BODY COMPOSITION OF CALVES INFECTED WITH *HAEMONCHUS PLACEI* ESTIMATED BY TRITIATED WATER TECHNIQUE

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SUMMARY: The total body water, fractional turnover rate, biological half-life of tritiated water, and total body solids (ash, protein, and body fat) were investigated in 3-to 6-months old Friesian calves. They were divided into two groups of 7 and 4 animals, infected and control respectivelly. The infected group received 500 *Haemonchus placei* L3 Kg^{-1} body weight, orally. Four weeks later, they were all housed in metabolic crates and injected with 2 MBq of tritiated water Kg^{-1} body weight. Blood samples were collected from 2 to 6h after injection and daily from day 1 to 7. The results showed that this level of infection, in calves, caused a moderate infection, without clinical signs. No statistical difference in the parameters studied was found between the two groups at the 5% level of significance. However there was evidence of an increase in the fractional turnover rate and reduction in the biological half-life of tritium in the infected animals.

RUNNING HEAD: VIEIRA-BRESSAN *et alii* - Body composition in calves infected with *H.placei*.
KEY-WORDS: *Haemonchus placei*, total body water, total body solids, fractional turnover rate, calves.

INTRODUCTION

There are several descriptions of field and experimental infections by *Haemonchus* sp in sheep and the effects on body weight and production (WHITLOCK *et alii* ,1966; DARGIE and ALLONBY, 1975; ALLONBY and URQUHART, 1975).

In heavy infections, its effects are easily recognized, with reduction in weight gain, haemorrhage into the host's abomasum, anaemia, increase in the rate of red cell production, alterations in the plasma volume, albumin pool sizes and turnover rates (DARGIE and ALLONBY, 1975; ABBOTT *et alii*,1985; ABBOTT *et alii*,1986). Whilst at low level or so-called subclinical infections, it is often difficult to demonstrate evidence of any adverse effects on productivity (ABBOTT *et alii*, 1984).

However, reduction in body weight gain is the most common and easily observed feature during the infection, and it is important to determine, not only the weight loss, but also changes in body composition.

In nematode infection a common feature is an increase in the water content relative to total body solids, consequently, reduction in body solids may be greater than estimated from body weight alone and the turnover of water in the body and its distribution could be alterated (HOLMES, 1987).

Tritiated water (TOH) can be used as a tracer to study the size and turnover of the body water pool and to indirectily estimate body composition in controlled and free-grazing situations (IAEA,1982). Although tritium is radioactive, it is relatively safe in tracer concentrations, is rapidly metabolized from total body water and its concentration can be accurately determined in body fluids (JOHRI *et alii*, 1975).

By the dilution principle using TOH it is possible to obtain total body water (TBW), biological half-life (T1/2) and fractional turnover rate (FTR). The T1/2 is the time interval required for the isotopic water concentration to be reduced to one-half as a result of processes other than radioactive decay (IAEA,1982). FTR expresses as the relative transfer of water per unit of time (HOLLEMAN *et alii*, 1982). TBW is the volume of body water as measured by the isotopic water method (IAEA,1982) and the total body solids can be calculated in the live animal by subtracting the TBW from live weight (RUSSEL, 1982; KING and FINCH, 1982). The methods normally used to estimate fat, protein and ash content of live animals are based upon the determination of TBW and the application of regression equations which relate the TBW to body fat, ash and protein contents (LITTLE and McLEAN, 1981; HEISSNER *et alii*,1980).

Loss of water by diarrhoea is not a common feature in haemonchosis, but sub-mandibular oedema and changes in the protein and plasma distribution are normally present. In calves, GENNARI *et alii* (1991) using a low level infection, reported changes in the distribution of albumin from the extra to the intravascular pool and an increase in the plasma and blood volumes of infected animals. Based on those observations, it was expected that the total body water and its distribution throughout the body could be alterated in calves infected with *H.placei*.

There has been little work on haemonchosis in cattle and there are no reports of the water and body solids in cattle infected with *H.placei*. This experiment was conducted to investigate the body composition of calves infected with 500 *Haemonchus placei* larvae Kg^{-1} body weight, estimated by the tritiated water technique.

MATERIALS AND METHODS

Experimental design

The experiment involved eleven 4-to 6-month-old male Friesian calves, that had been reared parasite-free from birth. The animals were divided into one group of seven calves that

received 500 infective third stage larvae (L3) of *H.placei* Kg^{-1} body weight *per os*, and the other group of four animals remained as non-infected controls. The calves were weighed prior to the infection, at the time of radioisotope injection, and at slaughter. The animals were fed a commercial balanced concentrate ration (750g twice a day) and hay.

Thirty days after infection, when the patency was established the calves were moved to metabolism stalls and injected with tritiated water. Blood samples were taken at hourly intervals for 6h after tracer administration, and thereafter one blood sample was removed daily for 7 days.

The radioactivity of plasma was measured and the radioactivity of all samples was determined. Total body water (TBW),fractional turnover rate (FTR), biological half-life (T1/2) of TOH, fat, protein and mineral contents were estimated.

Parasitological techniques

Infective larvae were obtained from culture of faeces from calves with a pure *H.placei* infection and larvae were collected using a Baermann apparatus. The infective dose was 500 larvae Kg^{-1} body weight, and the larvae were suspended in water and administered orally as a drench.

Faecal egg counts were carried out by the modified McMaster method of GORDON & WHITLOCK (WHITLOCK, 1948) once a week during all the experimental period.

At slaughter the abomasum was removed, opened, the contents collected and the surface of mucosae washed with water. The abomasum was placed in water bath with 0.85% sodium chloride solution at 42°C for 12h to digest the mucosa and release the worms. The worms present in the total abomasal contents and mucosal digest were counted.

Radioisotopic technique

Eighteen hours prior to the commencement of the radioisotope study, the calves 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 70 MBq ml^{-1}, 2MBq Kg^{-1} body weight of TOH was diluted in 0.85% sodium chloride solution. For each calf a 10ml syringe was filled with the respective amount of tritiated water solution and weighed.

The TOH was injected intravenously into the jugular vein using a multi-three-way tap assembly fixed to a catheter and subsequently flushed through with saline. After the injections the syringes were reweighed and the weight of the injected material determined. A 1.0ml syringe was also filled with TOH solution, weighed and the standard prepared using a volumetric flask and diluting in 1 liter of distilled water. The syringe was reweighed.

Blood sampling and radioisotope determination

The first blood sample was collected 2h after injection and further samples were taken at one-hour intervals over the next six hours, and daily from day 1 to day 7. The samples were collected from the jugular vein into 5ml vacutainer tubes containing di-potassium EDTA, centrifuged and the plasma removed. To one ml of each plasma sample was added 8ml of Patterson and Greene scintillation fluid (PATTERSON and GREENE, 1965). To the same amount of fluid, were added several 0.1 aliquots of standard solution plus 1 ml of plasma from a normal calf. The count rates of all samples were determined at the same time, at the end of the collection period, in a liquid scintillation counter (Beckman LS 5000TD).

Total body water, biological half-life and fractional turnover rate estimations

The body water was determined using the dilution principle (STOUFFER,1969). After the injection of the radioisotope an equilibrium period for the uniform distribution of TOH to TBW was allowed. Blood samples were taken and the radioactivity in the plasma determined. A curve plotting time after injection against plasma activity was made and extrapolated to zero time to give the equilibrium activity that was used to estimate the body water. The T1/2 of tritium was determined from the slope of this linear regression line and the water turnover rate was calculated as the product of TBW and the fractional turnover rate (K),wich was estimated as the product of ln2 (0.693) and the half-life of tritium.

Body solids

The protein, ash and fat content in the body of the live animals were estimated according to regression equations and corrections for body water suggested by the work of MEISSNER *et alii* (1980), using the TBW previously determined and the liveweight measured at TOH injection.

Statistical analysis

The variables studied were identified as means ± standard error or mean ± standard deviation and the observed parameters were investigated by analysis of variance. Whenever there were statistical significance, the means were compared by Tukey test.

RESULTS

Body weight and worm burdens

Body weight gains in the infected and control calves during the experimental period are shown in Table 1. A mean weight gain of 22.12 ± 5.82 Kg was recorded in infected calves compared with 24.42 ± 8.29 Kg in the control group. This difference was not statistically significant. Table 1 also shown worm burdens at necropsy. A mean of 4145 ± 1098 worms established in the infected animals, approximately 8% of the infected dose and no parasites were found in the control group.

Packed cell volume (%)

The packed cell volume (PCV) was determinated weekly from the infection to the end of the experimental period (Fig.1). The infected group showed an average of 26% and the control 32%, with no significant difference between groups.

Total body water, Biological half-life and Fractional

turnover rate

The total body water, biological half-life and fractional turnover rate estimations in the infected and control calves are shown in Table 2.

In the infected calves the total body water was higher and the half-life and turnover rate shorter than in the controls. However, these differences were not significant at the 5% level.

Body solids

The percentage of protein, ash and body fat in relation to body weight are shown in Table 3. There were no statistically difference between those parameters.

DISCUSSION

The infective dose of 500 *H.placei* L3 Kg^{-1} body weight used in the present study allowed the development of a moderate infection in the calves. Although there was a decrease in the PCV values in the infected group, this difference was not statistically significant and the infected calves did not show clinical signs of haemonchosis during the experimental period.

Table 1 - Weight gain, infective dose and worm burdens in calves of infected and control group (mean ±SD)

Experimental Group	Number calves	Weight gains (kg)	Infective dose	Worm burdens ($500L_3\ kg^{-1}$)
INFECTED	7	22.12±5.82	49.392±5304	4145±1098
CONTROL	4	24.42±8.29	-	-

Moderate or "sub-clinical" infection by *H.placei* is often encountered under natural grazing conditions during the dry period of the year in sub-tropical areas, however it is normally associated with others parasitic infections.

While there was no significant difference in the body water composition, some evidence of alterations was observed. Despite the very similar TBW values, the T1/2 of TOH in infected animals was shorter than in the controls (5.1 ± 0.3 and 6.0 ± 0.5 days respectively) and FTR was 17% higher than in the control group. These observations probably reflected a higher water intake and alterations in the water distribution in those animals, since TBW showed the same values in both groups and the change of water was faster and larger in the infected animals.

GENNARI *et alii* (1991) with the same level of *H.placei* infection used in this study, found a significant increase in the plasma volume and a change in the distribution of albumin from extravascular to the intravascular pool, in infected calves. These results implied alterations in the distribution of water.

BAKER *et alii* (1965) studied TBW and water turnover in normal and severely parasitized steers with TOH. The animals were naturally infected with *O.ostertagi*, *T.axei* and *Cooperia* spp. Their results showed a higher TBW volume and a shorter turnover rate in the infected group. The animals showed oedema, diarrhoea and clinical symptoms of parasitism, features

Table 2 - Total body water (TBW), biological half-life (T1/2), and fractional turnover rate (FTR) in calves infected with 500 *H.placei* L3 kg^{-1} body weight and respective controls (mean ±SE)

Experientall Group	Number of calves	TBW (l)	TBW (%BW)	T 1/2 (days)	FTR (ml/kg/day)
INFECTED	7	90.5±4.3	76.6±2.3	5.1±0.3	157±10.3
CONTROL	4	80.1±8.7	74.9±1.0	6.0 ±0.5	129.6±8.4

that were not present in this moderate infection by *H.placei*. In the same study, after therapy with anthelmintic, the increase in turnover rate coincided with cessation of diarrhoea and an increase in body weight.

HALLIDAY *et alii* (1965) in cattle infected with *Ostertagia ostertagi*,.observed no change in body composition expressed as a percentage of body weight when they studied type 1 ostertagiasis, but in the type 2 form of the disease, a great reduction in the percentage body solids was observed. These results contrast with work by TAYLOR *et alii* (1989) in which type 1 ostertagiasis showed an increase in the half life of TOH and a slight increase in TBW.

ENTROCASSO *et alii* (1986) also observed in steers exposed to natural trichostrongyle infection, by carcass measurement and analysis, that the protein deposition was affected by the course of type 1 and type 2 ostertagiasis.

In the experiments described (BAKER *et alii*, 1965; HALLIDAY *et alii*, 1965 and ENTROCASSO *et alii*, 1986) the differences in body water and composition were observed in animals with natural infection and after a long period of parasitism. The calves used in the present experiment, were at

Table 3 - Protein, ash and body fat as percentage of body weight (mean ±SE) in calves infected with 500 *H.placei* L3 kg^{-1} body weight and respective controls.

Experimental Group	Number of calves	Protein (% BW)	Ash (%BW)	Fat (%BW)
INFECTED	7	17.9±0.13	4.2±0.04	12.5±1.46
CONTROL	4	18.0±0.06	4.2±0.02	13.7±0.63

the beginning of patency, with a moderate infection and a good plane of nutrition. Some of the findings in this experiment may have been more statistically significant if a infection dose higher than 500 L3 kg^{-1} body weight had been used. Sheep infected with *O.circumcincta* showed a reduction in body water turnover and this reduction was associated with anorexia induced by the parasitic infection (HOLMES and BREMMER, 1971). This may be the main reason that in sub-clinical or moderate infection, few or no alterations are observed in the TBW.

Other experiments using higher infective doses of *H.placei* are planned and nitrogen and water balance studies should be

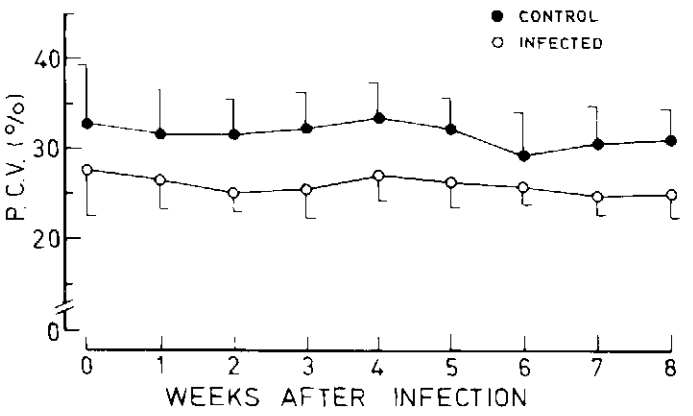

Fig.1 Mean weekly PCV(%) values of the calves infected with 500 *H. placei* larval kg^{-1} and the respective controls (mean ±SE) during the experiment.

conducted in calves on differing planes of nutrition.

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SUMÁRIO: A água corpórea total, taxa fracionada de turnover, meia vida biológica da água tritiada e sólidos corpóreos totais (matéria mineral, proteína e gordura corpórea) foram determinados em bezerros da raça Holandesa de 3 a 6 meses de idade. Estes foram divididos em 2 grupos de 7 e 4 animais, respectivamente infectados e controles. Os do grupo infectado receberam, oralmente, 500 larvas infectantes de *Haemonchus placei* por quilograma de peso vivo. Quatro semanas mais tarde, todos os bezerros foram colocados em gaiolas metabólicas e injetados com 2 MBq de água tritiada por quilograma de peso vivo. Amostras de sangue foram colhidas de 2 a 6 hs pós injeção e, uma vez ao dia, por uma semana. Os resultados mostraram que com o nível de infecção utilizada os bezerros desenvolveram uma infecção moderada, sem sinais clínicos. Nenhum dos parâmetros estudados apresentou diferença estatística ao nível de 5% de significância. Houve evidências de aumento na taxa fracionada de turnover e redução na meia vida biológica do trítio nos animais infectados.

FRASE CHAVE: VIEIRA-BRESSAN *et alii* - Composição corpórea em bezerros infectados com *H.placei*.

PALAVRAS CHAVE: *Haemonchus placei*, água corpórea total, sólidos corpóreos totais, taxa fracionada de turnover, bezerros.

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