

Effects of Dietary Protein Supplementation on the Performance and Non-Carcass Components of Goats Artificially Infected with *Haemonchus contortus*

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Abstract

This experiment was conducted to examine the effect of dietary protein supplementation on feed intake, body weight gain, FAMACHA scores, fecal egg count (FEC), packed cell volume (PCV), and non-carcass components of goats infected with *Haemonchus contortus*. Sixteen bucks with an average body weight of 23.69 ± 0.88 kg (Mean \pm S.E) were used in the study. The animals were randomly divided into infected and non-infected groups, then assigned to either a high (HP) or low (LP) protein diet. The infected groups were trickle infected with stage L₃ of *H. contortus* larvae at a dose of 600 units. Feed intake, body weight, FAMACHA scores, PCV and FEC were determined using standard procedures. After 140 days, 12 goats were randomly selected for slaughter. Non-carcass components were weighed and recorded. The results showed that diet and infection significantly ($P < 0.0001$) affected feed intake. Goat body weight was highly affected by diet, infection and time, while PCV was significantly influenced by infection and the interaction between diet and infection. There was a highly significant ($P < 0.0001$) difference in FAMACHA scores between HPI and LPI groups. FEC of goats on the LP diet was significantly higher than those on the HP diet. The infection had a significant ($P < 0.05$) effect on goat liver weight. It was concluded that a high protein diet may improve resistance and resilience to *H. contortus* infection in pen-raised goats fed a concentrate-only diet.

Keywords Growth performance, clinical parameters, *Haemonchus contortus*, non-carcass.

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Introduction

Internal parasitism of goat and sheep is the most important factor impacting animal health and production worldwide. Gastrointestinal nematodes (GINs) reduce growth and productivity (milk, meat, fiber) of adult animals and cause high mortality in kids resulting in large economic losses [1]. The conventional method for nematode control used by producers involves the repeated use of anthelmintic drugs. However, this approach has been rendered ineffective due to widespread anthelmintic drug resistance and consumer preference for organic meat products [2, 3]. Therefore, successful goat production requires an effective and sustainable control method against GINs.

Several studies have evaluated various alternatives to chemical control of GIN infection [1, 4]. The consensus seems to be that enhancement in host diet can improve the animal's ability to normalize GIN populations as well as the ability to endure the negative effects of GIN infection, thereby keeping a sound production level that would effectively reduce the use of anthelmintic drugs to control parasitism [4]. This method includes nutrient supplementation

(protein in particular) to boost host resistance and resilience to GINs. Despite the fact that the importance of meat animals is chiefly affiliated with carcass yield, most of the non-carcass components such as head, heart, liver, kidney, testicle and other organs are also eatable in most parts of the world [5]. As such, non-carcass components are becoming equally important in value with carcass components from meat animals. The growth and development of non-carcass components should be taken into account when raising meat animals. Documentations show that the productive efficiency of visceral and other non-carcass components of meat animals could be affected by the nutrient content of the diet [6]. However, in the cited studies, animals were either fed forages or kept in the pasture while receiving supplemental feed.

Therefore, the objective of this study was to assess the effects of two levels of dietary protein supplementation in the presence of *Haemonchus contortus*, one of the most pathogenic nematodes of ruminants, on the performance and non-carcass components of goats raised in pen sustained on concentrate only.

Materials and methods

Study site

The experiment was conducted at the Virginia State University (VSU) Randolph Research and Demonstration Farm (Small Ruminant Research Facility), located in the Tri-Cities area of Central Virginia (37.1° N; 77.3° W) at an elevation of 45 m above sea level. The climate in this area is temperate with cold weather from December to April and hot and humid conditions from June to October. Annual rainfall averages 1100 mm and the mean ambient temperature is 13 °C. The experiment was started in February 9, 2015 and ended on June 2, 2015.

Animals, diets and experimental design

The VSU animal use and care guidelines were strictly followed to treat animals used in this study. Sixteen 9-10 month old bucklings with an average body weight of 23.69 ± 0.88 kg were used in the study. Experimental animals were drenched for internal parasites using Levamisole (0.4 mg/kg BW) and divided into two groups (infected and non-infected) each with eight animals. Goats were housed in individual pens with concrete floors spread with wood shavings and allowed to acclimatize for 2 weeks before the experiment started. Each group was randomly assigned to two nutritional regimes of either high (HP) or low (LP) protein diets containing 25% and 16% crude protein (CP), respectively with soya bean meal as a major source of protein. The HP and LP diets contained 7.02 MJ/kg dry matter (DM) and 7.92 MJ/kg DM, respectively, of metabolizable energy. The experiment was a 2 × 2 (infected/not infected × HP/LP diets) factorial design. The amount of feed offered was based on 3% of live weight (LW) and animals were fed once a day at 0800h in the morning. Clean drinking water and licking salt blocks were available *ad libitum* throughout the study period. The licking salt blocks contain following nutrients: 96-98% NaCl, 2000 ppm Mn, 1000 ppm Fe, 1000 ppm Mg, 500 ppm S, 250 ppm Cu, 100 ppm Co, 80 ppm Zn and 70 ppm I.

Chemical analysis of diets

Samples of experimental diets were ground using a Wiley mill, pass through a screen (1 mm) and analyzed for moisture, crude protein (CP; Kjeldahl N×6.25), ash, organic matter, total digestible nutrients, lipids and other minerals according to published methods [7]. Neutral detergent fiber content was determined according to the method of

Van Soest et al. [8]. The metabolizable energy (ME) content was computed using the following equation [9]:

$$\text{ME (MJ/kg)} = \text{Total digestible nutrient (\%)} \times 0.15104$$

The ingredients and nutrient composition of experimental diet is given in Table 1.

Culturing and harvesting of *Haemonchus contortus* larvae

Larvae of *H. contortus* were cultured using procedures described by the World Association for Advanced Veterinary Parasitology (WAAVP). Fecal materials obtained from a donor goat pastured at the VSU Randolph Farm were placed in a labelled beaker (with set and harvest date) and incubated at 25 °C. Larvae were harvested every 10 days, stored at 4 °C, and used to infect the animals within a week.

Table 1 Ingredients and nutrient composition of experimental diets.

Item	Diets	
	High protein	Low protein
Ingredient (%)		
Alfalfa pellet	5.00	17.00
Cracked corn	63.00	73.00
Soybean meal	30.00	8.00
Limestone	1.00	1.00
Ammonium chloride	1.00	1.00
Nutrient composition		
Moisture (%)	11.00	10.90
Crude protein (%)	24.70	15.80
Lipid (%)	2.79	2.18
Acid detergent fiber (%)	14.58	15.90
Total digestible nutrient (% , TDN)	73.8	79.2
Metabolizable energy (MJ/kg)	11.15	11.96
Nitrogen (%)	3.95	2.52
Organic matter (%)	96.33	96.39
Ash (%)	3.67	3.61
Phosphorus (%)	0.44	0.32
Potassium (%)	1.25	0.94
Sulphur (%)	0.21	0.14
Calcium (%)	1.28	1.64
Magnesium (%)	0.22	0.18

$$\text{Metabolizable energy (MJ/kg)} = \text{TDN (\%)} \times 0.15104$$

Infection of the animals

On day 0 of the experiment, all animals in the infected group were orally trickle infected with a dose of 600 units third stage larvae (L₃) of *H. contortus* (96% pure). Thereafter, the treatment was repeated three times a week for three consecutive weeks with each animal in the infected group receiving a total of 5400 larvae. Fecal egg count (FEC) was used as the measure of resistance and body weight gain as a measure of resilience.

Data collection

Growth performance

On day one, the weight of feed offered and orts recovered were recorded. Feed intake for each individual animal was calculated by subtracting the ort from feed offered. Goats were weighed weekly using a scale prior to feeding in the morning.

Clinical parameters

The Faffa Malan chart (FAMACHA) was used to determine the level of anemia in the goats by matching the color of their ocular mucous membranes with the five categories in the FAMACHA eye color chart: 1 = red (non-anemic); 2 = reddish pink (non-anemic); 3 = pink (mildly anemic); 4 = pinkish white (anemic); 5 = white (severely anemic). Blood samples were collected from each animal by jugular venipuncture using a hypodermic needle and analyzed for packed cell volume (PCV) as a measure of anemia by calculating the percentage of red blood cells in the whole sample collected in hematocrit tubes after centrifugation. Fecal samples were directly collected from the rectum of each experimental animal and fecal egg count (FEC) was performed using the modified McMaster method.

Slaughtering procedure

At the end of the experiment, 12 goats were randomly selected for slaughter. The goats were fasted for 48 hours, with *ad libitum* access to drinking water and weighed. They were transported by truck from the study site at Randolph Farm, Virginia State University to Central Meats (Chesapeake, VA) for humane harvest. At the abattoir, each goat was stunned using a 500 V electric current then the jugular vein was severed, and the carcass was skinned. The heads were removed at the atlanto-occipital joint and the hooves cut off at the proximal end of the cannon bones, leaving the carpal and tarsal bones on the carcass. Non-carcass components (skin, head, heart, testicle, liver, lung, kidney, spleen, intestine, omental fat and kidney fat) were weighed and recorded.

Statistical analysis

Data were analyzed using SAS system version 9.4 (SAS Institute, Cary NC). Data on feed intake, body weight, FAMACHA scores, PCV, and FEC were analyzed using Proc-MIXED procedure for repeated measures analysis of covariance. The initial body weight of the animal was used as a covariate in the analysis. The FEC data were normalized by log

transformation ($\log + \text{FEC}$) prior to statistical analysis. The fixed effects in the model were diet, infection, and time, while each animal was considered as a random factor. Where interaction was observed, further analysis was carried out to ascertain the exact period during the course of study. Two-way analysis of variance (ANOVA) and Duncan's multiple range test (DMRT) were performed for non-carcass components using the General Linear Model (GLM).

Results

Feed intake

The results showed that feed intake trends were similar in the non-infected group, regardless of the CP content in the diet, while a similar trend but different pattern was observed in the infected group. However, a considerable variation in average daily feed intake (ADFI) was observed among the groups. The goats with the highest ADFI in the high protein non-infected (HPN), high protein infected (HPI), low protein non-infected (LPN) and low protein infected (LPI) groups consumed 7%, 12%, 12%, and 18% more than the mean, respectively.

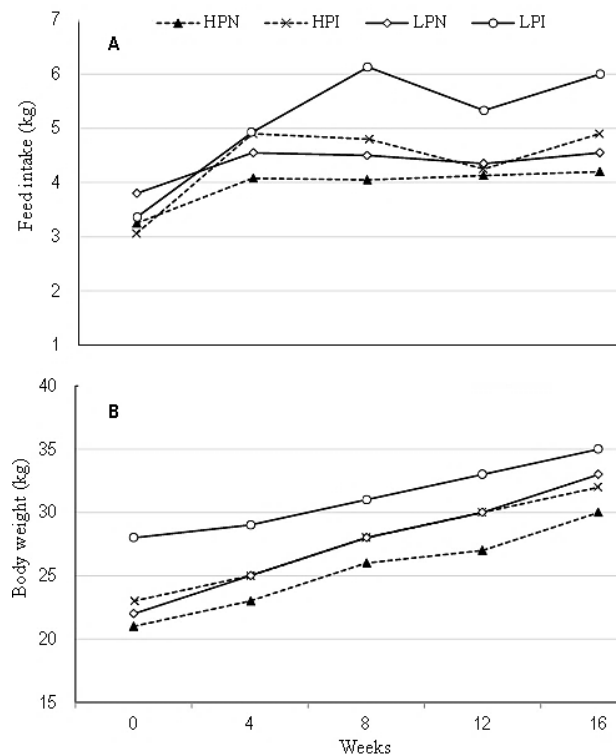


Fig 1 Feed intake (A) and mean body weight (B) of male goats (n=4) infected or non-infected with *Haemonchus contortus* and fed a high or low protein diet. HPN= high protein non-infected, HPI= high protein infected, LPN= low protein non-infected, LPI= low protein infected.

The goats with the lowest ADFI in HPN, HPI, LPN, LPI groups consumed 22%, 17%, 28%, and 6% less than the mean. The means of ADFI were 643, 607, 582, and 760 g/d for HPN, HPI, LPN and LPI groups, respectively (Fig. 1A). There was a significant effect of diet, infection and time on feed intake. The LPI group consumed significantly ($P<0.0001$) higher feed than LPN group (Table 2). There was no difference in ADFI among the three groups. The highest amount of feed consumed was recorded in LPI from week 12th to 16th and lowest in the LPN group from week 8th to 12th (Fig. 1A). There were no interactions between diet, infection and time for feed intake in the three groups.

Body weight

Time, diet and infection showed a highly significant ($P<0.0001$) effect on body weight gain (Table 2). All treatment groups recorded increased the body weight throughout the study period. However, there were differences in average daily gains (ADG) that were 84, 82, 100 and 60 g/day for HPN, HPI, LPN and LPI groups, respectively (Fig. 1B).

Generally, a higher increase in body weight was observed in the LPN group. At the end of the study, higher gains were observed in non-infected animals (HPN vs. HPI gained 45.09% vs. 40.93% and LPN vs. LPI gained 51.56% vs. 24.5% of their initial body weights, respectively) (Fig. 1B). The interactions between diet, infection and time were significant in all the groups (Table 2).

FAMACHA score

There was a highly significant ($P<0.0001$) effect of infection and diet × infection interaction on famacha scores (Table 2). The lowest FAMACHA score was recorded in the LPN group while the highest was in the LPI group (Fig. 2A). However, no difference was observed in FAMACHA scores between HPN, HPI and LPN groups (Table 2).

Packed cell volume (PCV)

Infection and diet × infection had a highly significant ($P<0.0001$) effect on PCV values (Table 2). The mean PCV values of the LPI group dropped from 30% to 20%, while those in the HPI group dropped from 32% to 30% in the post-infection period. No significant difference was observed in PCV between HPN, HPI and LPN groups (Table 2). Generally, the highest PCV values were recorded in the LPN group and the lowest in the LPI group (Fig. 2B).

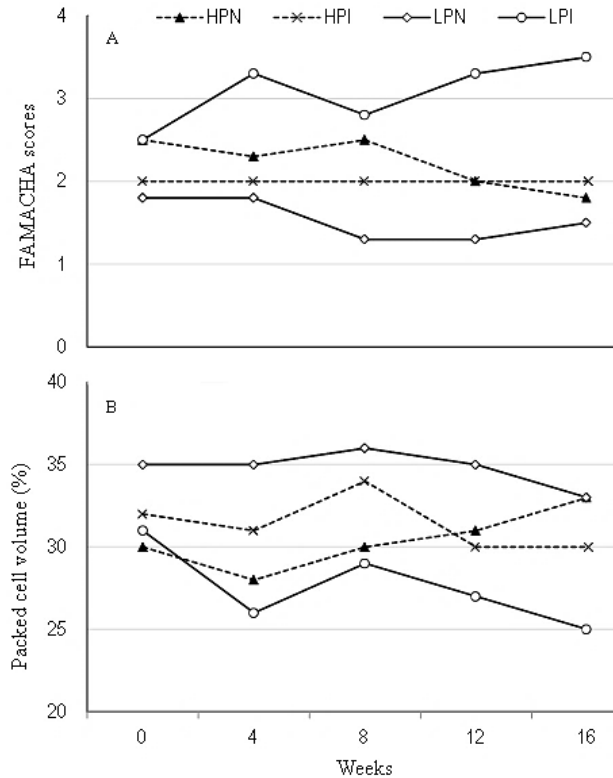


Fig 2 FAMACHA scores (A) and mean packed cell volume (B) for male goats (n=4) infected or non-infected with *Haemonchus contortus* and fed a high or low protein diet. HPN: high protein non-infected; HPI: high protein infected; LPN: low protein non-infected; LPI: low protein infected.

Fecal egg count (FEC)

Both HPI and LPI animals showed a gradual increase in FEC throughout the study period (Fig. 3). However, the LPI group had significantly higher ($P<0.0001$) FEC than the HPI group (Table 2). Goats in both HPI and LPI groups showed one major peak of FEC at the end of the study. There was no interaction observed between diet and time for FEC (Fig. 3).

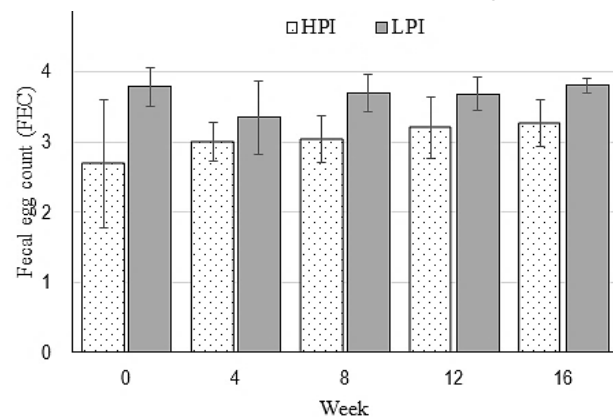


Fig 3 Mean Log transformed fecal egg count values for male goats infected with *Haemonchus contortus* and fed a high (HI) or low (LI) protein diet over a period of 16 weeks. Bars represent standard error (SE) of four replicates.

Table 2 Tests for fixed effects and specific interactions for performance and clinical parameters in goats receiving different levels of dietary protein after infection with *Haemonchus contortus*.

Effect	Feed intake	Body weight	FAMACHA score	PCV	FEC
	F-values				
Infection	29.87**	40.98**	19.10**	21.57**	-
Diet	33.06**	35.05**	0.12ns	0.00ns	24.27**
Diet × Infection	0.05ns	2.83*	46.22**	48.53**	-
Time	2.71*	11.51*	1.59ns	1.15ns	0.91ns
Infection × Time	1.31ns	0.35ns	0.97ns	0.39 ns	-
Diet × Time	0.42ns	0.08ns	0.40ns	0.19 ns	0.54ns
Diet × Infection × Time	0.98ns	0.19ns	0.40ns	0.56 ns	-

PCV = packed cell volume; FEC = fecal egg count. ns = Not significant; ** = Highly significant.

Table 3 Weight of non-carcass components from goats fed a high and a low protein diet and infected or not infected with *Haemonchus contortus*.

Parameter	High Protein		Low Protein	
	Infected	Non-infected	Infected	Non-infected
Blood (kg)	1.03 ± 0.07	1.04 ± 0.10	1.16 ± 0.16	1.04 ± 0.04
Head (kg)	2.38 ± 0.18	2.29 ± 0.05	2.39 ± 0.19	2.32 ± 0.07
Hide (kg)	3.6 ± 0.42	3.2 ± 0.16	3.6 ± 0.43	3.1 ± 0.15
Intestine (kg)	1.72 ± 0.19	1.56 ± 0.14	1.75 ± 0.08	1.61 ± 0.06
Kidney (kg)	0.39 ± 0.13	0.42 ± 0.11	0.55 ± 0.05	0.39 ± 0.03
Heart (kg)	0.15 ± 0.01	0.13 ± 0.03	0.14 ± 0.01	0.13 ± 0.01
Liver (kg)	0.52 ± 0.03a	0.47 ± 0.04b	0.51 ± 0.05a	0.39 ± 0.01b
Lung (kg)	0.62 ± 0.08	0.55 ± 0.05	0.61 ± 0.04	0.47 ± 0.02
Spleen (kg)	0.04 ± 0.01	0.05 ± 0.01	0.05 ± 0.00	0.04 ± 0.00
KPF (kg)	0.39 ± 0.13	0.42 ± 0.11	0.55 ± 0.05	0.39 ± 0.03
Omental fat (kg)	0.92 ± 0.34	0.98 ± 0.15	1.5 ± 0.17	1.39 ± 0.20
Testicle (kg)	0.22 ± 0.02	0.17 ± 0.00	0.22 ± 0.03	0.17 ± 0.02

The values are presented as mean ± standard error of three replicates.

KPF = kidney pelvic fat

Means within treatment in the same row with different letters are significantly different ($P < 0.05$)

Non-carcass components

The weight of non-carcass components in goats fed HP and LP diets were not significantly different. There were no interactions between dietary protein and infection for weight of non-carcass components (Table 3). However, the infection had a significant effect on goat liver weight with the infected groups recording heavier ($P < 0.05$) liver weights than non-infected animals (Table 4).

Discussion

Our results showed that high CP supplementation had no effect on feed intake of mature goats. This could be due to the fact that at maturity goats need $\leq 13\%$ of CP for body maintenance [10]. On the other hand, the higher feed intake observed in the LPI group could be attributed to increased demand for nutrients and CP content in the diet due to parasite infection. These results contradict the findings of Abbott et al. [11] who reported that infected male lambs given a low protein diet (88 g CP/kg DM) had more variable intakes with intake falling to about 600 g daily by day 21 post-infection.

Goats in the HPI group gained more weight than their counterparts in the LPI group over time in spite of nematode infection. Higher body weights attained within the HPI group likely resulted from adequate protein availability to meet the maintenance requirement and increased demand imposed by *H. contortus* infection, whereas protein availability for the LPI group was not sufficient to meet both demands. Additionally, it has been reported that the nematode infection reduced the growth of animal up to 50% at the same level of feed intake compared to non-infected and this reduction was caused by the shift in protein absorption away from the digestive tract [12]. The higher body weight value observed in the LPN group could be ascribed to the fact that the LP diet had adequate metabolizable protein for maintenance requirement of healthy goats. The results of this study agree with the findings of Mhomga et al. [13] and Phengvichith and Ledin [5] who reported higher body weights in infected goats fed HP diet compared to counterparts fed LP diet.

There was no difference in FAMACHA scores for HPI, HPN, and LPN groups. However, the FAMACHA score of the LPI group was significantly

higher than the other three groups, with FAMACHA scores for infected goats on LP diet increased throughout the study. The FAMACHA system is an indirect and cheap method for monitoring animal anemia resulting from nematode infection [14]. The system has been tested in most parts of the world with excellent results [15, 16]. In this study, the increase in FAMACHA score of the LPI group revealed the adverse effect of *H. contortus* infection under insufficient protein supplementation. Additionally, the high FAMACHA score in LPI group is consistent with the low body weight gain observed. These results are in agreement with that of Marume et al. [17] who also reported higher FAMACHA scores in infected, non-supplemented goats.

Table 4 Two-way analysis of variance for effects and interactions of dietary protein and infection on weight of non-carcass components of goat fed high and low protein diets.

Effect	Protein (P)	Infection (I)	P × I
Blood	0.546	0.584	0.518
Head	0.915	0.569	0.924
Hide	0.890	0.256	0.951
Intestine	0.860	0.309	0.987
Kidney	0.522	0.524	0.347
Heart	0.595	0.329	0.903
Liver	0.250	0.050	0.358
Lung	0.346	0.065	0.506
Spleen	0.924	0.436	0.147
KPF	0.523	0.523	0.346
Omental fat	0.061	0.923	0.731
Testicle	0.555	0.083	0.456

KPF = kidney pelvic fat; P × I = protein × infection interaction

The values shows P values of ANOVA and significant level is P = 0.05; Degree of freedom = 1

There was no significant difference in mean PCV among the various groups at week zero. However, there was a significant decrease in PCV in the LPI group after week 4 and 16. Infected goats on the HP diet were able to maintain high PCV levels throughout the study probably due to the enhancement of their immune systems by sufficient protein supplementation. Therefore, in response to HP supplementation goats on the HP diet showed evidence of resilience to *H. contortus* infection, a case that was not observed in the LPI group. These results were in agreement with those of Mhomga et al. [12] and Marume et al. [17] who reported high PCV in goats supplemented with a high CP source.

Goats on the LP diet had significantly higher FEC than their counterparts on the HP diet. The higher FEC in the LPI group is consistent with the low body weight gain observed in this group. This suggests that goats on the LP diet were less resistant to *H. contortus* infection. Similar results were reported by

Nnadi et al. [18] and Mhomga et al. [13] who reported significant differences in egg output of goats fed high and low protein diets.

The weight of non-carcass components in goats fed HP and LP diets were not significantly different, a result that contradicts findings of Phengvichith and Ledin [13], who reported a significant difference in non-carcass components of goats, fed high and low protein diets plus grass. In our study, the goats were only offered a concentrate based diet that varied in CP content once a day. Therefore, the lack of significance in the present study could be due to difference in feeding regime. There were no interaction between dietary protein and infection for weight of non-carcass components; however, the infection had a significant effect on goat liver weight with the infected group having heavier liver weight than non-infected animals. The heavier liver weight observed in the infected group could be ascribed to increase nutrient absorption by the liver resulting from nematode infection [11].

In conclusion, this study confirmed that protein supplementation leads to higher body weight gain and lower FAMACHA scores, FEC and increased PCV in goats. These results indicate that protein supplementation improves resilience and resistance to *H. contortus* in pen-raised goats sustained on a concentrate-only diet. Non-carcass components of goats were not affected by diet, but *H. contortus* infection significantly affected liver weight in goats.

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