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STUDIES ON PARASITIC GASTROENTERITIS

(With 7 Tables and 10 Figures)

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دراسات عن النزلات المعدية - المعوية الطفيلية في الأغنام

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تم إجراء هذه الدراسة للتعرف على بعض الجوانب الوبائية والإكلينيكية المتعلقة بالإصبابة بالطفيليات الداخلية في الأغنام خلال الفترة من يناير ٢٠٠٤ إلى ديسمبر ٢٠٠٤ في محافظه الغربية حيث شمات هذه الدراسة عند ٣٢٥ حيوان من السلالة المحلية للأغنام. أوضحت هذه الدر اسة أصابه عدد ٢٣٣ من الأغنام بالديدان الاسطوانية ممثلة نسبه أصابه قدرها ٧١،٦٩ % حيث كانت نسبه الأصابه في عمر ما بين ١ إلسي ٢ سنه ٨٠،٩٥ ونسبه الأصابه في العمر ما بين ٢ إلى ٣ سنوات بلغت ٩٦ ، ٦٦ % وأقل نسبه أصابه كانت في، عمر ما فوق ٣ سنوات بنسبه ٦٧،٢١% بالإضافه إلى أن نسبه الأصابه في الذكور كانت ٧٣٠٥٣ % بينما في الإناث بلغت ٧١٠٢١ % و أظهرت الدراسة أيضا أن نسب الاصابه كانت عالية في أغنام المراعي بنسبه ٨٩،٦٢ ثلتها الأغنام التي تربي بصورة منفردة في المنازل بنسبه أصابه ٢٧،٧٩% ثم الأغنام التي تربي بنظام متحكم في المزارع بنسبه أصابه ٢٥،٦٤ %. بالفحص المجهري تبين الكشف عن ديدان أفراد عائله التريكوسترونجيلس (Trichostrongylus spp.) ثم أفسراد السسترونجلويد (Strongyloides spp.) وأفسراد التريكيورس (.richuris spp). وأوضح الفحص الإكلينيكي للحيو انسات المصمابة أن أهم أعراض الأصابة كانت عبارة عن الهزال والإسهال وقله معدل النمو وسهوله تساقط الصوف بالاضافه إلى ابيضاض الأغشية المخاطية. وبالفحص التشريحي لبعض الحيوانات المصابة وجد أن المعدة الحقيقية بها طفيل هيمونكس (Haemonchus spp.) وأن الأغشية المخاطية بها احمرار وبعض التقرحات الصغيرة وبعض الأنزفه النقطيه مكان التصاق الطفيل بالغشاء المخاطي للمعدة الحقيقة ومحتويات المعدة الحقيقة كانت داكنة اللون. وبالفحص النسيجي تبين وجود تجمعات خلوية التهابية بين خلايا الغشاء المخاطى والغدد المعدية الموجودة به معم تهتك وسقوط لبعض الخلايا المبطنة. بدراسة تأثير نباتُ الشيكوريا (السريس أوالخس البلديُ) أو مستخلصه على طفيليات المعدة والأمعاء وجد أن نبات الشيكوريا له دور فعال في منع الأصبابه بالطفيليات.

SUMMARY

This study was carried out throughout one year from January 2004 to December 2004 at Gharbia governorate, Egypt, to determine some epidemiological and clinical features concerning parasitic gastroenteritis sheep. Moreover the efficacy of chicory against parasitic gastroenteritis was studied. Out of 325 native breed sheep examined, 233 animals proved to be infested with parasitic gastroenteritis representing an incidence rate of 71.69%. The highest infestation rate was recorded in mobile flocks among young age from 1 to 2 years during winter and autumn, whereas the susceptibility was not significantly affected by sex. Trichostrongylus spp., Strongyloides spp. and Trichuris spp. were recorded. Clinically, infested sheep showed signs of emaciation, diarrhea, poor growth rate, wool easily detached and pale mucous membrane. Necropsy finding of sheep infested with Haemonchosis revealed hyperemia, ulcers and petechial hemorrhages on infested abomasa. Whereas, histopathological examination revealed mononuclear cell infiltration in between mucosal glands and in the lamina propria in addition to necrosis and sloughing of mucosal epithelium. Chicory (Cichorium intybus) as green fodder had a good role in prevention of infestation with parasitic gastroenteritis, in addition it increase the live body weight gain. Moreover, its extract in vitro, decreased hatchability and development of nematode eggs and reduced the viability of obtained larvae at low concentration while the high concentration lead to death of these larvae.

Key words: Sheep, Parasitic gastroenteritis, Signs, Pathology, Chicory

INTRODUCTION

Sheep is considered as one of the most important farm animals in Egypt. Moreover, mutton and goat meats are preferable in Arabian region especially as ingredients for special dishes offered in Islamic festivals and other social occasions (El-Azazy, 1995).

Nutritional factors and helminthosis are the two main factors that may lead to decreasing sheep productivity (Dorny et al., 1996). Parasitic diseases remain a major constraint to livestock production systems in Africa, and gastrointestinal nematodes remain with a major economic importance in domesticated livestock throughout the world (Prichard, 1994). Parasitic infestations are worldwide distributed, particularly in the

third world countries. In animals, the infestation usually results in serious economic losses due to mortality, morbidity and/or decrease of performance (Lawrence, 2003). Moreover, gastrointestinal parasitism reduces the efficiency of production of ruminants and has adverse effects on their welfare.

Anthelmintic drugs and grazing management strategies are the main tools in controlling the parasitic infestations in sheep (Gauly et al., 2004). Mc Kenna et al. (1995) estimated that over 60% of sheep farms have detectable levels of resistance to at least one anthelmintic family. An increase in the emergence of parasites that are resistant to pharmaceutical anthelmintics (Jackson and Coop, 2000) has encouraged alternative approaches to parasite control (Marley et al., 2006).

There is also increasing public concern over drug residues in meat and milk products, and the potential for environmental contamination (Mc Kellar, 1997).

In recent years, there have been increasing interests in ethnomedical and ethnoveterinary practices across the world especially as it relates to the use of medicinal plants in treating various ailments. In developed world, this trend is in response to the production of animals free from industrial chemical inputs (Gasbarre et al., 2001) and the need to discover new therapeutic substances of natural origin with possibly low toxicity to man and animals (Guarrera, 1999). In livestock in Africa, this is borne out of the economics of affordability (Schillhorn Van Veen, 1997).

There have been great interests in the development of non-therapeutic approaches to control helminthes and insect pests of livestock in the last few years. Biological control will be an important part of livestock parasite control in the future (Padilha, 1999). There has been little study into the possibility that some forage species may be able to reduce production losses associated with internal parasitism (Niezen et al., 1995).

Some plants, which contain condensed tannins (CT), have nematicidal properties against free-living nematodes (Chandel and Mehta, 1990). The herb chicory (Cichorium intybus) and the condensed tannin-containing legumes have the potential to influence the nematode burden in sheep and other species (Hoskin *et al.*, 1999), and/or associated with an improved growth rates in lambs which have high fecal parasite egg counts (Niezen *et al.*, 1998a).

The aims of the present work were directed to determine some epidemiological, clinical and histopathological features concerning

parasitic gastroenteritis in sheep. Moreover, assessment the efficacy of chicory as an alternative method for nematode remedy as well as its aqueous extract on the development and hatchability of gastrointestinal nematode eggs in fecal culture was studied.

MATERIALS and METHODS

Animals:-

1. Field animals:-

At Gharbia Governorate, Egypt, a total of 325 sheep; 227 reared in mobile flocks, 59 reared individually and 39 reared in fixed farm with different ages and sex at were used in this study during the period from January, 2004 to December, 2004.

2. Experimental group:-

Fifteen parasite-free yearling sheep were used to study the efficacy of chicory in reducing the egg output of gastrointestinal nematodes in sheep.

Plant:-

- 1. Chicory (Cichorium intybus) as a green fodder.
- 2. Crude aqueous extract of chicory (Cichorium intybus):

The extract was prepared according to Alawa et al. (2003). Briefly, 1 Kg of the plant materials, in its herbal form, was extracted in water, filtered through filter paper in vacuum flask apparatus, and then dried in freeze drier. The dried extract was stored at -4° C to be checked regarding its effect on hatching and development of the larval stage of gastrointestinal nematodes in vitro.

Epidemiological investigation:-

Morbidity rate, age and sex susceptibility relationship as well as the seasonality of gastrointestinal nematodes were estimated according to Martin (1987).

Clinical examination:-

All examined sheep were subjected to clinical examination according to Kelly (1984).

Fecal examination:-

1. Macroscopic examination:-

Each fecal sample was examined by the naked eyes for the presence of any abnormalities (gross parasites, blood or mucous shreds, color, odor and consistency of the feces).

2. Microscopic examination:-

Concentration floatation technique using saturated salt solution, fecal egg counts, fecal culture and larval identifications were carried out according to Soulsby (1982).

Histopathological examination:-

At Tanta abattoir, 5 abomasa infested with Haemonchus spp. were examined grossly and histopathologically. After recovery of adult worms, suitable portions of the infested abomasa were collected and fixed in 10% buffered neutral formalin solution. These tissues were embedded in paraffin wax. Five microns thick paraffin sections were prepared and stained with Haematoxylene and Eosin (H&E) then examined microscopically according to Drurag and Wallington (1980).

Determination of chicory efficacy in reducing the egg output:-

Fifteen native lambs, free from gastrointestinal parasites, were divided into 3 groups. Animals of the first group were fed on berseem only. Those of the second group were fed on chicory - berseem (1:1) whereas animals of the third group were fed on chicory only for 2 months. Fecal samples were collected from each lamb after 2 weeks from feeding and then every 2 weeks. Intensity of infestation was determined by concentration floatation techniques and Mc-Master techniques according to Soulsby (1982). In addition, body weights were determined at day zero, after one month of the experiment and at the end of the experiment. The mean growth rate was recorded for each group.

Fecal hatch assay for determination the efficacy of chicory (Cichorium intybus) extract:-

It was done according to the Alawa et al. (2003).

Statistical analysis:-

The obtained results were statistically analyzed using Chi-square and student t- test according to Snedecor and Cochran (1980).

RESULTS

Table 1: Incidence of parasitic gastroenteritis in relation to type of rearing

Type of rearing	No. of examined sheep	No. of infested sheep	%		
Mobile flock	227	183	80.61		
Individual rearing	59	40	67.79		
Fixed farm	39	10	25.64		
Total	325	233	71.69		

Table 2: Incidence and percentage of different gastrointestinal nematode among infested sheep (No. of examined sheep = 325 & No. of infested sheep = 233)

Causing nematode	No.	%	Prevalence
Trichostrongylidae (single)	151	64.80	46.46
Strongyloides (single)	22	9.44	6.76
Trichostrongylidae + Strongyloides	58	24.89	17.84
Trichostrongylidae + Strongyloides + Trichuris	1	0.42	0.30
Trichostrongylidae + Trichuris	1	0.42	0.30

Table 3: Incidence of parasitic gastroenteritis in relation to age

Age	No. of examined sheep	No. of infested sheep	%	
Under 1 year	91	62	68.13	
1 year upto 2 years	84	68	80.95	
2 years upto 3 years	89	62	69.66	
Over 3 years	61	41	67.21	
Total	325	233	71.69	

N.B. No significant variation between different age groups.

Table 4: The effect of chicory in prevention of parasitic gastroenteritis

Group	Pre-experiment	After 2 weeks	After 4 weeks	After 6 weeks	After 8 weeks	
Group I	- ve	120 epg	190 epg	480 epg	790 epg	
Group II	- ve	60 epg	110 epg	200 epg	340 epg	
Group III	- ve	- ve	- ve	- ve	- ve	

Table 5: Mean growth rate in animals fed on berseem, berseem/chicory and chicory.

C	Mean body weights					
Group	Pre experiment	Post experiment				
Group I	26.6±1.83	27.2±1.16				
Group II	27±0.83	30.2±0.86				
Group III	30±0.83	36±0.83***				

Table 6: Effect of different concentrations of chicory extract on viability of larvae that obtained from fecal culture

Concentration	No. of obtained larvae	Viability of obtained larvae	% of dead larvae	
Fecal culture without extract	2 larvae/ml	High viability	9.5	
Fecal culture with 1% extract	1.5 larvae/ml	Moderate to low viability	20	
Fecal culture with 2% extract	l larvae/ml	Low viability	75	
Fecal culture with 4% extract	0.5 larvae/ml	Dead	100	
Fecal culture with 8% extract	0.25 larvae/ml	Dead	100	

Table 7: Effect of different concentration of extract on nematode eggs hatchability

Conc.		Egg remained unhatched									
	No, of eggs at 0 day	With embryonic cells		With larvac						Total	
		No.	%	With dead larvae		With viable larvae		Total		No.	%
				No.	%	No.	%	No.	%	1	ł .
Control	1160	100	8.62	-	-	-	-	-	-	100	8.62
1% extract	1160	500	62,5	204	25.50	96	12	300	37.50	800	68.96
2% extract	1160	510	63,75	218	27.25	72	9	290	36,25	800	68.96
4% extract	1160	1000	86,20	-	-	-	-	-	-	1000	86.20
8% extract	1160	1100	94.82	_		-	-	-	-	1100	94.82

FIGURE LEGENDS

- Fig. 1: Bunstomum phelopotomum larva.
- Fig. 2: Ostertagia ostertagia larva.
- Fig. 3: Strongyloid spp. larva.
- Fig. 4: Trichostrongylus spp. larva.
- Fig. 5: Sheep infested with parasitic gastroenteritis and showed severe emaciation.
- Fig. 6: parasite in between mucosal cells (H&E Stain X 200)
- Fig. 7: Mononuclear cells infiltration in between mucosal glands (H&E Stain X 200).
- Fig. 8: Mononuclear cells infiltration in the lamina propria (H&E Stain X 200).
- Figs. 9 and 10: Necrosis and sloughing of mucosal epithelium (H&E Stain X 200).

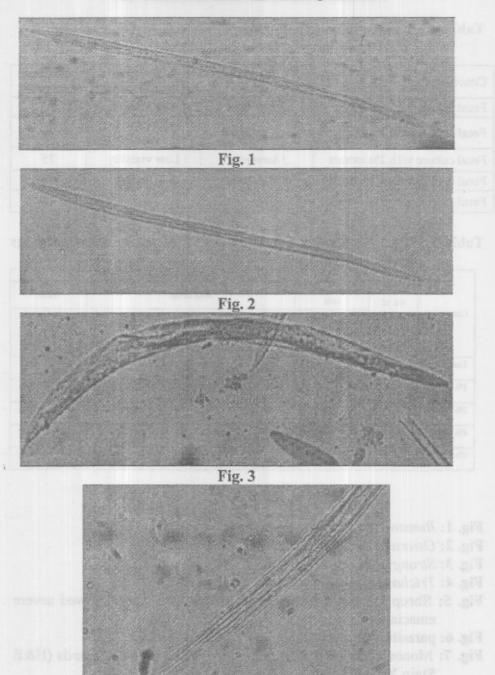
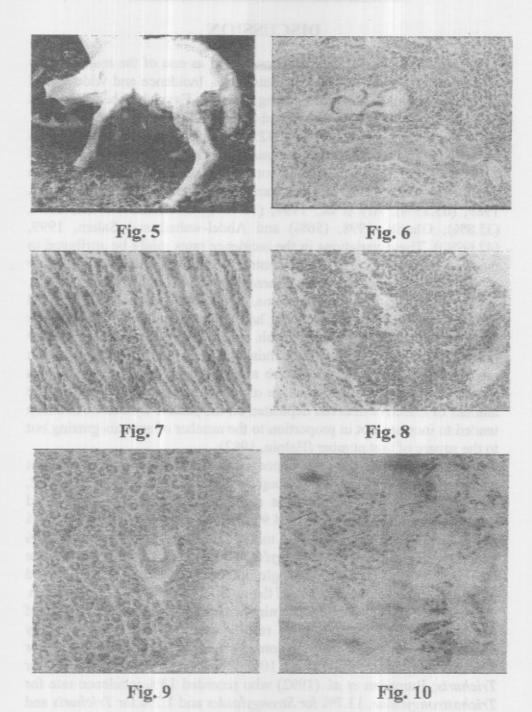


Fig. 4



DISCUSSION

Parasitic gastroenteritis is considered as one of the major causes of production losses in wet temperate areas. Incidence and wide spread of gastrointestinal nematodes among sheep in Egypt had been reported (Gharib, 1998 and Abdel-Wahed and Salem, 1999).

In this investigation out of 325 examined sheep 233 were proved to be infested with different nematodes representing an incidence of 71.69% (Table 1). Many studies concerning parasitic gastroenteritis in sheep were done in Egypt with variable incidence rates (El-Fayoumi, 1989, (65.83%); Aly et al., 1994, (18.13%); El-Shahawi et al., 1994, (35.8%); Gharib, 1998, (56%) and Abdel-wahed and Salem, 1999, (42.66%)). These variations in the incidence rates might be attributed to the type of rearing, hygiene and control measures applied in each study as well as the locality and environmental conditions in each locality.

Regarding the rearing systems, it was found that sheep that raised in flocks and depended on grazing had highest incidence with parasitic gastroenteritis (80.61%). This result agreed with the result of Pandey (1990). Whereas animals, which raised at farm had lowest infestation rate (25.64%). Grazing sheep were at risk of exposure to a dangerous level of challenge due to its nature of prehention. It was found that the amount of infective material deposited on the pasture by a flock of sheep tended to increase not in proportion to the number of animals grazing but to the square of that number (Halpin, 1982).

the incidence and percentage Concerning of different gastrointestinal nematodes among sheep as shown in table 2, Trichostrongylidae were the most common type passed from infested sheep where it was detected in 64.92% of the examined sheep (46.46% as single infection and 18.46% as mixed infection). It represents 90.55% of the total infested sheep. Strongyloides was detected in 24.92% of the examined sheep (6.76% as single infection and 18.15% as mixed infection). It represents 34.76% of the total infested sheep. *Trichuris* spp. was detected in 0.61% of the examined animals and represents 0.85% of the total infested ones. Similar results were reported previously by El-Favoumi (1989) who recorded 63.5% incidence rate for Trichostrongylidae followed by 10% for Strongyloides and 4.5% for Trichuris; Ramadan et al. (1992) who recorded 13% incidence rate for Trichostrongylidae, 13.7% for Strongyloides and 1.2% for Trichuris and Hashem and El-Sayed (1997) who recorded 46.4% incidence rate for Trichstrongylidae, 8% for Strongyloides spp. and 4% for Trichuris spp.

Our results were disagree with the results of Gharib (1998) who found that *Trichuris ovis* was the most common species of gastrointestinal nematode in sheep and Ghoniem and Amjad (1992) who found that *Strongyloides*, *Nematodirus* and *Trichuris* species were the only fecal parasites detected in sheep.

Concerning age susceptibility as shown in table 3, Sheep of the age group from 1 to 2 years was more susceptible to parasitic gastroenteritis with 80.95% infestation rate followed by sheep of the age group between 2 and 3 years (69.66%) then sheep of the age group under 1 year (68.13%), then group over 3 years (67.21%). Aly et al. (1994) and Chauhan et al. (2003) previously obtained similar results. On the contrary, Ramadan et al. (1992) mentioned that sheep and goats over 3 years old were mostly infested with parasitic gastroenteritis. The variation of age susceptibility might be immune dependant. This explanation agreed with the results of Grenfell et al. (1995) who indicated that the immunity increased and subsequent decline in the mean burden of parasites with host age.

Concerning sex predisposition, it was noticed that the variation of the infestation rate in males and females was non statistically significant (73.52% and 71.20% respectively). Gulland and Fox (1992) found that incidence and intensity of infection (fecal egg counts) were higher in males than females except during the lambing period.

The 3rd stage larvae which obtained from fecal culture (Figs. 1-4) were *Trichostrongylus spp.*, *Bunostomum phelopatomum*, *Strongyloides papilosus*, *Ostertagia ostertagi*, *Ostertagia triforcata* and *Haemonchus* spp. Similar results were reported previously by Abdel-Wahed and Salem (1999) and Theodoropoulos *et al.* (2000).

The relationship between seasons and 3rd stage larvae which obtained from fecal culture revealed that the predominant 3rd stage larvae obtained at winter were *Trichostrongylus* spp., *Bunostomum spp.* and *Strongyloides papilosus* while at spring the predominant larvae obtained were *Bunostomum* spp., and *Ostertagia* spp. At summer the obtained larvae were *Ostertagia* and *Haemonchus* and at autumn were *Ostertagia ostertagi, Ostertagia triforcata, Bunstomum phelopotomum* and *Strongyloid papillosus*. The obtained results were supported by the records of Cheah and Rajamanickam (1997) and Horak (2003) who recorded an increase of *Haemonchus* spp., at summer and *Ostertagia* spp., at summer and spring and with results of Gulland and Fox (1992); Ramadan *et al.* (1992) and Waller *et al.* (2004) who recorded an increase in the infestation with *Trichostrongylus* spp., at winter and *Strongyloid*

spp., at winter and autumn. Our results were disagree with the results of Suarez and Busetti (1995) and Vatta *et al.* (2002) who recorded that *Haemonchus* spp. infestation was more prevalent at winter.

Concerning the clinical signs, 22.31% of the infested sheep showed no apparent clinical signs whereas 77.69% showed clinical signs. The main clinical signs were emaciation (Fig. 5), diarrhea, soft feces, easily detached wool, pale mucous membrane and poor growth rate. These results were agree with the results recorded by El-Gharib et al. (1995); Theodoropoulos et al. (2000) and Vatta et al. (2002). It was noticed that the severity of the clinical signs was related to the intensity of infestation.

At abattoir, the gross examination of 5 abomasa obtained from 5 sheep infested with *Haemonchus* spp., revealed thickness, hyperemia and small ulceration. Histopathological examination revealed parasitic larvae in-between mucosal cells, mononuclear cell infiltration in-between the mucosal glands. In addition to mononuclear cell infiltration in the lamina propria (Figs. 6-8). Necrosis and desquamation of mucosal epithelium were also observed (Figs. 9-10). These results were similar to that obtained by Scott *et al.* (1998) and Hertzberg *et al.* (2000).

Forage chicory produce a large quantity of high quality feed in warm season under favorable conditions. Also animal performance on chicory is similar to that on legumes and superior to grass-based pastures. In addition, grazing chicory can decrease some internal parasites in live stock and therefore has potential to reduce the use of anthelmintics. (Guangdi and Kemp, 2005).

The efficacy of chicory in reducing the opportunity of infection with gastrointestinal nematodes was determined. It was found that the infestation began in all animals fed on berseem at the 2nd week of experiment and increased gradually till the end of experiment while animals of the 2nd group which fed on berseem with chicory, only 3 animals caught the infestation with low level till the end of the experiment and the last group which fed chicory no animal infested as shown in table 4. This result gave an indication that feeding on chicory can protect animals from gastrointestinal nematode infestation. These results agreed with that obtained by Marley *et al.* (2003 a.); Athanashi caiadou *et al.* (2005) and Tzamaloukas *et al.* (2005) who found that infected lambs that grazed on chicory had lower fecal egg counts and adult nematode population. Moreover, Hoskin *et al.* (1999) found that deers which grazed on chicory not required anthelmintic treatment. The anthelmintic activity of chicory may explained basing on its content of

condensed tannin 1.7g/Kg DM, sesquiterpene lactones 3.6g/Kg DM, cichoriin 0.5g/Kg Dm and chicoric acid 5.8g/Kg DM (Ress and Harborne, 1985; Scales et al., 1995 and Jackson et al., 1996). Moreover, Molan et al. (1999); Hoskin et al. (2000) and Molan et al. (2000 b.) found that the forage which contain condensed tannin have a good effect on reducing gastrointestinal nematode. In addition, sesquiterpene lactones have a direct effect on nematode and viability of the first and the third larval stages (Molan et al., 2000 a. and Schreurs et al., 2002). Also Moss and Vlassoff (1993) and Niezen (1998 b.) recorded that chicory lowered 3rd stage larvae (infective stage larvae) population than other grass.

Concerning life body weight gain as shown in table (5) it was observed that sheep which fed only on chicory showed an increase in the body gain followed by the 2nd group that fed on chicory mixed with berseem and lastly the group that fed on berseem only. These results were agree with the results that obtained by Hoskin et al. (1999) and Landau et al. (2005). Beside the efficacy of chicory against gastrointestinal nematodes, it also contain high nutritive value help to the increase of life body weight where Barry (1998) found that structural carbohydrate is particularly higher in chicory and metabolizable energy (ME) concentration is particularly high in vegetative chicory forage. Moreover, the digestibility of organic matter (OM) is higher for chicory whilst the converse is true for neutral detergent fiber (NDF) digestibility the later may be due to the much lower rumen pH of chicory-fed animals restricting the activity of rumen micro-organisms degrading cellulose and hemicellulose. Particle breakdown during rumination was much faster for chicory (Kusmartono et al., 1996). In addition chicory can increase microbial protein production as the increased rumen liquid for animals fed chicory could be expected to increase rumen outflow of microbial protein (Harrison et al., 1975). The high ratio of readily fermentable structural carbohydrate in chicory would also provide an increase in the energy for microbial growth (Barry, 1998).

Concerning the efficacy of chicory in reducing the fecal egg counts of gastrointestinal nematodes, our results were disagree with the results of Marley *et al.* (2003 b.) who found that lambs that grazed chicory did not have significantly lower fecal egg counts than lambs grazing other forages but its efficacy against the adult was proved where, these lambs were found to have fewer total adult abomasal helminthes than lambs grazing ryegrass/white clover.

The efficacy of aqueous extract of chicory in reducing the hatchability of nematode eggs was assessed using fecal hatch assay. Different concentrations of chicory's extract were used (1%, 2%, 4% and 8%). It was found that there was 2 larvae/ml obtained from control sample, 1.5 larvae/ml obtained at concentration 1%, 1 larvae/ml obtained at concentration 2%, 0.5 larvae/ml obtained at concentration 4% and 0.25 larvae/ml obtained at concentration 8% extract. The larvae obtained had high viability in control sample, moderate to low viability at concentration 1%, low viability at concentration 2% and found dead at concentration 4% and 8% extract. 9.5% of obtained larvae found dead in control sample, 20% of larvae found dead at concentration 1%, 75% of larvae found dead at concentration 2% and all larvae obtained found dead at concentration 4% and 8% (Table 6). These results were agreed with the results that obtained by Molan et al. (2000 a.) and Schreurs et al. (2002). Moreover the chicory's extract suppress the development of the nematode eggs where at concentration 1% & 2% of extract about 68.96% of egg remained unhatched and at 4% about 86.20% of eggs remained unhatched and at 8% extract concentration about 94.82% of eggs remained unhatched compared to 8.62% of eggs remained unhatched in control sample. It was found that, the rate of development of larvae in the remained unhatched eggs was greatly affected by the concentration of the added extract; at concentration of 1%, 37.5% of the eggs developed larvae while 36.25% of eggs had been developed at concentration of 2% extract. On the other hand the development was completely suppressed at concentration of 4% and 8% (Table 7).

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