The Use of Grape Products as a Natural Anthelmintic in Goats

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Project Information

Summary:

Gastrointestinal nematode parasitism is one of the greatest threats to economic goat production in the United States, costing the industry tens of billions of dollars annually. Furthermore, with increased frequencies of anthelmintic resistance, there is heightened interest in alternative natural dewormers, such as plants containing condensed tannins. Therefore, the objective of this study was to evaluate effects of fermented Chambourcin grape extract (CG) on parasite level and performance in mixed-breed goat kids. On October 14, 2014, mixed-breed male and female goat kids (n = 45; 17.17 kg ± 0.79 BW) were stratified by fecal egg count, weight, sex, and were allocated randomly to 1 of 3 treatments: 1) an oral dose (10 mL per 4.5 kg of BW) of CG at 7-d (D7) intervals, 2) the same dose of CG at 14-d (D14) intervals, or 3) control (C; 30 mL oral dose of water at 14-d intervals). Condensed tannins were extracted, purified, and standardized from CG and were found to have a concentration of 0.33 mg/mL. Kids were maintained on tall fescue [*Lolium arundinaceaum* (Schreb.) Darbysh] and mixed browse pasture with 14% crude protein corn-soybean meal based creep feed for the duration of the 63-d study.

Fecal egg counts, packed cell volumes, FAMACHA© scores, weights, and body condition scores were measured every 7 d. Data were analyzed by the PROC MIXED procedure of SAS. Two contrast statements were used to compare the mean of control versus D7 and D14 and the mean of D7 versus D14. Start BW, end BW, ADG, and gain did not differ ($P \geq 0.42$) across treatments. Start, final, and change from start to final body condition scores, fecal egg counts, and packed cell volumes did not differ ($P \geq 0.12$) across treatments. End FAMACHA© scores were higher ($P = 0.02$) for D7 and D14 as compared with C. White blood cell (WBC) count increased ($P = 0.04$) from beginning to end of study ($P = 0.08$) for C compared with D7 and

D14, whereas, D7 tended to be higher compared with D14. Start neutrophils tended to be higher ($P = 0.08$) in C compared to D7 and D14 and a change ($P = 0.05$) was found in neutrophils from start to end of study in C compared with D7 and D14. An increase ($P = 0.04$) was found in basophil concentrations from D7 as compared with D14. End of study hemoglobin and mean corpuscular hemoglobin concentrations tended to increase ($P = 0.07$ and $P = 0.06$, respectively) in C compared with D7 and D14. End and change from start to end of study mean corpuscular hemoglobin was decreased ($P = 0.04$) in C compared with D7 and D14. A change ($P = 0.02$) from start to end of study was found in platelets for C compared with D7 and D14. Other blood parameter counts were similar ($P \geq 0.10$) across treatments. Therefore, fermented Chambourcin grape extract may not be an effective natural anthelmintic for controlling nematodes in creep-fed goat kids.

Introduction:

Gastrointestinal nematodes (GIN) are the largest constraint to profitable goat production worldwide (Shaik et al., 2006). Since their introduction in the 1960's, broad-spectrum synthetic anthelmintics have been the primary defense against GIN infection in small ruminants worldwide (Hoste, 2011). However, due to widespread prevalence of anthelmintic resistance in goat GIN, alternative, natural control methodologies are needed to increase profitability of the small ruminant industry (Shaik et al., 2006; Terrill et al., 2009).

A compilation of research by Muir (2011) suggested that phyto-therapy or use of plants containing flavonoids, as a natural anthelmintic, should be evaluated. Found in nearly all families of plants, the most abundant flavonoid are polyphenols (Githiori, 2006). Polyphenols are tannins which manifest as plant secondary metabolites, and are closely associated with plant defense mechanisms against insects (Githiori, 2006; Oksana et al., 2012). Tannins are comprised of two groups: condensed tannins (CT) and hydrolysable tannins (Anthanasiadou, 2001).

High concentrations of CT have been measured in fruits with dark red, blue, or black pigmented skin such as grapes, many dark orange or red skinned vegetables, some legume cereals and beans, tree nuts such as almonds, pecans and hazelnuts, cocoa beans, wine, and spices such as cinnamon (King and Young, 1999; Gu et al., 2004; Mattivi et al., 2008). Components of pH, astringency or dryness, and bitterness, are indications of CT concentration (King and Young, 1999). Condensed tannins are compounds that possess high molecular weights (MW), 500-3,000, that demonstrate biological activities causing them to react and precipitate most proteins (Muir, 2011). As degree of polymerization and MW increases, astringency may also increase (Naumann et al., 2013). An increase in concentration of CT is also observed from red grape juice to red wine (King and Young, 1999), suggesting that fermentation may influence CT accessibility to the ruminant animal (Githiori, 2006).

Project Objectives:

Our objective was to evaluate effects of fermented Chambourcin grape extract on performance and parasite level in creed-fed goat kids.

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Research

Materials and methods:

Animals and experimental design

This project was conducted at the Lincoln University Allen T. Busby Farm in Jefferson City, Missouri and was approved by the Animal Care and Use Committee (14-4). Mixed-breed, male and female goat kids (n = 45; 17.17 kg ± 0.79 BW) were maintained on tall fescue [Lolium arundinaceaum (Schreb.) Darbysh] and mixed browse pasture with 14% crude protein corn-soybean meal based creep feed for 81-d post-weaning and were allowed to acquire a natural GIN infection. Kids were then weighed, assigned a body condition score (BCS), and fecal egg counts (FEC) were determined. Starting October 14, 2014, kids were then stratified by FEC, weight, and sex, and allocated randomly to 1 of 3 treatments: 1) drenched with Chambourcin grape extract (CG) every 7-d (D7) at a rate of 10-mL per 4.5 kg of BW; 2) drenched with CG every 14-d (D14) at a rate of 10-mL per 4.5 kg of BW; and 3) control; drenched with 30 mL of water every 14-d (C). Animals were removed from the study if they met 3 out of the following 4 criteria: 1) FEC of $\geq$ 4,000; 2)

FAMACHA© score of $\geq$ 4; 3) packed cell volume (PCV) of $\leq$ 21%; or 4) a BCS < 2. For the duration of the 63-d trial, kids grazed fescue and mixed browse pasture and had *ad libitum* access to water, mineral (Redmond Naturals, Redmond, UT), and a 14% crude protein corn soybean meal-based creep feed. Throughout the study, kids were maintained in a single group with ear tag numbers as the primary identification method.

Chemical analysis and quantification of condensed tannins

Condensed tannins were extracted and purified from CG by the CT isolation method using Sephadex LH-20 gel filtration (GE Healthcare Bio-Sciences Corp, Piscataway, NJ; Strumeyer and Malin, 1975) then quantified by the Protein-Precipitable Phenolic (PPP) method (Hagerman and Butler, 1978) which uses iron phenolate to detect tannins by UV Spectrophotometer (Beckman Coulter Inc., Model DU730, Fullerton, CA).

Condensed tannins were purified using Sephadex LH-20 for subsequent use as a standard from CG extract according to Naumann et al. (2013). The aqueous portion containing the CT was retained. The extract, along with enough 1:1 (v/v) methanol:water to form a slurry, were mixed with Sephadex LH-20, and the slurry was repeatedly washed with 1:1 methanol:water until cast off was near clear. Condensed tannins bound to the Sephadex were released by washing with 7:3 (v/v) acetone:water, followed by evaporation of residual acetone by air stream/vacuum. The aqueous phase containing CT was frozen at -80˚C and lyophilized (Strumeyer

and Malin, 1975; Cooper et al., 2014).

To determine PPP, 50 µl of supernatant from CG extracts were combined with 250 µl buffer A (0.20 M acetic acid, 0.17 sodium chloride, pH 4.9), 50 µl bovine serum albumin, and 50 µl 1:1 (v/v) methanol:water and incubated at room temperature for 30 min prior to centrifuging for 5 min. Supernatant was removed by vacuum aspiration and the protein-phenolic pellet was washed with 250 µl buffer A before re-centrifuging and aspirating. The protein-phenolic pellet was dissolved in 800 µl of SDS/TEA (sodium dodecyl sulfate [1% w/v]-triethanolamine [5% v/v] before adding 200µl FeCl3 (0.01 M FeCl3 in 0.01 M HCl). Absorbance was read at 510 nm after 30 min and quantified via external standards (Hagerman and Butler, 1978).

The concentration of protein bound by CT (PBCT) was determined as describe by Naumann et al. (2014). The procedure was carried out as described above, but the protein-phenolic pellet was analyzed for Nitrogen (N) to quantify precipitated protein. Rather than dissolving the protein-phenolic pellet in SDS/TEA, the pellet was dissolved in 500 µl of buffer A, and the solution was transferred into foil cups and allowed to dry. A Elementar Vario Macro Cube C-N Analyzer (Donaustraße 7, Hanau, Germany) was used to analyze the dried protein-phenolic residue for percent N, which was multiplied by 6.25 to calculate the amount of PBCT. To determine total phenolics (TP), 50 µl of supernatant from the crude plant extract was combined with 850 µl of SDS/TEA before adding 200 µl of FeCl3. Absorbance at 510 nm was read after 30 min and quantified via external standards as described for the PPP assay.

The procyanidin:prodelphinidin ratio of CT from CG extract was measured by High Performance Liquid Chromatography (Li et al., 2010) using a Thermo Fisher Dionex Ultimate 3000 UHPLC (Thermo Scientific, Indianapolis, IN).

Feedstuff analysis

Carbon (C), N, and crude protein (CP) were analyzed for pastures, by a C/N analyzer (Elementar Vario Macro Cube; Donaustraße 7, Hanau, Germany). Chambourcin grape extract and corn-soybean meal creep feed were analyzed for CP by the same method. Additionally, neutral detergent fiber (NDF), acid detergent fiber (ADF), and dry matter (DM) were determined on grab samples, at a 2.54 cm stubble height, taken from pastures pre-, mid-, and post grazing of the trial. Samples were freeze dried with a FreezeZone12 (Labconco Corp., Kansas City, MO), ground to pass through a 1 mm screen using a Wiley Mill (Arthur H. Thomas, PA, USA), and analyzed using the Van Soest (1991) method without α-amylase, using an ANKOM200 Fiber Analyzer (ANKOM Technology, Macedon, NY).

Parasitological procedure and measures

During the 63-d trial, individual fecal samples were taken from the rectum of each animal every 7-d. Fecal egg count was determined within 24 hr by the modified McMaster procedure (Whitlock, 1948; Mines, 1977) and quantified by using 2 g subsamples of fresh feces from each kid. Oocytes were counted under a microscope, but not identified by species. Every 7-d, individual blood samples were taken by jugular venipuncture into hematocrit tubes and PCV was determined using a HemataSTAT II Centrifuge (Separation Technology, Inc., Sanford, FL) within 6 hr of blood collection. Additionally, FAMACHA© scores (Hepworth et al., 2006) and BCS (Russell, 1991) were taken every 7-d.

Analysis of complete blood cell counts

Blood samples for complete blood cell (CBC) counts were taken by jugular venipuncture every 14-d into BD Vacutaine K3 EDTA 12-mg blood collection tubes (Fisher Scientific, Pittsburgh, PA). Samples were shipped to University of Arkansas in cold storage to maintain sample integrity, and CBC counts were analyzed by an Abbott Cell-Dyn 3700SL Automate Hematology Analyzer (GMI Inc., Ramsey, MN) within 24 hr of collection.

Statistical analyses

Data were analyzed using PROC MIXED procedure of SAS 9.3 (SAS Inst. Inc., Cary, NC). Animal was considered the experimental unit. Treatment means are reported as least squares means with the contrast statements of the mean of control versus D7 and D14 and the mean of D7 versus D14. Differences were considered significant at $P \leq 0.05$.

Research results and discussion:

Results

Nutrition and tannins

Pasture averages for all sample dates included: CP = 11.9 %; NDF = 65.6 %; ADF = 35.3 %; DM = 95%. Corn-soybean meal based creep feed analysis was: CP = 14%; NDF = 38.4%; ADF = 23.4%; DM = 98%. Chambourcin grape extract was found to have a concentration of 0.33 mg/mL of CT. Crude protein was 1.7 mg/mL by sample. The concentration of PBCT was determined and found to bind 3.5 mg/mL of protein with a 7.01% binding capability. The level of combined procyanidins and prodelphinidin was 0.0007 mg/mL with 12.5% Galloylated tannin.

Kid performance

Start BW, end BW, ADG, and gain did not differ ($P \geq 0.55$) across treatments. Start, end, and change from start to end of the study BCS averaged 2.9 and did not differ ($P \geq 0.12$) across treatments.

Parasite measurements

Natural GIN infection was apparent in all kids with an average FEC of 12.6 ± 6.47 eggs per g of feces. Three kids were removed from D7, three kids were removed from D14, and one kid was removed from C, because they met 3 of 4 health

threshold criteria. End FAMACHA© scores were higher ($P = 0.02$) for D7 and D14 as compared with control; however, start, end, and change from start to end FEC and PCV did not differ ($P \geq 0.12$) across treatments.

Kid immune response

White blood cell (WBC) count increased ($P = 0.04$) at end of study in C compared with D7 and D14, whereas end WBC tended ($P = 0.08$) to increase for D7 compared with D14. Start neutrophils (NEU) tended ($P = 0.08$) and start to end of study NEU were higher ($P = 0.05$) in C compared with D7 and D14. An increase ($P = 0.04$) was found in end basophil (BASO) concentrations from D7 compared with D14. Hemoglobin (HGB) and mean corpuscular hemoglobin concentrations (MCHC%) tended to be higher ($P = 0.07$ and $P = 0.06$, respectively) in C compared with D7 and D14. An increase ($P = 0.04$) was found in both end and change from start to end of study mean corpuscular hemoglobin (MCH) in C compared with D7 and D14.

Platelets (PLT) change from start to end of study was greater ($P = 0.02$) in C compared with D7 and D14. Other blood parameter counts were similar ($P \geq 0.10$) across treatments.

Discussion

The principal purpose of chemical anthelmintics is to achieve a >90% reduction of adult and larval parasites in the host animal (Ketzis, 2006). It is that small percentage that persist that creates the opportunity for resistant parasitic infections to occur (Ketzis, 2006). Evidence has suggested that differences in ruminant species and a lack of direct information about goats has led to dramatic errors in efficacy of GIN control (Hoste et al., 2010). It has been shown that goats metabolize anthelmintics faster than other ruminants (Hoste et al., 2010). Consequently, treating goats at the recommended sheep dosage rate has resulted in anthelmintic under-dosing, thus causing a reduced efficacy (Hoste et al., 2010). This could explain the prevalence of anthelmintic resistance and increased resistant GIN, in goats (Hoste et al., 2010). Subsequently, the purpose of natural anthelmintics involves a different approach towards the control of GIN in ruminants. Natural control methods do not always have a direct effect on the parasite, but instead use the animal's own ability to recover and assist in maintaining parasite infections below the economic threshold of the physical capabilities of the animal (Ketzis, 2006). This not only relates to the effectiveness of the control method used, but also to the epidemiology of the parasites, animal management program, ease of integration as a sustainable program, and climate in which production occurs (Ketzis, 2006). In the least, the precise mechanism by which CT acts as a natural anthelmintic needs to be better understood and a concerted effort on isolation, development, and validation of the effects needs to be undertaken before they are more widely accepted (Githiori, 2006).

In the current study, no change in performance in treated kids was apparent, which suggests there were no added benefits of CT in kids creep fed a 14% CP supplement, *ad libitum*, in addition to grazing fescue and mixed browse. The widely accepted explanation for positive effects of CT on protein digestion and metabolism is that CT-protein complexes escape ruminal degradation resulting in greater protein availability in the abomasum (Reed, 1995). In kids on a high plane of nutrition, additional protein-bound CT may not result in improved production and parasite control, because protein needs are already being met (Waghorn, 2008). Madibela and Jansen (2003) fed a diet containing Mistletoe, *Viscum verrucosum*, that supplied 8.9 g/d of CT. Similar to the current study, Mistletoe did not influence average daily gain in control goats as compared with treatment goats (Madibela and Jansen, 2003).

High CT content (Mattivi et al., 2008; Yang et al., 2009) and world-wide availability, make red grape products a potential source of natural anthelmintics (Kammerer et al., 2004). *In vitro* research conducted by LeShure (2014), revealed grape pomace extract resulted in 100% inhibition of egg hatching into third stage larvae. It was suggested that grape pomace had efficacy in decreasing hatchability of helminth eggs, as well as decreasing parasite viability in an *in vitro* setting (LeShure, 2014). However, the CG extract used in this study had a CT concentration of 0.33 mg/mL, but did not demonstrated a natural bioactive anthelmintic effect in pasture-grazed, creep-fed goat kids. Three experiments conducted by Whitley et al. (2009) to determine the influence of high CT grain sorghum on parasites suggested there was no influence of diet on PCV or FEC. The authors concluded that, high CT grain sorghum did not suppress GIN in goats (Whitley et al., 2009). Research by Paolini et al. (2005) used Quebracho, *Schinopsis* spp., extract and sainfoin hay, *Onobrychis*, at

a rate of 50% CT at 5% of DM diet and 3.2% CT, respectively. When compare with control animals, worm counts decreased, but differences where not significant. Furthermore, no differences were found in physiological measurements between the three groups (Paolini et al., 2005). In agreement with previously mentioned research, results from the current study exhibited similar response in FEC or PCV and end FAMACHA© scores. In contrast, Shaik et al. (2006) examined effects of sericea lespedeza (SL), *Lespedeza cuneata*, hay on FEC, PCV, morbidity of adult *Haemonchus contortus* (HC) worms, and larvae. On a diet with a total CT concentration of 22.4% on a DM basis, they found that FEC decreased starting at wk one and continued to decrease for the duration of the study. Also, PCV, number of larvae recovered, and HC recovered from fecal cultures were improved (Shaik et al., 2006). In a study by Mueller-Harvey (2006), grazing of SL forage (50 g CT/kg) achieved high reductions (57-100%) in FEC, total fecal egg output, and numbers of parasitic nematodes HC, *Teladorsagia* spp., and *Trichostrongylus* in goats. Further exploration is needed to determine the anthelmintic properties and biological processes by which CT influences response of the host to the nematode.

Most ruminants are grazers, in contrast, goats are browsers which in theory limits contact with infective stages of GIN (Hoste et al., 2010). Goat feeding behavior has evolved to browse on a high diversity of plants. This behavior might be involved in the regulation of parasite populations by a combination of self-medication with plant secondary metabolites and avoidance of GIN (Hoste et al., 2010). Goats have developed physiological adaptations to, and dependencies on plant CT, which have carved a browse niche that seeks out CT-containing plants (Muir, 2011).

Goats have a higher tolerance than most ruminants to high levels of CT, which are astringent or bitter and reduce palatability (Lamy et al., 2009). This difference could be the result of the existence of tannin-binding proteins in goat saliva (Lamy et al., 2009). For the majority of forage plants with moderate levels of CT, palatability to goats appears to be independent of CT presence and concentration, due to excretion of proline-rich proteins (PRP), in goat saliva (Lamy et al., 2009). Proline-rich proteins have been the most studied salivary proteins with defense functions against the potential harmful effects of tannins. Saliva of species which ingest high levels of tannins in their regular diet have been reported to have higher levels of PRP (Lamy et al., 2009). These salivary proteins are very reactive with CT and bind them as goats ingest forage CT. This may improve palatability of plants with moderate concentrations of soluble CT but, may also negatively affect the ability of CT to bind with proteins in the ruminant environment (Muir, 2011). Some forage CT may interfere with intestinal absorption of amino acids, even in a low pH environment where CT-protein bonds should be broken (Waghorn, 2008). This may be specific to goats only and could further explain the absence of a by-pass protein effect in this study.

Laboratory examination of the ruminant CBC can be an important addition to the physical examination (Jones and Allison, 2007). Consulting a CBC can often show an immune response to infection or virus before symptoms are presented in the animal. Research conducted by Hoste et al. (2008) illustrated that acquisition and expression of immune responses against GIN species are less efficient in goats and a fully expressed immune response appears delayed in goats as compared to other ruminants (Hoste et al., 2008). In this research, some changes were found in CBC results, including significantly increased WBC count at end of study from C compared with D7 and D14, whereas, a tendency for increase was found for D7 compared with D14. Increases in NEU can indicate stress related health responses (Jones and Allison, 2007). In the current study, start NEU tended to be higher and, a significant change was found in NEU from start to end of study in C compared with

D7 and D14. Increases were found in BASO concentrations from D7 compared with D14, which could indicate an allergic response or inflammation (Jones and Allison, 2007). An increase was found for HGB and MCHC% from C compared with D7 and D14, which could indicate an absence of anemia (Jones and Allison, 2007). An increase was found in both end and change from start to end of study in MCH in C compared with D7 and D14, again demonstrating no anemia (Jones and Allison, 2007). Positive changes in blood parameters involving iron, HGB and MCHC%, could be related to increased antioxidant properties of CT constituents (King and Young, 1999). A significant increase from start to end of study was found in PLT in C compared with D7 and D14. An increase in PLT may indicate infection or anemia (Jones and Allison, 2007).

Participation Summary

Educational & Outreach Activities

PARTICIPATION SUMMARY:

Education/outreach description:

This research paper will be submitted to the Sheep and Goat Research Journal in the near future. In addition, a poster presentation was submitted at the Journal of Animal Science Joint Meeting in Orlando, FL, July 2015.

JAM poster_2015

Project Outcomes

Project outcomes:

Fermented Chambourcin grape extract may not be an effective natural anthelmintic for controlling nematodes in creep-fed goat kids. Continued research is needed to understand why grape extract may be an effective natural anthelmintic in some ruminant animals, but not in the creep-fed goat kids in this study.

Economic Analysis

At this current time the economic benefits are not able to realized due to lack of reliable results. The availability of vineyards with castoff grape products and the ease of treatment for goats, as an oral drench, makes the use of CT in grape products as a natural anthelmintic, a more attainable goal . The use of natural anthelmintics is a more sustainable practice that should reduce the level of resistant parasitic infections. The information needed now is the required CT concentration level, to see an impact on GIN. Once this is established the economic impact can be assessed.

Farmer Adoption

We were unable to work with local farmers on this grant. We had difficulty obtaining producers working with goats. Due to factors of timing, lack of availability of animals, and equipment issues. Original grant submitted included an MOU with a local goat producer but he sold all of his animals before we were able to finish the research.

In replacement of field research the goal was to conduct an *in vitro* project on fermented grape extract on efficacy of GIN. We were unable to complete the study at this time because nematode levels were low enough we could not produce enough larvae to introduce to the grape extract. The plan is to continue this part of the project after the grant is complete because there are sufficient materials to continue without additional cost. This will become an undergraduate research project that can be used as a presentation in the future.

Recommendations:

Areas needing additional study

Impact of tannins on herbivory has been difficult to assess because of diversity in tannin chemistry and in animal physiology. Diversity of effects of tannins on digestion is due in part to differences in physiological capabilities of the animal to handle tannins and in part to differences in chemical reactivity of various types of tannins (Hagerman et al., 1992). Multiple factors may explain the conflicting results of feeding forages with similar concentrations of CT to ruminants, including differences in chemistry of CT from different plants, and laboratory methods used to determine CT concentrations.

Vast structural diversity of tannins complicates the study of parasitological aspects and leads to confusion in understanding the literature. It has been loosely interpreted that different forms of tannins may affect the usefulness of the compound and may directly affect the reduction of parasites (Whitley et al., 2009). Concentration and structure of CT present in different plant species seems to be the major factors modulating efficacy against GIN parasitism (Oksana et al., 2012).

Additional research needs to be completed to understand which function of CT is most important and the correct dosage to see reliable results in small ruminants.