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COPROCULTURE ASSAY FOR ESTIMATION OF *IN VITRO* LARVAL REDUCTION OF SHEEP GASTROINTESTINAL NEMATODES BY NEMATOPHAGOUS FUNGUS, *DUDDINGTONIA FLAGRANS**

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SUMMARY: Gastrointestinal nematodes in sheep represent serious problem in pastoral systems due to various production losses and significant cost in sheep industry worldwide. One of control options for these parasites in grazing sheep is application of nematophagous fungi to reduce number of available infective larvae, where Duddingtonia flagrans is one of the most promising candidates. In order to estimate effect on larval reduction in vitro, coproculture assay was performed with theoretically generated dose of chlamydospores added to faeces. Total reduction rate of larvae in fungus group compared to control was 32.45%, but without statistically significant difference in larval yields at 0.05 confidence level. Observed reduction percentage was lower in our study compared with results of other researchers. Possible reasons for low efficacy of fungus obtained in our study compared to other results are very complex and some factors are discussed.

Key words: *Duddingtonia flagrans, larval reduction, sheep, biological control, coproculture assay.*

INTRODUCTION

The importance of gastrointestinal nematodes (GIN) of sheep, beside various losses with significant cost for sheep industry, is much higher nowadays because of increase of resistance to antihelmintics routinely used for helminth control (Simin et

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al, 2011). Growing public demand for food without drug residues imposes introduction of innovative ways of controlling clinical and subclinical parasitism on pastoral farming systems, and one of options is biological control of free living stages of GIN using nematophagous fungi with *Duddingtonia flagrans* as one of most promising candidates (Lalošević et al, 2009; Simin et al, 2011).

Chlamydospores of fungus given *p/o* to animals need to survive passage through the gastrointestinal tract (GIT), to develop structures (traps) for capturing nematode larvae in feces and to catch these larvae before they leave feces and reach the surrounding vegetation and soil. To achieve this, the authors recommend the use of protocols that involve dosing with very high concentration of chlamydospores. According to this approach, higher concentration of chlamydospores increases the likelihood to catch a higher number of larvae. That is why the great effort was made to determine the optimal *p/o* dose for maximal reduction of larvae in sheep and goat feces, but clear and consistent dose-dependent effect was not confirmed (Ojeda-Robertos et al, 2008b).

In majority of experiments, the dosage for naturally infected sheep (and goats) was per kg body weight, and the most common dose used and recomended by other researchers was $5x10^5$/kg body weight (BW) (Chandrawathani et al, 2004; Epe et al, 2009; Fontenot et al, 2003; Larsen et al, 1998; Paraud and Chartier, 2003; Paraud et al, 2005; Peña et al, 2002).

In previous search for optimal dose, researchers failed to determine the final number of chlamydospores in feces, so Ojeda-Robertos and collegues (2008a) developed a technique for counting chlamydospores in feces, which allowed studies of the effect of different doses of fungus on final number of spores in feces and it's effectiveness in reducing the number of larvae in coprocultures.

Grønvold et al. (2004) and Ojeda-Robertos et al. (2009) determined that digestibility of *p/o* administered chlamydospores is about 90%, which means that only about 10% of total number of administered spores survive the passage through sheep GIT . If lamb of approximately 20 kg body weight produces an average of 1200 g of feces per day (da Silva et al, 2009), the final number of chlamydospores per gram of faeces (CPG) after passing through the GIT of sheep can be calculated. For the most used dose ($5x10^5$/kg BW), this number is approximately 830 CPG.

The aim of this study was to perform coproculture assay for determination of *in vitro* efficiency of biological control agent *D. flagrans* in reducing the number of larvae of naturally infected sheep with different levels of FEC by simulating the quantity of spores per gram of faeces which would survive passage through GIT of sheep, if animals were dosed with most commonly used dose of $5x10^5$/kg BW. This approach will provide insight into the effectiveness of fungus when dosing is based on the usual number of spores per kg body weight, which would be helpful for generating effective doses for *in vivo* studies.

MATERIAL AND METHODS

Faecal samples were taken directly from the rectum of seventeen naturally infected sheep randomly selected from flock of 120 Merinoland ewes grazing near the town of Srbobran (45.34° N; 19.48°E) in South Bačka region, Vojvodina province. The samples have been packed separately in small plastic cups, sealed and shipped to the laboratory until the end of the day, and refrigerated until examination in the following

two days. Samples were analyzed for determination of faecal egg counts (FEC) according to procedure described in Simin et al. (2012) and ten positive samples with different level of infection were selected for coproculture assay. Each sample was weighted and divided to two equal volumes (7 g) and added to experimental (with *D.flagrans*) and control group (without fungus).

D.flagrans (obtained from collection of Universite catholique de Louvian, Belgium) was cultured on potato decstrose agar (PDA) for four weeks at 25°C in standard plastic petri dishes. For collection of chlamydospores, five ml of sterile physiological saline was added to surface of culture in each petri dish, scraped with sterile platinum loop and collected to conical glass flask (volume 250 ml). Separation of chlamydospores of *D.flagrans* from mycelia was achieved by mixing, washing and sieving technique according to Ojeda-Robertos et al. (2005). After that procedure, satisfying separation of chlamydospores was achieved with only few chlamydospores in chains left as confirmed by microscopic examination.

Quantification of chlamydospores in the suspension was done by counting their number in ten subsamples of 5 µl taken after through mixing of the flask each time before sampling. Suspension was stored in refrigerator at 4°C until use.

Feacal samples of sheep in experimental group was thoroughly mixed with required number of chlamydospores by calculating appropriate volume of the suspension. Control faecal samples were mixed with equal volume of distilled water.

Coprocultures were made according to Fontenot et al. (2003) and Terill et al. (2004), with some modifications.

Briefly, 200 ml plastic cups were cut at half, and faecal samples were mixed with suspension of *D.flagrans*/distilled water in the lower half. After mixing, six small holes were perforated in the bottom of every cup; cups were covered with cheesecloth and turned upside down in new 200 ml plastic cup, previously filled with 10 ml of distilled water. The cultures were incubated at 25°C for ten days and mixed twice during that time. After incubation, the cups were filled with warm water and left overnight for baermanisation. The next day, cups with faecal samples were removed, level of water reduced to 50-60 ml without disturbing the sediment that contained larvae. The contents were then transferred to disposable plastic wine glasses with small reservoir at the base of the cup, and left in refrigerator for two hours. Again, water level was reduced to 1 ml, larvae were enumerated in ten subsamples of 5 µl, and data expressed as number of larvae per gram of faeces (LPG= larval counts x 20/ grams of faeces in culture).

Larval yield was calculated as the ratio between LPG and FEC x 100 (Paraud et al, 2005). Percentage of reduction (PR) of larvae was calculated according to Terill et al. (2004): PR= 100 – (mean larval yield in fungus group x 100/ mean larval yield in control group).

Two hundred and fifty larvae from control group were identified to the level of genera according to van Wyk et al. (2004) in order to determine which parasites infect sheep at that farm.

The comparison of larval reduction between samples was calculated with parametric t-test using Microsoft Excel 2007. The statistical significance of the variables was tested at the 0.05 confidence level.

RESULTS AND DISCUSSION

Larval populations after constant coproculture temperature (25°C) during ten days comprised of L_2 and L_3 larvae (Table 1.) and all larval counts were included in analysis. The results showed that total reduction of larval yield in experimental group was 32.45% compared to control, with the mean larval yield in the fungus treated group lower (10.98%; 95 % CI: 6.94 to 15.01) than in control group (16.25%; 95% CI: 9.51 to 22.99), but not statistically significant at 95% confidence level (p=0.07).

Table 1. Larval populations after coproculture in both groups

Fungus group		Control group	
L_2 (%)	L_3 (%)	L_2 (%)	L_3 (%)
5.38	94.62	4.84	95.16

According to identification keys provided by van Wyk et al. (2004), GIN that parasitise sheep at this farm belong to *Haemonchus contortus, Trichostrongylus spp.* and *Teladorsagia circumcinta* (54.8, 41.6 and 3.6 % of identified L_3 larvae, respectively). FEC values from ten sheep included in the experiment, LPG values, larval yield and individual larval reduction percentages are shown in Table 2. In four out of ten sheep faecal cultures there was not reduction of larvae following fungal treatment. Individual reduction rates ranged from 5.26 to 86.49 % in six remaining samples.

Table 2. FEC, LPG, larval yield values in both groups and individual larval reduction percentage

		LPG		Larval yield (%)		
Samples included in the study	FEC (epg)	Fungus group	Control group	Fungus group	Control group	Reduction percentage
1	100	20.00	22.86	20.00	22.86	12.50
2	1110	48.57	25.71	4.38	2.32	0.00
6	90	17.14	17.14	19.05	19.05	0.00
10	70	5.71	14.29	8.16	20.41	60.00
11	80	11.43	20.00	14.29	25.00	42.86
13	160	14.29	17.14	8.93	10.71	16.67
14	290	40.00	34.29	13.79	11.82	0.00
15	690	42.86	42.86	6.21	6.21	0.00
16	490	51.43	54.29	10.50	11.08	5.26
17	320	14.29	105.71	4.46	33.04	86.49
Average value	340	26.57	35.43	10.98	16.25	-

Total reduction of infective larvae in our study (32.45%) was low when compared to results obtained by other authors. For comparison of results, it is important to highlight that there are various designs of coproculture assays. There are a few available studies where authors directly mixed different quantities of different *D.flagrans* fungal

units (chlamydospores or conidia) with faeces of domestic ruminants that contained eggs of GIN: calves (Fernández et al, 1999; Grønvold et al, 2004), sheep (Silva et al, 2011) and goats (Sanyal et al, 2008). Some researchers preferred to dose animals *p/o*, and then to perform laboratory procedures of larval recovery (studies in sheep: Larsen et al, 1998; Peña et al, 2002; Fontenot et al, 2003; in goats: Terill et al, 2004; Ojeda-Robertos et al, 2005; Paraud et al, 2005, and in both species Waghorn et al, 2003), or to add already developed L_3 larvae to fungal cultures (Morgan et al, 1997; Mendoza de Gives et al, 1999). For that reason, only results where direct mixing was performed will be compared and discussed.

In calves, Fernández et al. (1999) added 6250 CPG of four different isolates of *D.flagrans* to study the effect on reduction of *Cooperia oncophora* (FEC=250 epg) larvae on different temperature regimes, and observed reduction percentage from 63.3 to 84% for these isolates at 20°C constant temperature. Grønvold et al, (2004) mixed increasing concentrations of chlamydospores ranging from 250 to 200000 CPG, and established reduction from 93% for only 250 CPG to 99% by increase of CPG (FEC=1050 epg, *C. oncophora*) at similar constant temperature.

In naturally infected sheep, Silva et al. (2011) mixed 1000 conidia with 20 g of *H. contortus* infected faeces and obtained 85.82% of reduction (FEC level not shown) at 26°C for 7 days. Campos et al. (2009) showed that conidia can also survive GIT passage, though reduction rate was significantly lower when compared to chlamydospores (23.89 vs. 61.23%). Since we used chlamydospores in our study, it is hard to compare effect with conidia even at the similar concentration (830 compared to 1000 fungal units).

True comparison can be made only with results of coproculture assay by Sanyal et al. (2008) in goats, although sheep were included in our investigation. Sheep and goats are parasitized with same species of GIN (Taylor et al, 2007) but goats do not develop strong immune response to nematode infection unlike other domestic ruminant hosts (Paraud et al, 2006). In their research, Sanyal and colleagues have mixed three levels of chlamydospores (1000, 10^4, and 10^5 CPG) with three levels of worm eggs (FEC=100, 500 and 1000 epg). We have extrapolated the reduction percentage from their data, and the values for 1000 CPG which was similar with our dose, were ≈17% for FEC=100 epg and ≈50% for FEC=500 and 1000 epg. These results were not much higher than ours (Table 2).

Reduction of larvae was different between studies, and it depended of initial FEC value and number of mixed units of fungal material. It is clear that increase of CPG results in higher reduction rate (Grønvold et al, 2004; Sanyal et al, 2008), but larval density is also important stimulus for trap formation of *D.flagrans* (Morgan et al, 1997; Sanyal et al, 2008). The percentage of larval development in control sheep cultures in our study (2.32-33.04%) was higher than percentage found by Ojeda-Robertos et al. (2005; 0.9-11.1%) and lower than the results of Terill et al. (2004; 3.9-100%) in goat coprocultures. Regardless of that, equal/ similar values of larval yields in four samples where reduction of larvae was absent showed that both tested groups were cultured under same conditions and that equal opportunity was provided for larval development. This makes fungal efficiency more obvious, even at low level of 32.45%. According to Larsen (2000), if species of known predacious fungi are tested in laboratory experiments, the researchers are more or less guaranteed some sort of positive response, depending on dose and number of nematodes involved. Also it is possible that isolate of *D.flagrans* used in our study is less effective than others, since Fernández et al. (1999)

showed different reduction rate of four isolates of fungus tested under same conditions.

Two samples yielded more larvae in fungus compared to control group. This is possible due to unequal distribution of helminth eggs in the faeces, where coefficient of variation of mean epg may range from 22 to 270% (Paraud et al, 2005) so different yields may be obtained. Although temperature is standardized variable in studies, other uncontrolled biotic or abiotic factors like consistency of faeces and thus oxygenation for example, may have influenced the larval development and variability (Paraud et al, 2005). Composition of larval species is different comparing to results obtained in august at the same farm, when there was 92%, 4%, 4% (n=100 larvae) *H.contortus, Trichostrongulus spp.* and *Chabertia ovina,* respectively (Simin, unpublished results). *H.contortus* is still predominate species, with 55% larvae in culture. This is very important fact, considering that this bloodsucking parasite is the most pathogenic species of sheep GIN (Sutherland and Scott, 2010), and that 5000 adults may cause loss of 250 ml of blood per sheep (Taylor et al, 2007). It must be repeated that the ultimate goal in dosing grazing animals with nematophagous fungi is reduction of larval number on pasture and thus lowering infection rate. Some field studies experienced great success with reduction of larvae up to 99% (e.g. Chandrawathani et al, 2003) while other obtained unsatisfying results (e.g. Rocha et al, 2007; Silva et al, 2010). *In vitro* studies are conducted in order to determine the best i.e. optimal performance of fungus species in certain conditions. Data obtained from these studies make good starting point for field application as well as reconsideration of study design if failure occurs.

In Serbia, we have recently started to test impact of *D.flagrans* on sheep GIN: the first trial tested effect on eggs (Lalošević et al, 2011) and this is our first study which examined effect on larvae. This coproculture assay design which theoretically generated final concentration of chlamydospores for most commonly used dose of $5x10^5$/kg BW is based on data obtained by others. Both Grønvold et al. (2004) and Ojeda-Robertos et al. (2009) independently determined 10% survival rate of chlamydospores after passage through sheep GIT, making survival rate constant variable. Total daily faecal output, which was one of variables included in calculation of final number of CPG in our study, is very variable and is directly related to dry matter intake and digestibility of the consumed herbage (Smith and Frost, 2000). It is measurable, but special faecal collection harness for sheep is required, if one wants to weight faeces for sheep at pasture. While our faecal collection harness is under construction, for lamb total faecal output, we have selected data from da Silva et al. (2009) since they have measured total faecal production in animal while grazing. One of the reasons for low level of larval reduction in our *in vitro* study may be to miscalculated number of final CPG concentration, due to different amount of total daily faeces produced by adult sheep at conditions at this farm.

Further studies are needed to investigate complex factors that influence reduction percentage of larvae. In the following experiments, all parameters will be measured in order to gain better insight for effect of fungus in given circumstances.

CONCLUSION

In this preliminary investigation, *in vitro* larval reduction percentage of GIN in naturally infected sheep by nematophagous fungus *D.flagrans* was estimated using coproculture assay, and the total reduction percentage was 32.45%. Although low, the results of larval reduction from this experiment are not discouraging. Since *Haemon-*

chus contortus is very present species at this farm, the fact that there can be even 30 % less larvae available at the pasture, may be the difference of life and death for some individuals, especially young, old and reconvalescent sheep. Also, this may be valuable fact for preventing and lowering subclinical losses, which are costly in grazing sheep due to parasitic gastroenteritis (West et al, 2009).

Further research is already in progress with modified protocols in order to improve *in vitro* efficiacy of this biocontrol agent, since the final aim is successful (*in vivo*) application in grazing sheep naturally infected with these parasites and measure of the effect of *D.flagrans* on pasture infectivity.

REFERENCES

CAMPOS, A.K., ARAÚJO, J.V., GUIMARÃES, M.P., & DIAS, A.S: Resistance of different fungal structures of *Duddingtonia flagrans* to the digestive process and predatory ability on larvae of *Haemonchus contortus* and *Strongyloides papillosus* in goat feces. Parasitol. Res., 105:913–919, 2009.

CHANDRAWATHANI, P., JAMNAH, O., WALLER, P.J., LARSEN, M., GILLESPIE, A.T., ZAHARI, W.M.: Biological control of nematode parasite of small ruminants in Malaysia using the nematophagous fungus *Duddingtonia flagrans*. Vet. Parasitol., 117:173-183, 2003.

CHANDRAWATHANI, P., JAMNAH, O., ADNAN, M., WALLER, P.J., LARSEN, M., GILLESPIE, A.T.: Field studies on the biological control of nematode parasites of sheep in the tropics, using the microfungus *Duddingtonia flagrans*. Vet. Parasitol., 120:177-187, 2004.

DA SILVA, A.S., ZANETTE, R.A., OTTO, M.A., SOARES, C.D.M., ALVES, S.H., MONTEIRO, S.G., SANTURIO, J.M.: *Duddingtonia flagrans*: Centrifugal flotation technique with magnesium sulphate for the quantification and qualification of chlamydospores in sheep faeces. Exp. Parasitol., 121:187-188, 2009.

FERNÁNDEZ, A.S., LARSEN, M., WOLSTRUP., J., GRØNVOLD, J., NANSEN, P., BJØRN, H.: Growth rate and trapping efficacy of nematode-trapping fungi under constant and fluctuating temperatures. Parasitol. Res., 85:661-668, 1999.

FONTENOT, M.E., MILLER, J.E., PEÑA, M.T., LARSEN, M., GILLESPIE, A.: Efficiency of feeding *Duddingtonia flagrans* to grazing ewes on reducing availability of parasitic nematode larvae on pasture. Vet. Parasitol., 118:203-213, 2003.

GRØNVOLD, J., WOLSTRUP, J., LARSEN, M., GILLESPIE, A., GIACOMAZZI, F.: Interspecific competition between the nematode-trapping fungus, *Duddingtonia flagrans*, and selected microorganisms and the effect of spore concentration on the efficacy of nematode trapping. J. Helminthol., 78:41-46, 2004.

LALOŠEVIĆ VESNA, JARAK MIRJANA, ĐURIĆ SIMONIDA, SIMIN, S.: Biološka kontrola helminata. Letopis naučnih radova, 33(1)118-125, 2009.

LALOŠEVIĆ VESNA, JARAK MIRJANA, ĐURIĆ SIMONIDA, OBRADOVIĆ, N.: The effect of nematophagous fungus *Duddingtonia flagrans* on the gastrointestinal parasites in sheep. Proc. Nat. Sci, Matica Srpska Novi Sad 120:243-248, 2011.

LARSEN, M., FAEDO, M., WALLER, P.J., HENNESSY, D.R.: The potential of nematophagous fungi to control the free-living stages of nematode parasites of sheep: Studies with *Duddingtonia flagrans*. Vet. Parasitol., 76:121-128, 1998.

LARSEN, M.: Prospects for controlling animal parasitic nematodes by predacious mi-

cro fungi. Parasitol., 120:121-131, 2000.
MENDOZA DE GIVES, P., DAVIES, K. G., CLARK, S. J., BEHNKE, J. M.: Predatory behaviour of trapping fungi against srf mutants of *Caenorhabditis elegans* and different plant and animal parasitic nematodes. Parasitol., 119:95-104, 1999.
MORGAN, M., BEHNKE, J. M., LUCAS, J. A., PEBERDY, J. F.: *In vitro* assessment of the influence of nutrition, temperature and larval density on trapping of the infective larvae of *Heligmosomoides polygyrus* by *Arthrobotrys oligospora, Duddingtonia flagrans* and *Monacrosporium megalosporum.* Parasitol., 115:303-310, 1997.
OJEDA-ROBERTOS, F. NADIA, DE GIVES, P.M., TORRES-ACOSTA, J.F.J., RODRÍGUEZ-VIVAS, R.I., AGUILLAR-CABALLERO, A.J.: Evaluating the effectiveness of a Mexican strain of *Duddingtonia flagrans* as a biological control agent against gastrointestinal nematodes in goat faeces. J. Helminthol., 79:151-157, 2005.
OJEDA-ROBERTOS, F. NADIA, TORRES-ACOSTA, J.F.J., AYALA-BURGOS, A., AGUILAR-CABALLERO, A.J., COB-GALERA, L.A., DE GIVES, P.M.: A technique for the quantification of *Duddingtonia flagrans* chlamydospores in sheep faeces. Vet. Parasitol., 152:339-343, 2008a.
OJEDA-ROBERTOS, F. NADIA, TORRES-ACOSTA, J.F.J., AGUILAR-CABALLERO, A.J., AYALA-BURGOS, A., COB-GALERA, L.A., SANDOVAL-CASTRO, C.A., BARRIENTOS-MEDINA, R.C., DE GIVES, P.M.: Assesing the efficacy of *Duddingtonia flagrans* chlamydospores per gram of faeces to control *Haemonchus contortus* larvae. Vet. Parasitol., 158:329-335, 2008b.
OJEDA-ROBERTOS, F. NADIA, TORRES-ACOSTA, J.F.J., AYALA-BURGOS, A., SANDOVAL-CASTRO, C.A., VALERO-COSS, R.O., DE GIVES, P.M.: Digestibility of *Duddingtonia flagrans* chlamydospores in ruminants: *in vitro* and *in vivo* studies. BMC Vet. Res., 5:46, 2009.
PARAUD, C., CHARTIER, C.: Biological control of infective larvae of a gastro-intestinal nematode (*Teladorsagia circumcinata*) and a small lungworm (*Muellerius capillaris*) by *Duddingtonia flagrans* in goat faeces. Parasitol. Res., 89:102-106, 2003.
PARAUD, C., HOSTE, H., LEFRILEUX, Y., POMMARET, A., VIRGINIE, P., PORS, I., CHARTIER, C.: Administration of *Duddingtonia flagrans* chlamydospores to goats to control gastro-intestinal nematodes: dose trials. Vet. Res., 36:157-166, 2005.
PARAUD, C., PORS, I., CHICARD, C., CHARTIER, C.: Comparative efficacy of the nematode-trapping fungus *Duddingtonia flagrans* against *Haemonchus contortus, Teladorsagia circumcinata* and *Trichostrongylus colubriformis* in goat faeces: influence of the duration and of the temperature of coproculture. Parasitol. Res., 98:207-213, 2006.
PEÑA, M.T., MILLER, J.E., FONTENOT, GILLESPIE, A ., M.E., LARSEN, M.: Evaluation of *Duddingtonia flagrans* in reducing infective larvae of *Haemonchus contortus* in faeces of sheep. Vet. Parasitol., 103:259-265, 2002.
ROCHA, R.A., ARAÚJO, J.V., AMARANTE, A.F.T.: Efficacy of the nematode-trapping fungus *Duddingtonia flagrans* against infections by *Haemonchus* and *Trichostrongylus* species in lambs at pasture. J. Helminthol., 81:387-392, 2007.
SANYAL, P.K., SARKAR, A.K., PATEL, N.K., MANDAL, S.C., PAL, S.: Formulation of a strategy for the application of *Duddingtonia flagrans* to control caprine parasitic gastroenteritis. J. Helminthol., 82:169-174, 2008.
SILVA, BRUNA. F., CARRIJO-MAUAD, J.R., BRAGA, F.R., CAMPOS, A.K., ARAÚJO, J.V., AMARANTE, A.F.T.: Efficacy of *Duddingtonia flagrans* and *Arthrobotrys robusta* in controling sheep parasitic gastroenteritis. Parasitol. Res., 106:1343-1350, 2010.

SILVA, A.R., ARAÚJO, J.V., BRAGA, F.R., ALVES, C.D.F., FRASSY, L.N.: Activity *in vitro* of fungal conidia of *Duddingtonia flagrans* and *Monacrosporium thaumasium* on *Haemonchus contortus* infective larvae. J. Helminthol., 85:138-141, 2011.
SIMIN, S., LALOŠEVIĆ VESNA, PAVIČIĆ LJILJANA, BOBOŠ, S. RADINOVIĆ, M.: Pasture contamination with strongyle eggs estimated with composite faecal egg counts in grazing sheep: report from a small flock. Contemporary Agriculture 59(1-2) 458-464, 2011.
SIMIN, S., PAVIČIĆ LJILJANA, SAVOVIĆ, M., CEGLEDI, I., LALOŠEVIĆ VESNA, BOBOŠ, S.: *Dicrocoelium dendriricum* in sheep flocks in Novi Sad region: occurrence and estimation of pasture contamination. Book of Abstracts of Second international epizootiology days, Belgrade, Republic of Serbia, 18-21 April, 2012, pp 131.
SMITH, K.A., FROST, J.P.: Nitrogen excretion by farm livestock with respect to land spreading requirements and controlling nitrogen losses to ground and surface waters. Part 1: cattle and sheep. Bioresource Technology 71:173-181, 2000.
SUTHERLAND, I., SCOTT, I.: Gastrointestinal nematodes of sheep and cattle. Blackwell Publishing, Oxford, 2010.
TAYLOR, M.A., COOP, R.M., WALL, R.: Vet. Parasitol., 3rd ed. Blackwell Publishing, Oxford, 2007.
TERRIL, T.H., LARSEN, M., SAMPLES, O., HAUSTED, S., MILLER, J.E., KAPLAN, R.M., GELAYE, S.: Capability of the nematode-trapping fungus *Duddingtonia flagrans* to reduce infective larvae of gastrointestinal nematodes in goat feces in the southeastern United States: dose titration and dose time interval studies. Vet. Parasitol., 120:285-296, 2004.
VAN WYK, J.A., CABARET, J., MICHAEL, L.M.: Morphological identification of nematode larvae of small ruminants and cattle simplified. Vet. Parasitol., 119:277-306, 2004.
WAGHORN, T.S., LEATHWICK, D.M., CHEN, L.-Y., SKIPP, R.A.: Efficacy of the nematode-trapping fungus *Duddingtonia flagrans* against three species of gastro-intestinal nematodes in laboratory faecal cultures from sheep and goats. Vet. Parasitol., 118:227–234, 2003.
WEST, D.M., POMROY, W.E., KENYON, P.R., MORRIS, S.T., SMITH, S.L., BURNHAM, D.L.: Estimating the cost of subclinical parasitism in grazing ewes. Small Rumin. Res., 86:84-86, 2009.

TEST KOPROKULTURE ZA PROCENU REDUKCIJE LARVICA ŽELUDAČNO-CREVNIH NEMATODA OVACA IN VITRO PRIMENOM NEMATOFAGNE GLJIVE, DUDDINGTONIA FLAGRANS

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Izvod

Želudačno-crevne nematode ovaca predstavljaju značajan problem primenom sistema napasanja zbog različitih proizvodnih i značajnih finansijskih gubitaka u ovčarskoj industriji širom sveta. Jedna od mera koje se primenjuju u kontroli ovih parazita kod pašnih ovaca je primena nematofagnih gljiva u smanjenju broja raspoloživih infektivnih larvica, među kojima je vrsta *Duddingtonia flagrans* kandidat koji najviše obećava. U cilju procene efekta redukcije larvica u *in vitro* uslovima, urađen je test koprokulture

sa teorijski generisanom dozom hlamidospora koja je dodata u feces. Ukupna redukcija larvica u oglednoj grupi je iznosila 32,45% u poređenju sa kontrolnom, ali nije bilo statistički značajne razlike u prinosu larvica na nivou značajnosti 0,05. Redukcija dobijena u našem ogledu je niža u odnosu na rezultate drugih autora. Potencijalni razlozi za nisku efikasnost gljive u ovom istraživanju u poređenju sa drugim rezultatima su veoma složeni i neki faktori su analizirani u diskusiji.

Ključne reči: *Duddingtonia flagrans,* redukcija larvica, ovce, biološka kontrola, test koprokulture.

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