Systemic and local effects of garlic administration in meat goats.

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Abstract Animal agriculture is a major contributor to the United States economy. Livestock contributes about 13% of the energy to the world's diet. Animal-source foods are good sources of high-quality protein and micronutrients that are essential for normal development and good health. Sustainable animal production requires that disease-causing pathogens be effectively controlled. Gastrointestinal parasites are a severe threat to goat production. The present study was carried out to determine the effect of garlic extracts on global gene transcription, protein secretion, and gut health indicators in meat goats. Garlic (Allium sativa) has been used as a medicinal herb since time immemorial in almost every known civilization. It has antibiotic, anti-helminthic, and anti-inflammatory properties. Twelve (N=12) adult female, clinically healthy Boer x Spanish goats were divided into 2 groups. The treatment group was drenched daily with a garlic barrier for 4 weeks. Fecal and blood samples were collected weekly. Total parasite Egg counts per gram of feces were evaluated using the Modified McMaster method. Total RNA was isolated using Trizol and evaluated for concentration and purity using the nanodrop spectrophotometer. Total plasma protein secretion was assessed using the BCA assay. All variables were analyzed using SAS 9.1 statistical analysis software ($P \leq 0.05$). The fecal egg count was reduced in animals treated with garlic over the 4-weeks. Garlic treatment significantly increased ($P<0.0374$) total plasma protein concentration. It also significantly reduced ($P<0.037$) the eggs per gram during the second and fourth weeks of treatment. The concentration of RNA varied during different weeks of treatment. There was a significant decrease in the transcription over time ($P<0.01$) but the purity remained almost similar. Garlic drenching can be an economic and eco-friendly approach to modulate global gene transcription, protein secretion, the gut microbiome in goats, or sustainable goat production. Temperature-controlled studies are needed. Target genes and protein pathways are under study.

Keywords: Animal Agriculture, Sustainable Animal Production, Garlic, Protein

Introduction

Globally, the livestock industry is very dynamic. It is currently one of the agricultural subsectors in developing countries with the quickest growth rates (Thornton, 2010). There is an enormous increase in demand for animal protein for human use worldwide (Van Boeckel et al., 2014). The demand for animal products, which is rising quickly due to population growth, urbanization, and rising affluence in developing nations, is what's driving this growth (Delgado, 2005). Goats are one of the most exploited species of farmed livestock because of their adaptability and ability to withstand unfavorable climates like high temperatures, low humidity, and limited feed supply (Zvinorova et al., 2016). Goat meat is a good source of proteins and also has health benefits when consumed in appropriate portions (Ivanovic et al 2016).

Meat goat production is affected by infectious diseases. Goat production may suffer from parasitic illnesses or infestations through different pathways. They also negatively impact animal

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well-being by having a variety of side effects, such as decreased feed intake (Fthenakis and Papadopoulos, 2018). Gastrointestinal (GI) nematode infection is considered the most critical limiting factor in goat production systems around the world and results in substantial economic losses to producers (Charlier et al., 2014). The predominant parasites that cause problems in goats are *Haemonchus contortus and* Coccidia. H. contortus is a blood-sucking worm that reduces goat productivity and causes loss through death (Roeber et al., 2013; Villarroel, 2013). Chemical anthelmintic medications have often been used to treat these parasitic infections (Torres-Acosta & Hoste, 2008). The resistance to these anthelmintic medications has, however, become universally recognized due to their continued usage (Wolstenholme et al., 2004; Rose et al., 2015). To get rid of GI nematodes, researchers have also looked at plant-based anthelmintics. The use of plant-based anthelmintics, including extracts from Sericea lespedeza, wormwood, neem, tobacco, cowpea (Worku et al., 2009; Adjei-Fremah et al., 2016), and garlic, is also being investigated for the treatment of gastrointestinal parasites.

Garlic is a member of the family Liliaceae and has the botanical name Allium sativum. It contains a variety of bioactive components, including organic sulfides, saponins, phenolic compounds, and polysaccharides that have a number of health advantages (Bose et al., 2014; Diretto et al., 2017). It has been used as a medicinal herb since time immemorial in almost every known civilization. Garlic functions as a natural antibiotic (Iciek et al., 2009). It is believed to provide health advantages due to its anti-inflammatory, antifungal, antiviral, and antibacterial properties (Cao et al., 2014). It is also advised as a treatment for intestinal parasites and other disorders in modern natural medicine (Iciek et al., 2009). The objective of this study is to determine the effect of garlic extracts on systemic and local effects of garlic administration in meat goats.

Methodology

Animals. Twenty-four (24) Boer x Spanish goats were selected from North Carolina Agricultural and Technical State University's Small Ruminant Research Unit in Greensboro, North Carolina. All animals were clinically healthy and not under any treatment prior to the study. All procedures were approved by the Institutional Animal Care and Use Committee. Body weight, body condition, and FAMACHA score, packed cell volume, and level of coccidian oocyst and H. contortus egg in fecal samples were all assessed during the initial screening process.

Experimental study. The animals were randomly placed into two groups of six each in the Experimental group and control group. Weekly sampling was performed.

Housing. The goats were housed in a semi-intensive unit at North Carolina Agricultural and Technical State University Farm, Greensboro, NC. The goats were placed in a pen in a barn with a sand rock floor. Each pen had its own feeder and watering system. The animals were sheltered in a barn during the evening hours and let out to pasture during the day.

Feeding. The animals were given balanced diet feed (Southern States Quality Kid and Goat Feed), which was enriched with vitamins and minerals such as ammonium chloride, copper, and zinc and comprised a high-protein, high-energy combination. Once a day, the goats received two pounds of grain from each animal. Hay was also freely provided to the animals.

Drench administration. Ten (10) ml of the undiluted garlic extract was given to each goat on a daily basis for 30 days. Ten (10) ml of distilled water was also given to the control group daily for

30 days. The drenches were administered orally using a 10ml BD syringe (Becton Dickinson, Franklin Lakes, NJ).

On Farm Evaluation

All samples were collected and evaluated once a week throughout the experiment.

Body weights. Body weights were collected before feeding using a chute scale measured in kilograms on the university farm.

Body condition Scores. Body condition was determined by physically assessing the rib areas using firm pressure with the fingers and running fingers down the goat's spine from the shoulders to the tail head, and the degree of fat cushion over these bones determines the score. Body condition refers to the fleshiness of the goat which is scored from 1 to 5, where 1 is very lean, 2 is lean, 3 is moderately good condition, 4 is fat, and 5 is obese (Mendizabel et al., 2010).

FAMACHA© score. The FAMACHA© scores were used to assess anemia in goats. This was measured by observing the color of the lower conjunctiva of the eye. The score is based on a scale of 1 to 5. A score of 1 = very red (not anemic), 2 = red/pink color (not-anemia), 3 = pink (questionable), 4 = pale pink (anemic), and 5 = white (very anemic) (Burke, 2007).

Collection of Fecal Samples. Fecal samples were collected by applying lubricant and inserting the index middle finger directly into the rectum wearing sterile gloves for each animal. Collected samples were placed in labeled clean zip-lock plastic bags to determine fecal egg count.

Fecal Egg Count. A modified McMaster technique was used for fecal egg count as described by Whitlock (Kaplan et al., 2004). To make parasite eggs float to the top of the solution, 28 ml of saturated sodium chloride was added to two (2) grams of feces before being dissolved in it. A spatula was used to break up the pellets. According to the Paracount-EPGTM Fecal Analysis Kit (Chalex Corporation, Wallowa, OR), aliquots of the solution were transferred into both McMaster slide chambers using a ten (10) ml syringe and then observed with a 10X Olympus B X40 microscope. The eggs per gram (EPG) of feces for each animal were determined by counting the Strongyle eggs to assess *H. contortus* and the coccidia oocyst in duplicate, averaging the results, and multiplying the result by 50 (Kaplan et al., 2004) to calculate eggs per gram(EPG).

Blood Collection. Whole blood (6 ml) was collected from the jugular vein using blood collection tubes containing the anticoagulant Acid Citrate Dextrose (ACD) (Becton Dickson, Franklin, N.J.). The blood was gently mixed with the anticoagulant and immediately placed on ice.

Plasma Preparation. Whole blood was centrifuged in a 1.5 ml tube at 12000 rpm for 15 minutes to separate plasma from blood cells. The plasma at the top surface was collected and transferred into a 10ml tube and kept at -80°C for storage.

Bicinchoninic Acid Assay (BCA). PierceTM BCA Assay kit (Rockford, IL) was used to determine the total protein concentration content from plasma isolated from whole blood. Protein standards were prepared (Appendix A). 25 µl of each standard and unknown sample was pipetted into a 96-microplate well (Thermo ScientificTM PierceTM, Rockford, IL). 50 parts of BCA

reagent A and 1 part of BCA reagent B were combined to prepare the working reagent (WR). Two hundred (200) µl of the WR was added to each well and mixed thoroughly on a plate shaker for 30 seconds. The plate was then covered and incubated at 370C for 30 minutes. Plates were cooled to room temperature (RT). A standard curve was created using the protein standard samples at 0µm/ml, 25µm/ml, 125µm/ml, 250µm/ml, 500µm/ml, 750µm/ml, 1000µm/ml, 1500µm/ml and 2000µm/ml and the absorbance was measured at 562nm on a plate reader (BioTek Instruments Inc, Winooski VT). By extrapolating the optical density from the standard curve and the line of best fit, unknown sample concentrations were calculated.

Isolation of RNA. The RNA was isolated using TRI Reagent® (Invitrogen, San Diego, CA) using the manufacturer's instructions. All precautions were taken to ensure no RNase contaminations. The cell pellet was homogenized by adding TRIzol™ Reagent at a 3:1 ratio and pipetting up and down. 200 µl of chloroform was added to each 1 mL of TRIzol™ after the tubes had been incubated on ice for five minutes. The sample was then centrifuged at 12,000 x g for 15 minutes at 4°C. New 1.5mL tubes were used to transfer the aqueous phase into. Isopropanol in the ratio of 500 µl/ 1 ml TRIzol™ was added to the cell pellet's aqueous phase. Then it was placed on ice for 10 minutes. The tubes were centrifuged for 10 minutes at 12,000 x g at 4°C after incubation. Using a micropipette, the supernatant was discarded. The TRIzol™ used for lysis was combined with 1 mL of 75% ethanol to resuspend the cell pellet. The tube was then vortexed and centrifuged at 4°C for 5 minutes at 7500 x g. The RNA pellet was air-dried for 10 minutes after the supernatant was removed. The pellet was resuspended in 20 µl of RNase-free water and incubated at 60 °C for 15 minutes in a water bath. RNA purity (260/280) and concentration (ng/µL) were determined using a Nanodrop Spectrometer ND 1000 (Thermo Scientific. Inc, Waltham, MA.).

Statistical Analysis. ANOVA was performed on all variables using the statistical analysis software package SAS (SAS Institute Cary, NC). The significance of the results was shown at 5% level of significance or $P<0.05$.

Results

No clinical changes were observed in goats following drench administration.

Fecal Egg Count. The EPG included oocysts of coccidia and eggs of Strongyles. A significant decrease ($P<0.037$) was observed in the egg per gram during the second and fourth weeks in the goats treated with garlic compared to day 0. Over time, the fecal egg counts of the control group increased and the mean average is 1083 EPG (figure 1).

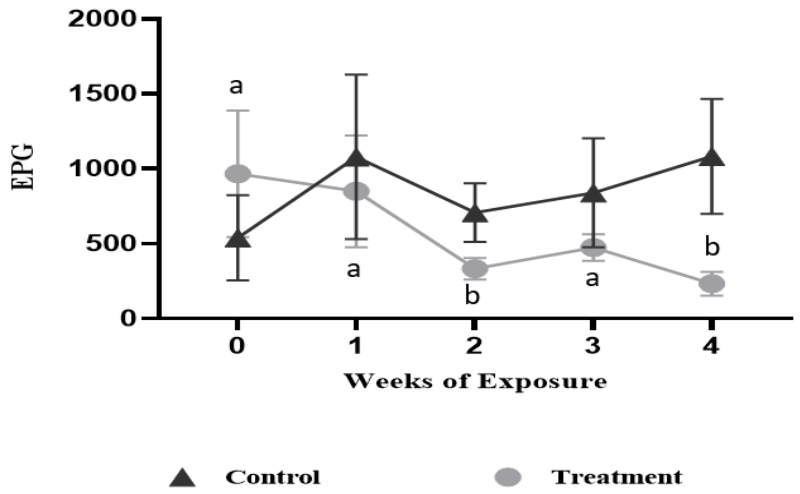

Figure 1. Effect of garlic barrier on parasite egg counts per gram (EPG) feces in goats. Means without common superscript differ P<0.05

Goat productivity is negatively impacted by parasitic diseases. The outcomes of this study are consistent with those of Worku et al. (2009), who found that a 10ml dose of garlic extract significantly reduced the quantity of coccidia in comparison to the control group. Garlic had anti-parasitic properties against GIN in goats, according to Hasan et al. (2015).

Microbial DNA concentration

The DNA isolated from fecal samples of kids during weeks 0 and 4 was analyzed. There was no significant difference between treatment and control (Figure 2). There was a reduction in the concentration from week 0 to week 4 in both groups. The presence of more microbes in the gut of animals during the first week after weaning might be due to the mother's milk. The reduction in the DNA concentration by week 4 may be the effect of dietary changes. Total DNA was not affected by garlic.

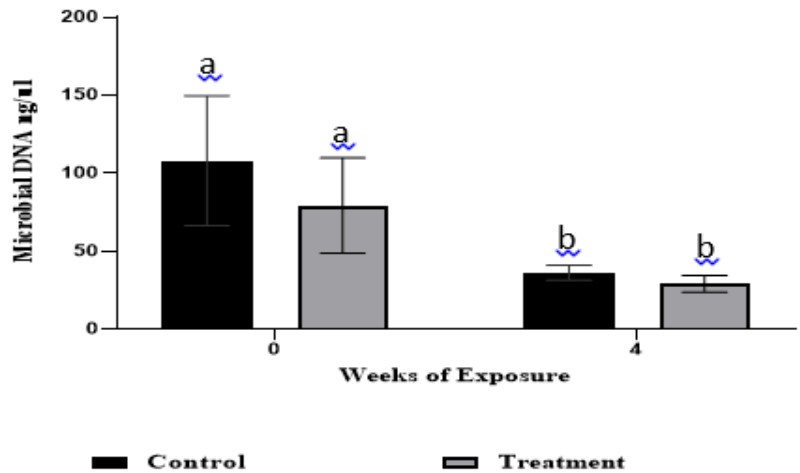

Figure 2. Effect of garlic barrier on fecal microbial DNA concentration (ng/µl) in weaned goats. Means with different superscripts differ P< 0.05.

Microbial DNA. The relative abundance of *Bifidobacterium* spp, *Lactobacillus* spp, total microbe (16S,) and *Haemonchus* spp was measured using PCR.

Total microbes decreased with garlic. No effect of garlic on those tested - *Bifidobacteria* and *Lactobacillus* spp were found in the fecal samples (Table 1).

Species	**Treatment**		**Control**	
	Week 0	Week 4	Week 0	Week 4
Bifidobacteria spp	0.38	0.3	0.27	0.27
Lactobacillus spp	0.4	0.39	0.29	0.26
16S	0.2	0.13	0.15	0.26
Haemonchus	0.25	0.22	0.27	0.2

Table 1. Relative abundance of microbes presents in the gut of weaned kids

There was no difference in the relative abundance of this spp (Table 1). *Haemonchus* was also observed in both treatment control groups. More beneficial microbes were present compared to *Haemonchus* in treatment. Garlic did not change the number of *Bifidobacteria*. There was no change in the level of *Haemonchus*. Treatment did not kill *Haemonchus* but may have targeted others such as *coccidia* (Worku et al., 2009), or stopped GIN from laying eggs.

Protein concentration

The protein concentrations increased in the treatment group over the study period as shown in Figure 3.

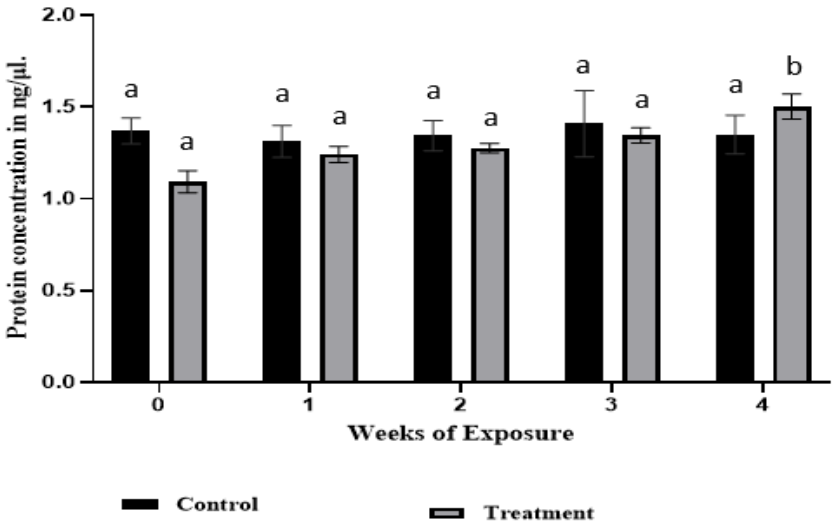

Figure 3. Effect of garlic on plasma protein concentration (ng/µl) in goats. Means without common superscript differ P<0.05

The protein concentrations increased in the treatment group over the study period as shown in Figure 3. A significant increase (P<0.0374) was observed in concentrations of total plasma proteins in adult goats. High protein concentrations are a sign that the body is battling infections. Adult animals may have previously been exposed, which is why they now react more strongly.

Total RNA transcription

The effect of garlic treatment on RNA concentration can be seen in Figure 4. There were no significant changes between the treatment and control groups in adult goats. There was a time effect (P<0.01). Treatment decreased the concentration of RNA during weeks 3 and 4. Garlic has not affected transcription initially but over time it reduced significantly (P<0.01).

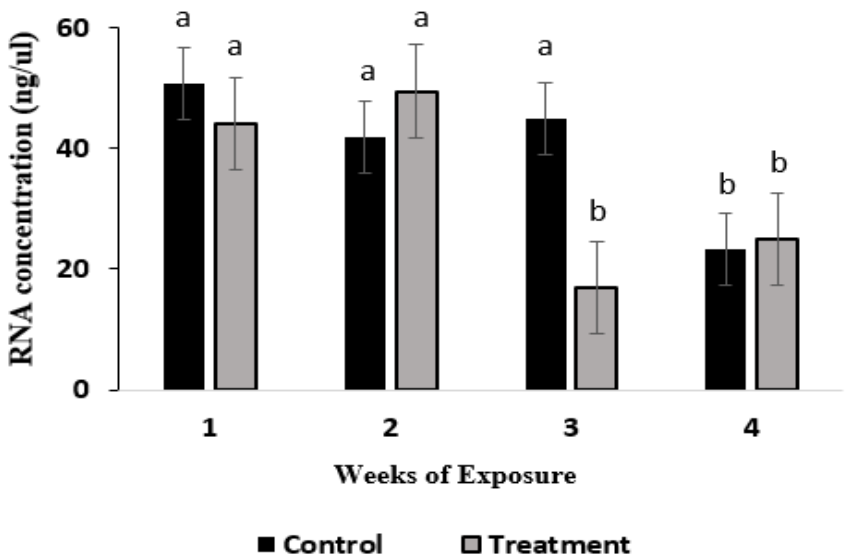

Figure 4. Effect of garlic on RNA concentration in goats. Means without common superscript differ P<0.05

Conclusion

Administration of fresh garlic as a drench in goats impacted global gene transcription, protein secretion, and gut health indicators in meat goats. Both systemic and local effects in the gut were observed. Garlic is a potential immuno-modulator.

Garlic Barrier extract reduced the parasite count, modulated the RNA levels, and increased protein secretion. Garlic is a potential immuno-modulator. It can be used as an alternative for anti-parasitic drugs in sustainable meat goat production. Variation was observed over time and in different animals and warrants further investigations. Studies are underway to further analyze the effect of gene expression.

References

1. Adjei-Fremah, S., & Worku, M. (2021). Cowpea polyphenol extract regulates galectin gene expression in bovine blood. *Animal biotechnology*, *32*(1), 1-12.
2. Bose, S., Laha, B., & Banerjee, S. (2014). Quantification of allicin by high performance liquid chromatography-ultraviolet analysis with effect of post-ultrasonic sound and microwave radiation on fresh garlic cloves. *Pharmacognosy magazine*, *10*(Suppl 2), S288.
3. Burke, J. M., Kaplan, R. M., Miller, J. E., Terrill, T. H., Getz, W. R., Mobini, S. & Vatta, A. F. (2007). Accuracy of the FAMACHA system for on-farm use by sheep and goat producers in the southeastern United States. *Veterinary parasitology*, *147*(1-2), 89-95.
4. Cao, H. X., Zhu, K. X., Fan, J. G., & Qiao, L. (2014). Garlic-derived allyl sulfides in cancer therapy. *Anti-Cancer Agents in Medicinal Chemistry (Formerly Current Medicinal Chemistry-Anti-Cancer Agents)*, *14*(6), 793-799.
5. Charlier, J., Velde, F. V., van der Voort, M., Van Meensel, J., Lauwers, L., Cauberghe, V., ... & Claerebout, E. (2015). ECONOHEALTH: Placing helminth infections of livestock in an economic and social context. *Veterinary Parasitology*, *212*(1-2), 62-67.
6. Diretto, G., Rubio-Moraga, A., Argandoña, J., Castillo, P., Gómez-Gómez, L., & Ahrazem, O. (2017). Tissue-specific accumulation of sulfur compounds and saponins in different parts of garlic cloves from purple and white ecotypes. *Molecules*, *22*(8), 1359.
7. Fthenakis, G. C., & Papadopoulos, E. (2018). Impact of parasitism in goat production. *Small ruminant research*, *163*, 21-23.
8. Hartung, J. (2013). A short history of livestock production. In *Livestock housing: Modern management to ensure optimal health and welfare of farm animals* (pp. 81-146). Wageningen Academic Publishers.
9. Iciek, M., Kwiecień, I., & Włodek, L. (2009). Biological properties of garlic and garlic-derived organosulfur compounds. *Environmental and molecular mutagenesis*, *50*(3), 247-265.
10. Ivanović, S., Pavlović, I., & Pisinov, B. (2016). The quality of goat meat and it's impact on human health. *Biotechnology in Animal Husbandry*, *32*(2), 111-122.

11. Kaplan, R. M. (2004). Drug resistance in nematodes of veterinary importance: a status report. Trends in Parasitology, 20(10), 477-481. doi: 10.1016/j.pt.2004.08.00
12. Kaplan. R,M., Burke, J.M., Terrill, T.H., Miller, J.E., Getz, W.R., Mobini. S., Valencia, E. Williams,M.J., Williamson, L.H., Larsen,M., & Vatta, A.F. (2004). Validation of the FAMACHA© eye color chart for detecting clinical anemia in sheep and goats on farms in the Southern United States. Veterinary Parasitology, 123:105-120.
13. Mendizabal, J., Delfa, R., Arana, A., & Purroy, A. (2011). Body condition score and fat mobilization as management tools for goats on native pastures. *Small Ruminant Research, 98*(1), 121-127.
14. Thornton, P. K. (2010). Livestock production: recent trends, future prospects. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *365*(1554), 2853-2867.
15. Roeber F, Jex AR, Gasser RB (2013) Impact of gastrointestinal parasitic nematodes of sheep, and the role of advanced molecular tools for exploring epidemiology and drug resistance – an Australian perspective. Parasites Vectors 6:153
16. Rose, H., Rinaldi, L., Bosco, A., Mavrot, F., De Waal, T., Skuce, P. & Morgan, E. R. (2015). Widespread anthelmintic resistance in European farmed ruminants: a systematic review. *Veterinary Record*, *176*(21), 546-546.
17. Torres-Acosta, J. F. J., & Hoste, H. (2008). Alternative or improved methods to limit gastro-intestinal parasitism in grazing sheep and goats. *Small Ruminant Research*, *77*(2-3), 159-173.
18. Villarroel A (2013) Internal parasites in sheep and goats. Extension Service, Oregon State University, Corvallis, pp 1–4
19. Van Boeckel, T. P., Brower, C., Gilbert, M., Grenfell, B. T., Levin, S. A., Robinson, T. P. & Laxminarayan, R. (2015). Global trends in antimicrobial use in food animals. *Proceedings of the National Academy of Sciences*, *112*(18), 5649-5654.
20. Worku, M., Franco, R., & Baldwin, K. (2009). Efficacy of garlic as an anthelmintic in adult Boer goats. *Biol.Sci*., 61: 135-140.