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The Study of Diversity of Ciliate Protozoa in Ghizel Sheep Fed in Pasture and Nourished by Dried Grape by-Product

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Abstract: Problem statement: Ciliate protozoa are one of the normal microorganisms that found in rumen of both domestic and wild ruminants. Several factors seem to influence the concentration and composition of the protozoal fauna in the rumen. The aim of the present study was to determine the rumen ciliates protozoa diversity in Ghizel sheep of East Azerbaijan province and evaluate effects of alfalfa replacement by multiple level of dried grape by-product (0, 15, 30 and 45% of diet) on ciliate protozoa population. **Approach:** In the first experiment, samples of rumen fluids were collected from 16 mature sheep. Selected sheep were belonged to 4 pure herds of Ghizel sheep in east Azerbaijan province. In the second experiment, sixteen mature Ghizel wether sheep of live weight 34 kg (±1.5) were used. Data obtained from study was subjected to ANOVA as a completely randomized design with 4 replicates by the GLM procedure and treatment means were compared by the Duncan test. **Results:** In experiment 1, different geographical locations affect total number of rumen ciliated protozoa ($p<0.05$), *Entodinium* spp. and *Dasytricha* spp. In experiment 2, the concentrations of *Entodinium* spp., *Diplodinium* spp., *Holotricha* spp. and *Opharyoscolex* species were higher when 15% dried grape by-product (DGB) was included in the diet. With increasing DGB in diets the number of *Epidinium* spp. and Euo*diplodinium* spp. reduced in treatments 2 (15% of diet replaced by DGB) and 3 (30% of diet replaced by DGB) ($p<0.05$). **Conclusion:** The different geographical locations affected total number of rumen ciliated protozoa, *Entodinium* spp. and *Dasytrisha* spp. The concentration of *Diplodinium* was observed to increase when sheep were fed with dried grape by-product.

Key words: Ciliate protozoa, dried grape by-product, East Azarbaijan, Ghizel sheep

INTRODUCTION

The microbial populations in the rumen consist mainly of bacteria, protozoa and fungi that involved in the digestion of feed in the rumen. Hydrolysis of lignocelluloses feeds in the rumen is accomplished by the collective effort of bacteria, protozoa and fungi. Studies on defaunated animals have shown that exclusion of protozoa from the rumen has a beneficial effect on the growth rate, wool growth and feed conversion efficiency of animals under certain feeding conditions[1-5]. Intrinsic factors such as the physiological status of the host (age, health, reproductive period, lactation, social behavior, feeding habits and competition among micro-organisms) influence the diversity of microfauna and the total number of ciliates. Yet above all, extrinsic factors, mainly food, determine the limits of the variability of microfauna. The food factor comprises the chemical composition of the diet, the amount given, the physical nature of the food, the number of meals and the time intervals between meals. Ciliate protozoa are one of the normal microorganisms that found in rumen of both domestic and wild ruminants. Several factors seem to influence the concentration and composition of the protozoal fauna in the rumen. These include type and amount of feed consumed[6], pH, turnover rate, frequency of feeding and feed level[7]. In animals that have been subjected to any kind of feed related stress, such as starvation or rumen acidosis, rumen ciliates may be eliminated[8]. Dogiel Dogiel Noirot-Thimothre (1959,1960) (cited by Ogimoto and Imai[9]) examined the problem of the specificity of the ciliates towards their hosts in a wide comparison of the fauna

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Ophryoscolecidae present in species of various ruminants occupying distinct geographical areas of various sizes. Ciliates follow definite geographical distributions. Some species are attached to one family of ruminants such as *Caloscolex camelis* with *Camelus dromedarus*, whereas others display a very wide dispersal. These differences in dispersal are probably due to the selection exercised by the food substrate chosen by the host, but inter specific competition is also involved. Some species therefore became dominant and cosmopolitan, whereas others are only represented in small numbers over a small area of distribution. Among domestic ruminants, the microfauna also vary depending on the geographical distribution of its hosts.

Ciliate protozoa play a diverse role in the ruminal metabolism of nutrients[8]. To improve the efficiency of feed protein utilization, considerable effort has been made to find a means of total elimination of protozoa from the rumen (defaunation), but a practical defaunation technique has not been established[10]. A massive reduction in the rumen protozoa population (reduced fauna) by chemical drenching of experimental animals has been found to improve milk production[11]. However, such a method to produce reduced fauna is not practical for use in ruminant production. Hristov *et al*.[12,13] tested a large number of substances in vitro and of those examined, tannins, saponin-based plant extracts and linoleic acid were particularly effective at reducing protozoal numbers. These bioactive compounds lowered numbers of protozoa without specifically inhibiting the activity of bacterial populations.

The aim of the present study was to determine the rumen ciliates diversity in Ghizel sheep of East Azerbaijan province and evaluate effects of alfalfa replacement by multiple level of dried grape by-product on ciliate protozoa population.

MATERIALS AND METHODS

Experiment 1: In the first experiment, samples of rumen fluids were collected from 16 mature sheep for studying of protozoa diversity in east Azerbaijan province. Selected sheep were belonged to 4 pure herds of Ghizel sheep in east Azerbaijan province. From each herds 4 sheep separated that they were approximately in same weight, age and condition. Rumen fluids were achieved by using stomach tube for counting protozoa.

Experiment 2 (*In vivo* study): Sixteen mature Ghizel whether sheep of live weight 34 kg (±1.5) were used. The animals were allocated individually in boxes with free-access to salt block and water. Four diets were used, one as basal diet (alfalfa) and the rest as experimental diets (DGB with alfalfa). The sheep were fed twice daily, at 0900 and 1600. For the first week, sheep received alfalfa for ad libitum intake. The amounts of consumed and refused feeds for every sheep were recorded. For the second and third week, DGB replaced 0, 15, 30, or 45% of the alfalfa DM. On the last day at 2 h after feeding, the digesta samples collected and were bulked for counting of rumen ciliate protozoa, VFA[14] and NH_3-N analyses.

Dried grape by-product: Dried grape by-product was obtained from raisin production factories of Tabriz, Iran. The DGB that was collected contained grape cluster stems and rejected raisins.

Chemical composition: Feedstuffs dry matter (DM, method ID 934.01), ash (method ID 942.05), ether extract (EE, method ID 920.30) and crude protein (CP, method ID 984.13) were determined by procedures of AOAC[15]. The NDF and ADF concentrations were determined using the methods of Van Soest *et al*.[16] without sodium sulphite. Total Phenolics (TP) were measured using the Folin Ciocalteau method[17]. Total Tannin (TT) was determined after adding insoluble polyvinylpyrrolidone and reacting with Folin Ciocalteau reagent[17]. Tannic acid was used as the standard to express the amount of TP and TT.

Total numbers and generic composition of ciliate protozoa were determined according to the procedures described by Dehority[18].

Statistical analysis: Data obtained from study was subjected to ANOVA as a completely randomized design with 4 replicates by the GLM procedure[19] and treatment means were compared by the Duncan test.

RESULTS

Experiment 1: In this study, 7 species of rumen ciliated protozoa were detected, identified and counted. Five species were belonged to the entodinomorphid family and they were *Entodinum*, *Epidinium*, *Diplodinium*, *Eudiplodinium* and *Opharyoscolex*. The other species were Isotricha and *Dasytricha* and belonged to Holoticha family.

The concentration ciliated protozoa in rumen of 16 experimental Ghizel sheep of East Azerbaijan of Iran ranged from 8.1×10^5-56.81×10^5 mL^{-1} (Table 1).

Based on analyzed data, all species of protozoa that expected to be counted in the samples were observed only in the samples of Osko area and samples of other areas had some of protozoa species.

Table 1: Effect of different geographical locations (East Azarbaijan) on rumen ciliated protozoa ($n\times10^6 mL^{-1}$)*

Items	Osko	Malekan	Sofiyan	Benab	SEM
Total protozoa	5.4690[a]	5.6810[a]	3.400[ab]	0.8130[b]	0.9747
Entodinomorph	2.3510[a]	2.0343[a]	1.237[b]	0.2781[c]	0.2475
Holoticha	0.2093	0.1812	0.1281	-	0.0351
Entodinium	4.1750[a]	3.4928[ab]	2.175[b]	0.4938[c]	0.4499
Epidinium	0.1312	0.3750	0.2625	-	0.0757
Diplodinium	0.0583	0.1375	-	0.0625	0.0421
Opharyoscolex	0.0562	-	0.0365	-	0.0117
Eu*diplodinium*	0.2812	0.0625	-	-	0.0825
Isotrisha	0.3688	0.1812	0.2063	-	0.0605
Dasytrisha	0.0500[b]	0.1812[a]	0.0500[b]	-	0.0319

*: The means within a rows without common letter differ (p<0.05)

Table 2: The chemical composition of feeds ($g\ kg^{-1}$ DM)

Feeds	DM	CP	NDF	ADF	Crude fat	OM	Total phenols	Total tannins
Alfalfa	931.4	122.3	548	436	28.0	930	-	-
Dried grape by-product	884.5	63.5	259	255	11.2	926	67	52.3

DM: Dry matter; CP: Crud Protein; NDF: Neutral Detergent Fiber; ADF: Acid Detergent Fiber; OM: Organic Matter

The minimum number of protozoa was observed for samples of Benab area and only ciliates were observed belonged to *Entodinium* orphid and ciliated protozoa that belonging to Holoticha wasn't observed. In the samples of this area, only species of *Entodinium* spp. and *Diplodinium* spp. were detected.

As we expected, *Entodinium* spp. were the highest ciliated protozoa that recognized, because *Entodinium* is dominant ciliated protozoa in the rumen in the normal conditions. There was significant difference between samples of different area for *Entodinium* (p<0.05). So highest numbers were belonged to Osko and Malekan areas and lowest number belonged to Benab area. Difference between other species of rumen Entodinom orphid ciliated protozoa were not significant (p<0.05).

In the ciliated protozoa that belonged to the family of Holoticha, there was no significant difference. But difference between number of *Dasytricha* was significant (p<0.05) and the highest number of *Dasytricha* was counted in the samples of Malekan area.

Experiment 2: The chemical compositions of feedstuffs are presented in Table 2. The CP, ADF, NDF and ash contents in DGB were 63.5, 255 and 74 g kg^{-1}, respectively. This shows that the DGB has greater levels of phenolic compounds compared with grape pomace.

Total numbers and distribution of ciliate protozoa in ruminal fluid from sheep fed diets containing alfalfa and DGB ($n\times10^6 mL^{-1}$) are shown in Table 3. Average total protozoal concentration increased when sheep were fed alfalfa plus dried grape by-product, but it was not significantly differ (p>0.05). Average total protozoal concentration in sheep fed only alfalfa was lower but not different from the other test diets. Within the given diets, there were no differences among sheep in total ruminal protozoa concentrations, but three of the treatments (numbers 2, 3 and 4) had a lower (p<0.05) average ruminal pH when fed the alfalfa plus dried grape by-product. Average ruminal pH decreased as the dried grape by-product percentage in the diet increased (6.41, 6.22, 6.14 and 5.84 for the all-alfalfa, 15 dried grape by-product, 30 dried grape by-product and 45% dried grape by-product diets, respectively). The concentration of *Diplodinium* was observed to increase when sheep were fed with dried grape by-product. No differences were found in either the concentration of *Entodinium* spp., *Holotricha* or *Opharyoscolex* species between the treatments. The concentrations of *Entodinium* spp., *Diplodinium* spp., *Holotricha* and *Opharyoscolex* species were higher when 15% dried grape by-product was included in the diet. With increasing DGB in diets the number of Epidinium and Euo*diplodinium* reduced in treatments 2 and 3 (p<0.05).

Table 3: Total numbers and distribution of ciliate protozoa in ruminal fluid from sheep fed diets containing alfalfa and DGB ($n\times10^6 mL^{-1}$)

	Treatments*				
Items	1	2	3	4	SEM
Entodinium	0.877780	1.041670	1.004170	0.988890	0.058
Diplodinium	0.050000	0.119440	0.087500	0.116670	0.029
Epidinium	0.061110[a]	0.000500[b]	0.025000[ab]	0.074070[a]	0.017
Holotricha	0.022220	0.044440	0.022920	0.022960	0.013
Euo*diplodinium*	0.016667[a]	0.000005[b]	0.000004[b]	0.011111[a]	0.003
Opharyoscolex	0.005556	0.011111	0.004167	0.000040	0.004
Total	1.033330	1.216670	1.143750	1.214810	0.057

*: The means within a rows without common letter differ (p<0.05). Treatments: 1 = 0% DGB, 2 = 15% DGB, 3 = 30% DGB and 4 = 45% DGB

Table 4: Rumen fermentation parameters in treatments

	Treatments				
Items	1	2	3	4	SEM
pH	6.41[a]	6.22[ab]	6.14[b]	5.84[c]	0.071
VFA (mmol)	103.70[a]	102.00[a]	97.20[b]	72.2[c]	1.160
$N-NH_3$ ($mg\ L^{-1}$)	130.00[a]	103.30[b]	77.50[c]	65.3[d]	2.350

[a,b,c and d]: Within a row, means without a common superscript letter differ (p< 0.05). Treatments: 1 = 0% DGB, 2 = 15% DGB, 3 = 30% DGB and 4 = 45% DGB

The concentrations of VFA and NH_3-N in the control treatment were greater than in the other treatments (Table 4; p<0.05).

DISCUSSION

Differences in protozoan populations due occur both among different ruminant species and different geographical locations[8]. This study indicated that different geographical locations affect total number of rumen ciliated protozoa, *Entodinium* spp. and *Dasytrisha* spp.

The concentration of protozoa in ruminal contents generally increases with the addition of concentrates to roughage diets[20-22] and the results of present study (Table 3) were in convenient with above reports.

The concentrations of VFA and NH_3-N in the control treatment were greater than in the other treatments. The difference in VFA and NH_3-N concentrations between treatments may have occurred because of a lower rate of fermentation (inhibition of microbial activity) as a result of tannin content.

Priolo *et al*.[23] reported the greater ruminal ammonia and a VFA concentration in PEG-vs. tannin-fed sheep indicates more rapid ruminal fermentation when PEG was given.

It seems that in addition to the lowering of ruminal pH, which occurs in all animals as a result of feeding high-concentrate diets, several other factors are involved in defaunation. These factors could include rate of feed consumption, rate of passage and salivary production. From the present study, these other factors seem to vary between individual animals and may be the determining factors of whether protozoa survive in the rumen.

CONCLUSION

The different geographical locations affect total number of rumen ciliated protozoa, *Entodinium* spp. and *Dasytrisha* spp. The concentration of *Diplodinium* was observed to increase when sheep were fed with dried grape by-product.

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