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EVALUATION OF CONTROL SCHEMES USING PROTEIN SUPPLEMENTATION AND ANTIHELMINITICS AGAINST GASTROINTESTINAL NEMATODES OF DAIRY EWES

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The purpose of the study was to investigate the possible influence of dietary protein supplementation and anthelmintic administration, in different schemes, for the control of gastrointestinal (GI) nematodes of dairy ewes under field conditions. Towards this end, 40 clinically healthy ewes of a local Greek dairy breed, grazing on infected irrigated pasture, were divided into 5 groups and treated as follows: Group (1S) supplemented with 300 g soybean meal 48%, twice per year (July and periparturiently), Group (2Alb) received albendazole twice as (Group 1S), Group (3S-Alb) supplemented with soybean as in (Group 1S) combined with the use of albendazole (as in Group 2Alb), Group (4Alb3) received albendazole three times (September, periparturiently and April) and Group (5C) (control group). Faecal samples were taken from each animal monthly for nematode egg count (FEC) and blood samples every two months for haematological and biochemical analysis. The results showed that the administration of albendazole twice reduced effectively the nematode FEC, whereas the extra protein supplementation did not significantly influence it. Haematocrit and total proteins were unaffected although they tended to be lower in the control Group (5C). Pepsinogen, calcium, inorganic phosphorus and magnesium were not influenced by the treatments.

Key words: albendazole, dietary protein, ewes, nematodes

INTRODUCTION

Sheep are most commonly bred under the semi-intensive system and therefore are infected with a large burden and variety of parasites resulting to mild or severe parasitosis. Among the parasites, the gastrointestinal (GI) nematodes may cause "invisible", but significant losses to animal production.

Gastrointestinal (GI) parasites alter blood parameters like total proteins (Kyriazakis *et al*., 1996), plasma pepsinogen (Stear *et al*., 1999), haematocrit (Amarante *et al*., 2004) and calcium (Ca), phosphorous (P) and magnesium (Mg) (Poppi *et al*., 1985).

Anthelmintics administered non-strategically result not only in direct economic losses (cost of anthelmintics), but also in a subsequent increase of the parasite burden, as expected after the disturbance of the host-parasite immunity balance (Theodoridis *et al*., 1992; Leathwick, *et al*., 2006). It is known that the supplementation of dietary protein improves the resistance of sheep to the GI nematode infection (Coop and Kyriazakis, 1999). However, most of these studies deal with the influence of protein supplementation in experimentally protein-depleted sheep. Therefore, it is challenging for researchers to integrate both these control options under field conditions, with animals receiving adequate protein levels in their diets, in order to investigate the possibility to develop a sustainable protection against GI nematodes.

The aim of this experiment was to study the possible influence of dietary protein supplementation and antihelmintic administration, in different schemes, for the control of GI nematodes of dairy ewes under field conditions, evaluated by parasitological, haematological and biochemical parameters.

MATERIALS AND METHODS

Experimental design

Forty clinically healthy ewes aged from two to four years, of a local Greek dairy breed, were equally divided into 5 groups, according to the nematode parasitic burden, body weight (b.w.) and age, and were treated as follows:

Group 1S: supplementation of 300g dietary protein (soybean meal 48%, Soya Mills S.A.), in the morning before grazing, twice: i) during summer for a two month period (July – August) and ii) for 20 days before and 20 days after parturition (November – December), approximately.

Group 2Alb: administration of albendazole (Albendazole oral susp.), at a recommended dose 7.5 mg/kg rate of b.w., twice: first in July and second in November, approximately two weeks before parturition.

Group 3S-Alb: supplementation of dietary protein as in Group 1S combined with the use of albendazole as in Group 2Alb.

Group 4Alb3: administration of albendazole three times: in September, periparturiently in November, and in April.

Group 5C: ewes received neither protein supplementation nor anthelmintics (control group).

The experiment lasted one year from July to June. All studied ewes were grazing on the same permanent pasture, known to be contaminated with a mixture of trichostrongyle larvae, throughout the experimental period. Additionally, they were all receiving on daily basis alfalfa hay and a commercial concentrate feed, offered in equal portions twice a day. The amount of this concentrate feed during lactation varied from 500 to 700 g according to milk yield, whereas in the non-lactating period all animals were fed 300 g of the above feed.

The health status of the animals, mainly the presence of diarrhea, was monitored monthly throughout the experiment. Faecal samples were taken from

each animal monthly for egg output evaluation and blood samples every two months for haematological and biochemical analysis.

Sampling, parasitological techniques and blood analyses

Faecal samples were collected directly from the rectum of each ewe and tested by the modified McMaster method for faecal egg counts (FEC) per g (epg) pooled faecal sample was cultured for larval development and identification (MAFF, 1986).

Blood samples were taken by jugular vein puncture, into evacuated glass tubes with and without anticoagulant. Serum and plasma were separated by low speed centrifugation and forwarded for biochemical analysis. In serum Ca and Mg were determined by means of flame atomic absorption spectophotometry (Perkin-Elmer Co, 1977), total proteins were determined colorimetrically (Weichselbaum, 1946) and inorganic P was evaluated by the heteropoly blue method (Boltz and Lueck, 1958). Plasma samples were analysed for levels of pepsinogen (indirectly through tyrosine measurement) by the modified method described by Edwards *et al*. (1960). Haematocrit was measured by the microhaematocrit apparatus according to Schalm *et al*. (1975).

Statistical analysis

The statistical analysis of the data included tests of one and two variables (associations) at 5% level of significance. Student *t*-tests, analysis of variance (ANOVA) and Duncan tests were performed using SPSS version 10 (SPSS Inc).

RESULTS

The animals were in good health, without diarrhea or other overt clinical disease, throughout the experimental period.

Parasitological findings

Faecal egg coutnts per g (Epg) counts of animals from the experimental groups are shown in Figure 1. At the start of the experiment it was not noted any significant difference in the mean faecal epg values among groups. Thereafter, and up to January, significant differences were noted between the albendazole treated and not treated groups ($p<0.05$). It was also obvious that FEC in Group 3S-Alb, tended to remain lower from the start of the study until February. Onwards, only FEC in Group 2Alb remained low up to the end of the experiment. Moreover, FEC in Group 4Alb3 after January started to increase and remained high until the end of the study. The soya treated group Group 1S did not differ statistically from the control group Group 5C throughout the experimental period.

Coprocultures included genera of *Teladorsagia* (*Ostertagia*) spp. (43.5%), *Haemonchus contortus* (11%), *Trichostrongylus* spp. (34.5%), *Chabertia ovina (*6.8%), *Oesophagostomum* spp. (2%), *Bunostomum* spp. (1.2%) and *Cooperia* spp. (1%).

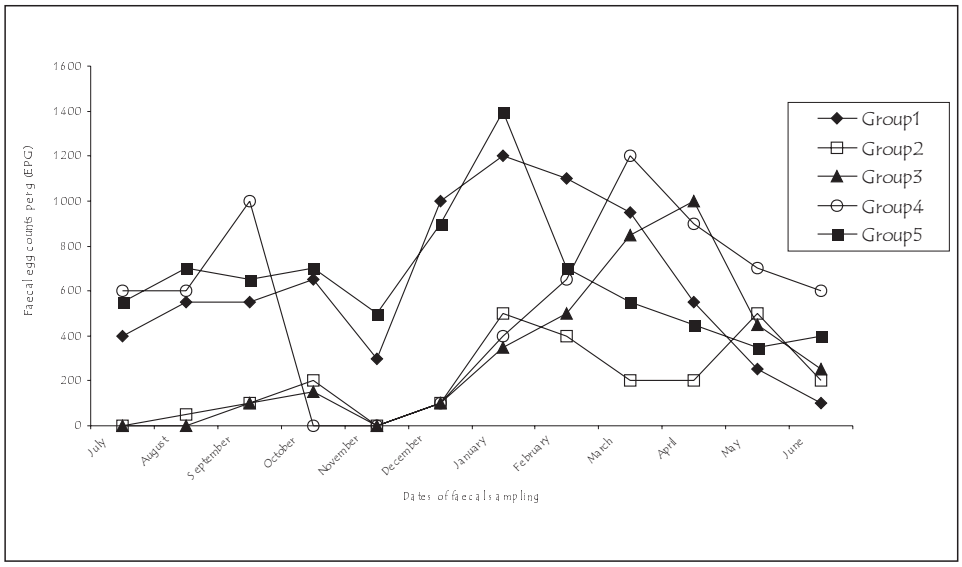

Figure 1. Mean faecal egg counts per g (EPG) of ewes of all groups throughout the experimental period

Haematological and biochemical findings

Mean haematocrit values of all groups of ewes remained within the normal range (27.05-32.00%) throughout the experiment. They were similar variations among groups (27.05-27.88%) in all groups at the beginning of the study, but thereafter that of the control animals Group 5C tended to be lower and that of Group 3S-Alb, but higher than the rest of the groups until the end of the experiment. Significant differences were only noted in November between Groups 1S and 5C and in January between Group 3S-Alb and Groups 4Alb3 and group 5C (Table 1).

Table 1. Mean hematocrit values for all groups throughout the experiment

Month	Groups	HCT (%)	SD
July	Group (1S)	27.25	1.04
	Group (2Alb)	27.05	3.02
	Group (3S-Alb)	28.01	2.09
	Group (4Alb3)	27.92	1.58
	Group (5C)	27.58	2.56
September	Group (1S)	30.00	2.14
	Group (2Alb)	31.25	4.35
	Group (3S-Alb)	31.31	3.51
	Group (4Alb3)	31.38	2.28
	Group (5C)	29.06	4.00
November	Group (1S)	31.69	3.23
	Group (2Alb)	28.75	3.89
	Group (3S-Alb)	30.56	1.97
	Group (4Alb3)	29.13	3.02
	Group (5C)	27.15	4.32
January	Group (1S)	29.63	3.16
	Group (2Alb)	28.19	3.88
	Group (3S-Alb)	32.00	4.17
	Group (4Alb3)	27.38	2.42
	Group (5C)	27.08	4.17
March	Group (1S)	27.81	2.58
	Group (2Alb)	27.14	3.06
	Group (3S-Alb)	29.00	3.10
	Group (4Alb3)	28.75	3.16
	Group (5C)	27.06	4.59
May	Group (1S)	28.63	3.35
	Group (2Alb)	27.16	4.05
	Group (3S-Alb)	28.25	2.34
	Group (4Alb3)	27.31	4.57
	Group (5C)	27.05	3.72

Mean plasma pepsinogen values of all groups were within the normal range throughout the experimental period. In September, pepsinogen values were significantly lower in Group 2Alb compared to Groups 1S, 4Alb3 and 5C, whereas in November it was lower in albendazole treated Groups 2Alb, 3S-Alb and 4Alb3 compared to the control Group 5C, as well as in Groups 2Alb and 3S-Alb compared to Group 1S ($p<0.05$) (Table 2).

Table 2. Mean plasma pepsinogen values for all groups throughout the experiment

Month	Groups	Pepsinogen (mU tyrosine/ml)	SD
September	Group (1S)	330.90	110.41
	Group (2Alb)	188.48	76.50
	Group (3S-Alb)	270.49	105.52
	Group (4Alb3)	423.75	181.20
	Group (5C)	411.85	127.27
November	Group (1S)	288.23	46.51
	Group (2Alb)	202.36	56.25
	Group (3S-Alb)	212.41	89.30
	Group (4Alb3)	206.18	110.13
	Group (5C)	370.90	161.81
March	Group (1S)	357.87	192.93
	Group (2Alb)	345.49	192.99
	Group (3S-Alb)	407.56	171.96
	Group (4Alb3)	530.15	194.55
	Group (5C)	494.73	299.68

Mean total protein values remained within the normal range throughout the experiment, but tended to be lower in the control Group 5C in comparison to the other groups, while in May a significant difference was noted in Group 3S-Alb compared with the other groups ($p<0.05$) (Table 3).

Mean serum Ca, P and Mg values were not significantly different among groups during the experimental period ($p>0.05$) (Table 4).

Table 3. Mean total protein values for all the groups throughout the experiment

Month	Groups	Total proteins (g/dl)	SD
July	Group (1S)	8.30	0.54
	Group (2Alb)	7.58	1.07
	Group (3S-Alb)	8.09	1.30
	Group (4Alb3)	7.28	0.85
	Group (5C)	8.01	0.67
September	Group (1S)	7.86	0.30
	Group (2Alb)	7.94	0.60
	Group (3S-Alb)	8.36	0.77
	Group (4Alb3)	8.00	0.83
	Group (5C)	7.68	1.01
November	Group (1S)	6.98	1.52
	Group (2Alb)	8.50	0.86
	Group (3S-Alb)	7.42	2.19
	Group (4Alb3)	6.75	2.28
	Group (5C)	6.09	1.40
January	Group (1S)	6.46	0.96
	Group (2Alb)	6.69	2.10
	Group (3S-Alb)	5.77	1.59
	Group (4Alb3)	6.06	1.28
	Group (5C)	5.98	1.95
March	Group (1S)	6.06	1.66
	Group (2Alb)	6.71	1.13
	Group (3S-Alb)	7.06	1.82
	Group (4Alb3)	6.88	0.94
	Group (5C)	6.19	0.99
May	Group (1S)	7.20	1.13
	Group (2Alb)	7.31	0.56
	Group (3S-Alb)	8.30	1.23
	Group (4Alb3)	7.16	0.72
	Group (5C)	6.35	0.74

Table 4. Mean serum Ca, P and Mg values for all groups throughout the experiment

Month	Groups	Ca (mg/dl)	SD	P (mg/dl)	SD	Mg (mg/dl)	SD
July	Group (1S)	9.57	0.63	6.42	0.62	2.20	0.35
	Group (2Alb)	9.06	1.19	7.53	2.06	2.23	0.46
	Group (3S-Alb)	9.67	1.73	6.54	1.15	2.51	0.49
	Group (4Alb3)	9.79	1.71	7.47	1.09	2.37	0.55
	Group (5C)	8.98	0.90	6.63	0.86	2.32	0.32
September	Group (1S)	8.69	0.50	4.35	0.56	2.34	0.14
	Group (2Alb)	8.74	0.46	5.06	0.96	2.40	0.30
	Group (3S-Alb)	8.80	0.53	5.36	1.15	2.43	0.20
	Group (4Alb3)	9.23	0.65	5.18	1.17	2.45	0.19
	Group (5C)	8.02	1.75	4.82	1.43	2.37	0.30
November	Group (1S)	6.75	2.14	4.96	2.00	1.97	0.60
	Group (2Alb)	8.67	0.29	5.04	1.30	2.36	0.25
	Group (3S-Alb)	7.35	2.09	5.89	1.21	2.02	0.55
	Group (4Alb3)	7.60	2.18	4.86	1.01	2.00	0.68
	Group (5C)	6.18	2.92	4.73	2.18	1.79	0.82
January	Group (1S)	6.20	0.74	6.26	1.73	1.87	0.31
	Group (2Alb)	6.73	1.83	6.68	0.98	1.89	0.37
	Group (3S-Alb)	6.80	1.71	6.27	0.84	1.69	0.41
	Group (4Alb3)	6.71	1.60	5.93	1.05	1.64	0.49
	Group (5C)	6.55	1.59	5.32	1.78	1.84	0.27
March	Group (1S)	7.18	1.32	5.69	1.06	2.16	0.52
	Group (2Alb)	7.50	0.93	6.24	1.17	2.27	0.31
	Group (3S-Alb)	6.98	1.38	6.53	2.37	2.08	0.46
	Group (4Alb3)	7.48	1.30	6.61	1.25	2.27	0.47
	Group (5C)	6.96	0.81	7.55	1.90	1.80	0.27
May	Group (1S)	8.07	1.28	4.99	1.41	2.31	0.24
	Group (2Alb)	7.75	0.75	6.43	0.71	2.38	0.19
	Group (3S-Alb)	7.95	0.49	6.10	1.54	2.27	0.16
	Group (4Alb3)	7.40	1.46	5.46	1.31	2.19	0.35
	Group (5C)	7.35	1.11	6.48	0.99	2.27	0.36

DISCUSSION

As it is seen from the results, at the beginning of the trial the faecal egg counts in all groups of ewes were similar. Then, the effect of albendazole treatment was obvious, as up to January the epg counts were significantly lower in the albendazole treated groups. Later on, was revealed that the administration of albendazole given twice tended to retain lower epg counts throughout the experimental period, in contrast to the 3 times albendazole treated ewes. A possible explanation to the above may be a suppression of the host immune response due to the more frequent albendazole administration, which may have led to a reduced reaction of the host to any subsequent nematode challenge. Host resistance to re-infection starts to increase the following 4-8 weeks and lasts up to 4 months (Benitez-Usher *et al*., 1977). Additionally, the frequent drug administration that enhances the development of anthelmintic resistant strains (Martin *et al*., 1984; Coles and Roush, 1992) may have contributed to some extent to the above results.. This is of particular interest in grazing animals in pastures contaminated with nematodes and consequently of high risk of re-infection, especially during the first two weeks post treatment.

Concerning dietary proteins, Theodoropoulos *et al*. (1998) observed higher mean faecal strongyle-type egg counts in the low protein than the high protein groups of sheep, indicating that FEC during grazing was influenced by the level of dietary protein. Coop and Kyriazakis (1999) studying the interaction between host nutrition and parasitism in small ruminants stated that the supplementation of protein always leads to improved resilience. Moreover, Houtert *et al*. (1995) by supplementing dietary protein in undrenched sheep found that FEC were significantly lower than in the unsupplemented group, but in drenched sheep they did not find any further reduction of FEC. On the contrary, Abbott *et al*. (1986 a, b) demonstrated that infected animals fed diets different in dietary protein levels, which had been given for either one or five months pre-infection, had similar FEC and worm burdens, indicating no influence on the parasite establishment among them.

The current study revealed that the soya treated Group 1S did not differ statistically from the control group 5C throughout the whole experimental period. This indicates that the level of protein intake does not influence the worm infestation, which may be due to the adequacy of the level of dietary protein in the basal diet of ewes. This finding supports the notion that further improvement of nutrition by adding protein will not always enhance the resilience to nematodes (Israf *et al*., 1996). On the other hand, the supplementation of ewes with soya concurrently with albendazole in Group 3 reduced FEC, but this result is rather attributed to the effect of albendazole and not to soya. This explanation is also supported by the low FEC noted in the soya unsupplemented Group 2Alb. However, attention must be paid that strict comparisons with other studies are subject to limitations because of the different genera of nematodes, breed and age of sheep, rations and, finally, the management system applied in different studies.

Haematocrit values remained within the normal range in all groups of ewes during the whole experimental period and values of the control ewes tended to be lower than the rest. This is attributed either to the albendazole treatment, which caused elimination of the nematode burden, and/or to soya supplementation. Moreover, this could explain the trend of the Group that received soya and albendazole concurrently to retain higher haematocrit values than the rest of the groups throughout the experiment. In agreement to these findings, earlier works indicated that animals on a low protein diet compared to animals on a high protein diet developed more severe anaemia, hypoproteinaemia and hypoalbuminaemia (Abott *et al*., 1988), while it is well known that the anthelmintic treatment contributes to the maintenance of haematocrit values within the normal range (Amarante *et al*., 2004).

Pepsinogen values of all groups were within the normal range throughout the experimental period, probably reflecting no severe abomasal damage from *Teladorsagia* spp. The results also indicated that the albendazole administration lowered or maintained stable the mean plasma pepsinogen values, which may be attributed to the control of *Teladorsagia* spp. This corresponds to the findings of Stear *et al*. (2003) stated that plasma pepsinogen depends upon the severity of abomasal infection and the nutritional status of the host.

Serum total protein values remained within normal range throughout the experiment, reflecting a mild effect of nematode parasitism on the ewes in this study. Furthermore, the fact that the total proteins in the control group tended to be lower than in the other groups, indicates that the long-term nematode infection resulted to a relatively constant low degree of hypoproteinemia in this group.

Serum Ca, P and Mg values remained within the normal range and no significant difference among groups was recorded throughout the experiment. Similarly, Sykes *et al.* (1979) reported that there were no significant changes in sheep serum Ca in chronic infections with *Trichostrongylus* spp. Serum P might be an indicator of subclinical intestinal parasitism under experimental conditions (Coop *et al*., 1984), however, hypophosphataemia in field conditions is influenced by the level of dietary P (Coop and Field, 1983). Mg metabolism was not affected by parasitism as also suggest the results of Brown *et al*. (1989).

In conclusion, the administration of albendazole twice in July and November-periparturiently, reduced effectively the nematode FEC, whereas the extra protein supplementation did not significantly influence it, probably due to the adequacy of protein in the basal diet of ewes. Haematocrit and total proteins were unaffected by any supplementation, although they tended to be lower in the control group. Pepsinogen, Ca, P and Mg were not influenced by any of the treatments.

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PROCENA KONTROLNIH SHEMA NADOKNADOM PROTEINA I PRIMENOM ANTIHELMINTIKA PROTIV GASTROINTESTINALNIH NEMATODA KOD MLEČNIH OVACA

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SADRŽAJ

Cilj ovog rada je bio da se ispita mogući uticaj dijetetske nadoknade proteina i primene antihelmintika prema različitim shemama radi kontrole GI nematoda kod mlečnih ovaca u terenskim uslovima. Klinički zdrave ovce lokalne grčke mlečne rase (40 grla) koje su pasle na inficiranom navodnjavanom pašnjaku su bile podeljene u 5 jednakih grupa i tretirane na sledeći način: Grupa 1S je kao dodatak hrani dobijala 300 g 48% sojinog brašna dva puta godišnje (u julu i oko jagnjenja). Grupa 2Alb je dobila albendazol dva puta. Grupa 3S-Alb je dobila kao dodatak hrani sojino brašno kao grupa 1S u kombinaciji sa primenom albendazola kao kod grupe 2Alb. Grupa 4Alb3 je dobila albendazol tri puta (u septembru, pred jagnjenje i u aprilu) a Grupa (5 C) je služila kao kontrola bez tretmana. Uzorci fecesa su uzimani svakog meseca od svih životinja zbog pregleda broja jaja nematoda (FEC) a uzorci krvi su uzimani svaka dva meseca zbog hematoloških i biohemijskih analiza. Dobijeni rezultati su ukazali da je dvokratna aplikacija albendazola efikasno smanjila FEC nematoda, dok dodatak proteina nije značajno uticao na ovo smanjenje. Nijedan od ovih dodataka hrani nije značajno uticao na vrednosti hematokrita i koncentraciju ukupnih proteia ali su te vrednosti bile nešto niže kod kontrolne grupe. Primenjivani tretmani nisu uticali na aktivnost pepsinogena i koncentraciju kalcijuma, neorganskog fosfora i magnezijuma.