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Impact of Transfaunation of Rumen Ciliate Cultures on Physical Examination, Selected Serum Parameters and Milk Profile in Defaunated Lactating Dairy Goats

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ABSTRACT

Rumen ciliates still have mysterious secrets and influences in ruminants. This study investigated the effect of transfaunation of pure and mixed cultures of rumen ciliates on physical clinical examination, selected serum parameters and milk profile in defaunated lactating dairy goats. A number of 8 Baladi native breed goats were randomly classified into two groups each one containing 4 goats. Pure culture group was transfaunated with 6 ml of pure culture of *Holotricha* spp., while mixed culture group was transfaunated with 6 ml of mixed culture of 81.85% *Holotricha* and 18.15% *Ophryoscolex* spp. once weekly for three consecutive weeks, after defaunation of both groups using 30 ml of 8% SLS for two consecutive days. Serum and milk samples were collected weekly for three successive weeks to study effect of type of ciliate culture, duration of transfaunation and their interaction. Results revealed that transfaunation of pure and mixed cultures of rumen ciliates had no effect on physical examination with minimal non-significant improvement of calcium, inorganic phosphorous, total protein and globulin in serum of defaunated goats. Transfaunation of pure or mixed cultures of rumen ciliates within three weeks could not improve significantly decreased milk fat % of defaunated goats without any effect on other measured milk profile parameters. It is concluded that further investigations on transfaunation without prior defaunation should be performed using different pure and mixed cultures of rumen ciliates for therapeutic and productive purposes.

Key words: Transfaunation, Defaunation, Rumen ciliate culture, Physical examination, Milk, Serum, Dairy goats.

INTRODUCTION

Goats play a substantial role in human nutrition. Their number increases more quickly comparatively to sheep, particularly in less developed parts of the world, indicating an increased role in food production systems (Skapetas and Bampidis, 2016).

In Africa, from 2000 to 2013 there was a conspicuous increase in goats total number representing 48.61% increase of total world increase (FAOSTAT, 2013). In Egypt, goats are considered one of the important economic sources for meat, milk, fibers and manure in rural and desert areas. Most Egyptian goats belongs to the Baladi breed (EL-SAYED *et al.*, 2016).

First described in 1843, Rumen protozoa with their striking appearance were assumed to be important for the welfare of their host. However, despite contributing up to 50% of the biomass in the rumen, the role of protozoa in rumen microbial ecosystem remains unclear. Even though protozoa make up a large portion of the rumen biomass, their exact role in ruminal fermentation and their contribution to the metabolism and nutrition of the hos tis still an area of substantial controversy (Williams and Coleman, 1997).

Defaunation is the removal of protozoa from the rumen and refaunation is the introduction of protozoa into the rumen. Scientific reports on the impact of presence or absence of rumen ciliates on ruminant performance have been contradicting each other; some researchers reported positive effect of defaunation, while others disagreed with these results with or without deleterious effect on ruminal vital functions and animal health (Walker, 2000). Thus, investigations on the effect of de- or refaunation have been continued hitherto in many dairy institutions all over the world (Gebeyehu and Mekasha, 2013).

Transfaunation or refaunation includes a broad spectrum of microorganisms including bacteria and protozoa that are transferred from rumen of a donor animal to the rumen of a recipient. Even though transfaunation has been an old practice for decades (Brag and Hansen, 1994), and is a common medical practice in food animal medicine

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to treat indigestion of ruminants (Smith, 2014), there is still a lack of scientific information regarding its benefits and mechanisms of actions (DePeters and George, 2014), particularly rumen ciliates transfaunation and its impact on animal health and productivity particularly milk fat % which is considered a measure of economics of milk and milk products (Mehta, 2014).

In Egypt, several authors; (Baraka, 2012), (Saber, 2016), (Saber *et al.*, 2016) and (Al-Azazi *et al.*, 2018) studied rumen ciliates of cattle, buffalos, sheep and camels from aspects of total protozoa count, morphology, proportions of ciliate families and ciliate culturing, and recommended future comprehensive studies to explain the impact of transfer of cultivated ciliate cultures into defaunated or faunated animals on wellbeing and productive status of dairy and fattening ruminants. So, the aim of this study is to evaluate the impact of transfaunation of pure and mixed cultures of rumen ciliates on physical clinical examination, selected serum biochemical constituents and milk profile, particularly milk fat %, in defaunated lactating Baladi goats.

MATERIALS AND METHODS

Animals and experimental design

Eight clinically healthy Baladi dairy goats were kept individually in separated pens (2 X 2) belonged to Department of Medicine and Infectious Diseases, Faculty of Veterinary Medicine, Cairo University. Their ages ranged from 2-3 years old and their mean bodyweight was 32.45±1.19 kg. Goats were exposed to clinical examination to confirm they are apparently healthy and free from signs of illness. Then, all of them were exposed to defaunation using sodium lauryl sulfate (SLS) administered orally in 8% solution at dose rate of 30 ml /animal /day for two consecutive days according to (Santra and Karim, 2000). At the beginning of experiment, goats were classified randomly according to type of transfaunated rumen ciliate cultures into two groups; pure culture group (TP) represented by 4 goats and mixed culture group (TM) represented by 4 goats. After defaunation of both groups, PC group was administered 100% culture of *Holotricha* counted 1.90±0.49 X 10^4/ml. MC group was administered mixed culture of 81.85% (±2.99) *Holotricha* and 18.15% (±2.99) *Ophryoscolex* with total count of 1.94±0.51 X 10^4/ml. Both groups were administered 6 ml of culture /head /day before morning meal for two consecutive days then once weekly for three successive weeks. In-vitro cultivation of these specific species of rumen ciliates were selected based on a previous study under reviewing performed by the same team of authors and concluded that *Holotricha* had the highest significant ($P<0.01$) positive correlation with milk fat % in Baladi breed, then *Ophryoscolex* ($P<0.05$) among all rumen ciliates concerning Spearman's correlation coefficient. Goats were fed locally manufactured concentrate mixture, containing vitamins and minerals premix, twice daily and wheat straw *ad libitum*. Nutritional analysis of offered concentrate mixture is presented in Table 1.

Table 1: Analysis of offered locally produced concentrate mixture fed to both groups of goats

Parameters [1]	%
Ash	8.93
OM	91.24
EE	12.54
CP	10.99
CF	14.72
NFE	52.95
TDN	88.69

[1]Feed Analysis results on dry matter basis: [2] DM; dry matter, OM: organic matter, EE: ether extract, CP: crude protein, CF: crude fiber, NFE: nitrogen free extract, TDN: total digestible nutrient.

Preparation and cultivation of pure and mixed culture of rumen ciliates

Rumen ciliate cultures were cultivated in certain media which prepared using the anaerobic culture technique described by (Hungate, 1950) and modified by (Dehority, 1969). Substrate for cultures was consisted of a suspension containing 1.5% ground wheat and 1.0% ground orchard grass (both were ground to pass through a 40-mesh screen) in distilled water followed by being gassed with O_2 free CO_2 for 20 min. One ml of substrate was inoculated daily and cultures were routinely maintained by transferring twice a week; 5 ml of cultures were transferred into a tube containing 5 ml of fresh medium plus 1 ml of substrate. Staining and counting of ciliate cultures were performed twice weekly before sub-culturing procedures.

Samples

All procedures of animal handling and sampling were approved by the Ethics of Animal Experiments Committee, Faculty of Veterinary Medicine, Cairo University, Egypt. Serum and milk samples were collected weekly at 8 a.m. before morning meal for three consecutive weeks. Serum samples were collected by puncture of jugular vein using plain vacutainers. Milk samples were collected manually.

Clinical examination and Laboratory investigation of samples

Physical clinical examination including pulse rate, respiratory rate, temperature, rumen motility and BCS were estimated according to (Pugh and Baird, 2012).

Analysis of blood serum samples including calcium (Ca), magnesium (Mg), inorganic phosphorous (P) total protein and albumin levels were carried out using specific kits produced by SPECTRUM Company, Egypt, according to the method described by (Young and Friedman, 2001). Serum globulin and albumin /globulin (A /G) were calculated. Milk composition including fat %, lactose %, solid not fat (SNF) %, protein % and salt % were estimated on the day of sampling using milk analyzer (LCD display-4 lines x 16 characters, 100-240V-1.6A max., Bulgaria).

Statistical analysis

Statistical analysis was performed with SAS® version 9.4 (SAS 2013, Cary, NC, USA). We used Two ways ANOVA, with Tukey as post hoc test, to test for effects of type of ciliate culture, duration of transfaunation and their interaction. Data were summarized as mean±standard error of mean and statistical significance between groups was considered at $P<0.05$.

RESULTS AND DISCUSSION

Parameters of clinical examination were presented in Tables (2&3) and revealed non-significant ($P>0.05$) variations. These values agreed with (Pugh and Baird, 2012) and (Constable *et al.*, 2016).

Table 2: Physical examination parameters under effect of duration of transfaunation and type of transfaunated ciliate culture in Baladi dairy goats

Variables	Overall mean						
	Duration of transfaunation					Type of ciliate culture	
	NF*	T0	T7	T14	T21	TP	TM
Mucous membrane	Rosy red						
Pulse rate /min**	82.90±1.44a	85.13±2.18a	81.50±1.41a	80.75±1.34a	81.17±1.64a	81.40±0.85a	83.35±1.25a
Respiratory rate /min.	24.10±1.44a	21.75±1.07a	24.63±1.51a	24.00±1.62a	23.33±1.73a	23.05±0.76a	24.15±1.11a
Temperature C^o	38.90±0.19a	39.14±0.08a	38.91±0.18a	38.91±0.18a	38.98±0.17a	38.97±0.10a	38.96±0.12a
Rumen motility /2 min.	2.40±0.25a	2.38±0.25a	2.75±0.15a	2.88±0.12a	3.00±0.00a	2.80±0.11a	2.50±0.15a
BCS	2.35±0.07a	2.13±0.15a	2.19±0.12a	2.19±0.12a	2.17±0.15a	2.30±0.05a	2.13±0.09a

*NF: normally faunated; T0: defaunated; T7: 7 days after transfaunation; T14: 14 days after transfaunation; T21: 21 days after transfaunation; TP: transfaunated with pure culture; TM: transfaunated with mixed culture: **Means have common letter in the same row (within duration or type) are not significantly different at P value less than 0.05.

Table 3: Physical examination parameters under effect of interaction between duration and type of transfaunated ciliate culture in Baladi dairy goats

Variables*	NF		T0		T7		T14		T21	
	NFP**	NFM	T0P	T0M	T7P	T7M	T14P	T14M	T21P	T21M
Mucous membrane	Rosy red									
Pulse rate	80.40±2.29a	85.40±0.73a	82.75±2.30a	87.50±3.29a	82.50±1.09a	80.50±2.51a	81.00±0.94a	80.50±2.51a	80.33±1.44a	82.00±2.87a
Respiratory rate	26.20±1.86a	22.00±1.74a	22.00±1.54a	21.50±1.48a	22.75±0.89a	26.50±2.56a	21.50±0.90a	26.50±2.56a	21.67±1.19a	25.00±2.94a
Temperature	39.00±0.24a	38.80±0.29a	39.13±0.11a	39.15±0.11a	38.85±0.25a	38.98±0.26a	38.85±0.25a	38.98±0.26a	39.03±0.03a	38.93±0.34a
Rumen motility	2.40±0.36a	2.40±0.36a	2.75±0.22a	2.00±0.35a	3.00±0.00a	2.50±0.25a	3.00±0.00a	2.75±0.22a	3.00±0.00a	3.00±0.00a
BCS	2.40±0.09a	2.30±0.11a	2.25±0.13a	2.00±0.25a	2.25±0.13a	2.13±0.21a	2.25±0.13a	2.13±0.21a	2.33±0.14a	2.00±0.24a

*Means have common letter in the same row are not significantly different at *P* value less than 0.05. **NFP: normally faunated pure culture group; NFM: normally faunated mixed culture group; T0P: defaunated pure culture group; T0M: defaunated mixed culture group; T7P: 7 days after transfaunation with pure culture; T7M:7 days after transfaunation with mixed culture; T14P: 14 days after transfaunation with pure culture; T14M: 14 days after transfaunation with mixed culture; T21P: 21 days after transfaunation with pure culture; T21M: 21 days after transfaunation with mixed culture.

Table 4: Selected serum biochemical constituents under effect of duration of transfaunation and type of transfaunated ciliate culture in Baladi dairy goats

* Variables	Overall mean						
	Duration of transfaunation					Type of ciliate culture	
	NF	T0	T7	T14	T21	TP	TM
Calcium mmol/l	1.27±0.17c	2.91±0.07a	1.87±0.17bc	2.08±0.22abc	2.47±0.35ab	2.23±0.17a	1.88±0.18a
inorganic Phosphorous mmol/l	0.50±0.08b	1.09±0.17b	2.94±0.26a	0.95±0.11b	2.24±0.31a	1.42±0.20a	1.50±0.27a
Magnesium mmol/l	0.87±0.08a	1.00±0.08a	0.67±0.12a	0.79±0.11a	0.80±0.15a	0.95±0.07a	0.71±0.07b
Total protein g/l	69.40±5.37ab	74.26±5.23ab	58.74±9.61b	90.32±8.16a	75.89±4.13ab	72.14±4.39a	74.66±5.45a
Albumin g/l	25.40±1.52a	30.37±1.20a	25.00±2.42a	25.33±2.45a	22.74±1.33a	24.99±1.38a	26.82±1.21a
Globulin g/l	44.01±5.43ab	43.89±5.02ab	33.75±9.06b	64.99±7.29a	53.15±4.10ab	47.15±4.50a	47.85±5.02a
Albumin/globulin ratio	0.85±0.26a	0.76±0.09a	2.14±1.08a	0.42±0.06a	0.45±0.05a	1.02±0.46a	0.86±0.18a

* Means have common letter in the same row are (within duration or type) not significantly different at P value less than 0.05.

Table 5: Selected serum biochemical constituents under effect of interaction between duration and type of transfaunated ciliate culture in Baladi dairy goats

Variables*	NF		T0		T7		T14		T21	
	NFP	NFM	T0P	T0M	T7P	T7M	T14P	T14M	T21P	T21M
Calcium mmol/l	1.62±0.21ab	0.91±0.13b	2.87±0.07a	2.95±0.12a	2.04±0.06ab	1.70±0.30ab	2.18±0.39ab	1.99±0.18b	2.75±0.65a	2.18±0.10ab
Inorganic phos-phorous mmol/l	0.59±0.13bc	0.40±0.09c	1.09±0.17bc	1.09±0.31bc	2.80±0.39a	3.09±0.34a	1.07±0.16bc	0.84±0.12c	1.84±0.15ab	2.63±0.52a
Magnesium mmol/l	0.93±0.12a	0.81±0.11a	1.02±0.11a	0.98±0.10a	0.98±0.07a	0.36±0.09a	0.83±0.19a	0.74±0.11	1.02±0.20a	0.58±0.11a
Total protein g/l	71.42±6.31ab	67.39±8.60ab	67.67±4.84ab	80.86±8.03ab	69.12±13.9ab	48.37±10.97b	78.86±12.16ab	101.79±7.25a	74.37±7.71ab	77.42±2.70ab
Albumin g/l	23.12±2.36a	27.68±1.25a	29.06±1.9 a	31.68±0.98a	27.98±2.50a	22.02±3.56a	21.90±3.80a	28.76±1.91a	22.79±2.44a	22.70±1.08a
Globulin g/l	48.30±6.52a	39.71±8.24a	38.61±3.5 a	49.18±8.63a	41.14±15.43a	26.35±7.96a	56.95±10.08a	73.03±8.89a	51.58±7.65a	54.72±2.64a
A/G ratio	0.55±0.12a	1.15±0.47a	0.77±0.05a	0.76±0.18a	2.90±2.03a	1.37±0.47a	0.41±0.09a	0.43±0.07a	0.48±0.10a	0.42±0.03a

*Means have common letter in the same row are not significantly different at *P* value less than 0.05.

Table 6: Milk profile parameters under effect of duration of transfaunation and type of transfaunated ciliate culture in Baladi dairy goats

Overall mean							
Variables*	Duration of transfaunation					Type of ciliate culture	
	NF	T0	T7	T14	T21	TP	TM
Fat %	6.47±0.30 a	5.18±0.22 b	3.60±0.30 c	3.80±0.18 c	2.86±0.26 c	4.56±0.37 a	4.57±0.30 a
Lactose %	4.21±0.11 a	3.97±0.20 a	3.85±0.08 a	4.13±0.16 a	4.26±0.11 a	3.95±0.07 b	4.22±0.11 a
SNF %	8.81±0.20 a	8.24±0.42 a	7.89±0.18 a	8.47±0.32 a	8.65±0.22 a	8.16±0.14 b	8.68±0.22 a
Protein %	2.73±0.09 a	2.64±0.14 a	2.66±0.06 a	2.87±0.11 a	3.03±0.09 a	2.67±0.05 b	2.87±0.08 a
Salt %	0.74±0.02 a	0.70±0.04 a	0.68±0.01 a	0.72±0.03 a	0.74±0.02 a	0.69±0.01 a	0.74±0.02 a
Freezing point	-0.49±0.02 a	-0.48±0.03 a	-0.45±0.01 a	-0.49±0.02 a	-0.50±0.01 a	-0.47± 0.01 a	-0.50± 0.02 a

*Means have common letter in the same row (within duration or type) are not significantly different at *P* value less than 0.05.

Table 7: Milk profile parameters under effect of interaction between duration and type of transfaunated ciliate culture in Baladi dairy goats

Variables*	NF		T0		T7		T14		T21	
	NFP	NFM	T0P	T0M	T7P	T7M	T14P	T14M	T21P	T21M
fat	6.84±0.45a	6.11±0.33a	5.07±0.20abc	5.28±0.39 ab	3.28±0.39cd	3.92±0.39bcd	3.68±0.20bcd	3.91±0.28bcd	2.96±0.47d	2.77±0.20d
lactose	4.19±0.08a	4.23±0.20a	3.76±0.12a	4.18±0.36a	3.68±0.09a	4.02±0.08a	3.84±0.06a	4.43±0.22a	4.30±0.15a	4.22±0.17a
SNF	8.81±0.15a	8.81±0.38a	7.82±0.25a	8.67±0.73a	7.53±0.18a	8.25±0.17a	7.87±0.10a	9.06±0.46a	8.73±0.27a	8.56±0.35a
protein	2.69±0.08a	2.77±0.17a	2.49±0.09a	2.79±0.25a	2.56±0.07a	2.77±0.05a	2.65±0.06a	3.08±0.16a	3.05±0.14a	3.00±0.12a
salt	0.74±0.01a	0.74±0.03a	0.66±0.02a	0.73±0.06a	0.65±0.01a	0.71±0.01a	0.67±0.01a	0.77±0.04a	0.75±0.02a	0.72±0.03a
freezing point	-0.49±0.02a	-0.48±0.03a	-0.45±0.02a	-0.51±0.05a	-0.43±0.01a	-0.48±0.01a	-0.45±0.01a	-0.53±0.03a	-0.51±0.02a	-0.50±0.02a

*Means have common letter in the same row are not significantly different at P value less than 0.05.

Serum constituents were presented in tables (4&5). Calcium level decreased significantly in T7 group than T0 group but increased significantly in T21 group than NF group, however it recorded significant increase in T21P, T0P and T0M groups than NFM group. The highest recorded value of Calcium in T0 group may be explained on basis of the highest rumen calcium which absorbed partially through rumen epithelium, while high value in T21 group may be attributed to high concentrate mixture intake containing dietary calcium. Calcium in NF group agreed with (Saber *et al.*, 2020), but disagreed with (Olafadehan *et al.*, 2014) and this could be referred to variations in breed, nutrition and raising circumstance.

Inorganic P increased significantly in T21 and T7 groups than T14, T0 and NF groups, whereas T21M, T7P and T7M groups revealed significant increase than T14M, T14P, T0M, T0P, NFP and NFM groups and the later decreased significantly than T21P, and this variation may be referred to variation in feed intake. Value of NF group disagreed with values reported by (Pugh and Baird, 2012) and (Olafadehan *et al.*, 2014) however, value of T21 group agreed with those previous authors.

Magnesium decreased significantly in TM group than TP group, while non-significant differences were recorded regarding the effect of duration-type interaction. Value of NF group agreed with (Casamassima *et al.*, 2007) and (Olafadehan, 2011), while (Mozaffari *et al.*, 2011) and (Pugh and Baird, 2012) recorded higher values.

Total protein and globulin increased significantly in T14 group than T7 group, whereas, regarding the effect of duration-type interaction, total protein increased significantly in T14M than T7M, but globulin showed non-significant differences. Total protein in NF group agreed with (Olafadehan, 2011) and (Pugh and Baird, 2012), while (Sadjadian *et al.*, 2013) reported higher value, but (Al-Habsi *et al.*, 2007) and (Sharma and Puri, 2013) recorded lower values and this variations could be attributed to different nutritional circumstances. Globulin in NF group agreed with (Olafadehan, 2011) and (Olafadehan *et al.*, 2014), while (Ikhimioya and Imasuen, 2007) mentioned higher value, but (Pugh and Baird, 2012) and (Sharma and Puri, 2013) estimated lower values and this could be referred to different nutrition and clinical conditions.

Albumin and A/G ratio revealed non-significant variation. Albumin in NF group agreed with (Latimer, 2011) and (Sharma and Puri, 2013), whereas (Sadjadian *et al.*, 2013) and (Al-Bulushi *et al.*, 2017) recorded higher values and this might be attributed to different nutrition and health statuses of goats. A/G ratio in NG group disagreed with (Sharma and Puri, 2013) who reported higher ratio.

The process of making milk fat includes not only the availability of acetate and butyrate, but also several enzymes that work in a coordinated fashion to synthesize fatty acids and use them to make "fat" (Bionaz and Loor, 2008). Milk fat synthesis also relies on the availability of dietary and rumen-derived fatty acids that are absorbed in the small intestine during digestion. These facts could easily explain the direct relationship between type and count of rumen microbiota and fat content and fatty acid profile in milk. From the standpoint of protozoa, the data concluded by (Loor *et al.*, 2016) supported the hypothesis of (Lima *et al.*, 2015) that the decrease in fungi and ciliate protozoa abundance leads to milk fat depression.

In our study, data presented in (table 6) showed the influence of rumen transfaunation on milk fat. Fat % in T7 group, T14 group and T21 group decreased significantly ($P<0.05$) than T0 group which decreased significantly ($P<0.05$) than NF group. On the other hand, there was no significant difference in fat % between pure and mixed ciliate culture used. However, T21P and T21M groups showed significant decrease in fat % than NFP, NFM, T0P and T0M groups. Also, fat % recorded significant ($P<0.05$) increase in NFP and NFM groups than T7M, T7P, T14P and T14M groups (table 7). These results could be explained on basis of certain correlation between fat % and rumen ciliate culture over time; fat % in both cultures treated groups was decreased every week to be finally significantly affected by the end of transfaunation period. Depending on our results, the use of *Holotricha* pure culture or in mixture with *Ophryoscolex* couldn't improve or even keep milk fat % away from decline, and this might be referred to failure of transfaunation to optimally restore rumen fermentation after it is being affected because of defaunation which caused significant decrease in milk fat content than normally faunated animals.

Depending on our results, even though milk fat % showed significantly sequential decline within the 21 days of transfaunation; lactose %, SNF %, protein %, Salt % and freezing point showed non-significant ($P>0.05$) differences between groups considering the effect of duration-type interaction, while regardless the effect of duration of transfaunation, lactose %, SNF % and protein % increased significantly ($P<0.05$) in TM group than TP group.

Conclusions

Transfaunation of pure culture of *Holotricha* or in a mixture of 82% *Holotricha* and 18% *Ophryoscolex* weekly for three consecutive weeks in defaunated goats had no effect on clinical examination with minimal non-significant improvement of serum calcium, inorganic phosphorous, total protein and globulin. Transfaunation of these types of ciliate cultures could not improve significantly decreased milk fat % of defaunated goats, without any effect on other measured milk profile parameters. It is recommended to

apply further investigations on transfaunation without prior defaunation using different pure and mixed cultures of rumen ciliates for therapeutic and productive purposes.

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