GARLIC FOR SUSTAINABLE PRODUCTION OF HAIR SHEEP IN THE US

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Abstract

St. Croix sheep are hair sheep whose production characteristics make them suitable for low-input, sustainable production systems. They are parasite resistant and can live in a wide variety of climates. Natural immune modulators such as garlic can enhance animal production, health, and well-being. Garlic (*Allium sativa*) is recognized as an eco-friendly alternative to antibiotics and contributes to a reduction in methane emissions. The present study was carried out to determine the effect of garlic extracts on global gene transcription, protein secretion, and gut health indicators in St. Croix sheep. Clinically, healthy adult, age-matched non-pregnant St. Croix sheep (n=12) from the NC A& T University farm were divided into a treatment group (n=6) and a control group (n=6). The treatment group was drenched with 5 ml garlic (Garlic Barrier (Glendale, CA) concentrate diluted 1:1 with sterile distilled water (10 ml total). The control group was drenched with 10ml sterile distilled water for four weeks. The body weight, body condition, FAMACHA scores, and blood and fecal samples were collected weekly. The fecal microbial DNA was evaluated using the fast DNA stool kit (Qiagen,CA). Total plasma protein was analyzed using the BCA assay. Total RNA was isolated using Trizol and the concentration was determined using a nanodrop spectrophotometer. Data were analyzed using t-test where $p \leq 0.05$ (SAS9.4 SAS Institute Cary NC). Garlic has shown no significant effect on gut microbial DNA abundance, increased total protein concentration ($p<0.004$) in the treatment group, and RNA concentration gradually decreased over time in the treatment group compared to the control ($P<0.05$). Oral administration of garlic has pleiotropic effects and no observed adverse effects. Determination of garlic's effect on specific gene expression and protein function will contribute to its sustainable use as an alternative anti-helminthic and immune modulator in sheep.

Keywords: Garlic Extracts, Protein Secretion, Sheep, RNA, DNA, Gene Expression

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Introduction

Sustainable production is the production for environmental, social, and economical benefits while protecting public health and the environment (Shurson, 2020). The sustainable model ensures the utilization of natural resources without exploitation in specific habitats and practices by humans (Jovanovic et al., 2011). The general use of antibiotics and anthelmintics in animal production has an adverse effect on public health and different environmental aspects (Lee et al., 2001). Sustainable animal products lead to the utilization of natural products which anthelmintic or antibiotic properties like garlic (Kumar et al., 2014). Sustainable product standards are voluntary standards. Although different certifications and labeling are present in different countries for various products.

Sheep are mainly reared for meat, milk, wool, and skin. They can convert low-quality feed and vegetation into nutritious meat, which is extremely helpful for small farmers (Elshazly & Youngs, 2019). The sheep are the most diversified animal species due to their wide range of genotypes and adaption to a number of environments (Gaafar et al., 2012; Benjelloun et al., 2021) and specific adaptation to environmental stress (Kypriotou, 2012). The sheep also adopted resistance to various parasites (Kalaldeh et al., 2019). The southeastern US has a humid and dry, subtropical climate with very warm summers and moderately cold winters. The climate is more suitable for hair sheep production whereas cooler temperatures are required for wool sheep production (Henry et al., 2018). Small producers in the southeastern US raise small ruminants such as sheep and goats to meet the demands of the ethnic and emerging niche markets (Lewis, 2015).

Hair sheep of Caribbean origin play an important role in the sheep industry of the U.S. The characteristics of hair sheep with adoption to various environmental conditions (tropical, arid, and semi-arid regions), parasitic resistance (resistant to *Haemonchus contortus*), and lack of wool (eliminates various health concerns and reduces the cost of production) (Bradford & Fitzhugh, 2019). The hair sheep have greater production although affected by drug-resistant gastrointestinal nematodes, which makes them suitable for a rising concern in the sheep industry (Torres-Acosta et al., 2012; Osei et al., 2018). They are ideal for organic production, reduced input and maintenance costs, decreased labor demands, and an overall greater return on investment than conventional wool breeds (Spangler et al., 2017).

St. Croix sheep are hair sheep whose production characteristics make them suitable for low-input, sustainable production systems (Baker & Gray, 2004; Osei et al., 2018). They are prolific, easy to manage, and resistant to climate (Davila et al., 2011). They are parasite resistant and can be managed under low input on pasture (Steger, 2005). There is an opportunity or small farmers to diversify their enterprise to meet the demand or meat from small ruminants (Haslin et al., 2022).

Internal parasites pose the greatest challenge to small ruminant production (Ekwemalor et al., 2017) in humid areas largely due to anthelmintic resistance (Osei et al., 2018). The GIN parasites like *Haemonchus contortus* are associated with increased mortality and poor weight gain, causing a loss of tens of billions of dollars along with *Coccidia* (Roeber et al., 2013). Such parasites are normally managed using anthelmintic medicines, however, due to their low effectiveness, alternative approaches are being investigated (Worku et al., 2009). The social push for non-

chemical (eco-friendly, green, and organic) farming has also accelerated the quest for feasible alternatives to anthelmintics drugs (Waller & Thamsborg, 2004). The natural immunomodulator acts as the major alternative for anthelmintics like garlic which controls GIN and also improves growth, health, and production (Vanwyk et al., 2006).

Garlic and its metabolites have antibacterial, anthelmintic, antifungal, and antiviral properties (Mikaili et al., 2013). The evidence for garlic efficacy against helminths, however, is ambiguous (positive effect: Strickland et al., 2009; no effect: Burke & Miller, 2004; Worku et al., 2009). Garlic is also used as an alternative to the available anthelmintic (Ivermectin) in the parasite management of sheep. Administering garlic extract has no effect on body weight, fecal egg count, and packed cell volume when compared to commercial anthelmintic drug-treated sheep (Curry & Whittaker, 2010). Dietary sources of garlic in feed can modulate immunity and gene expression (Worku et al., 2016). Garlic (*Allium sativum*) has antimicrobial, antioxidant, as well as antihypertensive properties, and has been used as a feed flavor in the livestock industry. Garlic has a positive effect on body condition scores, whereas some studies have shown that it has no effect (Strickland et al., 2009).

Natural food components have been shown in sheep and cattle to directly activate innate immune cells in the intestinal wall, irrespective of antigens, to control gastrointestinal parasites. (Dalton et al., 2013; Adjei-Fremah et al., 2017; Adjei-Fremah et al., 2016b). Supplementing the garlic barrier, which includes all bioactive components, might be an alternative and feasible way of delivering bioactive chemicals to animals. To improve innate immunity and determine toxicity, the potential systemic effect of a garlic barrier drench on gut health, global gene transcription, and protein secretion must be investigated. (Siddiqui et al., 2012). The effect of garlic at the molecular level is not studied, as it has effects on systemic and molecular effects in sheep.

The objective of this study was to assess the effect of garlic extracts on global gene transcription, protein secretion, and gut health indicators in St Croix sheep.

Materials and Methods:

Animals: Twelve clinically healthy, age-matched, adult female St. Croix sheep were selected from North Carolina Agricultural and Technical State University's Small Ruminant Research Unit in Greensboro, North Carolina.

Drench preparation & administration: Garlic Barrier is a concentrated garlic juice purchased from Glendale CA Company. Every week fresh drench was prepared for drenching by diluting the concentrated juice in a 1:1 ratio with distilled water. The prepared drench of 10ml was aliquoted into the 15ml polypropylene tubes for the treatment group (n=6), whereas distilled water of 10ml was aliquoted for the control group (n=6) for 30 days in the morning between 8 AM and 10 AM.

Sampling: Blood and fecal samples were collected before the starting of drenching as baseline or control samples and every week once after drenching for 4 weeks. Along with these phenotypic data such as Body weight, Body condition and FAMACHA scores were also collected on the farm

Evaluation of the concentration of total plasma proteins: Total secreted protein concentration in plasma was analyzed using the Pierce™ BCA Protein Assay Kit (Thermo Scientific, Waltham, MA) following the manufacturer's instructions. The absorbance of the plate was measured with an Epoch microplate reader (BioTek., Winooski, VT) at 562nm, and a standard curve was plotted with average blank and standard concentration; the concentrations of samples were determined.

Isolation of total RNA and assessment of concentration & purity: As described (Adjei-Fremah et al., 2017; Adjei-Fremah et al., 2016b) isolated the total RNA using the PureLink ® RNA Mini Kit (Invitrogen™ 12183018A) according to the manufacturer's protocol provided. The concentration and purity of the eluted RNA samples were determined using the Nanodrop Spectrometer ND 1000 (Thermo Scientific. Inc, Waltham, MA.) as per manufacturers' protocol. The concentration was determined at 260nm and Purity at 280nm as ug/ µL. The values along with graphs were recorded.

Isolation and relative abundance evaluation of total fecal microbial DNA: Fecal microbial DNA was isolated as described in previous studies (Osei et al., 2018). Using QIampFast stool DNA kit (Qiagen, CA) and following manufacturers' protocol. The concentration and purity of the eluted DNA samples were determined using the Nanodrop Spectrometer ND 1000 (Thermo Scientific. Inc, Waltham, MA.). The relative abundance is measured by real-time PCR using primers specific for *bifidobacterial* spp and *lactobacillus* spp. GAPDH is used as a housekeeping or internal control gene for normalization.

Statistical Analysis: Repeated measures and two-way ANOVA, SAS software version 9.4 (SAS Institute, Cary, NC) was used to analyze the Data. One-way analysis of variance and two-way analysis of variance was used to determine the significance of treatment, and treatment over time interactions for PCV, and white blood cell differential count. A standard curve was used to determine the Total protein concentration.

Results and Discussion:

Health Parameters: Garlic has shown different effects on health parameters. It has increased body condition scores significantly ($p<0.05$), whereas shown no effect on FAMACHA and body weight.

Relative abundance of gut microbial DNA: Garlic treatment has shown no significant effect on microbial DNA concentration in the feces. There is no significant difference between the control and treatment groups. The DNA concentration ranged from 19.215ng/µL to 31.5ng/µL. Using primers and real time PCR (Table 2) the effect of garlic on the relative abundanceof total microbe (16S), *Bifidobacterium spp*, *Lactobacillus spp D*NA, and *Haemonchus spp*. *Bifidobacterium spp* and *Lactobacillus spp* DNA, *Haemonchus,* was detected.
Although results were not statistically significant an increasing trend in *lactobacillus* abundance was observed (table 2).Gut microbial diversity and abundance is influenced by many factors(Ekwemalor et al., 2015; Osei et al., 2018).

Table 1: Relataive abundance of gut mircobial DNA

Relative Microbial abundance of Sheep				
	Bifi	Lacto	16S	Haem
C- Week - 0	0.245	0.251	0.296	0.208
T -Week - 0	0.359	0.328	0.201	0.313
C- Week - 4	0.257	0.310	0.151	0.282
T -Week - 4	0.299	0.373	0.193	0.298

Total RNA concentration: Total isolated RNA concentration was determined to assess transcriptional activation in blood. There was no statistically significant difference between treatment and control ($p>0.05$) observed in both adult non-lactating and lactating ewes. There was an observable decrease in RNA concentration in samples from treated sheep compared to controls. In male rats' garlic supplementation lowered mRNA levels of adipogenic genes and elevated the expression of mRNA encoding uncoupling proteins (Lee et al., 2011). In human cell s-allyl cyst, a garlic component showed the upregulation of Galectin 3 genes (Nirala & Gohil 2015).

Total plasma protein concentration: The total plasma protein concentrations indicate the release of protein by the cells in response to the activation and secretion of immune proteins such as cytokines. In adult ewes, garlic treatment resulted in a significant increase in total plasma protein concentration in the treatment group compared to the control group ($p<0.05$), and a significant time effect was also observed in this study ($p<0.05$) (figure2).

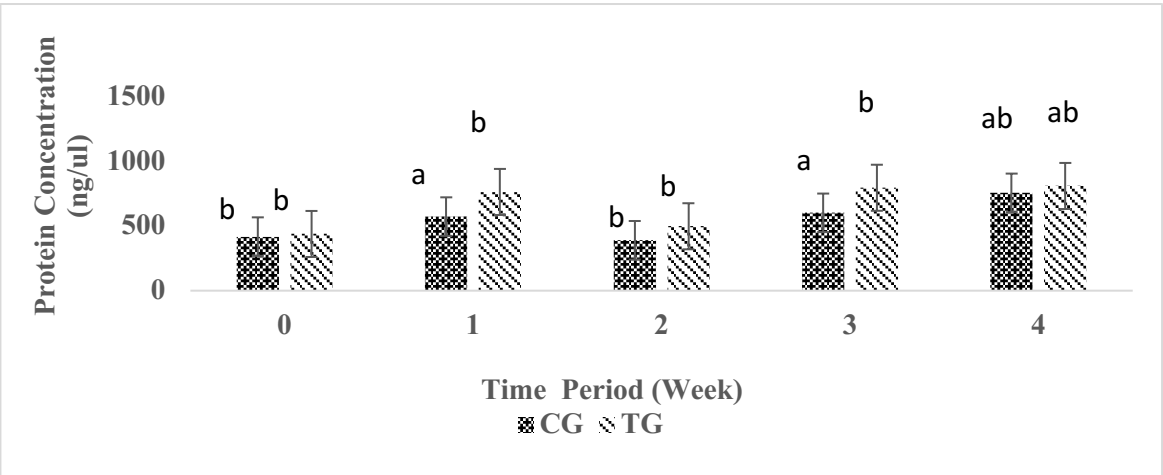

Figure 1: Effect of garlic on total plasma protein in ewes. The significance of least-square means at $P<0.05$ level is indicated by different letters. CG-control group; TG- Treatment Group.

Supplementation of garlic in the diet of Murrah buffalo calves has been found to significantly increase the total plasma protein (Duvvu et al., 2018).

Conclusion: Variability was observed among sheep indicating host-specific effects and Genetic Variability. Garlic has Increased Total plasma protein concentration and decreased Total RNA concentration and has no effect on gut microbial DNA. Garlic has no adverse effect on gut microbe as indicated by no change in fecal DNA. It regulates both transcription and translation differentially (RNA down protein up in a time-dependent manner). Garlic can be used to enhance immunity and production in St Croix sheep that are selectively resistant to parasites. Increased

protein secretion may enhance disease resistance and production efficiency. Gut health is important in feed efficiency and health**.** Thus, Garlic Can be used as an anti-parasitic and Immune Modulator.

Future Direction: Further studies are required to determine garlic's effect on specific gene expression, and protein function will contribute to its sustainable use as an alternative anti-helminthic and immune modulator in sheep

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