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Effect of Exogenous Enzyme Mixture on Growth Performance, Digestibility and Some Rumen Parameters of Finishing Lambs Fed High Whole Grain Hulled Barley Diet

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ABSTRACT

Background: Whole grain hulled barley has several nutritional limitations in diets for ruminants. Its fibrous hull which is resistant to ruminal microbial degradation and this could decrease performance. Therefore, tools that optimize the use of barley hull are required. Exogenous enzyme products including fibrolytic enzymes may be an important tool to improve the digestibility of whole grain, feed and improve the efficiency of lambs. Thus, this study aimed to investigate the effects of the supplementary exogenous enzyme mixture at three different levels on growth performance, digestibility and ruminal fermentation of male finishing lambs fed diets containing whole barley grain.

Methods: Twenty-one Karayaka lamb (29.36±1.32 kg initial weight) were assigned to three experimental treatments: (1) Control, diet without exogenous enzyme; (2) 5 exogenous enzyme, diet with 5 g exogenous enzyme/head/day) and (3) 10 exogenous enzyme, diet with 10 exogenous enzyme g/head/day). Forage-free finishing diets were composed of 70% whole barley and 30% protein supplement. The experimental diet was offered to the animals for 56 days. Nutrient digestibility was determined during the last 4 days of the experiment.

Result: The supplementation of exogenous enzyme did not affect dry matter intake. Increasing exogenous enzyme in whole barley grain-based diets did not alter digestibility of dry matter, organic matter, crude protein and neutral detergent fibre. Regarding, mean ruminal pH, NH_3-N and ruminal total volatile fatty acid, there was no difference between control and both exogenous enzyme mixture levels. The findings of the current study indicate that there was no effect of exogenous enzyme mixture on lamb performance, digestibility and ruminal fermentation fed whole grain hulled barley-based diets in Karayaka lambs. Therefore, supplementing such diets with exogenous enzyme for fatting Karayaka lambs is not recommended.

Key words: Digestibility, Exogenous enzyme, Finishing lamb, Hulled barley, Performance.

INTRODUCTION

Barley is an important feed for the fattening of lambs in Turkey as it is the most economical cereal grain as a source of dietary energy and also contributes the protein component of the diet. However, whole barley kernels with an intact pericarp are surrounded by a fibrous hull, which is resistant to ruminal microbial degradation (Rode and Beauchemin, 1995). Therefore, its energy digestibility is highly correlated with percent hulls. The digestible energy and digestible protein contents of hulled barley decrease as the percentage of hull increases (Bell *et al*., 1983). Considering that hulled barley has 15-25% fibrous hull levels of the total weight of barley grain dry matter (Grove *et al*., 2003), this could decrease animal performance. Therefore, tools that optimize the use of whole grain hulled barley are required.

Exogenous enzyme (EE) products including fibrolytic enzymes may be an important tool to improve the digestibility of whole grain, feed and improve the efficiency of lambs. Various studies have indicated that supplementing ruminant diets with EE increased the rate and extent of ruminal digestion of fiber and production efficiency (Arriola *et al*., 2017; Meale *et al*., 2014). Similarly, numerous researchers reported that enzymes added to the barley-based lamb diets improved digestibility of dry matter (DM), organic matter (OM), crud protein (CP), neutral detergent fiber (NDF), acid detergent fibre (ADF) (Riberio *et al*., 2019; Chung *et al*., 2012). However, in certain studies (Awawdeh and Obeidat, 2011; Muwalla *et al*., 2007), EE supplementa tion did not consistently improve DM intake, average daily gain (ADG) and feed conversion rate (FCR) of lambs. Diet and feeding conditions, enzyme application method, dose and

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rate are some of the factors that could cause inconsistency in EE applying (Gado *et al*., 2009). In addition, many commercial EE preparations are marketed as feed supplements; however these vary widely in their type and concentration of enzymes they contain and are not substrate specific (Valli *et al*., 2019).

Research on the effects of exogenous enzymes containing fibrolytic enzymes in whole barley grain-based diets for feeding growing lambs is limited. The experimental objective was to determine the impact of the supplementary EE mixture at three different levels on growth performance, digestibility and rumen fermentation parameters of growing lambs fed 70% whole barley-based diet. It was hypothesized that EE mixture would increase fiber digestibility and fermentation in lambs fed diets containing whole barley-based diet, subsequently growth performance.

MATERIALS AND METHODS

The experiment was conducted in the local farm in Bafra district of Samsun Province, Turkey at the period April 2019 to June 2019 with prior approval from the animal care committee of Ondokuz Mayıs University (Approval Number: 2018-68489742-600-E.8080).

Twenty-one male Karayaka lambs (29.36±1.32 kg of body weight (BW), 3.5 to 4 months old) were randomly distributed into 3 groups having 7 lambs in each. Experimental treatments were grouped as follows: (1) Control, diet without EE; (2) 5 EE, control diet added with 5 g EE/head/day and (3) 10 EE, control diet added with 10 EE g/head/day. Forage-free finishing diets were composed of 70% whole barley and 30% protein -vitamin-mineral premix and formulated using National Research Council (2007) nutrient requirement. The EE in powder mixture was applied as topdressed and hand-mixed into the diet. Ingredients and chemical composition of the diet are presented in Table 1. The enzyme selected for this study has been used in poultry. The selected EE levels in this study were above the manufacturer-recommended dose for poultry (1 g/kg of offered feed). The enzymatic activities were declared 4×10^6 IU of cellulose kg^{-1}, 1.8×10^6 IU of xylanase kg^{-1}, 3×10^5 IU of β-glucanase kg^{-1}, 2×10^5 IU of pectinase kg^{-1}, 1.2×10^6 IU of protease and 2×10^5 IU of alfa-amlyse kg^{-1} by the manufacturer (Farmazyme, Farmavet, İstanbul, Turkey).

Lambs were housed in individual pens. After a 2 week adaptation period to the diets, the experiment continued for 56 days. Whole barley and the protein-vitamin-mineral supplement were blended daily using a feed mixer and offered ad libitum (expected 10% refusual, DM basis every day). Also, lambs were offered fresh drinking water throughout the experiment. Lambs were fed once daily (07:30 h) and the amounts of feed offered and refused was recorded daily to determine DM intake.

To determine ADG, all animals were weighed trial initiation and 56 days (final day) of the experiment, after a 12 h fasting period. FCR (DM intake kg/ADG kg) was also calculated.

Nutrient digestibility was determined during the last 4 days of the experiment. Fecal samples, 4 lambs selected randomly from each treatment group for the digestibility, were collected by using fecal bags. Every morning throughout the digestion trial, fresh fecal daily samples for each animal were collected before offering of the feed, weighted, recorded, sampled (about 100 g) and frozen (-20°C) for later chemical analyses. About 50 mL rumen fluid was taken on day 56 from each lamb through stomach tube 4 h after feeding and filtered through double layers of cheesecloth. The pH of the ruminal fluid was immediately measured. Rumen fluid content was then divided into 2 portions for determination of NH_3-N concentration and total volatile fatty analysis acid (VFA). The rumen fluid samples were then centrifuged to remove suspended solids at 5000 × g for 10 min at 4°C. Five mL aliquot of the supernatant was mixed with one drop of 97% sulfuric acid for NH_3-N analysis. Another 5 mL of the supernat was preserved with 1 ml of 25% meta-phosphoric acid for TVFA estimation. Subsequently, all samples were stored at -20°C until analyzed.

Diet samples were collected weekly throughout the experiment and composited. Composited samples and feces were dried in a forced-air oven at 55°C for 72 h and ground through a mill to 1 mm screen. Proximate principles (AOAC, 1995) and detergent fiber were estimated (Van Soest *et al*., 1991) in the feeds and feces. Nutrient digestibility as a percentage was calculated according to the formula:

$$\frac{\text{Nutrient intake - nutrient excreted}}{\text{Nutrient intake}} \times 100$$

After thawing the supernatants at room temperature, they were used for NH_3-N analysis and total VFA analysis. The NH_3-N content on the samples was determined by direct distillation using a micro Kjeltech Auto Analyzer according to AOAC (1995). Concentrations of rumen total VFA were determined by titrating the steam distillate of rumen fluid with N/100 NaOH.

In SPSS 21.0 software package, one-way ANOVA was used to analyze various parameters. Differences were analyzed by statistical variance with a significance of $P<0.05$ and a comparison of the means was performed by the Tukey test.

RESULTS AND DISCUSSION

Feed intake and growth performance

Table 2 presents the results for supplementing different levels of EE on feed intake, growth performance and digestibility in lambs during the overall period. Supplementing different levels of EE in lambs fed whole barley with a protein supplement did not influence the DM intake. Similar to results in the experiment, Miller *et al*. (2008) reported no change in the DM intake when xylanase/endoglucanase enzyme was added to the barley grain-based diet of growing lambs. Also, the present results are

consistent with the results of McAllister *et al*. (2000), who reported that DM and OM consumption in lambs was not affected by the addition of mixed activity EE products to barley grain-based rations. However, growth performance results obtained from this research don't agree with the findings of Vallejo *et al*. (2016), which reported a significant effect of low and moderate rates of xylanase on feed intake in sheep but not on high concentration. A previous study also reported that the increase in feed consumption with low EE application reflects the increase in ruminal fiber digestion and digesta passage rate (Beauchemin *et al*., 2003). Moreover, Ahmed (2016) showed that feeding a high dose of EE had a beneficial effect on feed intake in lambs. Our research did not record substantive differences in the fiber digestibility of responses between control and treatment. As indicated by Ran *et al*. (2019), this situation may have been due to ad libitum feeding of lambs.

In this experiment, it is not surprising that there is no effect of EE supplementation on ADG. Adding EE to the diet containing whole barley and protein supplement did not increase both DM intake and nutrient digestibility and therefore did not provide more fermentable carbohydrates and available energy for synthesizing microbial protein in the rumen (Voelker Linton and Allen, 2009). Similarly, some previous studies have noted that EE supplementation did not affect ADG and FCR of lambs (Muwalla *et al*., 2007; Awawdeh and Obeidat, 2011), goats (Lu *et al*., 2015) and feedlot steers (He *et al*., 2014). This study observed no effect of EE application on growth performance and FCR. In contrast to the current study, Lopez-Aguirre *et al*. (2016) showed that the growth rate was improved by adding enzyme in Pelibuey lambs. Also, Togtokhbayar *et al*. (2017) and Salem *et al*. (2015) assessed the effect of different levels of enzyme supplementation on the growth performance of lambs and clarified that the digestibility increased, thereby increasing ruminal energy and/or nutrient availability and improving lamb performance.

Digestibility

In the current study, there was expectation improval *fiber degradation* of the outer layers of whole barley EE supplementation. However, contrary to our hypothesis, there were no differences in digestibilities of DM, OM, CP and NDF between diets including control or FE supplement (Table 2). Concerning digestibility, similar results are recorded by Miller *et al*. (2008) and Awawdeh and Obeidat (2011) who concluded that the use of EE had no effect on the digestibility of barley-based diets by lambs. Contrary to the present results, previous studies (Riberio *et al*., 2019; Chung *et al*., 2012) reported that adding EE in a barley based diet improved DM, OM, NDF and ADF digestibility in lambs. Some earlier studies (Beauchemin *et al*., 1997; Krause *et al*., 1988) reported that when feedlot cattle was fed a diet containing 4.9% barley silage and 91.6% barley and was supplemented with EE, DM and starch digestibility was improved, which may be related to an increased digestion of the hull in the rumen, thereby allowing accessibility of ruminal microorganism to the protein matrix in the endosperm, but this was not observed in the present study. The factors contributing to the lack of response to increasing enzyme levels on digestibility in the current study are probably complex. It is possible that ruminal cellulase from bacterial origin masked the effect of adding EE in lamb fed whole barley-based diets (Askar *et al*., 2006). Moreover, the activity and dose of adding EE can not increase the hydrolysis of fibre to serve as a substrate for ruminal cellulolytic bacteria (Morgavi *et al*., 2000). Further, suggests

Table 1: The compositions and nutrient content of basal diet.

Ingredients, % of DM	%
Whole barley grain	70
Protein-vitamin-mineral premix[1,2]	30
Analyzed composition, %	
DM	89.15
CP	16.03
Total ash	3.20
Crude fat	2.03
ADF	6.55
NDF	20.52
ME[3], kcal/kg	2.756

DM - Dry matter; CP - Crude protein; ADF - Acid detergent fibre; NDF - Neutral detergent fibre.
[1]Premix composition: DM, 88.44%; CP, 27.76%; Ashes, 6.65%; Fat, 1.67%; ADF, 7.42%; NDF, 13.86%; ME, 2186 kcal/kg. [2]Guaranted levels per kg of premix: 50.000 mg Mn, 50.000 mg Fe, 50.000 mg Zn, 10.000 mg Cu, 800 mg I, 150 mg Co, 200 mg Se, 50.000 mg Mg, 460.000 mg CaCO3, 10.000 mg Antioxidant, 10.000.000 IU vitamin A, 2.000.000 IU vitamin D3, 30.000 IU vitamin E[3] Calculated value.

Table 2: Effects of whole grain hulled barley based diet with exogenous enzyme supplementation at different levels on groth performance and nutrient digestibility in lambs.

	Diets[1]			SEM	P-value
	Control	5EE	10 EE		
İnitial BW (kg)	29.49	29.24	29.34	0.28	0.94
Final BW (kg)	41.40	41.02	40.85	0.38	0.85
DMI (kg/d)	1.36	1.37	1.38	0.02	0.89
ADG (g/d)	212.60	210.30	205.50	4.85	0.84
FCR	6.41	6.62	6.79	0.13	0.54
Digestibility (%)					
DM	68.31	67.75	65.81	0.81	0.48
OM	70.49	69.81	68.00	0.61	0.35
CP	70.96	71.36	70.80	0.31	0.79
NDF	43.92	44.48	42.61	0.65	0.55

BW: Body weight; DMI: Dry matter intake; ADG: Average daily gain; FCR: Feed conversion rate; OM: Organic matter; CP: Crude protein; NDF: Neutral detergent fibre; SEM: Standard error mean. [1]Diets= Control - diet without EE; 5 EE - Control diet added with 5 g EE/ head/day; 10 EE - control diet added with 10 EE g/head/day.

that ruminal fiber hydrolyzing capacity might be sufficiently high in lamb fed whole barley-based diets. On the other hand, the proportion of the potentially digestible NDF of the hull cementing to the outer layer of the grain is likely to be low as was found (about 14%) by Grove *et al*. (2003). Differences between present study and other studies could be related to dose and enzyme activity as well as the type of diet (Ran *et al*., 2019).

Rumen fermentation

The supply of increasing different levels of EE did not affect ruminal pH, NH_3-N and ruminal total VFA (Table 3). However, as expected, ruminal pH ranged from 6.35-6.37 for all groups, which were within a normal range acceptable for fiber digestion, normal rumen fermentation and microbial synthesis (Van Soest, 1994), Possible reasons for this may be associated with higher chewing, rumination time and salivation of lamb fed whole-barley based diet. Regarding the EE supplementation, the results of the present study were in line with the observation of Yuangklang *et al*. (2017), who concluded that the addition of EE which consisted of xylanase and glucanase did not affect ruminal pH in growing goats fed rice straw-based diet. However, the increase of ruminal pH value due to the benefical impact of EE on the degree of degradation of NDF of feeding ration were reported by Kholif *et al*. (2017) after EE supplementation in Nubian goats and by Elenin *et al*. (2016) after supplementation of cellulase in lambs. On the other hand, a decrease in ruminal pH was observed with supplemental cellulase and xylanase in growing goats by Lu *et al*. (2015) because of higher energy release by fibrolytic enzyme supplementation.

The results in this study indicate a lack of effects of enzymatic supplementation on NH_3-N utilization, which is in line with findings of other authors (Abid *et al*., 2020; Ribeiro *et al*., 2018). In contrast, some exogenous enzyme products have reported a decrease in the measured NH_3-N production (Kholif *et al*., 2017). It reported that this decreased NH_3-N level was a result of the incorporation of NH_3-N into microbial protein synthesis due to greater ADF and NDF digestibility in animals fed diets added with enzymes. Yuangklang *et al*. (2017) related the improvement in NH_3-N concentration to liberated by enzyme formulations some nitrogen that was bound to insoluble fiber.

Table 3: Effects of whole grain hulled barley based diet with exogenous enzyme supplementation a different levels on rumen fermentation in lambs.

	Diets[1]			SEM	P-value
	Control	5 EE	10 EE		
pH	6.35	6.37	6.37	0.02	0.97
NH3-N, mg/dl	15.45	15.60	15.58	0.24	0.96
Total VFA mol/ 100 mol	52.90	53.43	54.33	0.60	0.85

VFA: Volatile fatty acid; SEM: Standard error mean.

[1]Diets = Control - diet without EE; 5 EE - control diet added with 5 g EE/ head/day; 10 EE - control diet added with 10 EE g/head/day.

Some enzyme products decreased total VFA of lambs, whereas many other studies observed no effects. He *et al*. (2014) and Peters *et al*. (2010) found no changed TVFA production using exogenous fibrolytic enzyme supplement added to the diet as used in the present study. Also, Miller *et al*. (2008) reported no effect on total VFA concentration in the ruminal fluid adding fibrolytic enzymes to barley-based lamb diets. However, enzyme treatment of diets fed to lactating goats or sheep (40% berseem clover and 60% concentrate or 30% maize stover and 70% concentrate, respectively) had a positive effect on total VFA production in the study by Kholif *et al*. (2007) and Vallejo *et al*. (2016). Moreover, Yuangklang *et al*. (2017) reported that the hydrolysis of fiber by enzymes had provided more substrate for microbial production of total VFA and thus increased the concentration of total VFA in the rumen. Also, an increase in ruminal total VFA concentration was reported by Silva *et al*. (2016). They reported that EE supplementation ensured greater access of bacteria to feed particles. In the current study, EE had not affected the rate of fiber digestion of whole barley-based diet. This may be the reason why we did not find any change in total VFA concentrations.

CONCLUSION

The results obtained in this study demonstrated that adding exogenous enzyme mixture in high grain diets that contain mainly hulled barley grain did not affect dry matter intake, growth rate nutrient digestibility and ruminal fermentation parameters and thus not a feasible strategy for maximizing growth performance. Therefore, supplementing hulled-barley grain based diets with EE for fatting Karayaka lambs is not recommended.

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Conflict of interest

All authors declared that there is no conflict of interest.

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