INFLUENCE OF PROTEIN INTAKE ON CARCASS COMPOSITION OF CALVES INFECTED WITH HAEMONCHUS PLACEI

INFLUÊNCIA DO TEOR PROTÉICO DA DIETA NA COMPOSIÇÃO DA CARCAÇA DE BEZERROS INFECTADOS COM HAEMONCHUS PLACEI

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ADDITIONAL KEYWORDS

SUMMARY
Thirty, 2-3-month-old worm free male Holstein calves, were assigned to three groups each containing ten animals. Each group was offered one of three diets: High (HP), Medium (MP) and Low (LP) protein groups with 426, 257 and 91 g kg⁻¹ 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 the 7th and 10th rib joint was dissected to evaluate carcass composition and worm burdens. Faecal samples for egg counts and body weights were recorded once a week. The MP group had significantly higher mean adult worm burdens (11,900 ± 7,660) when compared with LP (5,450 ± 7,895) and both are similar to HP group (8,260 ± 2,847). As expected, calves that received more protein on diet had better food utilization efficiency and gained more weight, but when comparing uninfected with infected groups for the same diet no significant differences were observed. Chemical analysis of the carcass showed higher protein and lower fat deposition in the HP (C and I) and MP (C and I) than LP (C and I), but no influence on mineral composition was observed between all groups.

Feeding calves infected with H. placei and high protein diet tended to increase live gain weight and influence body composition including less water loss with more protein and less fat deposition despite showing a higher degree of infection.

RESUMO
Trinta bezerros holandeses com 2 a 3 meses de idade, machos criados livres de vermes foram divididos em 3 grupos de dez animais. Em cada grupo foi oferecido um concentrado distinto: Alto (HP), Médio (MP) e Baixo (LP) valor proteico com os seguintes teores de proteína 426, 257 e 91
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LOUVANDINI, GENNARI, ABDALLA AND MC MANUS

g kg⁻¹ na matéria seca, respectivamente. A dieta foi balanceada em energia e minerais. Após 4 semanas recebendo esta dieta, cada grupo foi subdividido em 2 subgrupos com 4 e 6 bezerros. O subgrupo que continha 6 animais foi infectado (I) com 5.000 L3 de *Haemonchus placei* duas fezes por semana durante nove semanas. Os outros quatro bezerros restantes não foram infectados compondo o grupo controle (C). Quatro semanas após a última infecção todos os animais foram sacrificados e retirada as costelas (7 a 10ᵃ) para avaliação da composição da carcaça, bem como contagem dos vermes. Durante todo o período experimental foram feitas as contagens de ovos por grama de fezes e pesagem dos animais uma vez por semana. O grupo MP apresentou maior número de vermes adultos (11.900 ± 7.660) em relação ao grupo de BP (5.450 ± 7.895), mas não houve diferença significativa com o grupo HP (8.260 ± 2.847). Como esperado, os bezerros que receberam maiores níveis de proteína na dieta apresentaram melhor eficiência alimentar e ganho de peso, mas quando foi comparado os grupos infectados e controle sob mesma dieta não houve diferença significativa entre eles. Na análise de composição química da carcaça os grupos HP (C e I) e MP (C and I) demonstraram maior teor de proteína e menor de gordura em relação aos grupos LP (C and I), mas não houve influência na composição mineral da carcaça entre todos os grupos. Bezerros alimentados com teores de proteína mais elevados e infectados com *H. placei* tendem a aumentar o ganho de peso com menor perda de água e apresentam maior teor de proteína e menor de gordura na carcaça, mesmo tendo maior grau de infecção.

INTRODUCTION

Nutrition fluctuations (quantity and quality) throughout the year, especially that of protein, form major constraint, for cattle production in tropical areas. This situation becomes worse when the animals present parasite infections. The young animal is more affected by parasite infection because their immune system is still in development (Adams, 1988). The effective control of parasites can improve bovine production by up to 15 percent (Cherrett et al., 1971). The fact that nutrition status of the host can influence the acquisition of immunity in ruminants, and that dietary protein is of particular importance, is well known (Coop and Holmes, 1996). Wallace et al. (1995) observed that sheep that received supplementation with soya-bean and were infected with *H. contortus* had more protein, mineral and water in their carcasses than those that received no supplementation. The infection with *H. placei* effected water metabolism in calves (Vieira Bressan et al., 1992 and Gennari et al., 1997). The changes in the body composition of calves suffering from chronic haemoncosis (trickle infection of *H. placei* larvae) have not previously been investigated. This experiment investigated whether the provision of a moderate to high protein diet would improve the live weight and carcass characteristic of young growing calves subjected to a trickle challenge with *H. placei* which more closely resembles the field situation.

MATERIALS AND METHODS

ANIMALS AND EXPERIMENTAL DESIGN

Thirty, 2-3-month-old, male Holstein calves, which had been reared indoors, worm-free from birth, were assigned to three groups, each contai-
ning 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 three diets, which differed in protein content: high (HP), medium (MP) 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 L3 was given twice a week for nine weeks (HP-I, MP-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, MP-C and LP-C). Four weeks after the last infection with larvae, all calves were slaughtered and their worm burdens determined. The 7th and 10th rib joint was dissected to evaluate carcass composition. Faecal samples and bodyweights were taken or recorded weekly.

**DIETS AND DIETARY ANALYSES**

Samples of the diets were analysed for dry matter (DM), crude protein, fibre and mineral content (Association of Official Analytical Chemists, AOAC, 1995) and their composition and mean analysis are given in table I. The diets consisted of Coast Cross grass (*Cynodon dactilon*) hay (basal diet) and concentrates with 43 percent crude protein (CP) for the HP group, 26 percent CP (MP group) and 9 percent CP (LP group) with 11, 11 and 10 MJ metabolisable energy kg⁻¹ DM respectively. The HP diet was prepared using 100 percent soyabean meal (a rumen by-pass protein); the MP diet used 50 percent soyabean meal and 50 percent ground corn and the LP diet comprised 100 percent 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⁻¹ day⁻¹, in accordance with their increase in bodyweight. Hay was offered *ad libitum* throughout the experimental period (1.0 to 1.5 kg) and water was freely available.

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. Food utilization efficiency was calculated by dividing total weight gain by total food intake.

**PARASITOLOGICAL TECHNIQUES**

Infective larvae were harvested using a standard Baerman technique from faecal cultures from calves with a monospecific infection of *H. placei*. Throughout the trial, the number of *Haemonchus* eggs per gram (EPG) of fresh faeces was estimated weekly, according to the standard modified McMaster method. Following slaughter, the abomasum was removed, opened and the digesta recovered and the mucosa incubated to liberate any larvae. Total worm burden was estimated from 10 percent of the subsample content (1 percent of total volume) and from the total abomasal digest fixed with 10 percent formalin.
Carcass Evaluation

The calves were slaughtered by exsanguination via jugular vessel. The carcases were dressed, and the viscera, head and skin removed for weighting, after which the carcases were divided into two parts. The 7th to 10th rib were removed from the left side and weighed (Ledger and Hutchison, 1962), and each was sealed in a polythene bag and stored at -20°C. For chemical analysis (protein, ether extract, ash, phosphorus and calcium) the 10th rib was dissected into muscle, fat and bone. It was then weighed, finely chopped and dried to a constant weight in high vacuum freeze drier (AOAC, 1995).

Statistical Analysis

Data were analysed as a 3 X 2 factorial with 3 protein levels and 2 infection levels (with or without) and their interaction as the main effects utilizing the general linear models (GLM) procedure of SAS (Statistical Analysis System Institute Inc. 1996). Mean comparisons were carried out using the Student's t test. EPG data was analysed using a univariate repeated measures analysis. The EPG data and worm burdens were logarithmically transformed using log (x+1). P values less than 0.05 were considered significant.

RESULTS

During the experimental period, two calves either died or were sacrificed. One calf (group HP-C) accidentally broke a leg at week 9 and was sacrificed. The other calf (group HP-I) showed signs of clinical haemonchosis from week 9 (high faecal egg counts, low PCV and haemoglobin concentration).

Table I. Chemical analyses of hay and the high protein (HP), medium protein (MP) and low protein (LP) concentrates offered to the calves. (Análise química do feno e dos concentrados com alta proteína (AP), média proteína (MP) e baixa proteína (BP), oferecidos aos bezerros (Controle e Infectado)).

<table>
<thead>
<tr>
<th>Dietary components</th>
<th>HAY</th>
<th>HP</th>
<th>MP</th>
<th>LP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dry matter (g kg⁻¹)</td>
<td>922</td>
<td>929</td>
<td>932</td>
<td>937</td>
</tr>
<tr>
<td>Crude protein*</td>
<td>65</td>
<td>426</td>
<td>257</td>
<td>91</td>
</tr>
<tr>
<td>Crude fiber*</td>
<td>361</td>
<td>75</td>
<td>52</td>
<td>28</td>
</tr>
<tr>
<td>Ether extract*</td>
<td>27</td>
<td>16</td>
<td>31</td>
<td>46</td>
</tr>
<tr>
<td>Metabolisable energy (MJ kg⁻¹ DM)</td>
<td>6</td>
<td>11</td>
<td>11</td>
<td>10</td>
</tr>
<tr>
<td>Ash*</td>
<td>58</td>
<td>100</td>
<td>69</td>
<td>54</td>
</tr>
<tr>
<td>Ca*</td>
<td>1.7</td>
<td>13.2</td>
<td>13.5</td>
<td>13.7</td>
</tr>
<tr>
<td>P*</td>
<td>1.1</td>
<td>7.6</td>
<td>7.6</td>
<td>8.6</td>
</tr>
</tbody>
</table>

*(g kg⁻¹ DM).
tration and decreased food intake) and died at week 10. As the death occurred in the last phase of the trial, the data were excluded. The effect of diet was observed compared the groups control, the infection was verified when compared the group infected with your group control. The interaction between Protein and infection was observed when compared all groups infected.

**PARASITOLOGICAL PARAMETERS**

Mean faecal egg counts for the HP, MP and LP infected groups are shown in **figure 1**. Infections were patent in all groups by four weeks after the beginning of trickle dosing. The mean egg counts for group LP-I were significantly lower than those from the other infected groups (p<0.05) through the trial.

The mean worm burdens are shown in **table II**. There were no significant differences between the groups in either total worms or L4 populations; the number of L5 + adults were significantly higher in MP-I calves than in LP-I animals (p<0.05). The highest percentage of L4 larvae (36.5 percent) were recovered from group LP-I.

**LIVE WEIGHT, EFFICIENCY FOOD UTILIZATION AND TOTAL WEIGHT GAIN**

**Table III** presents live weight, food utilization efficiency and the total weight gain in the six different groups.
At the beginning of the pre-infection period (allocation to diets) all calves showed similar body weights (p>0.05). However, at the end of the trial the calves received protein supplementation were more heavier than calves with low protein diet, but this differences were no significant (p>0.05). Infected calves that received more protein in their diet had better food utilization efficiency. The MP-I group had higher efficiency than the LP-I group (p<0.05) and HP-I tended to be higher than LP-I (P=0.0657). The total live weight gain was higher in HP-C (31.7 kg) and MP (C 34.1 kg and I 31.0 kg) when compared with LP (C 23.4Kg and I 23.2 kg) (p<0.05), but no difference significant with HP I (29.9 kg) (p>0.05).

CARCASS WEIGHT AND COMPOSITION

The MP-C group showed carcass weight higher than LP (C and I) groups (p<0.05), but there were no significant difference between the others groups.

The significant effect of infection was observed in the killing out percentages only for LP-C, which higher values than LP-I, but there were no significant differences between the other treatments when compared the group C with I (table III). The mean values of water were similar between HP-C and LP-I groups, but it was greater in MP-C and LP-C than the respective infected groups (p<0.05). The calves on the increased dietary protein HP (C and I) had proportionally more protein and less fat deposition (rib and muscle) than the LP groups (p<0.05). However, there were no effects on muscle, bone and mineral (either Ca or P levels) composition due to diet, infection or diet X infection status.

DISCUSSION

This study suggests that calves received additional protein supplement had no effect on the rate of worm establishment. However, the pathophysiological consequences are usually more severe in animals on lower planes of protein intake. This is in agreement with the general consensus from studies (Abbott et al., 1985, 1986; Wallace et al., 1995; Gennari et al., 1995; Coop and Holmes, 1996). The animals offered the LP diet had higher numbers of immature worms (L4), lower numbers of L5 and adult stages and lower faecal egg counts. It is difficult to interpret this result. One explanation may be that not only the quantity of protein, but the composition of the food may have adverse effects on the parasites development, such as alterations in rumen fermentation.

**Table II.** Mean worm burdens for HP, MP and LP infected groups (n=6). (Médias do número de vermes encontrados nos bezerros infectados (n=6) nos diferentes tratamentos AP, MP e BP).

<table>
<thead>
<tr>
<th></th>
<th>L4</th>
<th>L5 + Adult</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>HP-I</td>
<td>786±1242*</td>
<td>8260±2847*</td>
<td>9046±4938*</td>
</tr>
<tr>
<td>MP-I</td>
<td>1537±1754*</td>
<td>11900±7660*</td>
<td>1343±9194*</td>
</tr>
<tr>
<td>LP-I</td>
<td>3100±5017*</td>
<td>5450±8895*</td>
<td>8500±8430*</td>
</tr>
</tbody>
</table>

Values within columns without a superscript letter in common are significantly different (p<0.05).
availability of amino acids, changes in flow rates and pH of the digest.

Most of the studies found in the literature evaluating nutrition and endoparasites were carried out on sheep with few on cattle. Young animals are more susceptible to parasites and nutritional deficiency than older ones and any problem in this phase may have a lasting effect throughout the productive life of the animal. Various authors have shown that the principal effect of haemonchosis on production of ruminants is the reduction in the efficiency of use of the ration (Sykes, 1983 and Entrocasso et al., 1986).

It should be noted that total weight gain is associated with efficiency of use of the ration and is similar to the result presented here where protein had a beneficial effect on these parameters in both control and infected animals. Nonetheless the result for animals from groups HP (C and I) and MP(C and I) were similar, despite differences in the amount of crude protein offered, but the MP-I groups had higher total live weight and efficiency of food utilization than LP-I group. The amount of energy fed to the HP groups was insufficient to allow the animals to fully utilise the increased amount of protein.

Abdalla et al. (1996) showed that calves fed two levels of protein (97.8 and 175.3 g of crude protein per kg of dry matter) and infected with 100,000 Haemochus L3 (single dose), showed

**Table III.** Mean efficiency of food utilization, total weight gain and carcass composition in infected (n=6) and control (n=4) calves. (Valores médios da eficiência alimentar, ganho de peso total e composição da carcaça em bezerros infectados (n=6) e controle (n=4)).

<table>
<thead>
<tr>
<th></th>
<th>HP - C</th>
<th>HP - I</th>
<th>MP - C</th>
<th>MP - I</th>
<th>LP - C</th>
<th>LP - I</th>
<th>SED</th>
</tr>
</thead>
<tbody>
<tr>
<td>Live weight (kg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Efficiency of food utilization*</td>
<td>10.9*</td>
<td>11.3*</td>
<td>12.6*</td>
<td>11.8*</td>
<td>9.1*</td>
<td>8.1*</td>
<td>1.3</td>
</tr>
<tr>
<td>Total weight gain (kg)</td>
<td>31.7*</td>
<td>29.9*</td>
<td>34.1*</td>
<td>31.0*</td>
<td>23.4*</td>
<td>23.2*</td>
<td>4.3</td>
</tr>
<tr>
<td>Carcass weight (kg)</td>
<td>33.5*</td>
<td>34.6*</td>
<td>42.3*</td>
<td>34.0*</td>
<td>32.8*</td>
<td>28.8*</td>
<td>3.9</td>
</tr>
<tr>
<td>Killing out*</td>
<td>42.0*</td>
<td>44.0*</td>
<td>46.0*</td>
<td>44.6*</td>
<td>46.0*</td>
<td>41.2*</td>
<td>2.1</td>
</tr>
<tr>
<td>Rib water*</td>
<td>58.2*</td>
<td>57.3*</td>
<td>61.5*</td>
<td>56.2*</td>
<td>60.1*</td>
<td>55.7*</td>
<td>1.5</td>
</tr>
<tr>
<td>Rib muscle*</td>
<td>65.7*</td>
<td>66.2*</td>
<td>71.9*</td>
<td>71.5*</td>
<td>72.4*</td>
<td>68.5*</td>
<td>3.5</td>
</tr>
<tr>
<td>Rib bone*</td>
<td>32.2*</td>
<td>30.8*</td>
<td>26.2*</td>
<td>25.6*</td>
<td>24.7*</td>
<td>29.3*</td>
<td>3.1</td>
</tr>
<tr>
<td>Rib fat*</td>
<td>4.2*</td>
<td>3.9*</td>
<td>5.7*</td>
<td>4.9*</td>
<td>8.0*</td>
<td>7.3*</td>
<td>1.75</td>
</tr>
<tr>
<td>Rib ash**</td>
<td>6.3*</td>
<td>6.3*</td>
<td>5.8*</td>
<td>5.6*</td>
<td>5.6*</td>
<td>6.4*</td>
<td>0.8</td>
</tr>
<tr>
<td>Muscle: Crude protein**</td>
<td>72.5*</td>
<td>72.4*</td>
<td>71.7*</td>
<td>64.7*</td>
<td>60.5*</td>
<td>60.5*</td>
<td>3.9</td>
</tr>
<tr>
<td>Ether extract**</td>
<td>15.4*</td>
<td>10.2*</td>
<td>19.2*</td>
<td>19.8*</td>
<td>28.4*</td>
<td>24.7*</td>
<td>4.2</td>
</tr>
<tr>
<td>Bone: Ca**</td>
<td>17.1*</td>
<td>16.8*</td>
<td>16.6*</td>
<td>18.1*</td>
<td>16.1*</td>
<td>18.1*</td>
<td>1.0</td>
</tr>
<tr>
<td>P**</td>
<td>10.3*</td>
<td>10.5*</td>
<td>10.3*</td>
<td>10.5*</td>
<td>10.3*</td>
<td>10.4*</td>
<td>0.2</td>
</tr>
</tbody>
</table>

HP, high protein diet; MP, medium protein diet; LP, low protein diet; I, infected; C, control; values within rows without a superscript letter in common are significantly different (p<0.05).

*p.100; **p.100 DM.
a lower nitrogen balance for the LP group and the urine excretion was increased by infection. Armour et al. (1987) showed inappetence, weight loss, impaired nitrogen retention and a loss of plasma proteins into the gut on calves which had received daily infection of Cooperia oncophora.

These findings are in general agreement with haemonchosis in sheep offered a moderate/high protein diet (Holmes, 1993) and contrast with Ostertagia infection in sheep and cattle were the main contributor to reduced live weight gain is a reduction in appetite (Sykes and Coop, 1977; Fox et al., 1989).

A further problem due to haemonchosis has been the interference in water metabolism in the infected host (Vieira Bressan et al., 1992). In the present study, the infection caused a reduction in the water content of the carcass, for treatments MP-I and LP-I compared to the control but there was no difference between groups HP-C and HP-I, showing that high levels of protein may help in reducing water loss. It should also be noted that the infection of animals in the low protein group was less severe (fewer adult worms) but this group still showed significant water loss. This result is in agreement with Gennari et al., 1997, who studied water metabolism in calves fed high and low protein diets before and after acute infection with 100,000 L3 in tritiated water and found that calves infected with Haemonchus and received low protein showed and increase in water turnover and loss. Higher water retention was noticed in carcasses of sheep supplemented with soy and infected by Haemonchus (Wallace et al., 1995), thus confirming that protein exercises a positive effect on water loss during the infection process caused by these parasites.

The proportion of muscle and bone on the ribs was not significantly different between groups due to the large individual differences between animals, although fat (of rib and muscle) was higher and amount of protein in the muscle less in low protein ration animals, when only the diet effect was examined. This suggests that protein limitation in the diet of these animals reduces their growth, with low protein deposition in the muscle, thereby accumulating excess energy as fat in the carcass.

Wallace et al. (1999) demonstrated that the lambs infected with H. contortus on the increased dietary allowance had more muscle and fat on carcass, but in this case, the lambs received more protein and energy too.

In conclusion, the results of this trial indicate that feeding calves a high protein diet have influenced the body composition, reducing water loss, with more protein and less fat deposition despite showing a higher degree of infection.

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HAEMONCHUS PLACEI, PROTEIN AND CARCASS COMPOSITION

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