**Biochemistry** 

# The Effects of *Diatomaceous* Earth on Parasite Infected Goats

### Gregory Bernard\*, Mulumebet Worku\*\*, and Mohamed Ahmedna\*\*

\* Fungal Genomics Laboratory, North Carolina State University, 840 Main Campus Drive, Suite 1200 Raleigh, NC 27606, USA \*\* North Carolina Agricultural and Technical State University, B.C. Webb Hall, 1601 East Market Street, Greensboro, NC 27411, USA

(Presented by Academy Member N. Aleksidze)

**ABSTRACT.** *Diatomaceous* earth (DE) is the skeletal remains of single-celled algae, or diatoms from freshwater or marine sedimentary deposits. To evaluate the benefits of the use of DE in goats the effects of DE on internal parasite control was studied. Twenty Spanish and Spanish/Boer crosses were infected with 1.77 g (Group 1), 3.54 g (Group 2), and 5.31 g (Group 3) of DE. Body weights, fecal egg count (FEC), packed cell volume (PCV), white and red blood cell counts and body weights were recorded over 6 weeks. Highly significant differences in PCV, FEC, and white blood cell (WBC) and red blood cell (RBC) counts and body weights were observed between all groups. Over the course of the study increases in RBC counts over the course of the study. An anthelmintic effect of DE was not observed. The analysis of treatment effects on weights showed highly significant differences among treatments (P < 0.0001). A positive gain occurred in the mean weights of Group 1 and 2, which received the lowest dosage of DE. Group 3, which received the highest dose of DE, did not show statistically significant differences after the treatment. Exposure to DE in water may contribute to physiological changes in goats that impact performance. Further study is needed to evaluate the effect on performance characteristics in goats such as weight gain, attributable to treatment with DE. © 2009 Bull. Georg. Natl. Acad. Sci.

Keywords: diatomaceous; goat, nematodes, anthelmintic.

#### 1. Introduction

Infection with gastrointestinal nematodes such as *Haemonchus contortus*, and the development of anthelmintic resistance are major constraints to economic goat production in the southern USA [1]. The evaluation of alternative anthelmintics will provide scientific validation for commonly used approaches to address these constraints.

*Diatomaceous* earth (DE) is the skeletal remains of single-celled algae, or diatoms that formed sedimentary deposits when they died. *Diatomaceous* earth is comprised predominantly of silicon dioxide. It is a fine, crum-

bly substance used in insulating materials, abrasives, ceramics, as a food additive, in toothpaste, as an anticaking agent in artificial sweeteners, as a bio-filter and in grain storage among numerous applications [2-4]. Korunic [5] observed that *Diatomaceous* earth has low mammalian toxicity and is registered as a feed additive.

Use of DE to control internal parasites in organic livestock production is widely reported [6]. Studies have suggested that inclusion of a certain grade of DE in the diet of grazing ruminants may offer benefits in controlling internal parasites [2]. However there is a lack of scientific evidence supporting its efficacy [6] especially in goats. Goats present several unique metabolic, physiological and immunological attributes compared to those in sheep or cattle [7]. These differences could modulate the interactions between DE and nematode parasites. In light of the value placed by organic livestock producers in feeding DE and in response to the need for alternatives to anthelmintics currently in use, a study was conducted to evaluate the utility of DE for internal parasite control in goats.

#### 2. Materials and methods

#### 2.1. Animals and sampling

Twenty naturally infected Spanish, and Spanish/Boer crossed female goats at the North Carolina Agricultural and Technical State University meat goat herd were used. The animals were reared outdoors in sheltered pens containing automatic watering systems, and concrete flooring. The goats were fed a basal diet Southern States SSC – 31-911800 Goat Feed, 17% crude protein (Southern States Cooperative Incorporated). Water was administered on an *ad libtum* basis.

The goats were randomly assigned to four groups containing five per pen. The average body weight per group was 40 kg. The goats had acquired a natural parasite burden through pasture grazing. Each group received dosages of *Diatomaceous* earth (DE) (Perma-Guard Inc Gilbert, AZ) at different concentrations for a period of eight days. Group 1 received a drench of 1.77 g DE in 150 ml sterile distilled water (50 µg/kg body weight). Group 2 was drenched with 3.54 g DE in 150 ml sterile distilled water (100 µg/kg body weight). Group 3 received a treatment of 5.31 g DE in 150 ml sterile distilled water (150 µg/kg body weight). Group 4 was administered with 150 ml sterile distilled water and served as untreated controls.

The initial weights of all goats were recorded prior to the start of this study. Subsequent weekly measurements were recorded. Animals were weighed and fecal and blood samples were taken weekly for six weeks.

#### 2.2. Fecal egg counts

Fecal samples were collected from the rectum weekly to measure nematode egg excretion. Eggs per gram of feces were evaluated using a modified version of the McMaster's method. The egg counts were conducted in duplicate using McMaster slides (Paracount kit, Chalex Corporation, Issaquah, WA) as recommended by the manufacturer. Approximately 2 grams of fecal samples were collected and mixed with a supersaturated sodium chloride floatation solution. The combination was then loaded onto a McMaster slide and eggs counted using a microscope using a magnification of 100x. Total eggs per gram were determined for each treatment.

#### 2.3. White and red blood cell counts

Peripheral blood samples were collected weekly from the jugular vein in Vacutainer tubes (Becton Dickinson) containing 0.7 ml acid citrate dextrose anti-coagulant for hematological analyses. Blood samples with anticoagulant were prepared for automated electronic counting of total red cells (RBC) and total white blood cells (WBC) by using the Coulter Diluter Dispenser 10 and counter (Coulter Z Series Particle Counter and Size Analyzer, Beckman Coulter, Inc. Fullerton, CA).

The relative proportions of white blood cell types were calculated from blood smears stained with SureStain Wright Methanol Solution. A total of 100 white blood cells were counted. Basophils, lymphocytes, eosinophils, monocytes, and neutrophils were identified and counted in duplicate and averaged once per week throughout the experiment.

#### 2.4. Detection of packed cell volume

An aliquot of blood with anticoagulant from each goat was collected in micro-capillary tubes and then centrifuged for 10 minutes at 14 000 rpm in an IEC MB Micro Hematocrit centrifuge (Damon/IEC Division). After centrifugation, samples were analyzed for PCV in a micro-capillary reader (Damon/IEC Division).

#### 2.5. Statistical analysis

Statistical analyses were performed using the SAS statistical analysis software (SAS Institute Incorporated, Cary, NC). Analysis of Variance (ANOVA) was performed to determine significant differences among treatments. A Dunnett's T test was conducted for treatment comparisons, in which group 4 and week 0 were used as controls. Before analysis, outliers were removed from the original data using a special SAS macro program.

#### 3. Results and discussion

#### 3.1. Fecal egg count in goats following treatment

Figure 1 shows the effect of DE on the number of eggs per gram (EPG) of feces in goats. Haemonchus contortus, Eimeria, and Trichostrongylus spp. were the predominant species of nematodes present throughout the trial. There were highly significant differences among treatment groups (P < 0.0001). Variations in fecal egg counts for weekly measurements also proved to be highly significant (P < 0.0001). Group 2 showed the lowest mean fecal egg count increase while Group 3 showed the highest after the administration of DE. The results of this study coincide with previous experiments involving DE which confirmed no significant reductions of fecal egg counts in sheep [8]. Despite the promoted use of DE as an effective anthelmintic [6,8], the evidence presented in this study does not support DE as an effective alternative de-worming drench for goats.

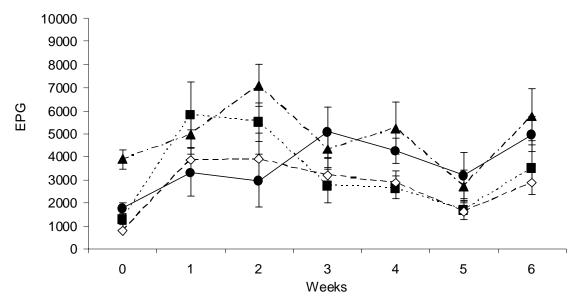


Fig. 1. Fecal egg count (EPG) of feces from goats treated with different doses of *Diatomaceous* earth. Group 1, treated animals with 1.77 g DE (●); Group 2, treated animals with 3.54 g DE (■); Group 3, treated animals with 5.31 g DE (▲); Group 4, untreated animals (◊). Error bars represent the mean±standard deviation (n=6). Weekly measurements P < 0.0001; treatment by Group P < 0.0001.</p>

# 3.2. Packed cell volume levels in goat blood following treatment

The PCV values observed ranged within the normal range for of goats (22% to 38%) [9]. The effect of treatments on PCV was found to be significant (P < 0.02; Figure 2). All groups showed decreases in PCV: -4.6413

(Group 1), -1.1560 (group 2), -3.1345 (group 3) when compared to the control (Group 4). There were no significant differences in the treatments by week and the week by group interactions (P < 0.1; P > 0.1, respectively). Treatment with DE did not improve the mean PCV. These results agree with previous studies in lambs [8].

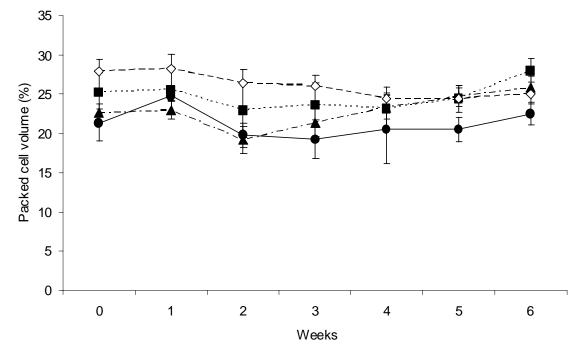


Fig. 2. Packed cell volume (%) of goats treated with different doses of *Diatomaceous* earth. Group 1, treated animals with 1.77 g DE ( $\bullet$ ); Group 2, treated animals with 3.54 g DE ( $\bullet$ ); Group 3, treated animals with 5.31 g DE ( $\bullet$ ); Group 4, untreated animals ( $\diamond$ ). Error bars represent the mean±standard deviation (n=6). Weekly measurements *P* < 0.1; treatment by Group *P* > 0.1.

3.3. Changes in white and red blood cell counts in goats following treatment

The treatments had a significant overall effect on the total white blood cell counts (P < 0.0001; Figure 3). The effect is due to the 'number of weeks' of infection following treatment (weekly measurements, P < 0.001). No statistically different effects were observed for the "group" by treatment interaction (P > 0.1). Each group showed an increase in the mean WBC count with the increase in the number of weeks from the first week (week 1) and up to the last week (week 6). The increased mean WBC may be a reflection of the increased parasite burdens indicated by the observed increased fecal egg counts [10].

The total RBC count was significantly different among treatment groups (P < 0.01; Figure 4); weekly measurements of mean RBC were significantly different over time (P < 0.0001). The mean RBC count decreased in all groups throughout the duration of the experiment. Anemia associated with increased FEC will reduce the number of red blood cells detected by the electronic particle counter and is an indicator of the presence of adult parasites.

Higher WBC counts and lower RBC counts were observed at the end of the study. The PCV accounts for the percentage of the cellular portion of blood relative to the total amount of blood. The RBC count is the actual number of cells found in a micro-liter of blood. The increase in WBC may have contributed to the observed PCV.

## 3.4. Changes in white blood cell differential counts in goats following treatment

Treatment had a significant effect on the proportions of lymphocytes (P < 0.01), basophils (P < 0.001), neutrophils (P < 0.01), and monocytes (P < 0.001) in goat blood. Treatments had no significant effects on eosinophil counts (P > 0.1).

Figure 5 shows the relative changes in white blood cell differential counts in goats treated with DE when compared to those from untreated controls. There was a dose- dependent difference in the neutrophil to lymphocyte ratio following DE treatment. Similar directional changes were observed in neutophils and monocytes as compared to lymphocytes.

All groups demonstrated a mild decrease in eosinophil counts from preliminary recordings after the administration of DE treatment. Eosinophilia is a feature of helminth infections [11]. Breed differences in the levels of eosinophils have been observed between Spanish and Spanish boer crosses [12]. Exposure to DE has an impact on the leukogram similar to those associated with genetic differences and environemtnal exposure in goats [12].

Reports on the systemic responses to *Haemonchus* contortus in sheep indicate a neutrophil leukocytosis no definitive eosinophilia and an antibody response [10].

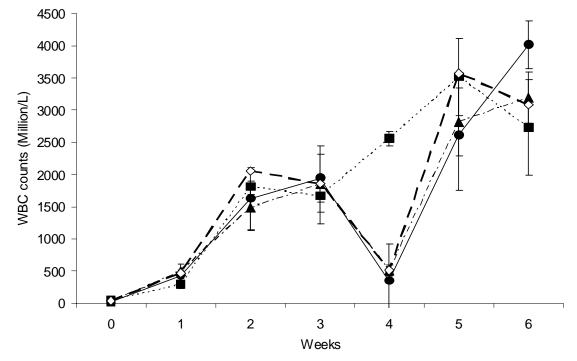


Fig. 3. Total white blood cell counts of goats treated with different doses of *Diatomaceous* earth. Group 1, treated animals with 1.77 g DE (●); Group 2, treated animals with 3.54 g DE (■); Group 3, treated animals with 5.31 g DE (▲); Group 4, untreated animals (◊). Error bars represent the mean±standard deviation (n=6). Weekly measurements P < 0.001; treatment by Group P > 0.1.

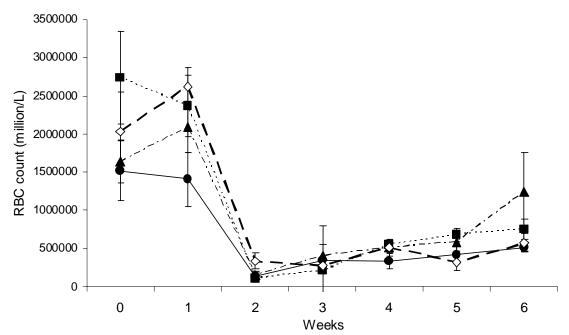


Fig. 4. Red blood cell counts of goats treated with different doses of *Diatomaceous* earth. Group 1, treated animals with 1.77 g DE ( $\bullet$ ); Group 2, treated animals with 3.54 g DE ( $\bullet$ ); Group 3, treated animals with 5.31 g DE ( $\bullet$ ); Group 4, untreated animals ( $\diamondsuit$ ). Error bars represent the mean±standard deviation (n=6). Weekly measurements *P* < 0.0001; treatment by Group *P* < 0.01.

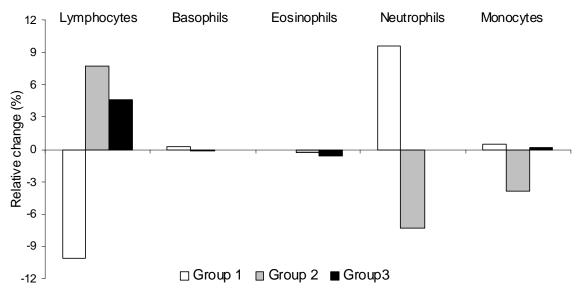


Fig. 5. Relative changes in goats' white blood cell differential count compared to counts from untreated animals. Dose of *Diatomaceous* earth used: Group 1, treated animals with 1.77 g DE; Group 2, treated animals with 3.54 g DE; Group 3, treated animals with 5.31 g DE. Positive value indicates a relative increase and negative values a relative decrease.

The systemic pattern observed in Figure 5 may reflect an inflammatory and immune response associated with DE administration, the levels of parasites and genetic variations between goats. This needs further investigation.

#### 3.5. Goat body weight changes following treatment

Highly significant differences were observed in body weight among treatments (P < 0.0001; Figure 6). A positive gain occurred in the mean weights of Group 1

and 2, which received the lowest dosage of DE. Group 3, which received the highest DE, did not show any statistically significant difference after the treatment. The results are similar to those of treatments of DE in sheep, where increases in weights were reported [8]. Further study is needed to evaluate the effect on performance characteristics such as weight gain attributable to treatment with DE in goats.

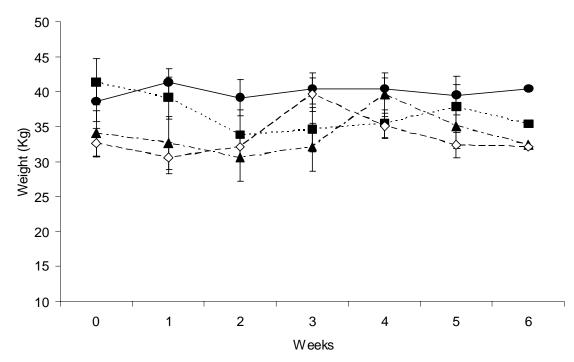


Fig. 6. Weight changes in goats treated with different doses of *Diatomaceous* earth. Group 1, treated animals with 1.77 g DE (●); Group 2, treated animals with 3.54 g DE (■); Group 3, treated animals with 5.31 g DE (▲); Group 4, untreated animals (◊). Error bars represent the mean±standard deviation (n=6). Weekly measurements and treatment by Group P < 0.0001.</p>

#### 4. Conclusions

The results of this study did not indicate significant effects of DE on the parasite load as measured by eggs per gram of feces. The progression of parasitemia was not affected. Variability was observed in the leukogram and may be associated with immune response, genetic variation or result from exposure to trace elements in DE. Further study is needed to evaluate the effect of DE in different breeds of goats, and its effect on performance characteristics such as weight gain and the immune response to support the use of DE in goat production.

#### ბიოქიმია

### დიატომიტების გავლენა პარაზიტებით ინფიცირებულ თხებზე

### გ. ბერნარდი, მ. ვორკუ, მ. აჰმედნა

\* ჩრდ. კაროლინას უნივერსიტეტი, რელეი, აშშ \*\* ჩრდ. კაროლინას აგრარული და ტექნიკური უნივერსიტეტი, გრინსბორო, აშშ

(წარმოდგენილია აკადემიის წევრის ნ. ალექსიძის მიერ)

-ჭლაკის ნემატომიტიმიტების, როგორც ანტიჰელმინტური პრეპარატის გამოყენების პერსპექტივა კუჭნაწლავის ნემატოღების Haemonchus contortus-ის საწინააღმღეგოდ. დიატიმიტისის გავლენა სხვადასხვა პარამეტრებზე შეისწავლებოდა საკვების დანამატის სახით დინამიკაში. თხების სხეულის წონა, პარაზიტის კვერცხების რაოდენობა, ლეიკოციტებისა და ერითროციტების რაოდენობა მნიშვნელოვნად იცვლებოდა მხოლოდ ექსპერიმენტის საწყის ეტაპზე. პრეპარატმა გავლენა იქონია თხების ერითროციტებისა და ლეიკოციტების რაოდენობაზე, მაგრამ კვლევის ამ პირობებში არ გამოვლინდა მისი ანტიჰელმინტური ეფექტი.

#### REFERENCES

- 1. L.L. Mortensen, L.H. Williamson, T.H. Terrill (2003), Evaluation of prevalence and clinical implications of anthelmintic resistance in gastrointestinal nematodes of goats. J. American Veter. Med. Assoc. 223(4), 495-500.
- B.M. L. McLean, D. Frost, D.E. Evans, A. Clarke, B. Griffiths (2005), The inclusion of Diatomaceous earth in the diet of grazing ruminants and its effects on gastrointestinal parasite burdens. Researching sustainable systems. Proceedings of the First Scientific Conference of the International Society of Organic Agriculture Research. Eds – U. Kopke, U. Niggli, D. Neuhoff, P. Cornish, W. Lockeretz, H. Willer, Adelaide, South Australia.
- 3. A. Nikpay (2006), Diatomaceous earths as alternatives to chemical insecticides in stored grain. Insect Sci. 13, 421-429.
- 4. V.S. Mohan, R.M. Krishna, P. Muralikrishna, S. Shailaja, P.N. Sarma (2007), Solid phase bioremediation of pendimethalin in contaminated soil and evaluation of leaching potential. Biores. Technol. 98(15), 2905-2910.
- 5. Z. Korunic (1998), Diatomaceous earths, a group of natural insecticides. J. Stored Prod. Res. 34 (2/3), 87-97.
- J. Sooby, J. Landeck, M. Lipson (2007), National organic research agenda, soils, and pests. Livestock, genetics. Outcomes from the Scientific Congress on Organic Agricultural Research (SCOAR). Organic Farming Research Foundation. Santa Cruz, CA. http://ofrf.org/publications/pubs/nora.pdf.
- 7. N. Silanikove (2000), The physiological basis of adaptation in goats to harsh environments. Small Rumin. Res. 35, 181–193.
- 8. G.D. Osweiler, T.L. Carson (Eds) (1997), Evaluation of *Diatomaceous* earth as an adjunct to sheep parasite control in organic farming. In: Leopold Center for Sustainable Agriculture Progress Reports. Vol. 6. Iowa State University, Ames, Iowa, USA.
- 9. O.M. Radostits, C.C. Gay, D.C. Blood, K.W. Hinchcliff (2000), Veterinary Medicine, 9th Ed., ELBS Bailliere Tindall, Philadelphia, USA, pp. 870-871.
- D.B. Adams (1993), Systemic responses to challenge infection with Haemonchus contortus in immune merino sheep. Veterin. Res. Commun. 17, 25-35.
- 11. K. Leder, P.F. Weller (2000), Eosinophilia and helminthic infections. Best Practice Res. Clin. Haemat. 13(2), 301-317.
- 12. G. Kannan, K.E. Saker, T.H. Terrila, B. Kouakoua, S. Galipallia, S. Gelayea (2007), Effect of seaweed extract supplementation in goats exposed to simulated preslaughter stress. Small Rum. Res. 73, 221-226.

Received October, 2008