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Effect of fibrolytic enzyme supplementation of urea-treated wheat straw on nutrient intake, digestion, growth performance, and blood parameters of growing lambs

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ABSTRACT

This research aimed to evaluate the effects of the supplementation of an exogenous fibrolytic enzyme (EFE; 1:1 v/v mixture of cellulase PLUS and xylanase PLUS, Dyadic International Inc, USA) to untreated (Control(WS)) and urea pretreated wheat straw (UWS) on feed intake, digestibility, nitrogen balance, ruminal fermentation, growth performance and blood metabolites of meat lambs. The diet contained 50 % wheat straw and 50 % concentrate (DM basis), and the EFE was applied at 1 mL/ kg DM to the straw. Lambs were arranged in a completely randomized design with four treatments (n = 5 lambs per treatment): Control(WS), WS+EFE, UWS, UWS+EFE. The lambs were housed individually in metabolic crates for 56 d divided into three experimental periods: $d_{0–21}$ adaptation period, $d_{21–28}$ digestibility trial and average daily gain (ADG) measurements and $d_{28–56}$ for only ADG measurements. All studied treatments have no effect on nutrients intake. As compared to the control WS, only the EFE supplementation affected the nutrients digestibility. The EFE addition improved the digestibility of dry matter by 9 % and 14 %, organic matter by 8 % and 13 %, crude protein by 7 % and 6 %, neutral detergent fiber by 24 % and 47 %, and acid detergent fiber by 30 % and 48 % for WS+EFE and UWS+EFE as compared to Control(WS) and UWS, respectively. The ruminal NH_3-N concentrations measured during d_{21}, d_{24} and d_{28} were lower for animals fed WS+EFE and UWS+EFE than those fed on Control(WS) and UWS. The nitrogen retention improved only for UWS+EFE. The EFE supplementation to straw had no effect on ruminal pH and blood metabolites. For the ADG, the highest total ADG improvement (22 %) was recorded for UWS+EFE. These results indicate that urea treatment alone was inefficient in improving straw digestibility and lamb growth performance. However, the EFE supplementation was efficient on lamb growth, especially when combined with urea treatment due to increased ruminal digestibility and nitrogen retention.

1. Introduction

Due to increased feed costs and limited feed resources, finding alternative feed sources is becoming increasingly crucial, especially during the dry season around the world. Agricultural by-products are produced in a huge quantity every year all over the world (4.7 billion tons) (OECD/FAO, 2018). Wheat straw (WS) is one of the most abundant lignocellulosic biomasses among agricultural residues (Kim and Dale, 2004), and has been widely used in ruminant diets, providing a cheap source of nutrients. However, the WS has a low nutritional value and consists mainly of cellulose (28–39%), hemicellulose (23–24 %), lignin (16–25 %), and fewer contents of ash and protein (3%) (Carvalheiro et al., 2009), with only 10–35 % of the straw energy content being available as net energy for animal maintenance and production requirements (Varga and Kolver, 1997). Researchers have applied various methods to optimize the use of this valuable byproduct for ruminant feed. Therefore, classical methods such as chemical treatments were widely used to improve the digestive use of fibrous feeds (Shreck, 2013; Sontakke, 2012) because of the ability of alkali pretreatments to cleave the esterified bonds within the plant cell wall, to improve the enzymatic

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hydrolysis during ruminal fermentation (Kontogianni et al., 2019), and to provide an additional source of non-protein nitrogen with urea treatment. Despite that, to have a balanced diet for animals, the use of a high level of cereal concentrate feed is necessary in most cases, which could limit fiber digestibility. On the other hand, the use of biotechnological methods such as exogenous fibrolytic enzymes (EFE) has proved its effectiveness to improve the availability of energy in fibrous feed by improving ruminal fermentation (So et al., 2020; Kholif et al., 2022), feed intake and fiber degradability, which is related to improvements in productive performance (Kholif et al., 2022). For lactating dairy cows, adding fibrolytic enzyme to the total mixed ration improved nutrient utilization, rumen fermentation, and milk production (Lunsin et al., 2021). For beef cattle fed low and high forage diets, the EFE supplementation improved the average daily gain (ADG) (Tirado-González et al., 2017) and the hot carcass yield (Vargas et al., 2013). However, the use of EFE for ruminants is related to many factors, including enzymatic products, the dose, the type of diet, and supplemented substrate (Tirado-González et al., 2021). It is of special relevance to study the effect of combining chemical pretreatments such as urea and EFE on diets based on agricultural byproducts such as WS, as the digestive use of WS is restricted due to its low nutritional value and reduced digestibility. So, our hypothesis is that combining chemical treatments like urea with exogenous fibrolytic enzymes will increase the straw intake and digestibility in growing lambs, resulting in improved performance. Therefore, the main objective of this study was to evaluate the effects of supplementing untreated and urea-pretreated wheat straw with cellulase-xylanase complex on the feed intake, nutrient digestibility, growth performance, and blood parameters of growing lambs.

2. Material and methods

The *in vivo* experiment was carried out at the experimental farm station of Higher School of Agriculture Kef (ESAK), located in the northwest region of Tunisia and lasted 56 days. The experimental animal procedures complied with the guidelines of IRESA (Institution de la Recherche et de l'Enseignement Supérieur Agricoles, Tunisia) and were conducted by specialized personnel in strict accordance with good animal practices as defined by national authorities and European Parliament and Council Directive, 2010/63/EU (2010) to ensure animal welfare.

2.1. Wheat straw preparation

The wheat straw was fed untreated Control(WS) or treated with 16 % urea (UWS) according to Chermiti et al. (1989). The urea treatment was performed in August with an ambient temperature of around 32 °C (during the daytime). The urea treatment was done on a flat ground in 5 layers of wheat straw bales and conducted by a special team from the "Office d'Elevage et des Paturages" Kef with the dose 40 g urea/kg diluted in 250 mL of water. The urea solution was sprayed over a straw layer using a gardener's sprinkler to ensure uniformity of urea treatment. Once the first layer has been treated, an additional layer is placed on top and sprayed with urea. This process is repeated until the end of the straw quantity, making a stack. At the end, the obtained straw stack was hermetically covered with a black plastic sheet to exclude the influx of oxygen and prevent ammonia from evaporating. In our case, around three weeks later, the urea treatment was done. The straw stack was kept covered to protect the urea-treated straw from direct sunlight and rainfall. Seven days before the beginning of the *invivo* trial, the UWS was uncovered and kept in the open air to remove the pungent smell of ammonia.

2.2. Enzymatic supplementation

The exogenous enzyme preparation (EFE) was a mixture (1:1, v/v) of two commercially available fibrolytic enzymes (Dyadic International Inc., Jupiter, Florida, USA). The enzymatic products are liquid concentrates and contained primarily the cellulase (Cellulase PLUS) and endo-1,4-β-D-xylanase (Xylanase PLUS), both produced by fermentation from non-genetically modified *Trichoderma longibrachiatum*. The enzymatic preparation was analysed in triplicate to determine the 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) under the closest possible conditions to ruminal environment (39 °C and pH of 6.6 under aerobic conditions) as described by Wood and Bhat (1988) and Bailey et al. (1992). The quantified fibrolytic activities showed that the Cellulase PLUS and Xylanase PLUS mixture (EFE) contained 2276 ± 152 IU of xylanase, 1160 ± 107 IU of endoglucanase and 113 ± 6.4 IU of exoglucanase. The EFE was applied to the Control(WS) and UWS at the dose 1 μL/g DM. The dosage of enzymes was chosen based on the result of our previous *in vitro* study using the same enzymes and the same substrate (Jabri et al., 2019).

The enzymatic solution containing 5 mL EFE and 2 L distilled water was prepared freshly each day and sprayed onto 5 kg DM straw, then incubated in aerobic environment for at least 8 h as recommended by Beauchemin et al. (1998). This was aimed to create a stable straw-enzyme complex against the proteolytic action of rumen during ruminal fermentation.

2.3. Animal preparation and experimental design

Twenty weaned male thin tail lambs, aged 6 months with an average initial body weight of 25.2 ± 3.8 kg, were randomly assigned into four uniform groups (n = 5 lambs per treatment) to receive one of the different diets: Control(WS), WS+EFE, UWS, UWS+EFE. The experiment lasted 56 days and was divided into two periods:

1) d_1-d_{28} animals were placed individually in metallic metabolic crates (1.2 m × 0.6 m) designed to separate feces, urine and refusals. During this period, the first 20 days were used to adapt animals to the metabolic crates and to the experimental diets (ensure an *ad libitum* intake (10% refusal)). Then, on the eight subsequent days ($d_{21–28}$), data and samples were collected to determine nutrients intake, digestion, blood parameters and growth performances.
2) $d_{28–56}$ each group of lambs (each receiving a different diet) was housed in a separate floor pen to collect average daily gain (ADG g/d) data.

Table 1
– Ingredients of the experimental diets and their chemical composition (g/kg dry matter, unless otherwise stated) (n = 3)[†].

	Control (WS)	UWS	CC[‡]	ED_1[§]	ED_2[§]
DM (g/kg)	890	790	910	900	850
OM	928	934	974	951	954
CP	32	145	171	101	158
EE	9	9	22	15	15
NDF	740	756	167	453	461
ADF	470	498	49	259	273
ADL	51	57	13	32	35
Hemicellulose	270	258	118	194	188
Cellulose	419	441	36	227	238

[†] Each value is the mean of three replicates. In all cases, coefficient of variation was < 3% of the mean.
[‡] The concentrate was composed of 850 g barley grain, 125 g soybean meal and 25 g of vitamin-mineral premix per kg concentrate. The composition of the vitamin-mineral premix (per kg) was 270 g Ca, 30 g P, 80 g Na, 30 g Mg, 2350 mg Zn, 5400 mg Mn, 40 mg Cu, 10 mg Co, 7 mg Se, 260 mg Fe, 10 mg I, 250,000 IU vitamin A, 50,000 IU vitamin D3 and 1000 IU vitamin E
[§] ED_1: experimental diet 1 (50 %WS+50 %CC); ED_2: experimental diet 2 (50 % UWS + 50 % CC).

2.4. Experimental diets

The experimental diets (ED_1 and ED_2, Table 1) were formulated to meet the nutritional requirements of lambs as recommended by the INRA (2007). The lambs received a diet consisting of 50% straw and 50% concentrate (DM basis). The concentrate feed (CC) was a mixture of barley grains (80%), soybean meal (17.5%) and vitamin-mineral premix (2.5%). The supplementation or not with the EFE and the presence or the absence of urea treatment made up four treatments. The treatments were: 1) untreated wheat straw with no enzyme Control(WS), 2) enzyme added to the untreated wheat straw (WS+EFE), 3) urea pretreated wheat straw with no enzyme (UWS) (control for UWS+EFE), 4) enzyme added to the urea pretreated wheat straw (UWS+EFE). The twenty lambs were offered two *ad libitum* equal meals at 09:00 am and 15:00 pm. Fresh and clean water was offered freely at all time.

2.5. Measurements

At the end of the adaptation period and during the eight measurement days, the feed refusals, total feces and total urine output of each lamb were daily measured and recorded before morning feeding to determine nutrients intake and digestibility. As explained by Abid et al. (2020), samples of feed refusals and total feces (10 % of total raw material) of each lamb were pooled daily. Feces samples were stored at − 20 °C until further chemical analysis (Abid et al., 2020). Total urine was daily collected into sterile plastic buckets containing 50 mL HCl (6 N) in order to maintain the urine pH below 3, in order to minimize ammonia volatilization and microbial growth. After volumes measurements for each lamb, an acidified urine sample (10 % of total acidified volumes) were daily pooled for each lamb and stored at − 20 °C until nitrogen concentration analysis (Abid et al., 2020).

For each lamb, diets, refusal, and feces samples were dried in a forced air oven at 55 °C until constant weight (AOAC, 1990). The samples were subsequently ground through a 1 mm-screen using a wiley mill. The samples were analyzed for dry matter (DM), organic matter (OM), crude fiber (CF), crude protein (CP) according AOAC (1990). For dietary ingredients, neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL) were determined according to Van Soest et al. (1991) method. The nitrogen content of urine was determined according to Kjeldahl method (AOAC, 1990). All chemical analyzes were performed in triplicate for each sample.

To study the effect of the different experimental diets (Control(WS), WS+EFE, UWS, UWS+EFE) on ruminal fermentation, samples of rumen liquor were collected before morning feeding from each lamb at d_{21}, d_{24} and d_{28} by using a smooth rubber stomach tube connected to a plastic syringe that was inserted into the rumen through the esophagus as described by Hassan and Almaamory (2016). Rumen liquor samples (approximately 30 mL) were strained through four layers of cheesecloth to discard solid unfermented particles. The pH was measured directly after sampling using a portable pH meter (Orion ™ StarA221, Thermo Scientific). Two mL of the rumen liquor were mixed with 2 mL 0.2 M HCl and stored at − 20 °C until NH_3-N was analyzed by Conway micro diffusion method (Conway, 1978).

Blood samples were drawn from jugular vein of each lamb before morning feeding on d_{28} by using an 18-gauge needle and vacutainer (Becton Dickinson and Co., Rutherford, NJ, USA) (SOP, 2017). Serum samples were obtained by centrifugation of the blood tubes for 20 min, *3000 x g* and immediately stored in sterile tubes, then frozen at − 20 °C until blood metabolites analysis. The total proteins, albumin, urea, glucose, triglycerides and cholesterol were analyzed by colorimetry with specific kits from Bio-Maghreb, Tunisia, according to the supplier's instructions using an automatic biochemical analyzer (Technicon RA-1000 Random Access Clinical Analyzer).

To study the growth performances, the ADG was calculated during the experimental periods d_{21} to d_{28} and d_{28} to d_{56}. Lambs were weighed before morning feeding at the beginning of the experiment (d_0) and every week until the end of the experiment period and thus to determine the weekly ADG. The total average daily gain (ADGt) was calculated for the entire experimental period (d_{21} to d_{56}).

2.6. Statistical analysis

The collected data were statistically analyzed according to a completely randomized design using the proc MIXED and the SAS® Studio 3.6 (2017) statistical analysis software according to the statistical models $Y_{ijk} = \mu + D_i + A_j + \varepsilon_{ijk}$ (1) for the first experimental period (d_1-d_{28}) and $Y_{ijk} = \mu + D_i + G_j + \varepsilon_{ijk}$ (2) for the second experimental period (d_{28}-d_{56}) for analysing the ADG, where: Y_{ijk} is the dependent variable; μ is the overall mean; D_i (i = 0–3) is the treatment effect, A_j is the random effect of animal (n = 20 lambs; (1)), G_j is the random effect of the experimental lamb's group (n = 4 groups; (2)), and ε_{ijk} is the residual error. Treatments effects were declared significant at P value < 0.05, and trends were discussed at 0.05 < P value< 0.1. The differences among groups were determined using Duncan's multiple-range test (Duncan, 1955).

3. Results

The daily feed intake (g kg^{-1} BW per day) was not affected by urea pretreatment and EFE supplementation whether added to the untreated (Control(WS)) or urea pretreated wheat straw (UWS) (Table 2). Except for CP intake for UWS lambs. The digestibility of DM and other nutrients (Table 2) increased only by adding EFE. For lambs receiving WS+EFE, the digestibility of DM, OM, CP, NDF, and ADF improved (P value<0.05) respectively by 9.1 %, 8.1 %, 7.5 %, 24.3 %, and 30 % as compared to the control diet (Control(WS)). As for lambs receiving UWS+EFE the association between urea pretreatment and EFE improved (P value<0.05) the digestibility of DM by 14.2 %, OM by 12.6 %, NDF by 46.8 %, and ADF by 47.7 % as compared to the diet UWS. Although not significant, the urea pretreatment of WS tended to decrease the digestibility of DM, OM, NDF, and ADF and to increase the CPD (Table 2).

The nitrogen intake and the excreted nitrogen (NU and NF) improved (P value<0.05) by urea pretreatment, whereas the EFE supplementation had no significant effect (Table 3) as compared to Control(WS) (Table 3). The retained nitrogen improved only by associating EFE and urea

Table 2
–Effect of urea treatment and EFE supplementation of untreated and urea pretreated wheat straw fed to fattening lambs on nutrients intake (g/ kg $BW^{0.75}$/ day) and digestibility (%) (mean values of all measurements, n = 5, lambs per treatment).

	Diets				SEM	P value
	Control (WS)	WS+EFE	UWS	UWS+EFE		
Feed intake						
DMI	63.6^{a}	60.6^{a}	63.8^{a}	60.6^{a}	2.6	0.27
OMI	60.3^{a}	56.5^{a}	60.6^{a}	57.6^{a}	2.5	0.33
CPI	7.91^{b}	7.5^{b}	12^{a}	11.9^{a}	1.1	0.05
NDFI	19.3^{a}	19.2^{a}	20.2^{a}	19.1^{a}	0.94	0.07
ADFI	15.1^{a}	15^{a}	15.8^{a}	14.9^{a}	0.74	0.09
ADLI	3.3^{ab}	3^{b}	3.9^{a}	3.6^{a}	0.33	0.05
Digestibility						
DMD	54.9^{ab}	59.9^{a}	50.5^{b}	57.7^{a}	2.4	0.04
OMD	57.7^{ab}	62.4^{a}	53.9^{b}	60.7^{a}	2.2	0.04
CPD	68.3^{b}	73.4^{ab}	73.2^{ab}	77.9^{a}	2.2	0.02
NDFD	36.5^{ab}	45.4^{a}	30.5^{b}	44.8^{a}	3.1	0.02
ADFD	33^{b}	42.9^{a}	28.7^{b}	42.4^{a}	2.6	0.01

DMI: dry matter intake, OMI: organic matter intake, CPI: crude protein intake, NDFI: neutral detergent fiber intake, ADFI: acid detergent fiber intake, ADLI: acid detergent lignin intake, DMD: dry matter digestibility, OMD: organic matter digestibility, CPD: crude protein digestibility, NDFD: neutral detergent fiber digestibility, ADFD: acid detergent fiber digestibility, SEM: standard error of the mean. a, b within the same row values with different letters are statistically different (P value <0.05).

Table 3
– Effect of urea treatment and EFE supplementation of untreated and urea pretreated wheat straw fed to fattening lambs on nitrogen intake (N_I), faecal nitrogen (N_F), Urinary nitrogen (N_U) and retained nitrogen (NR) (mean values of all measurements, n = 5 lambs per treatment).

	Diets				SEM	P value
	Control(WS)	WS+EFE	UWS	UWS+EFE		
N_I	15.3^b	14.6^b	22.3^a	23.2^a	1.8	0.027
N_F	4.9^{ab}	3.9^b	5.9^a	5.4^a	0.5	0.011
N_U	4.5^b	4.9^b	$_9a$	8.1^a	1	0.013
NR	5.9^b	5.8^b	7.3^b	9.8^a	0.8	0.010

NI: nitrogen intake (g/day), NF: fecal nitrogen (g/day), NU: urinary nitrogen (g/day), NR: retained nitrogen (g/day), SEM: standard error of the mean. a,b within the same row values with different letters are statistically different (P value <0.05).

pretreatment (Pvalue =0.01). For lambs receiving UWS+EFE the nitrogen retention (NR) increased by 66.1 % and 34.2 % respectively as compared to WS and UWS.

The effects of EFE on ruminal fermentation and serum biochemistry are presented in Tables 4 and 5. The Urea and EFE supplementation had no significant effects on ruminal pH. The ruminal NH_3-N concentrations (measured at d_{21}, d_{24} and d_{28}) decreased (P value<0.05) by EFE supplementation alone, and the association between EFE and urea. As for the biological assessment, the blood parameters were unaffected (P > 0.05) by the EFE addition, whereas a slight increase of urea and total protein blood concentration was recorded only for lambs receiving UWS.

The growth performances improved during each week of the experiment (P value<0.05) (Table 6) giving a higher ADG_T at the end of the experiment (P value =0.001). Both urea pretreatment and EFE supplementation used alone improved the total ADG as compared to WS, however, the greatest improvement of ADG_T was detected by associating the EFE with urea pretreatment (Table 6). The ADG_T of lambs fed UWS, WS+EFE and UWS+EFE increased by 12.6%, 9.4% and 22.3% respectively as compared to Control(WS) (P value<0.05). On the other hand, the ADG_T of lambs fed UWS+EFE diet increased by 8.6% as compared to UWS.

4. Discussion

In order to achieve optimal degradability and high efficiency of fiber digestion of untreated and urea-pretreated wheat straw in the rumen, a mixture of two exogenous fibrolytic enzymes (cellulase and xylanase,

Table 4
– Effect of urea treatment and EFE supplementation of untreated and urea pretreated wheat straw fed to fattening lambs on ruminal pH and ruminal ammonia nitrogen (NH_3-N) (mean values of all measurements, n = 5 lambs per treatment).

	Diets				SEM	P value
	Control (WS)	WS+EFE	UWS	UWS+EFE		
Ruminal p-H						
pH_{d21}	7.3^a	7.2^a	7.1^a	7.2^a	0.06	0.731
pH_{d24}	7.2^a	7.2^a	7.2^a	6.9^a	0.09	0.332
pH_{d28}	7.2^a	7.2^a	7.3^a	7.4^a	0.06	0.537
NH_3-N (mg/100 mL of rumen liquor)						
NH_3-N $_{d21}$	15.3^a	11.3^b	15.41^a	11.2^b	1.02	0.010
NH_3-N $_{d24}$	17.9^b	16.6^b	22.7^a	14.3^c	2.3	0.001
NH_3-N $_{d28}$	16.8^b	16.1^b	21.3^a	16.8^b	1.9	0.050

NH3-Nd21 ammoniacal nitrogen measured at d21, NH3-Nd24 ammoniacal nitrogen measured at d24, NH3-Nd28 ammoniacal nitrogen measured at d28, pHd21 ruminal pH measured at d21, pHd24 ruminal pH measured at d24, pHd28 ruminal pH measured at d28, SEM: standard error of the mean. a,b,c within the same row values with different letters are statistically different (P value <0.05).

Table 5 –
Effect of urea treatment and EFE supplementation of untreated and urea pretreated wheat straw fed to fattening lambs on blood biochemistry (mean values of all measurements, n = 5 lambs per treatment).

	Diets				SEM	P value
	Control (WS)	WS+EFE	UWS	UWS+EFE		
Glucose (mmol/L)	4.71^a	4.97^a	5.17^a	5.14^a	0.22	0.13
Triglycerides (mmol/L)	0.19^a	0.19^a	0.19^a	0.21^a	0.02	0.89
Urea-N (mmol/L)	4.7^b	5.15^{ab}	5.64^{ab}	6.4^a	0.46	0.05
Total protein (g/L)	54.6^b	57.4^b	62.9^a	59.8^{ab}	1.92	0.05
Albumin (g/L)	25.3^a	25.6^a	25.1^a	24^a	1.68	0.63
Cholesterol (mmol/L)	1.16^a	1.13^a	1.13^a	1.24^a	0.07	0.49

SEM: standard error of the mean. a,b within the same row values with different letters are statistically different (P value <0.05).

Table 6 –
Effect of urea treatment and EFE supplementation of untreated and urea pretreated wheat straw fed to fattening lambs on average daily gain (ADG) (g/day) (mean values of all measurements, n = 5 lambs per treatment).

	Diets				SEM	P value
	Control (WS)	WS+EFE	UWS	UWS+EFE		
Initial BW (kg)	25.4	25.7	24.2	25.4	0.8	0.262
Final BW (kg)	29.4^b	30.1^a	28.7^b	30.3^a	1.5	0.041
ADG_1	125^b	141.7^{ab}	143.3^{ab}	156.8^a	7.56	0.010
ADG_2	137.2^c	148.5^b	152^b	163.4^a	5.51	0.017
ADG_3	136.7^c	146.5^b	153^b	164.8^a	5.42	0.012
ADG_4	132.8^c	146.7^b	150.2^b	165.8^a	5.93	0.003
ADG_5	142^b	153.3^{ab}	159.8^{ab}	172.6^a	8.83	0.004
ADG_T	134.7^c	147.3^b	151.7^b	164.7^a	4.54	0.001

ADG1: ADG calculated between d21 and d28, ADG2: ADG calculated between d28 and d35, ADG3: ADG calculated between d35 and d42, ADG4: ADG calculated between d42 and d49, ADG5: ADG calculated between d49 and d56, ADGT: ADG calculated between d21 and d56, BW: body weight, SEM: standard error of the mean. a,b,c within the same row values with different letters are statistically different (P value <0.05).

EFE) at 1 mL/kg DM was added to the WS and UWS was incorporated at 50% into a growing lamb diet. This experimental protocol does not allow us to assess the effect of each enzyme. Indeed, Ribeiro et al. (2016) demonstrated that efficient degradation of plant cell wall polysaccharides requires a cocktail of enzymatic activities. Each enzymatic activity is necessary for a particular component of cell wall digestion (Beauchemin et al., 2004). Some studies have even proven a synergetic effect between ruminal and exogenous fibrolytic enzymes (Morgavi et al., 2000). Although nutrient intake was not significantly different among treatments, as previously reported by Arce-Cervantes et al., (2013), and Aboagye et al., (2015), However, the effect of EFE on nutrient intake depends upon forage quality (Assoumaya et al., 2007), the forage: concentrate ratio (Cruywagen and van Zyl, 2008), and the level of enzyme addition (Beauchemin et al., 2000).

There were no improvements in all studied nutrients' digestibility due to urea treatment of straw (Table 2). Similar to our results, Nurfeta et al. (2009) and Nimoniche et al. (2021) observed no effect of urea treatment of wheat straw on *in vivo* nutrient digestibility in sheep. Although there was enough evidence that urea-treated straw was more digestible (Kraidees, 2005). The alkali treatment of straw may increase the release of furfural and soluble phenolic compounds, which impede ruminal digestion and decrease microbial activity (Van Soest and

Mason, 1991; Liu et al., 1999). Furthermore, the distributed straw quantity, specifically the dietary physically effective NDF could affect the digestibility of dietary nutritional components (Zhao et al., 2009). Trach (2000) found that *adlibitum* urea treated rice straw decreased the digestibility of CP and OM in steers because urea treatment produced changes in the fiber and lignin fractions. Van Soest (2006) and Sadeghi and Shawrang, (2007) suggested that a 10% increase of lignin content indicating the formation of Maillard products that add to the lignin value, the ADF and silica, which reduce the CP and OM digestibility. This was supported by our finding of improved ADLI by 18 % and decreased OM digestibility after urea treatment (Table 2).

The EFE supplementation to Control(WS) and UWS improved the digestibility of DM, OM, CP, and CF. According to Beauchemin et al. (2004), the effect of EFE is not limited to fibre components and appears to be effective in improving digestibility of the non-fiber carbohydrate fraction. Indeed, Salem et al. (2013) showed that EFE supplementation improved CP digestibility in beef steers and lambs. Indeed, Kohn and Allen (1992) proved that the EFE facilitates the degradation of cell wall-bound proteins.

Since fibrolytic enzymes alter the cell wall structure of the feed (Beauchemin et al., 2004) and improve DM and CP solubility prior to feeding (Alvarez et al., 2009), it makes the diet more amenable to degradation. Then, the reduced sugars would provide energy that would lead to rapid microbial growth (Giraldo et al., 2008), thereby increasing ruminal hydrolytic capacity (Meale et al., 2014). Subsequently, EFE could enhance the complete diet's digestibility and its nutritive value rather than just being limited to the specific component targeted by the enzyme (Beauchemin et al., 2004). These findings highlight the importance of treating the substrate prior to feeding to initiate cell wall hydrolysis, to allow proper attachment of enzymes on feed particles, and to protect the enzyme from ruminal proteases, as discussed by Beauchemin et al. (2004). The creation of a stable enzyme-feed complex before feeding increases the potency of EFE.

The main constraint of straw incorporation in productive ruminant diets is the limited digestibility caused by the high content of lignified fibres (Flachowsky et al., 1999). The use of EFE supplementation for ruminant diets based on crop residues will increase its feeding value and achieve satisfactory levels of performance. As a result, low-quality local feed resources can be successfully used in ruminant feeding to produce enough meat to meet rising human demand. The use of WS by ruminants represents an efficient means to overcome economic problems by reducing feed costs, especially during food shortage periods and pollution problems linked to Straw burning.

The major finding in this study was the improvement of lamb's growth performance with EFE supplementation by increasing the average daily gain by 9.4 % (WS+EFE) and 22.3 % (UWS+EFE). This is possibly due to the improvement of nutrient digestibility and energy availability for ADG and production (Gado et al., 2011; Salem et al., 2013). Similarly, to our findings adding EFE to lamb diets improved nutrient digestibility and ADG by 13 % (López-Aguirre et al., 2016) and up to 15 % (Arce-Cervantes et al., 2013) without affecting intake. However, the use of high doses of EFE on lambs fed an oat straw-based diet decreased the intake linearly without changing the weight gain and digestibility (Bueno et al., 2013). On the other hand, Aboagye et al., (2015) proved that applying enzymes at feeding had no effect on fibre digestibility, and performance of sheep fed alfalfa hay. Hence, again, the effectiveness of EFE depends on several factors, including forage type (Beauchemin et al., 2004), the mode of application, and the level of enzyme addition (López-Aguirre et al., 2016).

Combined with urea pretreatment, the enzymatic supplementation affects mainly the CP digestibility and the NR. Commonly, the use of EFE in ruminant diets has no effect on protein utilization (Awawdeh and Obeidat, 2011). This is likely due to the presence of an additional source of non-protein nitrogen delivered by urea pretreatment. These results are consistent with our previous *in vitro* results on WS and those reported by Eun et al. (2006) that indicate the existence of a synergistic effect between ammonia pretreatment (NH3) and EFE supplementation that increases the *in vitro* ruminal fermentation and the degradability of rice straw. The mechanism by which urea pretreatment stimulates the EFE efficiency to improve the retained nitrogen and protein digestibility remains uncertain. However, it could be due to the association between the hydrolytic effect of EFE that improves fermentable carbohydrate amount (Eun et al., 2006) and the presence of an additional nitrogen source delivered by the urea pretreatment. Therefore, the energy-N equilibrium in the ruminal medium was ensured, which stimulated the microbial activity and, consequently, the increase in NR and nutrient digestibility (Yang et al., 1999; Wang et al., 2012). This might also explain the decrease ($P < 0.05$) of ruminal ammonia concentration by associating the urea pre-treatment of WS and EFE supplementation of WS for lambs. These results are in agreement with those shown by Silva et al. (2016) indicated that the addition of xylanase to the dairy cow diet decreased the NH3-N concentration in a quadratic manner ($P = 0.02$). Similarly, an *invitro* study conducted by Almaraz et al. (2010) proved that EFE supplementation of a diet (30% concentrated feed and 70% forage) decrease the ruminal NH3-N by 11%.

For ruminal pH, it remains stable and close to neutrality for all experimental diets (Control(WS), WS+EFE, UWS, UWS+EFE) during the entire measurement period (d21, d24 and d28) which ensures optimal activity of the microbial flora. These results are similar to those of Balci et al. (2007) on beef steers, Silva et al. (2016) on dairy cows and Arce cervantes et al. (2013) on sheep. For lamb blood biochemistry, the only significant effect was detected on blood urea concentration, especially when lambs received urea pre-treated wheat straw through the increased feed intake of urea treated roughage with a higher CP percentage, as proved by Sweeny et al. (2014). However, the remaining studied blood parameters are within a normal physiological reference range (Ismail et al., 2008; Kramer, 2000). These results confirm that the protein-energy ratio of the experimental diets was adequate and used effectively. The enzymatic supplementation alone or in combination with urea pre-treatment had no negative effect on lamb's health as proved previously by Peters et al., (2015) on dairy cows and Gomaa et al., (2012) on sheep.

5. Conclusion

These results proved that the EFE supplementation of untreated (WS) and urea pre-treated wheat straw (UWS), at the concentration 1 mL/kg DM, had the potential to increase the average daily gain by 9.4 % for WS+EFE and 22.3 % for UWS+EFE by increasing most of the nutrient digestibility. The EFE efficiency was improved by combining the EFE and urea pretreatment without any adverse effect on growing meat lamb health. This association of treatments for wheat straw will improve its feeding value, allowing it to be included at a higher level in ruminant diets.

Conflict of interest statement

The authors have no conflicts of interest to declare.All co-authors have seen and agree with the contents of the manuscript and there is no financial interest to report. We certify that the submission is original work and is not under review at any other publication.

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