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Effect of treating olive cake with fibrolytic enzymes on feed intake, digestibility and performance in growing lambs

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ABSTRACT

This research evaluated the effects of the addition of a blend of exogenous fibrolytic enzymes composed of a mixture of cellulase and xylanase (CX) to a high-fibre diet on growth performance of meat lambs. The diet contained olive cake (OC), and CX was added to this fibrous by-product at 0 (control, no addition of CX), 4 (CX04) or 16 (CX16) ml of the enzyme preparation per kg OC dry matter. The study was planned according to a quintuplicated 3 × 3 Latin square design, with three experimental treatments (control, CX04, CX16), three 21-day periods (14-day adaptation plus 7-day collection period) and 15 weaned male lambs (Queue Fine de l'Ouest) randomly assigned to the 5 replicates (3 lambs in each replicate). Intake of both dry matter and organic matter were increased ($P < 0.05$) by 4 % with CX addition. Fibre digestibility was increased ($P < 0.05$) by 6 % (CX04) and 8 % (CX16) as compared with control. Blood serum biochemistry were not affected ($P > 0.05$) by CX, except cholesterol concentration that was increased ($P < 0.05$). As compared with lambs fed the control diet, average daily weight gain was increased ($P < 0.05$) by 6 and 10 % when lambs were fed CX04 and CX16 diets, respectively. These results indicate that the supplementation of OC-based lamb diets with CX, even at relative low concentrations, improves lamb growth performance as a result of increasing feed intake and enhancing fibre digestibility with no adverse effects on animal health.

1. Introduction

Worldwide, the ruminant production systems rely on forage and roughage as the main feed ingredient, even though they are high in fibre and generally low in protein and energy contents (Wilkins, 2000). In Tunisia, forages and fibrous by-products, most often of limited nutritional value, are commonly used in small ruminant feeding systems. Olive cake (OC) is one of the by-products obtained from olive oil extraction. It represents 35 % of the olives weight pressed (Heuzé et al., 2015; Molina Alcaide and Nefzaoui, 1996), and

Abbreviations: BW, body weight; CX, cellulose plus xylanase enzyme preparation; CX04, diet in which CX was added to olive cake at 4 ml of the enzyme preparation per kg dry matter; CX16, diet in which CX was added to olive cake at 16 ml of the enzyme preparation per kg dry matter; DM, dry matter; EFE, exogenous fibrolytic enzyme; OC, olive cake; ME, metabolisable energy; SEM, standard error of the mean

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it has been widely utilized in small ruminant feeding (Awawdeh and Obeidat, 2013; Obeidat, 2017) as an inexpensive feedstuff precluding environmental pollution that would be caused by the disposal of waste (Awawdeh, 2011). However, OC is characterized by a high content of indigestible fibre (Molina Alcaide and Nefzaoui, 1996) and contains anti-nutritive compounds such as polyphenols and tannins (Molina-Alcaide and Yáñez-Ruiz, 2008) These fibrous feedstuffs can be digested by the rumen microorganisms but in most cases only 10–35% of the gross energy ingested is available as net energy (Varga and Kolver, 1997). A number of approaches have been used to improve its nutritive value such as chemical treatment with alkali (Molina-Alcaide and Yáñez-Ruiz, 2008) or acid (Awawdeh and Obeidat, 2013), or the addition of direct-fed microbials (Martin and Nisbet, 1992; Obeidat, 2017). The use of exogenous fibrolytic enzymes (EFE) to enhance quality and digestibility of fibrous forage is one of the most extensively applied biotechnological approach to attain practical benefits in ruminant production systems (Mendoza et al., 2014). Fibrolytic enzymes, such as cellulases and xylanases, are used to break ß1-4 glycosidic linkages in cellulose and xylan found in plant cell walls (Beauchemin et al., 2003; Dawson and Tricarico, 1999), thus facilitating the digestion of structural polysaccharides in the rumen. Several studies examining the effects of EFE have reported an increase of microbial activities in the rumen (see reviews by Meale et al. (2014) and Adesogan et al. (2019)), resulting in enhanced feed digestion and improvement of animal performance. These studies have shown that adding EFE to ruminant diets may result in increased milk production in dairy cows (El-Bordeny et al., 2015; Kung et al., 2000; Schingoethe et al., 1999) and average daily gains in feedlot cattle (Beauchemin et al., 1999; McAllister et al., 1999) or in growing lambs (López-Aguirre et al., 2016) in response to enhanced feed digestibility. Increased dry matter and fibre digestibility upon the use of EFE has been shown in situ, in vitro (Colombatto et al., 2003; Holtshausen et al., 2011; Yang et al., 1999) and in vivo (Beauchemin et al., 2000; Gandra et al., 2017; Kung et al., 2000; Yang et al., 1999). The relationship between the improvement in forage utilization and enzymatic activities is not fully understood and further studies in ruminants are warranted (Eun et al., 2007), as variable and inconsistent results with EFE addition to ruminant diets have been reported (Beauchemin et al., 2003; Colombatto et al., 2003). Some studies have shown substantial improvement of feed digestibility and animal performance (Bala et al., 2009; Cruywagen and Goosen, 2004; Nowak et al., 2003; Salem et al., 2013), while others reported either negative or no significant effects (Bowman et al., 2003; Peters et al., 2015; Vicini et al., 2003). It is of special relevance to assess the effects of the addition of EFE to diets with high-fibre ingredients such as OC, as the use of this by-product in ruminant feeding is constrained owing to its low digestibility. Our hypothesis is that the addition of exogenous fibrolytic enzymes to OC before feeding can enhance its digestibility and palatability in growing lambs, so that performance will be also improved. Therefore, the objective of this study was to evaluate the effects of adding a blend of EFE at different levels to growing lamb diets containing OC on feed intake and digestibility, nitrogen balance, blood biochemistry and growth performance.

2. Materials and methods

The study was conducted in the animal facilities at the research station of the Ecole Supérieure d'Agriculture du Kef (Tunisia) and lasted 12 weeks. The experimental animal procedures complied with the institutional guidelines of IRESA (Institution de la Recherche et de l'Enseignement Supérieur Agricoles, Tunisia) and were conducted by trained specialised 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. Treatment of olive cake with exogenous enzymes

Two months before the beginning of the experiment, fresh OC was obtained by pressure extraction of oil crushing the olives in an oil mill located in Sfax (South of Tunisia). The collected OC was spread on a plastic sheeting, sun dried with manual turning and tedding (3–4 times daily), and finally stored in plastic bags.

The preparation used as an additive was an equal volume mixture (50:50 by volume) of two fibrolytic enzyme commercial products manufactured by Dyadic International Inc. (Jupiter, Florida, USA). The products are concentrated liquids composed mainly of acid cellulase (Cellulase Plus) and acid-neutral endo-1,4-β-D-xylanase (Xylanase Plus), both produced by fermentation from non-recombinant *Trichoderma longibrachiatum* (formerly *Trichoderma reesei*). Both Cellulase plus and Xylanase plus are food grade products that were blended in an equal volume to obtain the cellulase + xylanase (CX) preparation used in the experiment. This 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-β-1,4-glucanase), and exoglucanase (EC 3.2.1.91, exo-β-1,4-glucanase) activities, according to the procedure of Wood and Bhat (1988). These activities were determined at a pH of 6.6 and a temperature of 39 °C, which reflect normal rumen conditions in sheep. The substrates (all purchased from Sigma Chemical Co., St Louis, MO, USA) used for the determinations of enzymatic activities were oat-spelt xylan (xylanase), carboxymethylcellulose sodium salt (endoglucanase) and cellulose (exoglucanase). The xylanase activity quantified in the CX mix was 2267 µmol xylose released/min per ml, the endoglucanase activity was 1161 µmol glucose released/min per ml, and the exoglucanase 113 µmol glucose released/min per ml.

The CX mix was diluted in distilled water at two different concentrations, either 20 ml CX preparation/L or 80 ml CX preparation per L. These solutions were freshly prepared every day and the CX treatment was applied by spraying one of the enzyme solutions (or only distilled water in the control) on the dry OC at 200 ml of solution per kg of OC dry matter (DM). The enzyme solutions were prepared and applied to the OC in an air-conditioned room at 26 °C. Treated OC was maintained in the same room at 26 °C for 12 h before it was fed to the lambs. Thus, the final concentration of CX mix in the olive cake was 0 (control), 4 (CX04) or 16 (CX16) ml per kg OC DM. These levels of addition were defined based on the outcome of a preliminary *in vitro* study carried out at the National School of Veterinary Medicine (Sidi Thabet, Tunisia) using the same enzymes. Steeping the OC in distilled water or in one of the CX

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

	Oat hay	Olive cake	Concentrate[2]
Dry matter (g/kg fresh matter)	890	950	910
Organic matter	923	938	974
Crude protein	80	72	171
Ether extract	140	112	22
Crude fibre	409	373	39
Neutral detergent fibre (aNDF)	581	579	167
Acid detergent fibre (ADF)	372	461	49
Lignin (sa)	81	222	13

[1] Each value is the mean of three replicates (one collected at each experimental period). In all cases, coefficient of variation was < 3 % of the mean.

[2] 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.

solutions for 12 h before feeding allowed for the formation of a stable enzyme-feed complex initiating the alterations of the fibre structure before proteolytic breakdown of the enzymes during fermentation in the rumen (Beauchemin et al., 2003).

2.2. Experimental design

Fifteen weaned "Queue Fine de l'Ouest" male lambs with average initial age of 5 months and body weight of 14.6 ± 0.45 kg were used. Lambs were housed individually in metabolism cages (1.2 × 0.6 m) with wire-mesh floor. All lambs received two equal meals at 09:00 am and 15:00 pm. In each meal, the lambs were fed 150 g DM of concentrate and 50 g DM of OC thoroughly mixed. Once this mix was consumed, oat hay was distributed to allow for ad libitum intake. Daily feed intake measurements were recorded for each individual animal. Chemical composition of the diet ingredients (concentrate, OC and oat hay) is shown in Table 1. Fresh water was available freely at all time.

The experiment followed a 3 × 3 Latin-square design in quintuplicate with three dietary treatments, three successive experimental periods and 15 lambs randomly assigned to the 5 replicates. A Latin square design was run for each replicate with 3 different lambs in each replicate. The replicates were run over the same three periods. The three experimental treatments were defined by the OC used in the diet, to which the CX enzyme mixture (cellulase and xylanase) was added at 0, (CONTROL), 4 (CX04) or 16 (CX16) ml/kg OC dry matter. Each period lasted for 21 days, with a 14-d adaptation period and the following 7 days as the measurement period. Every experimental period was followed by a 7-d washout period during which lambs were removed from the metabolic cages and all received the control ration.

2.3. Measurements and chemical analyses

Each lamb was weighed for three consecutive days before the morning feeding at the beginning and end of each period using a digital scale (range 0.01–50 kg, accuracy 10 g).

During the last 7 days of each experimental period, feed (oat hay) refusals were removed daily from the feeders, weighed out and dried. The mesh floor of the metabolism cages allowed faeces and urine to fall into a funnel placed beneath. A screen in the funnel was designed for the separate collection of faeces and urine (Wang et al., 2018). Daily faecal and urinary outputs were collected quantitatively and weighed for each lamb just before the morning feed distribution. A sample of faeces (10 % of the wet matter of the total output) was collected daily and all the samples of each lamb within each period were pooled and stored at − 20 °C. Urine was collected in buckets containing 50 ml of 6 N HCl to maintain urine pH < 3 in order to minimize NH_3-N volatilization and microbial growth. After weighing the daily output, an aliquot of acidified urine (10 %) was collected and all the samples of each lamb were mixed at the end of each period and the pooled sample was stored at − 20 °C.

Samples of dietary ingredients (oat hay, concentrate, and OC), feed refusals and faeces were oven-dried until constant weight for the determination of DM content (AOAC 930.15), and then ground through a mill using a 1 mm screen. Then, samples were analysed to determine the ash (AOAC 942.05), crude fibre (AOAC 962.09), crude protein (AOAC 954.01) and ether extract (AOAC 920.39) contents according to the methods of Association of Official Analytical Chemists (1995). Neutral detergent fibre (aNDF, assayed with a heat stable amylase and expressed inclusive of residual ash), acid detergent fibre (ADF, expressed inclusive of residual ash) and acid detergent lignin (Lignin (sa), after extraction with sulphuric acid) in dietary ingredients were determined by the methods proposed by Van Soest et al. (1991). Nitrogen of urine was determined using the Kjeldahl method (Association of Official Analytical Chemists, 1995).

The last day of each experimental period, samples of rumen contents (30 ml) were collected from each lamb 3 h after the morning meal using a rubber stomach tube introduced into the rumen through the oesophagus. The rumen samples were filtered immediately through cheesecloth, and pH was measured immediately using a digital portable pH meter (Thermo Scientific™ Orion™ Star A221pHPortable Meter). Then, 2 ml of filtered rumen fluid were mixed with 2 ml of 0.2 N HCl, and stored at –20 °C until the analysis of ammonia nitrogen concentration by the Conway microdiffusion method (Nocek et al., 1987).

Also the last day of each experimental period, blood samples were taken from the jugular vein of each lamb before the morning meal. Samples were centrifuged for 20 min at 3000 × *g* and serum (supernatant) was collected in Eppendorf tubes and frozen at –20 °C. Total protein, albumin, urea, glucose, triglycerides, cholesterol, aspartate aminotransferase and alanine aminotransferase were determined in serum using a clinical chemistry analyser (Technicon RA-1000 Random Access Clinical Analyzer). The concentration of serum globulins was calculated as the difference between total protein and albumin.

2.4. Calculations and statistical analysis

The metabolisable energy (ME, MJ/kg DM)) concentration of the diets was calculated from in vivo digestibility following the equation of GfE (1995):

$$ME = 0.0312 \times D_{fat} + 0.0136 \times D_{CF} + 0.0147 \times (D_{OM} - D_{fat} - D_{CF}) + 0.00234 \times CP,$$

where D_{fat} is digestible fat; D_{CF} is digestible crude fibre; D_{OM} is digestible organic matter and CP is crude protein (all in g/kg DM).

Data were subjected to analysis of variance using the PROC MIXED of SAS Institute Inc. (2011), according to the statistical model: $Y_{ijkl} = \mu + R_i + L_{j(i)} + D_k + P_l + DP_{kl} + \varepsilon_{ijkl}$; where Y_{ijkl} is an individual observation for each dependent variable, μ is the overall mean, R_i is the random effect of replicate (1–5), $L_{j(i)}$ is the random effect of animal (lamb 1–15) within replicate, D_k is the fixed effect of diet (CONTROL, CX04 or CX16), P_l is the effect of period (1, 2, 3), PD_{kl} is the interaction between diet and period, and ε_{ijkl} is the residual error. As feed intake measurements were taken for each animal, each individual lamb was regarded as the experimental unit. Polynomial contrasts (linear and quadratic) were used to evaluate the effects of increasing the concentration of CX. The polynomial coefficients for unequally spaced levels were determined by using the PROC IML of SAS Institute Inc. (2011). Significant effects of the treatment were declared at $P < 0.05$ and a tendency towards significance when $0.05 < P < 0.10$. The differences among means were determined using the Duncan's multiple-range test (Duncan, 1955).

3. Results

At the end of each experimental period, the average body weight of the lambs was 17.3, 19.1 and 21.3 kg for the first, second and third period, respectively. Daily feed intake (g/day or g kg^{-1} body weight per day), nitrogen intake and retention and average daily weight gain increased ($P < 0.05$) from the first to the last experimental period. All the other variables, in particular digestibility, feed to gain ratio and serum biochemistry, were not affected ($P > 0.05$) by experimental period. The interaction between period and treatment was not significant for any of the variables evaluated.

Treating OC with CX mix increased ($P < 0.05$) DM, organic matter and ME intake of lambs with no significant ($P > 0.05$) differences between CX04 and CX16 (Table 2). Daily weight gain in lambs fed diets with CX-treated OC was significantly increased ($P < 0.05$) by 6 % (CX04) or 9 % (CX16) compared with the CONTROL lambs. However, no significant effects ($P > 0.05$) of CX addition were observed on average body weight or on feed to gain ratio (Table 2).

No significant effect ($P > 0.05$) of CX addition on DM, organic matter and fat digestibility was observed (Table 3). However, protein digestibility tended ($P = 0.08$) to increase and fibre digestibility was significantly ($P = 0.03$) increased by 6 % (CX04) or 8 % (CX16) compared with the control diet (Table 3). Urine and faecal nitrogen were unaffected ($P > 0.05$) by adding CX to OC, whereas both N intake and N retained tended to increase ($P < 0.1$) by 16 % (CX04) and 13 % (CX16) as compared with the CONTROL diet (Table 3).

Table 2
Effect of adding increasing amounts of exogenous cellulose and xylanase enzymes to olive cake fed to growing lambs on feed intake, digestibility, nitrogen balance and growth performance.

	CONTROL	CX04	CX16	SEM (n = 15)	P-value	Linear	Quadratic
Feed intake							
g dry matter (DM)/d	745[b]	775[a]	776[a]	31.7	0.021	0.309	0.310
g DM kg^{-1} BW d^{-1}	40.0[b]	41.8[a]	41.7[a]	2.18	0.048	0.036	0.305
g DM kg^{-1} $BW^{0.75}$ d^{-1}	85.0[b]	88.5[a]	88.4[a]	4.37	0.042	0.018	0.184
g organic matter (OM)/d	707[b]	734[a]	738[a]	29.9	0.016	0.248	0.339
g OM kg^{-1} BW d^{-1}	38.0[b]	39.6[a]	39.7[a]	2.04	0.042	0.031	0.347
g OM kg^{-1} $BW^{0.75}$ d^{-1}	80.4[b]	83.8[a]	84.2[a]	4.08	0.032	0.022	0.199
MJ MEI/d	7.90[b]	8.22[a]	8.22[a]	0.298	0.030	0.010	0.671
Growth performance							
Average weight (kg)	18.8	18.7	18.7	0.51	0.786	0.758	0.537
Average daily weight gain (g/d)	137[b]	145[a]	150[a]	9.8	0.043	0.001	0.654
Feed to gain ratio (g DM intake/g weight gain)	5.47	5.35	5.18	0.091	0.091	0.032	0.681
Weight gain per feed (g weight gain/kg DM intake)	184	187	194	3.2	0.089	0.029	0.860

[a, b] within the same row, mean values not sharing a common superscript letter are statistically different ($P < 0.05$).
CONTROL: no enzyme added; CX04: 4 ml of the enzyme solution per kg olive cake dry matter; CX16: 16 ml of the enzyme solution per kg olive cake dry matter.
BW: body weight; MEI: metabolisable energy intake; SEM: standard error of the mean with 15 lambs per treatment (n = 15).

Table 3
Effect of adding increasing amounts of exogenous cellulose and xylanase enzymes to olive cake fed to growing lambs on digestibility and nitrogen balance.

	CONTROL	CX04	CX16	SEM (n = 15)	P-value	Linear	Quadratic
Digestibility							
Dry matter	0.688	0.684	0.688	0.0169	0.727	0.880	0.441
Organic matter	0.703	0.699	0.705	0.0167	0.541	0.744	0.293
Crude protein	0.677	0.716	0.720	0.0312	0.082	0.110	0.316
Crude fibre	0.598[b]	0.635[a]	0.646[a]	0.0282	0.029	0.010	0.434
Neutral detergent fibre (aNDF)	0.576[b]	0.595[ab]	0.615[a]	0.0069	0.002	<0.001	0.251
Acid detergent fibre (ADF)	0.524[b]	0.543[b]	0.572[a]	0.0080	0.001	<0.001	0.487
Ether extract	0.736	0.736	0.756	0.0115	0.604	0.382	0.612
Nitrogen balance							
Nitrogen intake (g/d)	14.1	14.7	14.8	0.66	0.050	0.059	0.383
Urinary nitrogen (g/d)	2.50	2.11	2.46	0.89	0.201	0.970	0.074
Faecal nitrogen (g/d)	4.57	4.40	4.30	1.22	0.811	0.531	0.910
Retained nitrogen (g/d)	7.04	8.20	7.99	1.24	0.094	0.134	0.321

[a, b] Within the same row, mean values followed by the same superscript letter or with no superscript are not significantly different at $P < 0.05$.
CONTROL: no enzyme added; CX04: 4 ml of the enzyme solution per kg olive cake dry matter; CX16: 16 ml of the enzyme solution per kg olive cake dry matter.
SEM: standard error of the mean with 15 lambs per treatment (n = 15).

The effects of treating OC with CX enzymes on ruminal fermentation and serum biochemistry are summarised in Table 4. Addition of CX mix had no significant effects ($P > 0.05$) on ruminal pH but tended to decrease ($P < 0.10$) ruminal NH_3-N concentration (both measured 3 h after feeding). Except for serum cholesterol, none of the other serum metabolites was affected ($P > 0.05$) by the addition of CX to the diet. Serum cholesterol was increased ($P < 0.05$) by 27 % (CX04) or 22 % (CX16) compared with the value recorded in control lambs.

4. Discussion

Two exogenous fibrolytic enzymes (cellulase and xylanase, CX) at increasing addition rates (CONTROL, CX04 and CX16) were used to treat OC incorporated at 100 g/kg to a standard growing lamb diet. A blend of two fibrolytic enzymes was used to achieve a more extensive effect on cell wall carbohydrates to enhance the digestion of a complex substrate (OC) in the rumen. With this approach, it is not possible to discriminate the specific effect of each enzyme. Commonly, mixtures of fibrolytic enzymes are used on the basis of their complementarity to degrade plant cell wall polysaccharides (Benvenutti and Pichard, 2014), even though some studies have shown no superiority of enzyme cocktails when compared to single enzymes (Ribeiro et al., 2018). Although DM and organic matter digestibility were not affected by CX addition, intake increased significantly due, probably, to the release of sugars caused by the hydrolysis of polysaccharides which may improve the palatability of the diet (Kholif and Aziz, 2014; Morgavi et al., 2004). The fibrolytic enzymes can also increase the rate of fibre degradation by the rumen microorganisms (Beauchemin et al., 2004; Yang et al., 1999), thus reducing the rumen fill and increasing voluntary intake of forage (Aitchison et al., 2005; Beauchemin and

Table 4
Effect of adding increasing amounts of exogenous cellulose and xylanase enzymes to olive cake fed to growing lambs on ruminal fermentation characteristics and blood serum biochemistry.

	CONTROL	CX04	CX16	SEM (n = 15)	P-value	Linear	Quadratic
Ruminal pH	6.89	6.89	6.92	0.17	0.825	0.632	0.716
Ruminal ammonia-N (mg/L)	189	157	148	29	0.091	0.080	0.751
Blood serum biochemistry							
Urea-N (mmol/L)	4.2	4.7	4.4	1.23	0.454	0.573	0.245
Total protein (g/L)	64.6	65.0	63.9	3.85	0.890	0.644	0.891
Albumin (g/L)	27.4	28.4	27.5	3.12	0.784	0.904	0.491
Globulin (g/L)	37.2	36.6	36.4	4.55	0.826	0.633	0.721
Albumin to globulin ratio	0.73	0.78	0.76	0.15	0.931	0.752	0.820
Cholesterol (mmol/L)	1.8[b]	2.3[a]	2.2[a]	0.61	0.024	0.022	0.106
Triglycerides (mmol/L)	0.57	0.45	0.53	0.34	0.771	0.753	0.519
Glucose (mmol/L)	4.2	4.6	4.5	0.63	0.323	0.362	0.233
Alanine aminotransferase (IU/L)	16.1	15.8	16.2	1.2	0.602	0.306	0.203
Aspartate aminotransferase (IU/L)	60.5	60.2	61.4	2.3	0.707	0.509	0.303

[a, b] within the same row, mean values not sharing a common superscript letter are statistically different ($P < 0.05$).
CONTROL: no enzyme added; CX04: 4 ml of the enzyme solution per kg olive cake dry matter; CX16: 16 ml of the enzyme solution per kg olive cake dry matter.
SEM: standard error of the mean with 15 lambs per treatment (n = 15).

Holtshausen, 2010; Mendoza et al., 2014). Similar results have been reported earlier by other authors (Pinos-Rodríguez et al., 2002; Vallejo et al., 2016). One of the critical limitation for the use of OC in ruminant diets is its low palatability (Heuzé et al., 2015), and there seems to be a potential to alleviate this restraint by the treatment of the by-product with exogenous enzymes. As the voluntary intake of roughage is increased with EFE, poor quality local resources can be economically and successfully used to produce meat and milk for human consumption. This could contribute widely to decrease production costs in ruminant systems by integrating poor quality roughage and fibrous by-products in ruminant diets.

Although fibre digestibility was increased by treating OC with CX, digestibility of DM and other fractions was not significantly improved. This finding was not consistent with other published results (Beauchemin et al., 2000; López-Aguirre et al., 2016). Discrepancies in the reported results can be due to differences in application procedures and rates of enzyme addition (Beauchemin and Holtshausen, 2010; Mendoza et al., 2014). However, our results showed a significant increase in fibre digestibility with the application of CX to OC. Enzymatic additives initiate the degradation of structural carbohydrates and act synergistically with the rumen microbiota thereby increasing the hydrolytic activity within the rumen environment (Beauchemin et al., 2004; Meale et al., 2014). Enzymes could also break the cell wall structure, stimulating the microbial colonization of feed particles and the attachment to polysaccharides (Nsereko et al., 2000). These results are in agreement with those reported by other authors using a wide range of forages in ruminant diets (Rode et al., 1999; Titi and Tabbaa, 2004). López-Aguirre et al. (2016) reported a significant effect of EFE on acid detergent fibre digestibility but not on neutral detergent fibre digestibility. Again, the mode of application and the amount of enzyme added can affect the magnitude of the effects, and it seems that the pre-treatment of feed with EFE is necessary to create a stable enzyme-feed complex (Kung et al., 2000).

The increased feed intake and digestibility resulted in improved growth performance increasing the daily weight gain by 6 %. Almaraz et al. (2009) observed no improvement of growth performance in lambs when the diet was treated with EFE immediately before feeding. In our study the enzyme preparation was mixed with OC 12 h before feeding the lambs, and thus it seems that the enzyme has to be applied in advance on the feedstuff some time before feeding to allow for an effective interaction between the enzyme and the substrate. Results reported elsewhere (Cruywagen and Goosen, 2004; López-Aguirre et al., 2016; Zhao et al., 2019) confirm this outcome. Krueger and Adesogan (2008) concluded that the preincubation of feeds with enzyme solutions is required to allow the maximum adsorption and binding of the enzyme to substrate and to develop a stable enzyme feed complex (Kung et al., 2000), so that the enzyme can initiate the substrate digestion before it can be degraded by rumen proteases (Beauchemin et al., 2003). Feed to gain was ratio not significantly affected, in agreement with the result reported by Awawdeh and Obeidat (2011), indicating that feed efficiency was not improved by the additive, and that the increased weight gain should be attributed to the improved feed intake. One of the main constrains in the use of OC as a feedstuff for ruminants is its low digestibility as results to its content in highly lignified fibre (Heuzé et al., 2015; Molina-Alcaide and Yáñez-Ruiz, 2008). The treatment of OC with fibrolytic enzymes will upgrade the feeding value of the by-product and would be in favour of its inclusion in ruminant diets to achieve satisfactory levels of performance in growing animals.

Although nitrogen retention was numerically increased (by 14–17 %) with CX compared with the control diet, the difference was not statistically significant. The enzymatic treatment of OC also did not affect protein digestibility. Although not significant, treatment of OC with CX tended to decrease ruminal ammonia concentration, likewise to the results of Almaraz et al. (2009), suggesting a slight increase of microbial protein synthesis by enhanced uptake of ammonia-N as a substrate by ruminal microbes. However, our results indicate a lack of effects of fibrolytic enzymes on efficiency of protein utilisation, in agreement with other authors (Awawdeh and Obeidat, 2011; Pinos-Rodríguez et al., 2002)

Addition of CX mix to feed had no significant effects on ruminal pH measured 3 h after feeding (values between 6.89 and 6.92), in agreement with Torres et al. (2013), showing that improved fibre digestibility will not cause a decline in pH or rise any risk of ruminal acidosis. There are almost no studies evaluating the effects of EFE on serum biochemistry in lambs. In our study all serum parameters were considered within the physiological range for healthy sheep (Boyd, 1984). The only significant effect of CX on serum biochemistry was the increased cholesterol concentration observed in lambs fed CX04 or CX16 diets compared with those fed the control diet. Zhao et al. (2019) found that cholesterol in lambs was not affected by adding a cellulase to a high-concentrate diet. A slight increase in cholesterol was observed in broilers fed a diet with 5 % OC supplemented with a multi-enzyme containing, among others, cellulase and xylanase (Al-Harthi, 2017). In contrast, decreased cholesterol has been reported in either dairy cows (Mohamed et al., 2013) or fattening lambs (Abdelrahman et al., 2016) fed diets supplemented with EFE. In the study with growing lambs, animals were heavier than in our study, and the experimental conditions (enzyme used, dose applied and substrate treated with the EFE –date pits–) differed substantially from those of the present study, and therefore the results are not directly comparable. Nevertheless, it is noteworthy that serum cholesterol in all the lambs were within the reference ranges reported in the literature for lambs (Fielder, 2016), suggesting that the observed increase can be regarded of no clinical relevance. Hence, our results indicate that the use of a blend of EFE has not adverse effects on liver or kidney function or on the general health status of lambs (Morsy et al., 2016; Vallejo et al., 2016).

5. Conclusions

These results indicate that the treatment of olive cake with non-ruminal cellulase and xylanase, even at relatively low concentration of the enzymes (CX04) has the potential to improve the growth performance of meat lambs (average daily weight gain increased by 6 %) by increasing feed intake and fibre digestibility, without adverse effects on animal health. The enzymatic treatment of olive cake will upgrade its feeding value allowing for increasing its level of inclusion in ruminant diets. Our results suggest that olive cake should be subjected to the action of the exogenous enzymes in advance to feeding, although further studies are warranted to optimise the mode of application and to determine the most suitable concentration of the enzymes to attain the most beneficial results.

CRediT authorship contribution statement

K. Abid: Investigation, Formal analysis, Visualization, Writing - original draft. **J. Jabri:** Software, Formal analysis. **H. Ammar:** Writing - review & editing. **S. Ben Said:** Investigation, Resources. **H. Yaich:** Software, Validation. **A. Malek:** Validation. **J. Rekhis:** Validation. **S. López:** Formal analysis, Validation, Writing - review & editing. **M. Kamoun:** Conceptualization, Methodology, Supervision, Project administration.

Declaration of Competing Interest

None.

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References

Abdelrahman, M., Sami, A., Suliman, G., Abudabos, A., 2016. Growth performance and economic efficiency of fattening Naimi lambs on unconventional ration enhanced with enzyme cocktail. Pak. J. Agric. Sci. 53, 467–471. https://doi.org/10.21162/PAKJAS/16.4536.

Adesogan, A.T., Arriola, K.G., Jiang, Y., Oyebade, A., Paula, E.M., Pech-Cervantes, A.A., Romero, J.J., Ferraretto, L.F., Vyas, D., 2019. Technologies for improving fiber utilization. J. Dairy Sci. 102, 5726–5755. https://doi.org/10.3168/jds.2018-15334.

Aitchison, E.M., Gill, M., Dhanoa, M.S., Osbourn, D.F., 2005. The effect of digestibility and forage species on the removal of digesta from the rumen and the voluntary intake of hay by sheep. Br. J. Nutr. 56, 463–476. https://doi.org/10.1079/bjn19860126.

Al-Harthi, M.A., 2017. The effect of olive cake, with or without enzymes supplementation, on growth performance, carcass characteristics, lymphoid organs and lipid metabolism of broiler chickens. Rev. Bras. Ciência Avícola 19, 83–90. https://doi.org/10.1590/1806-9061-2016-0311.

Almaraz, I., González, S.S., Pinos-Rodríguez, J.M., Miranda, L.A., 2009. Effects of exogenous fibrolytic enzymes on in sacco and in vitro degradation of diets and on growth performance of lambs. Ital. J. Anim. Sci. 9, 6–10. https://doi.org/10.4081/ijas.2010.e2.

Association of Official Analytical Chemists, 1995. Official Methods of Analysis of AOAC International, 16th e ed. AOAC International, Arlington, VA.

Awawdeh, M.S., 2011. Alternative feedstuffs and their effects on performance of Awassi sheep: a review. Trop. Anim. Health Prod. 43, 1297–1309. https://doi.org/10.1007/s11250-011-9851-z.

Awawdeh, M.S., Obeidat, B.S., 2013. Treated olive cake as a non-forage fiber source for growing Awassi lambs: effects on nutrient intake, rumen and urine pH, performance, and carcass yield. Asian-Australasian J. Anim. Sci. 26, 661–667. https://doi.org/10.5713/ajas.2012.12513.

Awawdeh, M.S., Obeidat, B.S., 2011. Effect of supplemental exogenous enzymes on performance of finishing Awassi lambs fed olive cake-containing diets. Livest. Sci. 138, 20–24. https://doi.org/10.1016/j.livsci.2010.11.021.

Bala, P., Malik, R., Srinivas, B., 2009. Effect of fortifying concentrate supplement with fibrolytic enzymes on nutrient utilization, milk yield and composition in lactating goats. Anim. Sci. J. 80, 265–272. https://doi.org/10.1111/j.1740-0929.2009.00634.x.

Beauchemin, K.A., Colombatto, D., Morgavi, D.P., Yang, W.Z., Rode, L.M., 2004. Mode of action of exogenous cell wall degrading enzymes for ruminants. Can. J. Anim. Sci. 84, 13–22. https://doi.org/10.4141/A02-102.

Beauchemin, K.A., Colombatto, D., Morgavi, D.P., Zang, W.Z., 2003. Use of exogenous fibrolytic enzymes to improve feed utilization by ruminants. J. Anim. Sci. 81, E37–E47. https://doi.org/10.2527/2003.8114_suppl_2E37x.

Beauchemin, K.A., Holtshausen, L., 2010. Development in enzyme usage in ruminants. In: Bedford, M.R., Partridge, G.G. (Eds.), Enzymes in Farm Animal Nutrition. CAB International, Wallingford, UK, pp. 206–230.

Beauchemin, K.A., Rode, L.M., Karren, D., 1999. Use of feed enzymes in feedlot finishing diets. Can. J. Anim. Sci. 79, 243–246. https://doi.org/10.4141/A98-124.

Beauchemin, K.A., Rode, L.M., Maekawa, M., Morgavi, D.P., Kampen, R., 2000. Evaluation of a nonstarch polysaccharidase feed enzyme in dairy cow diets. J. Dairy Sci. 83, 543–553. https://doi.org/10.3168/jds.S0022-0302(00)74914-9.

Benvenutti, M.A., Pichard, G.R., 2014. Fibrolysis in rumen mediated by a mixture of exogenous polysaccharidases. Anim. Feed Sci. Technol. 198, 130–140. https://doi.org/10.1016/j.anifeedsci.2014.10.009.

Bowman, G.R., Beauchemin, K.A., Shelford, J.A., 2003. Fibrolytic enzymes and parity effects on feeding behavior, salivation, and ruminal pH of lactating dairy cows. J. Dairy Sci. 86, 565–575. https://doi.org/10.3168/jds.S0022-0302(03)73635-2.

Boyd, J.W., 1984. The interpretation of serum biochemistry test results in domestic animals. Vet. Clin. Pathol. 13, 7–14. https://doi.org/10.1111/j.1939-165X.1984.tb00833.x.

Colombatto, D., Morgavi, D.P., Furtado, A.F., Beauchemin, K.A., 2003. Screening of exogenous enzymes for ruminant diets: relationship between biochemical characteristics and in vitro ruminal degradation. J. Anim. Sci. 81, 2628–2638. https://doi.org/10.2527/2003.81102628x.

Cruywagen, C.W., Goosen, L., 2004. Effect of an exogenous fibrolytic enzyme on growth rate, feed intake and feed conversion ratio in growing lambs. S. Afr. J. Anim. Sci. 34, 71–73.

Dawson, K.A., Tricarico, J.M., 1999. The use of exogenous fibrolytic enzyme to enhance microbial activities in the rumen and the performance of ruminant animal. In: Lyons, T.P., Jacques, K.A. (Eds.), Biotechnology in the Feed Industry: Proceedings of Alltech's 15th Annual Symposium. Nottingham University Press, Loughborough, Leics, UK. pp. 303–312.

Duncan, D.B., 1955. Multiple range and multiple F tests. Biometrics 11, 1–42. https://doi.org/10.2307/3001478.

El-Bordeny, N.E., Abedo, A.A., El-Sayed, H.M., Daoud, E.N., Soliman, H.S., Mahmoud, A.E.M., 2015. Effect of exogenous fibrolytic enzyme application on productive response of dairy cows at different lactation stages. Asian J. Anim. Vet. Adv. 10, 226–236. https://doi.org/10.3923/ajava.2015.226.236.

Eun, J.-S., Beauchemin, K.A., Schulze, H., 2007. Use of exogenous fibrolytic enzymes to enhance in vitro fermentation of alfalfa hay and corn silage. J. Dairy Sci. 90, 1440–1451. https://doi.org/10.3168/jds.S0022-0302(07)71629-6.

European Parliament and Council Directive, 2010. Directive 2010/63/EU of 22 September 2010 on the protection of animals used for scientific purposes. Off. J. Eur. Union L 276, 33–79.

Fielder, S.E., 2016. Serum Biochemical Reference Ranges [WWW Document]. MSD Vet. Manual; Merck Co., Inc., Kenilworth, NJ, USA. URL https://www.msdvetmanual.com/special-subjects/reference-guides/serum-biochemical-reference-ranges# (Accessed 11.1.19).

Gandra, J.R., Miranda, G.A., Goes, R.H.T.B., Takiya, C.S., Del Valle, T.A., Oliveira, E.R., Freitas Junior, J.E., Gandra, E.R.S., Araki, H.M.C., Santos, A.L.A.V., 2017. Fibrolytic enzyme supplementation through ruminal bolus on eating behavior, nutrient digestibility and ruminal fermentation in Jersey heifers fed either corn silage- or sugarcane silage-based diets. Anim. Feed Sci. Technol. 231, 29–37. https://doi.org/10.1016/j.anifeedsci.2017.06.009.

GfE, 1995. Mitteilungen des Ausschusses für Bedarfsnormen der Gesellschaft für Ernährungsphysiologie: Zur Energiebewertung beim Wiederkäuer. Proc. Soc. Nutr. Physiol. 4, 121–123.

Heuzé, V., Tran, G., Gómez Cabrera, A., Lebas, F., 2015. Feedipedia, a Programme by INRA, CIRAD, AFZ and FAO: Olive Oil Cake and By-products [WWW Document].

URL https://www.feedipedia.org/node/32 (Accessed 4.15.19). .
Holtshausen, L., Chung, Y.-H., Gerardo-Cuervo, H., Oba, M., Beauchemin, K.A., 2011. Improved milk production efficiency in early lactation dairy cattle with dietary addition of a developmental fibrolytic enzyme additive. J. Dairy Sci. 94, 899–907. https://doi.org/10.3168/jds.2010-3573.
Kholif, A.M., Aziz, H.A., 2014. Influence of feeding cellulytic enzymes on performance, digestibility and ruminal fermentation in goats. Anim. Nutr. Feed Technol. 14, 121–136.
Krueger, N.A., Adesogan, A.T., 2008. Effects of different mixtures of fibrolytic enzymes on digestion and fermentation of bahiagrass hay. Anim. Feed Sci. Technol. 145, 84–94. https://doi.org/10.1016/j.anifeedsci.2007.05.041.
Kung, L., Treacher, R.J., Nauman, G.A., Smagala, A.M., Endres, K.M., Cohen, M.A., 2000. The effect of treating forages with fibrolytic enzymes on its nutritive value and lactation performance of dairy cows. J. Dairy Sci. 83, 115–122. https://doi.org/10.3168/jds.S0022-0302(00)74862-4.
López-Aguirre, D., Hernández-Meléndez, J., Rojo, R., Sánchez-Dávila, F., López-Villalobos, N., Salem, A.Z.M., Martínez-González, J.C., Vázquez-Armijo, J.F., Ruíz, S., 2016. Effects of exogenous enzymes and application method on nutrient intake, digestibility and growth performance of Pelibuey lambs. Springerplus 5, 1399. https://doi.org/10.1186/s40064-016-3075-7.
Martin, S.A., Nisbet, D.J., 1992. Effect ofdirect-fed microbials on rumen microbial fermentation. J. Dairy Sci. 75, 1736–1744. https://doi.org/10.3168/jds.S0022-0302(92)77932-6.
McAllister, T.A., Oosting, S.J., Popp, J.D., Mir, Z., Yanke, L.J., Hristov, A.N., Treacher, R.J., Cheng, K.-J., 1999. Effect of exogenous enzymes on digestibility of barley silage and growth performance of feedlot cattle. Can. J. Anim. Sci. 79, 353–360. https://doi.org/10.4141/A98-099.
Meale, S.J., Beauchemin, K.A., Hristov, A.N., Chaves, A.V., McAllister, T.A., 2014. Board-invited review: Opportunities and challenges in using exogenous enzymes to improve ruminant production. J. Anim. Sci. 92, 427–442. https://doi.org/10.2527/jas.2013-6869.
Mendoza, G.D., Loera-Corral, O., Plata-Pérez, F.X., Hernández-García, P.A., Ramírez-Mella, M., 2014. Considerations on the use of exogenous fibrolytic enzymes to improve forage utilization. Sci. World J. 2014, 1–9. https://doi.org/10.1155/2014/247437.
Mohamed, D.E.-D.A., Borhami, B.E., El-Shazly, K.A., Sallam, S.M.A., 2013. Effect of dietary supplementation with fibrolytic enzymes on the productive performance of early lactating dairy cows. J. Agric. Sci. 5, 146–155. https://doi.org/10.5539/jas.v5n6p146.
Molina-Alcaide, E., Yáñez-Ruiz, D.R., 2008. Potential use of olive by-products in ruminant feeding: a review. Anim. Feed Sci. Technol. 147, 247–264. https://doi.org/10.1016/j.anifeedsci.2007.09.021.
Molina Alcaide, E., Nefzaoui, A., 1996. Recycling of olive oil by-products: possibilities of utilization in animal nutrition. Int. Biodeterior. Biodegrad. 38, 227–235. https://doi.org/10.1016/S0964-8305(96)00055-8.
Morgavi, D.P., Beauchemin, K.A., Nsereko, V.L., Rode, L.M., McAllister, T.A., Wang, Y., 2004. Trichoderma enzymes promote Fibrobacter succinogenes S85 adhesion to, and degradation of, complex substrates but not pure cellulose. J. Sci. Food Agric. 84, 1083–1090. https://doi.org/10.1002/jsfa.1790.
Morsy, T.A., Kholif, A.E., Kholif, S.M., Kholif, A.M., Sun, X., Salem, A.Z.M., 2016. Effects of two enzyme feed additives on digestion and milk production in lactating Egyptian buffaloes. Ann. Anim. Sci. 16, 209–222. https://doi.org/10.1515/aoas-2015-0039.
Nocek, J.E., Hart, S.P., Polan, C.E., 1987. Rumen ammonia concentration as influenced by storage time, freezing and thawing, acid preservative, and method of ammonia determination. J. Dairy Sci. 70, 601–607. https://doi.org/10.3168/jds.S0022-0302(87)80047-4.
Nowak, W., Kruczynska, H., Grochowska, S., 2003. The effect of fibrolytic enzymes on dry matter, ADF and NDF ruminal disappearance and intestinal digestibility. Czech J. Anim. Sci. 48, 191–196.
Nsereko, V.L., Morgavi, D.P., Rode, L.M., Beauchemin, K.A., McAllister, T.A., 2000. Effects of fungal enzyme preparations on hydrolysis and subsequent degradation of alfalfa hay fiber by mixed rumen microorganisms in vitro. Anim. Feed Sci. Technol. 88, 153–170. https://doi.org/10.1016/S0377-8401(00)00225-X.
Obeidat, B.S., 2017. The effects of feeding olive cake and Saccharomyces cerevisiae supplementation on performance, nutrient digestibility and blood metabolites of Awassi lambs. Anim. Feed Sci. Technol. 231, 131–137. https://doi.org/10.1016/j.anifeedsci.2017.07.006.
Peters, A., Meyer, U., Dänicke, S., 2015. Effect of exogenous fibrolytic enzymes on performance and blood profile in early and mid-lactation Holstein cows. Anim. Nutr. 1, 229–238. https://doi.org/10.1016/j.aninu.2015.09.001.
Pinos-Rodríguez, J.M., González, S.S., Mendoza, G.D., Bárcena, R., Cobos, M.A., Hernández, A., Ortega, M.E., 2002. Effect of exogenous fibrolytic enzyme on ruminal fermentation and digestibility of alfalfa and rye-grass hay fed to lambs. J. Anim. Sci. 80, 3016–3020. https://doi.org/10.2527/2002.80113016x.
Ribeiro, G.O., Badhan, A., Huang, J., Beauchemin, K.A., Yang, W., Wang, Y., Tsang, A., McAllister, T.A., 2018. New recombinant fibrolytic enzymes for improved in vitro ruminal fiber degradability of barley straw. J. Anim. Sci. 96, 3928–3942. https://doi.org/10.1093/jas/sky251.
Rode, L.M., Yang, W.Z., Beauchemin, K.A., 1999. Fibrolytic enzyme supplements for dairy cows in early lactation. J. Dairy Sci. 82, 2121–2126. https://doi.org/10.3168/jds.S0022-0302(99)75455-X.
Salem, A.Z.M., Gado, H.M., Colombatto, D., Elghandour, M.M.Y., 2013. Effects of exogenous enzymes on nutrient digestibility, ruminal fermentation and growth performance in beef steers. Livest. Sci. 154, 69–73. https://doi.org/10.1016/j.livsci.2013.02.014.
SAS Institute Inc, 2011. SAS/STAT® 9.3 User's Guide. Cary, NC, USA.
Schingoethe, D.J., Stegeman, G.A., Treacher, R.J., 1999. Response of lactating dairy cows to a cellulase and xylanase enzyme mixture applied to forages at the time of feeding. J. Dairy Sci. 82, 996–1003. https://doi.org/10.3168/jds.S0022-0302(99)75319-1.
Titi, H.H., Tabbaa, M.J., 2004. Efficacy of exogenous cellulase on digestibility in lambs and growth of dairy calves. Livest. Prod. Sci. 87, 207–214. https://doi.org/10.1016/j.livprodsci.2003.07.012.
Torres, N., Mendoza, G.D., Bárcena, R., Loera, O., González, S., Aranda, E., Hernández, P.A., Crosby, M., 2013. Effects of various fibrolytic enzyme extracts on digestibility and productive performance of lambs fed a forage-based diet. Anim. Nutr. Feed Technol. 13, 381–389.
Vallejo, L.H., Salem, A.Z.M., Camacho, L.M., Kholif, A.M., Mariezcurrena, M.D., Cipriano, M., Alonso, M.U., Olivares, J., López, S., 2016. Effects of xylanase supplementation on feed intake, digestibility and ruminal fermentation in Rambouillet sheep. J. Agric. Sci. 154, 1110–1117. https://doi.org/10.1017/S0021859616000216.
Van Soest, P.J., Robertson, J.B., Lewis, B.A., 1991. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. J. Dairy Sci. 74, 3583–3597. https://doi.org/10.3168/jds.S0022-0302(91)78551-2.
Varga, G.A., Kolver, E.S., 1997. Microbial and animal limitations to fiber digestion and utilization. J. Nutr. 127, 819S–823S. https://doi.org/10.1093/jn/127.5.819S.
Vicini, J.L., Bateman, H.G., Bhat, M.K., Clark, J.H., Erdman, R.A., Phipps, R.H., Van Amburgh, M.E., Hartnell, G.F., Hintz, R.L., Hard, D.L., 2003. Effect of feeding supplemental fibrolytic enzymes or soluble sugars with malic acid on milk production. J. Dairy Sci. 86, 576–585. https://doi.org/10.3168/jds.S0022-0302(03)73636-4.
Wang, S., Terranova, M., Kreuzer, M., Marquardt, S., Eggerschwiler, L., Schwarm, A., 2018. Supplementation of pelleted hazel (Corylus avellana) leaves decreases methane and urinary nitrogen emissions by sheep at unchanged forage intake. Sci. Rep. 8, 5427. https://doi.org/10.1038/s41598-018-23572-3.
Wilkins, R.J., 2000. Forage and their role in animal systems. In: Givens, D.I., Owen, E., Axford, R.F.E., Omed, H.M. (Eds.), Forage Evaluation in Ruminant Nutrition. CAB International, Wallingford, UK, pp. 1–14.
Wood, T.M., Bhat, K.M., 1988. Methods for measuring cellulase activities. Methods Enzymol. 160, 87–112. https://doi.org/10.1016/0076-6879(88)60109-1.
Yang, W.Z., Beauchemin, K.A., Rode, L.M., 1999. Effects of an enzyme feed additive on extent of digestion and milk production of lactating dairy cows. J. Dairy Sci. 82, 391–403. https://doi.org/10.3168/jds.S0022-0302(99)75245-8.
Zhao, M.D., Di, L.F., Tang, Z.Y., Jiang, W., Li, C.Y., 2019. Effect of tannins and cellulase on growth performance, nutrients digestibility, blood profiles, intestinal morphology and carcass characteristics in Hu sheep. Asian-Australasian J. Anim. Sci. 32, 1540–1547. https://doi.org/10.5713/ajas.18.0901.