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Research Article

The Effects of Exogenous Fibrolytic Enzymes and a Ferulic Acid Esterase-Producing Inoculant Treatment on Digestibility and Conservation Characteristics of Corn Stover [1]

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Abstract

The objective of the current study was to determine the effects of a mixed bacterial inoculant possessing ferulic acid esterase (FAE) activity and exogenous fibrolytic enzyme (EFEs) products on the chemical composition, conservation characteristics and digestibility of corn stover. The moisture level of corn stover was adjusted to 40% with deionized water and then treated with deionized water (control), EFEs (10 U cellulase + 60 U xylanase units g^{-1} of substrate dry matter) or EFEs + FAE inoculant (FAEI) ($1.3x10^5$ cfu g^{-1} fresh forage). The treated stover was then incubated in laboratory mini-silos for 30 days. After the incubation period, the corn stover treated with both EFEs and EFEs + FAEI had a lower pH, ADF and NDF (P<0.001), and higher acetic acid and lactic acid (P<0.001) than the control stover. In addition, moulds and yeasts were inhibited in stover treated with EFEs + FAEI. The in vitro true dry matter digestibility (IVTDMD) and *in vitro* true neutral detergent digestibility (IVTNDFD) of the stover treated with EFEs and EFEs + FAEI were higher than for the control (P<0.001) but there was no significant difference between the EFEs and EFEs + FAEI treatments. From economical point of view, the best treatment was EFEs + FAEI. These results suggest that EFEs played a major role in enhancing the digestibility of corn stover, alone or in combination with FAEI. Moreover the use of EFEs + FAEI promoted a positive response in the conservation characteristics.

Keywords: *Exogenous fibrolytic enzyme, Ferulic acid esterase producing inoculant, Corn stover, In vitro digestibility*

Eksojen Fibrolitik Enzim ve Ferulik Asit Esteraz Üreten Bakteriyal İnokulant Muamelesinin Mısır Samanının Sindirilebilirliği ve Konservasyon Özellikleri Üzerine Etkileri

Öz

Bu çalışmanın amacı, ferulik asit esteraz üreten bakteriyel inokulant (FAEI) ve ekzojen fibrolitik enzimlerin (EFEs) mısır samanının kimyasal kompozisyonu, konservasyon özellikleri ve sindirilebilirliği üzerine olan etkisini araştırmaktır. Mısır samanının nem içeriği deiyonize su ile %40'a ayarlandı ve daha sonra deionize su (kontrol), EFE's (10 U cellulase + 60 U xylanase units g^{-1} of substrate kuru maddesi) ve EFEs + FAEI ($1.3x10^5$ cfu g^{-1} taze kaba yem) ile muamele edildi. Muamele edilen mısır samanı, 30 gün süreyle laboratuvar mini-silolarında inkube edildi. İnkubasyon periyotundan sonra, EFEs ve EFEs + FAEI ile muamele edilen mısır samanının ADF, NDF miktarı ve pH kontrole kıyasla daha düşük (P<0.001), asetik asit ve laktik asit içerikleri daha yüksek (P<0.05) belirlendi. Buna ek olarak EFEs + FAEI ile muamele edilmiş mısır samanında maya ve küf tespit edilmedi. EFEs ve EFEs + FAEI ile üretilen mısır samanında in vitro gerçek kuru madde sindirilebilirliği (IVTDMD) ve *in vitro* gerçek nötral deterjan sindirilebilirliği (IVTNDFD) kontrolden daha fazla bulundu (P<0.001), fakat EFEs ve EFEs + FAEI arasında bir fark saptanmadı. Ekonomik bakımdan en iyi sonuç, EFES + FAEI grubundan elde edildi. Bu sonuçlar, mısır samanının EFEs'nin tek başına veya FAEI ile kombine edilmesinin mısır samanın sindirilebilirliğinin artırılmasında önemli bir rol oynadığını göstermiştir. Dahası, EFEs + FAEI kullanımı konservasyon özelliklerini olumlu etkilemiştir.

Anahtar sözcükler: *Eksojen fibrolitik enzimler, Ferulik asit esteraz üreten bakteriyal inoculant, Mısır samanı, In vitro sindirilebilirlik*

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INTRODUCTION

In Turkey, corn is planted on approximately 680.000 ha, which is approximately 5% of the land used for cereal crops, and corn stover production can reach more than six million tons per year [1]. Despite the abundant production of stover, the low digestion rate of cell wall material by rumen microbes is one of the main problems associated with its use as a feed for ruminants. Therefore, the issue of improvement of its digestibility has received considerable critical attention. Several chemical treatments have been long known to be effective [2]. However, despite these efforts, more than 50% of fiber is still not digested.

Recently, research on the subject has focused on the use of exogenous fibrolytic enzymes (EFEs) such as cellulase and xylanase to improve the nutritive value of low quality forage [3]. Several *in vitro* studies have demonstrated an increase in the fiber digestibility value and complex polysaccharide degradability when corn stover was treated with EFEs [4,5]. Compared with only cellulase or xylanase, the combined application of these enzymes was more effective due to synergism [6]. Moreover, the application of EFEs to forage prior to storage tended to produce a better response via a longer period of interaction between the enzymes and forage than application prior to feeding [7]. However, the pre-storage use of EFEs on forage with a high fiber content has been less well explored [8]. In one study, Mendoza et al.[9] reported that lower forage quality limits the effects of EFEs.

The extent of cell wall digestion is mainly determined by ferulic acid which is cross-linked to polysaccharides by ester bonds and to lignin mainly by ether bonds [10,11]. Other studies have shown that complete or partial hydrolysis of ferulic acid linkages in forage may directly increase the susceptibility of cell walls to ruminal digestion [12,13]. In addition, recent studies have showed that the degradation of ferulic acid linkages by athird-generation ferulic acid esterase producing bacterial inoculant (FAEI), namely *Lactobacillus buchneri*, may have the potential to partially break the ester linkage between ferulic acid and polysaccharides, thereby improving fiber digestibility [14,15]. However, more recent attention has focused on the use of EFEs in combination with FAEI as a potentially effective way to break down the cross linkages in carbohydrates incorporated in plant structural material [16,17]. A considerable amount of literature has been published on EFEs and FAEI [7,16,18]. Most of these studies have only focused on medium to good quality forages [7,16,17]. However, to date, no studies have been published on the effects of EFEs + FAEI as additives on the nutritive value of poor quality forages such as corn stover. Therefore, the principal objective of this study was to investigate the effects of the EFEs, cellulase and xylanase, alone or in combination with a mixed lactic acid bacteria inoculant containing FAE-producing *L. buchneri* on the chemical composition, fermentation and microbiological activity, and *in vitro* digestibility of corn stover.

MATERIAL and METHODS

Crop and Residue Collection

The stover from corn *(Zea mays indurata)* was collected from a farm in the Bafra district of Samsun Province, Turkey in September 2017. After the harvesting of the central rows of the field at 30 cm above ground level, samples of the whole corn stover (leaf, husk, and stalk) were sundried and then chopped into about 30 mm lengths with a straw chopper.

Preparation and Treatment

Deionized water was sprayed on the stover to achieve a final moisture level of approximately 40% of the fresh stover weight. The stover treatments were: 1) control (deionized water, no additives), 2) EFEs and 3) EFEs + FAEI. To ensure a homogeneous distribution, the EFEs and FAEI were dissolved in deionized water and then immediately sprayed on the stover in a 5 min period while it was mixed manually. The treated stover was packed into 1 L laboratory mini-silos (glass jars) which were then sealed, with five replications for each treatment. The silos were stored at room temperature (22ºC) for 30 d.

The EFEs used in the study were a mixture of cellulase and xylanase (Biovet, Bursa, Turkey). The declared enzymatic activities are 7300 IU of cellulose g^{-1} and 45000 IU g^{-1}. The combination of EFEs was applied (10 cellulase units/g of substrate DM + 60 xylanase units/g of substrate DM) according to the methodology of Zhao et al.[19], either alone or in combination with FAEI 11 GFT (Pioneer Hi-Bred, Int., Inc., USA). The FAEI (11GFT: 1.0 x 10^{11} cfu g^{-1} of *Lactobacillus buchneri* LN4017) applied to produce ferulic acid esterase, 2.0 x 10^{10} cfu g^{-1} of *Lactobacillus plantarum* LP7109 and 1.0 x 10^{10} cfu g^{-1} of *Lactobacillus casei* LC3200 were applied at the dosage recommended by the manufacturer, to achieve 1.3 x 10^{5} cfu g^{-1} fresh forage.

Chemical Analysis

After the incubation period, the silos were opened and the topmost spoiled portion of the corn stover was discarded. To determine the pH, 25 g of corn stover from each replicate was blended with 100 mL of distilled water for 10 min [20]. The homogenate was filtered through two layers of cheesecloth and the pH was immediately determined with a digital pH meter (Thermo Orion 710 A+, Thermo Electron Corporation). Two-three drops of toluene were added to approximately 10 mL of sample to prevent fermentation. The samples were stored at -20ºC until they were analysed. The acetic acid and butyric acid contents were determined according to Filipek and Dvarok [21] and the lactic acid content was measured according to the method described by Zhang et al.[22].

The stover samples were oven dried at 60°C for 48 h to achieve constant weight and the dried samples were milled through a 1 mm screen and preserved in labelled plastic containers before chemical analysis and *in vitro* digestion. Pre- and post-storage stovers were analyzed for their ADF, NDF (with alfa-amylase and sodium sulfite) and acid detergent lignin (ADL) contents, according to the methodology of Van Soest et al.[23] in an Ankom$^{200/220}$ Fiber Analyzer. Standard methods were used to determine the organic matter (OM) and ash contents [24].

Microbiological Analysis

When the silos were opened, samples of each treatment were taken for the determination of yeast and mould status. A composite sample (400 g) was taken using sterile gloves and polyethylene bags and sent directly to a laboratory (Samsun Food Control Laboratory Directorate, Turkey). The identities of yeasts and moulds were determined according to ISO 21527-2 criteria [25] and the microbial counts were log10 transformed.

In Vitro Digestibility

In vitro true digestibility (IVTD) was determined with an Ankom Daisy^{II} incubator (Ankom Technology Corp., Fairport, NY, USA) [26], with the unit consisting of a thermostatic chamber (39°C) and 4 rotating jars with a capacity of 2 L each, using an approximately 0.5 g sample of corn stover incubated for 48 h. The reagents, filter bags and samples were prepared according to the procedure specified for the Ankom Daisy^{II} *in vitro* system. The buffer solution consisted of 1.330 mL of buffer A (KH_2PO_4, 10.0 g/L; $MgSO_4.7H_2O$, 0.5 g/L; $CaCl_2{\cdot}2H_2O$, 0.1 g/L and urea, 0.5 g/L; and 266 mL of buffer B (Na_2CO_3, 15.0 g/L and $Na_2S.9H_2O$, 1.0 g/L), mixed in each digestion jar and the pH was adjusted to 6.8. In the analysis, F57 filter bags were used for incubation of the stover. The bags were rinsed with acetone for 3 min and the rinsed bags were then oven dried for 8 h at 60ºC. Rumen contents were collected from different sites within the rumen of two donor cows slaughtered in a private slaughterhouse in Samsun Province. The animals were fed with total mixed ration (TMR) as a basal diet. A basal diet (% dry matter [DM]) consisted of corn (38.2%), sun flowe meal (22.5%), barley (23.0%), wheat straw (12.5%), limestone (1.4%), dicalcium phosphate (1.4%), salt (0.8%) and vitamin-mineral premix (0.2%). The rumen contents were transported immediately to the laboratory. They were then strained through four layers of cheesecloth and held at 39ºC under a CO_2 atmosphere. The stover samples (0.5±0.01 g) were weighed into the four bags per treatment. The F57 filter bags were placed in the digestion jars filled with 1.596 mL (Buffer A: 1.330 mL + Buffer B: 266 mL) of buffer solution and 400 mL of rumen fluid. After 48 h of incubation, the bags were removed from the jars, and washed four times under running cold water. The bags were then placed in the Ankom$^{2000/220}$ Fiber Analyzer and the manufacturer's procedure for determining the neutral detergent fiber (NDF) amount was followed. The IVTD was calculated as the difference between the DM of the incubated material and the residue after neutral detergent treatment [23]. The IVTNDFD was calculated as the difference between the amount of fiber incubated and the amount recovered after the NDF analysis [26].

Economic Evaluation

According to Oba and Allen [27], 1 unit enhanced in forage NDFD *in vitro* was positively associated with 0.17 kg of dry matter intake (DMI) and 0.25 kg of 4% fat corrected milk yield. Therefore, economical return of the IVTNDFD was calculated for milk production. Price of 1 kg of enzyme and 25 gr of inoculant was 75.00 TRY and 308.00 TRY, respectively. 1 kg of 4% fat corrected milk was 2.10 TRY in year 2019. 1 US Dollar was valued at about 5.82 TRY in April, 2019.

Statistical Analysis

Firstly, the Shapiro-Wilk test was used to determine whether the population was normally distributed. Data management and analysis were carried out by repeated measurements. The difference between means was compared with Tukey's comparison test, with significance assumed for P values <0.05. Analyses were carried out by using IBM SPPSS V21.0 software (IBM Cooperation, Chicago, IL, USA) [28].

RESULTS

The pre-storage stover had high ADF, NDF and ADL contents (75.39, 51.34 and 12.41% respectively) and low IVTD and IVTNDF (55.06 and 37.02%, respectively) *(Table 1)*.

Table 2 displays the results of post-storage chemical composition of corn stover treated with fibrolytic enzymes and inoculant. The ADF and NDF contents were highly significantly (P<0.001) lower in the EFEs and EFEs + FAEI-treated stover than in the controls. However, none of the additives had a significant effect on the ADL content.

The EFEs + FAEI group had a significant (P<0.001) effect on the pH, lactic acid concentration, acetic acid concentration

Table 1. *Nutrient contents and in vitro dry matter digestibility of corn stover in pre-storage*

Item	Pre-storage Corn Stover (±SD)[1]
NDF[2]	75.39±0.34
ADF[2]	51.34±0.52
ADL[2]	12.41±0.10
IVTD[3]	55.06±0.37
IVTNDFD[3]	37.02±0.69

*[1] Means ± SD (n = 3) for corn stover samples collected before storage; [2] **NDF:** neutral detergent fibre; **ADF:** acid detergent fibre; **ADL:** acid detergent lignin (% dry matter); [3] **IVTD:** in vitro true digestibility, **IVTNDFD:** in vitro true neutral detergent digestibility*

and number of yeasts and moulds, compared with the other two groups *(Table 3)*. After the application of EFEs + FAEI, the pH level (4.69) declined and the concentration of lactic acid and acetic acid increased (1.71 and 2.02 g kg^{-1} DM, respectively). Moreover, moulds and yeasts were not detected in stover treated with EFEs + FAEI whereas appreciable numbers of yeasts and moulds were detected in the control and the EFEs.

After 48 h of incubation in the Ankom Daisy^{II} incubator, values of means for *in vitro* IVTD and IVNDFD of corn stover treated with EFEs and FAEI are shown in *Table 4*. The IVTD and IVTNDFD were significantly higher (P<0.001) for the EFEs treatment by 3.83 and 5.16 unit respectively, and EFEs + FAEI by 4.45 and 6.08 unit, respectively, compared to the control (data not shown in the table). However no difference (P>0.05) was found between EFEs and EFEs + FAEI treatments.

Regarding the economic evaluation *(Table 5)*, results indicated that the net profit for EFEs and EFEs + FAEI inclusion in corn stover was higher 2.63 and 3.10 TRY than control, respectively. Best net revenue was recorded for supplemented with EFEs + FAEI.

DISCUSSION

In this study, the NDF, ADF and ADL concentrations before storage of whole corn stover were were lower than the concentrations reported (51.34-55.19, 75.39-89.23 and 12.41-34.04% of DM basis, respectively) by Bhasker et al.[29] and higher than those reported by Cui et al.[30] and Zhao

Table 2. *Post-storage chemical composition of corn stover treated with fibrolytic enzymes and inoculant*

Item	Experimental Groups			SEM[3]	P-value
	Control	EFEs[1]	EFEs + FAEI[1]		
NDF[2]	69.65[a]	64.86[b]	65.04[b]	0.573	<0.001
ADF[2]	46.28[a]	42.92[b]	43.15[b]	0.211	<0.001
ADL[2]	12.10	12.24	12.16	0.222	0.840
Ash	9.20	9.33	9.45	0.167	0.372

*[1] **EFEs:** exogenous fibrolytic enzymes, **FAEI:** ferulic acid esterase-producing inoculant (1.0×10^{11} cfu/g of L. buchneri LN4017, 2.0×10^{10} cfu/g of L. plantarum LP7109, and 1.0×10^{10} cfu/g of L. casei LC3200); [2] **NDF:** neutral detergent fibre; **ADF:** acid detergent fibre; **ADL:** acid detergent lignin (% of dry matter); [3] Standard error of the mean, [a,b] Means (n =5) within a row with different superscripts differ significantly (P<0.001)*

Table 3. *Storage fermentation characteristics and microbial counts of corn stover treated with enzymes and inoculant*

Item	Experimental Groups			SEM[3]	P-value
	Control	EFEs[1]	EFEs + FAEI[1]		
pH	5.43[a]	5.01[b]	4.69[c]	0.043	<0.001
Lactic acid[2]	0.31[c]	0.84[b]	1.70[a]	0.167	<0.001
Acetic acid[2]	0.55[c]	0.84[b]	2.024[a]	0.177	<0.001
Butyric acid[2]	ND	ND	ND		
Microbiology, log_{10} cfu/g of fresh material					
Yeast	5.04[a]	2.3[b]	ND	0.732	<0.001
Mould	3.93[a]	2.90[b]	ND	0.325	<0.001

*[1] **EFEs:** exogenous fibrolytic enzymes, **FAEI:** ferulic acid esterase-producing inoculant (1.0×10^{11} cfu/g of L. buchneri LN4017, 2.0×10^{10} cfu/g of L. plantarum LP7109, and 1.0×10^{10} cfu/g of L. casei LC3200); [2] g kg^{-1} dry matter; [3] Standard error of the mean; [a,b,c] Means (n =5) within a row with different superscripts differ significantly (P<0.001); **ND:** none detected*

Table 4. *In vitro digestibility of corn stover treated with enzymes and inoculant*

Item	Experimental Groups			SEM[3]	P-value
	Control	EFEs[1]	EFEs + FAEI[1]		
IVTD[2]	59.87[b]	63.70[a]	64.32[a]	0.757	<0.001
IVTNDFD[2]	38.54[b]	43.70[a]	44.62[a]	0.968	<0.001

*[1] **EFEs:** exogenous fibrolytic enzymes, FAEI: ferulic acid esterase-producing inoculant (1.0×10^{11} cfu/g of L. buchneri LN4017, 2.0×10^{10} cfu/g of L. plantarum LP7109, and 1.0×10^{10} cfu/g of L. casei LC3200); [2] **IVTD:** in vitro true digestibility, IVTNDFD: in vitro true neutral detergent digestibility (% of dry matter); [3] Standard error of the mean; [a,b] Means (n =5) within a row with different superscripts differ significantly (P<0.001)*

Table 5. *Economic analysis of milk produced in terms of differences in IVTNDFD*

Item	Experimental Groups		
	Control	EFEs[1]	EFEs + FAEI[1]
Enzymes cost[2], TRY	0	0.08	0.08
Inoculant cost[3], TRY	0	0	0.008
Increase in IVTNDFD[4], unit	1 (38.54%)	5.16 (38.54% vs 43.70%)	6.08 (38.54% vs 44.62%)
Milk yield (4% FMC)[5], kg/day	0.25	1.29	1.52
Milk price[6], TRY/kg	2.10	2.10	2.10
Total profit, TRY/day	0.52	2.71	3.19
Net profit, TRY/day	0.0.52	2.63	3.10

*[1] **EFEs:** exogenous fibrolytic enzymes, **FAEI:** ferulic acid esterase-producing inoculant (1.0×10^{11} cfu/g of L. buchneri LN4017, 2.0×10^{10} cfu/g of L. plantarum LP7109, and 1.0×10^{10} cfu/g of L. casei LC3200); [2] Price of 1 kg of enzymes: 75.00 TRY, [3] price of 25 g of inoculant: 308.00 TRY; [4] Differences in in vitro true neutral detergent digestibility (IVTNDFD, % of dry matter) between control and treatments; [5] One-unit of enhanced NDFdigestibility was positively associated with 0.25 kg of 4% fat corrected milk yield [27]; [6] Price of 1 kilogram of 4% fat corrected milk: 2.10 TRY*

et al.[19]. In addition, the IVTD and IVNDFD of corn stover in the present study was higher than that of Zahao et al.[19] These different results are possibly resulted from different physical structures and chemical composition of various corn stover fractions (leaf, husk and stalk) [19].

In the current study, after 30 d of storage EFEs and EFEs + FAEI treatments had lower ADF and NDF contents than control. ADF and ADF concentration of corn stover produced using EFEs primarily reflects the hydrolysis of cell wall carbohydrates, and is in accord with previous studies by Ni et al.[31] and Sun et al.[32] who reported reduced NDF concentration of wheat straw silages produced using a cellulase additive. In contrast, Lync et al.[18] and Coblentz and Hoffman [33] showed that alfalfa hay produced using enzyme products generally had greater NDF concentration than the untreated hay. On the other hand, Lynch et al.[7] showed that the use of fibrolytic enzyme product at ensiling did not affect chemical composition of alfalfa haylage. With respect to chemical composition of corn stover stored in current study, no statistically significant difference was observed between EFEs and EFEs + FAEI. These results are likely to be releated to associated with inadequate breaking of the cross linking bonds in the cell wall by FAEI. In accordance with the present results, Lynch et al.[7] have demonstrated that the use of FAEI with EFEs did not forward the effects of fibrolytic-enzyme treatments.

pH values reflected a good silage conservation [34] and changes in the end pruducts of fermentation [14]. In this study, after incubation for 30 days, even if the pH values of all corn stovers with additive were not below 4.69, they were well preserved. It is attributed from this critical pH value varies with DM content [35]. Liu et al.[36] have found that stylo silage was well preserved at high pH of 5.0 when the DM was 542.9 g kg^{-1}. The DM level of corn strover in this study was approximately 600 g kg^{-1} which resulted in the pH at the level of 4.69 and 5.01. The lower pH (4.69) of corn strover produced using EFEs in combination with FAEI compared with corn stover produced using EFEs alone and control reflects the tendency for increased lactic acid production during storage. This result may be explained by the fact that the *L. plantarum* and *L. casei* populations included in the FAEI inoculant are commonly used as silage additives to increase lactic acid production [37] and/or the breakdown of cell walls by EFEs during incubation. The results also shown that EFEs alone was increased lactic acid and acetic acid concentrations compared to control. It may be that addition of EFEs increased WSC available for LAB from NDF degradation, and that propagation of LAB could be promoted in the stage of storage, which resulted in an increase in lactic acid [38]. The same positive effects of EFEs and EFEs + FAEI have been reported in other research [7,39]. However, Lynch et al.[18] found no effect on the pH and lactic acid content of alfalfa hay treated with EFEs + FAEI at baling compared to fibrolytic enzymes. Another important finding of the present study was that acetic acid production increased significantly in the corn stover treated with EFEs + FAEI compared to the other groups, suggesting that the *L. buchneri* component of FAEI may have converted lactic acid to acetic acid and 1,2-propanediol [40,41]. Similar effects were reported by Kang et al.[12] and Schmidt et al.[42] who treated alfalfa silage with *L. buchneri*. However, this finding contradicts that of Lynch et al.[17] who reported that the acetic acid concentration was unaffected by FAEI in combination with fibrolytic enzymes. These variations could be attributed to the differences in chemical composition of the stored material, seasonal conditions and method of storage.

Yeasts can create conditions favourable for the development of moulds and fungi, as well as other undesirable micro-organisms, which results in losses of organic matter. In the present study, the number of yeasts and moulds was not determined in EFEs + FAEI treatment compared with EFEs treatment and the control. The strain of *L. buchneri* found in the inoculant used in the present study is known to produce acetic acid which has strong antifungal properties [43]. In the present study, an increase in acetic acid production

in the EFEs + FAEI treatment led to the inhibition of yeast and mould multiplication. These findings are in agreement with those of Filya [41] and Muck [44]. Stored corn stover is susceptible to aerobic deterioration, causing loss of dry matter and the development of toxic substances [45]. Therefore, the findings of the present study could be useful in reducing the rate of aerobic deterioration during the storage of corn stover.

In vitro true digestibility and IVTNDFD are an important parameter of evaluating the digestion of forage [46]. Oba and Allen [27] reported that a one-unit of enhanced NDF digestibility *in vitro* was associated with 0.17 of dry matter intake, 0.23 kg of milk yield, and 0.25 kg of 4% fat-corrected milk yield. Therefore, the digestibility of DM and NDF were used to be the first parameter in this study. Results of the present study showed that treatment with EFEs and EFEs + FAEI inreased the amounts of IVTMD and IVNDFD in corn stover compared with the control. These results are likely to be related to fragile and susceptible to ruminal degradation of structural polysaccharides of corn stover during storage with EFEs and EFEs + FAEI groups [7,14]. In accordance with the present result, previous studies have demonstrated that in increase IVTDMD [4,29,47] and IVTNDFD [18,48] with the use of EFEs. In a study investigating *in vitro* DM and NDF digestibility of corn strover, Zhao et al.[19] reported that combination of 10 U/g DM of cellulose with 60 U/g DM of xylanase were screened out based on higher digestibility of DM and NDF, 49.3 and 37.7%, respectively that was lower in present study. In other study, Salem at al.[49] reported that *in vitro* DM digestibility in wheat straw was improved by addition of cellulose and xylanase by 4% in sheep. Similarly in the present study, IVTDM was increased by 6.39% with addition of EFEs compared with control. This outcome is contrary to that of Lynch et al.[17] and Krueger et al.[15] who did not increase DM digestibility or NDFD *in vitro* experiment. This inconsistency may be due to differences of the most digestible fraction of the structural polysaccharides, characteristics of material, enzyme composition and application rate, the period of interaction between forage and enzyme [7,48].

The specific objective of this trail was to examine whether the FAEI could complement EFEs products by increasing access to structural carbohydrates. Contrary to expectations, EFEs + FAEI did not increase the effect of fibrolytic enzymes and thus there was no significant difference between EFEs and EFEs + FAEI. The use of FAEI alone on corn strover was not investigated in this study. However, a possible explanation for this might be that ferulic acid esterase activity initiated by FAEI during storage was insufficient to hydrolyse esterified bonds in the polysaccharides in the cell wall [50]. Similar results, the detailed study of Lynch et al.[18] found that the use of FAEI in combination with EFEs did not improve IVTD and IVNDF of hay after storage compared to EFEs alone. However, the findings of the current study do not support the some previous research [13,14].

The use of IVTNDFD concept has also provided additional information in economic evaluation of forages [27]. The present study showed that the inclusion of EFEs and EFEs + FAEI as a feed additive into the corn stover achieved daily net profit 2.63 and 3.10 TRY respectively over control. These findings agreed with those obtained by Aboul-Fotouh et al.[51] who found that diets supplemented with cellulolytic enzymes economically better than control diet for feeding lactating goats because of higher yields of milk and milk components.

This study on the digestibility of stover showed that the use of EFEs alone and in combination with FAEI improved IVTD and IVTNDFD compared with the control. Another important finding was that FAEI in combination with EFEs did not affect the amounts of IVTDMD and IVTNDFD compared with the application of EFEs alone; however, they improved the fermentation of corn stover by reducing pH and inhibited mould and yeast multiplication compared with EFEs alone and the control. From economical point of view, the best ration was EFEs + FAEI. However, further studies should evaluate these additives under *in vivo* conditions with respect to digestibility, ruminal fermentation and feed intake.

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