

# Evaluating the relationship between fecal egg count, FAMACHA score, and weight in dewormed and non-dewormed Katahdin rams during a parasite challenge

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**ABSTRACT:** The objective of this study was to evaluate and to estimate the relationship between fecal egg counts (FECs) and FAMACHA score and the body weight of growing Katahdin rams during a parasite challenge. One of the largest factors negatively influencing reproduction and economics in the sheep industry is gastrointestinal nematode (GIN) parasites. Due to anthelmintic resistance of these parasites, animals are selected for parasite resistance using FEC and FAMACHA scores. Data were used from the Virginia Tech Southwest Agricultural Research and Extension Center Ram Test in Glade Spring, VA, from the year 2012 to 2018 in which animals were tested in 14-d intervals for 70 d. Mixed models for repeated weight measurements were made from backward stepwise selection to evaluate the relationships between weight and GIN FEC. A total of 576 animals within 23 contemporary groups derived from test year and pasture group were analyzed.

Ram, contemporary group, and consignor were considered random effects, and fixed effects were birth type, test day, age, age squared, starting weight, FEC, and FAMACHA score. Pairwise contrasts were used in the statistical analysis of parameters and their interactions. Weight and age were found to have a quadratic relationship. Increased FEC was associated with weight loss at a rate of 0.00030 kg/FEC ( $P < 0.0001$ ). Animals dewormed at any point during the trial weighed less than those that were not and increased with test day to a maximum difference of 4.66 kg ( $P < 0.001$ ). FAMACHA score was found to be significant ( $P < 0.05$ ), but a direct relationship with weight was not conclusive. Overall, rams with severe enough parasite load to require deworming had lesser weights, which could impact the profitability of sheep production and reinforced the need to select animals that had greater innate parasite resistance.

**Key words:** gastrointestinal nematodes, growth, ram test

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## INTRODUCTION

In 2018, there were 75,000 sheep and lambs in Virginia that had an average value of US\$195. This yielded an estimated value of over 14.5 million dollars in Virginia alone (USDA, 2018). In 2017, the sheep industry yielded an economic

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benefit of US\$5.8 billion to the U.S. economy (Shiflett, 2017). Given the impact sheep have on the economy, their health and maintenance should be closely monitored. One of the largest factors negatively influencing reproduction and economics in the sheep industry is gastrointestinal nematode (GIN) parasites. These GIN parasites can induce weight loss, decreased appetite, and cause death if not managed properly and in a timely manner (Lôbo et al., 2009; Saddiqi et al., 2010). One GIN of concern is *Haemonchus contortus* because of its prevalence and impact. Although GIN parasite load is known to be detrimental to health, its interactions with production traits are not completely understood and should be studied further (Burke and Miller, 2004; Ngere et al., 2018).

A common indicator of parasitism is fecal egg count (FEC), measured by the number of eggs per gram of feces (Ngere et al., 2018). Another valid method is the FAMACHA scoring system. This system consists of one to five subjective scores based on the color of the ocular conjunctival mucous membranes. The greater the number, the membrane is paler, and the animal is more anemic, which indicates greater possible parasitism (Burke et al., 2007). Although these measurements are common, the direct relationship between measures of GIN parasite load and performance are not well documented. The objective of this study was to quantify the relationship between FEC, FAMACHA score, and the body weight of growing Katahdin rams during a parasite challenge in field data.

## MATERIALS AND METHODS

Animal care and use committee approval was obtained through Virginia Tech (12-093, 15-133, and 18-161).

### *Animal Performance*

Data were used from the Virginia Tech Southwest Agricultural Research and Extension Center Ram Test in Glade Spring, VA, from the year 2012 to 2018. The purpose of the test was to provide producers with an avenue to compare the performance of their rams with those of other flocks. This was a forage-based ram performance test designed to evaluate the ability of rams to cope with GIN. Eligible rams were born from January 15 to March 15 of the year tested. In late May, rams ( $n = 722$ ) were delivered to the facility. On arrival, rams were treated according to label directions with three anthelmintics (levamisole, moxidectin,

and albendazole), and FECs were collected. After 12 d, a subsequent FEC was taken and analyzed to ensure FEC was less than 50 eggs/g. Three weeks after delivery, rams were allocated to three pastures based on heavy, average, and light body weight and were orally administered third-stage *H. contortus* larvae (150 larvae/kg body weight). Rams were tested for 70 d, had access to fescue-based pasture, and were fed 2% of body weight in grain supplement each day (74% total digestible nutrients and 14% crude protein).

Information was available for consignor, breed, birth date, birth type, and rear type. Weight was measured every 14 d (6 total dates), and weights at the beginning (0 d) and ending (70 d) of the test were the average of two weights taken on consecutive days. In 2012 and 2013, the ram test had not begun using two beginning and ending weights, and only one weight was available for each day in those years. Only Katahdin rams were retained for the study because of the limited numbers of other breeds and crosses. Age and age squared were determined for each test date to see if the quadratic form would significantly impact weight. Rams were removed if they had no information for birth and/or rear type. Furthermore, rams born as quadruplets were removed due to the limited number within the test ( $n = 5$ ). Possible levels for birth type were singlet ( $n = 92$ ), twin ( $n = 407$ ), and triplet ( $n = 77$ ). The same levels were used for rear type: singlet ( $n = 117$ ), twin ( $n = 407$ ), and triplet ( $n = 51$ ). Animals were removed if they had a weight per day of age of 0 because this was likely a data entry error. After all edits, 576 rams from 63 consignors remained. For analysis, animals were assigned to contemporary groups derived from the interaction between test year and pasture group. This yielded 23 unique contemporary groups with an average of  $25 \pm 8$  rams in each.

### *GIN Indicators*

FEC and FAMACHA score were measured to determine the levels of parasitism throughout the test. FEC was measured every 14 d starting at test day 28. The FECs were determined by the modified McMaster test with a detection limit of 50 eggs per gram. FAMACHA score was collected every 14 d starting at test day 14. Scores were determined by comparison of the animal's ocular conjunctival mucous membranes to the standard color chart, and values of one to five were assigned to colors ranging from red to white. The transition from red to white conjunctivae was indicative of

anemia associated with parasitism. Decisions on deworming during the trial were determined via FAMACHA scores, and rams with FAMACHA scores greater than or equal to 3 were treated with levamisole according to label directions. The dewormed status of animals was noted as a binary trait evaluating if a ram was ever dewormed during the 70-d test ( $n = 77$ ). Any ram that required multiple treatments was removed from the test and was not used in analyses because data did not exist for the entire 70-d test.

FEC and FAMACHA score were analyzed separately. Rams were removed if they did not have FEC and FAMACHA observations in their respective analyses. The FEC model had 559 observations and the FAMACHA model had 581 observations with no significant differences from the weight model in the distribution of observations between parameters. Statistical summary information for weight, FEC, and age at the beginning of the test is displayed in Table 1. Table 2 displays statistical summary information for FAMACHA scores.

### Statistical Analysis

Data were edited and analyzed with R software (R Core Team, 2019). To evaluate the relationship between FEC, FAMACHA score, and weight of Katahdin rams, three linear mixed models with weight as the dependent variable were developed with the lme4 (Bates et al., 2015) and car (Fox and Weisberg, 2019) packages in R. Models were developed for repeated weights, weights and FEC, and weights and FAMACHA score. Ram, contemporary group, and consignor were considered random effects and were assumed to be uncorrelated. Birth type, rear type, dewormed

status, test day, FAMACHA score, and interactions between them were tested as fixed effects; age, age squared, on-test weight, and FEC were tested as covariates.

A linear mixed model was first developed using all interactions of fixed effects in addition to covariates and random effects. To develop a model that best fits the data, random effects were first tested with a likelihood ratio test. All random effects were determined to be significant ( $P \leq 0.05$ ). For fixed effects and covariates, the backward stepwise selection was used to select the final models. The Akaike information criterion and Bayesian information criterion were compared between the two models; lower values indicated a better fit for the data.

The final models were as follows:

Model 1 depicted the factors associated with repeated measurements of weight:

$$y_{ijklmno} = \text{birth}_i + \text{day}_j * \text{dewormed}_k + \text{cg}_l \\ + \text{consignor}_m + \text{animal}_n + B_1 \text{age}_{ijklmno} \\ + B_2 \text{age}_{ijklmno}^2 + e_{ijklmno}$$

Model 2 depicted the relationship between weight and FEC:

$$y_{jklmnpq} = \text{day}_j * \text{dewormed}_k + \text{cg}_l + \text{consignor}_m \\ + \text{animal}_n + B_3 \text{FEC}_p + B_4 \text{swt}_{jklmnpq} \\ + e_{jklmnpq}$$

Model 3 depicted the relationship between weight and FAMACHA score:

$$y_{jklmnop} = \text{day}_j * \text{dewormed}_k * \text{FAMACHA}_o + \text{cg}_l \\ + \text{consignor}_m + \text{animal}_n + B_4 \text{swt}_{jklmnop} \\ + e_{jklmnop}$$

**Table 1.** Summary statistics for weight, FEC, and age of Katahdin rams evaluated in a 70-d growth test

Day	No. of observations	Mean	Min	Max	SD
Weight, kg					
0	576	34.7	16.3	59.4	7.8
14	576	36.9	17.7	60.3	8.0
28	576	39.3	20.4	65.3	8.5
42	576	41.8	21.8	66.7	8.4
56	576	43.3	19.0	68.9	8.6
70	576	44.9	20.4	71.2	9.1
FEC, eggs/g feces					
28	562	574	0	7,415	691
42	557	474	0	7,166	690
56	559	549	0	11,043	1,147
70	564	403	0	7,937	911
Age at the start, d					
0	576	130	88	200	18

**Table 2.** Number of observations for each FAMACHA score on each test day of a 70-d ram growth test<sup>a</sup>

Day	FAMACHA			
	1	2	3	4
14	482	19	0	0
28	460	39	2	0
42	505	68	8	0
56	413	117	41	10
70	425	96	55	5

<sup>a</sup>FAMACHA scores were determined by comparing animals' ocular conjunctivae to a color chart assigning severity of anemia (1 normal to 5 severely anemic) based on coloration. No animals had severe enough anemia to warrant a score of 5.

Where,  $y$  is the weight; birth is the birth type ( $i = 1, 2, \text{ or } 3$ ); day is the test day ( $j = 0, 14, 28, 42, 56, \text{ or } 70$ ); dewormed is the dewormed status ( $k = \text{Yes or No}$ ); FAMACHA is the FAMACHA score ( $o = 1, 2, 3, 4$ );  $B_1, B_2, B_3, \text{ and } B_4$  are regression coefficients relating records to linear age, squared age, FEC, and starting on-test weight, respectively;  $cg$  is a random effect for the contemporary group ( $n = 23$ ); consignor is a random effect for the ram's consignor ( $n = 63$ ); animal is a random effect for repeated observations on the ram ( $n = 576$ ); and  $e$  is the random residual. Random effects were all assumed to be normally distributed and uncorrelated.

After the weight model (model 1) was developed, residuals were tested via histograms and boxplots against fixed effects. All effects were normally distributed except for the interaction between test day and dewormed status. To correct this, heterogenous residual variances by day were modeled. This new model was used as the base to develop the models with FEC (model 2) and FAMACHA (model 3) to ensure that residuals were normally distributed across days and dewormed status.

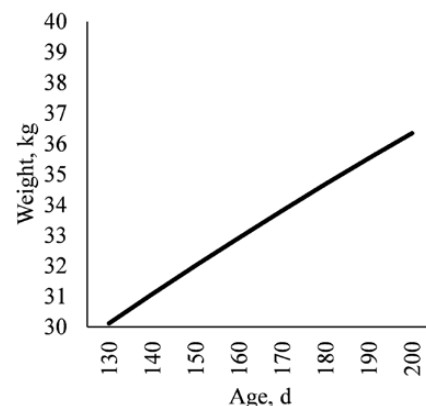
Contrasts were analyzed using the emmeans package in R (Lenth, 2020). Pairwise comparisons were made for birth type in the weight analysis with a Tukey adjustment for multiple comparisons. For the FAMACHA model (model 3), grouping and contrasts were conducted separately due to the three-way interaction of test day, dewormed status, and FAMACHA score and were adjusted with the Bonferroni correction. Two groups of pairwise contrasts were made: 1) for dewormed status within day and FAMACHA score and 2) for FAMACHA score within day and dewormed status. The contrasts for FAMACHA score were only observed between scores of 1 and 2 due to those being the only

scores present within all test days. For all contrasts, parameters were declared significant at  $P < 0.05$ .

## RESULTS AND DISCUSSION

### Model 1: weight

All fixed effects retained in the models were significant. A quadratic relationship existed between weight and age where the linear coefficient  $\pm$  SE was  $0.28 \pm 0.05$  kg/d of age and the quadratic coefficient  $\pm$  SE was  $-0.0002 \pm 0.0001$  kg/d of age<sup>2</sup>. This positive relationship with age and negative relationship with age squared indicates that average daily gain (ADG) decreased as rams got older. Yet, this quadratic relationship may not be numerically significant and pertinent within the scope of this trial as seen in Figure 1. In this figure, weight was plotted as a function of the mean on-test weight and the ages represented in this study. Previous models of rams also found a positive linear relationship between weight and age. A study in Arkansas found that unchallenged Katahdin lambs had an ADG of 0.24 kg/d from birth to weaning (60 d of age). The ADG decreased to 0.13 kg/d from weaning to slaughter (210 d of age) (Burke and Apple, 2007). Lambs grazed on bermudagrass pasture and were fed a corn-soybean meal supplement (680 g) once a day. Animals in the present study achieved a comparable ADG of  $0.15 \pm 0.07$  kg/d at approximately 200 d of age (data not shown). Al-Kawmani et al. (2014) studied the growth rate and sexual development of Najdi rams in Saudi Arabia from 1 to 9 mo of age. They found rams to gain 3.94 kg/mo, which amounts to 0.13 kg/d. The Najdi rams were fed a standard growing ration and mineral blocks ad libitum. Variation in weight gain between the two trials could be attributed to breed differences, age of rams, and/or environment.



**Figure 1.** Prediction of weight from the combined effect of age and age squared using regression coefficients from model 1 where  $weight = \bar{x}_{start\ weight} + B_1 * age + B_2 * age^2$  in growing Katahdin rams.

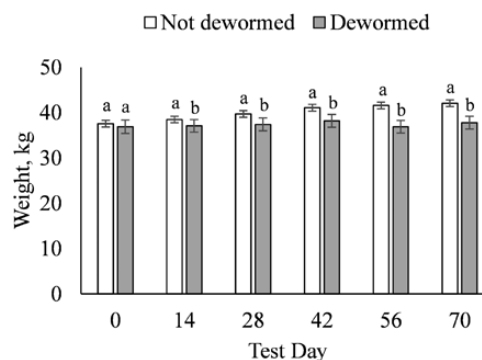
López-Carlos et al. (2010) studied the growth performance of hair sheep including Katahdin rams in north-central Mexico for 90 d starting at an average of  $146 \pm 11$  d of age. They found rams to gain  $20 \pm 3$  kg during the 90-d trial, which equates to a gain of 0.22 kg/d. Rams were kept in pens and fed a total mixed ration (72% total digestible nutrients and 14.5% crude protein) ad libitum. Elevated ADG compared with the present study could be caused by diet because rams in our study were restricted in their concentrate intake.

Three comparisons were made within the birth types of animals (Table 3). The comparison of singlet and triplet produced the greatest difference in weight with the singlet being  $2.25 \pm 0.58$  kg heavier. The smallest weight difference was between triplet and twin with the twin tending to be heavier by  $1.06 \pm 0.46$  kg. The contrast suggests that weight decreases as birth type increases from singlet to twin to triplet as we expected. A 17-yr study conducted in the United States found a similar trend for Katahdin lambs to be heavier when reared in smaller litters (Ngere et al., 2017). Ngere et al. (2017) saw within lambs reared as singlets, those that were born as twins weighed 1.5 kg more than triplets, and those that were born as singlets weighed 5.8 kg more than quadruplets. Lambs were raised and observed in 100 flocks across the United States with different management systems that may have introduced greater weight variations than the present forage-based system. Several other studies varying in breed and environment confirm this general trend in body weight in young animals, but vary in magnitude (Carrillo and Segura, 1993; Akpa et al., 2006; Stafford et al., 2007; Mellado, 2016). Some studies propose that birth type primarily influences birth weight and becomes less important as animals age (Jucá et al., 2016; Pesántez-Pacheco et al., 2019).

The weight difference was evaluated via six comparisons of dewormed and non-dewormed rams by day. The contrast was developed by subtracting dewormed rams' weights from those of non-dewormed rams within each test day (Figure 2). All estimated differences were positive, and all were significant except for day 0. This result at day 0 was

**Table 3.** Pairwise contrasts for weight differences based on birth type in growing Katahdin rams

Contrast	Weight, kg	SE, kg	P-value
Singlet–triplet	2.25	0.58	<0.01
Singlet–twin	1.20	0.44	0.02
Triplet–twin	-1.06	0.46	0.06



**Figure 2.** Least squared means (SE) for weight in Katahdin rams that were not dewormed and those that were dewormed anytime during a 70-d growth test. Rams were dewormed with levamisole if the FAMACHA score was greater than or equal to 3. Different letters within day indicate significant differences ( $P < 0.01$ ).

expected because rams received anthelmintic treatment upon delivery and had only just been given the controlled dose of *H. contortus*. Infected animals that were not dewormed weighed more than those that were by at least 0.71 kg to a maximum of 4.66 kg by day 56. The difference between dewormed and not dewormed rams increased steadily through day 56 but stabilized for the end of the test. Previous research comparing the performance of sheep that were or were not dewormed is limited in field data.

### Model 2: FEC

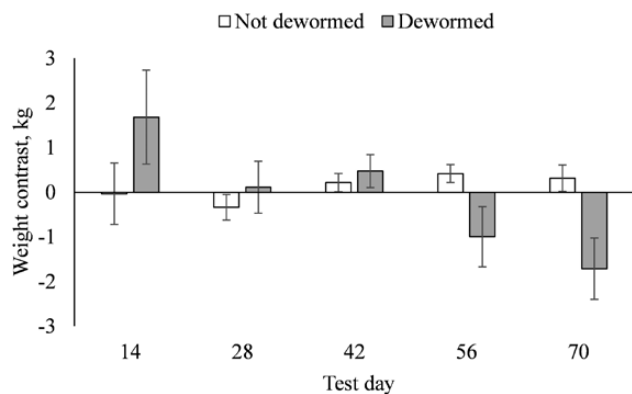
All fixed effects and covariates were significant. Weight  $\pm$  SE decreased by  $0.00030 \pm 0.00005$  kg per unit of FEC. Based on the average FEC at the end of the test, the average challenged ram would weigh approximately 0.12 kg less than a ram with no GIN parasites. Although statistically significant, this result was not biologically significant because of the small reduction in weight. Previous research found few, small phenotypic correlations between body weight and FEC depending on age (weaning vs. postweaning), litter effects, and maternal additive effects (Ngere et al., 2018). They found correlations between weaning FEC and weaning and post-weaning weights to be -0.07 and -0.10, respectively; however, results for postweaning FEC were not significant. Further studies are needed to evaluate the relationship between FEC and postweaning growth because this study was only applicable to forage-based systems in Southwest Virginia.

Given that the regression coefficient for FEC was negative, the GIN parasites could be causing weight loss in the animals. Most of the rams in the study would have a negligible reduction in weight gain based on FEC. However, rams with more

extreme FEC could have much greater reductions in weight gain and would be good candidates for treatment. Animals were dewormed throughout the study based on subjective observance of FAMACHA score. As such, dewormed rams were those identified as suffering from anemia and had the greatest physiological response to their GIN infection. Limited studies were found to directly compare FEC to body weight of sheep. A study conducted in Ethiopia found that sheep with poor body condition scores, associated with emaciation, had a greater proportion of GIN and larger mean FEC (Seyoum et al., 2018). In this case, the GIN parasites caused not only a reduction of weight gain but also a net loss in weight. The rams in the present study had access to better nutritional resources and were in good body condition explaining why we did not see such extreme cases.

### Model 3: FAMACHA

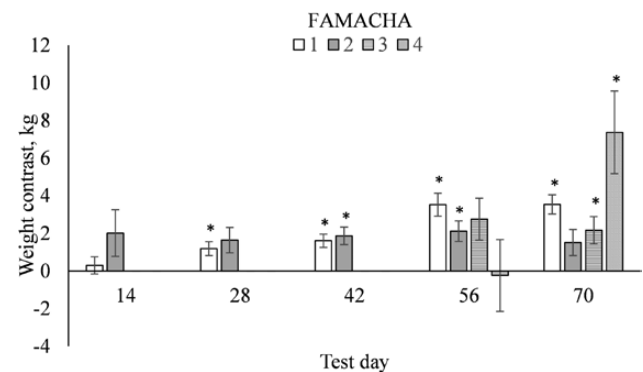
All fixed effects were significant. Two contrasts were compared for the FAMACHA model to test the three-way interaction of test day, dewormed status, and FAMACHA score. One set of contrasts was conducted to determine the relationship between FAMACHA score and weight within a given day and dewormed status (Figure 3). Hence, the difference between weights of rams with FAMACHA scores of 1 and 2 was evaluated for every test day and was further separated based on if the ram was dewormed or not. These contrasts were not significant, indicating the subjective difference between FAMACHA scores 1 and 2 may not have much bearing on weight differences in rams. This result justified the practice of not



**Figure 3.** Weight contrasts (SE) for the difference between FAMACHA scores 1 and 2 based on test day and if the ram was dewormed anytime during the test. Rams were dewormed with levamisole if the FAMACHA score was greater than or equal to 3. Contrasts were made by subtracting FAMACHA score 2 from FAMACHA score 1, producing 10 different contrasts. No contrasts were significant ( $P > 0.01$ ).

treating those animals. The FAMACHA scoring system has a lower sensitivity for anemia detection in lambs than adults (Cintra et al., 2018). Although there is a general positive relationship with increasing FEC, the levels of FEC within FAMACHA score are quite variable (Cintra et al., 2018). Given no weight difference between FAMACHA scores of 1 and 2, producers should continue to not deworm these animals.

The other contrast determined whether deworming an animal or not influenced weight based on the combination of test day and FAMACHA score (Figure 4). For all test days and FAMACHA scores (except score 4 on day 56), the estimated differences were positive. These results supported the other findings that dewormed animals weigh less than those that are not. Most contrasts were significant by day 28. However, both contrasts for test day 14 were not significant, possibly due to GIN parasite immaturity until about 3 wk after infection. FAMACHA score measures the level of anemia of the animal (1 = normal and 5 = severe anemia) and thus would increase as the GIN parasites matured, caused greater blood loss, and began reproducing to increase the infection after several weeks (Burke et al., 2007). By the end of the test, the not dewormed rams were anywhere from 1.2 to 7.4 kg heavier than the dewormed rams within the same FAMACHA score. The greatest contrast existed for rams with a score of 4 on test day 70. This was likely because these rams were classified as having the most anemic reaction to GIN. Animals that were dewormed at some point during the trial but were still anemic enough by test day 70 to warrant a score of 4 can be determined as animals that were highly affected by GIN and were especially susceptible to GIN infection.



**Figure 4.** Contrasts (SE) for the weight difference between Katahdin rams that were and were not dewormed anytime during the test based on day and FAMACHA score. Rams were dewormed with levamisole if the FAMACHA score was greater than or equal to 3. Dewormed animals were subtracted from those that were not to develop contrasts, and \* indicates a significant contrast ( $P < 0.01$ ).

Previous researches both support and contradict these findings. Studies found that FAMACHA score was highly related to FEC and reduced weight gain, and deworming animals based on this parameter led to lower weights compared with other selection methods, such as daily weight gain, FEC, or a controlled monthly treatment (Leask et al., 2013; Rizzon Cintra et al., 2019). However, another study found that weight loss did not significantly differ between treated and non-treated animals (Stafford et al., 2009). The deworming of the animal and its relationship with weight loss are complex and vary with other factors, such as nutritional status, age, and breed, which were all consistent in our dataset. Further studies should evaluate the correlation between elevated FAMACHA scores and weight in other environments and types of sheep to continue to parse the relationship between GIN infection and performance.

## CONCLUSIONS

The objective of this study was to evaluate the relationship between FEC, FAMACHA score, and body weight of growing Katahdin rams to determine performance differences associated with indicators of GIN parasites. The FEC could potentially have negative implications in flocks with elevated GIN parasite loads and poor nutrition, but the weight loss for average GIN parasite loads was minimal in this study. Although FEC is an objective indicator of GIN parasites, selecting for reduced FEC may not significantly change the growth in moderately challenged animals. Katahdin rams that needed to be treated for GIN parasites were generally 5 kg lighter than their contemporaries after 70 d of parasite challenge. Rams that did not require treatment were likely more profitable given the treatment cost and better weight gain. As such, it is beneficial to select for animals that do not require treatment for GIN parasites and subsequently would be less prone to reduced weight gain. Results with FAMACHA scores were most dramatic for more extreme cases of anemia, but no weight differences existed between scores of 1 and 2 as expected. Additional economic modeling would be beneficial for producers to better understand the financial implications of treatment for GIN parasites and animal performance.

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*Conflict of interest statement.* There was no conflict of interest.

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