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EFFECTS OF EXOGENOUS ENZYME PREPARATIONS ON PROTOZOAN POPULATION AND CELLULOLYTIC ACTIVITY IN THE RUMEN OF YEARLING RAMS

N. GRIGOROVA[1], T. SLAVOV[1], P. TODOROVA[2], V. RADEV[1] and I. VARLYAKOV[1]
[1]Trakia University, Faculty of Agriculture, BG - 6000 Stara Zagora, Bulgaria
[2] Institute of Mountain Stockbreeding and Agriculture, BG - 5600 Troyan, Bulgaria

Abstract

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The effects of supplementing diets for yearling rams with exogenous enzyme preparations *Hostazym C 100* and *Hostazym X 100* on the total number of infusoria, their genetic composition and cellulolytic activity in the rumen were investigated. Nine ruminally fistulated yearling rams, divided into three groups of three animals each, were fed diets containing 1kg meadow hay and 1 kg barley-based concentrate, the concentrate of first group consisting of 1kg barley (ration I), the second – 0.8 kg barley and 0.2 kg sunflower meal and the third – 0,8kg barley (ration II) and 0,2kg sunflower expeller (ration III). The study was conducted in three periods – a control and two experimental ones. *Hostazym C 100*, respectively *Hostazym X 100,* was added to the rations during the experimental periods at a dose of 1g.kg^{-1} concentrated feed. *Hostazym C 100* is a multienzyme product and has a predominant endo-1,4-β-glucanase and secondary cellulose, α-amylase, protease and hemicellulase activity. *Hostazym X 100* has a predominant endo-1,4- β-xylanase activity and the same secondary activities. It has been found that: *Hostazym C 100* and *Hostazym X 100* did not affect the studied parameters in the ration with 93.2g.kg^{-1} crude protein and 18 g.kg^{-1} crude fats content (ration I). *Hostazym X 100* increased the total number of infusoria, protozoa count from genus *Dyplodinium* and reduced the rate of *Holotricha* population in the diets with a 115g.kg^{-1} crude protein and 18,0g.kg^{-1} crude fats content (ration II) ($p<0.05$-0.001). The observed effects were lower by adding *Hostazym X 100* in the same ration and at a higher level of crude fat in the diet (ration III) ($p>0.05$ – $p<0.001$). *Hostazym C 100* reduced the degradation of cellulose in ration II ($p<0.001$). *Hostazym X 100* increased the studied indicator in ration II ($p<0.01$) and in ration III ($p<0.05$).

Key words: exogenous enzymes, protozoa, cellulolytic activity, rumen

Introduction

The use of exogenous enzymes in ruminant diets is a widely discussed theme. There are many studies, in which the enzymes benefit feed digestion and improve animal performance (Yang et al., 1999; Kung et al., 2000; Cruywagen et al., 2007; Sivkova, 2007; Flores et al., 2008), but the mecha-

* *E-mail: todor_slavov@abv.bg*

nism whereby those additives work is not clear yet and appears to be very complex. Researchers have shown that the increased feed digestion is likely not simply the result of a supplemental enzymic activity, because the contribution of added exogenous enzymes to total ruminal activity is relatively small (Beauchemin et al., 2001). In a study on the relationship between rumen and exogenous enzymes from Trichoderma longibrachiatum, Morgavi et al. (2000) found that the final hydrolytic effect greatly exceeded the normal enzymic activity in the rumen. The authors attributed that result to the presence of synergistic interaction between exogenous enzyme preparations and rumen microorganisms. Because of enzymatic enrichment of ration, many studies found an increased number of microorganisms, changed composition of microbial population in the rumen (Wang et al., 2001; Nserko et al., 2002; Lee et al., 2004) and promoted microbial adhesion to food particles (Yang et al., 1999; Wang et al., 2001). However, there are only few studies investigating the effect of the exogenous enzyme on the protozoan population. It is known that infusoria represent 40-80 percentages of the total microbial mass in the rumen (Harrison and Mc Allan, 1980) and have an important role in rumen fermentation (Prins, 1990; Hristov et al., 2001). They can provide one third of cellulolytic activity in rumen and predetermine the cellulolytic activity of the rumen bacteria (Allison and Leek, 1993). The number and type of infusoria population are influenced by age and physiological status of the animal, frequency of eating, physical form of ration, and especially type and composition of the fed diet (Willams and Coleman, 1992; Hristov, 2001).

Therefore, the objective of this study was to investigate the effects of exogenous enzyme preparations *Hostazym C 100* and *Hostazym X 100* on the total number and genetic composition of infusoria, and cellulolytic activity in the rumen of yearling rams fed ration with different amount of crude protein and fat.

The multienzyme preparations *Hostazym* C 100 and *Hostazym* X 100 have not been tested in ruminants yet. This study was a part of a larger research to obtain their effect on the digestion, health status, quantity and quality of production in ruminants.

Materials and Methods

The experiment was conducted with 9 male, Blackface Pleven breed, yearling rams having average live weight of 45.2kg at the beginning of the trial. The animals were fistulated on the dorsal sac of rumen, twenty days prior to the experiment, by the method of Aliev (1960). The test yearling rams were grown individually. They were divided into three groups of three animals each and fed a diet containing 1kg meadow hay and 1 kg barley-based concentrate. The concentrate of the first experimental group consisted of 1kg barley (ration I), the second – 0.8 kg barley and 0.2 kg sunflower meal (ration II) and the third – 0.8 kg barley and 0.2 kg sunflower expeller (ration III). The ration was given twice a day – at 8.00 and 13.00 h. The chemical composition of the fed forages is presented in Table 1.

The study was conducted in three periods – a control and two experimental ones. Multienzyme supplements *Hostazym C 100* and *Hostazym X 100* were added to the described rations during the experimental periods at a dose of $1 g.kg^{-1}$ concentrated feed, mixed with concentrate 10 days before feeding. *Hostazym C 100* has a predominant endo-1,4-β-glucanase and secondary cellulose, α-amylase, protease and hemicellulase activity. *Hostazym X 100* has a predominant endo-1,4- β-xylanase activity and the same secondary activities.

Samples of rumen content were taken four days in each period, three times a day – before feeding, 2.5 h and 5 h after feeding. The following parameters were studied: total number and genetic composition of infusoria, and cellulolytic activity. The infusoria were counted by using Fuchs-Rosental

Table 1
Chemical composition of the fed forage, g.kg^{-1}

Forage	Dry mater	Chemical composition, g.kg^{-1} of DM			
		Crude protein	Crude fiber	Crude fat	Ash
Meadow hay	882.0	90.3	283.0	19.0	10.0
Barley	899.0	96.0	50.0	17.0	13.0
Sunflower meal	888.0	325.0	275.0	15.0	57.0
Sunflower expeller	897.0	311.0	169.0	88.0	62.0

counting chamber as described by Petkov at al. (2001). Protozoan genera were identified according to the criteria described by Dogiel (1929). Cellulolytic activity analysis was performed by the Balch and Jonson's method (1950).

The results were processed with a computer package for statistical analysis "Statistic for Windows 7".

Results and Discussion

Total number and genetic composition of infusoria

The data concerning the total number and genetic composition of rumen infusoria is presented in Tables 2 and 3, respectively.

The effect of the studied enzyme products on infusoria total number significantly differed depending on crude protein and fat amount in the rations. No significant effect was observed when adding the enzyme preparations to the first ration (93.2 g.kg^{-1} CP and 18 g.kg^{-1} CF). The investigated multienzyme additives increased the number of infusoria in ration II (116 g.kg^{-1} CP and 17.8 g.kg^{-1} CF) – *Hostazym C 100* in all three hours of study ($p<0.001$), and *Hostazym X 100* only 2.5 h after feeding ($p<0.05$). Both enzyme products increased the count of infusoria before feeding in ration III (114.8 g.kg^{-1} CP and 25.2 g.kg^{-1} CF) ($p<0.05$).

The addition of *Hostazym C 100* to ration I increased the percentage of infusoria of genus *Entodinium,* before feeding and 2.5 h after feeding ($p<0.05$). No statistically significant differences were found in the other two rations and by adding *Hostazym X 100* in the diets. The studied products provoked an increase the percentage of infusoria of *genus Diplodinium* in the rations with higher content of crude protein (ration II and ration III) - *Hostazym C 100* significantly increasing their percentage at the three studied hours in ration II ($p<0.05$-0,001) and 2.5 h after feeding in ration III ($p<0.05$), and *Hostazym X 100* before feeding and 2.5 h after feeding in ration II ($p<0.01$) and at the three studied hours in ration III ($p<0.01 – 0.001$). In this study there was no significant effect of the exogenously applied enzyme preparations on the infusoria of *genera Epidinium* and *Ophryoscolex.*

The enzyme preparations decreased the percentage of infusoria of *genus Isotricha* at the hours after feeding in ration II ($p<0.05 – 0.001$). The tendency was also preserved in the other two rations, but the differences were nonsignificant. The percentage of *genus Dasytricha* changed significantly only under the influence of *Hostazym C 100*. In ration II and ration III was recorded a decrease of the amount of *genus Dasytricha* before feeding and 2.5 h after feeding ($p<0.05$).

The enzymic profile of infusoria of *order Holotricha* shows that they contain amylase, invertase, pectin esterase and polygalacturonidase in a relatively great quantity, which allows them to use the starch, pectin and soluble sugars as a main energy source (Perez, 2004). The cellulose - and hemicellulose-degrading enzymes are mainly contained in infusoria of *order Entodiniomorpha* (Perez, 2004).

Table 2
Effect of *Hostazym C 100* and *Hostazym X 100* on the total number of rumen infusoria

After time of feeding	Group I /Ration I/			Group II /Ration II/			Group III /Ration III/		
	control	+Host. C 100	+Host. X 100	control	+Host. C 100	+Host. X 100	control	+Host. C 100	+Host. X 100
Total number of infusoria (n.10.cm^{-3})									
0h	110.96	125.00	141.50	171.29	293.29***	213.96	131.92	201.42*	214.20*
2.5h	87.08	86.75	82.04	107.38	180.83***	152.54*	101.04	126.42	95.15
5 h	104.08	106.83	105.17	148.58	276.13***	161.75	145.46	165.08	141.95

* - significant difference between control and enzyme-supplemented ration; *-p<0,05; **-p<0,01; ***-p<0,001)

Table 3
Effect of *Hostazym C 100* and *Hostazym X 100* on genetic composition of rumen infusoria

After time of feeding	Group I (Ration I)			Group II (Ration II)			Group III (Ration III)		
	Control	+Host. C 100	+Host. X 100	Control	+Host. C 100	+Host. X 100	Control	+Host. C 100	+Host. X 100
Genus Entodinium (n.100^{-1})									
0h	89.58	94.21*	89.25	91.75	89.54	88.50	89.42	89.30	89.10
2.5h	88.42	95.00*	85.21	90.25	90.00	88.00	87.38	87.54	88.15
5 h	91.46	93.63	87.42	90.75	91.67	81.13	89.88	90.95	86.60
Genus Diplodinium (n.100^{-1})									
0h	3.75	2.29	3.25	1.83	5.17***	6.17**	1.50	4.04	6.40***
2.5h	3.63	2.21	4.21	2.25	6.21***	7.04**	2.21	4.92*	6.95**
5 h	3.46	4.00	3.92	3.21	6.50*	5.79	1.83	3.29	8.45**
Genus Epidinium (n.100^{-1})									
0h	1.92	0.00	4.21	0.00	0.00	0.00	3.08	2.92	0.40
2.5h	4.08	0.00	7.33	0.00	0.00	0.00	4.71	3.67	1.10
5 h	0.96	0.00	4.58	0.00	0.00	0.00	3.00	2.88	0.10
Genus Ophryoscolex (n.100^{-1})									
0h	0.00	0.00	0.13	0.00	0.00	0.00	0.00	0.00	0.00
2.5h	0.00	0.08	0.04	0.00	0.00	0.08	0.04	0.00	0.10
5 h	0.00	0.00	0.00	0.00	0.00	0.96	0.00	0.00	0.10
Genus Isotricha (n.100^{-1})									
0h	3.88	3.36	2.75	6.00	5.29	5.08	5.42	3.71	4.00
2.5h	3.38	2.21	2.88	7.21	3.79**	4.58*	5.08	3.83	3.50
5 h	4.00	2.25	0.58	5.88	1.83***	3.38*	5.04	2.88	3.35
Genus Dasytricha (n.100^{-1})									
0h	1.29	0.13	0.42	0.42	0.00*	0.25	0.50	0.00*	0.10
2.5h	0.42	0.58	0.33	0.25	0.00*	0.29	0.58	0.00*	0.20
5 h	0.13	0.17	0.50	0.17	0.00	0.42	0.25	0.00	0.70

* - significant difference between control and enzyme-supplemented ration; *-p<0,05; **-p<0,01; ***-p<0,001)

The increase of the number of infusoria of *genus Diplodinium* and the decrease of the percentage of *genera Isotricha* and *Dasytricha* in ration II and III found in this study confirmed the view that the effect of the enzyme preparations in ruminant animals is a result of occurring complex interrelationships between the exogenously applied enzyme additive, the available microorganisms and the fed ration. *Hostazym C 100* and *Hostazym X 100* are fibrolytic enzyme preparations and their addition to the ration support the degradation of the complex carbohydrates, increasing also the quantity of the liberated readily available sugars. If their effect after application to the rumen is only hydrolytic, then the enzyme products ought to benefit the multiplication of microorganisms absorbing readily available sugars – in this case – infusoria of *genera Isotricha* and *Dasytricha.* The increase of the percentage of *genus Dasytricha* after addition of the fibrolytic enzymes could be due to promoted adhesion of the microorganisms to the forage particles. It is also possible that due to their proteolytic activity the two enzyme preparations contribute to degradation of the structural proteins in the cell wall and in this way, according to Colombatto and Beauchemin (2008), a possibility is provided to the ruminal microorganisms to hydrolyze the available fiber faster and easily.

When comparing the effect of *Hostazym C 100* and *Hostazym X 100* in the different rations, slightly significant differences were found.

Cellulolytic activity

The degree of cellulose crystallization varies depending on its origin and is most often between 300 and 700 $g.kg^{-1}$ (Wood, 1988). The cotton cellulose, which is used in this analysis, is about 700 $g.kg^{-1}$ crystallized (Bhat et al., 2001). This study followed up the influence of the enzyme preparations on the hydrolysis of not readily degradable crystalline cellulose that is one of the most difficult barriers of the plant walls to overcome by the ruminal microorganisms.

The results of the performed analysis showed that the addition of the enzyme preparations to ration I provoked no statistically significant difference in the studied indicator (Figure 1). *Hostazym C 100* decreased the cellulose degradation in ration II ($p<0.001$), whereas *Hostazym X 100* increased its degradability in ration II ($p<0.01$) and ration III ($p<0.05$).

The cellulolytic enzymes are most generally divided into two types – endoglucanases (EG, E.C.3.2.1.4), degrading the cellulolytic chain internally and exoglucanases (cellobiohydrolases, CBH, E.C.3.2.1.91), that are capable to degrade the crystalline cellulose. Both enzyme preparations in this study contain cellulase (cellobiohydrolase) secondary activity and we expected their addition to the rations to increase the degradation of the crystalline cellulose from cotton threads, as compared to the control groups We supposed also that *Hostazym C 100* would have a stronger effect on the cellulolytic activity due to its leading endo-1,4-β-glucanase activity. Contrary to our expectations, *Hostazym C 100* did not influence the degradation of the cellulose from the externally applied cotton threads and even it decreased the percentage of the studied indicator in ration II, whereas *Hostazym X 100* increased the cellulose degradation in the rations with higher content of crude protein.

It is clear from the obtained results that the exogenously applied enzyme preparations influenced the crystalline cellulose degradation indirectly, most probably through a change in the abundance and ratio of the microbial population.

Nsereko et al. (2002) found that the addition of cellulolytic enzyme products to the ration for dairy cows composed of 52% barley concentrate, 29% maize silage and 19% chopped lucerne hay increased the total number of bacteria that use hemicellulose or secondary products from the cellulose degradation, but they did not influence the number

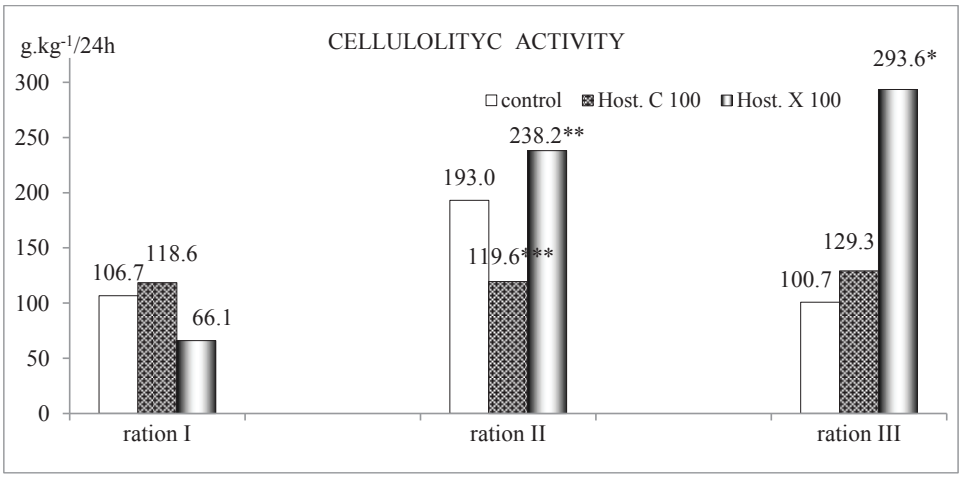

Fig. 1. Effect of Hostazym C 100 and Hostazym X 100 on the cellulolytic activity in the rumen

of cellulolytic bacteria. Similar results in a study of the effect of fibrolytic enzyme preparations in a rumen simulation technique (Rusitec) were obtained by Wang et al. (2001) and Colombatto et al. (2003a).

Colombatto et al. (2003b) assumed that the addition of exogenous enzymes probably provided the hemicellulolytic bacteria with a supplemental growth factor that they use quickly for growth and substrate degradation. The same authors assumed that the effect of the enzymes would be greater when maintaining a higher pH of the rumen content.

Non-cellulolytic bacteria also exist, such as for instance *Prevotella ruminocola*, which when putting them in suitable in vitro conditions degrade native cellulose (Russell and Wilson, 1996).

The conditions of the maximum cellulolytic effect of the rumen fungi are not completely clarified yet. Joblin et al. (1989) reported that the rumen fungi could reach to some not readily available plant polysaccharides more easily than the rumen bacteria. According to Morgavi et al. (2000), possibly in certain conditions the fungi in the rumen show stronger cellulolytic activity than the available bacteria. Wood et al. (1994) found a synergistic effect between cellulase enzymes from *Trichoderma koningii* and the rumen anaerobic fungus *Neocallimastix frontalis* in degradation of the crystallized cellulose.

Conclusion

The results of the present study lead to the conclusion that exogenous products *Hostazym C 100* and *Hostazym X 100* did not affect the studied parameters in the ration with 93.2g.kg^{-1} crude protein and 18 g.kg^{-1} crude fats content (ration I). *Hostazym X 100* increased the total number of infusoria, protozoa count from genus *Dyplodinium* and reduced the rate of *Holotrich* population in the diets with 115g.kg^{-1} crude protein and 18 g.kg^{-1} crude fats content (ration II) ($p<0.05$-0.001). The observed effects were lower by adding *Hostazym X 100* in the same ration and at a higher level of crude fat in the diet (ration III) ($p>0.05 – p<0.001$). *Hostazym C 100* reduced the degradation of cellulose in ration II ($p<0.001$). *Hostazym X 100* increased the studied indicator in ration II ($p<0.01$) and in ration III ($p<0.05$).

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