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Some nutritional and metabolic aspects of calves maintained under two different dietary protein and experimentally infected with *Haemonchus placei*

Alguns aspectos nutricionais e metabólicos de bezerros mantidos com dois níveis diferentes de proteína na dieta e infectados experimentalmente com *Haemonchus placei*

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SUMMARY

The experiment was carried out to study the effects of the dietary protein and the infection with *H. placei* on nutritional parameters in six-months-old calves, based on their response to an infection of 100,000 infective larvae. Treatments consisted of two protein levels, 97.8 (G1) and 175.3 (G2) g of crude protein per kg of dry matter. After three months receiving the diet, the animals were infected. Five days prior and 30 d after infection, animals were kept in metabolic cages to measure diet digestibility, water and N balance. Thirty-five days after infection, animals were slaughtered, abomasum removed and the worm burdens counted. There were no statistical differences in live weight changes, but animals from G1 showed a tendency to decrease weight after infection. The protein level in diet showed an effect ($p < 0.01$) on plasma, urea and protein. The infection affected ($p < 0.05$) haematocrit, haemoglobin, albumin and total plasma protein. Crude protein digestibility was lower ($p < 0.05$) in G1. Dry matter and crude protein digestibilities were not affected by infection. Nitrogen balance was lower for G1 and urine nitrogen excretion was increased ($p < 0.01$) by infection. There was no effect on water balance. It is suggested that some of the nutritive parameters studied were affected by protein level in diet, as well as by the infection with *H. placei*.

UNITERMS: Calves; *Haemonchus*; Dietary protein D; Digestibility; Nitrogen balance; Water balance.

INTRODUCTION

Haemonchosis in cattle represents one of the biggest problems in Brazil[7], where wide variations in dietary protein can be observed in pastures throughout the year. It is thought that this variation in protein intake might have influences on the impact of the parasite host metabolism and productivity[1].

Previous studies have shown that *Haemonchus contortus* infection in sheep fed different dietary protein might affect the nitrogen metabolism in such animals, varying according to breed, level of protein in the diet and the level of parasite infection[1,2,9]. According to Sykes[11] (1994), parasitic infection reduces efficiency of food utilization and thus affects productivity.

This experiment was conducted to investigate the influence of dietary protein and the infection with *Haemonchus placei* upon nutritional parameters in six-months-old calves, based on their response to an infection of 100,000 larvae.

MATERIALS AND METHOD

Experimental design

In a 127-day trial, eight worm free, male, Holstein calves, four to six-months-old, were divided into two treatment groups of four calves each according to their age and weight. Group G1 was fed with a diet containing 97.8g crude protein (CP) kg^{-1} dry matter (DM) and group G2 received a diet containing 175.3 g CP kg^{-1} DM.

Approximately three months after the beginning of the trial (day 87), the animals were moved to metabolic cages for digestibility studies and water and nitrogen balance assays prior to the infection. At the end of the five days collection period (from day 87 to day 92), all animals were given a single dose of 100.000 *Haemonchus placei* infective larvae *per os*. Four weeks later, another period of collection was run for digestibility studies after infection (from day 120 to day 125).

Blood samples were collected at days 32, 62, 92 and 127 after beginning of the diet treatment. Body weights were recorded at the same interval, and all calves were slaughtered for abomasal worm counts on day 127.

Diets

Animals from group G1 received a concentrate mixture 80% corn grain, 6% cotton seed meal and 14% rice polishing. The concentrate mixture offered to animals from group G2 was 65% corn grain, 19% cotton seed meal and 16% protenose (protein supplement). All animals received 2 kg $head^{-1}$ d^{-1} grass hay *(Cynodon dactylon)*, 500 g $head^{-1}$ d^{-1} of the respective concentrated mixture and were watered *ad libitum*. Proximate analysis[3] of hay, G1 and G2 diets is given in Table 1.

Table 1

Proximate analysis (g kg^{-1}) of hay and G1 and G2 concentrates offered to calves during the experiment. Piracicaba - SP, 1995.

fractions	hay	G1	G2
dry matter	831	917	900
crude protein	67	141	298
crude fiber	329	46	47
ether extract	24	38	55
phosphorus	2	5	5

Blood analysis

Blood samples for haematological and biochemical analyses were collected by jugular venipuncture in vacutainer coated with heparin. Haemoglobin (Hb) concentration was measured by cyanomethemoglobin method (Hemometer - L. Jungberg & Co) and haematocrit was measured by the microhematocrit method. Total plasma protein, albumin, β-hydroxy-butyrate and urea were determined by kits*.

Parasitological techniques

Infective larvae were harvested from faecal cultures, maintained at room temperature (25°C) and used within five weeks after harvesting. The larvae were suspended in water, the number of L3 calculated, and administered orally to each animal.

At necropsy the abomasum was removed intact, turned inside-out and contents collected in a graduated bucket. The surface of the mucosa was washed with tap water and the residue added to the respective buckets. The abomasum was then placed in a water bath at 37°C for 6h to recover the worms. After this period, the total content was sedimented and fixed with formalin. Ten per cent homogenized samples from abomasum content were collected and fixed with formalin. The worms present in the total mucosal digest were counted. From the contents, the total number of worms was determined by multiplying the number found in 1% aliquot by the dilution factor.

Faecal egg counts were performed by modified McMaster technique[12].

Nutritional studies

Two, five-day collection periods for digestibility, water and nitrogen balance were conducted five days prior and 28 days after the infection[5]. A 20 per cent sub sample of daily feed offered, faecal and urinary output for each calf was retained and stored at -4°C for laboratory analysis. Urine was collected in vessels previously acidified with concentrated sulphuric acid.

After bulked according to period, feed and faecal dry matter content were estimated following air-drying at 60°C for 48 hours, and nitrogen determinations were performed by micro Kjeldahl procedures.

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Statistical analyses

Statistical analyses were carried out using the software Sas[10] (1991). A linear model for analyses of covariance was used to compare the end points (at infection or post infection), adjusting for initial points (at day zero and at infection, respectively). The means were adjusted by least squares and the data from worm burdens were logarithmically transformed. Body weight changes were fit in a regression model and gradients compared by analyses of variance.

RESULTS AND DISCUSSION

Clinical and body weight changes

After worm infection, varying degrees of sub-mandibular oedema were observed in calves and it was most market in those animals from group G1, which also showed weakness. Despite these clinical signs, calves exhibited no inappetence throughout the experiment. According to Parkins; Holmes[9] (1989), the degree of inappetence may vary with the level and duration of parasitism and protein intake. In this study, none of those factors appear to have affected the voluntary feed intake in the calves.

Abbott *et al.*[1] (1985), working with lambs fed a similar proportion of CP in diet, also reported that protein levels did not affect the voluntary feed intake, and only one out of seven lambs became inappetent. They also reported that only an acute haemonchosis affected feed intake in lambs[2].

At slaughter, mucosal oedema was easily detectable in the abomasum of all calves, but particularly in the G1 group, that showed the most obvious changes. Although the infection induced a clinical haemonchosis, this was not so severe in terms of affecting feed intake.

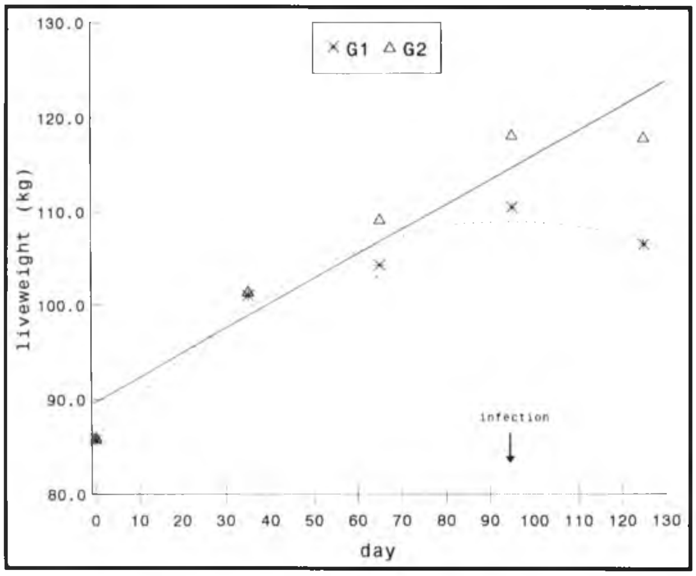

Figure 1
Mean liveweight changes (kg) in calves fed two dietary protein levels and infected with *H. placei*.

Sykes[11] (1994) reported that total inappetence may occur after a massive acute infection, despite proportional reductions in feed intake having been recorded in animals showing no clinical symptoms of helminth infection.

A linear regression ($r = 0.92$, $p < 0.01$) was fit to describe body weight changes in group G2. For group G1, a quadratic regression described better the body weight changes ($r = 0.86$, $p < 0.01$). These findings suggest that the animals in G1 started to gain weight in less time after the infection, and probably this tendency would be more significant if the observation period after infection were longer.

Fig. 1 shows weight changes for each group. According to regression models, there were no statistical differences ($p > 0.05$) between the two gradients (0.26 kg day^{-1} for group G2 and 0.17 kg day^{-1} for group G1), but despite the fact that both groups started the trial with the same live weight, at the end of trial (day 127) calves in group G1 had a live weight 10% less than calves in group G2.

The present findings are in accordance with Devaney *et al.*[6] (1992), who have reported that mean weight gain in calves infected with nematodes was about 10 kg less than that of uninfected controls during 20 weeks. Khan *et al.*[8] (1988) have found that sheep infected with *Haemonchus contortus* gained significantly less weight than controls, despite the level of infective larvae given.

Although there were no statistical differences in feed intake, nor in changes, these observed parameters may indicate the potential effect of infection upon productivity, which is in accordance to the review of Sykes[11] (1994).

Worm burdens

Faecal egg production commenced 30 days after infection. The eggs per gramme of faeces (epg) values at slaughter were 875 and 500 epg, respectively, for G1 and G2 ($p > 0.05$).

Table 2
Least square means for haematological and biochemical parameters of calves fed with two different dietary protein diets (G1 and G2) at the beginning of diet (day 0); at infection with *H. placei* (day 92) and at slaughter (day 127). Piracicaba - SP, 1995.

parameter	group	day 0	day 92	day127	SE	infection effect*
haemoglobin (g%)	G1	7.7	9.6	5.1	2.1	**
	G2	8.3	9.4	5.5	2.3	**
haematocrit (%)	G1	26.3	25.1	15.0	6.6	**
	G2	29.3	24.8	14.0	7.1	**
albumin (g%)	G1	1.4	2.0	1.5	0.4	**
	G2	1.8	2.5	1.6	1.8	**
total protein (mmol/l)	G1	79.6	70.7^a	48.9	13.5	**
	G2	86.3	78.5^b	51.8	16.4	**
urea (mmol/l)	G1	2.9	2.7^b	2.9	0.8	ns
	G2	4.4	4.7^b	4.3	1.3	ns
β-OH-butyrate (mmol/l)	G1	0.12	0.19	0.28	0.1	ns
	G2	0.13	0.23	0.23	0.1	ns

* = significancy for the difference between pre and post infection;
** = p<0.01; different letters in a column mean statistical difference between G1 and G2;
SE = standard error of the means;
ns = not significant.

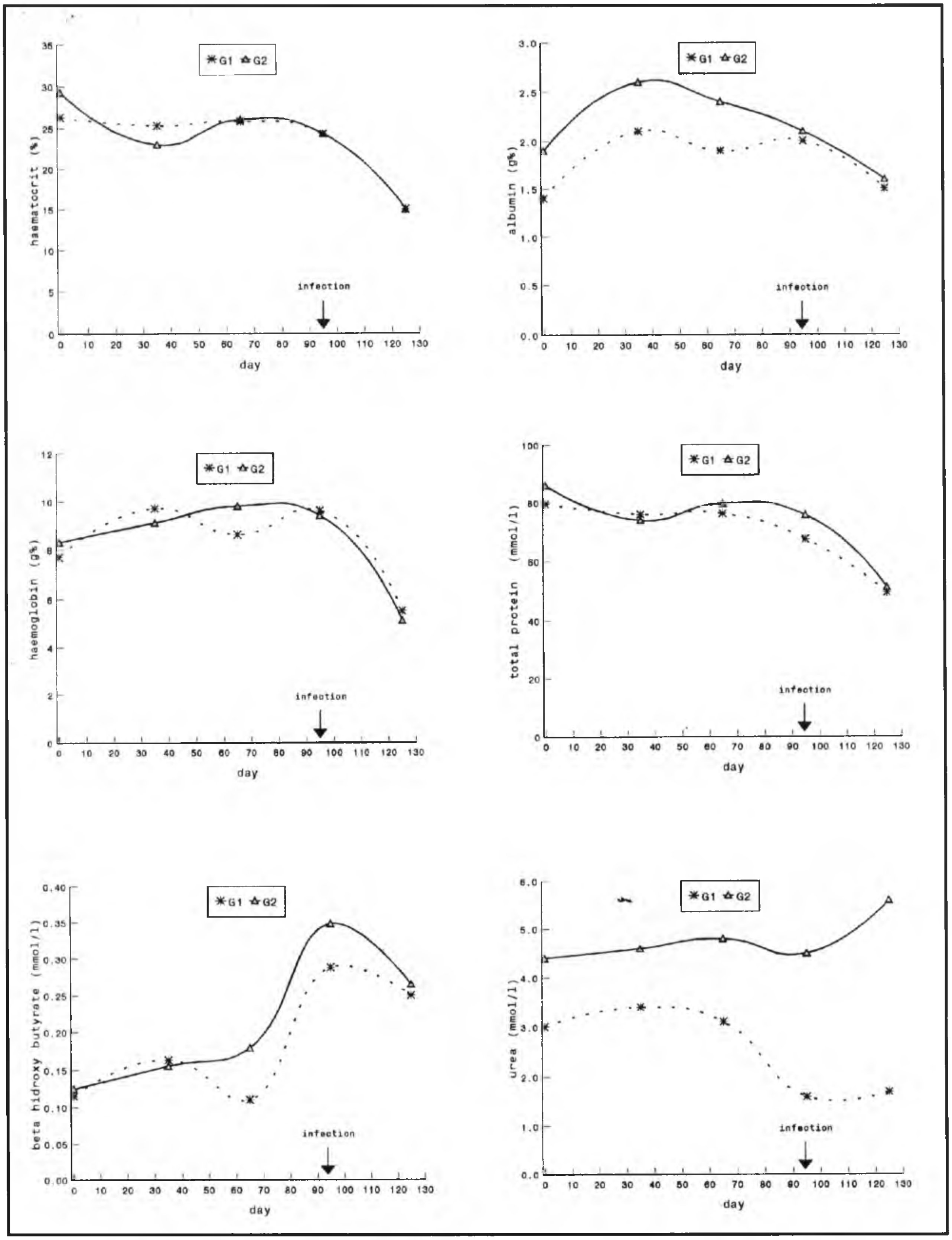

Figure 2

Metabolic parameters in calves fed two dietary protein levels and infected with *H. placei*.

At slaughter the worm burdens (mean ± standard error) for G1 was 41,500 ± 14,870 and for 52,850 ± 21,300 *H. placei*. The difference was not significant, and it probably occurred due to the great difference in worm burdens among animals.

The composition of diet did not influence the worm establishment. According to Abbott *et al.*[1,2] (1985, 1986) and Berry; Dargie[4] (1976), the proteïn content of the diet did not appear to influence the parasite establishment, however it influenced the host's ability in the immune-mediated expulsion of worm.

Haematological and biochemical changes

Table 2 shows the least square means for the haematological and biochemical parameters. Haematocrit values were within the normal limits until day 92, when the animals received the infection. The haemoglobin values were not statistically different ($p < 0.05$) between groups. On day 127 these parameters showed a significant fall ($p < 0.01$) but there were no statistical differences between groups, reflecting an effect of infection and not the protein level in the diet (Fig. 2).

Abbott *et al.*[1] (1985) did not find differences either, on haematocrit of lambs fed low or high protein diets, in a moderate haemonchosis.

Plasma albumin was significantly ($p < 0.01$) affected by infection in both dietary groups, with levels dropping from 2.0 and 2.5 at day 92 to 1.5 and 1.6 g l^{-1} at day 127, respectively for groups G1 and G2 (Table 2), and again the level of protein in diet did not affect ($p > 0.05$) plasma albumin levels. Abbott *et al.*[2] (1986) have found significant differences in serum albumin concentrations in lambs fed high or low protein diets, and given an acute infection of *H. contortus*. However, Abbott *et al.*[1], studying the pathophysiology of a moderate haemonchosis in lambs, did not find differences in serum albumin levels among animals on high or low dietary protein levels.

The above mentioned results agree with Parkins; Holmes[9] (1989), and the albumin level in plasma of *Haemonchus sp* infected ruminants is affected by the extension of infection and dietary protein.

Total plasma protein and plasma urea were significantly higher ($p < 0.01$) in calves from group G2 than in those from group G1 (Table 2), and the infection affected total plasma protein, with concentrations significantly ($p < 0.01$) reduced in both G2 and G1 animals. β-hydroxy-butyrate concentration in plasma was not affected ($p > 0.05$) by level of dietary protein, nor by the infection with *Haemonchus placei* (Table 2).

The metabolic parameters studied indicate that the diets were nutritionally different in terms of protein content, but their energy content were similar throughout the trial (Fig. 2). Differences in plasma protein, and plasma urea concentration are explained by the different content of crude protein of the diets. However, similar plasma β-hydroxy-butyrate concentration in the groups indicate that the amount of energy offered in both diets was enough to keep the calves in positive energy balance, even though in group G1 there was a tendency for loosing weight at the end of trial.

Nutritional studies

Results for apparent digestibility of DM and CP fractions are shown in Table 3, while data for nitrogen and water balance studies can be seen in Table 4 and Table 5, respectively.

DM digestibility was not different ($p > 0.05$) between dietary protein groups, however, the apparent digestibility coefficients of CP were significantly ($p < 0.05$) lower in calves fed G1 diets than calves on G2 diets. The infection did not affect ($p > 0.05$) either DM, or CP apparent digestibility coefficients (Table 3).

These findings are in accordance with the results reported for lambs fed on high or low dietary protein levels, indistinctive to a moderate or acute haemonchosis[1,2].

The nitrogen intake was respectively 29.4 and 63.3 g N d^{-1} for G1 and G2 groups, during both observation periods (prior and after infection) (Table 4). Nitrogen excretion via faeces was not different ($p > 0.05$) between dietary protein groups, neither was it affected by the infection. However, the urinary N

Table 3

Apparent digestibility coefficient of dry matter and crude protein fractions of diets given to calves, prior and after infection with *H. placei*. Piracicaba - SP, 1995.

fraction	group	day 92	day 127	SE	infection effect*
dry matter	G1	0.58	0.54	0.07	ns
	G2	0.62	0.61	0.05	ns
crude protein	G1	0.38[a]	0.43[a]	0.11	ns
	G2	0.68[b]	0.69[b]	0.04	ns

* = significancy for the difference between pre and post infection; different letters in a column mean statistical difference between G1 and G2;
SE = standard error of the means;
ns = not significant.

Table 4

Nitrogen balances (g N day^{-1}) of calves fed with two different dietary protein diets (G1 and G2), prior (day 92) and after (day 127) infection with *H. placei*. Piracicaba - SP, 1995.

fraction	G1 day 92	G1 day 127	G1 SE	G2 day 92	G2 day 127	G2 SE	infection effect*
intake	29.4	29.4	0	63.3	63.3	0	ns
faeces	18.2	16.5	2.5	20.1	19.1	3.3	ns
urine	4.3	16.4	7.3	6.1	22.3	9.4	**
retained	6.9	-3.6	7.0	34.5	21.9	9.0	**

* = significancy for the difference between pre and post infection;
** = $p < 0.05$;
SE = standard error of the means; ns = not significant

Table 5

Water balance (l day^{-1}) of calves fed with two different dietary protein diets (G1 and G2), prior (day 92) and after (day 127) infection with *H. placei*.Piracicaba - SP, 1995.

fraction	G1 day 92	G1 day 127	G1 SE	G2 day 92	G2 day 127	G2 SE	infection effect*
intake	9.7	10.3	0.4	9.8	10.5	0.5	ns
faeces	3.7	3.2	0.4	3.4	3.2	0.1	ns
urine	1.8	2.7	0.6	1.6	2.4	0.6	ns
balance	4.2	4.3	0.1	4.8	4.9	0.1	ns

* = significancy for the difference between pre and post infection;
SE = standard error of the mean; ns = not significant.

output was significantly higher ($p < 0.01$) after infection, on both G1 and G2 groups.

These observations led to a decrease in then balance after infection of 23.5 to -12.2% in the low dietary protein group, and of 58.8 to 34.9% in the high dietary protein group ($p < 0.01$).

The water balance study showed that neither the dietary protein, nor the infection affected the intake, excretion or water retention in the studied calves (Table 5). However, the calves were dehydrated with submandibular oedema. Maybe a more accurate technique, based on isotope dilution, rather than on simple water intake and excretion balance, should be used.

CONCLUSIONS

It was concluded that some of the metabolic parameters studied in calves were affected by the level of protein in the diet, and or by the infection with *H. placei*. There was a tendency for animals on a higher protein content in diet to perform better than the animals on a lower protein content in diet.

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RESUMO

O presente experimento teve por objetivo o estudo dos parâmetros nutricionais em bezerros mantidos sob dietas com dois níveis protéicos e infectados com 100.000 larvas infectantes de *H. placei*. Os animais do grupo 1 (G1) receberam uma dieta com 97,8 g de proteína bruta (PB) por kg de matéria seca (MS) e os do grupo 2 (G2), 175,3 g PB kg^{-1} MS. A infecção foi realizada três meses após o início da dieta. Cinco dias antes e 30 dias após a infecção, os animais foram colocados em gaiolas metabólicas para estudos de digestibilidade e balanços hídrico e de nitrogênio. Trinta e cinco dias pós-infecção os bezerros foram abatidos e os vermes recuperados. A variação de peso nos dois grupos não foi estatisticamente diferente ($p > 0,05$) e houve uma tendência, nos animais do G1, a reduzir o ganho de peso após a infecção. O nível protéico da dieta teve efeito significativo ($p < 0,01$) nos teores de uréia e proteína plasmática; por outro lado, a infecção afetou os teores de hematócrito, hemoglobina, albumina e proteína total ($p < 0,05$). A digestibilidade aparente da PB foi inferior ($p < 0,05$) no G1 e a digestibilidade da MS e PB não foram afetadas pela infecção ($p > 0,05$). O balanço de nitrogênio foi inferior no G1 ($p < 0,01$) e a infecção alterou a excreção de nitrogênio via urina ($p < 0,01$). O balanço hídrico não mostrou significância ($p > 0,05$) para as fontes de variação estudadas.

UNITERMOS: Bezerros; *Haemonchus*; Digestibilidade; Dieta protéica; Equilíbrio nitrogenado; Equilíbrio hídrico.

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