

## Effect of dietary protein intake on calf resilience to *Haemonchus placei* infection

### Efeito da proteína da dieta no desempenho de bezerros infectados com *Haemonchus placei*

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#### SUMMARY

Twenty, 2-3-month-old worm free male Holstein calves, were assigned to two groups each containing ten animals. Each group was offered one of two diets: High (HP) and Low (LP) protein with 257 and 91 gkg<sup>-1</sup> dry matter respectively, balanced for energy and minerals. After an initial period of 4 weeks on the diets, the calves from each group were subdivided into two groups of four and six calves. A trickle infection of 5,000 *Haemonchus placei* L3 was given twice a week for nine weeks to the sub group of six calves (I). The remaining four calves from each dietary group were used as non-infected control (C). Four weeks after the last infection, all calves were slaughtered and worm burdens counts. Carried out Biochemical determinations, faecal egg counts and body weights were carried out once a week. The HP group had significantly higher mean adult worm burdens (11,900 ± 7,660) when compared with BP (5,450 ± 7,895). Faecal egg counts were higher in the HP than LP group. Despite higher worm burdens, resilience was increased in the HP calves, with higher packed cell volume values as well as body weight when compared with the LP group.

**KEY-WORDS:** *Haemonchus placei*. Protein . Diets.

#### INTRODUCTION

Gastrointestinal parasitism is predominantly a problem in grazing ruminants, and there is limited information on the influence of nutrition on the ability of growing calves to accommodate nematode infection<sup>15</sup>. *Haemonchus placei* is one of the most prevalent and pathogenic endo-parasites of cattle in tropical and sub-tropical areas<sup>14</sup>. In these regions the herds, particularly the beef animals, depend almost entirely on pasture for their food supply and wide fluctuations in nutrient availability and quality occur over the different seasons. In Brazil, the beef herd is concentrated mainly in the sub-tropical regions of the country. Epidemiological studies conducted in these areas<sup>5,16,18</sup> have shown that *Haemonchus placei* is the second most prevalent gastrointestinal parasite.

In ruminants the nutritional status, particularly the availability of protein and minerals, is an important factor in optimising animal productivity and resilience of the host to gastrointestinal parasites<sup>7,15</sup>. However, few studies have been conducted in young cattle to investigate the interaction between nutrition and the pathogenic effects of *Haemonchus*

infection and resistance to parasitism<sup>3,11</sup>. In these experiments, the authors studied the influence of nutritional supplementation on worm establishment and pathogenesis in growing calves previously immunised with repeated single infections of *H. placei* larvae. The current experiment investigates the effect of dietary protein intake on the resilience of young growing calves subjected to a 'trickle' infection of *H. placei* larvae, which mimic more closely the field situation.

#### MATERIAL AND METHOD

##### Animals and experimental design

Twenty, 2-3-month-old, male Holstein calves, which had been reared indoors, worm-free from birth, were assigned to two groups each containing ten animals, to provide uniformity of body-weight (weight range for the groups at week 0; 60.6 to 65.9 kg). All animals were housed in individual pens on a slatted floor. Each group was offered one of two diets, which differed in protein content: high (HP) and low protein (LP) groups, which were balanced for energy and mineral content. After an initial acclimatisation

period of 4 weeks on the diets, the animals from each dietary group were sub-divided into two groups comprising four and six calves. A trickle infection of 5,000 *Haemonchus placei* L<sub>3</sub> was given twice a week for nine weeks (HP-I and LP-I) to the larger sub-group (n=6). This regimen was designed to establish a moderate number of worms which reflects the field situation of haemonchosis in cattle in Brazil. The remaining four calves from each dietary group were used as non-infected control (HP-C and LP-C). Four weeks after the last infection with larvae, all calves were slaughtered and their worm burdens determined. Blood, faecal samples and bodyweights were recorded or taken weekly.

### Diets and dietary analyses

Samples of the diets were analysed for dry matter (DM), crude protein, fibre and mineral content<sup>4</sup> and their composition and mean analysis are given in Tab. 1. The diets consisted of Coast Cross (*Cynodon dactylon*) grass hay (basal diet) and concentrates with, 26% CP (HP group) and 9% CP (LP group) with 11 and 10 MJ metabolisable energy kg<sup>-1</sup> DM respectively. The HP diet was prepared using 50% soyabean meal and 50% ground corn and the LP diet comprised 100% ground corn.

At the beginning of the experiment the calves were offered 1.0 kg of concentrate, five weeks later 1.3 kg and from week 10 until the end of the trial 1.5 kg calf<sup>-1</sup> day<sup>-1</sup>, in accordance with their increase in bodyweight. Hay (1.0 to 1.5 kg) and water were offered *ad libitum* throughout the experimental period. Food refusals were collected daily, pooled weekly for each group and dry matter determined by drying a sample at 60°C for 48 h followed by 100°C for 24 h.

### Parasitological techniques

Infective larvae were harvested using a standard Baerman technique from faecal cultures from calves with a monospecific infection of *H. placei*. Larvae were used within two weeks of harvesting. Larvae were suspended in water; the dose calculated and administered orally to each animal.

Throughout the trial, the number of *Haemonchus* eggs per gram of fresh faeces was estimated weekly using a saturated NaCl solution, according to the standard modified McMaster method.

Following slaughter, the abomasum was removed, opened, digesta recovered and the mucosa washed. The abomasum was then incubated in 0.9% NaCl at 40°C for 6 h to liberate any larvae from the mucosa. Representative sub-samples of abomasal contents (digesta + washings) were collected (10%) and passed through a 38 mm aperture sieve and the retentate fixed with 10% formalin. The total abomasal digests were fixed with 10% formalin.

Total worm burden was estimated from 10% of the sub-sample content (1% of total volume) and from the total abomasal digest.

### Measurement of worm length

From each animal, 100 worms (50 males and 50 females) were picked out at random from the abomasal contents and measured using an image analysis system (Kontron-KS 300, Carl Zeiss Vision, Germany) at x 15 magnification.

### Blood analysis

Blood was taken by jugular venepuncture weekly before feeding. For determination of packed cell volume (PCV) and haemoglobin concentrations blood was taken into vacutainer tubes containing ethylenediaminetetraacetate (EDTA) and for other analyses blood was clotted and serum stored at -20°C.

Haemoglobin concentration was measured by the cyanomethaemoglobin method<sup>8</sup> and PCV by microhaematocrit. Total serum protein and albumin concentrations were determined fortnightly using commercial kits (Labtest, Brazil).

### Statistical analysis

The experiment was analysed using a mixed model procedure with repeated measures using the SAS system.<sup>20</sup> The effects of protein levels, infection and time were analysed as well as all interactions between these factors. The data was analysed within phase where: Phase 1 (0-4 weeks after infection), Phase 2 (5-9 weeks after infection) and Phase 3 (10-13 weeks after infection). These phases represent the stage where worm eggs start to appear in the faeces (pre-patent phase)(P1), P2 represents phase where the animals receiving continue larvae (chronic infection) and in the last 4 weeks there is no active infection of the calves (P3). Data from faecal egg counts and worm burden were logarithmically transformed as Log (x+1). Changes within groups were analysed by Student's t test with the hypothesis (H<sub>0</sub>) established as  $\mu_1 = \mu_2$  and P values less than 0.05 were considered significant.

## RESULTS

### Feed intake

The quantity of dry matter, crude protein and metabolisable energy intake by HP and LP infected and control groups during P1, P2 and P3 are shown in Tab. 2. In P1, all groups showed the similar dry matter intake, except for LP-C was which significant higher (p<0.05). In P2, the dry matter intake was significantly higher for HP-C than the others. In P3, the HP-I was significantly lower than the other groups. The effect of infection was observed in the HP groups in P2 and P3, with lower dry matter intake in the infected calves (P<0.05).

### LIVEWEIGHT

At the beginning of the pre-infection period

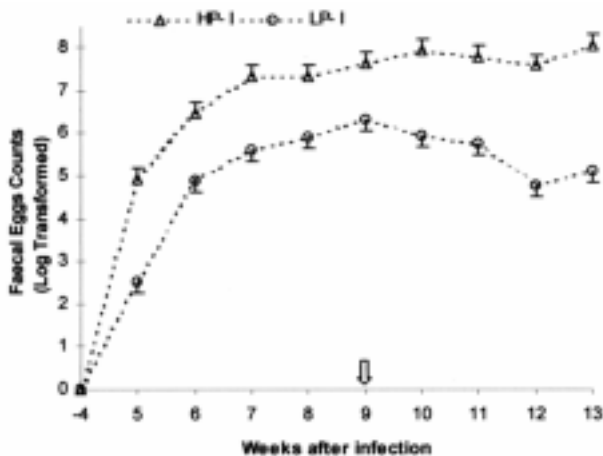
(allocation of animals to diets) all calves showed similar live weights ( $P>0.05$ ). The mean LW is shown in Tab. 2. In P1 the HP-I group (69.0 kg) was significantly heavier than HP-C (64.0 kg) and LP-I (62.8 kg) ( $p<0.05$ ), but not significantly different from LP-C (65.5kg). In P2 no differences were found between HP-C (72kg) and HP-I (76kg), but HP-I was significantly heavier than LP control and infected groups ( $p>0.05$ ). In P3 the HP control and infected groups were heavier than the LP control and infected groups ( $p<0.05$ ), but no difference was found between the control and infected groups on the groups same diet.

### Parasitological parameters

Mean weekly faecal egg counts for the HP and LP infected groups are presented in Fig. 1. Infections were patent in all groups by four weeks after the beginning of trickle dosing and remained high until end of the experiment. The statistical analysis showed that there were significant differences among between groups for (HPI vs LPI) over time ( $p< 0.05$ ). Those on restricted protein regimes showed lower mean egg counts over time. The mean worm burdens and size of the adult parasites are shown in Tab. 3. There were no significant differences between the groups in either total worm count or L4 populations; the number of L5 + adults were significantly higher in HP-I calves than in LP-I animals ( $P<0.05$ ). The highest percentage of L4 larvae (36.5%) were recovered from group LP-I which also showed the lowest adult female and male worm lengths ( $P<0.05$ ).

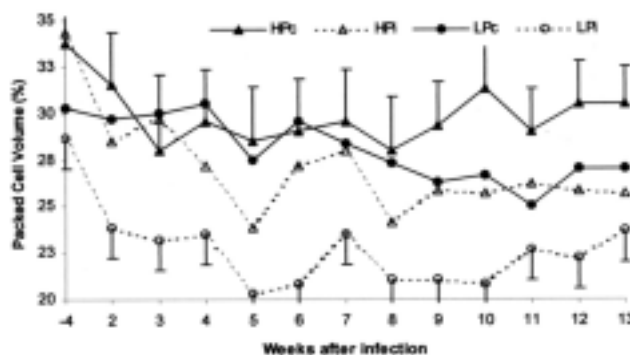
### Haematological and biochemical changes

Changes in PCV over time are presented in Fig. 2. During the full experimental period, there was no significant effect of



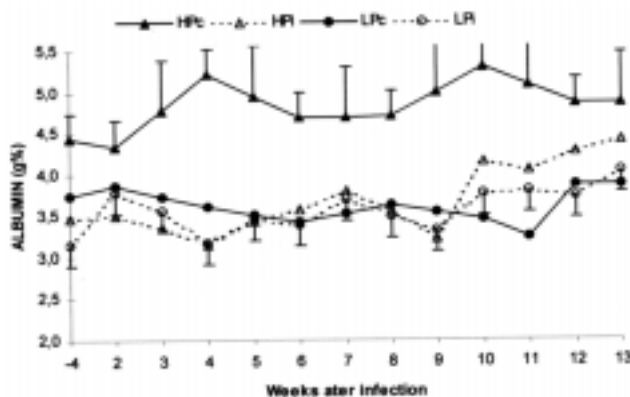
**Figure 1**

Faecal egg counts following infections. The open circles represent infected group with high protein (HP-I) while the open triangle represent infected group with low protein (LP-I). Egg counts have been transformed to  $\log_{10}(x + 1)$ .



**Figure 2**

Packed red cell volumes following infection. The closed circles represent uninfected group with high protein (HP-C), the open circles represent infected group with high protein (HP-I), while the closed triangle represent uninfected group with low protein (LP-C) and the open triangle represent infected group with low protein (LP-I).



**Figure 3**

Albumin concentration following infection. The closed circles represent uninfected group with high protein (HP-C), the open circles represent infected group with high protein (HP-I), while the closed triangle represent uninfected group with low protein (LP-C) and the open triangle represent infected group with low protein (LP-I).

**Table 1**

Chemical analyses of hay and the high protein (HP) and low protein (LP) concentrates offered to the calves, Pirassununga 1997.

	Dietary components		
	HAY	HP	LP
Dry Matter ( $\text{g kg}^{-1}$ )	922	932	937
Crude Protein*	65	257	91
Crude Fiber*	361	52	28
Ether Extract*	27	31	46
Metabolisable Energy ( $\text{MJ kg}^{-1}$ DM)	6	11	10
Ash*	58	69	54
Ca*	1.7	13.5	13.7
P*	1.1	7.6	8.6

\* ( $\text{g kg}^{-1}$  DM)

**Table 2**

Body Weight, Dry matter, crude protein and metabolisable energy intakes (per day), in calves under different feeding treatment, infected (n=6) with *H. placei* and controls (n=4), in the different experimental phases, Pirassununga 1997.

	Body Weight (kg)			Dry Matter (g kg <sup>-1</sup> BW)			Crude Protein (g kg <sup>-1</sup> BW)			Metabolisable Energy (MJ kg <sup>-1</sup> DM)		
	Phase 1	Phase 2	Phase 3	Phase 1	Phase 2	Phase 3	Phase 1	Phase 2	Phase 3	Phase 1	Phase 2	Phase 3
HP – C	64.0 <sup>b</sup>	72.0 <sup>a b</sup>	83.0 <sup>a</sup>	27.8 <sup>a</sup>	29.8 <sup>a</sup>	29.5 <sup>a</sup>	4.9 <sup>a</sup>	5.1 <sup>a</sup>	5.1 <sup>a</sup>	15.4 <sup>a</sup>	18.3 <sup>a</sup>	21.0 <sup>a</sup>
HP – I	69.1 <sup>a</sup>	76.0 <sup>a</sup>	82.9 <sup>a</sup>	27.1 <sup>a</sup>	27.3 <sup>b</sup>	27.0 <sup>b</sup>	4.6 <sup>a</sup>	4.7 <sup>b</sup>	4.9 <sup>a</sup>	16.0 <sup>a</sup>	17.9 <sup>a</sup>	19.93 <sup>a b</sup>
LP – C	65.5 <sup>a b</sup>	70.5 <sup>b</sup>	75.5 <sup>b</sup>	30.3 <sup>b</sup>	27.6 <sup>b</sup>	30.6 <sup>a</sup>	2.4 <sup>b</sup>	2.2 <sup>c</sup>	2.5 <sup>b</sup>	15.9 <sup>a</sup>	16.3 <sup>b</sup>	19.2 <sup>b c</sup>
LP – I	62.8 <sup>b</sup>	68.9 <sup>b</sup>	73.5 <sup>b</sup>	27.4 <sup>a</sup>	28.4 <sup>b</sup>	29.7 <sup>a</sup>	2.2 <sup>b</sup>	2.3 <sup>c</sup>	2.4 <sup>b</sup>	14.4 <sup>b</sup>	16.5 <sup>b</sup>	18.6 <sup>c</sup>
SED	0.788	0.705	0.733	0.286	0.257	0.266	0.051	0.046	0.047	0.216	0.194	0.201

HP, high protein diet; MP, medium protein diet; LP, low protein diet; I, infected; C, control; BW, body weight; DM, dry matter.

**Table 3**

Mean worm burdens and worm lengths (± SD) for HP and LP infected groups (n=6), Pirassununga 1997.

	Worm Burden (mean ± SD)			Adult Worm Size (mm) (mean ± SD)	
	L4	L5 + Adult	Total	Male	Female
HP-I	1537 ± 1754 <sup>a</sup>	11900 ± 7660 <sup>a</sup>	13437 ± 9194 <sup>a</sup>	17.92 ± 1.63 <sup>a</sup>	22.35 ± 1.83 <sup>a</sup>
LP-I	3100 ± 5017 <sup>a</sup>	5450 ± 7895 <sup>b</sup>	8550 ± 8430 <sup>a</sup>	16.77 ± 3.88 <sup>b</sup>	19.61 ± 1.79 <sup>b</sup>

Values within columns without a superscript letter in common are significantly different (P<0.05).

nutrition (HP-C vs LP-C) on PCV. For HP-C and HP-I there were no significant differences between groups over the full experiment period. There were significant differences in the overall mean values for PCV between the LP-C and LP-I (p<0.05) up to week 10, after which there were no significant differences observed. In this case, the effect of infection was significant (p<0.05). The interaction of diet and infection was also significant (p<0.05), the high protein infected calves had higher values PCV than low protein infected group most of the time (p<0.05), up week 11.

With respect to haemoglobin concentrations (data not shown) there were significant differences due to infection effects (p<0.05). This occurred when infected groups were compared with their respective controls, the LP-C calves showing significantly higher haemoglobin concentration (p<0.05) throughout the experiment except for weeks 3, 11 and 12. The same results were observed for HP-C vs HP-I except in weeks 3 and 7. There were no significant effects of interaction of diet X infection (P>0.05), the mean haemoglobin values were higher for HP-I than LP-I during full experimental period, but the difference was not significant.

The albumin levels are presented in fig. 3. There was a significant effect of diet, infection and the interaction of diet and infection. The globulin values were higher for HP-C group when compared with LP-C from week 4 until to the end of the trial. There were no significant differences between LP-C and LP-I for albumin values, but for HP-C the mean

albumin values are higher than HP-I (p<0.05), from week 2 to week 9. The infected groups had no significant difference with respect to albumin content.

Total serum protein concentration showed similar behaviour to the globulin levels (data not shown). The LP-C group had significantly lower serum protein values (p<0.05) than HP-C from week 2 until the end of the trial, showing the effect of the diet (p<0.05) on this parameter. The total serum albumin concentrations showed no significant differences between the infected and control LP groups during the experiment. For HP-C group, the mean of total protein values was significantly higher than HP-I from week 8 to the end of the trial, except for weeks 6 and 12. There was a significant difference for diet X infection interaction, but when comparing LP-I with HP-I for total serum protein, there were no significant differences, both groups were low.

## DISCUSSION

No obvious clinical disease such as inappetance, marked weight loss or submandibular oedema, was observed despite the high faecal egg counts and anaemia observed in many of the calves. Previous experiments with haemonchosis in calves have shown that infection is usually present as a subclinical disease, particularly in zebu cattle and that the outcome of infection in Holstein-Friesian cattle is dependent on the dose of infective larvae administered<sup>10,17,19</sup>. In the

majority of these experiments calves received an adequate balanced diet and a single infection with *Haemonchus* larvae. The aim of the present study was to investigate whether the provision of a moderate to high protein diet would improve the resilience of young growing calves subjected to a 'trickle' challenge with *H. placei* which more closely resembles the field situation.

The dietary treatments had no significant effect on the total worm burdens which is in agreement with the general consensus from studies with single and 'trickle' infections of abomasal nematodes in sheep and cattle where the main influence of protein intake is on the ability of the animal to cope with the consequences of the parasite infection, there being no marked protein effect on parasite establishment<sup>1,2,7,11,22</sup>. However, the animals offered the LP diet had higher numbers of immature worms ( $L_4$ ), lower numbers of  $L_5$ , adult stages and smaller adult worm size. This situation was reflected in lower faecal egg counts. One possible interpretation is that the corn diet was the responsible for this effect, not only in the quantity, but also the quality of the protein (specific amino acid) or that the rumen fermentation could have influenced larval development. Abbott et al.<sup>1</sup> found that Scottish Blackface female lambs fed a low protein diet had maximum faecal egg counts of 12000 and a mean worm burden of 550 against 15000 and 816 respectively with high protein intake. Gennari et al.<sup>11</sup> observed no differences in worm populations in calves given a single *H. placei* infection and fed two different corn diets (80% corn, 6.5% cotton seed meal and 13.5% rice polishing) and (65% corn, 19% cotton seed meal and 16% protenose). Further experiments are required to determine whether a chemical diet composition can affect worm populations in cattle. There is evidence in pigs that worm populations may be influenced by the physical characteristics of diets which are very similar in chemical composition.<sup>6</sup>

*H. placei* infection reduced the growth rate of the calves on a high protein diet when compared to the uninfected control. At P1, the HP- I group was heavier than

HP-C, though by the end of the trial (P3) they had similar weights. The results indicate that the effect of *Haemonchus* infection was to decrease voluntary feed intake from in the HP-I group during P2 and P3, but the same effect not observed in calves on the low protein diet. The reason for these differences are unclear as often the impact of gastrointestinal nematode infection is more marked in animals which are offered a low protein diet,<sup>7,15</sup> but they could result from differences in the structure of the parasite populations.

These findings are in general agreement with haemonchosis in sheep offered a moderate/high protein diet<sup>13</sup> and contrast with **Ostertagia** infection in sheep and cattle were the main contributor to reduced live weight gain are reductions in appetite.<sup>9,21</sup>

The results clearly showed that the effects of *H. placei* on haematological parameters were more severe in the calves which received the lower protein ration. Infection had little effect on serum albumin concentrations, the changes being mainly attributable to diet. In an experiment in which calves were challenged with single doses of 50,000 to 100,000 *H. placei*  $L_3$ , after feeding for three months on a low (98g  $kg^{-1}$  DM) or high (175g  $kg^{-1}$  DM) protein diet, Gennari et al.<sup>11</sup> found a similar tendency for greater pathogenesis on the lower protein ration. However, the decrease in the serum albumin and protein concentrations were more pronounced, particularly in the LP calves, following challenge which was probably due to the higher doses of larvae administered.

In conclusion, in this trial it could be observed that the protein supplementation increased some characteristics of the resilience such as packed cell volume and body weight on calves.

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## RESUMO

Vinte bezerros com 2 a 3 meses de idade criados livres de vermes foram divididos em 2 grupos com 10 animais cada alimentados com alta proteína (HP) e baixa proteína (LP) com 257 e 91 g  $kg^{-1}$  de proteína na matéria seca respectivamente, devidamente balanceado em energia e minerais. Após 4 semanas submetidos a estas duas dietas cada grupo original foi subdividido em 2 grupos, um contendo 4 animais não infectado (C) e 6 animais infectados (I). O grupo infectado recebeu 5.000 larvas de *Haemonchus placei* duas vezes por semana por um período de 9 semanas, após 4 semanas da última infecção todos os animais foram sacrificados e realizada a contagem de vermes. Semanalmente foram feitas as pesagens dos animais, número de ovos por grama de fezes e colheita de sangue para determinação do hematócrito, hemoglobina, albumina e proteína total. A contagem de ovos por grama de fezes foi superior no grupo de HP em relação ao grupo de LP, tendo em vista que o número de vermes adultos no grupo HP (11.900  $\pm$  7.660) foi maior que o grupo de LP (5.450  $\pm$  7.895). Apesar do número superior de vermes encontrado no grupo HP, observou-se valores superiores de hematócrito e peso vivo quando comparado com o grupo de LP ( $p < 0,05$ ), demonstrando que a suplementação protéica possibilita uma melhor resiliência em bezerros infectados com *Haemonchus placei*.

**PALAVRAS-CHAVE:** *Haemonchus placei*. Proteína. Dieta.

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