

EFFECTS OF GRAZING MANAGEMENT AND
TARGETED ANTHELMINTIC ADMINISTRATION ON
THE PERFORMANCE, PARASITE BURDEN, AND
HEMATOLOGIC PROFILES OF HAIR SHEEP

By

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Abstract: The objective of this experiment was to determine if extensively managed rotational grazing would impact the performance, fecal egg counts, and hematologic profiles of hair sheep ewes and lambs. Crossbreed hair sheep ewes and lambs were assigned to 2 experimental treatments that included a continuous (CON) grazing treatment and a rotational (RTL) grazing treatment. The experiment was conducted during the summer months from 2015 to 2019. Blood and fecal samples were collected from the ewes only and body weight (BW), body condition score (BCS), and FAMACHA™ score (range = 1 to 5) were collected from all animals. An anthelmintic was administered if FAMACHA™ score was ≥ 4 . There was no difference in ewe ($P \geq 0.47$) or lamb ($P \geq 0.57$) BW between treatments at any collection period. Average BCS in late summer tended to be greater ($P = 0.06$) for the RTL treatment. The FAMACHA™ score at the end of the summer regardless of yr tended to be greater ($P = 0.07$) for the RTL treatment. White blood cell (WBC) count was greater in early summer ($P = 0.03$) and tended to be greater in mid-summer ($P = 0.07$) for the RTL treatment. Eosinophil (EOS) count tended to be greater in early summer ($P = 0.06$) for the RTL treatment. Neutrophil (NEU) count for the RTL treatment tended to be greater in late spring ($P = 0.10$) and was greater in early summer ($P < 0.02$) than the CON treatment. No differences in parasite count were observed between treatments during the experiment ($P \geq 0.28$). The percentage of lambs that score a BCS $\geq 2+$ tended to be greater for the CON treatment ($P = 0.07$) in late summer. No difference in average FAMACHA™ score was observed in lambs between treatments ($P \geq 0.24$) at any collection. There were no differences between treatments for anthelmintic use in ewes ($P \geq 0.36$) or lambs ($P \geq 0.37$) at any collection. This experiment indicates that there are minimal differences between CON and RTL grazing in extensively managed hair sheep, and demonstrates that regardless of grazing system, targeted parasite management can improve herd health and decrease parasite burden.

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CHAPTER I

LITERATURE REVIEW

Introduction

Gastroenteric diseases due to parasite infections have a great economic impact in the sheep industry worldwide (Mugambi et al., 1997; Jackson et al., 2012; Kandasamy et al., 2013; Ruiz-Huidobro et al., 2019), and have demonstrated to affect productivity by increasing economic losses, mortality rates, costs of treatments, and reduced growth (Jackson et al., 2012; Walkden-Brown et al., 2013). Australia has reported to spend an estimate of 400 million (USD) in parasite control per year, and in Kenya, South Africa, and India cost about 26, 46 and 103 million USD, respectively (Sackett et al., 2006).

Parasite control is primarily based on the administration of anthelmintics, however due to the massive use of these products, multiple anthelmintic resistance is now established in gastrointestinal nematode parasites of small ruminants in many regions in the world (Kaplan, 2004; Crook et al., 2016), and the increase of anthelmintic resistance is a developing concern (Burke and Miller, 2020).

Haemonchus contortus is the most important gastroenteric nematode in sheep (Balic et al., 2000; Cachat et al., 2010; Besier et al., 2016), and it is catalogued as the main cause of economic losses in many regions of the world (Zajac and Garza, 2020). Lately, some alternative methods that have been researched to control *H. contortus* are good nutrition, rotational grazing, multispecies grazing (Terrill et al., 2012), intensive rotational grazing (IRG; Walkden-Brown et al., 2013), use of biological control (Waller, 2006), cooper oxide needles (Vatta et al., 2009), medicinal plants (Qadir and Dixit, 2010), vaccination (ArunKumar et al., 2012), use of targeted selective treatments (TST; Jackson et al., 2012; Kenyon and Jackson, 2012; O'Shaughnessy et al., 2015; Mohammed et al., 2016), and use of resistant breeds or genetic resistance (Baker et al., 1991).

Internal parasites

The internal parasites responsible of most of the problems in sheep and goats are gastrointestinal nematodes (GINs), and belong to the order Strongylida, superfamily Trichostrongyloidea. Sheep are infected with a community of these strongylid nematodes, whose disease is known as parasitic gastroenteritis or (PGE; Bowman, 2014). To design effective management programs, it is important to have a basic knowledge of the biology of parasites (Zajac and Garza, 2020). Nematodes that belong to other orders include *Aoncotheca* and *Strongyloides*, mainly found in the small intestine, *Skrjabinema*, and *Trichuris* in the large intestine (Bowman, 2014; Smith and Sherman, 2009), but are not considered important because they only cause disease in unusual circumstances.

All sheep that graze are at some point infected with GINs, and the severity of infection is determined by parasite burden. Low worm burdens usually have minimal

impact on animal health, and as the burden increases, subclinical effects such as reduced BW gain and anorexia occur. When heavier burdens are present, clinical signs such as weight loss, anemia, submandibular edema, and diarrhea can develop (Bowman, 2014; Smith and Sherman, 2009). The most important of the strongylid nematodes in North America and many other regions, and known as the most challenging, and economically important gastrointestinal nematode in the sheep industry is *Haemonchus contortus*, colloquially called the barber pole worm or wireworm (Zajac and Garza, 2020).

The normal niche of adult stage of *H. contortus* is the abomasum of small ruminants (Balic et al., 2000). Females reach about 3 cm in length, what makes this species to be one of the largest strongylid nematodes in ruminants (Zajac and Garza, 2020). *H. contortus* has a distinguishing barber pole appearance that is easily seen in the abomasal contents when a necropsy is performed (Bowman, 2014; Sutherland and Scott, 2010), different from other species that are smaller and can only be identified using a microscope. *H. contortus* is highly fecund, meaning that a female can produce 10,000 eggs daily, and larvae on pastures can accumulate quickly during the grazing season (Levine, 1980; Bowman, 2014; Besier et al., 2016). The length of time elapsing between the time of infection and parasite maturity to the egg-laying stage is called the prepatent period, which usually last 17 to 21 days (Zajac and Garza, 2020). Adult stage worms are short lived and typically survive in the host for a few months, depending on animals and specific circumstances (Zajac and Garza, 2020). *H. contortus* has an important and distinct pathogenic potential that results from its blood-feeding behavior in the abomasum (Besier et al., 2016; Zajac and Garza, 2020). When heavy infections are present, *H. contortus* can cause fatal anemia. While most of the parasites produce weight

loss and growth reduction, *H. contortus* has demonstrated to cause high mortality rates, and is not a primary cause of diarrhea (Levine, 1980; Bowman, 2014; Besier et al., 2016). Haemonchosis and PGE occurs most in young animals whose immunity has not been developed, or is compromised, or in animals that are exposed to very high levels of infection (Levine, 1980; Bowman, 2014; Besier et al., 2016). Although other nematodes can affect small ruminants, *H. contortus* is the primary cause of parasite diseases in most of the US and several areas in the world (Fleming et al., 2006; Whitley et al., 2014).

Because of the major importance of *H. contortus*, it is the most common studied nematode. Other species of the genus *Trichostrongylus* also contribute to the PGE. Some of these species infect small ruminants and include *Trichostrongylus axei* and *Teladorsagia circumcincta* in the abomasum and *T. colubriformis* and *T. vitrinus* in the small intestine (Zajac and Garza, 2020). Clinical signs of *T. circumcincta* are diarrhea, anemia, and hypoproteinemia. *T. circumcincta* is found in the U.S. and Canada; it is most important in northern areas because of the presence for colder climatic conditions (O'Connor et al., 2006).

Life cycle

In life cycle of strongylid parasites, adult female parasites living in the abomasum or intestines produce eggs that are passed in the manure. The development occurs in the fecal matter that provides protection from environmental conditions. After eggs are expelled, a first-stage larva (L1) forms and hatches out of the egg; after hatching, larvae molt to the second stage (L2), then molt for a second time to reach the infective stage (L3). This third stage moves out of the fecal matter and crawls up to the forage, where

they are ingested by sheep or goats while grazing (Levine, 1980; Sutherland and Scott, 2010; Bowman, 2014; Zajac and Garza, 2020).

Meteorological conditions and parasites

The chances of strongyle larvae in the microenvironment of the fecal pellets and forage determine if parasites will be challenging to overthrow during the grazing season. By knowing the biology of the larvae stages, a prediction of parasite distribution and formulation of parasite management programs can be made (Zajac and Garza, 2020).

The development of larvae in the fecal matter from egg to third stage occurs at a temperature of 10°C to 36°C (Levine, 1980; O'Connor et al., 2006), and moisture is equally vital for the successful development of eggs to L3. Moisture levels in feces during the period of larval development is affected by moisture at the time of feces deposition, soil moisture levels, and precipitation, and all these factors determine if successful parasite development occurs (Levine, 1963; O'Connor et al., 2006; O'Connor et al., 2008; Leathwick, 2013; Besier et al., 2016; Molento et al., 2016).

Of all common nematodes of small ruminants, *H. contortus* has the most remarkable capacity for severe pathogenic effects on a large scale and over a wide climatic range. Hus and Levine (1977) discussed the interactions between moisture and temperatures that ranged from 20-35°C, and no egg development occurred at low humidity levels (70-85%). Still, most eggs produced infective larvae when humidity increased up to 100% (Besier et al., 2016). Maximum hatching rates and mortality occur at high temperatures, but it has been demonstrated to survive at low temperatures. Several authors have found that under conditions of extreme cold, freshly or embryonated eggs in

fecal pellets cannot survive. From only 24 hours at 0°C (Jasmer et al., 1986; Shorb, 1944a; Veglia, 1915), a few days at 4-5 °C (Shorb, 1944b; Smith-Bujis and Borgesteede, 1986). Larvae stored in water at < 0°C survives only a few days (Jasmer et al., 1987; Rose, 1963; Todd et al., 1967), but for some months at temperatures close to the freezing point, although in minimal numbers (Boag & Thomas, 1985, Misra, 1978; Rose, 1963; Veglia, 1915). Chances of survival at high water temperatures are considerably lower, only 16-33 days at 40°C (Jehan and Gupta, 1974; Sood and Kaur, 1975). A study where differences in egg hatching from different geographical regions were compared suggested environmentally mediated survival differences. Crofton and Whitlock (1965) reported that the minimum egg hatching temperature was 9°C in the UK, 15°C for New York and Ohio isolates, and morphological characteristics were also related to temperature preferences (Besier et al., 2016).

Studies have confirmed that rainfall is the main limitation for *H. contortus* development, and when year-round rain occurs, it allows continual *H. contortus* development. After four days of fecal deposition, moisture is essential for the successful development of infective larvae (O'Connor et al., 2008), and rain is important in releasing larvae from the manure. When several rain events occur results in the larval release; therefore, when a period of drought is about to end, producers can monitor animals a few weeks after the end of the drought and apply targeted treatments to reduce infections and promote health and productivity in the flock (Bullick and Andersen, 1978; O'Connor et al., 2006; Besier et al., 2016; Molento et al., 2016; Van Dijk et al., 2018; Wang et al., 2018; Zajac and Garza, 2020).

Several authors have concluded that fecal desiccation and rehydration rates on pasture could explain temporal patterns of larval availability, and that sheep feces may act as a larval reservoir in dry conditions, with peaks of infection following rainfall. Observations of worm burdens in grazing animals have indicated relationships between nematode development and environmental factors and provide an epidemiological environment. These investigations include structured studies using ‘tracer’ animals grazing small pasture areas contaminated with worm eggs at specific times, worm counts from flocks or herds grazing repeatedly contaminated pastures and slaughterhouse surveys. Total worm counts from grazing animals also indicate the presence of hypobiotic larvae and their relative importance as a survival mechanism during adverse environmental conditions (Besier et al., 2016).

T. circumcincta has the greatest ability to tolerate cold weather. Eggs of *H. contortus* and *T. circumcincta* were exposed to a 15-hr period of 18°C, more than 87% of *T. circumcincta* but less than 4% of *H. contortus* were able to hatch (Jasmer et al., 1986), and when L3 of both species were exposed to -18°C for 5 hrs., the viability of *H. contortus* was less than 4%; however, the viability of *T. circumcincta* was 85% (Jasmer et al., 1987). Hoste and Cabaret, (1992) reported that ewes grazing during winter are probably infesting a more significant proportion of *T. circumcincta* L3. In spring, already established *T. circumcincta* adult worms could negatively impact the establishment and development of newly ingested *H. contortus* L3 (Hoste & Cabaret, 1992).

L3 can migrate laterally and vertically; however, this migration is affected by air temperature, soil moisture, and relative humidity. Some *H. contortus* larvae can migrate up to 90 cm away from fecal pellets, but the majority stay within 10 cm of the feces

(Skinner and Todd, 1980; Molento, 2016). Most GIN cannot migrate more than 5 cm vertically from the ground (Skinner and Todd, 1980; Molento et al., 2016). This is an important limitation that implicates how pastures can be managed; when animals are forced to graze too short, ingest more larvae than when grazing tall forage (Zajac and Garza, 2020). As mentioned before, in periods of drought larvae cannot migrate to the forage (Skinner, 1980). Nematodes are covered by a hard semipermeable outer layer called the cuticle. The cuticle gets replaced with each molt, but the L3 maintains the L2 cuticle, carrying a double cuticle that protects the parasite (Sutherland and Scott, 2010; Besier et al., 2016). While the double cuticle provides greater resistance to the environment, it also prevents feeding, and once L3 uses the energy stored, they die. Therefore, the best conditions to survive are cool, dry weather due to the reduction in larvae movement and the use of the reserves of energy. Cool, humid weather also supports survival for several months. Hot weather rapidly affects the survival rate because it accelerates the metabolic rate, which rapidly uses the energy reserves. Other factors that affect larval survival include parasite species, location on pasture, and forage height (Zajac and Garza, 2020). In a study performed by Chylinski et al. (2014), *H. contortus* had the lowest capacity to survive desiccation, followed by *T. colubriformis* and *T. circumcincta*; which means that *Haemonchus contortus* species could be more affected than other species by long rest periods. It could be a challenge for *H. contortus* to survive 10 or 15 days more in a cell grazing system than in a rotational grazing system before host comes back in a given plot, especially during summer periods (Ruiz-Huidobro et al., 2019).

Anthelmintics and resistance

Anthelmintics have been available since the 1960s, and producers have relied on these products to control GINs since then. In areas that were mostly affected and caused extensive losses, frequent treatments were recommended; nevertheless, all the overuse of these products has contributed to resistance in parasite populations, specifically for *H. contortus* (Fleming et al., 2006; Mortensen et al., 2003; Falzon et al., 2013; Crook et al., 2016). Currently, most producers continue to include some level of anthelmintic use. However, the importance of alternative parasite control methods such as genetic selection and pasture management and reduced use of anthelmintics needs to be addressed (Burke and Miller, 2020). Anthelmintics approved for small ruminants belong to three major drug groups: the benzimidazoles, cholinergic agonists, and macrocyclic lactones. If a population becomes resistant to one of the groups, it automatically becomes resistant to all groups (Lanusse et al., 2018; Zajac and Garza, 2020). Albendazole and fenbendazole belong to the benzimidazole group, and the primary mode of action is by inhibiting the microtubule formation (Lanusse et al., 2018; Lifschitz et al., 2017). The macrocyclic lactones include 2 products approved for oral administration in sheep. These products are ivermectin and moxidectin and have activity against both nematodes and arthropods, and the mechanism of action is to interfere with chloride channel neurotransmitters (Lanusse et al., 2018; Lifschitz et al., 2017).

The overuse of anthelmintics has caused an increase in the incidence of resistance of parasites to anthelmintics, limiting the efficacy of pharmacological control of parasites (Howell et al., 2008; Kaplan and Vidyashankar, 2012; Leathwick and Besier, 2014; Ngere et al., 2018). Conder and Campbell (1995) defined anthelmintic resistance as “a

heritable reduction in the sensitivity of a parasite population to the action of a drug” (Jackson et al., 2012). When an animal is exposed to anthelmintics, the parasites that survive after that treatment are considered “resistant.” Resistant parasites carry resistant genes to their offspring; this increases the number of eggs released to the environment and, subsequently, makes it a critical situation that needs to be managed (Jackson et al., 2012). More than 40 countries have reported anthelmintic resistance cases involving several drug groups and species, and when compared to cases of anthelmintic resistance in cattle, more reports of multiple resistance in sheep and goats have been reported throughout the world (Kaplan et al., 2004; Jackson et al., 2012). Several of the common parasites in sheep said to be part of the anthelmintic resistant group are *Haemonchus contortus*, *Trichostrongylus species* and *Telardosagia circumscincta*, *Fasciola hepatica*, and *Nematodirus species* (Jackson et al., 2012).

FAMACHA system

The most significant sign of anemia is the pallor of the mucous membranes, mainly seen in the conjunctivae. This specific sign is associated with the degree of anemia in small ruminants. This association is based on the FAMACHA™ system, a system developed in South Africa. FAMACHA is an abbreviation that originated from the name of the creator of the idea, Dr. Faffa Malan (FAffa Malan Chart). It is expressed as “a score of 1-5, that ranges from a red-pink (normal) color to an extreme white in terminal situations”(Vatta, 2001; Besier et al., 2016). Kaplan et al. (2004) validated the use of the FAMACHA system in the U.S.

Lately, the FAMACHA system has been used to slow the development of anthelmintic resistance. Several authors have considered it as the “best-known example” and “best developed health-based” targeted selective treatment (TST) indicator (Besier, 2012; Marques et al., 2018). Using the FAMACHA system has demonstrated that the number of treatments can be reduced by 50% in sheep and goat flocks (Vatta, 2001; Kaplan et al., 2004) with a reduction in fecal egg count between 35% and 83% depending on the principle used to apply the treatment (Kaplan et al., 2004). Another significant advantage is that this method can identify animals that are resistant because resistant animals are usually associated with low FAMACHA scores, so this demonstrates that it can be used to select animals for resistance and therefore eliminate susceptible animals from the flock (Burke and Miller, 2008; Jackson et al., 2012).

Hematological parameters

Anemia is a decrease the number of red blood cells resulting in the decline in oxygenation of tissues. The amount of red blood cells in an organism is determined by measuring the packed cell volume (PCV) or hematocrit, the amount of hemoglobin, and the number of erythrocytes (Thrall et al., 2012). In North America, PCV is mainly analyzed as the primary value for the interpretation of anemia; however, when automated cell counters calculate it, hemoglobin concentration is more accurate. Anemia occurs as an indication of a disease that causes an increase in erythrocyte loss through hemorrhage, decreased production of red blood cells, or a combination of these manifestations (Thrall et al., 2012)

Clinical signs of anemia are related to a reduction in oxygenation or other mechanisms associated with compensation. Some of these signs include changes in the color of the mucous membranes, fatigue, reduced exercise tolerance, dyspnea, tachycardia, and other nonspecific signs such as weight loss, anorexia, fever, or increase in the size of lymph nodes in case of a major systemic illness. Signs may vary depending on the situation; however, animals that experience acute blood loss or destruction might die (hematology book). Clinical signs could be specific or unspecific; therefore, it is crucial to perform an appropriate diagnosis and therapy to determine the cause of anemia.

Anemia can be briefly classified by volume (MCV; Mean cell volume) and the amount of hemoglobin within erythrocytes (MCHC; Mean corpuscular hemoglobin concentration). Anemia can also be hypochromic when erythrocytes contain less hemoglobin than normal and normochromic when they contain normal hemoglobin concentration. When using laser-detection technology, hemoglobin concentration is reported as corpuscular hemoglobin concentration mean (CHCM). Anemia is classified as regenerative and non-regenerative. When the bone marrow is trying to compensate for blood loss, it increases its production, releasing immature red cells before being ready. Increasing the production and immature red blood cells indicates regenerative anemia, and an increase in reticulocyte concentration could identify this type of anemia. Regenerative anemia is present when there is blood loss or destruction from an external or internal source. It may be acute or chronic losses such as bleeding lesions in the body, especially in the GI tract and internal or external parasites (Thrall et al., 2012).

Several authors have recognized anemia as the main sign of *Haemonchus contortus* in sheep (Baker et al., 1959; Le Jambre, 1995). Anemia caused by *H. contortus*

is mainly of hemorrhagic origin due to the blood loss that the parasite causes in its blood-feeding activity (Baker and Douglas, 1966, Besier et al., 2016). Depending on the size of the parasites is the amount of blood that is lost. Adult worms have been observed to suck blood for up to twelve minutes at a time following detachment; the hemorrhage could continue for up to 7 minutes (Boughton and Hardy, 1935; Le Jambre, 1995). Besides the blood-sucking activity, larvae migration also causes hemorrhages into the abomasum, and the average blood loss due to *H. contortus* infection is 0.03 ml per parasite per d (Kumar et al., 2015). Dargie and Allonby (1975) reported a variation between sheep in blood loss per worm and attributed this to a variation in worm size with larger parasites consuming way more blood and occurring in the animals with the smallest burdens. Other authors estimated that adult worms could remove from 30-50 μ L of blood per day (Clarke et al., 1962; Dargie and Allonby, 1975). Le Jambre (1995) reported that there is a strong correlation between blood loss and the number of adult stage worms, therefore showing that blood loss depends mostly on the number of larvae that are ingested and the ability of the host to reject the parasite and the capacity to produce new blood to compensate for the blood being lost (Besier et al., 2016).

Anemia is reported to be first detectable about 10-12 days post-infection (Dargie and Allonby, 1975; Hunter and McKenzie, 1982). When blood loss is severe, packed cell volume remains normal mostly because cells and plasma are lost; nevertheless, after a few hours later, PCV and plasma proteins decrease since water that is in the interstitial space is added to the blood. After 72 hours, reticulocytes start to appear in blood, recognizing that its concentration peaks around one week (hematology veterinary book). Erythropoietic responses and immunological controls on worm numbers and egg

production are more commonly invoked after an extended period than at the beginning of infection (Barger and Dash, 1987).

Regardless of important findings between red blood cells and hemoglobin concentrations, there has not been enough research to consider the relationship between blood components and gastrointestinal nematodes. One study demonstrated that as the degree of anemia in sheep is not significant, the RBC decreases and hemoglobin concentration increases, going the opposite way when a state of severe anemia (Ferreira et al., 2017).

The loss of blood during infection with *H. contortus* also reduces leukocytes (Le Jambre, 1995). Studies of total leukocytic count revealed significant leukocytosis due to neutrophilia, lymphocytosis, monocytosis, and eosinophilia (Kumar et al., 2015). Early changes in the leukogram when acute inflammation is present are considered the most distinctive features of ruminant white blood cell responses. In the first 24-48 hours of acute inflammation, neutropenia occurs due to the recruitment of neutrophils from the blood to the inflammation site (Lumsden et al., 1974; Valli, 2007).

When inflammation becomes chronic, it may be associated with neutrophilia or normal neutrophils. However, it is not easy to predict chronic inflammation and acute inflammation when it is less severe, making plasma hyperfibrinogenemia a better indicator of these conditions in ruminants (Weiss et al., 2010). Several authors reported the presence of inflammatory neutrophilia in bacterial, viral, fungal, protozoal, and parasitic infections of cattle, goats, and sheep (Adah et al., 1993; Bauer et al., 2005;

Mandal and Gupta, 1993; Seimiya et al., 2005; Singh et al., 1997; Summers et al., 2002; Tennant et al., 1974).

Another change that is sometimes present in either acute or chronic inflammation cases in ruminants is monocytosis or increased monocytes (Weiss et al., 2010). However, it is not a consistent change, and it is sometimes associated as part of a stress response in ruminants (Weiss, 1992). Monocytes can also be seen as immature cells that become macrophages after migration to the tissues (Thrall et al., 2012). Lymphocytosis is not common in ruminants; however, it may occur in chronic inflammation (Weiss et al., 2010).

Eosinophils are considered crucial cells in parasitic infections, however, the mechanisms of eosinophils during infections are not well defined (Pernthaner et al., 1995; Saddiqi et al., 2010; Shakya et al., 2011; Alba-Hurtado and Munoz-Guzman, 2013; Toscan et al., 2017). Eosinophils are responsible for providing a defense mechanism against larval stages of parasite infections, and contain proteins that bind to the parasite's membranes (Thrall et al., 2012). Eosinophils are involved in immune responses to parasites and in parasite rejection. Since *H. contortus* is located in the abomasum during infection, eosinophils are recruited into the abomasal tissue during initial infections (Balic et al., 2002, 2003). Peripheral eosinophilia or an increase in eosinophils circulating in blood is commonly considered an inconsistent parameter only associated with gastrointestinal nematodes in some circumstances (Douch et al. 1996; Woolaston et al. 1995). Kassuku et al. (1986) reported that eosinophilia is not always present in parasite infections even when eosinophils respond in tissues. Meeusen et al. (2005) reported that the activation mechanisms of eosinophils in the tissues have not been determined; however, some

results suggested that the cytokine ecosystem in the abomasum may be an important factor to determine the activation of eosinophils to kill parasites (Balic et al., 2005).

Hohenhaus et al. (1998) reported that normal eosinophil levels during parasite infection might be associated with an innate ability of sheep to resist the stress caused by the effects of parasite burden and other types of stress such as lactation and handling. Amarante et al. (1999) reported no apparent association between eosinophil or globule leukocyte numbers with parasitological parameters. Adams (1981) noted that a reduction of eosinophils or eosinopenia was present in sheep on d 105 of infection with *H. contortus*. Dalton and Selye (1939) reported that eosinopenia is also characteristic in animals exposed to different stressors. Buddle et al. (1992) reported that eosinophilia is associated with the expression of resistance to nematode infection in lambs rather than as an indicator of nematode infection. However, Amarante et al. (1999) found no association between worm burden and eosinophil counts. Wollaston et al. (1996) found in experimental and field infections that eosinophils can be genetically related to resistance under some circumstances, but not all. The association appeared stronger during experimental infection than during field infections. According to some authors, eosinophils offer no advantage over FEC as a selection for resistance (Amarante et al., 1999). Pernthaner et al. (1995) showed a trend of eosinophilia to a reduction of FEC. The eosinophil levels of resistant and susceptible lambs remained at the same levels as a control group of lambs but increased throughout the experiment.

Eosinophilia, both in blood and in tissues, is reported to be generally associated with higher protection and resistance against nematodes (Toscan et al., 2017). Some researchers who studied eosinophilia with seasonal parasitism observed that it is present

during earlier infections and greater in some sheep. Their hypothesized that the severity of the eosinophilia reflects the familial immunological resistance to nematode infection (Pernthaner et al., 1995; Pernthaner et al., 2005a; Weiss et al., 2010).

Basophils react similarly to eosinophils as they tend to increase in response to parasite infections; however basophils are not frequently reported in ruminants (Weiss et al., 2010). Basophilia has been reported in cattle tick infestations (Williams et al., 1978; Brown, 1984) and goats infected with nematodes (Richard and Cabaret., 1993). Sheep are reported to be less likely to display basophilia than other species (Rothwell et al., 1994).

Refugia concept

The need to maintain those genes that are susceptible to anthelmintic treatment in parasites has been recognized for many years (Barnes et al., 1995; Michel, 1985; Prichard et al., 1980), but the interest has been increasing in the last 10 years (Jackson et al., 2012). Recently, the “refugia” concept has been identified as an essential tool in managing resistance (Besier, 2012). This concept is based on leaving populations of nematodes that have not been exposed to treatments and allow them to survive. By doing this on purpose, the offspring provide a source of non-resistant parasites, which dilute the number of resistant worms in the environment. This reduces the contribution of developing resistance (Van Wyk, 2001; Kenyon et al., 2009; Besier, 2012). The way to implement the refugia concept is either by modifying the way treatments are implemented to ensure the survival of some proportion of the infective larvae on pasture or to avoid treating animals that can be identified as parasite tolerant. Strategies such as “targeted treatment” that are based on estimates of worm-burdens, and “targeted selective

treatments” which are based on clinical signs are some examples of different approaches to utilize refugia and benefit from the application of this concept (Besier, 2012).

Factors such as the increased risk of parasitism, production losses, the effectiveness of reducing the development of resistance, feasibility of implementation, and the direct effects on cost and labor efficiency are many factors that could affect the use of refugia. Environment, nematode species, and resources available are the main variables that affect its use. Some studies suggest that using these strategies could be an appropriate way to manage resistance in different situations (Besier, 2012), that “it is vital to manipulate refugia by the integration of the use of anthelmintics with other control strategies that utilize pasture and nutritional management, as well as host immunity and behavior to reduce our reliance upon anthelmintics”(Jackson et al., 2012).

Resistance to parasites

Resistance vs Resilience

Albers et al., (1987), reported that there was a genetic component in the ability of sheep to keep gaining weight and wool while being infected with *H. contortus*. This ability was defined as “resilient” to *H. contortus*, and it was pointed that selection for resilience would have the advantage of reducing pasture contamination, but Le Jambre (1995) found that sheep that were genetically resilient were found to be more resistant to *H. contortus*, nevertheless it was found that genetic control of resilience was partially independent of that for resistance and that it would be possible to select for resilience without an associated increase in resistance.

When an animal is resistant, it induces the initiation and maintenance of a host response that prevents, reduces, or clears parasitic infection (Hooda et al., 2007). This means that the disease is not entirely eradicated, but a lower parasitic load than the animals that are susceptible exists. Resistance is based on the immunological capabilities of each animal when exposed to parasites (Gill, 1991). Resilience on the other hand, is the ability of the animal to compensate for the effects of parasitism by maintaining its productivity (Paolini et al., 2005). Sheep are reported to be simultaneously highly resistant and resilient to haemonchosis. Even breeds that are not highly resistant, but high resilient, are allowed to be as productive as breeds that are naturally resistant.

Immune response to Haemonchus contortus

During infection, *H. contortus* has various life stages during its cycle (L3, L4, L5, and adult). These stages are different in size and expression, and there are specific antigens for L3 and L4 that are not expressed during the adult stage (Bowles et al., 1995). This is the reason why it is so hard to identify the changes in antigens that are present, because each developmental stage is immunologically a different organism (Meeusen et al., 2005). Therefore, the larval antibody response does not cross-react with the adult stage (Alba-Hurtado and Munoz-Guzman, 2013). Resistance to haemonchosis has been associated with alleles of the ovine MHC (OMCH-188) and with some surface molecules of ovine leukocytes (OLADRB2-282), this suggests that the mechanisms of antigen presentation differ between breeds (Castillo et al., 2011). Dendritic cells can internalize antigens similar to those of *H. contortus* (Vervelde et al., 2003), thus the specific response to *H. contortus* may be induced by the dendritic cell-mediated presentation of parasite antigens to helper T lymphocytes. Eosinophils also function as antigen-

presenting cells, particularly in the case of helminth infections. Eosinophils exposed to *Strongyloides stercoralis* antigens had increased expression of CD69, CD86, and MHC class II similar to dendritic cell controls, these eosinophils transformed in vitro naïve CD4⁺ lymphocytes to IL-5-producing CD4⁺ Th2 cells (Padigel et al., 2006).

Natural and experimental infections with *H. contortus* induce the production of specific antibodies. Serum antibody response has been studied extensively even though results have been variable. Some studies show an association between serum IgG levels and resistance (Munoz-Guzman et al., 2006), others have found associations with infection but not with resistance (Gómez-Muñoz et al., 1999; Amarante et al., 2005). Abomasum antibodies have been reported as more important than serum antibodies in gastroenteritis caused by nematodes. High specific IgA levels in the abomasal mucus are reported to decrease the fertility and length of *Teladorsagia circumscincta* (Martinez-Valladares et al., 2005). Amarante et al. (2005) reported a negative correlation between the amount of specific IgA in abomasum mucus and the parasite burden in *H. contortus* infection.

A typical characteristic of helminth infections is the induction of specific IgE, which results from a Th2-type response (Pernthaner et al., 2005b; Pernthaner et al., 2006). IgE induces antibody-dependent cytotoxicity in eosinophils, mast cells, and macrophages. An increase in local IgE levels has been associated with resistance to gastroenteric nematodes in sheep and goats (Alba-Hurtado and Munoz-Guzman, 2013).

Alba-Hurtado and Munoz-Guzman (2013) concluded that resistance to *Contortus* infection is an inheritable genetic characteristic associated with some breeds of sheep.

The immune response against *H. contortus* is the expression of this genetic resistance. Genetically resistant sheep have innate defense mechanism that prevent their colonization by larvae during their first infection. In addition, they establish a Th2 type immune response in the abomasum mucosa that protects them. However, susceptible sheep are not as efficient to establish this type of immune response. The immune response and the associated resistance can be modified by the type of antigen that is being recognized and by age, nutrition, and the number of infections.

Immunological tolerance

“Nematodes induce several forms of immunological tolerance to allow their longer survival” (Dineen, 1963, 1978; Maizels and Lawrence, 1991). The development of immunity against helminth infections relies heavily on sufficient numbers of parasites in the host to exceed the required threshold. It generally develops slowly, being dependent upon good nutrition, but could be repealed by any form of stress experienced by the animal (Waller, 2006).

Dineen et al. (1965) reported that nematode burdens should produce a minimum threshold level of antigenic information before immunological recognition and mobilization of host responses affecting nematode survival in the host occurs.

Manifestations of resistance to adult nematode parasites

Immunity against adult stages of nematodes is manifested as an expulsion of the adult nematode population, changes in the morphology of adult nematodes, and reduction in the fecundity of female worms (Balic et al., 2000). Expulsion of adult nematodes in ruminants is a function developed from the acquired immunity as a consequence of

repeated infection and is a common indicator of developing immunity to GIN infections (Balic et al., 2000), and is demonstrated in sheep infected with *H. contortus* (Barger et al., 1985), *O. leptospicularis* (Hertzberg et al., 1995), *T. circumcineta* (Seaton et al., 1989), and *T. colubriformis* (Barnes and Dobson, 1990).

The ability to expulse nematodes following the development of immunity to *H. contortus* and *T. colubriformis* after persistent larval infections is dependent on the level of parasite burden (Barger et al., 1985; Barnes and Dobson, 1990). Expulsion of *H. contortus* adult nematodes has occurred only in sheep given weekly doses of 2400 or 4800 L3, but not at lower frequencies of 600 or 1200 L3 per week (Barger et al., 1985). Experiments performed indicate that the ability to expulse is dependent on the level of infection; though, experiments do not differentiate if the loss of adult stages is a result of the development of immunity against larval stages or if higher larval levels result in the more rapid accumulation of adult nematodes and the development of adult-specific expulsion mechanisms (Balic et al., 2000). This mechanism of expulsion resulting from immune mechanisms activated by larval stages has been reported in ruminants, and it has been attributed to an immediate hypersensitivity response in sheep against incoming larva, affecting the adult nematode population without being specific (Miller, 1984; Rothwell, 1989). There is evidence that the expulsion of adult nematodes occurs due to the development of acquired immunity, particular to the adult stage. It is independent of the development of resistance to larval establishment as the expulsion of adult nematodes in ruminants develops at a different rate to other manifestations of immunity in continuously infected animals, and the development of immunity to adult nematodes in the absence of larval infections (Balic et al., 2000).

The change in morphology of gastrointestinal nematodes of ruminants have been observed and described the reduced size of adult nematodes or the loss of vulval flaps in adult female worms. These changes have been observed in sheep infected with *T. circumcincta* (Smith et al., 1985; Seaton et al., 1989; Stear et al., 1995), *H. contortus* (Coyne and Smith, 1992), and *T. colubriformis* (Douch, 1988). Coyne and Smith (1992) described that lambs exposed to 30 000 L3 *H. contortus*, after removing a training infection of 30 000 L3 three weeks before, had reduced adult burdens when compared to control animals, and the adult worms collected in the group were smaller in size. Sheep that had low FEC also had significantly smaller female *Trichostrongylus* spp. Compared to sheep with high FEC.

Hair sheep breeds

Hair sheep breeds have several advantages that make them a perfect fit for sustainable production systems. Some of these advantages include resisting extreme hot temperatures, do not require shearing, and may be more resistant to gastrointestinal nematode infections (GIN) than most wool breeds (Burke and Miller, 2002). In many studies, it has been mentioned that hair sheep breeds are resistant to gastrointestinal parasites; however, the primary mechanism of resistance has not been identified. Some hair sheep breeds such as St. Croix have developed what is called “a natural resistance to parasites,” which is reported to be relatively mediated by a particular increase in their humoral immune response to parasites (Gamble and Zajac, 1992). Bowdridge et al. (2013) revealed that parasite-resistant sheep have a distinct advantage in developing a more effective and specific acquired immune response as evidence shows an increased number of local and circulating antibodies in the blood.

The resistance of sheep against gastrointestinal infections has been recognized for both wool and hair sheep breeds. Both breeds have demonstrated to poses some degree of resistance, but there is strong evidence that suggests hair sheep breeds are more resistant than wool sheep breeds (Bishop, 2012). For many years, several authors have been trying to determine which breeds are more resistant due to the crisis of the sheep industry that is mainly threatened by gastrointestinal nematodes that are resistant to the available anthelmintics (Burke and Miller, 2002).

Alba-Hurtado and Munoz-Guzman (2013) created a summary of comparative studies of susceptibility and resistance to *Haemonchus contortus* or gastrointestinal nematodes between sheep breeds. They presented breeds that were resistant and susceptible to a specific parasite or type of parasite infection if infections were artificial or natural and the variety of evaluated parameters. Main breeds resistant to *H. contortus* are Florida Native (Zajac et al., 1990), Saint Croix (Courtney et al., 1985; Gamble and Zajac, 1992), German Merino (Gauly et al., 2002), Katahdin, and crossbred Blackbelly x Saint Croix (Burke and Miller, 2002; Vanimisetti et al., 2004), Crioula Lanada of Brazil (Bricarello et al., 2004), Red Maasai (Mugambi et al., 2005), Santa Ines (Bricarello et al., 2005), Blackbelly (Munoz-Guzman et al., 2006), Lohi (Saddiqi et al., 2010), Criolla Native to the Central Mexican Plateau (Alba-Hurtado et al., 2010), Gulf Coast Native (Shakya et al., 2011), and Canaria Hair (Gonzalez et al., 2008).

Breeds that are resistant to other gastrointestinal nematodes are Red Maasai (Mugambi et al., 1997), Pelibuey (Diaz-Rivera et al., 2000), Sabi (Matika et al., 2003), Saint Croix (Burke and Miller, 2002), and Texel breed (Good et al., 2006). Rhön breed is classified as resistant to *Trichostrongylus spp.* (Gauly and Erhardt, 2001), and

Blackbelly to *H. contortus* and *Trichostrongylus colubriformis* (Gruner et al., 2003).

These determinations were made in several studies using different parameters that included fecal egg count, histological cellular count in the abomasum (eosinophils, leukocytes, mast cells, etc.), immunological detection of antibodies, hematological and biochemical analysis, use of FAMACHA score, and live weight (Alba-Hurtado and Munoz-Guzman, 2013).

Grazing systems

Increasing forage production, forage quality, and efficiency of utilization, with a consequent increase in production per area per animal are the goals when thinking of grazing management systems (Santos et al., 2017). Stocking rate is one of the most critical management decisions for both grazing and pasture management as it influences a wide range of production characteristics such as the prevalence of pasture, diet selectivity, animal production rates, soil compaction, and in cases of wool animals, wool quality and quantity (Cottle et al., 2013).

Continuous grazing

Continuous grazing is a type of grazing that involves grazing a particular pasture throughout the grazing season without resting periods (Holecheck, 1983). The primary problem associated with continuous grazing is overgrazed areas due to plant selectivity and preferred areas. These areas receive excessive even when using light stocking rates and are usually areas where water, shelter, or better quality of forage are in close proximity.

Continuous grazing is considered the most common grazing system in the United States, and it often results in overgrazing and having an increase of less-desirable plant species (Beetz and Rinehard, 2004). Some plants are repeatedly grazed, while others are not being grazed at all. When this happens, plants that are being eaten cannot recover and die, while plants not being eaten mature and go to seed, therefore increasing populations of undesirable plants, reduces the overall quality of forage in the pasture. On the other hand, continuous grazing has the benefit of low investment since fewer fencing and water facilities are required than with rotational grazing systems. Management is simpler because livestock is moved less frequently. Some studies demonstrate that rotational grazing and continuous grazing have similar effectiveness on rangelands (Briske et al., 2008). However, it has been reported that using grazing systems on rangeland has reported an increased range of health and animal performance (Sayre, 2001). Continuous grazing has been reported to result in higher per-animal gains than other grazing systems, as long as enough forage is available to maintain increased growth rates (Beetz and Rinehard, 2004).

Rotational grazing

Rotational grazing is a type of grazing management strategy characterized by having a division of several paddocks with a periodical livestock movement to allow pastures to rest and regrow before being grazed again. Some popular rotational grazing systems include Management-intensive Grazing, multi-pasture rotation, and short-duration grazing (Gerrish, 2004; Hanselka, et al., no date). Minor differences between how each type of system is described, but they are established on rest periods to have satisfactory forage regrowth (Beetz and Rinehard, 2004). Rotational grazing increases

pounds of animal production per acre, but the production level depends on how the system is managed. Although rotational grazing increases stocking densities in the short term, it includes resting periods when the vegetation is allowed to recover from grazing (Hao et al., 2013). This may maintain or even increase the short-term as well as long-term grassland productivity (Virgona et al. 2000).

Effects of rotational vs continuous grazing systems on the performance of sheep

Briske et al. (2008) reported that 87% of 32 studies on vegetation response found a similar or lower herbage production in rotational grazing than continuous grazing; they also reported that 92% of 38 experiments that analyzed the effects of grazing systems on animal performance determined a similar or lower animal live weight gain in rotational grazing than in continuous grazing. Hao et al. (2013) reported results of four study years and showed that herbage quality, feed intake, and weight gain of sheep grazing the Inner Mongolian steppe at a moderate grazing intensity is inferior in rotational grazing than in continuous grazing. However, higher energy intake in continuous grazing did not result in a corresponding increase in weight gain, because animals used a greater amount of ingested energy for purposes other than maintenance and growth, likely for grazing and walking. They reported that the differences between grazing systems might vary between years according to the amount of rainfall and thus herbage mass on offer and differences in the ecosystem, the animal species used and the applied stocking rates.

Derner and Hart (2007) reported that rotational grazing did not show greater live weight gain compared with continuous grazing. They concluded that rotational grazing could help achieve conservation goals desired by society or produce benefits through

modifications of seasonality and intensity of use in the paddocks to create good vegetation management, generating a more even use. Wang et al. (2009) analyzed the effects of rotational and continuous grazing on herbage as well as on feed intake and live weight of sheep and found that the higher feed intake and digestibility did not result in improved daily live weight gain of sheep because of the extra energy required for grazing and walking which reduced the amount of energy available for growth.

Burke et al. (2009) reported similar weight gains between continuous bermudagrass pasture and rotational bermudagrass pasture grazing systems with the exception of a period during the first 28 days for the rotational group in 2007. They noted that managing a rotational plot based on forage height could lead to poor weight gains if animals are forced to graze poorer quality forages.

Hunt et al. (1958), in a three-year study, reported that ewes gained slightly more weight on continuous grazing than on the rotational grazing in two out of the three years; however, one season showed an advantage for the rotational pastures. Overall, they reported that sheep gained an average of 1.16 lbs. more when using rotational grazing, but two out the three years, sheep gained more when grazed continuously. They also reported that lambs were not consistent in their performance, but they averaged .02 lbs. per day more gain when using rotational grazing. They concluded that from a practical standpoint, would not pay for the cost of extra fencing required to segregate and rotate animals.

Santos et al. (2017) reported that sheep grazing simultaneously with cattle had higher live weight gains than the sheep that grazed after cattle, while the grazing system

with sheep only showed intermediate results, which was not different from the others. Sheep that were grazing with cattle and sheep only had better forage utilization, as the same dry matter production and the forage available for the animals was richer in leaves, where most digestible nutrients are concentrated. This difference was explained by the fact that sheep grazing with cattle and sheep only had a higher stocking rate during the period of occupation since all the animals allocated to their respective grazing systems consumed the available forage in one week while when sheep that were grazed after cattle, the same paddock was occupied for 14 days, and 7 days for each of the cattle and sheep categories, which have different grazing habits.

Grazing management systems to control parasite infections

When looking for economic profitability in production systems, the only possible option is to have grazing pressure and short grazing intervals (Uriarte and Valderrábano, 1989). That is why intensive rotational grazing systems are mainly established to improve pasture utilization and animal performance. Increasing stocking rate is often accompanied by improved pasture production, fertilizer and irrigation to improve pasture growth (Waller, 2006). However, enhanced productivity of pasture following fertilizer often fails to improve overall animal production (Speedy, 1980), and a several studies have shown a direct relationship between increased stocking rates and increased levels of parasitism of livestock (Beveridge et al., 1985; Brown et al., 1985; Downey, 1969; Southcott et al., 1967, 1970; Thamsborg et al., 1966; Zimmerman, 1965).

“Environments favorable for the establishment and maintenance of improved pasture species also favor the development, survival and transmission of

nematode parasites of ruminant livestock. The distribution and abundance of nematode parasites varies considerably within and between species, with the principle determinant being the prevailing weather conditions – most importantly rainfall and temperature” (Waller, 2006)

The degree of contamination acquired by grazing animals relies on many inter-related factors. These include weather conditions, which are reported to determine the accessibility of infection and pasture, grazing behavior, previous infections, and the physiological state of the animal. The effects of parasite infection can be modified by nutrition but are not directly related to the number of parasites available in the environment. Therefore, parasitism on pasture utilization is influenced by the effects on the physiological and metabolic processes in the infected animals and by management decisions taken to reduce the degree of infection on pasture (Waller, 2006). Some authors suggest that when a lower worm egg count in intensive rotational grazing is observed, it may be due to a greater resistance regulated by better nutrition (Colvin et al., 2012).

The use of grazing management strategies, combined with anthelmintic treatment, may result in better parasite control at lower costs but may not significantly reduce the selection pressure for resistance development. It has been argued that the selection for resistance in any control strategy involving anthelmintics will be more closely related to the degree of success of the control strategy than to the frequency of treatment it involves. (Barger, 1995; Waller, 2006). New methods of grazing management have been weakly investigated. Grazing management should be integrated as part of a control program of parasites (Ruiz-Huidobro et al., 2019). The development of successful

grazing systems should utilize management alternatives combined with anthelmintic treatments (Uriarte and Valderrábano, 1989).

Briefly, Michel (1985) classified grazing management strategies aimed to control nematode infections in ruminants as Preventive, which are strategies that rely on having worm-free animals on clean pastures, or by suppressing worm egg count yield by treating with anthelmintics early in the grazing season; Evasive, which do not attempt to avoid contamination of the pasture but rely on movement to another pasture just before larvae are likely to appear in substantial numbers on the original pasture; and diluting, which are strategies that manipulate grazing of susceptible animals with a greater population of animals with acquired natural resistance to parasites of the same livestock species or different to reduce invasion resulting from their fecal yield of worm eggs.

The combination of anthelmintics with these strategies is highly recommended, but even though combination strategies were highly effective at controlling infections, they also proved to select potentially for anthelmintic resistance (Besier, 1999).

Populations that exist normally on pastures will make a disproportionate contribution to the anthelmintic resistance status of parasite generations (Barnes et al., 1995). In a study performed by Colvin et al. (2012), intensive rotational grazing sheep showed significantly higher worm egg count than those on high intensive grazing or typical grazing that consisted in long grazing periods, a high proportion was anemic, had lower numbers of circulating eosinophils and lower bodyweights, but similar bodyweight gain. Colvin et al. (2012) reported that the effects of intensive rotational grazing were due to a disruption of the parasite lifecycle outside of the host rather than differences in the host immunity, meaning that the impact of intensive rotational grazing on worm egg count is

mediated by reduced larval challenge rather than increased resistance to infection. They also reported that shorter rest periods between grazing have demonstrated to ensure the maintenance of higher L3 burdens on pasture, with less time to die off.

Short-term rotational grazing using weekly rotation across four paddocks showed no effect on worm burdens (Roe et al. 1959), but there has been circumstantial evidence of low drenching requirements on some areas that practiced more intensive rotational grazing with a shorter period of grazing and long rest periods with more typical management strategies (Walkden-Brown et al., 2013). Ruiz-Huidobro et al. (2019) noted that the cell grazing system reduced fecal egg count in lambs and ewes during a six-year-long survey. Ewes in the cell grazing system excreted more eggs than ewes in the rotational grazing system in May-August 2017 and May 2018. When considering the whole study, the type of grazing system did not influence significantly in the intensity of egg excretion. They concluded that difference in weather and seasonality may influence the results of this application, and that specific studies are necessary to prove that intensive rotational grazing works in different environments. Ruiz-Huidobro et al. (2019) reported that the proportion of ewes excreting more than 500 EPG was significantly higher in cell grazing (34%) than in the rotational grazing system (23.3%).

Forage biomass and nutrient composition in rotational grazing

Hao et al. (2013) found in a four-year study that the mean herbage mass on offer in rotational grazing and continuous grazing was 628 kg DM/ha, and it did not differ between grazing systems, but the chemical composition of herbage was influenced by type of grazing system. Crude protein concentrations were lower ($P < 0.05$) in the

rotational grazing than the continuous grazing, whereas concentrations of acid detergent fiber (**ADF**) were greater in rotational grazing ($P < 0.05$) than in continuous grazing, but the grazing system did not affect concentrations of neutral detergent fiber (**NDF**).

Correlations between parasite burden, FAMACHA score and hematological parameters

The estimation of correlations between parasite resistance, performance, and other characteristics are necessary to develop management programs. The ability to select for resistance when breeding and have no adverse effects in other traits is the main goal to improve animal health and economic efficiency in sheep production (Nieuwhof and Bishop, 2015; Ngere et al., 2018). Some studies demonstrate a positive correlation between FEC values and the mean hemoglobin concentration (MCH; 0.70) and mean corpuscular hemoglobin concentration (MCHC; 0.65) (García et al., 2015). García et al., 2015 reported no direct relationship between parasitic infestation, the physical conditions, BW, BCS, and MCH values. Other studies observed a negative correlation for PCV (-0.61) and positive for MCV (0.32) compared to FEC (Sampaio, 2007).

Burke et al. (2007) evaluated the accuracy of the FAMACHA system used on farms by small ruminant producers in the southeastern United States during the summer months. The correlation between FAMACHA scores and PCV or FEC and BCS was calculated. Two anemia threshold levels (FAMACHA ≥ 3 versus ≥ 4) and PCV values of (≤ 19 versus $\leq 15\%$) were used to provide alternative views of the data; since no specific values for PCV have been established for anemia, then sensitivity, specificity, and predictive values were calculated according to Vatta et al., (2001).

“Sensitivity was defined by the proportion, true positives/(true positives + false negatives); specificity by true negatives/(true negatives +false positives); predictive value of a negative (PVneg) by true negatives/(true negatives + false negatives); and predictive value of a positive (PVpos) by true positives/(true positives +false positives).” (Burke et al., 2007)

A true positive was defined as an animal that was anemic with a PCV value of ≤ 15 or $\leq 19\%$ with FAMACHA score of ≥ 3 or ≥ 4 . A false positive was defined as an animal that was not anemic but presented high FAMACHA score. A false negative was defined as an animal that was truly anemic but were assigned low FAMACHA score. A true negative was defined as an animal that was not anemic with low FAMACHA score. Correlations and regressions between eye scores and FEC were 43.5 and 18.9% ($P < 0.001$) for sheep and 14.9 and 2.2% ($P < 0.001$) for goats. PCV values for sheep ranged from 8 to 47% for sheep and 7 to 49% in goats. The correlation and regression between eye scores and PCV were 28.8 and 5.7% ($P < 0.001$) in sheep and 19.9 and 3.9% ($P < 0.001$) in goats. Burke et al. (2007) found a significant relationship between FAMACHA scores and FEC and PCV. However, they noted that this relationship was not as close-fitting as when scores were assigned by more experienced scientists in a previous study (Kaplan et al., 2004).

Di Loria et al. (2009), in an experiment performed in Southern Italy, compared FAMACHA score values to Hematocrit and Hemoglobin values, considering a score of 3 as a borderline value for anemia and anemic animals were considered in the categories of 4 and 5. Anemia due to nematode infection was considered when hematocrit and hemoglobin values were under 27% and 9 g/dl. The correlation between FAMACHA

score and hemoglobin/hematocrit was significant ($p < 0.001$) and negative; however, low correlation coefficients indicated a weak negative association between FAMACHA and either hemoglobin or hematocrit parameters. Di Loria et al. (2009) concluded that the FAMACHA system showed a loss of sensitivity in detecting anemic sheep. Findings indicate that under the experimental condition in Southern Italy, FAMACHA may represent an additional element that can integrate clinical examinations. It is important to evaluate the system in each region before the system is implemented, as there could be issues that affect the accuracy of the system.

Mohammed et al., (2016) reported a significant correlation ($r = 0.289$; $p = 0.042$) between the severity of the infection and the FAMACHA score in Damara and Barbados Blackbelly cross sheep in Malaysia. However, it showed no correlation between EPG and FAMACHA score ($r = 0.237$; $p = 0.097$), meaning that an increase in anemia does not necessarily increase EPG; however, the severity of infection was correlated with the FAMACHA score ($r = 0.289$; $p = 0.042$).

Stear et al., (1995) in an experiment during infections with *Ostertagia circumscincta* in Scottish blackface sheep FEC, peripheral eosinophil count and plasma pepsinogen concentrations were measured and reported no correlation between eosinophil count and adult nematodes at slaughter in most sampling dates of the experiment. However, there were negative associations with adult worm burdens, and eosinophil counts 17-31 and 48-52 days after infection during the second time of infection. The correlation matrix revealed a significant negative correlation between transformed eosinophil count ($r = -0.46$; $P < 0.05$).

Notter et al. (2017) reported correlations between log transformed FEC and FAMACHA scores ranging from 0.25 in Katahdin lambs at approximately 90 d to 0.31 at approximately 120 d of age. These associations were obtained with geometric means for FEC of approximately 1,000 eggs/g and means for FAMACHA scores of approximately 1.7. These results are consistent with increasing FAMACHA scores in sheep, and a correlation of 0.44 between FAMACHA score and FEC in lambs with arithmetic mean FEC of approximately 945 eggs/g. However, in that study, PCV ranged from 8 to 47%, but the correlation between FAMACHA scores and PCV was only 0.24. Results indicate that, with uniform training, consistency in the relationship between FAMACHA outcome groups and FEC can be achieved among flocks (Notter et al., 2017). FAMACHA scores have the potential to both facilitate selective deworming of highly parasitized individual lambs and improve breeding value estimates in programs designed to genetically improve parasite resistance (Notter et al., 2017).

Ngere et al. (2018) noted that evidence for additive genetic variation in both innate and acquired resistance to internal parasites was observed in Katahdin sheep. A strong genetic correlation between weaning and post-weaning fecal egg count indicated that selecting one would improve the other. Relationships between FEC and BW revealed no significant genetic antagonism between these traits. Breeding for increased resistance to internal parasites in Katahdin lambs would not be expected to have adverse effects on BW.

CHAPTER II

EFFECTS OF GRAZING MANAGEMENT ON PERFORMANCE, PARASITE BURDEN, AND HEMATOLOGICAL PARAMETERS OF HAIR SHEEP EWES AND LAMBS

INTRODUCTION

Gastroenteric diseases due to nematode infections have a significant economic impact on the sheep industry (Waller, 2006; Miller et al., 2011; Jackson et al., 2012; Walkden-Brown et al., 2013; Ruiz-Huidobro et al., 2019). Gastrointestinal nematode infections have an obligatory free-living stage that reside within the pasture (Waller, 2006). The free-living stage is considered the infective stage for animals to become parasitized from the nematodes (Waller, 2006). Therefore, pasture management is crucial for the management of internal parasites in grazing livestock.

Additionally, hair sheep breeds have demonstrated an increased resistance to parasites compared to wool sheep breeds (Burke and Miller, 2002). The control of parasitic diseases in sheep is dependent on the administration of anthelmintics; however, anthelmintic resistance has become a significant concern worldwide due to the overuse of

these products. Anthelmintics have been used to control and limit the effects of parasites (Marques et al., 2018), and as a result, there has been an increase in reported cases of anthelmintic resistance in sheep and goats worldwide (Kaplan et al., 2004; Jackson et al., 2012). This anthelmintic resistance has caused alternative control parasite strategies to gain more attention. Strategies such as intensive rotational grazing (Walkden-Brown et al., 2013) and the use of targeted selective treatments (Kenyon et al., 2009; Jackson et al., 2012; O’Shaughnessy et al., 2015; Mohammed et al., 2016) such as the use of the FAMACHA™ system are considered alternative approaches to parasite management.

Parasites that survive anthelmintic treatments are considered resistant “when a greater frequency of individuals in a parasite population, normally affected by a certain dose or concentration of compound, are no longer affected, or greater concentration of drug is required to reach a certain level of efficacy.”(Prichard et al., 1980; Wolstenholme et al., 2004). These resistant parasites have demonstrated the ability to carry resistant genes to the offspring, increasing the number of resistant eggs released to the environment (Jackson et al., 2012). The need to maintain genes that are susceptible to anthelmintic treatments has increased over time (Jackson et al., 2012), and recently, the “refugia” concept has been considered a fundamental tool when managing resistance (Besier, 2012). The “refugia” concept consists of letting populations susceptible to treatment or that are unexposed to treatment survive. This has demonstrated to reduce resistance development (Van Wyk, 2001; Kenyon et al., 2009; Besier, 2012). The refugia concept can be implemented by modifying how treatments are applied or by avoiding treating animals that can be identified as parasite tolerant (Besier, 2012). Therefore, it is fundamental to manipulate refugia by integrating the use of anthelmintics with other

strategies such as pasture management, nutritional management, and host immunity to reduce the dependence on anthelmintics (Jackson et al., 2012). Ruiz-Huidobro et al. (2019) suggested that grazing management should be integrated as part of a comprehensive parasite control program.

We hypothesized that implementing an extensively managed rotational grazing system similar to how a common sheep producer would implement could improve performance and decrease the dependence on anthelmintics in hair sheep breeds due to a reduction of parasite burden while implementing the use of FAMACHA™ scores to maintain refugia population. The objective of this experiment was to determine if extensively managed rotational grazing would impact the performance, fecal egg counts (FEC), and hematologic profiles of hair sheep ewes and lambs.

MATERIALS AND METHODS

All procedures were approved by the Institutional Animal Care and Use Committee at Oklahoma State University (Animal Care and Use Protocol number: AG-11-16).

Animals and experimental treatments

A total of 577 crossbred hair sheep ewes ($n = 305$) and lambs ($n = 272$) were used in a 5-yr experiment comparing 2 pasture management systems. Initially ewes were delivered to the Oklahoma State University Sheep and Goat Center in Stillwater, Oklahoma for yrs. 1 and 2, and ewes, and replacement ewe lambs that remained from the previous yr were used again in the following yrs. Upon arrival, all ewes were ear-tagged and placed in common grazing plot until the experiment started. The ewes remained in

this plot and were managed as a single group when not on experiment for the following yrs. Table 1 shows the constitution of the experimental groups of ewes and lambs per yr as well as average initial BW each yr.

Ewes and lambs were randomly pairwise assigned to 1 of 2 experimental treatments by lamb status, initial BW, and initial FAMACHA™ score. Experimental treatments included a continuous (**CON**) grazing treatment where animals continuously grazed a primarily bermudagrass (*Cynodon dactylon*) single pasture (4.4 ha.) and a rotational (**RTL**) grazing treatment where ewes were rotated every 7 d among 5 different primarily bermudagrass (*C. dactylon*) pastures (1.06 ha. average per pasture). At the end of the last collection of each yr, all ewes were turned out together to a common pasture and lambs were weaned. Ewes were then managed as a single group and rotated among pastures until the experiment was resumed the following yr.

Meteorological data

Weather data were collected and summarized from the Oklahoma Mesonet Stillwater station (Ronald L. Elliott, 1993). In 2015, 2017, and 2019, the study occurred during the months of June, July, August, and September, and in 2016 and 2018, the study occurred during the months of May, June, July, and August. Figure 1-4 show the average values per collection period for each yr and include the average maximum and minimum temperature (C) and the average daily precipitation (mm) during the 5 yr of experiment.

Data collection

For the first 2 yr, collections were performed every 35 d, and for the following 3 yr, data were collected every 28 d. Due to the variation in d between collections from yr

to yr, the number of collection (collection 0 = late-spring, 1 = early-summer, 2 = mid-summer, or 3 = late-summer) were used instead of the actual d of the experiment. Each collection d represents approximately 1 collection per month (May through August or June through September) conducted approximately every 28 or 35 d. More specifically, in yr 1 and 2 performance data collection occurred on d 0, 35, 70, and 105; however, in yr 3, 4, and 5 collection of data occurred on d 0, 28, 56, and 84.

Every collection period, ewes were weighed (WW Paul Scale Model 70SX; WW Livestock Systems, Thomas, OK), body condition scored (BCS) using on a 1-5 scale (Russel, 1984; where 1 is emaciated and 5 is fat), FAMACHA™ scored (Vatta et al., 2001; where 1 represents “not anemic” and 5 represents “severely anemic”) and had blood and fecal samples collected.

Blood samples were collected via venipuncture from the jugular vein into ethylenediaminetetraacetic acid (EDTA) vacutainer tubes (BD; Franklin Lakes, NJ), and kept on ice until analysis. Within 6 hr. of collection, samples were analyzed for complete blood count using an Idexx ProCyte Hematology Analyzer (IDEXX Laboratories; Westbrook, ME). The hematology analyzer measures blood parameters using laser flow cytometry, optical fluorescence, and laminar flow impedance. Data collected from the hematology analyzer included white blood cells (**WBC**), red blood cells (**RBC**), hemoglobin (**HGB**), hematocrit (**HTC**), mean cell volume (**MCV**), mean cell hemoglobin (**MCH**), mean cell hemoglobin concentration (**MCHC**), platelet count (**PLT**), neutrophils (**NEU**), lymphocytes (**LYM**), monocytes (**MONO**), eosinophils (**EOS**), and basophils (**BASO**).

Fecal samples were collected directly from the rectum via palpation and fecal egg counts (**FEC**) were performed using a modified McMaster technique (Whitlock, 1948).

At each collection, ewes with a FAMACHA™ score of 4 or 5 were treated with an anthelmintic. Anthelmintics were alternated to reduce parasite resistance. First anthelmintic treatment utilized albendazole (7.5 mg/kg; Valbazen™, Zoetis LLC, Parsippany, NJ); second anthelmintic treatment utilized ivermectin (0.2 mg/kg; Ivermectin Sheep Drench, Durvet Inc, Blue Springs, MO), and third anthelmintic treatment utilized fenbendazole (5 mg/kg; Safeguard™, Merk & Co., Kenilworth, NJ).

Forage analysis

Five random samples from the continuous pasture and 1 random sample from each rotational pasture (total = 5) were obtained to determine DM yield and forage nutrient composition. Samples were dried in a 40°C oven for 24 hr. to determine DM yield. Laboratory DM was calculated by weight difference when samples were dried at 105 °C for 24 hr. Acid detergent fiber (**ADF**) and neutral detergent fiber (**NDF**) were analyzed using an ANKOM 2000 automated fiber analyzer (ANKOM Technology; Macedon, NY). Percent N was determined using combustion analysis in a crude nitrogen analyzer (TruSpec CN; LECO; St. Joseph, MI).

Crude protein was calculated using the following equation:

$$\% \text{ N} \times 6.25 = \% \text{ CP}$$

Proximate analysis was performed for each sample every yr and average nutrient composition per yr can be found in Table 2.

Statistical analysis

Performance, fecal, and blood data were analyzed as a completely randomized design using the PROC MIXED procedure of SAS (SAS 9.4), where the model included the fixed effects of treatment. Year was included as a random effect, and means were separated using the pdiff option. All values were averaged over the 5 yr for each collection date. Statistical significance was declared at $P \leq 0.05$ and a trend was considered at $0.05 < P \leq 0.10$.

RESULTS AND DISCUSSION

Meteorological conditions, forage yield, and nutrient composition

The average maximum temperatures varied from 30.2 to 34.4 °C (Figure 1) during the experiment, with mid-summer recording the highest maximum temperatures during the experiment in every yr. The fourth yr (2018) had the highest temperature in June with 34.4 °C in mid-summer. The average minimum temperatures varied from -2 to 23.1 °C (Figure 2) during the experiment, with late-spring recording the minimum temperatures every yr. The yr that presented the lowest temperature was 2018 in late-spring with an average minimum temperature of -2 °C. In general, the coldest temperatures were observed during late-spring, and the yrs. when the experiment initiated in May had the lowest temperatures overall.

The average daily precipitation varied from 0.25 up to 12.44 mm during the experiment (Figure 3). The first yr (2015) had 7.62 mm during early summer; however, less than 4 mm of precipitation were recorded during the remaining collection periods for that yr. The highest precipitation recorded for the second yr (2016) were 3.81 mm during

mid-summer, with lower precipitation during the rest of collection periods. The third yr (2017) had 5.08 mm during early-summer and 4.82 mm during late-summer, with less than 3 mm of precipitation during late-spring and mid-summer. The fourth yr (2018) had 0.45 mm during late-spring, then presented 6.60 mm during early-summer, 1.5 mm during mid-summer and 6.09 mm during late-summer. The last yr (2019) had the highest precipitation among yrs. and collection periods during late-spring, with 12.44 mm; however, 1.27 mm and 1.77 mm were recorded during early and mid-summer, and then 5.58 mm were recorded during late-summer. Overall, average precipitation varied among yrs. and collection periods; however, highest precipitation is likely to be reported during late-spring and late-summer, depending on the months of experiment.

The average daily humidity varied from 55.5% to 76.5% during the experiment. The lowest average daily humidity corresponded to the lowest temperature and precipitation reported during late-spring in 2018. Overall, average relative humidity remained between 65.7 to 71.1% during the experiment in all yrs.; however, mid-summer had less than 70% relative humidity in all yrs.

Forage production ranged from 2,335 to 6,384 kg/ha during the 5 yr of the experiment (Table 2). In 2015 and 2016, no fertilizer was applied to the research pastures; however, in 2017, 2018, and 2019, 100 kg/ha of 46-00 urea fertilizer were applied. Forage production in 2015 and 2016 was similar, varying from 2,335.3 to 3,691.8 kg/ha with similar yield in both treatments. Forage production increased in 2017, corresponding with the start of the use of fertilizer and the increase in rain. Overall, the yr when fertilizer was applied had greater production than the first 2 yr. The greatest production per ha was in 2018.

Neutral detergent fiber % (NDF) varied from 62.9 to 72.4 %. In the first 2 yr, NDF was lower in the RTL pastures than the CON pasture, with 62.9% for the RTL pastures and 72.4% for the CON pasture. During the following 3 yr, average NDF content remained steady approximately 66.1 to 67 % range for both treatments. Acid detergent fiber % (ADF) varied from 28.6 to 37.0 %. The highest percentages of ADF were observed in the first 2 yr, and % ADF was lower in the following 3 yr. The year with the lowest ADF % was 2017, with 28.6% for the RTL treatment and 30.3 % for the CON treatment.

Percentage CP in the forage samples varied from 8.1 to 12.2% on average during the 5 yr of the experiment. In the first yr, similar CP % was observed between treatment pastures, having an average of 8.1 % CP in the CON, and 8.8 % CP in the RTL treatment (Table 2). In the second yr, the RTL pastures had greater % CP (10.3) than the CON pastures (7.8); however, in the third yr the CON pasture had greater % CP (12.2) than the RTL pastures (10.3). This is similar to what Eich horn et al. (1972) reported where the N rate positively affects bermudagrass yield and CP. Eich horn et al. (1972) reported that CP content was around 8.6 % when no fertilizer was used, which is similar to this experiment, and when 100 kg of N per ha were used, CP increased to 10.8%. In general, average nutrient composition remained similar in both treatment pastures, with some yr-to-yr variation due to weather, temperatures, and a positive impact due to the use of fertilizer.

Performance of ewes

No difference in ewe BW was observed between treatments ($P \geq 0.47$; Table 2) at any collection period. This result is similar to Hunt et al. (1958) that discovered in a 3-yr experiment where ewes gained slightly more weight on continuous grazing during 2 yr of experiment, but there was an advantage during 1 yr of experiment for ewes on rotational grazing. Hunt et al. (1958) mentioned that from practical standpoint, the difference in gain would not pay for the extra cost and labor that rotational grazing requires. Wang et al. (2009) established that grazing systems did not affect live weight gain in sheep ($P = 0.41$) when analyzing different grazing systems, and even though there were greater feed intake and digestibility in the continuous grazing system, this did not result in improved live weight gain of sheep due to the extra energy required for walking a larger area due to plant species selectivity. Santos et al. (2017) also reported no differences between different grazing systems in BW, carcass traits, and retail commercial cuts. Unlike Wang et al. (2009), Santos et al. (2017) concluded that grazing systems did not affect DMI of sheep, and observed that the best forage production and animal performance was observed when using continuous grazing with both cattle and sheep at the same time or grazing only sheep continuously. Briske et al. (2008) reported that 87% of 32 studies on vegetation response had a similar or lower herbage production in rotational grazing than continuous grazing, and 92% of 38 experiments that analyzed the effects of grazing systems on animal performance determined a similar animal live weight gain in rotational grazing than in continuous grazing.

Average BCS in late-summer tended to be greater ($P = 0.06$; Table 2) for the RTL treatment (2.78) compared to the CON treatment (2.70). The percentage of animals that

had a BCS $\geq 2+$ was not different between treatments ($P \geq 0.44$) at any collection. However, the percentage of ewes that scored a $\geq 2+$ increased over time, with approximately 87% of the ewes scoring $\geq 2+$ in late-spring and 94% of the ewes with a BCS $\geq 2+$ in late-summer. These results can be attributed to improved forage quality, nutritional composition, and the management implemented with targeted selective treatments.

The FAMACHA™ score in late-summer tended to be greater ($P = 0.07$; Table 3) for the RTL treatment (2.55) than CON treatment (2.41). The percentage of animals with a FAMACHA™ score of ≥ 4 was not different between treatments ($P \geq 0.34$) at any collection, but a reduction in the percentage of animals with critical FAMACHA™ score ≥ 4 decreased over time. At the beginning of the experiment, 16.8% of the ewes in the CON treatment and 16.2% in the RTL treatment had a FAMACHA™ score of ≥ 4 , while at the last collection, 5.45 % of the ewes in the CON treatment and 7.57% in the RTL treatment had FAMACHA™ scores ≥ 4 . There were no differences between the percentage of animals that were treated with an anthelmintic between treatments ($P \geq 0.40$; Table 3) at any collection. However, a decrease in the percentage of animals treated over time was also observed. On average, in late-spring, about 15.1 % of ewes on the CON treatment were treated with an anthelmintic while on the RTL treatment, 16.0% of the ewes were treated. In late-summer, only 4.84 % of the ewes in the CON group were treated, and 2.79 % of the ewes in the RTL were treated, which indicates that the percentage of ewes treated with an anthelmintic decreased by 10.2% from late-spring to late-summer for the CON treatment, and 13.2% for the RTL treatment during the same interval. The reduction in treatments is similar to other experiments where the

FAMACHA™ system demonstrated a reduction of 50% of anthelmintic treatment in sheep and goat flocks (Vatta, 2001; Kaplan et al., 2004).

No differences in mortality were observed between treatments ($P = 0.67$; Table 3). From the 305 ewes, 26 mortalities and 32 mortalities from the 281 lambs were reported during the 5 yr of the experiment. The yr that presented the majority of the mortalities was 2016.

Performance of lambs

No difference in BW between treatments was observed ($P \geq 0.57$; Table 4) at any collection. This is in agreement with Colvin et al. (2008), where lambs under different grazing systems did not differ in BW. Burke et al. (2009) also reported similar BW gain between lambs that were grazing continuous and rotational bermudagrass pastures on a multiple-yr study. Burke et al. (2009) reported an exception where the rotational group performed better during the first 28 d in one of the yr of the study, however, no differences were established between continuous or rotational grazing after that. The percentage of lambs that scored a BCS of 2+ or higher tended to be greater for the CON treatment ($P = 0.07$) in late-summer. There was no difference in average FAMACHA™ score between treatments ($P \geq 0.24$; Table 5) at any collection. There were no differences between treatments for the anthelmintic use in lambs ($P \geq 0.37$) at any collection. However, there were changes in the percentage of lambs treated throughout the experiment. In late-spring, less than 10.0% of the lambs were being treated in each treatment group. The percentage increased through time, and around 20.0% of the lambs were being treated in each

treatment group on early-summer and mid-summer. However, at the end of the study, the percentage of lambs that were being treated decreased to less than 10.0%.

Fecal egg count

In this experiment, mainly 2 parasite species were identified among FEC: *Coccidia* oocysts and *Strongyle* eggs. *Coccidia* oocysts per g were counted, but not managed in the experiment as a treatment for *coccidia* was not implemented. Small numbers of *Nematodirus* were also observed, but not statistically analyzed. *Haemonchus contortus* was the dominant nematode observed in this experiment.

Fecal egg count results differed in *coccidia* oocytes per g (**OPG**) in early-summer ($P = 0.05$; Table 6) with CON treatment being greater than RTL treatment. However, no differences were observed after grazing treatments were implemented ($P \geq 0.28$). No difference in FEC between treatments ($P \geq 0.25$) were observed at any collection for *Strongyle* eggs per g (**EPG**). There were numerical differences between treatments, but due to variation in the data, no statistical differences were observed between treatments. Roe et al. (1959) reported that short-term rotational grazing using weekly rotation across four paddocks showed no effect on worm burdens. Colvin et al. (2012) reported that sheep under intensive rotational grazing had significantly higher EPG than those on high intensive grazing or typical grazing that consisted in long grazing periods, a high proportion of animals was anemic, had lower numbers of circulating eosinophils and lower BW, but similar BW gain. Colvin et al. (2012) reported that the impact of intensive rotational grazing on worm egg count is mediated by reduced larval challenge rather than an increase in resistance to infection. Ruiz-Huidobro et al. (2019) analyzed a 6-yr long

survey where different grazing systems were implemented and noted that cell grazing system reduced FEC in lambs and ewes. Ewes in cell grazing excreted more eggs than the ewes in the rotational grazing system in May-August in 2017 and May in 2018. Overall, the type of grazing did not have a significant influence in the intensity of egg excretion and concluded that differences in weather and seasonality may influence worm egg counts. Multiple studies in different environments are needed to determine the system that works best for a particular ecosystem (Ruiz-Huidobro et al., 2019).

Meteorological conditions and parasites

Average FEC per animal varied every yr during the experiment (Figure 5). The second yr of experiment (2016) had the highest average FEC per animal in all the collection periods. For most of the yrs., average FEC per animal was low in late-spring, increased during early-summer and mid-summer and decreased in late-summer. In general, peaks of FEC per animal were observed after humidity increased in some years; however, yrs. with dry and warm conditions did not have as high peaks of infection as other yrs. but continual presence and survival of the eggs in the environment.

As mentioned before, average maximum temperatures varied from 30.2 to 34.4°C, and average minimum temperatures from -2 to 23.1°C during the experiment every yr. Levine, (1980) and O'Connor et al. (2006) reported that larvae can develop in the fecal matter from egg to L3 at a temperature of 10 to 36°C, and moisture is fundamental for the development. Several authors reported that eggs in the feces cannot survive conditions of extreme cold. Jasmer et al. (1986) and Shorb, (1944) reported that eggs can only survive for 24 hrs. at 0°C, Shorb, (1944) and Smith-Bujjis and Borgesteede,

(1986) reported that a few days at 4-5°C and only a few days stored in water at < 0°C (Jasmer et al., 1987; Rose, 1963; Todd et al., 1967). This justifies the levels of parasitism that were presented in this experiment, having larvae decreasing in periods of cold temperatures during late-spring and late-summer and increasing during early and mid-summer. Several authors reported that moisture levels in the feces during larval development is affected by factors such as moisture at the time of feces deposition, soil moisture, precipitation and these factors determine if parasite development will successfully occur (Levine, 1963; O'Connor et al., 2006; O'Connor et al., 2008; Leathwick, 2013; Besier et al., 2016; Molento et al., 2016).

Hus and Levine (1977) discussed the interactions between moisture and temperatures that ranged from 20-35°C, and no egg development occurred at low humidity levels (70-85%); however, most eggs produced infective larvae when humidity levels increased up to 100% (Besier et al., 2016). Rainfall is the main limitation for the development *H. contortus* and when rain occurs all yr round, it allows continual development. After 4 d of fecal deposition, moisture is essential for the successful development of infective larvae, and rain is important in the release of larvae from the feces (O'Connor et al., 2008). Some authors have reported that several rain events result in the release of larvae; and therefore, when a period of drought is about to end, producers can monitor animals a few weeks after the end of the drought and apply targeted treatments to reduce infections and promote health and productivity in the flock (Bullick and Andersen, 1978; O'Connor et al., 2006; Besier et al., 2016; Molento et al., 2016; Van Dijk et al., 2018; Wang et al., 2018; Zajac and Garza, 2020). Fecal desiccation and rehydration rates on pasture could explain sequential patterns of larval availability,

and sheep feces may act as a larval reservoir in dry conditions, with peaks of infection following rainfall (Besier et al., 2016). In this experiment, average humidity levels varied from 55 to 76% of relative humidity; however, having some average degree of precipitation during every yr explains the continuance of infection.

Observations of worm burdens in grazing animals have indicated relationships between nematode development and environmental factors and provide an epidemiological environment. Total worm counts from grazing animals also indicate the presence of hypobiotic larvae and the importance as a survival mechanism during adverse environmental conditions (Besier et al., 2016).

Migration of larvae from the fecal pellets to the forage is affected by air temperature, soil moisture, and relative humidity. The majority of larvae of *H. contortus* stay within 10 cm of the feces (Molento et al., 2016). This is an important limitation that implicates how pastures can be managed; when animals are forced to graze too short, ingest more larvae than when grazing tall forage (Zajac and Garza, 2020). Parasite species, location on pasture, and forage height are other factors that affect larval survival (Zajac and Garza, 2020). Chylinski et al. (2014) reported that *H. contortus* had the lowest capacity to survive desiccation, followed by *T. colubriformis* and *T. circumcincta*; meaning that *H. contortus* could be more affected than other species by long rest periods. Ruiz-Huidobro et al. (2019) reported that it could be a challenge for *H. contortus* to survive 10 or 15 d more in a cell grazing system than in a rotational grazing system before the host comes back, especially during summer periods.

The degree of infection relies on many inter-related factors such as weather conditions that determine the accessibility of infection of pasture, grazing behavior, previous infections, and the physiological state of the animal (Waller, 2006). Parasitism on pasture utilization is influenced by the effects of the physiological and metabolic processes in the infected animals and by management decisions taken to reduce the degree of infection on pasture (Waller, 2006). Overall, in this experiment the yr with the highest average EPG per animal was 2016, with the lowest precipitation among other yrs., temperature ranging from 11.14 to 34.2°C, and average daily humidity ranging from 68.7 to 73.5%, and these observations are well justified by what has been previously stated in the literature.

Blood analysis

There were no differences in RBC count ($P \geq 0.30$; Table 7) at any collection, but values increased over time. In late-spring, RBC values were considered low or anemic for sheep (IDEXX Laboratories, Inc., 2017). Values reached normal values at the end of the experiment each yr. No differences were found on HGB ($P \geq 0.28$), but values increased for both treatments in early-summer and kept increasing until the end of the experiment for each yr. Hematocrit values did not differ between treatments ($P \geq 0.14$) at any collection, however values increased through time. Toscan et al. (2017) also found no difference in hematocrit concentration during an experiment where sheep grazed a cultivated pasture that consisted primarily of oats (*Avena sativa*) and ryegrass (*Lolium multiflorum*), and a native pasture where sheep were weekly evaluated for clinical, parasitological and hematological conditions in Brazil.

White blood cell count was greater in early-summer ($P = 0.03$; Table 8) for the RTL treatment compared to the CON treatment and tended to differ in mid-summer ($P = 0.07$) with RTL treatment being greater as well. Eosinophil count tended to be greater in early-summer ($P = 0.06$) for the RTL treatment compared to the CON treatment. Average values from all ewes were in the normal range (IDEXX Laboratories, Inc., 2017) for sheep, and this illustrates a similar detection to Hohenhaus et al. (1998) where normal EOS levels were observed during parasitic infection and were associated with “an innate ability of sheep to resist the stress” including factors such as parasitism and handling. Neutrophil count greater in early-summer in the RTL treatment ($P < 0.02$) than the CON treatment.

The results of this experiment indicate that there are limited differences between continuous and rotational grazing in hair sheep ewes and lambs. Areas of sheep production including those adapted for hair sheep can be limited for rotational grazing due to the long survival periods of infective L3 larvae in temperate regions (Eysker et al., 2005). The complexity of parasite management in a temperate region will require flock managers to include pasture management, nutritional management, alternative anthelmintic treatments including TST using the FAMACHA™ system, development of vaccines, and selective breeding to combat the increasing production of haemonchosis. This experiment demonstrates that the FAMACHA™ method is a useful tool to treat only those animals that need to be treated and reduce resistance to anthelmintics while reducing parasite burden, costs, and maintaining health in the flock.

Table 1. Constitution of the experimental groups of ewes and lambs per year.

	Year	Treatment		Total	BW, ³ kg
		CON ¹	RTL ²		
Ewes					
	2015	24	23	47	26.0
	2016	36	36	72	30.3
	2017	26	26	52	32.0
	2018	30	29	59	36.6
	2019	38	37	75	33.9
Lambs					
	2015	10	9	19	7.87
	2016	42	38	80	11.75
	2017	11	9	20	12.67
	2018	32	32	64	12.33
	2019	45	44	89	10.82

¹Continuous treatment (CON) represents ewes that continuously grazed on a single pasture

²Rotational treatment (RTL) represents ewes that were rotated among 5 different pastures

³Represents initial body weight at the start of the experiment

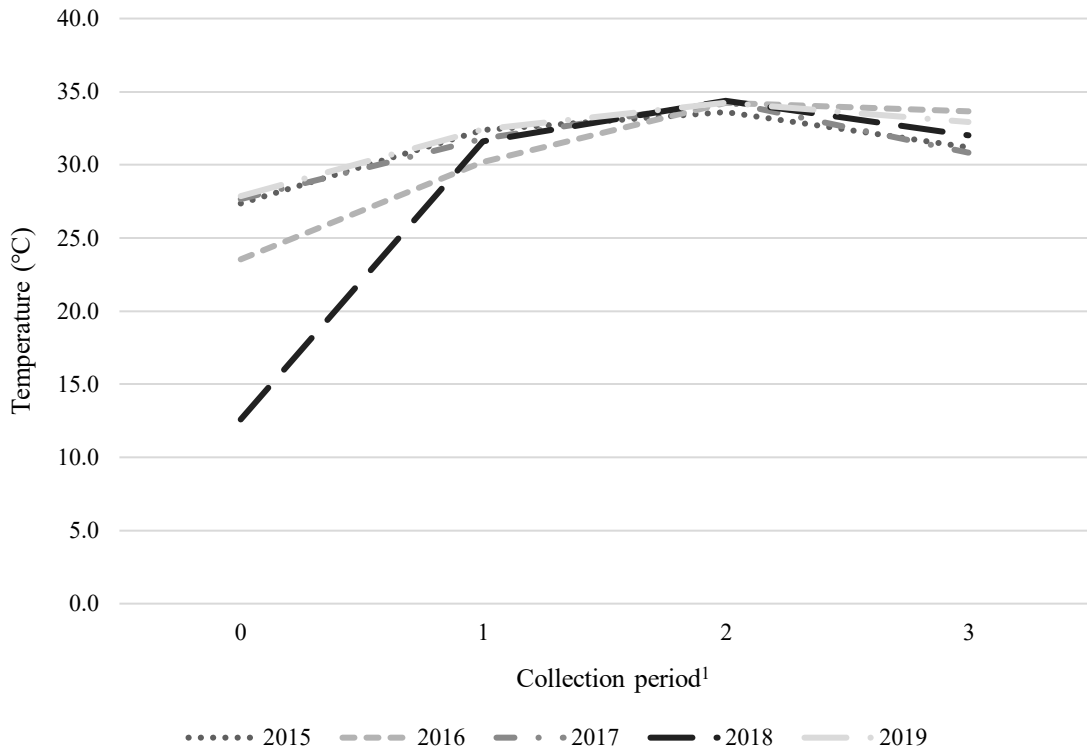


Figure 1. Average maximum temperatures during the experimental period

¹Collection periods occurred every 35 d (d0, d35, d70, d105) for 2015 and 2016 and then changed to every 28 d (d0, d28, d56, d84) for 2017, 2018, and 2019. The collection periods (0, 1, 2, or 3) represent 0 = late-spring, 1 = early-summer, 2 = mid-summer, and 3 = late-summer. In 2015, 2017, and 2019, the experiment occurred during June, July, August and September, and in years 2016 and 2018, the study occurred during May, June, July and August.

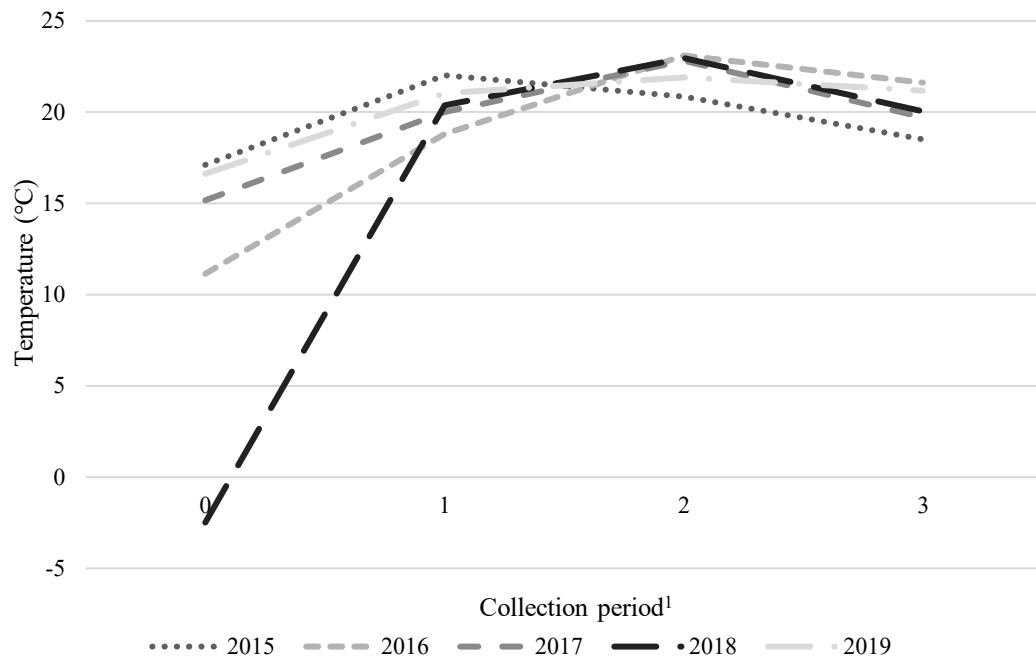


Figure 2. Average minimum temperatures during the experimental period

¹Collection periods occurred every 35 d (d0, d35, d70, d105) for 2015 and 2016 and then changed to every 28 d (d0, d28, d56, d84) for 2017, 2018, and 2019. The collection periods (0, 1, 2, or 3) represent 0 = late-spring, 1 = early-summer, 2 = mid-summer, and 3 = late-summer. In 2015, 2017, and 2019, the experiment occurred during June, July, August and September, and in years 2016 and 2018, the study occurred during May, June, July and August.

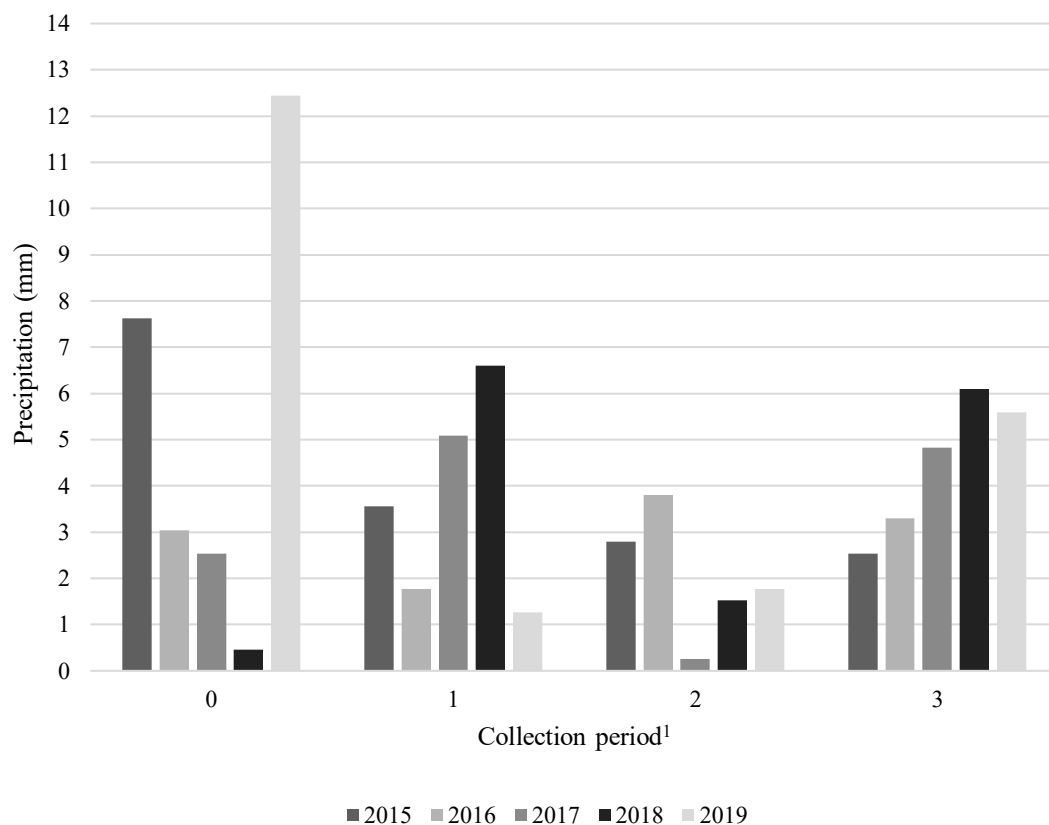


Figure 3. Average daily precipitation during the experimental period

¹Collection periods occurred every 35 d (d0, d35, d70, d105) for 2015 and 2016 and then changed to every 28 d (d0, d28, d56, d84) for 2017, 2018, and 2019. The collection periods (0, 1, 2, or 3) represent 0 = late-spring, 1 = early-summer, 2 = mid-summer, and 3 = late-summer. In 2015, 2017, and 2019, the experiment occurred during June, July, August and September, and in years 2016 and 2018, the study occurred during May, June, July and August.

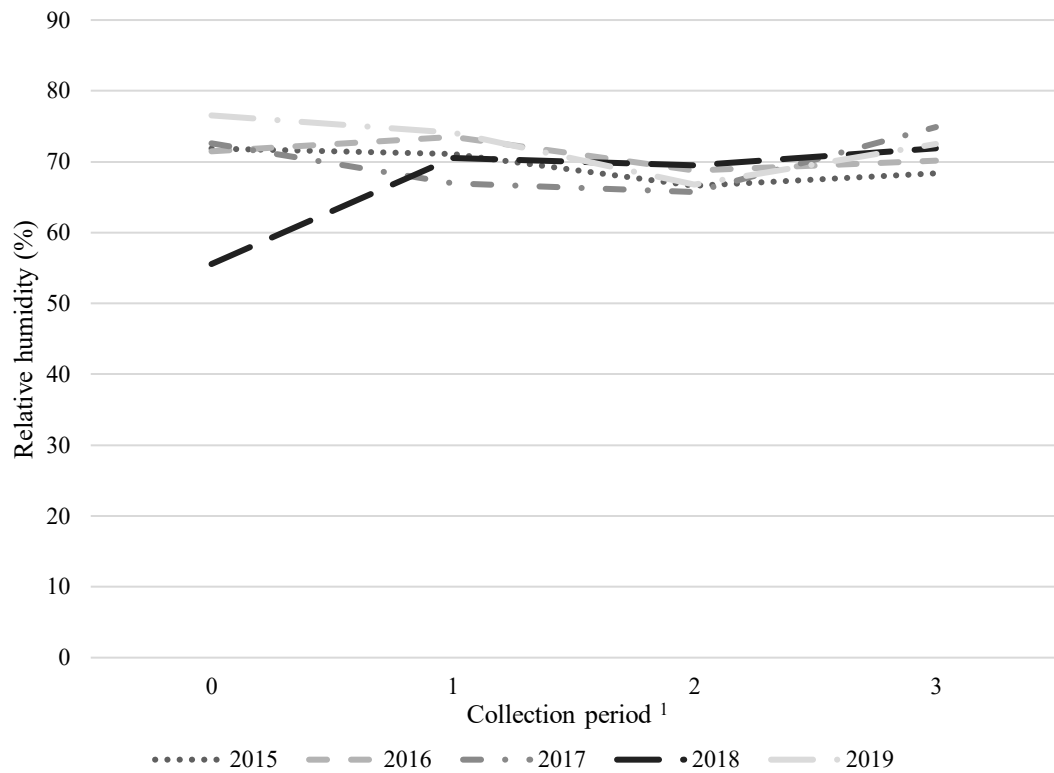


Figure 4. Average daily humidity during the experimental period

¹Collection periods occurred every 35 d (d0, d35, d70, d105) for 2015 and 2016 and then changed to every 28 d (d0, d28, d56, d84) for 2017, 2018, and 2019. The collection periods (0, 1, 2, or 3) represent 0 = late-spring, 1 = early-summer, 2 = mid-summer, and 3 = late-summer. In 2015, 2017, and 2019, the experiment occurred during June, July, August and September, and in years 2016 and 2018, the study occurred during May, June, July and August.

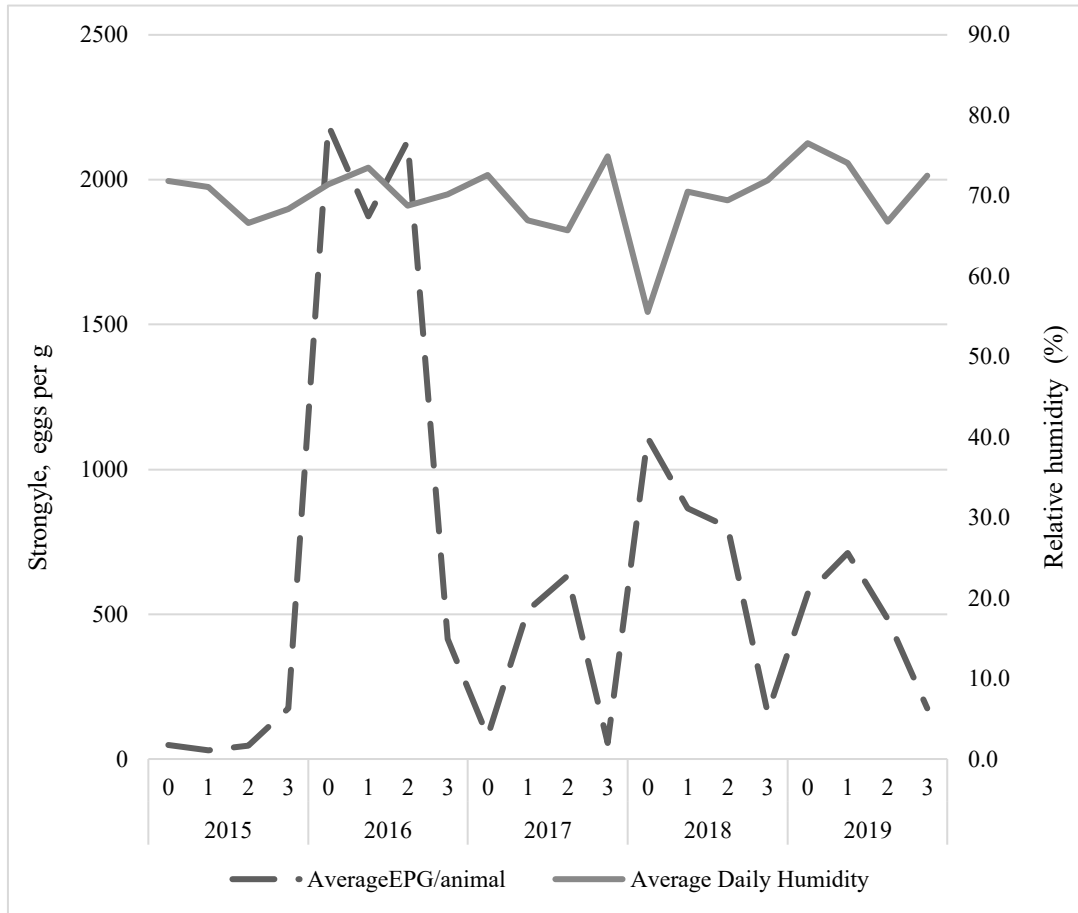


Figure 5. Average EPG per animal, average maximum temperature and daily humidity during the experimental period

¹Collection periods occurred every 35 d (d0, d35, d70, d105) for 2015 and 2016 and then changed to every 28 d (d0, d28, d56, d84) for 2017, 2018, and 2019. The collection periods (0, 1, 2, or 3) represent 0 = late-spring, 1 = early-summer, 2 = mid-summer, and 3 = late-summer. In 2015, 2017, and 2019, the experiment occurred during June, July, August and September, and in years 2016 and 2018, the study occurred during May, June, July and August

Table 2. Average nutrient composition of pastures and forage production

	Year									
	2015		2016		2017		2018		2019	
	Treatments ¹									
	CON	RTL	CON	RTL	CON	RTL	CON	RTL	CON	RTL
Dry matter, %	94.2	92.2	92.5	92.5	91.3	91.3	91.2	91.4	89.7	89.8
Ash, %	6.43	9.02	6.00	8.70	8.01	8.21	9.30	10.7	8.82	9.27
Neutral detergent fiber, %	72.3	62.8	70.3	63.7	67.0	66.0	66.1	66.3	64.5	63.1
Acid detergent fiber, %	35.3	37.0	35.2	35.5	30.3	28.6	33.5	31.5	30.2	31.1
Crude protein, %	8.15	8.82	7.88	10.3	12.2	10.3	10.3	10.2	9.83	12.0
Forage production, kg/ha	2335	2536	3692	3279	4354	4210	6385	4769	4120	4466

¹ Treatments included a continuous (CON) grazing treatment where animals continuously grazed a primarily based bermudagrass (*Cynodon dactylon*) single pasture, and a rotational (RTL) grazing treatment where animals were rotated every 7 d among 5 different primarily based bermudagrass (*C. dactylon*) pastures.

Table 3. Effects of grazing treatment on BW, BCS, and percentage of ewes and lambs that scored a BCS of $\geq 2+$

	Ewes				Lambs			
	Treatment ¹		SEM	<i>P</i> -value	Treatment ¹		SEM	<i>P</i> -value
	CON	RTL			CON	RTL		
BW, kg								
Collection 0 ²	33.1	33.0	1.12	0.92	11.4	11.2	0.94	0.85
Collection 1	33.5	33.8	0.92	0.76	15.3	15.1	1.11	0.75
Collection 2	36.7	36.5	1.79	0.88	19.0	8.7	1.59	0.69
Collection 3	37.7	38.3	1.52	0.47	22.0	22.5	1.84	0.57
BCS³								
Collection 0	2.56	2.56	0.099	0.94	2.94	2.92	0.105	0.66
Collection 1	2.45	2.48	0.127	0.62	2.70	2.64	0.127	0.27
Collection 2	2.45	2.41	0.090	0.53	2.50	2.44	0.160	0.15
Collection 3	2.70	2.78	0.068	0.06	2.62	2.68	0.114	0.30
BCS $\geq 2+$,⁴ %								
Collection 0	87.3	86.7	7.17	0.87	100.0	98.2	1.02	0.13
Collection 1	66.4	70.3	9.58	0.44	88.4	82.2	8.66	0.16
Collection 2	75.1	72.3	10.27	0.56	80.0	80.4	13.81	0.93
Collection 3	92.9	94.9	2.60	0.47	93.3	87.1	5.82	0.07

¹Treatments include a continuous (CON) treatment where ewes continuously grazed on a single pasture and a rotational (RTL) treatment where ewes were rotated among five different pastures

²Collection periods occurred every 35 d (d0, d35, d70, d105) for 2015 and 2016 and then changed to every 28 d (d0, d28, d56, d84) for 2017, 2018, and 2019. The collection period (0, 1, 2, or 3) represent 0 = late-spring, 1 = early-summer, 2 = mid-summer, and 3 = late-summer.

³Body condition score rated on a scale of 1 to 5 with 1 being emaciated and 5 being extremely fat

⁴BCS $\geq 2+$ % represents the percentage of animals that scored 2+ or higher

Table 4. Effects of grazing treatment on FAMACHA score, percentage of ewes and lambs that scored a FAMACHA of ≥ 4 , use of anthelmintics and mortality of hair sheep ewes and lambs

	Ewes				Lambs			
	Treatment ¹		SEM	P-value	Treatment ¹		SEM	P-value
	CON	RTL			CON	RTL		
FAMACHA²								
Collection 0 ³	2.81	2.81	0.110	0.99	2.64	2.74	0.221	0.24
Collection 1	2.60	2.67	0.147	0.49	2.85	2.91	0.274	0.54
Collection 2	2.68	2.68	0.069	0.98	2.95	2.91	0.178	0.74
Collection 3	2.41	2.55	0.175	0.07	2.49	2.58	0.218	0.34
FAMACHA ≥ 4,⁴ %								
Collection 0	16.80	16.25	4.935	0.89	10.4	13.6	8.823	0.45
Collection 1	13.13	15.91	4.607	0.50	23.7	22.8	8.355	0.86
Collection 2	11.43	8.08	3.496	0.34	23.9	19.2	7.251	0.39
Collection 3	5.45	7.57	4.106	0.46	10.5	9.50	7.292	0.81
Anthelmintic,⁵ %								
Collection 0	15.10	15.93	4.527	0.84	5.34	8.43	4.234	0.37
Collection 1	12.81	16.57	4.705	0.36	23.68	22.92	8.347	0.88
Collection 2	10.76	8.79	3.543	0.57	23.72	20.69	8.074	0.59
Collection 3	4.84	2.79	1.916	0.37	7.41	10.30	6.911	0.46
Mortality,⁶ %	7.30	8.60	4.206	0.67				

¹Treatments include a continuous (CON) treatment where ewes were continuously grazed on a single pasture and a rotational (RTL) treatment where ewes were rotated among five different pastures

³FAMACHA™ score to estimate the level of anemia associated with parasite infections where category 1 represents “not anemic” and category 5 represents “severely anemic”

³Collection periods occurred every 35 d (d0, d35, d70, d105) for 2015 and 2016 and then changed to every 28 d (d0, d28, d56, d84) for 2017, 2018, and 2019. The collection periods (0, 1, 2, or 3) represent 0 = late-spring, 1 = early-summer, 2 = mid-summer, and 3 = late-summer.

⁴FAMACHA™ ≥ 4 , % represents percentage of animals that scored 4 or higher using the FAMACHA™ system

⁵Anthelmintic % represents percentage of animals treated with an anthelmintic

⁶Mortality % represents percent of mortality during the experiment

Table 5. Effects of grazing treatment on fecal egg count of hair sheep ewes

	¹ Treatment		SEM	P-value
	CON	RTL		
<i>Coccidia</i>,² OPG				
Collection 0 ³	365	261	133.7	0.05
Collection 1	168	203	73.8	0.28
Collection 2	112	170	65.4	0.32
Collection 3	255	254	148.4	0.99
<i>Strongyle</i>,⁴EPG				
Collection 0	768	1149	518.9	0.25
Collection 1	934	780	345.7	0.52
Collection 2	1020	784	419.7	0.42
Collection 3	219	242	77.1	0.71

¹Treatments include a continuous (CON) treatment where ewes were continuously grazed on a single pasture and a rotational (RTL) treatment where ewes were rotated among five different pastures

²*Coccidia*, oocysts per g

³Collection periods occurred every 35 d (d0, d35, d70, d105) for 2015 and 2016 and then changed to every 28 d (d0, d28, d56, d84) for 2017, 2018, and 2019. The collection periods (0, 1, 2, or 3) represent 0 = late-spring, 1 = early-summer, 2 = mid-summer, and 3 = late-summer.

⁴Strongyle, eggs per g

Table 6. Effects of grazing treatment on the hematological parameters of hair sheep ewes

	Erythrogram				Leukogram			
	Treatment ¹		SEM	P-value	Treatment ¹		SEM	P-value
	CON	RTL			CON	RTL		
RBC,² M/μL					WBC,¹⁰ k/μL			
Collection 0 ³	8.57	8.44	0.288	0.63	8.44	8.68	0.279	0.39
Collection 1	9.36	9.23	0.583	0.63	7.41	7.98	0.469	0.03
Collection 2	9.22	9.49	0.755	0.30	7.28	7.70	0.250	0.07
Collection 3	10.11	10.20	0.859	0.65	7.76	7.99	0.232	0.38
HGB,⁴ g/dL					EOS,¹¹ k/μL			
Collection 0	9.76	9.59	0.439	0.56	0.37	0.42	0.057	0.32
Collection 1	10.36	10.07	0.423	0.28	0.52	0.64	0.158	0.06
Collection 2	10.11	10.27	0.612	0.52	0.52	0.61	0.161	0.12
Collection 3	10.95	11.04	0.543	0.66	0.74	0.70	0.090	0.49
HTC,⁵%					LYM,¹² K/μL			
Collection 0	31.7	31.0	2.52	0.48	3.39	3.43	0.192	0.78
Collection 1	34.7	33.3	3.30	0.14	3.22	3.41	0.159	0.14
Collection 2	33.3	33.7	3.82	0.68	3.24	3.41	0.134	0.17
Collection 3	36.9	37.8	3.96	0.28	3.21	3.30	0.144	0.45
MCV,⁶ fL					MONO,¹³ K/μL			
Collection 0	37.2	36.8	2.76	0.46	1.13	1.08	0.108	0.39
Collection 1	37.1	36.4	2.86	0.16	1.03	1.06	0.115	0.56
Collection 2	36.3	35.8	3.11	0.45	0.91	0.98	0.062	0.16
Collection 3	36.9	37.4	2.76	0.34	0.91	0.93	0.050	0.69
MCH,⁷ pg					NEU,¹⁴ K/μL			
Collection 0	11.4	11.4	0.38	0.96	3.69	4.02	0.241	0.10
Collection 1	11.3	11.1	0.44	0.37	2.76	3.04	0.257	0.02
Collection 2	11.0	11.0	0.48	0.60	2.75	2.76	0.098	0.93
Collection 3	11.0	11.0	0.57	0.83	2.96	3.07	0.164	0.49
MCHC,⁸ g/dL					BASO,¹⁵ K/μL			

Collection 0	31.7	32.0	2.41	0.39	0.039	0.024	0.013	0.18
Collection 1	31.2	30.9	2.43	0.58	0.025	0.026	0.008	0.94
Collection 2	31.6	31.7	2.66	0.74	0.020	0.020	0.009	0.92
Collection 3	30.6	30.4	2.36	0.23	0.027	0.020	0.006	0.21
PLT,⁹ K/μL								
Collection 0	423	434	74.7	0.48				
Collection 1	390	380	54.7	0.59				
Collection 2	384	383	61.7	0.96				
Collection 3	357	375	58.7	0.31				

¹Treatments include a continuous (CON) treatment where ewes were continuously grazed on a single pasture and a rotational (RTL) treatment where ewes were rotated among 5 different pastures

²RBC: red blood cells

³Collection periods occurred every 35 d (d0, d35, d70, d105) for 2015 and 2016 and then changed to every 28 d (d0, d28, d56, d84) for 2017, 2018, and 2019. The collection periods (0, 1, 2, or 3) represent 0 = late-spring, 1 = early-summer, 2 = mid-summer, and 3 = late-summer.

⁴HGB: hemoglobin

⁵HCT: hematocrit percentage

⁶MCV: mean cell volume

⁷MCH: mean cell hemoglobin

⁸MCHC: mean cell hemoglobin concentration

⁹PLT: platelet count

¹⁰WBC: white blood cell count, k/ μ l

¹¹EOS: eosinophils, k/ μ L

¹²LYM: lymphocytes, k/ μ L

¹³MONO: monocytes, k/ μ L

¹⁴NEU: neutrophils, k/ μ L

¹⁵BASO: basophils, k/ μ L

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