The Influence of Some Nutritional Factors and of Some Biophysical Parameters on the Ruminal Fluid Infusorians

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Abstract
The microflora and the microfauna that inhabit the stomach compartments, develop under conditions of physiological constants closely related, under the control of regulating mechanisms such as: the thermoregulator system, anaerobiosis condition, nutrient substrate, ionic composition, (pH) buffer system. Any change in these constants leads to a decrease or increase of microorganisms. The experiment had in view to study the influence of Yea Sacc^{1026} yeast on ruminal infusorians, on some biophysical parameters and the correlations between them. In all experimental variants, the protozoa density was higher than in the control sample and the amount of small protozoa, like Entodinium, with over 95% of the total protozoa. It was ascertained that for all experimental variations there are very small fluctuations between viability values and depending on the time of harvest, the medium viability values was close. When yeast was present in the feed ration, the density of protozoa has a moderate influence on the pH of ruminal fluid (R = 0.7056).

Keywords: rumen, sheep, protozoa, yeast

1. Introduction

The microflora and the microfauna that inhabit the stomach compartments, develop under conditions of physiological constants closely related, under the control of regulating mechanisms such as: the thermoreglator system, anaerobiosis condition, nutrient substrate, ionic composition, (pH) buffer system. Any change in these constants leads to a decrease or increase of microorganisms.

The experiment had in view to study the influence of Yea Sacc^{1026} yeast on ruminal infusorians, on some biophysical parameters and the correlations between them.

2. Materials and methods

In the experiments, we used a 40 kg ram, provided with a ruminal cannula. The ram was fed 12 days with ratios based on alfalfa hay 100% (M) and after that with rations with various proportions of barley (R_1=20%; R_2=40%; R_3=60%; $R_{1'}$=20% + Yea Sacc^{1026}; $R_{2'}$=40% + Yea Sacc^{1026}; $R_{3'}$=60% + Yea Sacc^{1026}). 1.5 g/day live yeast *Saccharomyces cerevisiae Yea Sacc^{1026} strain,* obtained from Alltech Company, was added to the rations. Water was administrated abundant.

The ruminal content was harvested every 2 days, before feeding and at various moments after feeding (at 2, 3 and 5 hours) and immediately was determined the ruminal density of infusoria, the viability, the identification of protozoa species using standard methods [1].

Measurements were made in infusion physiopathology laboratory by direct counting slide and slide the magnifying stereoscopic, number of infusoria (N) by calculating with the formula:

$$N = \frac{n \cdot 5 \cdot 1000}{3.2}$$

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where: N = number of infusoria per mm^3; n = number of counted infusoria; 5 = dilution; 3.2 = room volume (cm^3); 1000 = correction coefficient (cm^3 /mm^3). Density and proportions of genera of ruminal protozoa were determined by counting and identification under a microscope, ob. 10, ruminal fluid fresh.

Protozoa viability was assessed as follows: fresh ruminal fluid was filtered through four layers of cheesecloth, a drop was taken, deposited on a heated glass slide and covered with a warmed slide and then examined with the objective of 20 or 40. Infusoria movement and counting fall into one of the following five categories:

1 – many infusoria, all live with lively movements

2 – many infusoria some dead, less lively movements;

3 – fewer infusoria, 50% dead, with slow movements;

4 –very few infusoria, for 50% of deaths, with slow movements or still;

5 – absent infusoria or all infusoria are dead.

The laboratory determinations of the protozoa were carried out in the Laboratory of Pathophysiology.
The chemical composition of the diets was determined using standard methods (DM – drying in oven at 105°C, CB - Kjeldahl method, Ash-sample calcination between 450°C şi 600 °C).
The chemical analysis of the nutritive value was carried out in the Laboratory of Nutrition.
Statistical program used was origin 3.0.

3. Results and discussion

The chemical composition of the fodder used in the structure of the diets is presented in Table 1.
In Tables 2 and 3 are presented the total number of protozoa / mL of ruminal fluid and the gender structure of ruminal protozoa.
In the case of addition of the Yea Sacc^{1026} yeast in the three types of ration, it was observed a decrease in the number of protozoa compared to where no yeast is added.
In the case of an addition of yeast Yea Sacc^{1026} in the three types of rations, we observed a decrease in the number of protozoa compared with the case when yeast was not added.

Table 1. Chemical composition of the fodders (%)

Specification	DM (%)	CP (%)	OS (%)	Ash (%)
Alfalfa hay	89.40	13.51	80.73	8.67
Barley	90.08	10.69	87.73	2.35

Table 2. Density of the ruminal protozoa (protozoa/mL of ruminal fluid) and the proportion of the genera of protozoa in the sheep ruminal fluid depending on the diet type at different time intervals from the feeding

Diet type	sampling time (hours)	viability	protozoa/mL of ruminal fluid	genera of protozoa					
				1.	2.	3.	4.	5.	6.
M	0	3	321312.5	96.09	0.48	-	-	2.92	-
	2	2/3	212500	97.11	-	-	-	0.88	-
	3	2/3	339062.5	98.41	-	-	-	1.58	-
	5	3	220312.5	96.44	0.44	-	-	-	-
R_1 = 20%	0	3	362500	96.27	0.46	-	0.46	2.32	0.46
	2	2	462500	98.01	-	-	-	1.48	0.49
	3	3	359375	98.29	0.85	-	-	2.85	-
	5	2	252125	94.78	-	-	-	3.79	1.42
R_2 = 40%	0	2	1909375	99.11	0.44	-	-	0.44	-
	2	1	1121174.5	98.14	0.46	-	-	0.46	0.92
	3	3	342187.5	98.65	0.44	-	-	0.44	0.44
	5	2	521875	94.32	0.35	-	-	2.83	2.83
R_3 = 60%	0	1	2512500	100	-	-	-	-	-
	2	1	1929687.5	97.22	0.92	-	-	0.92	0.92
	3	2	1545312.5	99.04	-	-	-	0.95	-
	5	1	1528125	98.26	0.2	-	-	1.43	0.1

1.=Entodinium; 2.=Diplodinium; 3.=Epidinium; 4.=Ostracrodinium; 5.=Dasytricha; 6.=Isotricha

Table 3. The effect of the Yea Sacc[1026] yeast on the ruminal protozoa density (protozoa/ml of ruminal fluid) and the proportion of genera of protozoa in the ram ruminal fluid, depending on the diet at different moments from feeding

Diet type	sampling time (hours)	viability	protozoa/ml of ruminal fluid	genera of protozoa					
				1.	2.	3.	4.	5.	6.
R`$_1$ = 20%+1.5 g SC	0	4	778125	95.65	1.73	-	-	0.86	1.73
	2	4	153125	89.07	1.68	-	-	7.56	1.68
	3	4	315625	89.32	2.42	-	-	9.22	1.45
	5	3/4	489062.5	95.15	2.96	-	-	2.4	0.48
R`$_2$ = 40%+1.5 g SC	0	4	1295312.5	97.70	1.37	-	-	0.45	0.45
	2	3/4	617817.5	90.90	4.78	-	-	2.39	1.91
	3	3	365625	95.61	1.75	-	-	1.75	0.87
	5	3	289062	98.73	-	-	-	0.84	0.42
R`$_3$ = 60%+1.5 g SC	0	2/3	1142187.5	95.21	1.43	-	-	0.95	2.39
	2	2/3	934375	96.35	1.09	-	-	0.36	2.18
	3	2	757812.5	98.69	-	-	-	0.32	0.97
	5	3	962500	96.20	1.68	-	-	0.87	1.26

1.=Entodinium; 2.=Diplodinium; 3.=Epidinium; 4.=Ostracrodinium; 5.=Dasytricha; 6.=Isotricha

Tables 2 and 3 also show that the average density of protozoa in the ruminal fluid was 321312.5 in M and 362500 in R_1 at sampling time 0. Before feeding R_2 and R_3 values are 1909375 and 2510500. In the case of yeast Yea-Sacc [1026] addition (1.5 g / day), the values for R`$_1$ was 778125, for R`$_2$ was 1295312.5 and for R`$_3$ was 1142187.5. In all experimental rations ($R_1$, $R_2$, $R_3$, R`$_1$, R`$_2$, R`$_3$) the density of protozoa was higher than in control sample, accordance with the literature data.

The structure of the protozoa types is dominated in M in small protozoa, like Entodinium with over 95% of the total protozoa. For R_1, R_2, R_3, the small protozoa represent over 97%. R`$_1$, R`$_2$, R`$_3$ show a slight reduction in the percentage of small protozoa and especially Entodinium species, probably as a result of the addition of yeast in the ration: from 2512500 ($R_3$) before feeding to 11421875 (R`$_3$) before feeding and from 1909375 (R_2) to 1295312.5 (R`$_2$). Literature data concur that the highest ratio of Entodinium gender of the protozoa population is especially highest in the dorsal sack (DS) of the rumen.

Figure 1 shows the dorsal rumen sack in the case of 40% barley proportion. Through folding the mucous generates large and narrow conical papillae with an average height of about 1140 µ middle papillae 712.5 µ and small papillae 408.5 µ. The mucous, free from glands, is lined with a cornified multilayered epithelium, with the thickness of approx. 150 µ (laterally), 112.5 µ (towards the base of the papilla) and 100 µ (at the tip of the papilla). The epithelium stands on a slightely curled basal membrane. The cornification process of the epithelium is obvious on the lateral sides and at the tip of the papillae. The corion is represented by connective lax tissue formed from fine and condensed collagen fibres, among which display themselves numerous fibroblasts and cellular elements with defensive mechanisms. In the corion from the ax of the papilla, blood vessels can be observed, which penetrate the folds generated by the basal membrane.

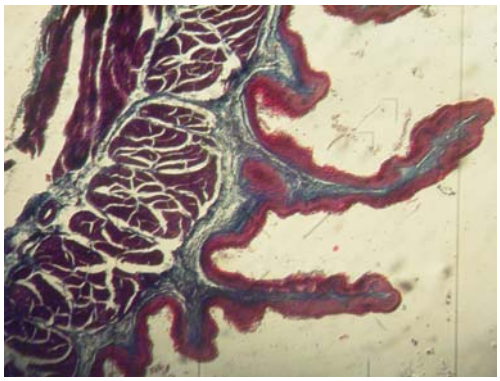

Figure1. Dorsal ruminal sac LE (Tricromic Mallory-Ob. 4)

The basal corion, well represented, contains collagen fibres, elastic fibres, numerous fibroblasts and blood vessels with a large lumen.
The muscular tunica is organized on two layers of smooth muscular cells: the internal layer is oriented longitudinally (more developed) and the external layer is oriented circularly [2].
It is noted that for all experimental variations there are very small fluctuations between viability values and by the time of harvest, medium viability values are close.
Very high values of protozoa could be related to the increased viscosity with the increase of grain proportion. No literature data have been found regarding such studies, but there are studies regarding the relationship between ruminal bacteria growth and increased ruminal fluid viscosity [3], that suggested that an increase of ruminal fluid viscosity and "in vitro" results appear from lyses of bacterial cells, which release deposits/accumulations of carbohydrates, proteins and other intracellular components in the rumen fluid [4] and heavy residue production by surviving bacteria [5,6,7,1,8,9].

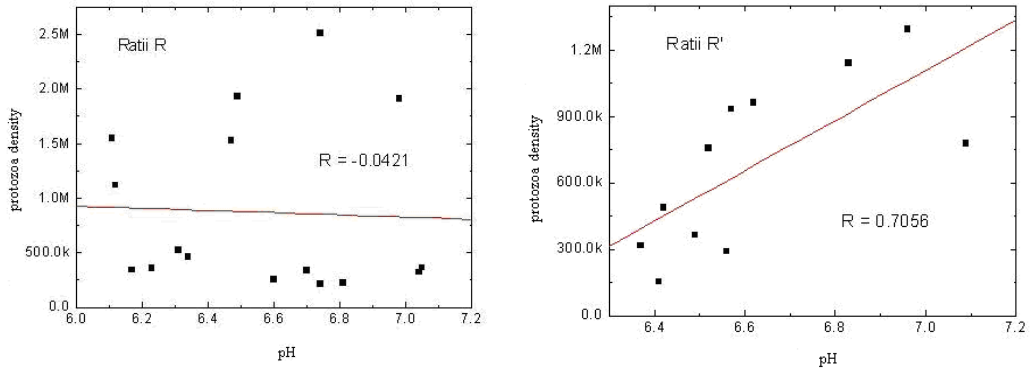

Figure 2. The influence of the protozoa density on the pH of the ruminal fluid for the R and R' feeding diets

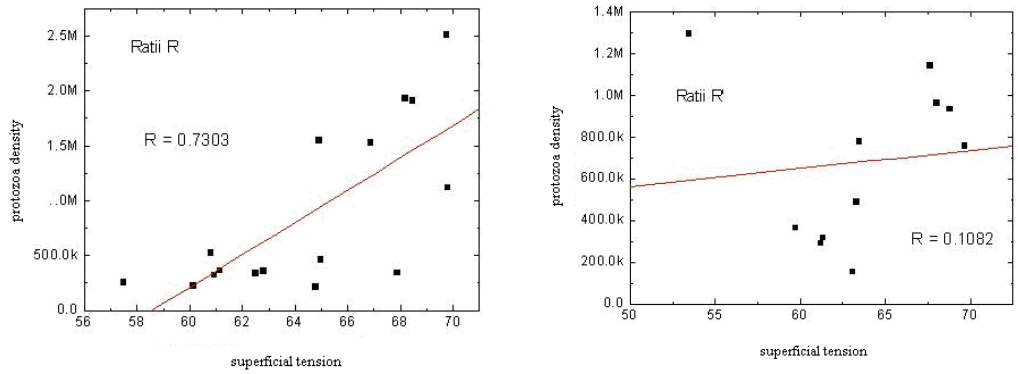

Figure 3. The influence of the protozoa density on the surface tension of the ruminal fluid for the R and R' feeding diets

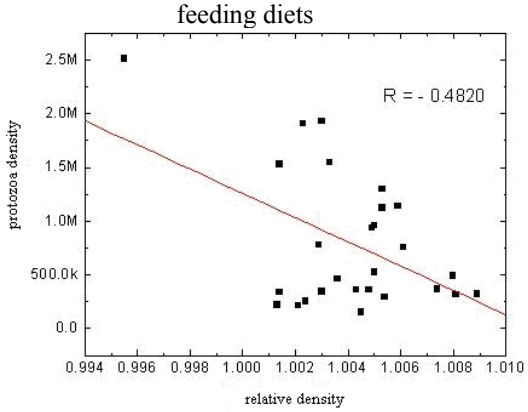

Figure 4. The influence of the protozoa density on the relative density of the ruminal fluid for the R and R' feeding diets

The influence of protozoa density on pH (Figure 2) and the surface tension of ruminal fluid (Figure 3) were treated separately for R, respectively R' rations. Ruminal fluid pH in the absence of yeast in fodder ration is unaffected by the density of protozoa (R = - 0.0421). However, in the case of the presence of yeast in the diet, the density of protozoa has a moderate influence on the pH of ruminal fluid (R = 0.7056).

Surface tension is moderately influenced by the density of protozoa (R = 0.7303) from ruminal fluid (Figure 3). That may explain the increasing surface tension at an increase of the barley proportion in the diet (Table 2.). However, in the case of yeast presence in the fodder ration, there is no correlation between the protozoa density and the surface tension.

The influence of protozoa density on the relative density of the ruminal fluid (Figure 4) was studied statistically for all experimental points offered by both types of fodder rations. The protozoa density has low influence on ruminal fluid (R =- 0.4820).

4. Conclusions

1. In the case of all types of fodder rations, the protozoa density was higher than in control sample and it increased with increasing the grain proportion from the ration: from 320312.5 (M) to 2510500 (R_3) before feeding and from 212.500 (M) to 1929687.5 (R_3), at 2 hours from feeding.
2. On the genera structure of protozoa, a small protozoa was prevailed, in particular Entodinium genera and for all types of ration was over 95%.
3. From the total protozoa populations, in R_1, R_2, R_3 small protozoa represent over 97% and in $R`_1$, $R`_2$, $R`_3$ we observed a slight reduction in the proportion of small protozoa and especially Entodinium genera.
4. It is noted that for all experimental variations there are very small fluctuations between viability values and by the time of harvest, medium viability values are close.
5. Ruminal fluid pH in the absence of yeast in fodder ration is unaffected by the density of protozoa (R =- 0.0421). However, in the case of the presence of yeast in the ration of fodder, the density of protozoa has a moderate influence on the pH of ruminal fluid (R = 0.7056).
6. Surface tension is moderately influenced by the density of protozoa (R = 0.7303) from ruminal fluid at the fodder rations without yeast in them.
7. However, in the case of yeast presence in the fodder ratio, there is no correlation between protozoa density and surface tension.

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