Title

Assessment of a multimodal herbal feed additive on reducing trichostrongyle burden in an alpaca fiber operation

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Abstract

With ever-growing drug resistance and significant global prevalence, gastrointestinal nematodes are an increasingly common cause of death on small ruminant and camelid operations, threatening the productivity and economic health of those industries. An alternative to traditional anthelmintics is a combination of botanical ingredients that address the multifaceted parasite infection. The objective of this trial was to evaluate a novel compound of whole herbs, including constituents previously shown to be successful in nematode control,,asa viable alternative to traditional anthelmintics. Thirty-six Huacaya alpacas of mixed genders and reproductive statuses were evaluated on the basis of reproductive stress, body condition score, FAMACHA score, and naturally occurring eggs per gram on fecal testing without any intervention. Supplement was top dressed to the daily feed delivery in the form of a coarse powder rendered from 20 whole herbs for a total dose of 500-2000 mg over a 14 d period. This treatment was repeated a total of three times, with two weeks off between each treatment period. Alpacas were continuously evaluated for body condition score, FAMACHA score, quantitative fecal testing, and changes in reproductive stress. The herd experienced an overall decrease in FAMACHA score from 3.01 to 2.37 ($P < 0.0001$) and a decrease in mean fecal egg count from 111 to 25 ($P < 0.001$). Overall, the percent of alpacas that had a positive fecal egg count decreased from 39% to 3% ($P < 0.001$).The herbal blend successfully prevented further regeneration of the existing parasitic populations, thereby eliminating the need for traditional anthelmintics during that time period and bolstering the overall sustainability of the fiber operation.

Introduction

Drug resistance following anthelmintic treatments in ruminants leaves producers with few effective options to protect their herds against parasites, which can incur economic and productivity losses. Small ruminant and camelid industries are in need of a sustainable alternative to anthelmintic usage, one that aids in both healthier pastures and leads to significant production improvements without threat of resistance. An alternative method that prevents a herd-wide rise in gastrointestinal nematodes would allow small ruminant and camelid operations to use anthelmintics less frequently, thereby delaying parasite resistance.

Gastrointestinal nematodes (GIN), such as the trichostrongyle: *Haemonchus contortus*, commonly known as the barber pole worm, are the most prevalent threat to small ruminant and camelid operations throughout the world. In the Mid-Atlantic region of the United States, a 2016 study detected *Haemonchus contortus* in 79% of the participating sheep and goat farms (Crook, 2016). Additionally, the National Animal Health Monitoring System named internal parasites the leading cause of non-predatory goat losses in the United States (Zajac, 2020). Increased parasite populations are typically the result of casual overuse of commercial dewormers, ensuring that the organisms become drug resistant. As climate change affects global temperatures, these worms are expected to become more prevalent and resistance to dewormers is likely to expand (Kaplan, 2017).

Currently in the United States, there are six commercially available deworming products representing three drug classes. Trichostrongyles have been reported to be resistant to all three classes of dewormers (Kotze, 2016). In the Mid-Atlantic, Crook (2016) reported a 100% resistance of *Haemonchus* to benzimidazoles, an 82% resistance to ivermectin, a 24% resistance to levamisole, and a 47% resistance to moxidectin (Crook, 2016). However, anthelmintics are not the only available method to control parasite populations..

The current strategy to manage GINs is to maintain the naivety of the worm population to drugs, which prevents their ability to mutate and build resistance. This is referred to as the preservation of “refugia”, and is the prevailing recommendation of the American Consortium for Small Ruminant Parasite Control (ACSRPC, 2020). Related practices include appropriate stocking density, pasture rotation, quarantining new animals, fecal testing surveillance, FAMACHA scoring, appropriate nutritional practices, and body condition scoring. However, in regions where acreage comes at a premium, practicing appropriate stocking density and pasture rotation can present significant barriers to appropriate pasture use.

Anecdotal descriptions of botanical anthelmintics and antidiarrheals for afflicted livestock species can be found across the globe (Gray, 2004). Tannins are frequently discussed throughout caprine and camelid communities, and tannin supplementation has resulted in reduced fecal egg counts and a reduction in fecundity (Paolini, 2003). Similar studies have explored the efficacy of various plants and potential mechanisms of action. Thyme, for example, was reported to have anthelmintic activity equivalent to thiabendazole and levamisole with respect to *H. contortus* (Ferreira, 2016). Veterinary parasitologists have been able to build a scientific foundation for practical use of various herbs by elucidating chemical structures of compounds and their pharmacokinetics (Mengistu, 2017).

~~Available commercially, a well-known herbal deworming agent consisting of a single plant tincture, failed to control GINs in goats, and in some test populations, resulted in an increase of fecal egg counts (Burke, 2009).~~ Parasite infestations are multifactorial, and involve poor absorption of nutrients, poor gut motility, diarrhea, abdominal inflammation, poor red blood cell regeneration, and many other comorbidities. It is reasonable to believe that products that utilize only one ingredient fail to be efficacious because it is not able to address all the factors involved in chronic parasitism. An appropriate product will not simply have only anthelmintic properties, it will also apply itself to the various comorbidities of this parasite and prevent the parasite from reestablishing. A similar strategy was employed during a field trial of neonatal lamb diarrhea, with the treatment combining five medicinal plants. Results were favorable, with the treated group experiencing a shorter duration of clinical illness, a higher cure rate, lower mortality, and higher average body weight upon recovery (Shengkun, 2015).

The comprehensive blend used in this trial combines plants with anthelmintic, anti-diarrheal, appetite stimulating, anti-inflammatory, mucosal-protectant, red blood cell stimulating, and promotility constituents. It is prepared as dried, whole herbs in order to maintain the complex branching cellular structure similar to the fibrous roughage of their natural diet. Administering a supplement in this medium will ensure that the active constituents of these plants are used to their fullest bioavailability, in the unique environment of the ruminant’s gastrointestinal physiology. An herbal antiparasitic diminishes drug safety concerns with respect to pregnancy, reproductive risks, and meat or milk withdrawal. Thus, this trial aimed to evaluate the efficacy of a new herbal supplement, containing 20 whole, dried herbs, in improving overall GINegg counts,fecal quality, body condition score, and FAMACHA score of alpacas experiencing different levels of reproductive stress.

Methods

Thirty six Huacaya alpacas (26 adult females, 9 intact males, and 1 gelding) were used for supplement evaluation. Alpacas were obtained from a hobby operation in Waretown, NJ.The ten males were housed together on 0.75 acre dry lot soil pen containing mature pine trees. emales were housed in a 1.1 acre dry lot soil pen containing mature pine trees. The two areas were separated by 0.25 acre and several visual barriers, including a tree line and barns. At the beginning of the trial, two females were lactating with cria at their side, two females were pregnant and lactating with cria at their side, and seven females were pregnant and not lactating. Nursing cria (n = 5) were not included in this trial. All animals had *ad libitum* access to second cutting orchard grass hay, ¼ lb of Nutrena grain, and 1 tsp Stillwater Minerals.

Throughout the trial, alpacas were evaluated for FAMACHAⓒ scores, body conditions scores (**BCS**), fecal quality scores, fecal egg counts (**FEC**), and reproductive stress (**RDS**). FAMACHA scores were used using the 1-5 scale by evaluating the color of the ocular membranes as an indicator of anemia. Lower FAMACHA scores are indicative of a lower parasite load, whereas higher FAMACHA scores indicate a parasitic infection. Body condition scores were measured using a 1-5 scale where 1=emaciated, 3=optimal, and 5=obese (Van Saun, 2013). Reproductive stress was defined as alpacas in maintenance (MNT), nursing a single or pair of crias (NRS), pregnant (PR), or nursing while pregnant (NRSPR).

Fecal egg counts were obtained using the Modified McMaster fecal egg counting technique, which is described as follows. Four grams were taken from each individual's sample, weighed using a digital scale rated for accuracy to 0.001 gram, and placed into a plastic cup. Phoenix's sodium nitrate fecal float solution, which represents a specific gravity between 1.25 and 1.30, was added to each representative sample in 28mL aliquots. Each sample was allowed to sit undisturbed in 28mL of solution for three minutes. Fecal pellets were then digitally agitated to form a more homogenous mixture, then the solution sat undisturbed for an additional three minutes. Two 4x4" 12-ply woven gauze sponges were laid across an additional cup, and the solution was poured over top of the gauze and into the new cup to achieve straining. The gauze and the material strained by the gauze were discarded. A pipette was used to transfer the solution into both chambers of the McMaster slide. Slides sat on a level surface undisturbed for ten minutes and then were promptly read. Eggs within grid lines were counted in both chambers, both totals were added together, and the sum was multiplied by 25 to achieve a figure of "eggs per gram" for each species of egg identified. Results for each individual were recorded alongside the individual's identification, body condition score, FAMACHA score, and production notes.

On day 0, all alpacas received an identifying colored collar and an individual number for the duration of the trial. At that time, each animal received a physical exam to ensure wellness prior to entering the study;no signs of infectious disease and no abnormalities were detected at that time. individual, RDS, and FAMACHA scores were recorded by one trained observer. Fecal samples were collected per rectum from each individual, stored in individual plastic cups with lids, refrigerated within 30 minutes of collection, and stored for 12-24 h prior to testing.

Table 3.1: Schedule of Treatment Cycles and Fecal Egg Counts

Day	Data Collected	Treatment or No Treatment
0	FEC, BCS, FAMACHA, Prod	No Treatment
14	FEC, BCS, FAMACHA, Prod	No Treatment
28	FEC, BCS, FAMACHA, Prod	No Treatment
42	FEC, BCS, FAMACHA, Prod	Immediately after 14 days of first treatment cycle
56	FEC, BCS, FAMACHA, Prod	Two weeks after 14 days of first treatment cycle
70	FEC, BCS, FAMACHA, Prod	Immediately after 14 days of second treatment cycle
84	FEC, BCS, FAMACHA, Prod	Two weeks after 14 days of second treatment cycle
98	FEC, BCS, FAMACHA, Prod	Immediately after 14 days of third treatment cycle
112	FEC, BCS, FAMACHA, Prod	Two weeks after 14 days of third treatment cycle

Body weights, BCS, FAMACHA score, and FEC were collected on d 0, 14 and 28. After sample collection on d 28, alpacas began receiving treatment, described as the treatment period. Treatment period is defined as a two-stage addition of the supplement, with each stage lasting 7 days, resulting in a 14 d treatment period. The supplement consisted of blended whole herbs that were rendered into a coarse powder. The first phase consisted of Areca catechu, Prunus mume, Atractylodes lancea, Codonopsis pilosula, Fructus quisqualis, Zingiber officinale, Torreya gandis, Raphanus sativus L., and Omphalia lapidenscens, achieving a total of 2.0g of supplement administered to each alpaca as a feed additive. The second phase consisted of Avena sativa, Astragalus membranicus, Curcurbita pepo L., Withania somnifera, Althea officinalis, Urtica dioica L., Medicago sativa, Centella asiatica, Foeniculum vulgare, Silybum marianum, and Calendula officinalis acheiving a total of 2.0g of supplement administered to each alpaca as a feed additive. The authors refer to the resulting product as Early Bird.

Table 3.2: Whole herbs included in the treatment product

Table 3.2.1 - First Phase				
Chinese Name	**Taxonomic Class**	**Common Descriptors**	**Mechanism of Action**	**Scientific Reference(s)**
Lei Wan	Omphalia lapidenscens	fungus	anthelmintic	Zhang, Song, Chen
Da Fu Zi	Areca catechu	palm seed	anthelmintic	Tangalin, Zhang, Li
Fei Zi	Torreya gandis	Conifer nut	anthelmintic	Li
Wu Mei	Prunus/Fructus mume	Flowering plum	astringent	Li
Shi Jun Zi	Fructus quisqualis/ Quisqualis indicum L./ Combretum indicum	Flowering vine	expels parasites	
Dan Shen	Codonopsis pilosula	Flowering vine	Immunomostimulatory: heightens activity of macrophages, promotes RBC generation, increases blood flow and oxygen consumption of small intestine	Sun, Gao

Bai Zhu	Atractylodes lancea (A. macrocephalia)	rhizome	anti-inflammatory, carminative, prokinetic	Li, Han
Gan Jiang	Zingiber officinale	ginger root	invitro anthelemnic activity	Qadir
Lai Fu Zi	Raphanus sativus L.	radish	anti-inflammatory, prokinetic	Sham, Choi, Ghayur
Total Dose per alpaca	**2.0 grams (2000mg)**			

Table 3.2.2 - Second Phase				
Chinese Name	**Taxonomic Name**	**Common Descriptors**	**Mechanism of Action**	**Scientific Reference(s)**
Yan Mai	Avena Sativa	Oatstraw	Prebiotic, antiparasitic	Sargautiene, Doligalska, Liu
Nan Gua Zi	Curcurbita pepo L.	Pumpkin seed	Anthelmintic, antioxidant	Zhang, Li, Paul
Huang Qi	Astragalus membranicus	Flowering plant	Immunomodulatory, anti-oxidant, anti-inflammatory	Auyeung
Nan Fei Zui Jia	Withania somnifera/ Ashwagandha	Cherry root	Adaptogenic, anti-inflammatory, immunomodulatory, hematopoetic	Bhattacharya, Chandrasekhar, Santhi, Sharma, Mishra
Yao Shu Qui	Althea officinalis	Marshmallow root	Biofilm dissolution, gastrointestinal antiinflammatory	Varadyova, Aminnezhad
Xun Ma	Urtica dioica L.	Nettle leaf	Immunomodulatory, monocyte chemoattractant protein-1 expression, oncogene release from intestinal epithelium, MyD88/NF-κB/p38 signaling	Gülcin, Hajhashemi, Namazi
Zi Mu	Medicago sativa	Alfalfa	Mineral rich, gastrointestinal anti-inflammatory, hematopoetic	Bora, Huyghe, Vyas
Gotu Kola	Centella asiatica	Pennywort	Intestinal mucosal protectant, antiinflammatory	Gohil, Jana, Paocharoen, Sainath, Ullah, Wanasuntronwong
Xiao Hui Xiang	Foeniculum vulgare	Fennel seed	Anti-inflammatory, antispasmodic, galactogogue, carminative	Varadyova, Choi, Bensch
Shui Fei Ji	Silybum marianum	Milk Thistle seed	Antifibrotic, antioxidant, anti-inflammatory, hematopoetic	Abenavoli, Kalatari
Jin Zhan Ju	Calendula officinalis	Flowering plant	Intestinal mucosal protectant, anti-inflammatory, gastroprotectant, antispasmodic	Al-Snafi, Bashir, Cwikla, Mehrabani, Ukiya
Total Dose per Alpaca	**2.0 grams (2000mg)**			

The first phase was administered at a dose of 500 mg/alpaca once a day for two days, then increased to 1000 mg/alpaca for two days, then increased to 2000 mg /alpaca for three days. This was achieved by sprinkling the supplement on top of each animal's daily grain allowance. The second phase was administered at a dose of 1000 mg /alpaca once a day for three days, then increased to 2000 mg/alpaca once a day for four days. The first treatment period occurred from d 28 to 42. On d 43, addition of the supplement was discontinued, and each animal was examined, body condition scored, FAMACHA scored, and fecal samples were collected. No treatment was given from Day 43 to Day 56. On d 56, each animal was examined, body condition scored for body condition, and FAMACHA scored, and fecal samples were collected.

Table 3.3: Dosing Schedule

Day of Treatment	First Phase	Second Phase
1	500mg/head/day	-
2	500mg/head/day	-
3	1000mg/head/day	-
4	1000mg/head/day	-
5	2000mg/head/day	-
6	2000mg/head/day	-
7	2000mg/head/day	-
8	-	1000mg/head/day
9	-	1000mg/head/day
10	-	1000mg/head/day
11	-	1000mg/head/day
12	-	2000mg/head/day
13	-	2000mg/head/day
14	-	2000mg/head/day

On d 57, alpacas began another two week treatment period from d 57 until d 70. Alpacas were dosed as described above, with seven days of phase 1 followed immediately by seven days of phase 2. On d 71, each animal was examined, body condition scored, FAMACHA scored, and fecal samples were collected. From d 1 until d 84, no supplement was administered. On d 84, each animal was examined, body condition scored, FAMACHA scored, and fecal samples were collected. On d 85, alpacas began a third treatment period lasting from d 85 to 98, dosed as described above. On Day 98, supplement was discontinued, each animal was examined, body condition scored, FAMACHA scored, and fecal samples were collected No supplement was given from d 98 to 112. On d 112, each animal was examined, body condition scored, FAMACHA scored, and fecal samples were collected.

Table 3.4: Changes in Production Status During Study

Day	Adult Males (10)	Adult Females (26)
0	3 breeding studs	2 lactating, 2 lactating while pregnant, 7 pregnant
14	No Change	2 lactating, 2 lactating while pregnant, 7 pregnant
28	No Change	4 lactating while pregnant, 9 pregnant
42	No Change	4 lactating while pregnant, 9 pregnant

56	No Change	4 lactating while pregnant, 3 lactating, 6 pregnant
70	No Change	4 lactating while pregnant, 3 lactating, 6 pregnant
84	No Change	4 lactating while pregnant, 3 lactating, 6 pregnant
98	No Change	6 lactating while pregnant, 1 lactating, 5 pregnant
112	No Change	6 lactating while pregnant, 1 lactating, 5 pregnant

Results

There was no treatment by RDS interaction for BCS or a main effect of treatment (Table ;$P \geq 0.16$). There was a significant RDS effect, where BCS was lower in NRS females than PR and NRSPR females ($P \leq 0.001$). There was no treatment by RDS interaction for FAMACHA or a main effect of RDS (Table ;$P \geq 0.35$). There was a significant effect of treatment, where FAMACHA decreased with each additional treatment ($P \leq 0.001$).

There was a significant treatment by RDS effect on fecal quality (Figure 1; $P \leq 0.001$). During CTL, fecal quality score was lowest in the MNT alpacas, followed by PR, and then NRS and NRSPR ($P \leq 0.02$). During both TRT1 and TRT3, MNT alpacas continued to have a lower fecal quality score than all other RDS groups ($P \leq 0.05$). During TRT2, there was no difference in fecal quality scores between RDS groups ($P \geq 0.13$).

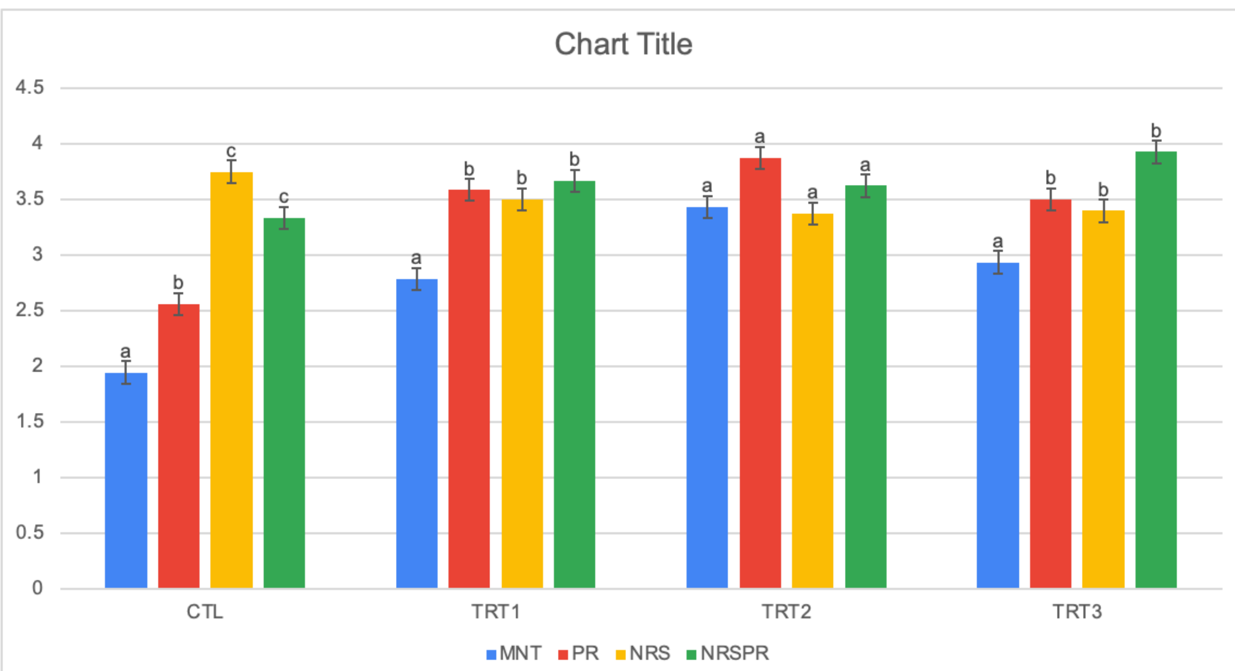
There was a significant decrease in the total percent of alpacas with a positive FEC throughout the course of the trial (Figure 2; $P \leq 0.01$). During CTL when alpacas were not treated, approximately 39% of alpacas had a positive FEC, which significantly declined to less than 3% of animals following the third treatment ($P \leq 0.01$). Of the subjects that had a positive FEC, the most commonly observed species was trichostrongyles. Additional species that were observed were Trichuris, Nematodirus, Moniezia, and Eimeria species.

Because of the significant decline in the percent of alpacas with a positive FEC, total FEC is displayed in two ways (Figure 3). First, the total FEC of all alpacas, including those with a negative test, decreased from CTL to TRT1 and TRT2 ($P \leq 0.01$), but had a slight increase during TRT3. There was no effect of RDS on this measure ($P = 0.24$). Second, while only accounting for the alpacas that tested positive on the FEC, there was no effect of treatment or RDS on FEC (Figure 3; $P \geq 0.80$).

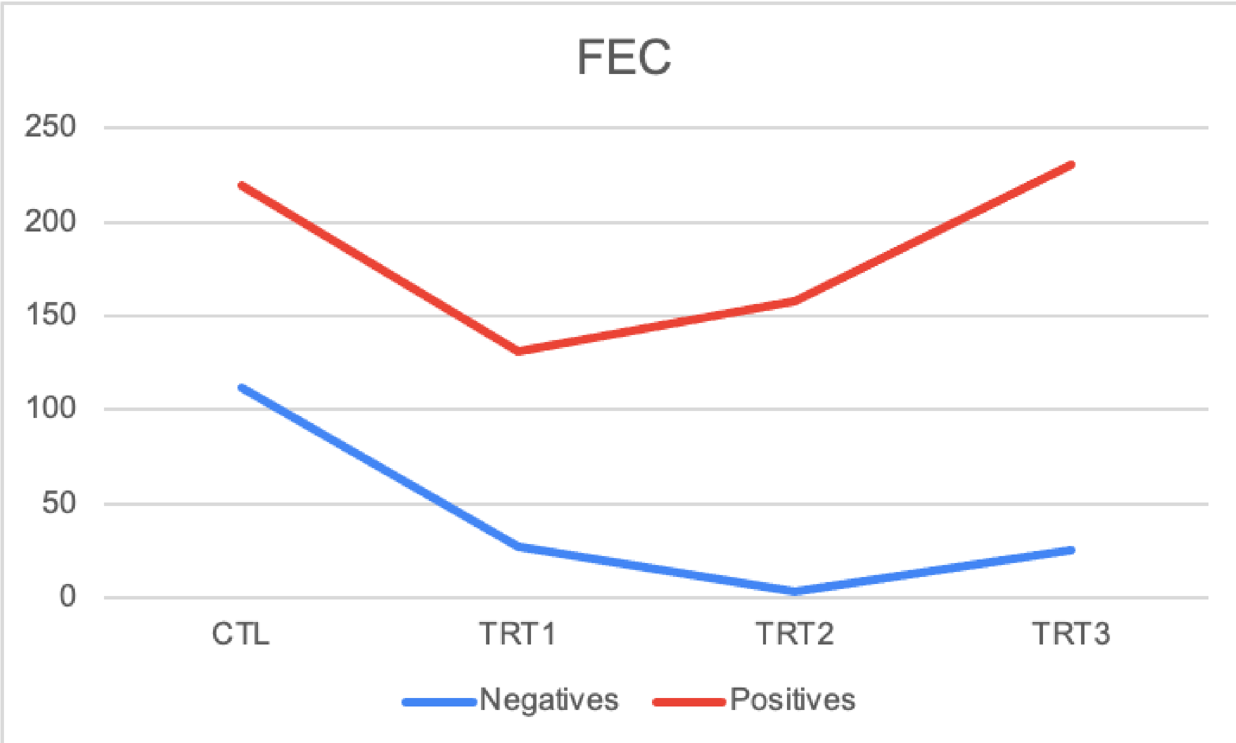
	Collection Time	Mean	SEM	P-value
BCS	**CTL**	3.06	0.092	0.39
	TRT1	3.17	0.105	
	TRT2	3.23	0.099	
	TRT3	3.28	0.099	
FAMACHA	**CTL**	3.01	0.098	<.0001
	TRT1	2.99	0.111	

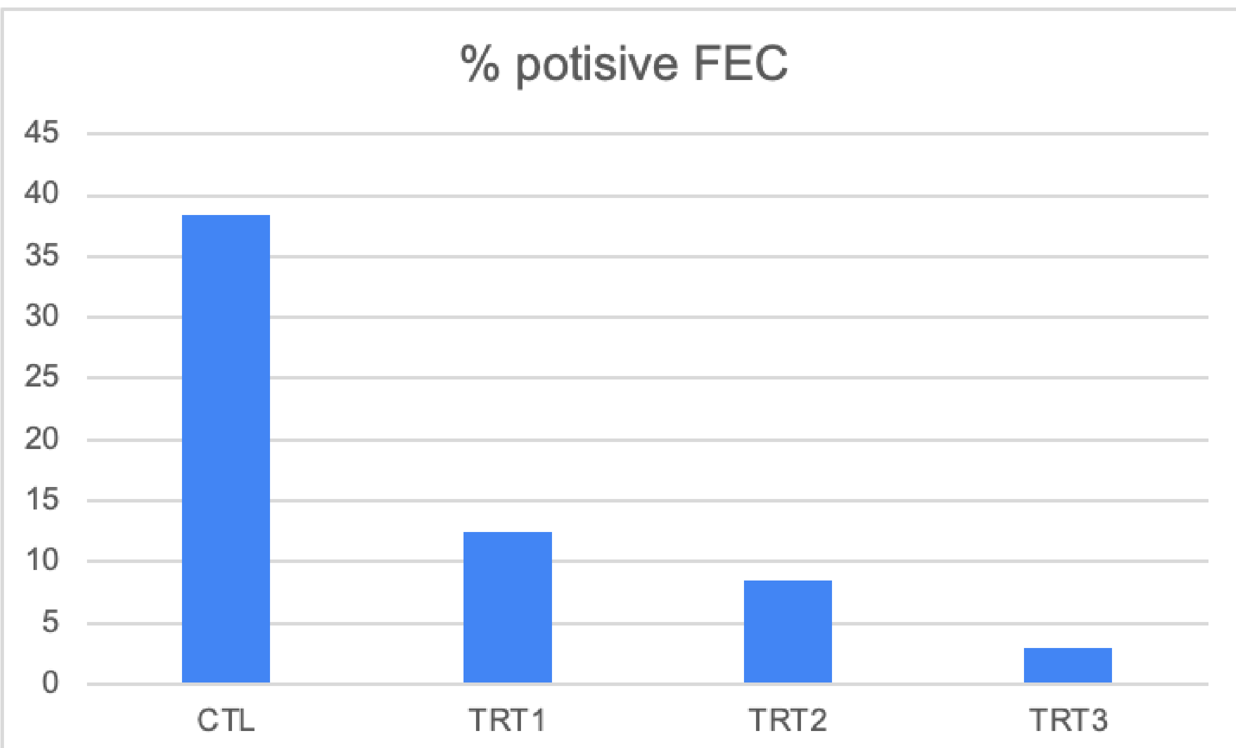
	TRT2	2.80	0.105	
	TRT3	2.37	0.105	

	Production Stage	Mean	SEM	P-value
BCS	**MNT**	3.61	0.042	<.0001
	PR	3.18	0.090	
	NRS	2.77	0.132	
	NRSPR	3.18	0.109	
FAMACHA	**MNT**	2.74	0.045	0.58
	PR	2.84	0.095	
	NRS	2.72	0.140	
	NRSPR	2.88	0.115	



Fecal quality





Discussion

The feed additive was not only successful in preventing further parasitism in an alpaca operation, but it out-peformed expectations in several capacities. With respect to the product's ability to arrest trichostrongyle propagation, the expectation was that there would not be a significant rise in eggs per gram of trichostrongyles. However, in all production categories, eggs per gram of trichostrongyles decreased, with many animals converting to completely negative fecal egg counts. Previously negative animals remained negative.

Since body condition and parasitism are undeniably associated, the maintenance of body condition was a very important parameter for this study. The physical demands on a healthy adult alpaca during conception, gestation, and lactation are expected to manifest as a decrease in body condition. One collection of data from the La Raya Llama and Alpaca Research Station demonstrates that a dam will experience 11-15% loss of body weight during the first three postpartum months. From an additional study, one can expect that a healthy adult dam will lose an average of 9kg from the prepartum period to the postpartum period (Burton 2003). In our experimental herd, half of the females were adults under reproductive stress (see Table 3.4) and one quarter of the females were growing weanlings, leaving only one quarter of the females in a maintenance stage of life. While our expectation for the product was that it would stabilize the herd's body condition, despite significant reproductive demands, body condition increased for all production groups. The ability to decrease trichostrongyle population herd-wide and support healthy body condition during periods of high demand makes this feed additive a strong alternative to traditional dewormers. If a producer can reduce drug usage in instances where they would typically need it, then that producer is delaying drug resistance both on their own farm, and on the farms with which they trade.

The value of an alternative to traditional dewormers cannot be understated given how the topography of livestock is changing. While prudent pasture practices are the keystone of parasite management, there are very real barriers to these strategies. In underserved regions without access to food animal veterinarians, many operations haven't been historically judicious with their drug usage, disarming themselves for the future. In regions where development continues to creep into rural areas, the amount of acreage available for farmers decreases. Delaying the need for traditional dewormers either in the short term or the long term will achieve dividends in terms of overall production gains and pasture health.

Since the priority of this study was to determine if a composite herbal feed additive could be efficacious against a mixed-species GIN population, budgetary concessions were made. Now that this mode of parasite control has performed appreciably, resources for more elevated trials can be expanded. The most notable shortcoming of this study was the usage of the McMaster technique for egg detection without the added diagnostic support of either larval culture, DNA detection, or peanut agglutination tagging. A follow up trial, using the same product, is planning on using a population of sheep who are profoundly afflicted by H. contortus, and face ongoing morbidity and mortality. With this more challenging environment, quantitative flotations will be complemented by both larval culture and peanut agglutination identification. After this trial, further analyses could address corollary questions, such as the possibility of GIN resistance to this feed additive, or establishment of toxic doses.

There are more questions to address, however this study was successful in showing that a multimodal herbal formula has a scientifically sound position in the toolbox of trichostrongyle weapons. Despite considerable reproductive stress, and in the absence of no other medications or dewormers administered, three treatments of the herbal blend increased body condition scores, decreased FAMACHA scores, and decreased trichostrongyle eggs per gram in a way that was statistically significant in comparison to the control.

Conclusion

The goal of this feed additive is to prevent further regeneration of the existing trichostrongyle population in any given operation in spite of significant production demands, thereby delaying or eliminating the operation's need for traditional anthelmintics during peak reproductive stress.The feed additive utilized in this trial, in conjunction with appropriate management practices, increased the overall sustainability of the herd, decreased clinical illness and production losses, eased economic strain on the operation, and aided in the delay of anthelmintic resistance. A composite herbal feed additive can be an efficient and effective way to inhibit the replication of trichostrongyles present in an individual or the herd, therefore preventing an increase in fecal egg counts. This results in reduced usage of traditional anthelmintics to "knock down" trichostrongyle populations in an operation. In turn, this increases the overall health of the herd, increasing the meat, fiber, and/or dairy dividends of the operation, as well as decreasing mortality and expenses related to morbidity. Without the promise of any novel anthelmintics, this could be the next logical step towards a solution for this particularly devastating problem.

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