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RUMEN LIQUOR PHYSICAL, CILIATES AND BIOCHEMICAL COMPOSITION IN HOLSTEIN-FRIESIAN DAIRY CATTLE FED ON CORN SILAGE IN EGYPT

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ABSTRACT

Strained rumen liquor (SRL) examination in 37 dairy Holstein–Fresian cattle, at El-Gharbia Governorate, fed on corn silage; for physical characteristics showed that pH level was 6.64 ± 0.086, with green to olive green color, slimy consistency and aromatic odor; total protozoa count, *Entodinium*, *Diplodinium*, *Epidinium*, *Holotricha* and *Ophryoscolex* percentages were 9.3 ± 1.87×10^4, 89.09 ± 1.92, 4.62 ± 1.32, 0.37 ± 0.03, 4.55 ± 1.33 and 1.38 ± 1.38% respectively. Mean levels of ammonia concentration, total volatile fatty acids (TVFAs), total protein, calcium, phosphorus, copper and zinc were 364.66 ± 23.08 mmol/L, 85.10 ± 14.69 mmol/L, 10.51 ± 1.47 g/L, 1.11 ± 0.69 mmol/L, 2.01 ± 1.06 mmol/L, 4.31 ± 1.78 µmol/L and 16.59 ± 2.52 µmol/L respectively. Compared with 19 Holstein-Friesian dairy cattle from selected farms fed on traditional feed stuffs, significant changes were found for total protein, phosphorus, copper concentration and *Epidinium* % ($p < 0.001$); zinc concentration, Diplodinium %, TVFAs concentration, pH, total protozoa count and calcium concentration ($p < 0.01$) and ammonia nitrogen concentration ($p<0.05$). The microscopic examination of stained SRL samples in both groups revealed the identification of 4 families, 3 subfamilies, 12 genera, 39 species and 9 forma with significant variations in the percentages of each family composition. Two new genera (*Buetschlia* and *Ophryoscolex*), and 20 new species belonging to 7 genera (one species in genus *Buetschlia*, 9 in *Entodinium*, 2 in *Diplodinium*, 3 in *Metadinium*, 2 in *Epidinium*, 1 in *Elytroplastron* and 2 in *Ophryoscolex*) were recorded in dairy cattle in Egypt. All genera were demonstrated in figures and their dimensions were measured. These results should be put in consideration during the physical, ciliates and biochemical examination and evaluation of SRL status in dairy cattle.

Keywords: Dairy cattle, rumen, ciliates, biochemical constituents.

INTRODACTION

Silage is a method of forage preservation through stabilizing fermentation process by decreasing the pH within minimum fermentation period. In silage, lack of oxygen and the accumulation of lactic acid inhibit its microbial metabolism and preserves nutrients (Ranjit and Kung 2000). It is essential to investigate the effect of corn silage on rumen physical status, biochemical constituents (Zehra and KILIÇ 2009); and ciliates composition (Baraka 2006). Importance of rumen ciliates is referred to that; they constitute about 50% of rumen biological population, represent about 20% of gained protein by host with digestibility at abomasum of 91%, detoxify toxins of poisonous plants and eliminate some toxins out of the digestive tract, stabilize number of Streptococci to reduce harmful lactic acid, and *Entodinium* types of ciliate protozoa digest starch and protein to produce amino acids which are essential for bacteria and protozoa. Until now, no sufficient comprehensive recording of rumen ciliates in different ruminants in Egypt was established. This work was carried out to investigate the changes in rumen liquor physical, ciliates and biochemical composition in Holstein-Friesian dairy cattle fed on corn silage in Egypt; and to be put in consideration during the physical, ciliates and biochemical examination and evaluation of rumen liquor status in dairy cattle.

MATERIALS AND METHODS

Thirty seven Holstein-Friesian dairy cattle; belonging to a private milk production farm, at El-Gharbia Governorate, fed on corn silage (Approved by Central Laboratory of Ministry of Agriculture –Egypt)) were used in this study to investigate the physical, ciliates and biochemical constituents in their rumen. Another 19 Holstein-Friesian dairy cattle from different farms fed on traditional feed stuffs were used as control group for comparison. From each cow 50ml of rumen juice were collected using a rubber stomach tube connected to a suction pump and wooden mouth gag. Samples were examined at Laboratory of Rumenology, Department of Medicine and Infectious Diseases, Faculty of Veterinary Medicine, Cairo University. Samples were examined immedietly for pH (using SMP1 pH-meter), color, odor and consistency; then divided and stored for determination of total protozoal count according to the method described by Dehority 1984, generic protozoal composition according to Dehority 1993, ammonia concentration according to Zapletal 1967 and volatile fatty acids concentration according to Cottyn and Boucque 1968.

The identification and description of rumen ciliate protozoa were applied according to Nassar 1971, Dehority 1974, Dehority 1979, Ogimoto and Imai 1981, Dehority1984, Norman 1985, Williams 1986, Sakr 1988; Dehority 1993; Akira et al. 1994; Selim et al 1996; Selim et al 1999;

Bayram 2000; Bayram et al 2001; Baraka and Dehority 2003; Mermer et al 2003; Baraka , et al 2005; Bayram and Karaoglu 2005; Baraka 2006 (1); Baraka 2006 (2); Bayram and Sezgen 2006. Ciliates dimensions were measured and identified using research microscope (Boeco-Germany), micrometer eye piece (MOB-1-16$^{\times}$) and digital camera (Canon A650 IS). The biochemical constituents (total protein, calcium, phosphorus, copper and zinc) were analyzed in the supernatant of centrifuged strained liquor using Apel PD-303S spectrophotometer and the specific chemical kits. The obtained data were statistically analyzed using the SPSS Statistical Computer Software. Copyright (c) SPSS Inc., 2007 version 16.0.

RESULTS

Physical examination of strained rumen liquor samples in dairy cattle fed on corn silage revealed that rumen juice color ranged between green to olive green with slimy consistency and aromatic odor; on the other hand in dairy cattle fed on traditional feed stuffs the color varied according to the type of given rations, with slimy consistency and aromatic odor.

Table 1: Rumen physical, ciliates and biochemical constituents in Holstein-Friesian dairy cattle fed on silage and others fed on different traditional rations:

Parameters	Cattle fed on silage (37)	Control cattle (19)
pH	6.64±0.086 ^{b}	7.27±0.19
Total protozoa count (×10^4)	9.3±1.87 ^{b}	13.08±1.71
Entodinium (%)	89.09±1.92	89.52±10.73
Diplodinium (%)	4.62±1.32 ^{b}	2.08±1.46
Epidinium (%)	0.37±0.03 ^{a}	3.17±4.01
Holotricha (%)	4.55±1.33	6.10±3.18
Ophryoscolex (%)	1.38±1.38 ^{a}	0.15±1.87
Amm. Conc.* (mmol/L)	364.66±23.08 ^{c}	269.85±24.63
Volatile fatty acids (mmol/L)	85.10±14.69 ^{b}	59.89±5.87
Total protein (g/L)	10.51±1.47 ^{a}	6.29±0.396
Calcium (mmol/L)	1.11±0.69 ^{b}	5.36±0.80
Phosphorus (mmol/L)	2.01±1.06 ^{a}	10.01±1.14
Copper (µmol/L)	4.31±1.78 ^{a}	11.8±1.85
Zinc (µmol/L)	16.59±2.52 ^{b}	10.75±1.35

*Amm. Conc.: Ammonia concentration

a: $p<0.001$ b: $p<0.01$ c: $p<0.05$

Significant changes (Table 1) were found in total protein, phosphorus, copper concentration and *Epidinium* % ($p < 0.001$); zinc concentration, Diplodinium %, TVFAs concentration, pH, total protozoa count and calcium concentration ($p < 0.01$) and ammonia nitrogen concentration ($p < 0.05$); which can be explained by the interaction between these constituents (Table 2).

Table 2: Correlation between physical, cellular and biochemical rumen constituents in Holstein-Friesian dairy cattle fed on corn silage:

	Tprz.	Ent.	Dpl.	Holo.	Ophr.	Am.	VFA.	Tprt.	Ca.	Phos.	Cu.	Zn.
pH	-0.05	0.16	0.37	-0.05	-0.73	0.34	0.08	-0.21	-0.50	0.55	-0.76	0.34
T prz.		0.16	-0.68	-0.58	0.73	0.61	-0.71	0.82	-0.76	-0.24	-0.50	0.61
Ent.			-0.68	-0.89	0.00	0.55	0.29	0.41	-0.29	0.76	-0.13	0.55
Dpl.				0.89	-0.73	-0.55	0.24	-0.82	0.39	-0.13	0.03	-0.56
Holo.					-0.36	-0.71	0.08	-0.72	0.55	-0.50	0.29	-0.71
Ophr.						0.18	-0.54	0.71	-0.18	-0.54	0.18	0.18
Amm							0.08	0.31	-0.50	0.55	-0.76	0.34
VFA.								-0.72	0.68	0.68	0.16	-0.58
Tprt.									-0.72	0.21	-0.15	0.82
Ca.										-0.05	0.68	-0.89
Phos.											-0.37	0.16
Cu.												-0.37

Tprz: Total protozoa count.
Ent.: *Entodinium.*
Dpl.: *Diplodinium.*
Holo.: *Holotricha.*
Amm.: Ammonia concentration.
VFA.: Volatile fatty acids.
Tprt.: Total protein.
Ca.: calcium.
Phos.: Phosphorus.
Cu.: Cupper.
Zn.: Zinc.

Table 3: Total ciliates concentrations and distribution of total number of genera, species and forms of rumen ciliates in cattle at various localities around the world:

Locality	Total protozoa count $\times 10^4$/ml	Total no. of genera	Total no. of species	Total no. of forms	Number of animals and breeds	References [a]
Brazil	26.4±17.7	14	55	4	4 Zebu cattle	(1)
Canada	6.9 [b]	12	28	- [b]	11 H. F.*	(2)
China	30 [b]	17	20	6	45 Chinese cattle	(3)
Egypt	45 [b]	10	28	11	7 H. F.	(4)
Iran	29±18.2	5	10	- [b]	37 cattle	(5)
Japan	40.3±1.9	15	48	25	125 H. F.	(6)
Kenya	15.1 [b]	13	51	19	13 Zebu cattle	(7)
Libya	81 [b]	9	27	6	9 H. F.	(8)
Mexico	8.3 [b]	13	38	15	10 Hereford cattle	(9)
Philippine	15.8 [b]	10	26	3	70 H. F.	(10)
Sri Lanka	2.9±4.9	16	53	19	20 Zebu cattle	(11)
Tanzania	22.2 [b]	15	46	- [b]	10 Tanzanian cattle	(12)
Thailand	7.1±2.8	17	56	4	46 Zebu cattle	(13)
Turkey	52.4±20.7	13	52	36	28 Domestic cattle	(14)
Egypt	9.3±1.87	12	39	9	37 H. F.	Present study
	13.08±1.71	12	39	9	19 H. F.	

[a] (1) Dehority 1986a, (2) Imai, et al. 1989, (3) Rong and Imai 2002, (4) Selim, et al. 1996, (5) Talari, et al. 2004, (6) Ito , et al. 1994, (7) Imai 1988, (8) Selim, et al. 1999, (9) Imai and Kinoshita 1997, (10) Shimizu et al. 1983, (11) Imai 1986, (12) Mishima, et al. 2009, (13) Imai and Ogomoto1984, (14) Bayram, et al. 2003.

[b] Data not reported. * H. F.: Holstein-Friesian cattle.

The total protozoa count, number of genera, species and forms recorded in both groups (Table 3) were compared with data in other countries (Shimizu et al. 1983, Imai and Ogomoto1984, Dehority 1986a, Imai 1986, Imai 1988, Imai, et al. 1989, Ito, et al. 1994, Selim, et al. 1996, Imai and Kinoshita 1997, Selim, et al. 1999, Rong and Imai 2002, Bayram, et al. 2003, Talari, et al. 2004, Mishima, et al. 2009) .

Although the microscopic examination of stained strained rumen liquor samples in both groups revealed identification of 4 families, 3 subfamilies, 12 genera, 39 species and 9 forma (Fig. I); marked variations in the percentages of each family composition were obvious. This is the first illustration of identified and measured species and forma of rumen ciliates in strained rumen liquor of dairy cattle in Egypt:

ORDER: PROSTOMA

FAMILY: ISOTRICHIDAE

GENUS: *ISOTRICHA*

1. *Isotricha prostoma:*

The body is oval and measures 80-100 × 50-120 µm. Cilia uniformly covers the body which is tapered at the level of cytostome which is sub-terminal. Macronucleus is kidney shape and connected with micronucleus by fibrils forming the karyophore. The mouth is located at the end opposite the leading or anterior end. This location has elicited speculation as to what is actually the anterior end.

2. *Isotricha intestinalis:*

The body is oval and measures 90-200 × 45-150 µm. Macronucleus is kidney shape. The cytostome is more sub-terminal at the level of macronucleus. The mouth is on one side of the cell equidistant between the posterior end and the middle.

GENUS: *DASYTRICHA*

3. *Dasytricha ruminatum:*

Body is oval, flattened and measures 45-100 × 25-50 µm. It is smaller than the isotricha and commonly occurs in greater numbers in the rumen. The mouth is at the posterior end. Elliptical macronucleus is in middle or posterior third of the body. Cilia are in spiral longitudinal rows. There are no contractile vacuoles.

FAMILY: BUETSCHLIIDAE

GENUS: *BUETSCHLIA*

4. *Buetschlia polymorphella bovis:*

Body is generally ovoid with anterior one third tapered like a flask and measures 25-40 × 20-25 µm. Uniform cilia are present in two ciliary zone; the large one on the tapered anterior one third area and the smaller one is a small tuft consists of a few cilia near a cytoproct on the posterior end of the body. Macronucleus is sub-spherical and situated at central part of the body. Spherical micronucleus is near the margin of macronucleus. Contractile vacuole is at posterior end of body and concretion vacuole close to body surface at middle of the body.

FAMILY: BLEPHAROCORYTHIDAE
GENUS: *CHARONINA*
5. *Charonina ventricularis:*
The body is cylindrical and wide at anterior end with two ciliary tufts near the posterior end. The body measures 24-36 × 12-15 µm. The esophagus is very long and directed to the macronucleus, which is spherical to globular and located in middle to posterior part of the body.

ORDER: ENTODINOMORPHIDA
FAMILY: OPHRYOSCOLECIDAE
SUBFAMILY: ENOTODININAE
GENUS: *ENTODINIUM*
6. *Entodinium caudatum f. caudatum:*
The body is truncated anteriorly and measures 25-70 × 25-50 µm with single adoral zone. Macronucleus is cylindrical to wedge shaped and is nearly ½ of body length broader anterior than in posterior with a contractile vacuole at anterior pole. Pointed to slight rounded lobes are on both upper and lower posterior left side.
7. *Entodinium cauadatum f. lobospinosum:*
The truncated body measures 30-70 × 30-60 µm. Macronucleus is nearly ½ of body length broader anterior than in posterior with a contractile vacuole at anterior pole. Pointed to slight rounded lobe is on posterior left side.
8. *Entodinium williamsi f. turcicum:*
The body is ovoid or quadric-angular to ellipsoid and measures 35-65 × 28-50µm and generally wider at mid-point. There are two spines and a spinated lobe at the posterior end of the body. Micronucleus is usually ellipsoid or ovoid in shape and situated in left ventral posterior edge of the macronucleus. The contractile vacuole lies to the ventral side and to the left of the macronucleus.
9. *Entodinium caudatum f. dubardi:*
Oval body truncated anteriorly, the body measures 25-45 × 25-35µm. Contractile vacuole at edge of triangular macronucleus. Anus is on right side of small posterior left lobe.
10. *Entodinium longinucleatum:*
Body is ellipsoid, flattened and measures 45-110 × 25-80 µm. Macronucleus is as long as body length. The contractile vacuole is close to upper side of macronucleus.

11. ***Entodinium longinucleatum f. spinolobum:***
Body is ellipsoid in shape and measures 45-60 µm × 30-40µm. there are two spines and one lobe at posterior end of body. One spine on right side and the second at upper left side, while the lobe is at lower left side. Right spine is longer than left one. Esophagus is relatively short. Macronucleus is very long and extends along right body side. Micronucleus is ellipsoid and present at upper third of macronucleus. Contractile vacuoles are at upper left side of macronucleus.
12. ***Entodinium yunnense f. yunnense:***
The body is ellipsoidal and measures 40-60 × 28-40 µm. the macronucleus extends along the right side of the body; from near rectum to anterior end at anterior one sixth of its length. Micronucleus is ellipsoid and lies on the left side of the anterior third of macronucleus. One contractile vacuole is at left upper side of macronucleus.
13. ***Entodinium yunnense f. spinonucleatum:***
Body is ellipsoidal in side view, measuring 26-41 × 24-33µm, both sides are convex, widest part is at middle of cell. Left body side extends at the end with single sharp spine; and may curve slightly to the right. Posterior right side ranges from smooth rounded lobe to sharp spine. Short esophagus of funnel shape is at a distance from mid of macronucleus. Macronucleus extents along entire right side near the anterior end at the rectum. Micronucleus is ellipsoid and located at left of macronucleus and at anterior 1/3 of it. Contractile vacuole lies to the left of upper surface of macronucleus, just anterior to micronucleus.
14. ***Entodinium nanellum:***
The body is ovoid, flattened. The body measures 22-32 × 12-18µm. Thin macronucleus of wedge-shape and longer than ½ of body length. Esophagus curves to the macronucleus.
15. ***Entodinium constrictum:***
The body is ellipsoid or ovoid in side view. The body measures 30-40 × 20-30 µm and has a convex right side. Left side has indentation at the level of base of adoral membranelle zone. macronucleus is occasionally spherical and lies on the right side in the middle to posterior half of the cell.
16. ***Entodinium bovis:***
The body is ellipsoid in side view, measuring 24-44 × 18-33µm, both sides are convex. Small left lobe is present. Adoral membranelle zone slants away from the macronucleus and the esophagus is bending sharply

to the right, terminating posterior to the micronucleus. Macronucleus is triangular to club shaped, lies on right side, its anterior part bends to left. Micronucleus is small ellipsoidal lies to the left of macronucleus below the level of adoral zone. Contractile vacuole lies at left upper part of macronucleus just anterior to the micronucleus.

17. *Entodinium bursa:*

Flattened body measuring 80-120 × 75-100 µm, macronucleus is 4/5 of body length; dense granular cytoplasm, contractile vacuole is anterior, body surface has longitudinal striation.

18. *Entodinium exiguum:*

Elongated oval body measures 20-35 × 15-25 µm, straight esophagus, parallel with long body axis, macronucleus irregular shaped (short and thick) shorter than ½ of body length and generally lies in middle third of body.

19. *Entodinium imaii*:

The body is ovoid and widest at 1/4 of the body level. The body measures are 20-35 × 20-25 µm; dorsal side is convex and humpbacked anteriorly. The ventral side is almost straight but slightly depressed on the mid-surface. In the posterior part of the body, there is one dorsal spine, extending outwardly but sometimes bending dorsally and towards the anterior part. Another triangular spine and a back-shaped lobe on the right side are also present. The right lobe is shorter than the secondary spine on the left. Macronucleus, which is bean-shaped, mostly concave on the ventral side and convex on the dorsal side, is located at almost the anterior tip of the body. The micronucleus is ellipsoidal and is situated close to the left posterior of the macronucleus. The contractile vacuole lies left posterior or left of the macronucleus.

20. *Entodinium oktemae:*

The body is ovoid-ellipsoidal and is widest at the midpoint. The body measures 50-75 × 35-46 µm. A spine and two matching spinated lobes are present at the posterior end. The spine is on the dorsal side, whereas the lobes are located ventrally on both sides. The dorsal spine is bending sometimes dorsally or towards the left side of the body. The macro- and micronuclei are spherical and the micronucleus is situated posterior or anteriorly on the left of the macronucleus, generally in its vicinity. The contractile vacuole is located before or at the level of macronucleus on its ventral side.

SUBFAMILY: DIPLODININAE

GENUS: *DIPLODINIUM*

21. *Diplodinium anisacanthum:*

The body is oval to triangular and measures 150-210 × 90-120 µm with posterior oblique and truncated end. Macronucleus is sausage in shape with anterior curved end toward the ventral aspect.

22. *Diplodinium monocanthum:*

The body is oval to triangular and measures 60-90 × 40-60 µm; with posterior oblique and truncated end. Only single posterior spine is present. Macronucleus is sausage in shape with anterior curved end toward the ventral aspect.

23. *Diplodinium tetracanthum:*

The body is oval to triangular and measures 60-80 × 40-60 µm; ends posteriorly with four spines.

24. *Diplodinium dentatum:*

The body measures 60-80 × 50-65 µm with six heavy incurved posterior spines. Spine on right side is the longest one. The macronucleus is long; it is heavy and rod like. The anterior end is curved. Two contractile vacuoles are on left side.

25. *Diplodinium lobatum:*

The body measures 40-60 × 24-40 µm. Three prominent lobes are on left side of the macronucleus. Two contractile vacuoles are at both poles of the macronucleus.

GENUS: *EUDIPLODINIUM*

26. *Eudiplodinium magii:*

The body measures 110-220× 75-150 µm. One narrow skeletal plate extends downward near the posterior end of macronucleus. Macronucleus is hook or pistol like. Two contractile vacuoles. The rectum is large.

GENUS: *OSTRCODINIUM*

27. *Ostracodinium clipeolum:*

The body is ellipsoidal and measures 60-130 × 40-70 µm with one large skeletal plate board shaped and three contractile vacuoles between macronucleus and left body wall. Macronucleus has two left lobes. The body ends with large lobe.

GENUS: *METADINIUM*

28. *Metadinium banksi:*

Body is ellipsoid, measuring 118-162 × 75-118 µm, both body sides are slighty convex; posterior end issmoothly rounded. Two skeletal plates on upper side generally fused posteriorly, the plates are not parallel. Rectun is wide and lined with longitudinal fibrils; anus is on upper side, slightly to right of main bodyaxis. Macronucleus consists of 3 lobes; while micronucleus is ellipsoid and lies in a depression anterior to middle lobe of macronucleus. Two contractile vacuoles between macronucleus and lower left body margin, one anterior to micronucleus and one in the depression between middle and posterior lobes.

29. *Metadinium esalqum:*

Flattened ellipsoidal body measures 70-100 × 50-70 µm, left side is convex while right one is nearly flat. Two skeletal plates fused posteriorly at posterior three fourth of the macronucleus; which consists of main two lobes and two contractile vacuoles. The rectum is large.

30. *Metadinium medium:*

The body measures 150-250 × 90-175 µm. two skeletal plates, fused at posterior end of macronucleus which consists of three lobes. The body ends with large rectum. ELYTROPLASTRON:

GENUS: *ELYTROPLASTRON*

31. *Elytroplastron bubali:*

The body measures 110-165 × 65-100 µm. Two medium width skeletal plates are on upper side, long skeletal plate on lower side and small plate on right side; while four contractile vacuoles between left edge of macronucleus and left side of body.

SUBFAMILY: OPHRYOSCOLECINAE

GENUS: *EPIDINIUM*

32. *Epidinium caudatum:*

Elongated twisted body with Left ciliary zone below anterior end of the cell and not parallel with adoral zone. Body around the main axis measures 80-140 × 35-55 µm. Macronucleus is club shaped. The body ends with one caudal spine.

33. *Epidinium bicaudatum:*

Elongated twisted body around the main axis measures 80-140 × 35-55 µm. Macronucleus is club shaped. The body ends with two caudal spines.

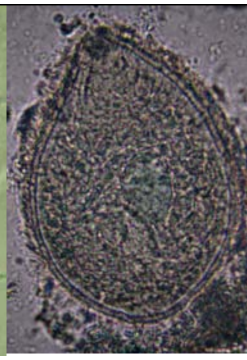
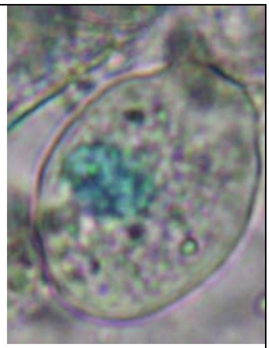
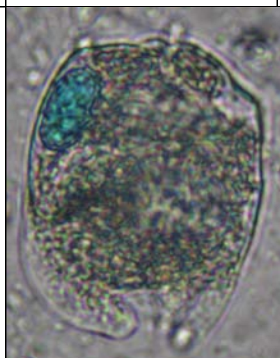
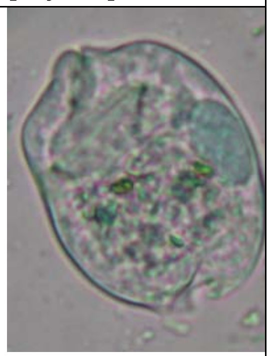
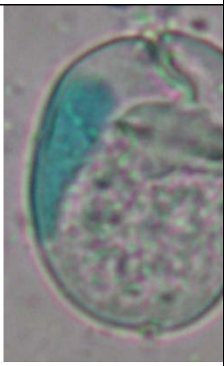
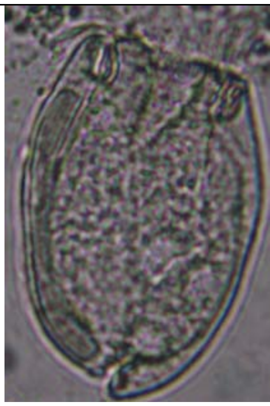
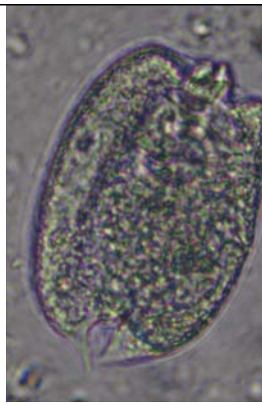
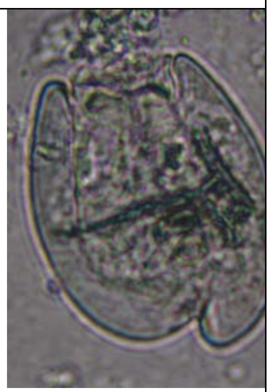
34. *Epidinium graini f. graini:*

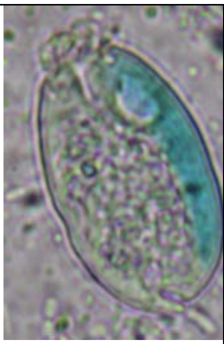
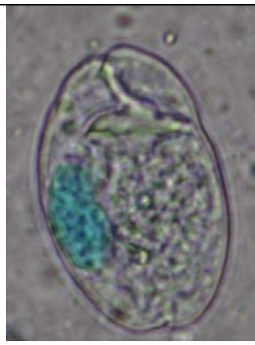
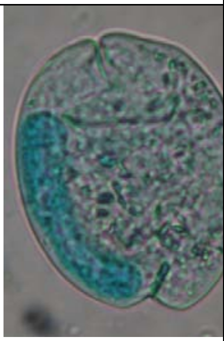
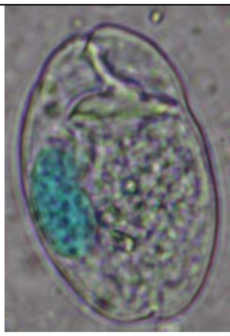
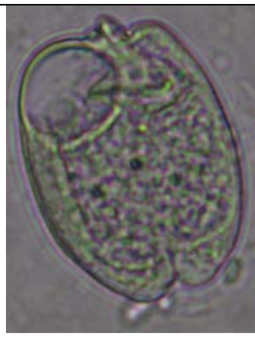
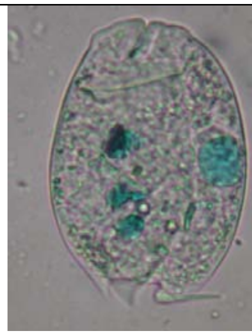
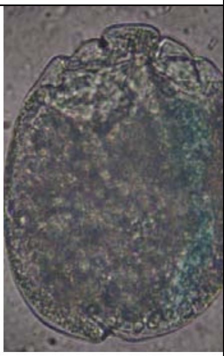
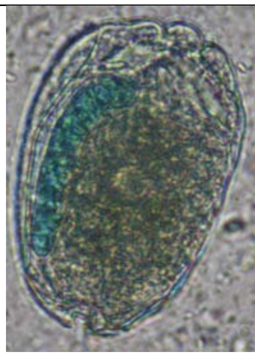
The body is elongated and measures 70-125 × 35-50 µm. There are two transverse periplastic pellicle foldings resembling coronets. Skeletal plate complex is composed of three plates lying close together from left ventral edge of adoral zone to the end of cytoprocalt tube.

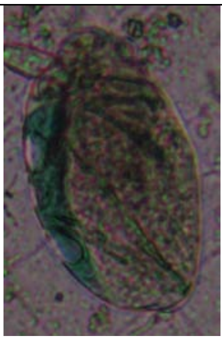
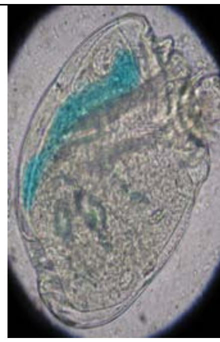
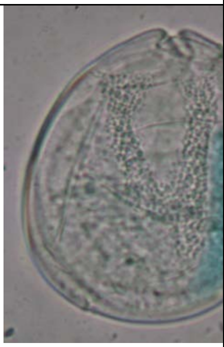
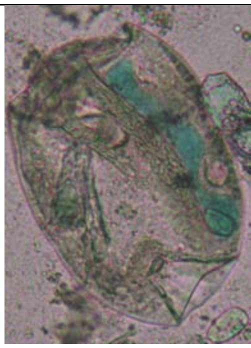
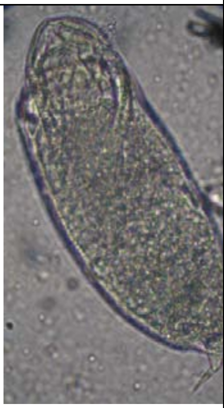
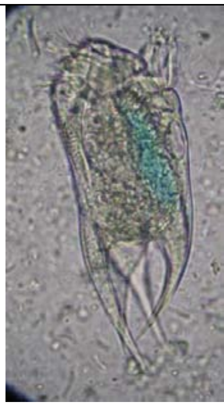
35. ***Epidinium graini f. caudatricoronatum:***
There are three transverse periplastic pellicle foldings resembling coronets.
36. ***Epidinium ecaudatum:***
It has an elongated twisted body around the main axis measures 100-150 × 35-60 µm. Macronucleus is club shaped. The body ends without caudal spine.
37. ***Epidinium ecaudatum f. cattanei:***
Body is elongated and twisted around the main axis measures 80-120 × 40-70 µm. Macronucleus is club shaped. The body ends with five caudal spines one on the right side, two on the left and one each on upper and lower side, body is relatively short.

GENUS: ***OPHRYOSCOLEX***
38. ***Ophryoscoles caudatus:***
The body is large measures 140-160 × 80-100 µm; and characterized by complicated spination and the long caudal spine which is nearly half length of the body.
39. ***Ophryoscolex purkynje***
The body measures 140-220 × 70-150 µm; and characterized by the long caudal spination which may be in two or three groups.

Fig. I: Photographic illustration of the identified species of rumen ciliates in dairy cattle:

			
1. Isotricha prostoma	*2. Isotricha intestinalis*	*3. Dasytricha ruminatum*	*4. Buetschlia polymorphilla bovis*
			
5.Charonina ventricularis	*6. Entodinium caudatum f. caudatum*	*7. Entodinium caudatum f. lobospin.*	*8. Entodinium williamsi f. turcicum*
			
9. Entodinium caudatum f. dubardi	*10. Entodinium Longinucleatum f. Longinucleatum*	*11. Entodinium Longinucleatum. f. spinolobum*	*12. Entodinium Yunnense f. yunnense*

			
13. Ent. Yunnense f. spinonucleatum	*14. Entodinium nanellum*	*15. Entodinium constrictum*	*16. Entodinium bovis*
			
17. Entodinium bursa	*18. Entodinium exiguum*	*19. Entodinium Imaii*	*20. Entodinium oktemae*
			
21. Diplodinium anisacanthum	*22. Diplodinium monocanthum*	*23. Diplodinium tetracanthum*	*24. Diplodinium dentatum*

			
25. Diplodinium lobatum	*26. Eudiplodinium magii*	*27. Ostrachodinium clipeolum*	*28. Metadinium banksi*
			
29. Metadinium esalqum	*30. Metadinium medium*	*31. Elytroplastron bubali*	*32. Epidinium caudatum*
			
33. Epidinium bicaudatum	*34. Epidinium ecaudatum*	*35. Epidinium ecaudatum f. cattanei*	*36. Epidinium graini f. graini*

		
37. Epidinium graini f. caudatricoronatum	*38. Ophryoscolex caudatus*	*39. Ophryoscolex purkynjie*

DISCUSSION

The available previous papers dealing with the rumen function were mainly focusing on the evaluation of pH, ammonia nitrogen concentration, volatile fatty acids percentages and fermentation indexes (Insung, et al. 1998, Bosi, et al. 2002, Melendez, et al. 2004 and Chung, et al. 2009). In comparison between SRL constituents of the dairy cattle fed on corn silage and other group fed on traditional rations (Table 1); the significant decrease in pH ($p<0.01$) was within the levels recorded by Insung, et al. 1998, Bosi, et al. 2002, Melendez, et al. 2004, Arelovich, et al. 2008 and Khampa, et al. 2009. The rumen ammonia nitrogen concentration showed a significant increase ($p<0.05$) with higher levels than that recorded by Yang, et al. 2001 and Masoera, et al. 2006. The significant increase in the level of total volatile fatty acids ($p<0.01$) was lesser than the levels mentioned by Yang, et al. 2001, Melendez, et al. 2004, Laugalis, et al. 2007 and Chung, et al. 2009. The significant increase in the level of rumen total protein ($p<0.001$), both of zinc and *Diplodinium* percentage ($p<0.01$); on the other hand the significant decrease ($p<0.001$) in the levels of phosphorus, copper and *Epidinium* percentage; with the significant increase in both of total protozoa count and calcium level ($p<0.01$) can be explained on the basis of interaction between these constituents (Krzywiecki et al 2006).

High negative correlation was recorded between pH and all of *Ophryoscolex*, calcium and cupper levels (Table 3); with high positive correlation with phosphorus. The total volatile fatty acids showed high negative correlation with total protozoa count, which highly negative correlated with *Diplodinium*, *Holotricha*, calcium and copper levels and positively with *Ophryoscolex*, ammonia, total protein and zinc. In SRL biochemical constituents, high positive correlations between zinc, total protein and between calcium and copper were recorded; while high negative correlation between calcium and total protein and between calcium and zinc were obtained. It was interesting to find that in both groups the ratio between calcium and phosphorus levels was 1:2.

Rumen ciliates showed high negative correlation between total protozoa count and *Diplodinium* and *Holotricha* levels. High negative correlation between *Entodinium*, *Diplodinium* and *Holotricha* and inter-between *Diplodinium* and *Ophryoscolex* was present. The only high positive correlation was recorded between *Ophryoscolex* and total protozoa count. These correlations were in agreement with that recorded by Baraka and Dehority 2003 and Krzywiecki et al 2006.

Total protozoa count in dairy cattle fed on silage was nearly in the ranges recorded in Canada, Mexico and Thailand; while higher number was recorded in Egypt by Selim, et al. 1996. The number of genera and species was in agreement with that in Brazilian, Canadian, Kenyan and Turkish cattle (Table3). Even though, Genus *Polyplastron multivesiculatum* was not recorded and 16 species belonging to 6 genera were absent in all samples; It was interesting to record two new genera (*Buetschlia* and *Ophryoscolex*), and 20 new species belonging to 7 genera (one species in genus *Buetschlia*, 9 in *Entodinium*, 2 in *Diplodinium*, 3 in *Metadinium*, 2 in *Epidinium*, 1 in *Elytroplastron* and 2 in *Ophryoscolex*) in dairy cattle in Egypt.

CONCLUSION

The feeding of dairy cattle on corn silage reduced significantly total number of protozoa ($p<0.01$), increased significantly total protein level ($p<0.001$), both of total volatile fatty acids and zinc levels ($p<0.01$) and ammonia concentration ($p<0.05$) in rumen liquor; and highlighted the importance of investigating its effect on the blood biochemical constituents. Two new genera (*Buetschlia* and *Ophryoscolex*), and 20 new species belonging to 7 genera (one species in genus *Buetschlia*, 9 in *Entodinium*, 2 in *Diplodinium*, 3 in *Metadinium*, 2 in *Epidinium*, 1 in

Elytroplastron and 2 in *Ophryoscolex*) were recorded in dairy cattle in Egypt. All genera were demonstrated in figures and their dimensions were measured to be used in the investigation and banking of cattle ciliates. These data should be put in consideration during the physical, ciliates and biochemical examination and evaluation of rumen liquor status in dairy cattle.

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