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Rumen *Protozoa* of adult bovine during autumn in the east of Algeria

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Abstract. The two main pregastric compartiments of ruminants: rumen and reticulum work as anaerobic fermenter which allows them to use energetic and nitrogen substrates which are indigestible by monograstric animals. They have a major importance in this fermenter, also these microbes : bacteria, protozoa and fungi, on the degradation of cell-wall carbohydrates: cellulose, hemicelluloses, pectic substances, on nitrogen digestion and finally on the utilisation of the major minerals and trace elements. The objective of this study is to identify and quantify the protozoa populations in the rumen of Algerian bovines local breed, adult animals living in a relatively humid climate, basic ration based on straw and pastures during hot days. Whereas in cold weather rations are based on straw, cereals and grass forage. Usually not 2 to 5 × 10^6 ml of rumen containing organisms however, under certain feeding conditions, they represent at least 50 % of the rumen microbial biomass. Due to the difficulties in cultivate *in vitro*, their role and their metabolism are less known than those of bacteria. Ciliated protozoa are capable of transforming a large number of food and bacterial constituents in metabolites and cellular compounds which will then be used by the host animal. The importance of these activities is however still very controversial since in the absence of protozoa in the rumen, Isolation carried out on rumen fluid sampled of 20 cows. Rumen fluid was diluted in formaldehyde 10 % solution, and distributed in Sedgewick Rafter chamber. Then observed in optical microscope using Lugol's iodine coloration. Classified in three genders according to size. 11 different genders were identified; a considerable population of rumen protozoa was identified and classified for first time on Algerian local breed (Atlas).

Keywords: Algeria; bovine; climate; gender; *Protozoa*; rumen.

Склад мікрофлори рубця великої рогатої худоби сходу Алжиру в осінній період

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Анотація. Завдяки багатокамерному шлунку, зокрема його основних відділів рубця і сітки, жуйні тварини можуть використовувати енергентичні і азотні компоненти корму, що не засвоюють тварини з однокамерним шлунком. Основну функцію перетравлення при цьому, виконує мікрофлора – бактерії, найпростіші та гриби, які забезпечують руйнування і засвоєння вуглеводів клітинної оболонки рослин: клітковини, геміцелюлози, пектинових речовин, а також метаболізмі азоту, мінеральних речовин і мікроелементів. Мета цього дослідження – виявлення і кількісне оцінювання популяцій найпростіших у рубці дорослії великої рогатої худоби алжирської місцевої породи у відносно вологому кліматі. За жарких погодних умов раціон тварин складається з соломи і випасу, в той час, як за холодних – солома, концентрати і трава. Зазвичай у рубці міститься від 2 до 5 × 10^6 мл найпростіших, проте за певних умов годівлі вони становлять щонайменше 50 % біомаси вмісту. Через труднощі культивування найпростіших *in vitro* їх роль у метаболізмі менш відома, ніж бактерій. Найпростіші можуть трансформувати більшу кількість харчових і бактеріальних компонентів у метаболіти та клітинні сполуки, які у подальшому використовує тварина-господар. Дослідження проведено на 20 коровах. Відібраний уміст рубця розбавляли у 10 % розчині формаліну і розподіляли у камері Sedgewick Rafter. Під світловим мікроскопом ідентифікували та описали 11 різних родів найтростіших. Вперше ідентифікована і класифікована популяція найпростіших рубця місцевої алжирської породи великої рогатої худоби (Atlas).

Ключові слова: Алжир; велика рогата худоба; клімат; стать; *Protozoa*; рубець.

Introduction

Algeria has one local bovine breed, Known as Brown Atlas, which has a population of about 1.4 million head (Bouzebda, 2007). Different species of microorganisms presents in the rumen present interactions allow the fermentation, in which rumen ciliates were the first microorganisms to be described in this environment (Guyader et al., 2014). The ciliates They mainly belong to two groups the

Holotrichs and the Entodiniomorphs. Among the holotrichs, the genera lsotricha and Dasytricha: family of lsotrichidae are the most abundant; among the *Entodiniomorphs*, they are the genera *Entodinium*, *Diplodinium*, *Epidinium*, *Ophryoscox*, *Polyplastron* and *Eudiplodinium* which are the most frequent (Hungate, 1966; Grain, 1967; Coleman, 1967; Fonty et al., 1986). Flagellates They have been the subject of only a very limited number of studies and their role in the ecosystem is unknown. Only five species were described: *Monocercomonas ruminantium*, *Monocercomonoides caprae*, *Chilomastix caprae*, *Tetratrichomonas buttreyi* and *Pentatrichomonas homimis*. Three genera: Callimastix, Sphaeromonas and Piromonas originally identified as flagellated protozoa are actually zoospores of fungi phycomycetes (Orpin et al., 1971). Flagellates are particularly abundant in the rumen of young ruminant during the period before the appearance of ciliates (Eadie, 1962). In adults, due to their low number (< 105 / ml) and their reduced size, their role in the rumen is insignificant compared to that ciliates and bacteria. With a population that varies between 104 and 106 protozoa per ml of rumen content and these eukaryotes can represent up to
50 % of the rumen bi-mass. Allow also the degradation of forage cell wall which is essential for ruminant complete digestion, refers to the importance of enzymes and microorganisms; bacteria, protozoa and fungus (Russell & Rychlik, 2001). Any imbalance may lead to digestive disorders, the protozoa elimination can increase microbial protein supply and reduces methane production (Guyader et al., 2014), protozoa's population was identified and well-studied in many cattle breeds around the world (Agarwal et al., 2015), since they were first discovered by Gruby and Delafond (1843), studies on rumen protozoa have relied on morphologic identification by optical microscopy. There are currently no cultures collections of rumen ciliates, so researchers have to use photomicrographs for identification (Williams & Coleman, 1997) or line drawings (Dehority, 2017). No study has been yet published about Algerian local bovine rumen protozoa. In this research, we aim to identify and quantify the protozoan population present in the rumen of adult bovine raised during autumn.

Material and methods

This study was carried out in the laboratory of Parasitology, University of Mohamed Cherif Messaâdia, Souk Ahras, Algeria. Rumen fluid was collected from 20 cows fed on extensive pastures of *Alfalfa spp*, and barley crop residues, in addition of low diary quantity of cereals (IFB 249). The daily rumen pH values were about 6.45 in studied animals, these values were higher than those reported by Williams & Coleman, (1997). So, the extension of rumen pH over the 24 hour is probably the most relevant factor in the survival of ciliate protozoa in the rumen. Cows were older than 5 years and were multiparous, and were lactating. The study was carried out in the region of Souk Ahras in the Algerian east 36° 17′ 15″ north, 7° 57′ 15″ east. In Mediterranean climate, bit humid. Ruminal fluid was collected between late August and early December. After 8 h fasting, bovines were presented and immobilized in order to collect rumen fluid using esophageal tube with manual pipetting. Approximately 15 ml of rumen fluid was collected and received in waterproof container. Samples were transported in insulated boxes and stored for up to 1 h in a sealed sterile test tube kept at 4 °C. Tubes were homogenized in a vortex for 1 min, and an aliquot of 1000 µL was transferred to tubes containing 9 ml of 10 % formalin. Counting chambers (S52 glass; Pyser-SGI, Edenbridge, Kent, UK). The numbers of small (up to 40 × 60 µm), medium (up to 100 × 150 µm) and large (larger than 100 × 150 µm) ciliates per ml of rumen fluid were determined by light microscopy at 10 × magnification (Finlay et al., 1994) For protozoa identification, subsamples were placed on slides with cover slips with a drop of Lugol iodine solution (D'Agosto & Carneiro, 1999). The identification was performed in the optical microscope at 40 × objective to characterise protozoa (Rufino et al., 2011).

Results

In this study, protozoa sizes as well as their genders were determined on the population of rumen raised in autumn .Populations of small protozoa were the highest while the concentration of large protozoa was the lowest. The small ciliate protozoa are the most resistant while the largest ones are the most sensitive. Like this, the lower concentration of large ciliates observed in cows evaluated in this study must be justified by nutrition conditions with mature pasture, composition of the diet offered to the young animal influences directly the rumen development (Table 1).

Table 1. Protozoa proportion according to size

Category	Small	Medium	Large	Total	CV
Gender	8.61×10^4	3.31×10^4	2.64×10^4	14.56×10^4	2.36
CV (%)	9.85	7.65	10.45	9.10	

Note: CV – coefficient of variation

On the 20 female bovines evaluated, we identified 11 genera, with variable concentrations; *Charonina spp* with concentration of 36.97 %, *Entodinium spp* with a concentration of 17.00 %, *Eodinium spp* with a concentration of 15.12 %, *Isotricha spp* with concentration of 12.44 %, *Diplodinium spp* with a concentration of 5.65 %, *Buetschilia spp* with a concentration of 6.49 %, *Eremoplastron spp* with a concentration of 3.74 %, *Ostracodinium spp* with a concentration of 1.74 %, *Ophryoscolex spp* with a concentration of 0.37 %, *Elytroplastron spp* with a concentration of 0.20 %, and finally *Caloseolex spp* with a concentration of 0.29 % Ciliates of *Charonina spp* (Fig. 9), *Isotricha spp* (Fig. 5) and *Entodinium spp* (Fig. 3), *Eodinium spp* (Fig. 2) and *Diplodinium spp* (Fig. 6), *Buetschilia spp* (Fig. 1), *Eremoplastron spp* (Fig. 7), *Ostracodinium spp* (Fig. 8), *Ophryoscolex spp* (Fig. 4), *Elytroplastron spp* (Fig. 10), *Caloseolex spp* (Fig. 11).

The diversity of proportions shown in, indicate a healthy ruminal environment, that no gender in dominating the others, and huge number of protozoas indicate that the healthy nutrition token even in dry periods is adequate to keep the rumen in healthy situation, The most frequent genus was *Charonina spp* (36.97 %), followed by *Entodinium spp* (17.00 %) and *Eodinium spp* (15.12 %). In this research, *Entodinium spp* was identified in high proportion (Table 2; Fig. 12).

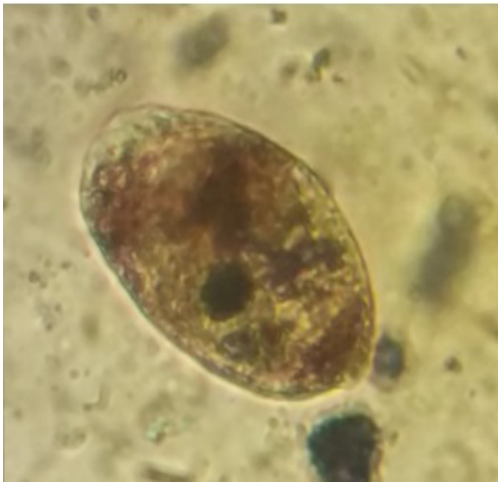

Fig. 1. Genus *Buetschilia*

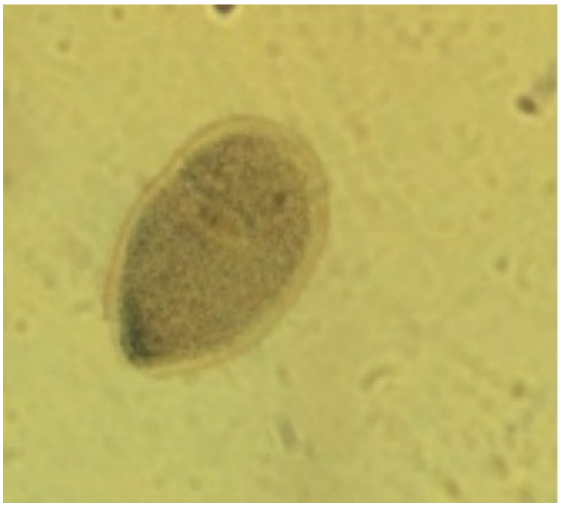

Fig. 2. Genus *Eodinium*

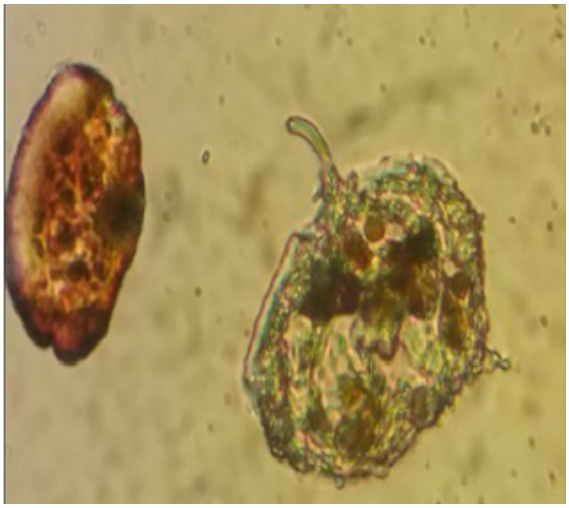

Fig. 3. Genus *Entodinium*

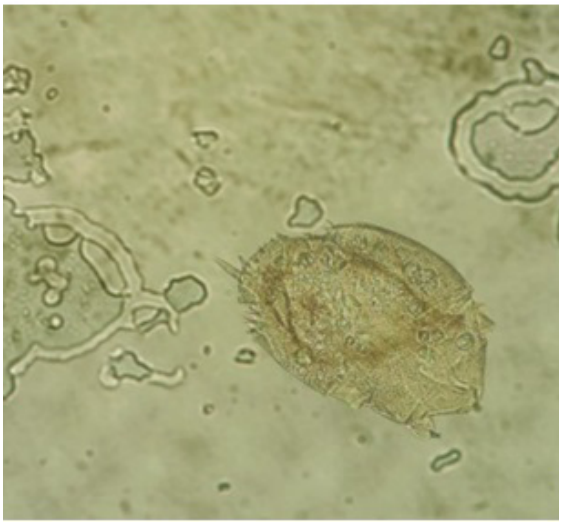

Fig. 4. Genus *Ophryoscolex*

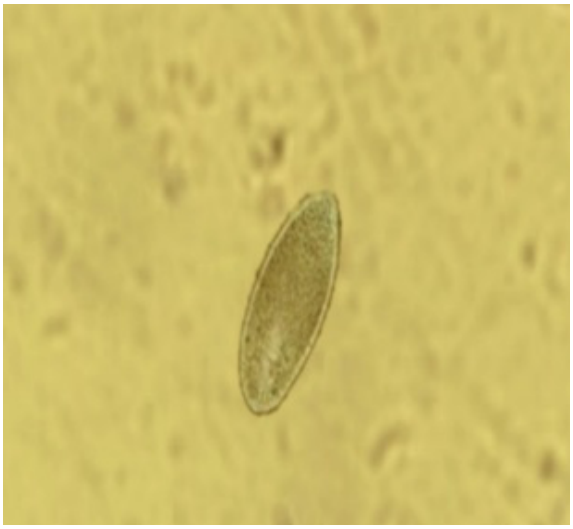

Fig. 5. Genus *Isotricha*

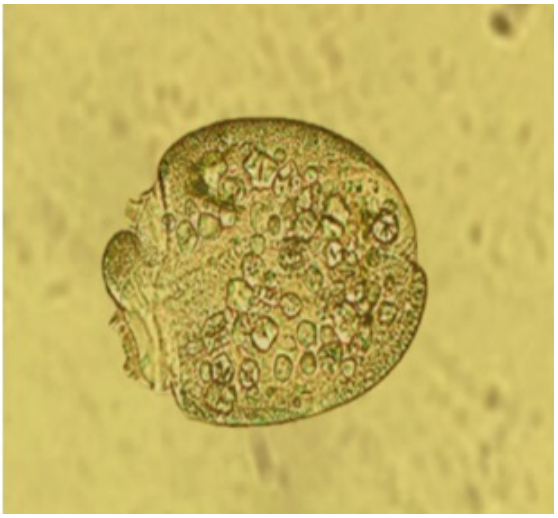

Fig. 6. Genus *Diplodinium*

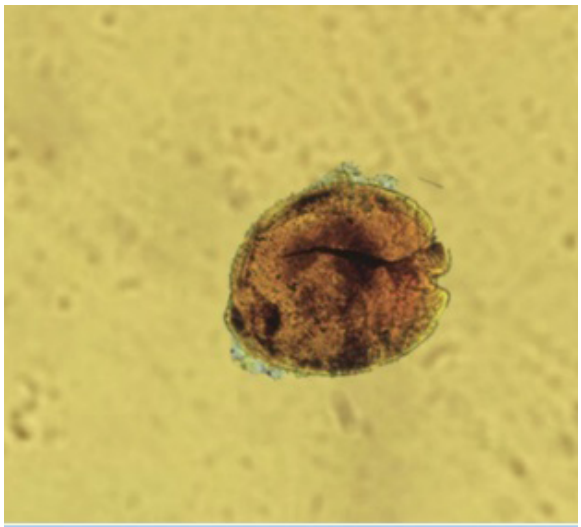

Fig. 7. Genus *Eremoplastron*

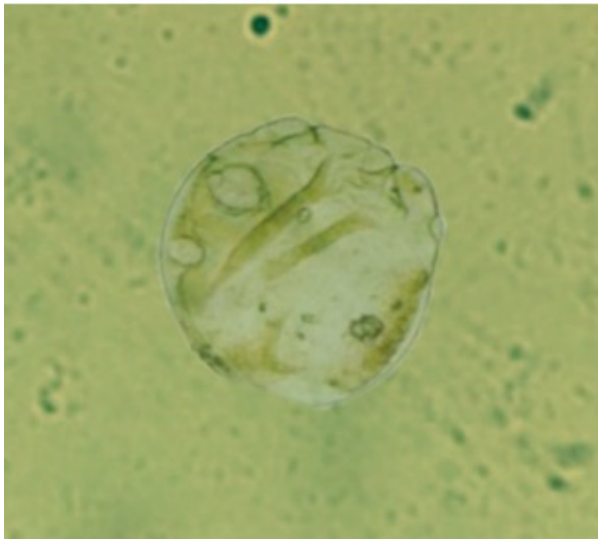

Fig. 8. Genus *Ostracodinium*

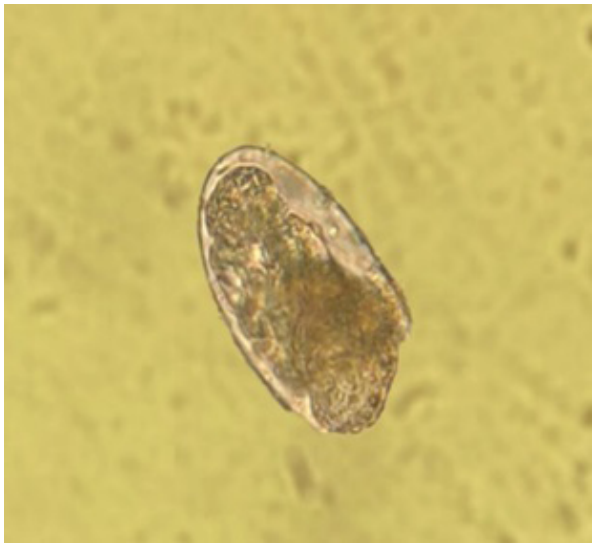

Fig. 9. Genus *Charonina*

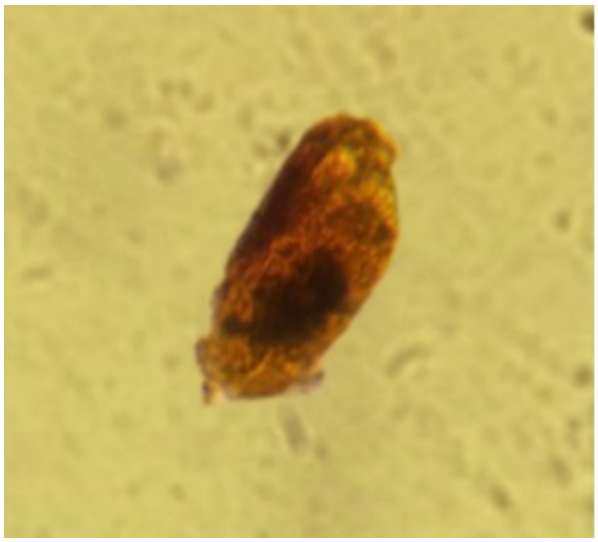

Fig. 10. Genus *Elytroplastron*

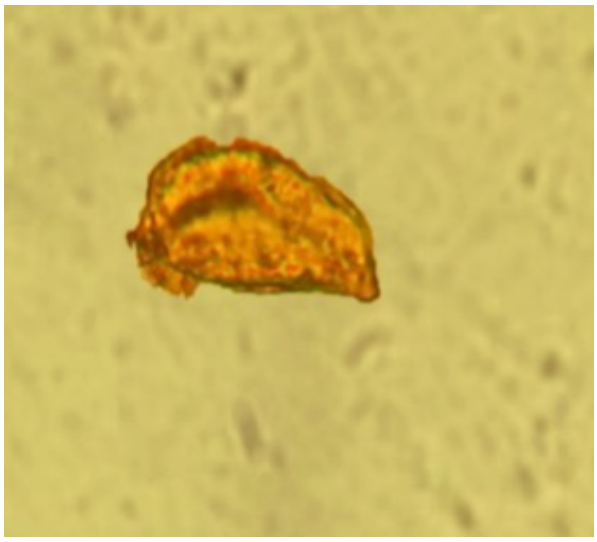

Fig. 11. Genus *Caloseolex*

Table 2. Protozoa proportion according to genders

Genera	Bovins (n / %)
Charonina spp.	18460 / 36.97
Entodinium spp.	8490 / 17.00
Eodinium spp.	7550 / 15.12
Isotricha spp.	6210 / 12.44
Diplodinium spp.	2820 / 5.65
Buetschilia spp.	3240 / 6.49
Eremoplastron spp.	1870 / 3.74
Ostracodinium spp	870 / 1.74
Ophryoscolex spp	170 / 0.37
Elytroplastron spp	110 / 0.20
Caloseolex spp	145 / 0.29
Total	49935 / 100

Discussion

The addition of concentrate and grains increases the speed of rumen development (Suárez et al., 2007) in this study, these ingredients were offered. The rumen protozoa are present in a larger population (> 1.106 protozoa per mL) when the diet is composed of mixed proportions of forage/concentrated (Ushida et al., 1990). Multiple comparisons were included from an individual publication with multiple studies. For each comparison included in the analysis, the effect size was calculated as the natural logarithm of the response ratio (mean value in the defaunated treatment divided by mean value in control treatment) and variance of the ratio calculated based on the reported standard deviation or standard error of the mean for each comparison (Viechtbauer, 2010). Other authors (Silva et al., 2014), found that during long dry period, rumen ciliates increase while population of small and medium rumen protozoa decreased at the end of the dry season, the large protozoa population was constant during all the dry season (Silva et al., 2014). Beef cattle fed Straw, with high maturation level presented higher concentration of the rumen protozoa than those fed

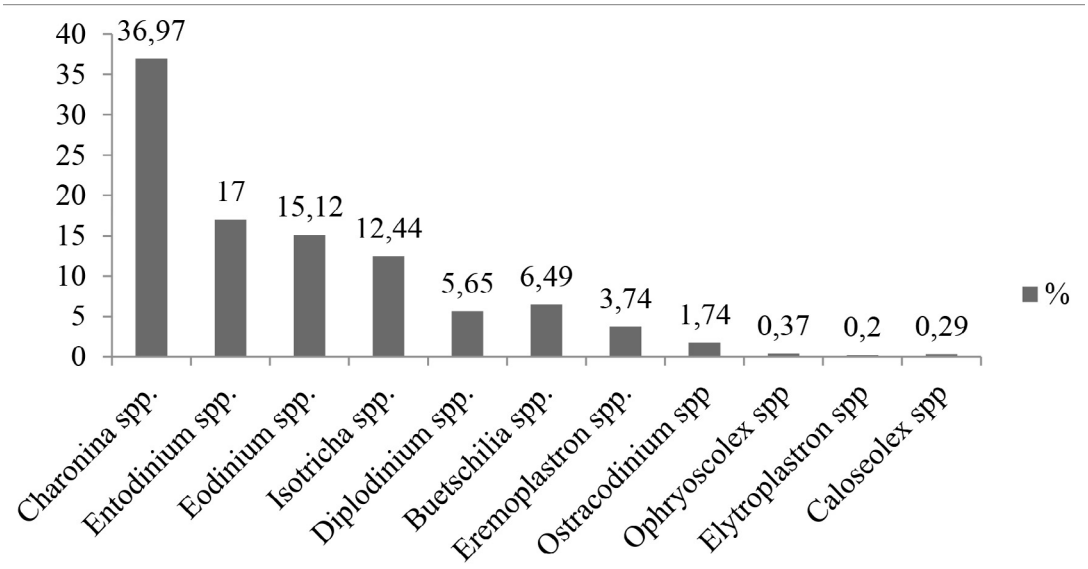

Fig. 12. Protozoa proportion according to genders

high grain and without forage. The acid pH of the rumen reduced large ciliates in cattle fed without roughage (Nigri et al., 2017). Researchers demonstrated hemicellulolytic and cellulolytic activity in the rumen ciliates, especially for the group of large *Entodinium* (Nigri et al., 2017). Principally the large protozoa may colonize fiber fragments and directly ingest plant tissues favoring the action of cellulolytic bacteria (Williams et al., 1997). Such development of protozoa population during autumn with pasture with small dose of cereals, its effect would be degradative for the vegetal cell wall and will contribute to better utilization of the pastures. Specifically, the large protozoa its enzymes constitute have important role in fiber digestion (Santra & Karim, 2002). About the activity of xylanases and carboxymethyl celluloses among different species of rumen protozoa, a set of carboxymethyl cellulases and xylanases is produced by the large ciliates *Elytroplastron spp*.

The most frequent genus was *Charonina spp* (36.97 %), followed by *Entodinium spp* (17.00 %) and *Eodinium spp* (15.12 %). In this research, *Entodinium spp*. was identified in high proportion (17.00 %). Which is different than other studies were this gender was in low proportion (Abe et al., 1981). Isotricha spp has a propotion of (12.44 %), this level was recorded with such a nutritional aptitude in region with same climate of Wilaya of Souk Ahras in Algerian east, Beef cattle fed Alfalfa spp. with high maturation level leads to concentration of the rumen protozoa than those fed high grain and without forage. The acid pH of the rumen reduced large ciliates in cattle fed without roughage. (Nigri et al., 2017), for *Diplodinium spp* (5.65 %), *Buetschilia spp* (6.49 %) and *Eremoplastron spp* (3.74 %). Considering the activity of xylanases and carboxymethyl celluloses among different species of rumen protozoa, a set of carboxymethyl cellulases and xylanases is produced by the large ciliates *Diplodinium*, *Eremoplastron* and *Buetschilia*, this refers to the results found by (Béra-Maillet et al., 2005)

Also researching Holstein calves fed with Alfalfa and concentrate, recorded a higher frequency of this protozoa genus. We believe that the diet offered by these researchers, including concentrated. Other research has also recorded higher occurrences of *Entodinium spp*. in adult cattle (Abrar et al., 2016). Observed a large concentration of this protozoa (80 %) in adult animals (Holstein × Wagyu). also recorded for (Martinele & D'Agosto, 2008), in crossbred Dutch-zebu cows receiving different concentrations of elephant grass. The presence of different protozoa genera in the rumen environment is directly related to the type of diet (Dehority, 2017).

Conclusion

The rumen protozoa concentration is high in Algerian local breed fed with Alfalfa and cereals compared to adult animals. The considerable concentration and genus diversity of rumen ciliates, detected for cattle of same age in this study, may indicate the ecologic and nutritional importance of these eukaryotes for the rumen environment of local bovine.

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