INFLUENCE OF DIETARY PROTEIN ON WATER METABOLISM IN CALVES INFECTED WITH *HAEMONCHUS PLACEI*

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SUMMARY: The influence of protein level in the diet and haemonchosis on the water metabolism was examined in calves. At five months of age, the calves were divided in two groups, and received a diet with low level of protein, 97.8 9 kg $^{-1}$ dry matter (group 1) and high level of protein, 175.0 g kg $^{-1}$ dry matter (group 2). Four months later they were kept in metabolic cages, the total body water estimated by tritium injection and they were all challenged with 100,000 *Haemonchus placei* L_3. Five weeks later, the tritium was injected again to estimate the water metabolism after infection. A week later the calves were slaughtered and their worm burdens and total body water determined by the 10th rib method. Although their mean worm burdens were similar, the body weight and total body water were influenced by the diet. The infection significantly affected the total body water in the low protein group. The animals with a higher level of protein in diet also improved the body condition when estimated by the 10th rib technique.

KEY WORDS: *Haemonchus placei,* total body water, protein diet, tritiated water, 10th rib technique.

INTRODUCTION

Infection by gastrointestinal helminths forms a major constraint to cattle production throughout the world. The young animals are particularly more susceptible to nematode infection, and the reason is not completely clear (SOULSBY, 1981).

The nutritional status is, in part, responsible by differences in the production and development of immunity in the host to a variety of pathogenic agents (SOLOMONS & NEWBERNE, 1981; EARTHING & KEUSCK, 1984; COOP *et alii, 1995).*

BAWDEN (1969) and DOBSON & BAWDEN (1974) first documented the effect of nutrition in parasitized sheep. Recent experiments by BOWN *et alii* (1991) have shown that three-and-a-half-month-old lambs that received an infusion of protein into the abomasum, developed immunity to *T.colubriformis* more rapidly than the non supplemented controls. In the same experiment they observed that the nitrogen content of the protein was the responsible for the acceleration in the immunity, rather than energy.

ABBOTT *et alii* (1985, 1986, 1988) observed that sheep infected with *H.contortus* and under a dietary protein supplementation, presented less severe pathophysiological and pathological alterations. WALLACE *et alii (1995)* also in sheep infected with *H.contortus,* showed that the animals with a protein supplement, have less severe pathological alterations and a better carcass composition.

Body weight reduction is the most common factor in nematode infection. According to KEMPSTER *et alii* (1982), the body weight is a good indicator of the corporal composition, only when we compare animals of the same breed, sexes and under the same management. Meanwhile, factors as nutrition, diseases and hormonal alterations, limiting the efficiency of this variable. Besides to the body weight changes, alteration in the body composition are observed during parasitic infections (HOLMES, 1987).

Using tritiated water technique, VIEIRA BRESSAN *et alii* (1992) found alterations in the water distribution in cattle infected with *H.placei.* ENTROCASSO *et alii* (1986) using the carcass analyses to estimate the body composition, observed that in naturally infected cattle, the muscles were reduced in weight and presented more water content than the non infected controls.

Tritiated water technique can be used as tracer to estimate the size and turnover of body water (IAEA, 1982). The tritium

is safe when used in tracer concentrations, rapidly metabolized and its concentration can easily be measured in body fluids (JOHRI *et alii*, 1975).

Experiments from the beginning of the century, suggesting the utilization of carcass samples joints as predictors of carcass composition (LUSH, 1926). Later, HOPPER (1944) showed that the utilization of the ninth to 11th rib inclusive produced prediction equations with the smaller residues. Many authors tested the equations and concluded that the rib technique allowed a stable and sensitive estimate (POWELL & HOFFMAN, 1968; LANNA, 1988).

Despite *H.placei* been a prevalent parasite of cattle in Brazil (HONER & VIEIRA-BRESSAN, 1992), few are the informations about metabolic alterations during the infection. VIEIRA-BRESSAN *et alli* (1992) working with young calves with a subclinical *H.placei* infection, reported evidence that the water turnover is higher and the tritium biological half-life is reduced, suggesting alterations in the water metabolism. POMPEU *et alii* (1997) also in calves infected with two different doses of *H.placei*, observed a series of alterations in the water metabolism after clinical infection.

The aim of this study was the estimate of water metabolism in calves under two different dietary protein levels and infected with *H.placei*, using the isotopic dilution and the 10th rib technique.

MATERIALS AND METHODS

Experimental design: Eight male calves reared worm-free from birth, were divided into two groups of four animals each, according to body weight. When they were 5-month old, to the group 1 calves were offered a low protein diet, and the group 2 calves received a high protein diet. Eleven weeks after the start of the diet, the calves were placed in metabolic cages and the tritium was injected for the body water estimate, at pre infection period. A week later, the animals were infected with 100,000 *H.placei* L_3 and five weeks later they were allocated again in the cages, for the estimate of the water metabolism after infection. Five days after the tritium injection, they were killed, the abomasum removed for worm counts and the 10th rib was separated and stored at -20° C for posterior analyses. The body water content was estimated by both methods.

During the experimental period the calves were weighed fortnightly and faecal samples from parasitological exams were collected weekly.

Diet: The low protein concentrate (Group 1) contains 97.8 g of crude protein kg^{-1}, dry matter and were composed by 80% corn grain, 6% cotton seed meal and 14% rice polishing.

The high protein concentrate (group 2) contains 175.0 g of crude protein kg^{-1}, dry matter and was composed by 65% corn grain, 19% cotton seed meal and 16% protenase (protein supplement). All animals received 500 g heady day $^{-1}$ of the respective concentrate mixture and 2 kg heady day $^{-1}$, grass hay *(Cynadon dactilon)*, and water *ad libitum*. Proximate analyses of hay and concentrate followed AOAC (1980) recommendations and is showed in Table 1.

Table 1 - Proximate analyses (g kg -1) of hay and concentrate offered during the experimental period to group 1 and 2 calves.

	HAY	CONCENTRATE	
		group 1	group 2
Dry matter	831	917	900
Crude protein	67	141	298
Crude fiber	329	46	47
Ether extract	24	38	55

Parasitological Techniques: The larvae were of a strain of *H.placei* which has been maintained at the Department of Parasitology, Instituto de Ciências Biomédicas da Universidade de São Paulo, since 1987. They were used within two weeks after fecal culture collection and the infective larvae were suspended in water and administered orally as a drench.

Fecal samples were taken from the rectum and the number of eggs per gram of feces (EPG) estimated by the McMaster technique (WHITLOCK, 1948).

The calves were killed and the abomasum was ligated, removed and contents collected. The surface of the mucosa was washed with tap water and the washing was added to the contents. The abomasa were then soaked in a solution of 0.85% NaCl and incubated in a water bath at 42° C for six hours, with the mucosal surface down to loosen the larvae from its surface. The mucosa was washed again, discarded and the contents sedimented for 2 hours. This was then fixed with formal in a 10% (v:v) proportion. Representative samples of abomasal contents were collected (10%) and fixed with formal. The total worms present in the mucosae and contents were found by counting 10% of the samples and multiplying by 100.

Tenth rib technique for body water determination: The 10th rib was collected according the procedures described by LEDGER & HUTCHINSON (1962). It was removed the 10th rib from the left side of the split carcass, sealed in a polythene bag and stored at -20° C. Afterwards it was dissected into

muscles and bones, grounded under liquid nitrogen refrigeration, lyophilised and analyzed according AOAC (1980).

Isotopic dilution technique: Two days before tritium (TOM) was injected, the calves were placed in metabolic cages and 18 h prior the injection, they were kept without food and water and only received them six hours after the injection of the tracer, when the last blood samples were taken.

From a stock solution of tritiated water containing 7 Mbq ml $^{-1}$, aliquots corresponding to 0.7 Mbq kg $^{-1}$ body weight were taken and diluted in 0.85% NaCl. The injection of TOM, blood collection and the total body water, biological half-life and turnover rate estimations were done as described by VIEIRA-BRESSAN *et alii (1992)*.

Statistical methods:

Total body water by isotopic dilution: It was used the covariance analysis to compare the means obtained prior and after the infection. The means were adjusted to the least square mean and the effect of treatment (level of protein in the diet) compared by F test. The T test was used to detect the infection effect in each group comparing the null hypotheses that there were no differences between values obtained prior and after the infection (SAS, 1991).

Total body water by the 10th rib method: It was used the analysis of variance using the general linear model procedures to evaluate the effect of treatment (level of protein in the diet) and the means, adjusted to the least square mean, compared by F test (SAS, 1991).

Comparisons were made using the Pearson Correlation method and values for $P<0.05$ were considered statistically significant.

RESULTS

The results were expressed as mean ± standard error (SE) and it is summarized in Table 2.

Clinical observations and body weight changes: After challenge various levels of a sub-mandibular oedema was observed in the calves and the same occurred in the gastrointestinal tract during the necropsy. The gelatins layer was more evident in group 1 calves. Animals from both groups showed no innapetence throughout the experiment.

The initial mean body weights for groups 1 and 2 were 84.5 kg and 86.0 kg ($P>0.05$). At the 13th week after the beginning of diet, at the *H.placei* infection, the mean body weight was 107.8 kg and 134.3 kg, respectively for groups 1 and 2 ($P<0.05$) and five weeks later, at slaughter the mean body weight was 106.5 kg for group 1 and 133.5 kg for group 2 ($P<0.05$).

Table 2 - Body weight (kg), T 1/2 (hs), Turnover (ml day $^{-1}$ BW $^{-1}$) and Total body water - TBW (liter and %BW) in calves from group 1 (G1) and group 2 (G2) before and after *H.placei* infection estimated by the tritiated water technique.

PARAMETER	GROUP	DIET EFFECT (Pre infection)	DIET and INFECTION EFFECT (Post infection)	P*
BODY WEIGHT	G1	107.8[a]	106.5[a]	0.60
(KG)	G2	134.3[b]	133.5[b]	0.67
	SE	7.92	2.53	
T 1/2	G1	143.4	160.8[a]	0.56
(hs)	G2	113.9	123.0[b]	0.60
	SE	19.27	10.43	
TURNOVER	G1	89.0	67.9[a]	0.24
(ml d -1 BW1)	G2	95.4	92.3[b]	0.81
	SE	11.29	6.99	
TBW	G1	74.5	75.5[a]	0.01
(liter)	G2	86.6	83.0[b]	0.32
	SE	4.76	1.82	
TBW	G1	70.1[a]	63.7[a]	0.009
(% BW)	G2	64.6[b]	68.4	0.343
	SE	1.26	1.77	

SE - standard error
* = significance for the difference between pre and post infection
Different letters in a column means statistical difference between G1 and G2

Worm numbers: The mean renumber of *H.placei* recovered from group 1 was 41,500 ± 14,870 and from group 2 was 52,850 ± 21,300. This difference was not statistically significant. The percentage of immature stages was 0.18% for group 1 and 0.33% for group 2 ($P>0.05$).

Total body water (TBW), tritium half-life (T1/2) and water turnover: There was no significant difference between groups in the tritium T1/2 before infection. However, the mean values of T1/2 after infection were significantly higher in group 1 when compared with group 2 calves (160.8 h and 123.0 h respectively). This difference reflects in the water turnover after the infection, with a turnover rate 26.5% lower for group 1 and mean values of 67.9 ml kg $^{-1}$, days for group 1 and 92.3 ml kg $^{-1}$, day, for group 2.

Even with the T1/2 and turnover alterations, the TBW, at the same period, showed no difference ($P>0.05$) between groups when calculated in percentage of body weight (BOO) (63.6 % BW for group 1 and 68.4% BW for group 2). However the TBW, in liters, for the high protein group was 83.9 l and for the low protein group 75.5 l, a significant difference ($P<0.05$).

When the infection effect was measured, only group 1 calves showed a significant decrease in the TBW (%BW) with values following from 70.1% before the infection to 63.7% five weeks after infection.

Table 3 - Rib weight (g), rib water (g and %BW), total body water (TBW) (kg and %BW) in calves from group 1 and group 2 estimated by the 10th rib technique.

PARAMETER	GROUP 1	GROUP 2	SE
RIB WEIGHT (g)	312.73	509.35	27.52 *
RIB WATER (g)	223.08	355.18	19.85 *
RIB WATER (% BW)	71.30	69.70	0.83
TBW (kg)	71.0	90.35	2.60 *
TBW (% BW)	67.03	68.60	3.21

SE = standard error
* = significance between groups (P<0.01)

Total body water - 10th rib technique: The results from the 10th rib analyses were showed in Table 3.

The rib weights, in grams, were statistically different (P<0.01) for group 1 (312.73 g) and 2 (509.35 g). The same was observed with the total rib water and TBW when analyzed in grams. The mean values of 223.08 g and 71.0 kg for group 1 and 355.18 g and 90.35 kg for group 2, respectively rib weight and TBW were different (P<0.01) for the observed variables. However, the same parameters when analyzed in percentage BW showed no significant differences (71.3 g and 69.7 g for group 1 and 2 rib water; 67.0 % BW and 68.6 % BW for group 1 and 2 TBW).

The statistical analysis used to compare TBW values obtained by each of the techniques, showed a significant correlation, P<0.05 and r = 0.84.

DISCUSSION

There was no significant difference between the prepatent periods in animals from group 1 and 2. WALLACE *et alii* (1995) working with lambs, also observed the same and suggesting that the protein levels did not influence the rate of larval development in a prime infection.

Despite the big amount of *H.placei* found at necropsy and the clinical alterations during the experiment, the calves showed no innapetence, evidencing a relatively resistance of cattle to *H.placei* infection and confirming previous observations (POMPEU *et alii*, 1997).

As expected, the diet influenced the weight of the calves. It was found lower values in the animals that received less protein in diet. However, the infection did not influence the body weight. The observed period of five weeks is a short interval, the patency had started only a week before the necropsy, and some influence on the appetite or body weight probably will be more evident if the measurements continue for some more weeks. However, in the same interval, the calves from both groups presented a significant decrease in the total serum protein, albumin and PCV values (GENNARI *et alli*, 1995).

The higher protein level in diet was responsible for a significant increase in urea levels (P<0.01) and digestibility of dry matter (P<0.05) in calves for group 2 (ABDALLA *et alii*, 1997).

The protein level in the diet altered the water metabolism before and after the *H.placei* infection. Despite TBW values had been calculated in liters, the results in % BW are more adequate, since body weight was significantly different at infection and necropsy for group 1 and 2 calves.

By the TOH technique, the diet influenced the TBW (%BW). Group 1 calves, before the infection, showed higher water content, evidencing a lower amount of muscle mass. However, already at the first week of patency, the more debilitated and lighter calves, group 1, showed lower body water than group 2 calves, and these values were significantly lower than those found in the same animals before the infection. That loss of water from group 1, reflecting an effect of both, infection and diet at this time. This loss was not present in calves under high protein diet, who kept similar TBW values after infection. The TBW in group 1 calves was considerably lower than those recorded before the infection, even with an increase in the T1/2 and decrease in the water turnover at this period. Those results also suggesting that, despite the infection and poor protein diet, until this phase of the disease, an efficient mechanism was regulating the water loss.

POMPEU *et alii* (1997) also using TOM technique in young calves under balanced diet, observed a significant increase in the TBW after infection with 1000 *H.placei* L_3 kg -1 BW. However in the same experiment the TBW showed no alteration after infection with 2000 L_3 kg -1 BW. Meanwhile, comparisons with the present results are difficult once the TBW values were only expressed in liters/ day. VIEIRA-BRESSAN *et alii* (1992) also found an increase in the TBW values in calves with a sub-clinical *H.placei* infection. However, in both reported studies, the calves were younger and body composition is highly associated with age, mainly in growing calves and in the present study the diet represents another important source of variation.

The estimate of TBW by the indicator joint revealed the effect of diet and infection. When TBW and total rib water were calculated in grams, the effect of nutrition was significant,

showing the best conditions of group 2 calves. However, when those parameters were calculated based on BW, no difference was found between the two treatments.

WALLACE *et alli* (1995) analyzing the influence of supplemental dietary protein by the determination of carcass composition, also observed that the supplemented lambs retained more water within the carcass.

Comparing the techniques that were used in this trial, it is possible to conclude that both gave very similar TBW values, despite the TOM technique had allowed a more accurate measurement and the use of alive animals.

RESUMO

A influencia do nivel de proteína na dieta e infecção por *Haemonchus placei*, sobre o metabolismo da água, foi estudada em bezerros. Aos cinco meses de idade, os animais foram divididos em dois grupos e receberam dieta com 97,8 g de proteína kg^{-1} de matéria seca (grupo 1) e com major teor protéico, 175,0 g de proteína kg^{-1} de matéria seca (grupo 2). Onze semanas mais tarde, os animais foram colocados em gaiolas metabólicas, a água corpórea total estimada através da injeção de água tritiada e após foram desafiados com 100.000 L_3 de *Haemonchus placei*. Cinco semanas depois, o trítio foi injetado e o metabolismo da água novamente estimado. Após uma semana, os bezerros foram sacrificados e o numero de vermes e água corpórea total determinados, pelo método da 10ª costela. Apesar do numero total de nematóides ter sido semelhante, o peso vivo e a água corpórea sofreram influencia da dieta. A infecção afetou significativamente a água corpórea no grupo sob dieta com baixo teor protéico. Animais com alto teor protéico na dieta, também demonstraram melhor desenvolvimento corporal quando estimado pela técnica da 10ª costela.

PALAVRAS-CHAVE: *Haemonchus placei*, água corpórea total, dieta protéica, água tritiada, técnica da 10ª costela.

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