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SUPPLEMENTATION WITH PELLETED STINGING NETTLE IMPROVES HEMATOLOGICAL PARAMETERS, AND REDUCES TOTAL PARASITE LOAD AND GUT *ESCHERICHIA COLI* COUNTS IN PASTURED GOATS

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ABSTRACT

Stinging nettle (*Urtica dioica*), is widely consumed as a vegetable in many parts of the world and also touted for many health benefits. Although the nutritional value has been known and medicinal value purported for years, research findings are scarce on its value to ruminants. This study evaluated the hematological and gut health benefits of supplementing pelleted stinging nettle to pastured yearling goats for twelve weeks. Overall health, hematocrit, total serum protein, and anemia scores using (FAffa MAlan CHArt) FAMACHA© scoring system were evaluated. Total gut parasite load was determined by both fecal egg count and molecularly using a PCR protocol. Total fecal *Escherichia coli*, *Bifidobacterium* and *Lactobacillus* spp were also determined molecularly. Overall, pelleted stinging nettle was highly palatable to goats and no negative health effects were detected in supplemented goats. Nettle supplemented goats exhibited significantly increased hematocrit, FAMACHA© scores, and decreased total parasite load. A significant decrease in total gut *E. coli* in the supplemented goats was also detected. The *Bifidobacterium* and *Lactobacillus* spp counts were very low in all goats irrespective of treatment group. These findings indicate potential of stinging nettle pellets in circumventing the negative health impacts of *Hemonchus contortus* and other gut parasites while reducing the potential gut microbial pathogen loads. Thus stinging nettle seems a good candidate for further research on potential use as a bioactive feed supplement for ruminants.

Keywords: Stinging nettle, supplement, goats, hematocrit, FAMACHA©, *Hemonchus contortus*, parasites, *E. coli*
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INTRODUCTION

Gastrointestinal health in farm animals is critical to animal performance and also the production of safe animal products. Impaired gut health due to gastrointestinal (GIN) parasitism and microbial infections are among the challenges that reduce the economic viability of small ruminant production in the US and worldwide (Torres-Acosta & Hoste, 2008). The blood-sucking parasite *Hemonchus contortus* by far remains the most economically important gut parasite in small ruminants although other GIN parasites cause significant losses too. Small ruminant gastrointestinal tract is also host to some pathogens that include bacteria like *E. coli*, *Salmonella,* and *Campylobacter* spp that may cause diarrhea in animals and also have food safety implications (40). The use of anthelmintic and antibiotics has been the mainstay of treatment and control for parasites and microbial infections respectively for decades. However, the development of resistance to many of the currently used drugs and demand for organically raised animal products has called for research on alternatives and/or complementary strategies to control parasites and gut pathogens (Athanasiadou *et al*., 2007; Terrill, Miller, Burke, Mosjidis, & Kaplan, 2012). In ruminants, forage-based strategies that have the potential to improve performance and improve gut health are advantageous and desirable since they can easily be adapted to on-farm applications and inclusion in feeds (41). They are also in line with the global call for reduced use of chemical and antibiotic-based health control methods and therefore can be beneficial in organic and naturally grown production systems that consumers have currently been keen on. The use of plants with nutritional benefits and potentially bioactive compounds that have anti-parasitic and antimicrobial properties that could control parasites or reduce their negative health impact on animals has gained attention lately. Several studies have shown that plants including *Lespedeza* spp, pine bark (*Pinus* spp), *Trifolium* spp, sulla (*Hedysarum coronarium* L), and Chicory (*Cichorium intybus*) (Athanasiadou *et al*., 2007; Herve Hoste *et al*., 2015; H. Hoste & Torres-Acosta, 2011; Marley, Cook, Barrett, Keatinge, & Lampkin, 2006; Min, Wilson, Solaiman, & Miller, 2015; Peña-Espinoza *et al*., 2018; Terrill *et al*., 2012) have anti-parasitic effects when included in ruminant diets. These plants are thought to control parasites by the presence of high levels of plant secondary metabolites (PSM) mostly of the condensed tannin or sesquiterpene lactone types (Hervé Hoste, Jackson, Athanasiadou, Thamsborg, &

Hoskin, 2006; H. Hoste & Torres-Acosta, 2011). Although their specific mechanisms are still under intensive research, it has been postulated the effects may either result from direct interaction with the parasites or by enhancing the host resiliency (Hervé Hoste *et al*., 2006).

Stinging nettle (*Urtica dioica*) is a plant that is widely known and has been consumed in the western world and many parts of the developing world for its nutritional and purported medicinal effects in people (Upton, 2013; Westfall, 2001; Yarnell, 1998; Zeipiņa, Alsiņa, & Lepse, 2014). It has been described as "food and medicine for a millennia" that is nutrient-dense and contains all the essential amino acids (Rutto, Xu, Ramirez, & Brandt, 2013; Westfall, 2001). Water extracts of stinging nettle were found to have antimicrobial, antiulcer, antioxidant and analgesic effects (Gülçin, Küfrevioğlu, Oktay, & Büyükokuroğlu, 2004) *in vitro*. Other studies have also shown stinging nettle to contain compounds with anti-inflammatory (Francišković *et al*., 2017), anti-hyperlipidemic (Daher, Baroody, & Baroody, 2006), and cell anti-proliferative (Schneider & Rübben, 2004) effects. Other studies have shown that stinging nettle improves immunity, growth, and blood parameters in fish (Adel, Caipang, & Dawood, 2017; Awad & Austin, 2010; De Vico, Guida, & Carella, 2018; Ngugi *et al*., 2015) and blood parameters in pregnant women (Westfall, 2001). The various compounds responsible for the myriad of benefits of stinging nettle have recently been reviewed (Ahmed & Parsuraman, 2014; Said, Otmani, Derfoufi, & Benmoussa, 2015; Yarnell, 1998).

The potential of stinging nettle as a livestock feed has been known for over sixty years. In Britain, stinging nettle as a potential forage for feed was described in 1941 as having the same chemical composition as good quality hay and having the same protein content as Lucerne (*Medicago sativa*) and sanfoin (*Onobrychis viciifolia*) and lower fibre content than good quality hay (Wilson, 1941). Although no research or widespread use of stinging nettle as animal forage followed this report, the potential remains. In recent years, however, the search for alternative crops, feeds, and plants with beneficial potential like stinging nettle have seen renewed interest (Bernhoft, 2010). In fish and farm animal production including poultry, pigs, goats, and cattle, a few studies have been carried out. In poultry, stinging nettle improved egg quality and antibody response in laying hens (Poudel & Khanal, 2011) were observed in Nepal while stinging nettle enhanced the antibody response in indigenous chicken (Bwana *et al*., 2018) and cell-mediated immunity (Şandru *et al*., 2016) in Kenya. In Iran, stinging nettle extracts improved growth, hematocrit, hemoglobin, and also antibody response to Newcastle vaccine in broiler chicken (Hashemi, Soleimanifar, Sharifi, & Vakili, 2018) while serum cholesterol, triglycerides and LDL were reduced in chicken receiving stinging nettle compared to controls (Mansoub, 2011). Supplementation of broiler chicken with stinging nettle root increased feed intake, *Lactobacillus* spp counts, and also reduced the *E. coli* counts in the gut. In the same study, combined nettle root and pumpkin seed oil also increased the overall feed conversion ratio (Tabari, Ghazvinian, Irani, & Molaei, 2016). In cattle, a study in Nepal reported increased milk production, increased fat and protein content in milk, and also higher weight gain in cattle supplemented with dried stinging nettle than controls(Khanal, Tiwari, Bastola, & Upreti, 2017). In one study, stinging nettle addition decreased fecal egg count and increased the hematocrit in yearling goats fed corn-soybean meal supplemented with stinging nettle (Davis, Corley, & Rutto, 2018). Another study in dairy cattle in the UK reported that lactating dairy cattle consuming stinging nettle hay had their rumen pH better regulated and rarely reached 5.5 compared to those consuming ryegrass silage (Humphries & Reynolds, 2014). In producer surveys done in Canada and Austria, stinging nettle was used by organic farmers for control of parasites in chicken and was also reported as having medicinal and health benefits in pigs, cattle, and chicken in Austria (Lans & Turner, 2011; Vogl, Vogl-Lukasser, & Walkenhorst, 2016). While these few studies and surveys indicating a potential use of stinging nettle in farm animals, much remains to be explored in terms of full benefits in the different species and groups of farm animals.

In this study, we explored further the effect of supplementation with pelleted stinging nettle on intestinal health and select blood parameters in pastured goats.

MATERIALS AND METHODS

Location and experimental layout: The study was conducted at the Virginia State University (VSU) research and demonstration farm, the Randolph Farm, in Chesterfield County, Virginia. Nine paddocks, measuring a total of about 8 acres, mainly composed of eastern gamagrass (*Tripsacum dactyloides*) pasture with volunteer common Bermuda grass (*Cynodon dactylon*), and herbaceous annual grasses and legumes were used. The study assessed the overall health, parasite load, blood parameters (hematocrit, FAMACHA© scores, and total serum protein), and changes in select gut microbial populations (*E. coli*, *Lactobacillus* spp and *Bifidobacterium* spp) of pasture-raised 16 myotonic (females-6, males-10) and 12 Spanish (6/sex) yearling goats in four experimental units (two for females and likewise for males) with or without air-dried pelletized vegetative biomass of stinging nettle. For six pre-experiment weeks, the females and males were kept separately on the same pastures being moved onto fresh units every week. This was intended to acclimatize the animals to the experimental protocols and also

contaminate the pastures with GIN-loaded feces. Fecal samples were collected from all animals before initiation of the treatments and were all confirmed to be infected through microscopy. Throughout the twelve-week grazing experiment, each of the treatment groups grazed a separate pasture unit rotationally and had free access to shade along the tree line, *ad libitum* commercial mineral licks, and fresh drinking water. The nettle supplemented groups received about 350 g/head/day supplemental stinging nettle pellets, offered around noon on shared spacious feeders that were equally accessible to each animal. Supplemental nettle was grown and pelleted at the Virginia State University Randolph farm by the specialty crop program. Shoots were harvested at the beginning of flowering and dried. Proximate analysis values for the stinging nettle pellets are presented in Table 1 (Appendix 1). The animals always finished all stinging nettle pellets offered rather quickly.

Sample and data collection

Field animal evaluation and sample collection: Biweekly, animals were moved onto a work area where each was weighed and screened for GIN status based on FAMACHA© scores. FAMACHA© scores were recorded on a scale of 1– red or healthy to 5 – severely anemic by examining ocular mucous membranes (Kaplan *et al*., 2004). The fecal egg count (FEC) was determined according to Whitlock, 1948 (Whitlock, 1948) at a sensitivity of 50 eggs/g on fecal samples collected directly from the rectum using a lubricated glove. After every sampling and weighing and based on FAMACHA© scores and FEC, animals deemed incapable of completing the study without treatment were appropriately dosed with Cydectin® (Bayer Animal Health), the same chemical anthelmintic in use at the farm, and incidences of return to deworming treatment recorded.

At 30, 58, and 79 days post-treatment (DPT), whole blood and fecal samples were collected. Whole blood samples were evaluated for hematocrit using the packed cell volume (PCV) determination by microhematocrit method, and also serum extracted for total protein determination. Fecal samples were processed for total parasite load by fecal egg count (FEC) and total fecal parasite DNA detection, and also microbial detection and quantification by polymerase chain reaction (PCR) protocol. Experimental protocols and husbandry practices were reviewed and approved by VSU's Animal Care and Use Committee.

Laboratory sample processing

Serum extraction: Blood samples for serum collection were collected from the jugular vein and allowed to clot at room temperature for about an hour. Subsequently, samples were centrifuged at 2000 rpm for 15 minutes to separate the clot and serum. The serum was transferred to microcentrifuge tubes and stored at -80 °C until further processing.

Total protein determination: Total protein was determined using a Vet360® digital refractometer (Reichert Analytical Instruments, USA). Frozen serum samples were thawed and brought to room temperature before analysis. A filtered water sample was used as the control. The recommended protocol indicated by the manufacturer was followed to dispense the sample onto the refractometer° and determine total serum protein in each sample.

Fecal DNA extraction: To extract all DNA, including that of parasite eggs, a bead beating method for extraction of total fecal DNA was utilized. The EZNA stool fecal DNA extraction kit and recommended protocol were utilized using 200 mg of fecal sample. Extracted DNA concentration and purity were measured using a Nanodrop. The DNA was stored at -80 °C until further processing for *E. coli*, *Bifidobacterium* spp, *Lactobacillus* spp, and total parasite DNA detection and quantification.

Quantification and detection of E. coli, Bifidobacterium spp, and Lactobacillus spp in fecal samples: For each sample, extracted fecal DNA was diluted to between 10 and 100ng of DNA. A SYBR green Qpcr protocol (Applied Biosystems by Life technologies) was used that utilized each species gene-specific primers. For *E. coli* the UID gene primers described in (Heijnen & Medema, 2006) were used while for *Bifidobacterium* spp and *Lactobacillus* spp, the 16s ribosomal RNA gene primers described in (Rinttilä, Kassinen, Malinen, Krogius, & Palva, 2004) were used. The samples were run in duplicate and a cycle threshold (Cq) was used to calculate and quantify the log count of each bacterium in each sample. Evaluation of the melting curve was used to detect and confirm specific amplification of each bacteria spp in each sample. A standard curve was generated using PCR products generated from amplification of ATCC 25922 UID gene for *E. coli* and (ATCC 4356) for *Lactobacillus*. *Bifidobacteria* spp, PCR amplicons were generated from DNA extracted from a purchased commercial probiotic capsule containing a mix of *Bifidobacteria* spp (Lifted Naturals-mood boosting probiotic) using the conserved primers. The specific amplicon was visualized on the gel and the PCR product purified. The limit of detection of *E. coli* genome, *Bifidobacterium* spp, and the *Lactobacillus* spp was 10 genome copies.

For all bacteria detection and quantification, the amplification protocol followed the Applied Biosystems PowerUp ™ SYBR ™ Green Master Mix reaction set up recommendations except for the annealing and extension temperature that were unique for each primer pair. The total reaction volume was 10µl for all reactions. The annealing temperature for *E. coli* was 56C for 15 sec

followed by an extension of 72C for 1minute, quantification data were collected at both annealing and extension steps. *Lactobacillus* spp annealing temperature was 58C for 15sec, extension 72 for 30sec, and final extension at 80C for 30sec with data collection being at 80C only. For *Bifidobacterium* spp, annealing temperature was 53C for 15sec, extension at 72C for 1minute with data collection at both annealing and extension temperatures. The program was for forty cycles for all bacteria tested.

Quantification of total parasite DNA: DNA extracted as described above was used for total parasite DNA determination in each sample utilizing conserved primers previously published (Bisset, Knight, & Bouchet, 2014). To compare the relative total parasite DNA in each sample, a standard curve using a PCR amplified conserved fragment was generated using pooled fecal DNA extracted from animals on-farm confirmed parasite-infected by microscopy. The limit of detection and quantification of the standard was 10 copy numbers of total parasite DNA. Extracted fecal DNA was diluted 1:10 before use and between 10-100ng of DNA was used in a total of 10µl SYBR green Qpcr reaction. Cycle threshold was used to estimate the equivalent log total parasite egg DNA while melting curve analysis was used to determine the specificity of the reaction. Similar to the bacterial quantification protocol, the Applied Biosystem SYBR green Qpcr master Mix recommended protocol was followed except for annealing temperature which was set at 53C for 15sec and an extension of 72 for 1 minute. The program was for 40cylces and data was collected at both annealing and extension steps.

Statistical analysis: The data were analyzed using Students t-Test comparing the measured parameters in nettle treated vs control groups (independent t-Test), all groups at different time points (dependent t-Test), and also between the different goat breeds where possible (independent t-Test).

RESULTS

Overall health and performance: The stinging nettle pellets were highly palatable to the goats and the feeding troughs were empty within 15-20 minutes each day. Apart from a wild dog attack after the second sampling point, the experimental animals remained healthy throughout the study. Four goats (three Spanish (controls) and one Myotonic (nettle supplemented) were dewormed once each with Cydectin® (Bayer Animal Health) during the study period. The percentage changes in live body weight at each sampling point in the nettle supplemented groups were slightly higher but not significantly different from the control group (data not shown).

Hematocrit and FAMACHA© scores: The mean baseline hematocrit values in all experimental animals ranged from 20 to 25. Overall the mean hematocrit in the control group remained relatively constant ranging from 24 at baseline to a high of 26.3 at 79 DPT (Figure 1). On the other hand, the nettle treated group showed significant (P=0.01) increases in mean hematocrit from a baseline value of 22.4 to 28.2, 29, and 31.5 at 30DPT, 58dPT, and 79DPT respectively (Figure 1).

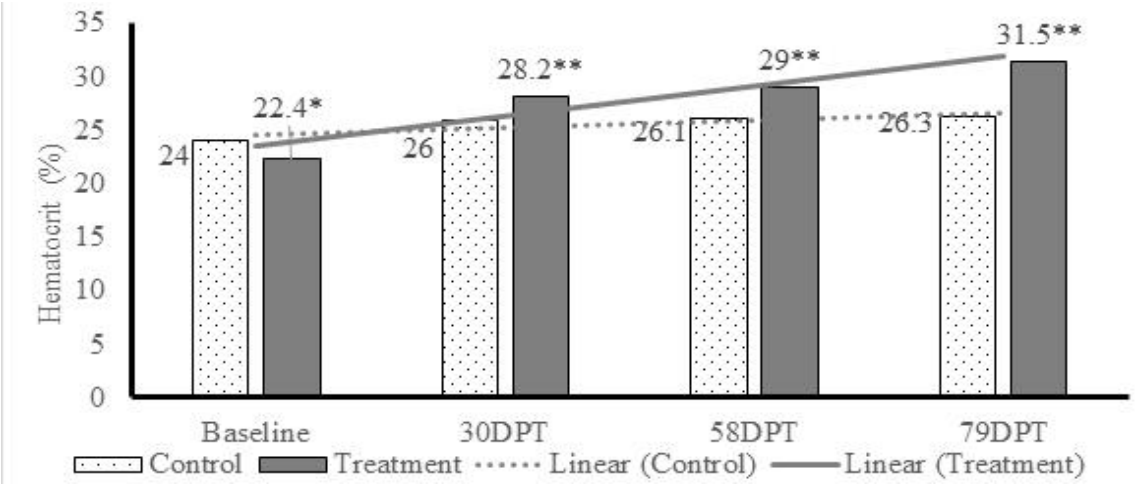

Figure 1: Overall hematocrit values in control and nettle supplemented goats at different sampling times. *vs **: Hematocrit values within the treatment group at different sampling are significantly(P<0.05) different.

At the beginning of the experiment, the mean hematocrit values for the Myotonic were lower (21.5) than the Spanish (25). However, at the next three sampling points, hematocrit values for the Myotonic control group were generally higher than for their Spanish counterparts although no significant differences were detected. The effect of stinging nettle supplementation in goats was evident in both breeds but

more pronounced in the Spanish group. In both breeds, nettle supplemented groups showed a sustained increase in the hematocrit values (Figure 2) from baseline to 79DPT. In the Myotonic group, the nettle supplemented group showed a significant increase ($p<0.05$) in the hematocrit values from baseline as early as 30DPT and remained high until the end of the experiment. The nettle supplemented Spanish group showed a significant ($P<0.05$) increase in the hematocrit values at 30, 58, and 79 DPT compared to the controls and also a significant ($P<0.05$) increase from baseline at 79DPT (Figure 2).

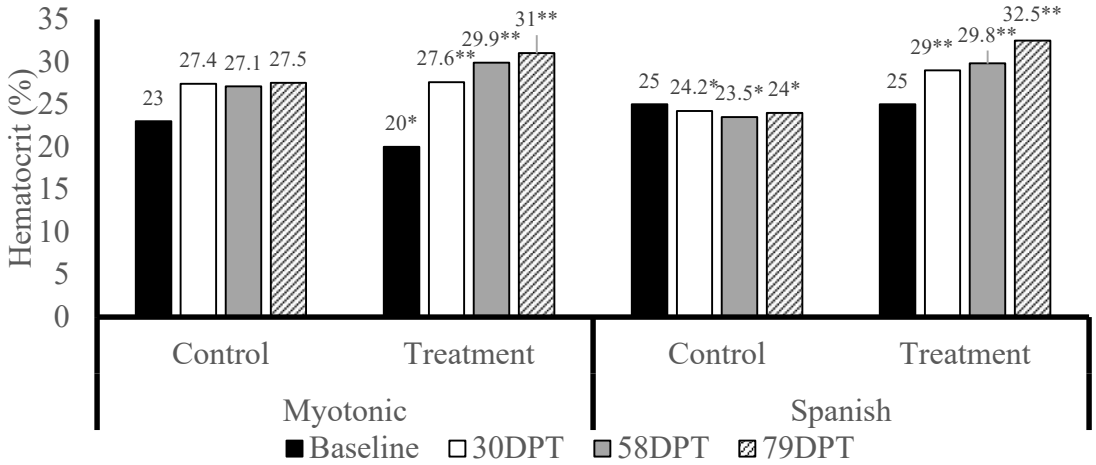

Figure 2: Comparison of hematocrit values between and within breeds at different sampling points *vs: hematocrit values at different sampling points in the same treatment group within breed or between treatment groups within breed are significantly different**

The FAMACHA© mean score was 2.71 for the nettle treated group to 2.79 for the control group at the beginning of the experiment. At all the days sampled, nettle improved the FAMACHA© scores in all supplemented goats compared to controls starting at a high of 2.71 at baseline to a low of 1.63 at 79DPT. The control group scores remained relatively constant ranging from 2.79 at the beginning of the experiment to 2.67 at 79DPT. The difference between nettle supplemented and control groups were significant as early as 30DPT($P=0.02$) and also significant at 79DPT ($P=0.003$) (Figure 3).

Total serum protein: The mean total serum protein of the control and nettle supplemented groups changed between sampling points but was similar at baseline and one month after treatment (Figure 4). In both groups, a significant increase ($P=0.007$) in total protein from the values detected at 30DPT was detected at 44DPT. Although at 58 and 79DPT the serum protein in the nettle supplemented group tended to be higher, the differences were not significant ($P>0.05$). For both control and nettle supplemented groups there was a decrease in the total serum protein at 58DPT from values detected at 44DPT.

This could be due to the wild dog attack that left the animals in shock and probably disrupted their grazing durations. Interestingly, the decrease in total serum protein was significant for the control group ($P<0.05$) but not for the nettle supplemented group. During the next sampling (79DPT), the total protein increased significantly in both the control group ($P=0.04$) and the nettle supplemented group ($P=0.0003$). In comparison to the Spanish group, the total serum protein of the Myotonic group was generally higher irrespective of the treatment group (Figure 5). However, the pattern of change in serum protein was similar in both breeds except for the Spanish nettle supplemented group that did not show a decrease in total protein after the wild dog attack.

Total parasite load by fecal egg count and total parasite DNA quantification: Parasite eggs were detected in all animals at the beginning of the experiment with a mean egg count of 1520FEC/g and 1586 FEC/g feces in control and nettle supplemented goats respectively. At 58 and 79DPT, the total fecal egg count in the control tended to be higher than the nettle group but there was high variability in the egg count between individual animals (data not shown). Parasite DNA was detected by PCR in both control and nettle supplemented goats throughout the experimental period. The mean log total parasite DNA detected in nettle supplemented goats was lower than in the control groups at all sampling points (Figure 6). Despite the nettle supplemented group had lower mean parasite DNA than controls early on at 30DPTP the differences were not significant. At 58 DPT, the total parasite DNA increased in both control and nettle supplemented groups again probably due to a stress response from the wild dog attack and disrupted feeding. However, the total parasite detected in the nettle

supplemented goat was significantly lower (P=0.008) than the control group. These significant differences were maintained until the last sampling at 79DPT (P=0.03) (Figure 6).

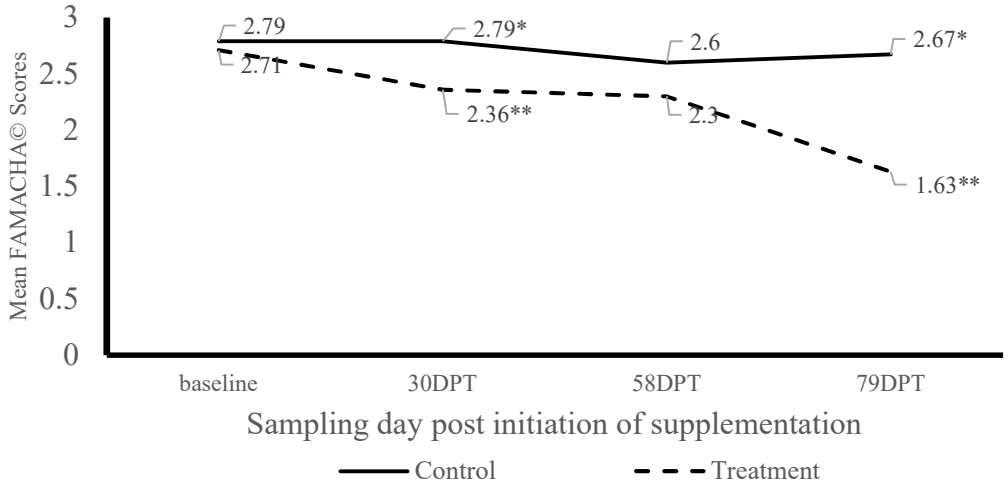

Figure 3: Mean FAMACHA© scores in control and nettle supplemented yearling goats at different sampling points

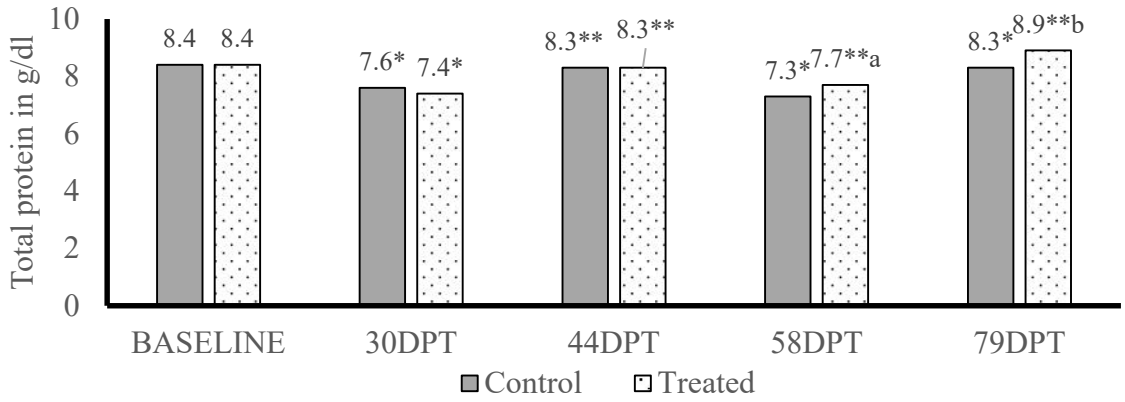

Figure 4: Total serum protein in control and nettle supplemented yearling goats at different sampling points. *vs or [a] vs [b]: Values within the same treatment groups are significantly different(p<0.05) between sampling points**

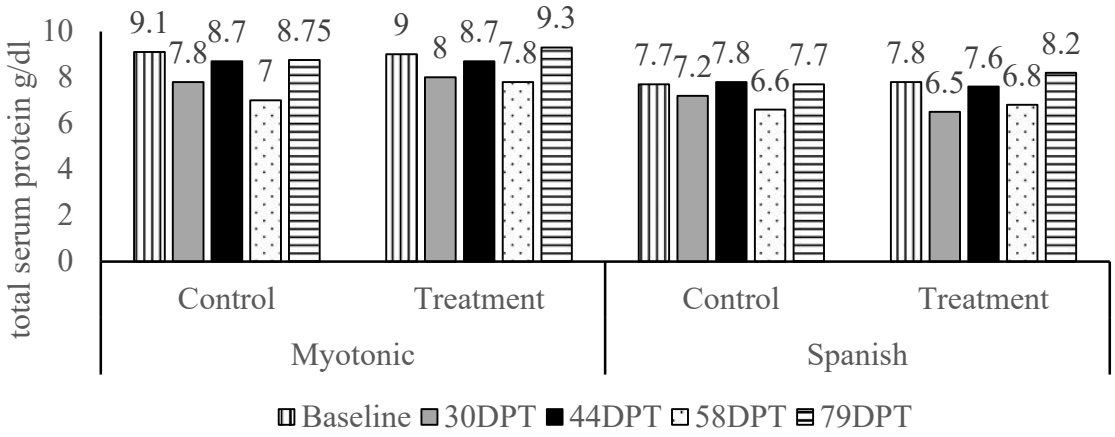

Figure 5: Serum protein values in control and nettle supplemented groups in the two goat breeds.

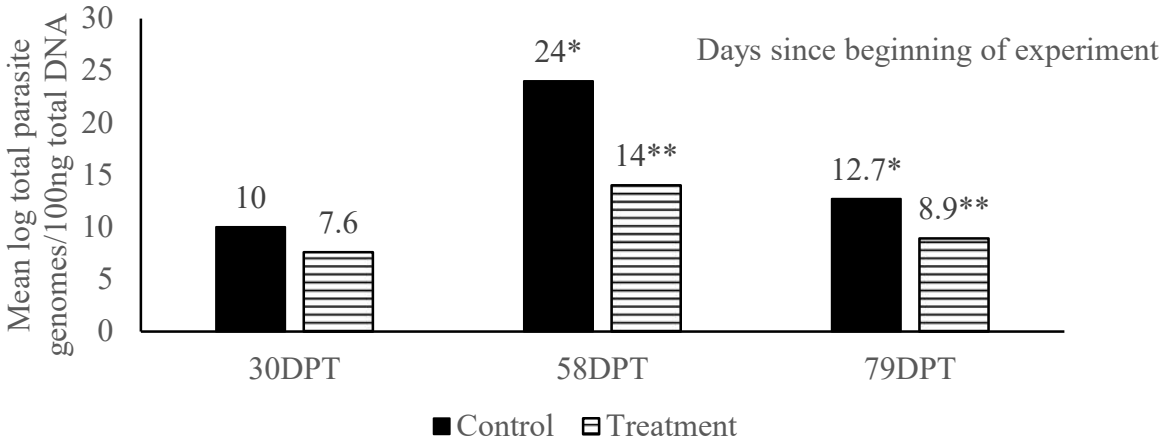

Figure 6: Mean total fecal parasite DNA in control and nettle supplemented goats at different sampling points. * vs **: Values at the same sampling point between treatment groups are significantly different (P<0.05).

The Spanish breed tended to have a higher parasite load detected at all sampling points evaluated compared to the Myotonic. At 30DPT, the parasite load was significantly higher in the Spanish control goats (P=0.004) and nettle supplemented (P=0.003) than Myotonic control and nettle supplemented goats respectively (data not shown). The reduction in parasite load by nettle supplementation was however detected in both breeds.

Total gut E. coli quantification: The mean *E. coli* counts were determined in both control and nettle supplemented goats at baseline, 30, 58, and 7DPT. *E. coli* counts were higher in control than nettle supplemented goats but, at 58 and 79 DPT, the counts were significantly lower in nettle supplemented goats compared to controls (P<0.05) (Figure 7). The *E. coli* counts decreased over time in both control and nettle supplemented goats and the lowest counts were detected at 79DPT. For both control and nettle supplemented groups, the counts detected at 79DPT were significantly lower than those detected at 30DPT (P<0.05). This pattern was similar in both Myotonic and Spanish breeds although counts detected in Myotonic were lower than those detected in the Spanish breed at all sampling points. At 30DPT, *E. coli* counts detected in Myotonic were significantly lower than those detected in Spanish goats for both control and nettle supplemented groups (data not shown).

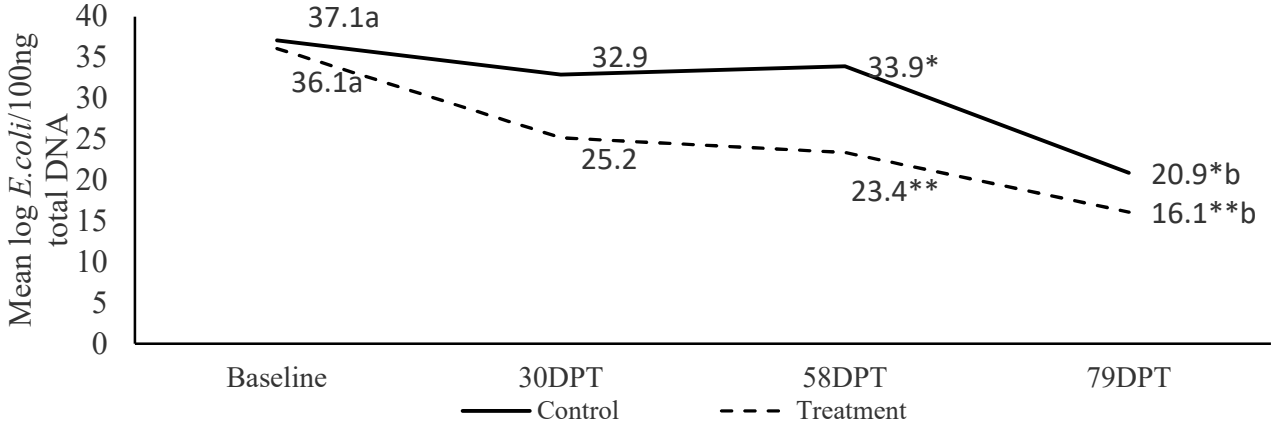

Figure 7: Mean total *E. coli* DNA in fecal samples of control and nettle supplemented yearling goats at different sampling points. * vs: Values at the same sampling point between treatment groups are significantly (P<0.05) different. [a] vs [b]: Values within the treatment group at the two different sampling points are significantly (P<0.05) different.**

Proportion of gut Lactobacillus and Bifidobacterium spp positive goats: The presence and total *Lactobacillus* and *Bifidobacterium* spp counts were evaluated in both control and nettle supplemented goats at three sampling points after initiation of treatment. The proportion of goats with detectable *Bifidobacterium* or *Lactobacillus* spp tended to be higher in the control group than in the nettle supplemented goats. *Lactobacillus* spp was detected in less than half of all the goats for this age group (yearlings) at all sampling points and counts were very low (less than 100 copies per animal, data not shown) irrespective of treatment group (Figure 8). The proportion of goats with detectable *Lactobacillus* spp in the control group tended to decrease over time and ranged from 57 to 30% in the control group while the number in nettle supplemented group increased from 14 to 36% during the same period although these changes were not significant. At 30DPT the proportion of goats with detectable *Lactobacillus* spp in the control group was significantly higher (P=0.03) than in the nettle supplemented groups but this difference was not detected at the next two sampling points (Figure 8). The *Bifidobacterium* spp counts were also low in this group with most goats having less than 1000 copies per 100ng total DNA throughout the sampling period. However, at 79DPT, there was an increase in the proportion of goats in both control and nettle supplemented groups that had counts higher than 1000 copies per 100ng total DNA (data not shown). On the other hand, the proportion of goats with detectable *Bifidobacterium* spp increased significantly from 36% at 30DPT to 80% at 58DPT(P=0.04) and 88% at 79DPT (P=0.02) in the control groups while the proportion in their nettle supplemented goats did not change between 30 and 58DPT but increased to 55% at 79DPT. At 58DPT, the proportion of goats with detectable *Bifidobacterium* spp in the control group was significantly higher than in the nettle supplemented goats (P=0.03). There were no differences between the different goat breeds in bacterial counts or proportions with detectable bacteria for any of the two genera evaluated.

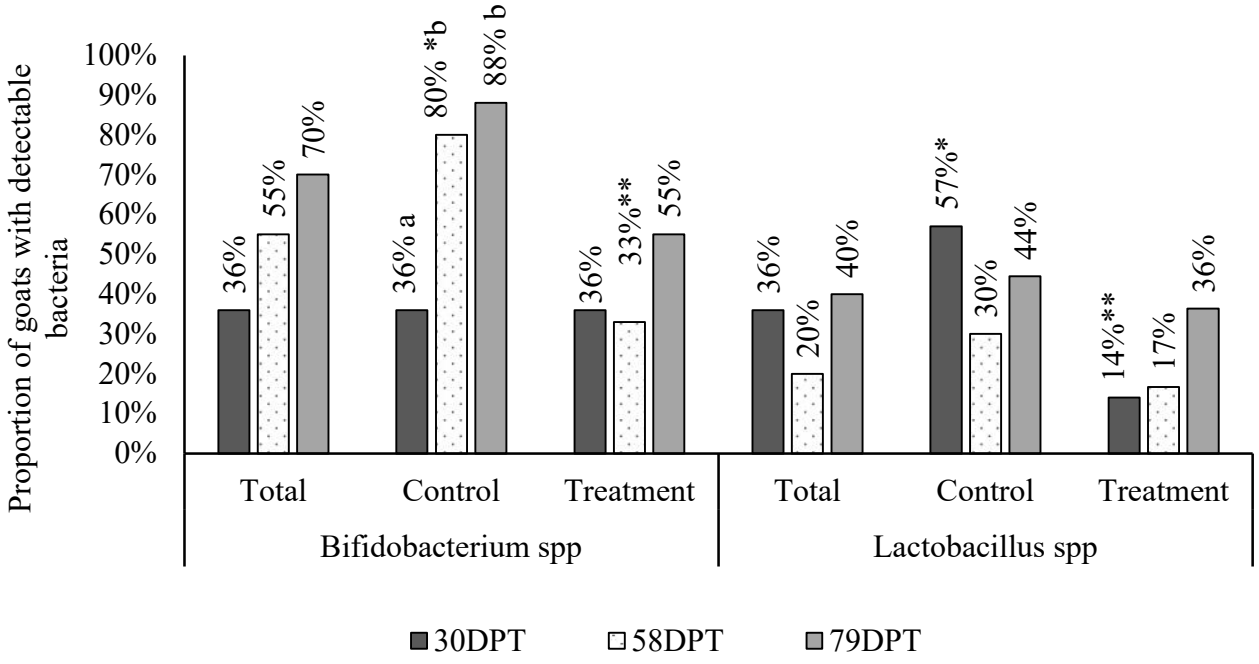

Figure 8: Proportion of goats with detectable *Bifidobacterium* and *Lactobacillus* spp in control and nettle supplemented yearling goats at different sampling points. *vs **: Proportions of specific bacteria species between treatment and control at these sampling points are significantly (P<0.05) different. [a] vs [b]: Proportions between sampling points within the treatment group are significantly (P<0.05) different.

DISCUSSION

In this study, the potential benefits of pelleted stinging nettle supplementation on select blood and gut health parameters in two breeds of pastured goats were evaluated. We observed that stinging nettle improved the packed cell volume (hematocrit) in supplemented goats, significantly, compared to the control and this difference was noted throughout the treatment period. While not many experimental studies have so far reported similar findings in people and animals, stinging nettle has

traditionally been recommended for pregnant mothers and young children in developing countries and is indicated as a good vegetable for hematological improvement including treatment of anemia (Koc, Sağlam, & Topatan, 2017; Said *et al*., 2015). Stinging nettle is known to contain higher amounts of iron (Kara, 2009; Kregiel, Pawlikowska, & Antolak, 2018; Rutto *et al*., 2013), calcium, potassium, and magnesium, among other important minerals, compared to other commonly used herbs (Kara, 2009). This could explain the observed increase in hematocrit in nettle supplemented animals. Similar to our study, a beneficial effect of stinging nettle in ameliorating blood loss in a rabbit experimental model was demonstrated over eighty years ago and the effect was found to simulate the effect of iron containing supplements (reviewed in (Upton, 2013). Additionally, in agreement with our findings, another study using carbon tetrachloride (CCL4) treated rats observed that stinging nettle oil increased hematocrit, total white blood cells, and also the total hemoglobin (Meral & Kanter, 2003) that had been reduced by CCL4 treatment. Recently, a few experimental studies that evaluated hematological and immune benefits of stinging nettle in chicken, fish, and goats (Awad & Austin, 2010; Davis *et al*., 2018; Hashemi *et al*., 2018; Mansoub, 2011; Ngugi *et al*., 2015) have also reported similar improved hematocrit in the three species. Another parameter that was improved in nettle-supplemented goats was the FAMACHA© scores. These scores are an indirect measure of severity of anemia in sheep and goats as a result of gastrointestinal parasitism especially the level of blood-sucking *Hemonchus contortus* (Kaplan *et al*., 2004; Van Wyk & Bath, 2002). Thus it is promising that stinging nettle supplementation increased the hematocrit and FAMACHA© scores in goats which presumably would protect them from the negative effects of blood loss due to parasitism. While these results were observed in goats, the implications go beyond this species. Similar hematological benefits could likely be achieved in other animal species including people. In this study, we also detected an increase in total serum proteins in nettle-supplemented goats compared to the controls. This finding is in agreement previous reports showing that the chemical composition of stinging nettle is similar to good quality alfalfa, clover, or sainfoin (Wilson, 1941) and that stinging nettle is rich in proteins and essential amino acids that could be beneficial to people and animals (Kregiel *et al*., 2018; Rutto *et al*., 2013). Similar to our studies, stinging nettle increased the total protein, albumin, and overall survival rate in fish infected with *Aeromonas hydrophila* (Ngugi *et al*., 2015) and also hematocrit, hemoglobin, and increased survival in those infected with *Yersinia ruckeri* (Adel *et al*., 2017). In other studies involving chicken and cattle, although no total serum protein was evaluated, stinging nettle inclusion in the diet had a growth-promoting effect (Hashemi *et al*., 2018) and caused an increase in total milk protein (Khanal *et al*., 2017). These findings may indirectly be related to increased total serum protein by stinging nettle in these two latter experiments. These results call for further research on the potential of *U. dioica* as a forage or herbal supplement with medicinal and nutritional benefits.

Improved gut health parameters were detected in goats receiving stinging nettle compared to the control, throughout the treatment period. In particular, we observed a significant decrease in total *E. coli* and gut parasite DNA in goats that were supplemented with stinging nettle compared to the control. No other study was found in the literature that investigated this potential of stinging nettle in any animal models using molecular detection methods. Thus these are novel and significant findings that warrant further studies to confirm this effect and possible nettle constituents that may affect gastrointestinal parasites and their mechanism of action. However, in a survey of farmers in British Columbia, stinging nettle was one of the herbal preparations used in the control of endoparasites in organic production systems in poultry (Lans & Turner, 2011). In Kenya, stinging nettle is also one of the herbs mentioned in literature as being used by some communities for parasite control in poultry (Kaingu *et al*., 2010) while in Nordic countries, stinging nettle whole plant and seeds were reported as being used in human and sheep for control of helminths (Waller *et al*., 2001). Another study in goats reported a decreased fecal egg count in the nettle-fed group (Davis *et al*., 2018) while another study in plants reported that stinging nettle reduced the number of soil nematodes within a radius of two meters. In the latter study, tomato plants are grown in pots with stinging nettle as green manure had fewer nematodes detected (Nasiri, Azizi, Hamzehzarghani, & Ghaderi, 2014). It is also worth noting that stinging nettle has been reported to have anti-parasitic effects against Cutaneous Leishmaniasis in experimental mice although the specific mechanism of action or active compounds have not been elucidated (Badirzadeh *et al*., 2020).

In many studies, most plants that have been shown to have anti-parasitic effects in small ruminants have been attributed to high levels of tannin content (Herve Hoste *et al*., 2012). The most studied of these are the *Lespedeza* species that have been shown to reduce the level of parasitism and fecal egg count in supplemented goats (Shaik *et al*., 2006). In the literature, stinging nettle is reported to contain tannins but the specific nature and relative amounts have not been described (Đurović *et al*., 2017; Jan & Singh, 2017; Joshi, Mukhija, & Kalia, 2014; Ramic, Murko, & Alibabic, 1987; Upton, 2013). It is, therefore, possible that the effect we report for stinging nettle in this study may be due to tannins or other unknown bioactive components in this plant. These results are promising and further research on mechanistic

effects of stinging nettle in parasite reduction is worth examining. This would further validate the inclusion of stinging nettle, as a plant with anti-parasitic benefits, in animal and plant agricultural systems.

A reduction in the total *E. coli* counts in the gut as a result of including stinging nettle to the feed of goats was also observed in this study. Reports of the potential of stinging nettle as a plant with antimicrobial effect either anecdotally from traditional uses or experimentally have been reviewed (Di Virgilio *et al*., 2015; Jan & Singh, 2017; Kregiel *et al*., 2018; Said *et al*., 2015; Zeković *et al*., 2017). Similar to our findings, water extracts of stinging nettle were shown to have antimicrobial activities against several bacteria species including *E.coli, in vitro* and *in vivo,* in several studies (Gāliņa & Valdovska, 2017; Gülçin *et al*., 2004; Mahmoudi, Amini, Fakhri, & Alem, 2020; Tabari *et al*., 2016; Zeković *et al*., 2017) while hydro alcoholic stinging nettle root extract supplementation reduced *E.coli* counts and increased *Lactobacillus* spp counts in broiler chicken (Tabari *et al*., 2016). However, contrary to our studies, the antibacterial activity in nettle extracts using organic solvents was found to have only minimal effect in some studies (Kukrić *et al*., 2012; Modarresi Chahardehi, Ibrahim, Fariza Sulaiman, & Aboulhassani, 2012). The differences in the latter studies may be due to the organic solvents used in the extraction since our study utilized dried pelleted nettle without any chemical extraction. Our findings are also in agreement with other studies in fish, poultry, and cattle that reported improved performance and resistance to bacterial diseases after inclusion of stinging nettle in the diet (Adel *et al*., 2017; Bilen, Ünal, & Güvensoy, 2016; Hashemi *et al*., 2018; Khanal *et al*., 2017; Mansoub, 2011; Ngugi *et al*., 2015). In our study, *Lactobacillus* species were rarely detected in the supplemented goats and, therefore, no effect of stinging nettle on these bacteria could be reported. This could be due to the age of the animals used in the study that were over one year (yearlings) which our recent data shows to be devoid of any *Lactobacillus* spp or rarely detected compared to goats three months old and below (unpublished data). Except for the study described above, which showed an increased number of *Lactobacillus* spp in broiler chicken gut by stinging nettle root powder (Tabari *et al*., 2016), no other studies were found in the literature that evaluated this phenomenon. We also report findings on the effect of stinging nettle supplementation on *Bifidobacterium* spp in the gut of goats. The findings in this study that showed a lower proportion of goats with detectable *Bifidobacterium* spp in the gut in the nettle supplemented group needs further studies for confirmation.Stinging nettle may have broad-spectrum antibacterial compounds that may not be specific to *E. coli* as discussed above. The significance of these findings is not clear since no negative effect on growth rate was detected in nettle-supplemented groups despite fewer numbers of them having no detectable *Bifidobacterium* spp. Given stinging nettle has no toxicity effects when consumed and its high nutritional benefits, these findings need further exploration for the potential of stinging nettle to be used as an alternative or complement to antimicrobial additive in animal feed.

These results indicate a great potential of stinging nettle in small ruminants in not only alleviating the negative effects of specific blood-sucking gut parasites like *Hemonchus contortus* but also overall gastrointestinal parasitism by reducing parasite loads and increasing total serum proteins. The results also show there is also potential in using stinging nettle as a feed supplement that may reduce potentially pathogenic bacteria like *E. coli* in the gut.

In conclusion, we report potential benefits of supplementing pelleted stinging nettle in pastured goats that include improvement of hematological parameters including hematocrit, FAMACHA© scores, and total serum proteins. We also report a decreased level of parasite load and total *E. coli* in supplemented goats. While these results were observed in pastured goats, there is potential that similar and many other benefits reported in other studies are achievable in other animal species. We therefore, recommend further experimental studies to explore and exploit all potential medicinal and nutritional benefits of this species including the specific compounds involved.

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Appendix 1

Table 1: Pelleted stinging nettle analysis.

Analysis	%	ppm
Moisture	1.95	
Crude Protein	17.57	
Soluble Protein	27.28	
Acid detergent fiber (ADF)	34.86	
Neutral detergent fiber(NDF)	45.37	
Acid detergent insoluble nitrogen (Adin (HD))	3.08	
Neutral detergent insoluble protein (NDIP)	5.74	
Starch	2.02	
Oil	0.16	
Nitrates	379.67	
In vitro dry matter digestibility (IVDMD)	60.21	
In vitro total digestibility (IVTD)	76.32	
Neutral digestible fiber digestibility(NDFD-30 hr)	47.76	
Lignin	11.24	
ASH	11.08	
CA	1.58	
P	0.51	
MG	0.45	
K	3.32	
S	0.34	
NA	0.05	
CL	0.46	
FE	NA	262.50
CU	NA	8.00
MN	NA	43.50
ZN	NA	23.17