylanases, which have varying efficiencies in degrading plant cell wall components [16].

This renders the need for further dedicated research efforts for the broad generalization of exogenous enzyme usage in ruminant nutrition. In this study, it was hypothesized that the association between both chemical treatment and enzymatic supplementation could improve the ruminal fermentation of faba bean straw. The objective of this study was to evaluate the effect of two different exogenous fibrolytic enzyme complexes supplemented with the untreated and alkali pre-treated faba bean straw on the in vitro ruminal fermentation and digestive use parameters.

2 Materials and methods

2.1 Plant material and alkali pretreatments

After the pod's harvesting, samples of faba bean straw (FBS) were randomly collected from private fields located in the northwest region of Tunisia, then manually chopped into small stands of 5–6 cm to allow alkali treatments and improve samples homogeneity. Once well mixed, the obtained FBS biomass was divided into 9 subsamples of 2 kg each, which were used for alkali treatments. The first three sub-samples were kept untreated (CFBS) as the control group. The second three sub-samples were subjected to NaOH treatment as described by Dulphy et al. [17] for NFBS. The last three sub-samples were treated by urea as described by [18] for UFBS. A 48 h prior to the in vitro assay, the UFBS was kept in the open air to remove the pungent smell of ammonia. When all alkali pretreatments were ready, samples of 500g from each FBS preparation (CFBS, NFBS, and UFBS) were dried in a forced air oven overnight at 55°C until constant weight, then grounded through a 1-mm sieve using a Retsch SK 100 standard, Giessen, Germany, for subsequent analysis.

2.2 Chemical analysis

The FBS preparations (CFBS, NFBS, and UFBS) were analyzed for dry matter (DM, method ID 930.15), organic matter (OM, method ID 942.05), ether extract (EE, method ID 920.30), crude protein (CP, ID 954.01), and crude fiber (CF, ID 962.09) contents according to the methods of the Association of Official Analytical Chemists [19]. The neutral detergent fiber (NDF, assayed with a heat stable amylase and expressed inclusive of residual ash), acid detergent fiber (ADF, expressed inclusive of residual ash), and acid detergent lignin (ADL, after extraction with sulfuric acid) were determined using the ANKOM fiber analyzer (ANKOM, A2001, New York, NY, USA) in a fiber filter bag (F57-ANKOM Technology Corporation, Macedon, NY, USA) according to VanSoest et al. [20]. Calcium content (Ca) was measured using an atomic absorption spectrophotometer (Varian AA140, Varian, Australia) (method ID 968.08). Total phosphorus (P) contents were analyzed by the molybdovanadate colorimetric method (method ID 965.17) using a spectrophotometer (Shimadzu UV-1201 UV-Vis). All chemical analyses were performed in triplicate for each sample ($n = 3$) and are presented in Table 1.

2.3 EFE products and their enzymatic activities

The enzymatic supplementation was ensured by commercial exogenous fibrolytic enzyme (EFE) products, which are cellulase PLUS and xylanase PLUS supplied by Dyadic International Inc. (Jupiter, Florida, USA) in liquid form and MaxFiber supplied by Provita Supplements SCHAUMANN GmbH, Bad Laasphe, Germany, in powdered form. The cellulase PLUS and xylanase PLUS were produced by the fermentation of non-genetically modified *Trichoderma longibrachiatum* and are composed primarily of endo-1,4-β-D-xylanase (E.C. 3.2.1.8) and endoglucanase (EC 3.2.1.4), in addition to other side additional activities such as pectinase, mannanase, and protease. The MaxFiber was a protein-rich by-product from solid-state fermentation of five different fungi species: *Aspergillus niger*, *Aspergillus tubingensis*, *Aspergillus orzyae*, *Aspergillus sojae*, and

Table 1 Chemical composition (g/kg dry matter, unless otherwise stated) of untreated (CFBS), NaOH (NFBS), and urea-treated faba bean straw (UFBS) (n=3)

	CFBS	NFBS	UFBS
Dry matter[1]	914[a]	335[c]	796[b]
Ash	50[b]	91[a]	96[a]
Crude protein	48[b]	47[b]	165[a]
Crude fiber	560[a]	520[b]	520[b]
Neutral detergent fiber (aNDF)	753[a]	716[a]	635[b]
Acid detergent fiber (ADF)	675[a]	622[a]	595[a]
Acid detergent lignin	109[a]	98[ab]	87[b]
Hemicellulose	78[a]	73[a]	40[b]
Cellulose	466[a]	524[a]	423[a]
Calcium	4.1[c]	13.1[a]	7.5[b]
Phosphorus	0.47[b]	0.68[a]	0.56[a]

[1]The dry matter was expressed as g/kg fresh matter of oat straw preparation

[a,b,c]Means within a row with different superscripts differ significantly (p-value < 0.05)

Neurospora intermedia incubated on four defined substrates: rapeseed meal, sugar beet molasses, corn gluten, and corn powder. The MaxFiber contained 315 g/kg CP and possessed xylanase, endoglucanase, and exoglucanase activities.

The dyadic products were studied in combination between cellulase PLUS and xylanase PLUS in a ratio 1:1 v/v (DCX) and supplemented to the FBS preparations according to increasing dose levels as recommended by Jabri et al. [21]: 0, 1, 2, 5, and 10 µL/g DM CFBS, NFBS, and UFBS. The diluted DCX (tenfold) was directly sprayed onto the ground FBS preparation with the appropriate dose/g DM. The MaxFiber was supplemented to CFBS, NFBS, and UFBS according to the manufacturer's instructions as follows: 0, 0.5, 1, 2, and 4 mg/g DM.

The enzymatic complexes DCX and the MaxFiber were assessed in triplicate, in three runs, for their xylanase (EC 3.2.1.8, endo-β-1,4-xylanase), endoglucanase (EC 3.2.1.4, endo-β1,4-glucanase), and exoglucanase (EC 3.2.1.91, exo-β-1,4-glucanase) activities, according to Wood and Bhat [22] and Bailey et al. [23] methods. The used substrates are, respectively, xylan of oat spelt (poly-D-xylopyranose (1-->4) at 1%), carboxymethylcellulose (CMC at 1 %), and cellulose (sigmacell cellulose (type 20, 20 µm) at 1%). The enzymatic activities were determined under the closest possible conditions to the ruminal environment at 39°C and a pH of 6.6. The absorbance was read at 540 nm against glucose and xylose standard curves. One international unit (IU) was defined as the amount of enzyme required to release 1 µmol of released reducing sugar (glucose or xylose) per minute from the corresponding substrate.

2.4 In vitro ruminal fermentation

The in vitro batch culture using gas-tight fermentation bottles (117 mL) was used to assess the effect of EFE supplementation on untreated and alkali pretreated FBS [24]. To prepare the fermentation inoculum, two cannulated non-lactating cows (600–650 kg body weight) were used during the experiment as ruminal fluid donors. The cows were fed a diet composed of oat hay ad libitum and 2 kg of commercial concentrate formulated for dairy cows (Alfa® 7 standard) with free access to water and mineral/vitamin licks to meet the nutritional requirements as recommended by INRA [25]. The ruminal fluid was collected via an electric pump before morning feeding, from different sites within the rumen. The collected ruminal fluid was immediately transferred to the lab under anaerobic conditions in prewarmed insulated flasks and then strained through 4 layers of cheesecloth. Then, the fermentation inoculum was prepared by mixing the freshly collected ruminal fluid and anaerobic buffer medium (from pH 6.5 to 6.8) prepared in advance as described by Menke and Steingass [24] in a ratio of 1:2 (ruminal fluid: buffer medium). The fermentation inoculum preparation steps were all done under continuous flushing with CO_2 at 39°C water bath.

To set up the in vitro fermentation, samples of 200 ± 10 mg DM grounded CFBS, NFBS, and UFBS were weighed into fermentation bottles, then received the corresponding enzymatic dosage 20h before the in vitro incubation to create a stable straw-enzyme complex against the proteolytic action of the rumen as recommended by Beauchemin et al. [26]. The control samples (0µL/g DM) were sprayed in the same manner with distilled water. Each treatment was performed in triplicate in three consecutive runs.

Twenty hours later, each fermentation bottle was loaded with 30 mL of fermentation inoculum under continuous CO_2 flushing, then immediately sealed with a butyl rubber stopper and an aluminum crimp cap and incubated at 39°C for 96 h.

Negative (fermentation inoculum without substrate) and positive controls (without enzymatic supplementation) were incubated in six replications. Second positive controls containing inulin as reference were used to ensure the repeatability of the results and the effectiveness of the used inoculum for a total of 66 bottles. The in vitro ruminal fermentation was repeated three times with the same procedure (n=9). The incubation was repeated each time the difference in inulin gas production was larger than 5% between runs.

The gas production (GP) was measured for each bottle after 2, 4, 6, 8, 12, 24, 48, 72, and 96 h of incubation by inserting a 23-gauge (0.6 mm) needle attached to a pressure transducer connected to a visual display. For each fermentation bottle, after recording the produced gas pressure, the

transducer was immediately removed, leaving the needle in place to permit venting.

2.5 Calculations and statistical analysis

The recorded gas pressures were converted to gas volume using the following equation:

$$GP\ (ml) = GPr \times \frac{Vf - Vi}{Patm}$$

where GPr is recorded gas pressure (bar); Vf, volume of serum bottle (=117.39 mL); Vi, volume of inoculum added to each bottle; and Patm, atmospheric pressure (= 1.01325 bar).

The metabolizable energy (ME), organic matter digestibility (OMD), and volatile fatty acids (VFA) were estimated based on in vitro gas production after 24 h of incubation according to Menke and Steingass [24] and Getachew et al. [27]:

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

$$\text{OMD (\%)} = 14.88 + \left(0.889 \times GP_{24h}\right) + (0.45 \times CP) + (0.0651 \times Ash)$$

$$\text{VFA (mmol/200 mg DM)} = 0.00425 + \left(0.0222 \times PG_{24h}\right)$$

The recorded GP was fitted according the model of Groot et al. [28] by using the residual least square method using the reduced generalized gradient algorithm of the solver function in Microsoft Excel software.

$$GP\ [ml] = \frac{A}{\left[1 + \left({}^{B}/_{t}\right)^{C}\right]} \quad (1)$$

where A is the estimated potential GP (mL/g DM); B is the required time to produce ½ A (h); C is the sharpness of the switching characteristic of the curve sharpness. The parameter maximum rate of GP (Rmax) and the time at which Rmax is attained (Tmax) were calculated according to Bauer et al. [29] and Groot et al. [28] as (2) and (3):

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

$$Tmax\ [h] = B\left[\frac{C-1}{C+1}\right]^{1/c} \quad (3)$$

The experiment was conducted in a completely randomized design, where the results were subject to least square analysis of variance by using the GLM procedure of SAS studio 3.6. The feed additive types, doses, and the interaction between additive type × additive doses were considered as fixed factors. The in vitro ruminal fermentation run was repeated three times (n=3), and the mean value of each sample in each run was considered as the experimental unit. All collected data were analyzed as a completely randomized design and were conducted using the MIXED procedure of SAS Studio 3.6 (2017) including the incubation replication as a random effect and analyzed according to the statistical model:

$$Yijklm = \mu + Ri + EFEj + Dk + Tl\ (T * D)kl + \varepsilon ijkl$$

where Y_{ijklm} is an individual observation for each dependent variable, μ is the overall mean, R_i is the random effect of replicate (1–3), EFE_j is the fixed effect of enzymatic complex, Dk is the fixed effect of EFE dose rate, Tl is the fixed effect of FBS alkali treatment, $(T * D)_{kl}$ is the interaction between the FBS treatment and the EFE dose rate, and εijkl is the residual error.

The polynomial contrasts were used when the EFE effect was significant to determine the linear and quadratic response to increasing EFE dose rates. As the tested enzymatic doses are unequally spaced, the Proc IML from SAS® studio was used to generate coefficients for polynomial contrasts.

The differences between control (without enzyme addition), enzymatic supplementation, and dose rates were detected by a multivariate test of Duncan [30]. Means were considered significantly different when the p-value was less or equal to 5% and tendencies were declared at $0.05 < p\text{-value} < 0.1$.

3 Results

3.1 Effect of alkali treatments on FBS chemical composition

The studied faba bean straw is fibrous biomass containing high concentrations of cell wall components with 466 g/kg DM cellulose, 78 g/kg DM hemicellulose, and 109 g/kg DM lignin, and relatively moderate crude protein concentration by 48 g/kg DM. The chemical composition of FBS was affected by alkali treatments as presented in Table 1. The urea treatment caused significant solubilization of the hemicellulose and lignin fractions by 48.7% and 20.2%, respectively, and improvements of ash, crude protein, phosphorus, and calcium concentrations by 47.9%, 243%, 19%, and 45.3%, respectively. As for the NaOH treatment, only a significant increase of ash and calcium concentrations was recorded with a slight improvement of hemicellulose and lignin solubilization by 6.4%, and 10.1%.

3.2 Effect of EFE supplementation on in vitro ruminal fermentation of untreated and alkali-treated FBS

Both studied EFE complexes supplied xylanase, endoglucanase, and exoglucanase activities under ruminal conditions (pH= 6.6, T= 39°C) as presented in Table 2.

The effects of EFE supplementation at increasing doses on the different FBS preparations are presented in Tables 3, 4, 5, and 6. The response to the studied enzymatic complexes (DCX and MaxFiber) on the FBS preparations was dependent on the supplemented EFE dose and the chemical treatment of the straw. For both enzymatic complexes, the considered optimal dose was the minimum dose required to obtain the greatest significant improvement for the studied fermentation parameters as suggested by Eun et al. [31].

The DCX supplementation to the CFBS at increasing dose rates exerted a quadratic response (p-value < 0.01) for the GP kinetic throughout the incubation period (Table 3). This resulted in a quadratic improvement of the fermentation and digestive use parameters (Table 4). As compared to the control (D0), the optimal improvements were recorded by supplementing the optimal DCX dose D5 = 5µL/g DM, which caused improvements by 43.6%, 60.2%, 27%, 25.9%, and 43.5% for A, Rmax, ME, OMD, and VFA, respectively. As for the MaxFiber complex, the supplementation of CFBS by increasing dose rates exerted a linear response (p-value < 0.01) for the GP kinetic during the 96h (Table 5) of incubation, fermentation, and digestive use parameters (Table 6). As compared to the control (D0), the optimal improvements were recorded by supplementing the highest MaxFiber dose M4 = 4µL/g DM, which caused improvements by 24.6%, 37.6%, 14.3%, 14.8%, and 24.2% for A, Rmax, ME, OMD, and VFA, respectively.

As compared to the untreated FBS, the alkali treatments decreased the EFE effect. Indeed, except for the NFBS, the D5 improved slightly the GP production kinetic from 8h of incubation, the extent of GP by 15.8%, the rate Rmax by 15.3%, the ME by 9.7%, the OMD by 9.5%, and the VFA by 17% as compared to the control (0µL/g DM). The association between DCX and MaxFiber complexes with both NaOH and especially urea treatments had no significant effects on the GP kinetics throughout the 96h of incubation and on the estimated fermentation and digestive use parameters (Tables 3, 4, 5, and 6).

Table 2 Fibrolytic activities of supplied enzymatic complexes, measured under experimental conditions close to the ruminal environment ($n = 9$)

Enzymatic complexes[1]	Enzymatic activities[2]			Ratio[3]
	Xylanase	Endoglucanase	Exoglucanase	
DCX	2573 ± 131	1554 ± 76	160 ± 10.2	1.50
MaxFiber	5118 ± 6	75 ± 1	74 ± 0.3	0.75

[1]DCX (50% cellulase-PLUS et 50% xylanase-PLUS)

[2]Xylanase, endoglucanase, and exoglucanase activities are expressed as µmol of xylose and glucose, respectively, released by 1 ml of undiluted enzyme per minute (IU)

[3]Ratio of fibrolytic activities "Xylanase to Cellulase"

4 Discussion

The present study indicated that CFBS contains high amounts of cell wall components. Indeed, as presented in Table 1, the CFBS contained high fiber (aNDF = 753 g/kg DM), lignin (109 g/kg DM), and CP content by 48 g/kg DM. These results were in the range of NDF and CP values reported by Wegi et al. [1] for different Ethiopian faba bean varieties. According to Jabri et al. [32], the NDF content was similar to a typical cereal straw which is commonly used in ruminant's diet. However, the CFBS was 33% and 53% richer in CP and lignin, respectively. So, the CFBS could be regarded as a fibrous feed source with better nutritional value than cereal straw, which could significantly contribute to ruminant livestock feeds. The used alkali treatments modified the chemical composition of FBS as presented in Table 1. Indeed, the urea treatment caused a significant decrease in NDF and ADF contents because of hemicellulose and lignin solubilization. The NaOH treatment exhibited minor solubilization effects. These findings were similar to those obtained from treated bagasse and rice straw [33]. Vorlaphim et al. [34] and Nayan et al. [35] suggested that the dissolution of lignin and hemicellulose after alkali treatments were initiated by the swelling of cellulose, which resulted in the weakening of hydrogen bonding between cellulose and hemicellulose. This process renders the fiber content more flexible, leading to an improvement in the feeding value of low-quality forage. In other hand, the urea treatment provides an additional source of non-protein nitrogen, which improved the CP content by 243%. Indeed, during urea treatment, the urea was hydrolyzed by the urease enzyme into NH_3. The latter was transformed to NH_3–N as an end-product [36], then transformed to microbial protein which could contribute to the improvement of the CP content of the treated forage [37]. The ash contents of NFBS and UFBS improved (p-value < 0.05) over the untreated FBS (Table 1). For the NFBS, the high ash level could be attributed to the residual NaOH. As for UFBS, the high ash levels could be due to the decrease of UFBS organic matter content after lignin and hemicellulose dissolution. These findings were equivalent to those reported previously by Rasool and Gilani [38] and Mesfin and Ktaw [39].

As the in vitro ruminal fermentation is one of the most used methods to evaluate the nutritional value of feeds [40–42], it proved that the rate of in vitro GP and the

Table 3 Effect of DCX supplementation at increasing dose levels (D1=1; D2=2; D5=5; and D10=10 µL/g DM) on gas production (GP) kinetic of untreated (CFBS), NaOH (NFBS), and urea (UFBS)-treated faba bean straw during 96h of in vitro ruminal fermentation incubation (n=9)

	2h	4h	6h	8h	12h	24h	48h	72h	96h
Untreated faba bean straw (CFBS)									
Control	23.6^{d}	38.5^{c}	55.1^{c}	70.4^{c}	95.6^{c}	140.7^{c}	143.9^{c}	145.4^{c}	146.4^{c}
D1	30.9^{c}	50.7^{b}	73^{b}	89.3^{b}	121^{b}	167.5^{b}	171.2^{b}	173.2^{b}	174.2^{b}
D2	33.5^{bc}	55.5^{ab}	79.4^{ab}	96.9^{ab}	124.31^{b}	167^{b}	170.4^{b}	172^{b}	172.9^{b}
D5	40.5^{a}	63.7^{a}	89.8^{a}	112.1^{a}	144.4^{a}	202.5^{a}	207.3^{a}	209.3^{a}	210.3^{a}
D10	38.9^{ab}	59.1^{ab}	81.2^{ab}	100.3^{ab}	130.1^{ab}	178.1^{b}	181.7^{b}	183.8^{b}	184.7^{b}
Linear	0.12	0.32	0.22	0.14	0.19	0.23	0.14	0.23	0.22
Quadratic	<0.001	0.01	0.003	<0.001	0.002	<0.001	<0.001	<0.001	<0.001
NaOH-treated faba bean straw (NFBS)									
Control	22.9^{a}	30.7^{a}	40^{a}	51^{b}	65.9^{c}	120.9^{bc}	126.8^{b}	129^{bc}	129.9^{b}
D_1	23.1^{a}	28.9^{a}	37.4^{a}	45.6^{b}	68.2^{bc}	119.8^{c}	126.4^{b}	127.5^{c}	129.3^{b}
D_2	20.5^{a}	28.7^{a}	39.3^{a}	48.5^{b}	68.3^{ab}	138.5^{abc}	144.1^{ab}	145.5^{abc}	146.6^{ab}
D_5	23.5^{a}	32.6^{a}	44.1^{a}	59.3^{a}	83.8^{ab}	141.7^{a}	147.3^{ab}	148.9^{ab}	150.5^{a}
D_{10}	24.8^{a}	33.4^{a}	43.1^{a}	58.5^{a}	87.7^{a}	140.6^{ab}	148.05^{a}	151.7^{a}	153.1^{a}
Linear	0.18	0.20	0.12	0.007	0.007	0.05	0.03	0.03	0.01
Quadratic	0.21	0.15	0.35	0.18	0.22	0.26	0.19	0.17	0.15
Urea-treated faba bean straw (UFBS)									
Control	14.3^{a}	10.1^{b}	18^{b}	21.5^{b}	46.3^{a}	128.1^{a}	138.3^{a}	140.1^{a}	142.1^{a}
D_1	13.7^{a}	21.7^{ab}	36.2^{ab}	39.3^{ab}	66.3^{a}	126.6^{a}	136.1^{a}	137.8^{a}	142.7^{a}
D_2	12.8^{a}	24.6^{ab}	38.1^{ab}	41.6^{ab}	65.7^{a}	128.9^{a}	138.3^{a}	138.3^{a}	142.9^{a}
D_5	11.5^{a}	21.7^{ab}	40^{a}	45.8^{a}	66.5^{a}	124.4^{a}	133.9^{a}	134.7^{a}	138.7^{a}
D_{10}	15.6^{a}	27.9^{a}	42.8^{a}	47.2^{a}	68.9^{a}	130.4^{a}	139.7^{a}	140.4^{a}	142.5^{a}
Linear	0.12	0.06	0.06	0.05	0.11	0.18	0.23	0.24	0.23
Quadratic	0.19	0.23	0.24	0.28	0.32	0.26	0.31	0.25	0.42
SEM	6.5	9.2	12.3	15.4	17.3	14.8	14	14.2	13.9
D^1	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
T^2	0.006	0.006	0.005	<0.001	<0.001	0.004	0.004	0.004	0.004
T× D^3	0.02	0.023	0.13	0.05	0.22	0.003	0.03	0.02	0.002

[1]D is the enzymatic dose

[2]T is the alkali treatment of FBS

[3]T × D is the interaction between the EFE complex and the FBS treatment

[a,b,c]Means within a row with different superscripts differ significantly (p-value < 0.05)

estimated ME, OMD, and VFA (Tables 3 and 4) of FBS are higher than cereal straw [32, 43] and sugarcane bagasse [44]. Compared to other crop residues, FBS could serve as an important nutrient source for ruminants. However, it has a relatively lower energy content, which may not be adequate to meet the dietary requirements of ruminants if used alone. As a result, it is recommended to supplement faba bean straw with other feeds, such as grains, to provide a balanced diet for ruminants. Using alkali treatments had variable effects on ruminal fermentation and digestive use parameters. Indeed, the urea treatment improved slightly the OMD and the ME. As for the NaOH treatment, we obtained an unexpected decrease in the rate and the extent of in vitro GP throughout the 96h of incubation coupled with a decrease in the estimated digestive use parameters (Tables 3 and 4). Sundstol [45] reported that the presence of a high concentration of residual NaOH may limit the digestive use of the treated forage by disrupting the pH balance in the ruminal environment leading to reduced feed efficiency and impaired digestion.

The EFE supplementation to the CFBS adjusted significantly the in vitro ruminal fermentation profile of CFBS, NFBS, and UFBS. For both studied EFE, the extent and the rate of GP, then the estimated digestive use parameters improved significantly (p-value < 0.05). The plant's cell walls are composed mainly of complex polysaccharides, such as cellulose, hemicellulose, and pectin which are held together by covalent bonds and noncovalent interactions. Fibrolytic enzymes such as xylanase, endoglucanase, and exoglucanase should act synergistically to hydrolyze these

Table 4 Effect of DCX supplementation at increasing dose levels (D1=1; D2=2; D5=5; and D10=10 µL/g DM) on the digestive use and in vitro ruminal fermentation parameters of untreated (CFBS), NaOH (NFBS); and urea (UFBS)-treated faba bean straw (*n*=9)

	Fermentation parameters					Digestive use parameters		
	A	B	C	Rmax	Tmax	ME	OMD	VFA
Untreated faba bean straw (CFBS)								
Control	146.4^{c}	7.7^{a}	1.7^{a}	11.8^{c}	3.6^{a}	6.3^{c}	42.4^{c}	0.62^{b}
D1	174.2^{b}	7^{b}	1.7^{ab}	15.2^{b}	3.1^{b}	7.02^{b}	47.1^{b}	0.74^{b}
D2	172.9^{b}	6.4^{c}	1.6^{bc}	16.6^{ab}	2.6^{bc}	7.01^{b}	47^{b}	0.73^{b}
D5	210.3^{a}	6.8^{bc}	1.6^{bc}	18.9^{a}	2.8^{bc}	$_{8}$a	53.4^{a}	0.89^{a}
D10	184.7^{b}	6.5^{bc}	1.6^{c}	17.2^{ab}	2.5^{c}	7.3^{b}	49^{a}	0.78^{b}
Linear	0.22	0.18	0.20	0.19	0.18	0.17	0.15	0.21
Quadratic	<0.001	0.03	0.05	0.002	0.04	<0.001	<0.001	<0.001
NaOH-treated faba bean straw (NFBS)								
Control	129.9^{b}	9.33^{a}	1.7^{a}	8.8^{ab}	4.3^{a}	5.8^{bc}	39.1^{bc}	0.53^{c}
D1	129.3^{b}	9.8^{a}	1.8^{a}	8.2^{b}	4.8 ^{a}	5.7^{c}	38.9^{c}	0.53^{c}
D2	146.6^{ab}	10.5^{a}	1.9^{a}	9.01^{ab}	5.9 ^{a}	6.2^{abc}	42.2^{abc}	0.61^{abc}
D5	150.5^{a}	9.3^{a}	1.8^{a}	10.15^{a}	4.8 ^{a}	6.3^{a}	42.8^{a}	0.62^{a}
D10	153.1^{a}	9.4^{a}	1.8^{a}	10.14^{a}	4.75 ^{a}	6.3^{ab}	42.6^{ab}	0.62^{ab}
Linear	0.02	0.18	0.23	0.003	0.11	0.03	0.04	0.02
Quadratic	0.19	0.13	0.16	0.26	0.33	0.14	0.26	0.15
Urea-treated faba bean straw (UFBS)								
Control	142.1 ^{a}	13.9^{a}	3.23^{a}	9.04 ^{a}	11.47^{a}	6.62 ^{a}	45.7 ^{a}	0.56 ^{a}
D1	142.7 ^{a}	11.5^{b}	2.05^{b}	8.23 ^{a}	6.73^{b}	6.58 ^{a}	45.5 ^{a}	0.56 ^{a}
D2	142.9 ^{a}	11.3^{b}	2.05^{b}	8.46 ^{a}	6.64^{b}	6.64 ^{a}	45.9 ^{a}	0.57 ^{a}
D5	138.7 ^{a}	10.9^{b}	2^{b}	8.35 ^{a}	6.21^{b}	6.52 ^{a}	45.1 ^{a}	0.55 ^{a}
D10	142.5 ^{a}	10.4^{b}	1.96^{b}	9.05 ^{a}	5.8^{b}	6.68 ^{a}	46.1 ^{a}	0.57 ^{a}
Linear	0.23	0.003	0.003	0.23	0.02	0.12	0.11	0.15
Quadratic	0.11	0.06	0.16	0.15	0.18	0.22	0.27	0.30
SEM	23.3	0.4	0.07	13.9	1.3	0.25	2.2	1.44
D[1]	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
T[2]	0.003	0.002	0.004	0.002	0.03	0.01	0.002	0.002
D× T[3]	0.04	0.04	<0.001	0.002	0.004	0.03	0.03	0.05

[1]D is the enzymatic dose

[2]T is the alkali treatment of FBS

[3]T × D is the interaction between the EFE complex and the FBS treatment

[a,b,c]Means within a row with different superscripts differ significantly (p-value < 0.05)

bonds into more readily soluble molecules [46]. This process facilitates the extraction of valuable compounds such as sugars and other biomolecules from the plant material and helps to improve the nutritional value of feedstocks by providing an additional energy source for the ruminal microorganisms' growth and preparing the cell wall surface for microbial attachment [47].

The DCX supplementation at increasing dose levels improved quadratically the in vitro ruminal fermentation parameters with the optimal dose D5=5µL/g DM. At higher doses, the DCX effect could be detrimental to the ruminal fermentation as proved by Jabri et al. [21] for oat straw and Yang et al. [9] for whole plant faba bean silage, whereas adding the second enzymatic complex (MaxFiber) at increasing doses exhibited a positive linear effect on CFBS ruminal fermentation with an optimal dose of M4= 4mg/g DM. So, the in vitro gas production and the digestive use of CFBS increased with increasing MaxFiber dosage. These findings were similar to those reported by Sakita et al. [48] for tropical forages (Aruana grass) and Pech-Cervantes et al. [49] for the dairy cow diet. Accordingly, making a conclusive statement about the typical effect of increasing doses of EFE supplementation on ruminant feeding is challenging due to the variation in effects caused by several factors. These factors include the type and source of the enzyme being used, the activity of the enzyme, the type of animal being fed, the

Table 5 Effect of MaxFiber complex supplementation at increasing dose levels (M0.5=0.5; M1=1; M2=2; and M4=4 µL/g DM) on gas production (GP) kinetic of untreated (CFBS), NaOH (NFBS), and urea (UFBS)-treated faba bean straw during 96h of in vitro ruminal fermentation incubation (n=9)

	2h	4h	6h	8h	12h	24h	48h	72h	96h
Untreated faba bean straw (CFBS)									
Control	23.6^{c}	38.5^{c}	55.1^{b}	70.4^{b}	95.6^{b}	140.7^{b}	143.9^{b}	145.4^{b}	146.4^{b}
M0.5	30.2^{b}	46.5^{ab}	62.4^{ab}	79.8^{ab}	103.8^{ab}	150.8^{ab}	154.2^{b}	155.8^{b}	156.5^{b}
M1	36^{ab}	53^{ab}	69.8^{a}	86.6^{a}	118.1^{a}	163^{ab}	166.6^{ab}	167.6^{ab}	168.3^{ab}
M2	37.1^{a}	53.2^{ab}	74^{a}	88.1^{a}	115.7^{a}	157.1^{ab}	159.9^{ab}	161^{ab}	162^{ab}
M4	38.2^{a}	56.3^{a}	76.4^{a}	94.7^{a}	121.9^{a}	176^{a}	180^{a}	181.4^{a}	182.5^{a}
Linear	<0.001	0.004	0.004	0.006	0.04	0.005	0.003	0.003	0.002
Quadratic	0.13	0.23	0.25	0.18	0.19	0.21	0.13	0.25	0.29
NaOH-treated faba bean straw (NFBS)									
Control	22.9^{a}	30.7^{ab}	40^{a}	51^{a}	65.9^{a}	120.9^{a}	126.8^{a}	129^{a}	129.9^{a}
M0.5	22^{a}	30^{ab}	38^{a}	49^{a}	75.9^{a}	128.3^{a}	133.5^{a}	135.5^{a}	136.2^{a}
M1	28.1^{a}	37.7^{a}	46.6^{a}	54.3^{a}	76.2^{a}	130.5^{a}	135.9^{a}	137.2^{a}	138.4^{a}
M2	28.3^{a}	33.4^{b}	41.9^{a}	55^{a}	83.1^{a}	135.2^{a}	140.7^{a}	143.6^{a}	145^{a}
M4	28.3^{a}	34.4^{ab}	42.3^{a}	57.8^{a}	87.1^{a}	143.2^{a}	147.6^{a}	150^{a}	150.5^{a}
Linear	0.11	0.05	0.18	0.14	0.06	0.05	0.05	0.07	0.08
Quadratic	0.22	0.26	0.24	0.19	0.15	0.13	0.25	0.29	0.33
Urea-treated faba bean straw (UFBS)									
Control	4.3^{b}	10.1^{c}	18^{b}	21.5^{b}	46.3^{b}	128.1^{a}	138.3^{a}	140.1^{a}	142^{a}
M0.5	9.9^{ab}	22.9^{a}	34.9^{a}	39.3^{a}	62.9^{a}	124.4^{a}	132.8^{a}	134.9^{a}	136.8^{a}
M1	6.2^{b}	12^{bc}	19.4^{b}	23.7^{b}	45.1^{b}	122.4^{a}	130.5^{a}	131.8^{a}	134.9^{a}
M2	13.1^{a}	20.5^{ab}	29.6^{a}	36.3^{a}	56.7^{ab}	127.2^{a}	136.4^{a}	137.8^{a}	140.9^{a}
M4	7.9^{ab}	17.1^{abc}	37^{a}	42.7^{a}	66.3^{a}	133.8^{a}	142.1^{a}	143^{a}	150.2^{a}
Linear	0.22	0.18	0.002	<0.001	0.005	0.23	0.12	0.23	0.26
Quadratic	0.02	0.13	0.14	0.11	0.19	0.16	0.18	0.14	0.19
SEM	6.6	8.7	10.8	13.3	14.8	10.8	10.1	9.9	9.9
D[1]	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
T[2]	<0.001	<0.001	<0.001	<0.001	<0.001	0.003	0.02	0.03	0.006
D× T[3]	0.04	0.002	0.004	0.02	0.17	0.23	0.19	0.17	0.11

[1]D is the enzymatic dose

[2]T is the alkali treatment of FBS

[3]T × D is the interaction between the EFE complex and the FBS treatment

[a,b,c]Means within a row with different superscripts differ significantly (p-value < 0.05)

composition of the fermented feed, the specific simulated rumen conditions, and the rearing conditions of the animal. In some cases, there may be no significant effect of enzyme supplementation on the animal's performance [50].

Comparing the effect of both studied EFE, the MaxFiber exerted lower efficiency than the DCX enzymatic complex regarding the GP kinetic, the fermentation parameters, and the estimated digestive use parameters ME, OMD, and VFA. These results could be due to the higher activity of the xylanase, endoglucanase, and exoglucanase enzymes present in the DCX complex (Table 2), as compared to the MaxFiber complex. Also, the effectiveness between the enzymes produced by *Trichoderma longibraciatum* and the mix produced by *Aspergillus strains* and *Neurospora intermedia* could be due to some possible factors that could influence the effectiveness of these enzymes including their specific enzyme activities, stability in the rumen environment, interaction with ruminal microorganisms, and compatibility with other dietary components [41, 51, 52]. It was also proved that the supplementation with the appropriate proportions of xylanase and cellulase activities (xylanase to cellulase ratio) is the key to determining the optimal enzyme dosage for an optimal effect on ruminal fermentation [21, 46]. The association between alkali treatments (NaOH and urea) and EFE supplementation did not affect significantly the in vitro ruminal fermentation and the digestive use parameters as the OMD and VFA of FBS despite the lignin solubilization after alkali treatment

Table 6 Effect of MaxFiber complex supplementation at increasing dose levels (M0.5=0.5; M1=1; M2=2; and M4=4 µL/g DM) on the digestive use and in vitro ruminal fermentation parameters of untreated (CFBS), NaOH (NFBS), and urea (UFBS)-treated faba bean straw (n=9)

	Fermentation parameters					Digestive use parameters		
	A	B	C	Rmax	Tmax	ME	OMD	VFA
Untreated faba bean straw (CFBS)								
Control	146.4^{b}	7.7^{a}	1.7^{a}	11.7^{b}	3.6^{a}	6.3^{b}	42.4^{b}	0.62^{b}
M0.5	156.5^{b}	7.2^{ab}	1.6^{ab}	13.4^{ab}	2.9^{b}	6.6^{b}	44.2^{b}	0.66^{b}
M1	168.3^{ab}	6.8^{bc}	1.6^{b}	15.1^{a}	2.7^{bc}	6.9^{ab}	46.3^{ab}	0.72^{ab}
M2	162^{ab}	6.3^{c}	1.5^{b}	15.6^{a}	2.3^{c}	6.7^{ab}	45.3^{ab}	0.69^{ab}
M4	182.5^{a}	6.9^{b}	1.5^{b}	16.1^{a}	2.6^{bc}	7.2^{a}	48.7^{a}	0.77^{a}
Linear	0.006	0.36	0.03	0.005	0.005	0.003	0.002	0.001
Quadratic	0.22	<0.001	0.16	0.06	0.13	0.19	0.33	0.41
NaOH-treated faba bean straw (NFBS)								
Control	129.9^{a}	9.3^{a}	1.7^{a}	8.8^{a}	4.3^{a}	5.7^{a}	39.1^{a}	0.53^{a}
M0.5	136.2^{a}	9.6^{a}	1.9^{a}	9^{a}	5.1^{a}	6^{a}	40.4^{a}	0.56^{a}
M1	138.4^{a}	8.8^{a}	1.6^{a}	$9.\ 7^{a}$	3.5^{a}	6^{a}	40.8^{a}	0.57^{a}
M2	139.9^{a}	9.7^{a}	1.9^{a}	9.2^{a}	5.2^{a}	6^{a}	40.8^{a}	0.57^{a}
M4	150.5^{a}	9.2^{a}	1.8^{a}	10.3^{a}	4.8^{a}	6.4^{a}	43.1^{a}	0.63^{a}
Linear	0.05	0.21	0.16	0.08	0.18	0.08	0.09	0.09
Quadratic	0.21	0.11	0.23	0.36	0.35	0.41	0.36	0.33
Urea-treated faba bean straw (UFBS)								
Control	142.1^{a}	13.9^{a}	3.2^{a}	9.04^{a}	11.5^{a}	6.62^{a}	45.7^{a}	0.56^{a}
M0.5	136.8^{a}	11.3^{c}	2.1^{b}	8.03^{a}	6.8^{b}	6.52^{a}	45.1^{a}	0.55^{a}
M1	134.9^{a}	13.7^{a}	3^{a}	8.24^{a}	10.8^{a}	6.47^{a}	44.7^{a}	0.54^{a}
M2	140.9^{a}	12.3^{b}	2.2^{b}	7.93^{a}	8^{b}	6.6^{a}	45.6^{a}	0.56^{a}
M4	149.9^{a}	11.8^{bc}	2.2^{b}	8.66^{a}	7.1^{b}	6.77^{a}	46.7^{a}	0.59^{a}
Linear	0.11	0.006	0.006	0.09	0.005	0.13	0.13	0.36
Quadratic	0.25	0.31	0.33	0.1	0.09	0.53	0.42	0.22
SEM	18.2	0.3	0.05	9.9	1.44	0.3	1.7	1.7
D^{1}	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.004
T^{2}	<0.001	0.005	0.006	0.004	0.003	0.002	0.003	0.003
D× T^{3}	0.22	0.21	0.15	0.18	0.002	0.005	0.006	0.003

[1]D is the enzymatic dose

[2]T is the alkali treatment of FBS

[3]T × D is the interaction between the EFE complex and the FBS treatment

[a,b,c]Means within a row with different superscripts differ significantly (p-value < 0.05)

(Table 1). It is possible that the supplemented enzymes may have already been saturated with the available substrate, meaning that further supplementation could have a significant effect on digestibility. In other hand, other factors, such as changes in the chemical composition of the straw or the activity of endogenous enzymes, may have influenced the digestibility and volatile fatty acids content [53].

Furthermore, the alkali treatment decreased the EFE efficiency for both studied enzymatic complexes (DCX and MaxFiber). These findings were similar to those reported by Jabri et al. [54] for sunflower head by-products using the same enzymatic complexes. However, Wang et al. [55] found a synergetic effect between 5% NaOH treatment of wheat straw and EFE on the effect of in situ dry matter digestibility. Indeed, based on earlier studies and our findings, we hypothesize that the alkali treatment modifies the pH conditions which may denature enzymes causing them to lose their native structure and activity. This can lead to a decrease in the efficiency of exogenous fibrolytic enzymes [56, 57]. Moreover, alkali treatment can alter the structure of plant cell walls by breaking down the lignocellulosic biomass, which may generate by-products such as lignin-derived phenolic compounds (e.g., vanillyl alcohol, coniferyl alcohol, and sinapyl alcohol) which reduce the efficiency of enzymatic hydrolysis [58]. However, the potency of lignin inhibition is dependent on its content, type of lignin, its crosslinked, and phenolic and polymeric structure [59]. Overall, it is important to carefully optimize the conditions of the alkali treatment process to maximize the efficiency of exogenous fibrolytic enzymes and maximize the yield of fermentable sugars from plant biomass.

5 Conclusion

The EFE supplementation of faba bean straw with exogenous fibrolytic enzymes composed mainly of cellulase and xylanase has the potential to improve the extent and the rate of GP, resulting in an improvement of the estimated digestive use parameters ME, OMD, and VFA. Studying the effect of two different EFE proved differences in supplementing doses effect, optimal dose, efficiency to improve the ruminal fermentation, and sensitivity to other chemical treatments caused probably by the supplemented fibrolytic activity, to the xylanase-cellulase ratio, and the specificity enzyme-substrate. The association between EFE and alkali treatment of faba bean straw decreased the efficiency of EFE which may be attributed to the modification of pH conditions and the release of antinutritional factors after chemical treatments. Overall, further research is needed to determine the optimal dose level of enzyme supplementation and the specific circumstances under which the enzymatic effect will be optimal.

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

Declarations

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

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

Competing interests The authors declare no competing interests.

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