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Effects of exogenous cellulase and xylanase enzyme preparations on feed intake, nutrient digestibility, growth, and economics of rearing Mongolian lambs

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Abstract

In a completely randomised design, twenty four 12-months old Mongolian breed male lambs averaging 21.6 ± 0.48 kg of body weight (BW) were used to evaluate the effects of exogenous cellulase and xylanase enzyme preparations on feed intake, digestibility of nutrients, growth and economics of rearing lambs. The lambs were randomly assigned to six treatment groups with four animals per treatment. The treatment combinations comprised: i) two enzyme preparations, i.e. cellulase *vs.* xylanase (ENZc, ENZx), ii) two ration types, i.e. wheat straw + wheat bran (diet W) *vs.* barley straw + wheat bran (diet B) and iii) two control diets (diet W and diet B without enzyme preparations, –ENZ). Lambs were fed the cellulase and xylanase treated wheat and barley straws ad libitum whereas the wheat bran was offered at 400 g DM per day. Significant effects were observed for nutrient intake of diet B +ENZc, and for crude protein (CP) and neutral detergent fibre (NDF) digestibility of diet W +ENZc. The average daily gain (ADG) increased in all enzyme treated groups with highest values found in diet B +ENZc, but without significant differences found between the two enzyme preparations. Both enzyme preparations had positive effects on feed conversion ratio (FCR) of both diets, where by the highest values were found for diet B +ENZc. Enzyme preparation had no effect on the total feed cost for both diet types and showed positive effects on other economic parameters, where by cellulase yielded better results than xylanase. These results suggest that cellulase addition is effective for improving digestibility of nutrients, growth performance and net revenue ingrowing lambs.

Keywords: feed intake, nutrient digestibility, exogenous enzyme preparations, average daily gain, Mongolian lambs

1 Introduction

Mongolia is a landlocked country between China and Russia in Central Asia and lies between latitudes 41°N and 52°N and longitudes 87°E and 120°E (Fernandez-Gimenez, 1999; Barzagur, 2002). The climate is continental with long, cold winters and short summers (July and August). The growing season is therefore limited to about 90 days. In Mongolia, all types of livestock are kept outdoors and the vegetation of natural pastures is the main source of feed throughout the year including winter. The nutritive value of summer and autumn pasture is good, but it declines strongly in winter and spring; therefore, winter and spring pastures provide only about half of the nutrient requirements of the animals (Nergui *et al.*, 2011). Bynie *et al.* (2012) reported that Mongolian sheep lost 20.6–34.0 % of their live

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weight during winter and spring due to lack of supplemental feeding.

Traditionally, only a small amount of supplements consisting of hay, cereal straws and bran, cracked and mixed grains are offered during the winter and spring seasons and only to weak animals (Batjargal *et al.*, 2014).Wheat and barley straw have become valuable low cost forage. The straw can be used effectively, especially in extensive low-input ruminant production systems (Lopez *et al.*, 2005). In Mongolia straws are commonly used as survival feed for animals during the long winters when other types of feeds are short in supply; more than 7900 tons of cereal straws were used nationwide for animal feeding in 2014 (NSOM, 2015). However, because the digestibility of these straws is low, the energy potentially stored in these cannot be fully exploited. Thus, a number of physical, chemical and enzymatic treatments are used to improve the nutritive value of straws by increasing their digestibility through structural modifications of the polysaccharide–lignin crosslinks of the cell wall (Lopez *et al.*, 2005).

Several studies with exogenous fibrolytic enzymes have reported increased microbial activities in the rumen, which resulted in enhanced animal performance (Gado *et al.*, 2009; Khattab *et al.*, 2011; Salem *et al.*, 2013; Alsersy *et al.*, 2015; Rojo *et al.*, 2015; Salem *et al.*, 2015; Valdes *et al.*, 2015). Rode *et al.* (1999), Yang *et al.* (1999), and Salem *et al.* (2011) showed that feeding enzyme treated roughages have beneficial effects on dry matter intake, feed digestibility and growth performance in ruminants. Khattab *et al.* (2011), Salem *et al.* (2013), and Rojo *et al.* (2015) showed that feeding dairy animals on diets supplemented with fibre-degrading enzymes improved feed utilisation and animal performance. However, effects of feeding enzymes on animal performance in Mongolia have not been documented so far. The objective of the current study was therefore to evaluate the effects of exogenous cellulase and xylanase enzyme preparations on feed intake, digestibility of nutrients, body weight gain and economics of rearing lambs fed wheat and barley straw diets supplemented with wheat bran.

2 Materials and methods

2.1 Straw provenience, enzyme preparations and enzymatic activities

The wheat straw (*Triticum aestivum* variety no 141) and barley straw (*Hordeum vulgare* variety noI166.79) used in this trial were harvested at the Research Institute of Plant Science and Agriculture fields in the Darkhan-Uul province. The temperature at the study site ranged from 5 to 39°C during summer and from –30 to –7°C during winter with over 260 sunny days per year.

Two exogenous fibrolytic enzyme products were tested: Cellulase (Dyadic® cellulase PLUS; Dyadic International, Inc. Jupiter, FL, USA) and xylanase (Dyadic® xylanase PLUS; Dyadic International, Inc. Jupiter, FL, USA). As per the manufacturer, the activities of the enzymes were determined as described by Robyt & Whelan (1972) and Bailey *et al.* (1992) at pH 6.5 and 39°C. Briefly, the enzyme products were assayed for endoglucanase and xylanase activity by catalytic hydrolysis of xylan from oat spelt; the liberated reducing groups were determined using alkaline copper reagent. The cellulase product contained 30,000 to 36,000 units of cellulase ml^{-1} and 7,500 to 10,000 units of beta-glucanase ml^{-1}. The xylanase product contained 34,000 to 41,000 units of xylanase ml^{-1}, 12,000 to 15,000 units of beta-glucanase ml^{-1}, and 45,000 to 55,000 units of cellulase ml^{-1}.

2.2 Experimental design and treatments

In a completely randomised design, twenty four 12-months old Mongolian breed male lambs averaging 21.6 ± 0.48 kg of body weight (BW) were used. The lambs were randomly assigned to six treatment groups (two controls and four experimental) with four animals per treatment. The treatment combinations comprised: i) two enzyme preparations, i.e. cellulase *vs.* xylanase (+ENZc, +ENZx), ii) two ration types (Table 1), i.e. wheat straw + wheat bran (diet W) *vs.* barley straw + wheat bran (diet B) and iii) two control diets (diet W and diet B without enzymes; -ENZ). The straws were chopped to 3–5 cm length and treated with the xylanase and cellulase, respectively, at a dose of 1.0 $\mu l\, g^{-1}$ for 16 hours before feeding to allow enzyme and straw to interact (Beauchemin *et al.*, 2003). The enzyme preparations were diluted in water at a ratio of 1:3 (v/v). Lambs were fed the cellulase and xylanase treated wheat and barley straws ad libitum whereas the wheat bran was offered at 400 g DM per animal and day.

2.3 Digestibility trial

The feeding and digestibility trial was conducted in February to March 2015 at "Nart" training and experimental centre in Bornuur sum of Tov province; it followed the experimental protocol approved by the Animal Care and Use Committee of the Mongolian Univer-

Table 1: *Chemical composition (g kg^{-1} DM) of feed ingredients and experimental diets.*

Constituent	*Wheat straw*	*Barley straw*	*Wheat bran*	*Diet W*	*Diet B*
Crude protein	52	46	165	100.8	97.2
Crude ash	68	94	71	69.2	84.0
Ash-free neutral detergent fibre	607	626	329	487	498

DM: dry matter

sity of Life Science (Mongolian University of Life Science, 2009). The feeding and digestibility trials comprised in total 48 days, where of the first 10 days were for adaptation, the last 10 days for measurement (digestibility determination) and remaining days constituted the feeding trial. The lambs were individually offered the treated wet straws and the bran in feeding boxes; their daily diet was offered in two equal meals, at 9:30 am and at 17:30 pm. The amount (fresh weight) of feed offered, of refusals and of total faeces was determined at every feeding, the latter by using collection bags, and quantified daily for the 10 days to determine the effect of enzyme preparations on the apparent digestibility. All refusals were removed right after feeding and the feeding place was cleaned daily before next feeding. Representative samples of straws, bran, diet refusals and faeces were collected daily and dried at 60°C in a forced air oven prior to processing the samples. Lambs had free access to fresh water at all times.

2.4 Chemical analyses

Dried samples of diet components, feed refusals and faeces were ground to pass a sieve of 1.5 mm diameter (Wiley mill, Model 4, Thomas Scientific, Swedesboro, NJ, USA) and analysed for dry matter (DM; #934.01), ash (#942.05) and crude protein (CP; #954.01) according to AOAC (1997). Neutral detergent fibre (NDF) concentration was determined according to the method of Van Soest *et al.* (1991) using an ANKOM^{200} Fibre Analyser (ANKOM Technology Corporation, Macedon, NY, USA). The NDF was assayed without use of alpha amylase but with sodium sulphite in the neutral detergent solution. The NDF concentration is expressed without residual ash.

2.5 Calculations and statistical analysis

The body weight (BW) gain was determined by weighing animals on the first and last day's morning of the experimental period, before feeding, using a digital electronic parcel postal scale (range 0.5–150 kg, accuracy 0.1 kg). Average daily gain (ADG) was calculated using the formula (final weight – initial weight) / (end date – date at commencement). Feed conversion ratio (FCR) was calculated as total body weight gain (kg) divided by total amount of consumed feed (kg DM) during the experimental period. The total body weight gain was assumed to only consist of meat/muscle and was multiplied by the price of mutton (7000 MNT[1]/kg; NSOM, 2015) to determine the price per kg of body weight gain. The net revenue was calculated as the difference between revenue (price of weight gain) and the cost of feed consumed. The economic feed efficiency (EFE) was determined as the quotient of net revenue over total feed costs and expressed in percent. The relative economic feed efficiency was defined as the difference in EFE between the control and the enzyme-fed groups and was expressed in percent of the control's EFE. Wheat and barley straws and wheat bran were bought from Mongolian markets with prices of 120 and 350 MNT per kg, respectively (Altantaria Ltd, Ulaanbaatar, Mongolia; price lists for the straws and bran are available from: http://www.altantaria.mn/).

Multivariate analysis of variance was carried out to determine the effects of enzyme preparations (cellulase, xylanase) and straws (wheat, barley) on the different dependent variables. The data were analysed with the PROC MIXED procedure of SAS (SAS Institute Inc., 2011) using the model:

$$Y_{ijk} = \mu + ENZYME_i (n = 3) + DIET_j (n = 2) + (ENZYME \times DIET)_{ij} + \varepsilon_{ijk}$$

Where Y_{ijk} is the *ij* observation of the *j*-th lamb assigned to the *i*-th treatment, and ε_{ijk} is the error. Mean comparisons were performed using Tukey's test and significant differences were declared at $p \leq 0.05$.

[1]MNT is abbreviation of Mongolian National Tugrik, 1 US$ = 1950.7 MNT

3 Results

3.1 Feed intake and digestibility

Significant effects were observed for the intake of nutrients with diet B +ENZc and for the CP and NDF digestibility of diet W +ENZc, whereas no effects were found for xylanase addition (Table 2). The DM, CP and NDF intake of B +ENZc increased by 10.9 %, 5.7 % and 16.6 %, respectively, in comparison to B –ENZ (Table 2). Compared to diet W +ENZc, the CP intake was 7.5 % lower ($p \leq 0.05$) and NDF intake was 22.9 % higher ($p \leq 0.05$). The DM and organic matter (OM) intake of B +ENZc were higher ($p \leq 0.05$) than of B +ENZx.

In general, the digestibility of nutrients of both diets was not affected by enzyme treatments ($p > 0.05$) except for CP and NDF digestibility of diet W, which was 14 % and 50 % higher, respectively, with +ENZc than with –ENZ. Moreover, DM, OM and CP digestibility of diet W +ENZc were 14 %, 17 %, and 19 % higher ($p \leq 0.05$), respectively, than those of diet B +ENZc. In general, nutrient intake was highest with diet W +ENZc except for NDF intake, which was highest with diet B +ENZc (Table 2).

Table 2: *Average feed intake and digestibility of nutrients by Mongolian lambs fed diet W (wheat straw + wheat bran) and diet B (barley straw + wheat bran) without (–ENZ = control) or with cellulase (+ENZc) or xylanase (+ENZx) addition.*

Variable	*Diet*	*–ENZ*	*+ENZc*	*+ENZx*	*SEM*	*p=*
Dry matter						
Straw intake (g d^{-1})	W	447	509	476	12.8	0.244
	B	448 ^{b}	497 ^{a}	456 ^{ab}	8.4	0.032
Wheat bran intake (g d^{-1})	W	340	375	351	6.2	0.111
	B	338 ^{b}	377 ^{a}	344 ^{b}	5.3	0.004
Total intake of ration (g d^{-1})	W	788	885	827	14.4	0.022
	B	786 ^{b}	875 ^{a}	800 ^{b}	9.9	0.001
Digestibility of ration (g kg^{-1})	W	564 ^{A}	636 ^{A}	581	12.9	0.055
	B	540 ^{B}	560 ^{B}	556	11.3	0.759
Organic matter						
Intake (g d^{-1})	W	717	734	704 ^{A}	8.9	0.384
	B	694 ^{ab}	733 ^{a}	661 ^{B,b}	8.9	0.003
Digestibility (g kg^{-1})	W	585 ^{A}	653 ^{A}	591	13.0	0.060
	B	542 ^{B}	558 ^{B}	551	11.4	0.922
Crude protein						
Intake (g d^{-1})	W	93 ^{A}	100 ^{A}	94 ^{A}	1.6	0.061
	B	88 ^{B,b}	93 ^{B,a}	90 ^{B,ab}	0.7	0.009
Digestibility (g kg^{-1})	W	66 ^{A,b}	75 ^{A,a}	69 ^{A,ab}	1.0	0.022
	B	59 ^{B}	63 ^{B}	61 ^{B}	1.1	0.243
Neutral detergent fiber (ash-free)						
Intake (g d^{-1})	W	276 ^{B}	309 ^{B}	288 ^{B}	8.5	0.285
	B	326 ^{A,b}	380 ^{A,a}	338 ^{A,ab}	7.6	0.009
Digestibility (g kg^{-1})	W	93 ^{b}	139 ^{a}	106 ^{b}	7.0	0.040
	B	108	141	137	7.2	0.140

W and B represent diet W and diet B, respectively. SEM is standard error of means; p is probability of a variable effect. ^{A}, ^{B} Capital letters within one column indicate significant difference between the two diets for the given variable. ^{a}, ^{b}, ^{c} Superscripts within one row indicate significant differences within a diet treated with different exogenous enzyme preparations and/or untreated diet.

3.2 Growth performance and feed efficiency

The ADG increased inall enzyme treated groups as compared to the control, with the largest increase found for diet B +ENZc. However, there were no differences between the two enzyme preparations with an exception of diet W +ENZc (Table 3).

The ADG of lambs fed diet W +ENZc was higher ($p \leq 0.05$) in comparison to W –ENZ, whereas diet type (W or B) had no effect on ADG. Both enzyme preparations had positive effects on feed conversion ratio (FCR) for both diet types and the best values within diet types were found with +ENZc. Diet type had a significant effect on FCR, whereby the values were higher for diet B (+ENZc, +ENZx) than for diet W with both enzyme preparations. However, without enzyme treatment (–ENZ) diet B yielded a lower FCR than diet W.

3.3 Economic evaluation

Enzyme application had no effect on the total feed cost for both diet types but in both cases showed positive effects on daily gain, net revenue, economic and relative economic feed efficiency (Table 4). The cellulase enzyme treatment showed the highest improvements in the above parameters compared to the xylanase treatment. Cellulase and xylanase treated diet B showed numerically higher BW gain, price for BW gain, net revenue and feed efficiency than diet W, but differences were only significant for net revenue and relative feed efficiency.

On the contrary, without enzyme treatment, diet B yielded lower values than diet W for all economic variables.

4 Discussion

The use of exogenous enzyme preparations in ruminant diets is an efficient technology for improvement of forage quality and has gained lot of interest in recent years (Beauchemin *et al.*, 2003; Salem *et al.*, 2015). It is well known that supplementing animal rations with fibre degrading enzymes can improve feed utilisation by enhancing fibre degradation *in vitro* (Diaz *et al.*, 2013; Salem *et al.*, 2015; Togtokhbayar *et al.*, 2015) and animal performance *in vivo* (Gado *et al.*, 2009; Khattab *et al.*, 2011; Salem *et al.*, 2013; Alsersy *et al.*, 2015; Rojo *et al.*, 2015; Valdes *et al.*, 2015).

In the current study the general results on nutrient intake and digestibility were similar to those reported by Hussain *et al.* (2008) and Meale *et al.* (2014) who concluded that the application of exogenous enzyme preparations to the diets of small ruminants has little impact on nutrient intake and production. However, the application of exogenous enzyme preparations had a positive effect on body weight gain and was therefore in agreement with findings of *in vivo* studies with cattle (Feng *et al.*, 1996; Beauchemin *et al.*, 1999) and lambs (Giraldo *et al.*, 2008) who reported that enzyme supplementation of the daily diet resulted in a higher average daily gain. In general the digestibility of the various proximate constituents was higher for diet W +ENZc than for diet B +ENZc. This is in agreement with Wang & McAllister (2002) and Jafari *et al.* (2004) who also found that wheat straw treated with a fibrolytic enzyme enhanced CP, NDF and ADF digestibility (*in vivo*). Also

Table 3: *Average daily gain (ADG) and feed conversion ratio (FCR) of Mongolian lambs fed diet W (wheat straw + wheat bran) and diet B (barley straw + wheat bran) without (–ENZ) or with cellulase (+ENZc) or xylanase (+ENZx) addition.*

Variable	*Diet*	*–ENZ*	*+ENZc*	*+ENZx*	*SEM*	*p=*
Body weight (kg)						
Initial	W	22.1	20.3	20.6	0.5	0.231
	B	23.4	21.8	21.1	0.4	0.597
Final	W	24.4	23.9	23.5	0.3	0.627
	B	25.5	25.9	24.2	0.4	0.672
Average daily gain (g/d)	W	50 ^{b}	80 ^{a}	66 ^{ab}	4.7	0.004
	B	46	93	68	6.3	0.060
Feed conversion ratio (FCR)	W	15.7 ^{B,a}	11.1 ^{A,c}	12.3 ^{A,b}	0.3	0.000
	B	17.1 ^{A,a}	9.4 ^{B,c}	11.8 ^{B,b}	0.4	0.000

W and B represent diet W and diet B, respectively. SEM is standard error of means: p is probability of a variable effect. ^{A}, ^{B} Capital letters within one column indicate significant difference between the two diets. ^{a}, ^{b}, ^{c} Superscripts within one row indicate significant differences within a diet treated with different exogenous enzyme preparations and/or untreated diet.

Table 4: *Economic evaluation of feeding Mongolian lambs without (–ENZ) or with cellulase (+ENZc) or xylanase (+ENZx) treated diet W (wheat straw + wheat bran) and diet B (barley straw + wheat bran).*

Variable	*Diet*	*–ENZ*	*+ENZc*	*+ENZx*	*SEM*	*p=*
Total feed cost (MNT)	W	7780	8664	8100	237.1	0.348
	B	7749	8631	7883	173.2	0.065
Body weight gain (kg)	W	2.3 ^{c}	3.6 ^{a}	3.0 ^{b}	0.2	0.001
	B	2.1 ^{c}	4.2 ^{a}	3.1 ^{b}	0.3	0.001
Price for BW gain (MNT)	W	15867 ^{c}	25200 ^{a}	20767 ^{b}	1416.2	0.001
	B	14525 ^{c}	29225 ^{a}	24525 ^{b}	1975.1	0.001
Net revenue (MNT)	W	8086 ^{c}	16536 ^{B,a}	12665 ^{b}	1293.5	0.001
	B	6776 ^{c}	20595 ^{A,a}	13642 ^{b}	1858.6	0.000
Economic feed efficiency (%)	W	104 ^{b}	193 ^{a}	157 ^{ab}	8.3	0.013
	B	88.6 ^{b}	238 ^{a}	173 ^{a}	21.0	0.001
Relative economic feed efficiency (%)	W	100 ^{b}	186 ^{B,a}	151 ^{A,ab}	15.0	0.006
	B	100 ^{c}	268 ^{A,a}	195 ^{B,b}	12.9	0.001

W and B represent diet W and diet B, respectively. SEM is standard error of means; *p* is probability of a variable effect. ^{A}, ^{B}: Capital letters within one column indicate significant difference between the two diets. ^{a}, ^{b}, ^{c}: Superscripts within one row indicate significant differences within a diet treated with different exogenous enzyme preparations and/or untreated diet.

Rode *et al.* (1999) reported that total digestibility of nutrients was increased by enzyme treatment and that supplementing dairy cow diets with a fibrolytic enzyme mixture has the potential to enhance milk yield and nutrient digestibility of cows in early lactation without affecting feed intake.

In general, the addition of cellulase and xylanase enzyme preparations increased the average daily gain of lambs by 60–102 % and 32–48 %, respectively, over their control groups. This was probably due to the improved nutrient digestibility and thus enhanced feed efficiency. Kung *et al.* (2000) noticed that treatment of feeds with enzyme preparations just prior to feeding can improve digestibility via different mechanisms, among them enhanced microbial attachment, changes in gut viscosity, complementary actions with ruminal enzymes, changes in the site of nutrient digestion and improvements in palatability and changes in the patterns of feed consumption.

These results are consistent with findings of Beauchemin *et al.* (1997), Lewis *et al.* (1999), Titi & Lubbadeh (2003), and Salem *et al.* (2011) who suggested that the improved animal performance through feeding of enzyme treated straw was due to increased digestibility, which enhances energy and/or nutrient availability to rumen microbes. However, other studies in sheep (McAllister *et al.*, 2000; Miller *et al.*, 2008) and cattle (Krueger *et al.*, 2008) reported no effects of enzyme treatments applied at feeding on average daily gain. Although the results of using fibrolytic enzyme preparations in lambs' rations in the current study are encouraging in general, the results of such applications in animal trials have been inconsistent due to varying enzyme doses, different ways of enzyme preparations, different feed rations and animal species.

Using the cellulase enzyme preparation improved the feed conversion ratio of diet W and diet B by 30 % and 45 % over the control, and the xylanase treatment increased the feed conversion ratio of diet W and B by 22 % and 31 %, respectively, which is in agreement with Brossard *et al.* (2006).The improved feed conversion ratio observed in the current study might be attributable to the enhanced NDF digestibility; a similar trend was observed by Holtshausen *et al.* (2011). For both diets, calculated net revenue and economic feed efficiency were substantially higher for both enzyme treatments as compared to the control diets. The cellulase enzyme addition showed higher positive effect on net revenue and feed efficiency in comparison to the xylanase treatment. Without enzyme treatment, diet B yielded lower values than diet W for all economic variables. In short, our results suggested that economically, the cellulase enzyme preparation performs better than the xylanase preparation for both types of diets. These results agree with those of Tozer *et al.* (2003) who found that although costs per kilogram of milk produced were lowest for pasture-concentrate feeding, an enzyme-treated total mixed ration yielded the highest net income per cow per day because of higher yields of milk and milk components.

5 Conclusions

The treatment of wheat and barley straw with the cellulase enzyme improved the digestibility of organic matter, crude protein and NDF, increased average daily body weight gain, feed efficiency and net revenue as compared to untreated control diets. The higher economic feed efficiency with low feed cost per kg live weight gain of lambs fed the cellulase enzyme treated wheat and barley straws in the current study suggest economic feasibility of this enzyme preparation and point to its potential for the production of good quality winter feed for ruminants in Mongolia.

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