BODY WATER METABOLISM, WATER AND NITROGEN BALANCE IN CALVES HARBORING DIFFERENT INFECTION LEVELS OF *HAEMONCHUS PLACEI.*

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SUMMARY: Water metabolism, nitrogen and hydric balance were evaluated in calves experimentally infected with two levels of *Haemonchus placei*. Nine calves, males, Holstein-Friesan, five months old received by oral route 1000 (n=4) and 2000 (n=5) L_3 of *H. placei* per kg of body weight. Physiopathologic studies were conducted in two phases, before and after infection. In each phase of five days, animals were kept in metabolic cages and injected with tritiated water. During this period, samples of blood, feces and urine were daily collected for laboratory assays; the intake of food and water, and the output of feces and urine was thoroughly measured. Animals were sacrificed 35 days after infection, worms in the abomasum were collected, counted and classified. Results showed not many significant differences in helminth burdens related to the infective dose, except for L_5 which population was larger than that of adults in both dose levels. There was some decrease in the packed cell volume after two weeks of infection, particularly in calves infected with 2000 L_3 which showed lower and more persistent hematocrit values than those infected with 1000 L_3. Calves infected with 1000 L_3 also showed significant ($p<0.05$) increases of the total body water and water turnover rates and reduction of its biological half-life. Animals infected with 1000 L_3 reached the hydric equilibrium. Only the decrease of body weight gain was significant ($p<0.05$) in animals infected with 2000 L_3. Other slight changes suggested only water retention for longer periods. Excreted nitrogen did not overcome ingested levels because of the high protein levels in feed. Reduction in the proportion of body fat in the patent period of infection with 1000 L_3 was interpreted as a large energetic demand produced by the immune response and parasite spoliation.

KEY WORDS: *Haemonchus placei*, water metabolism, nitrogen balance, hydric balance, levels of infection, cattle.

INTRODUÇÃO

Haemonchus placei is an important nematode causing parasitic gastroenteritis in cattle, particularly in calves of tropical or subtropical areas because of its wide distribution, prevalence and pathogenicity. In Brazil it ranks second after *Cooperia* spp. showing a mean prevalence of 80%, according to epidemiological studies in different regions; it is the main responsible for the high helminth summer infections (HONER & VIEIRA-BRESSAN, 1992).

A variety of gastrointestinal helminths is usually involved in the gastroenteritis process reducing absorption and utilization of nutrients. They cause anorexia, weight loss, impaired development, faulty reproduction and consequently reduce animal value (BREMNER, 1982).

The degree of physiological disturbances affecting the productivity of animals suffering from parasitism is related to the level of infection, parasite genus, age, nutritional and immunological condition of the host. There is also interference in the retention and balance of nitrogen, reduction of metabolism and of protein synthesis; changes and reduction

of the energetic metabolism whose mechanisms have not yet been precisely determined and interference in the water and electrolytic balance (BRENMER, 1982; HOLMES, 1987).

The most common method to evaluate the effect of parasitism in animals, is through live body weights or the weight of carcasses after slaughtering, in beef cattle.

According to HOUPT (1984), there is some variation in the body water of animals. The amount of body water depends upon animal species, age, sex and nutritional condition. The quantity of water in the organism remains constant day after day, being regulated by the ingestion, or indirectly through the excretion of urine, feces, through the skin and by breathing.

Some studies have already shown that water and body composition as well as water consumption and its retention by the organism are subjected to changes in ruminants exposed to natural mixed infections by gastrointestinal nematodes or by single induced infections (BAKER *et alii* 1965; HALLIDAY *et alii* 1965; ENTROCASSO *et alii* 1986). The mechanisms of these changes have not yet been examined in detail, but such changes clearly show that tissue losses attributed to parasitic infections cannot be determined only by changes in body weights. Some other intrinsic factors such as nitrogen absorption and the energetic metabolism are also involved.

VIEIRA-BRESSAN *et alii* (1992), using tritiated water, studied the body composition of calves infected by oral route with 500 L_3 *H. placei*/kg/body weight in comparison with non infected controls. In this study there were not very much differences between the groups. However, even the subclinical infection of *H. placei* produced some increase in the water turnover. There was also a decrease in the half-life of tritiated water with a discreet increase in the volume and proportion of body water.

Based in those observations this study was designed to evaluate the pathological and physiological effects in young calves exposed to two levels of infection by *H. placei*. This was made through the body water study, body composition, and water and nitrogen balance by using the technique of isotope dilution (tritiated water).

MATERIALS AND METHODS

Experimental design: Two trials (Experiments I and II) were conducted with calves, experimentally infected by oral route with 1000 and 2000 L_3 *H. placei* infective larvae/kg/ body weight, respectively.

Four calves (N°s 1, 2, 3 and 4) were used in Experiment I and five calves (N°s 5, 6, 7, 8 and 9) in Experiment II. Tritiated water, nitrogen and water balances were performed in the two phases of both experiments, as stated by VIEIRA-BRESSAN *et alii* (1996). In the first phase, animals were under normal condition without infection. In the second phase, they were infected and showed clinical signs of haemonchosis. Intervals between the two phases correspond to the development of the prepatent period of infection.

Each phase study with tritiated water was conducted for five days, in which calves were kept in metabolic cages. During this period, samples of blood, feces and urine were daily collected; the intake of food and water, and the output of feces and urine was thoroughly measured.

The daily clinical monitoring of animals was made throughout the experimental period. After the second phase, animals were sacrificed and helminths recovered from the abomasum.

Animals: Nine Holstein-Friesan worm-free calves, three to five months old, kept under isolation in metabolic cages at the "Biotério de Grandes Animais do Instituto de Ciências Biomédicas da Universidade de São Paulo (ICB-USP)", Brazil. Each calf was fed twice a day with 500 g of a commercial pelleted concentrate containing 16% of protein (Ternerina-Purina S.A.) and one kg of hay (Coast -cross, *Cynodon dactylon)*. Water was supplied in 5 liter recipients and the daily consumption recorded.

Parasites: Infective third stage larvae (L_3) of *H. placei* from a strain colonized in the Laboratory of Veterinary Helminthology of ICB/ USP since 1987. Larvae were stored at 4° C up to the moment of infection. The storage period was no longer than 2 weeks. The infective dose was of 1000 (Experiment I) or 2000 (Experiment II) L_3 per kg of live body weight.

The establishment of infection was monitored by examination of fecal samples collected directly from the rectum. Egg counts were conducted by using a McMaster modified technique (WHITLOCK, 1948).

Necropsy and helminth recovery: After finishing the second phase of utilization of tritiated water, on Day 35 post infection, calves were sacrificed and necropsied following the procedures described by VIEIRA-BRESSAN *et alii* (1995). Recovery of eventual immature stages attached to the abomasum was obtained by placing this organ with the mucosal surface down in a water bath of physiological saline solution at 42° C for six hours. After stirring up, ten percent aliquots of the total contents were taken and helminths found were counted and classified according to their development stage and the criteria of DOUVRES (1957).

Radioactive assay: The radioisotope injection followed the protocol used by VIEIRA-BRESSAN *et alii* (1992), with few alterations. In this present study was used approximately 1 Mbq of tritiated water per kilogram of body weight.

After the radioisotope injection, samples of blood were collected at 2, 3, 4, 5, 6, 24, 48, 72, 96 and 120 hours. During the second phase, an additional sample was collected at time 0

(To), i. e., before the injection of tritiated water to deduct from the results the residual activity of tritiated water which was injected in the first phase.

From the blood of each animal a sample was used for the hematocrit, and after it, the plasma was separated. At the end of each experimental phase the activity of plasma samples was measured in the scintillation solution of PATTERSON & GREENE (1965) by means of a scintillation spectrometer (Beckman Instruments, LS Analysis-Version 3.1 - A).

Water balance: Metabolic water and losses by evaporation of body water were calculated by indirect methods recommended by VERMA *et alii* (1980).

Sampling of feed, feces and urine: The alimentary regimen remained equal to that one used in the individual cages. Samples of feed were collected for bromatologic assays and the food and water left over, were weighed or measured in graduated cylinders, respectively.

Urine was measured and 10% aliquots of the total volume, twice a day, were kept in vials containing concentrated sulfuric acid. Samples of feces, urine and food were preserved at -20° C up to the moment of assay.

Nitrogen balance: Determinations of dry matter of hay samples and feed concentrate were estimated after drying samples in a ventilated oven at 60° C. After grinding, samples were homogenized and put into the oven at 100° for 24 hours. The two daily samples of each animal were pooled for a single assay. After homogenized they were weighed and put into the ventilated oven at 60° for 48 hours.

Total nitrogen was determined by the Kjeldahl methods. The macro Kjeldahl was used in the fecal and food assays, the micro Kjeldahl in the urine assays (A.O.A.C., 1980).

Statistical analysis: The Mann-Withney's non parametric test for the comparison of two samples was used to analyze parasitological and hermatocrit data. The paired *t*- Student test included in the software SAS (1982) was used to analyze variables occurring in the studies of body water, body composition and nitrogen balance. The *t*-Student's test was also used to compare data of experiments I and II after infection. In all tests the significance level used was $p < 0.05$.

RESULTS

Clinical observations: Pallor of the visible mucous membranes was gradually apparent followed by loss of hair gloss at the beginning of the second week post infection. By the end of the third week these symptoms reached a climax, particularly in those calves infected with 2000 L_3. Feces of animals infected with 1000 L_3 became less consistent, like a paste, throughout the third week, while in the group infected with 2000 L_3 this occurred only in a single animal. However, diarrhea was observed on Day 34 post infection, this sign persisting up to the end of the trial.

Three calves out of four animals belonging to Experiment I developed a submaxillary oedema. This sign was noticeable at the beginning of the fourth week post infection. From this time onwards, this oedema was clearly apparent up to the time of slaughter.

All animals of Experiment II showed the submaxillary oedema which begun on Day 23 post infection gradually developing up to the end of the trial.

Fecal egg counts: Figure 1 shows the results of fecal egg counts. All animals of experiments I and II passed eggs of *H. placei* in feces beginning on days 30 and 32, respectively, up to the end of trial. Only on Day 30 there was a significant difference ($p<0.05$) between egg counts of the two experiments.

Helminth burden: There were no significant differences between the helminth burdens of *H. placei* in experiments I and II on Day 35, in spite of the infective larval dose of 1000 and 2000 L_3, respectively (Table 1). The parasite burden of most development stages within the mean of the total population was similar among the animals in both experiments. The only exception was the population of L_5, significantly ($p<0.05$) larger than the population of adults (Fig. 2).

Table 1 - Worm burdens and total number of nematodes at different development stages per animal, 35 days after infection with 1000 L_3 and 2000 L_3 *H.placei*/kg of live body weight.

Experiment I (1000 L_3/Kg)						Experiment II (2000 L_3/Kg)					
Animal	Adults	L_5	L_4 late	L_4 early	Total	Animal	Adults	L_5	L_4 late	L_4 early	Total
1	3100	35800	19000	200	58100	5	400	5400	1200	[illegible]	[illegible]
2	2400	14500	4600	0	21500	6	6400	38700	15800	[illegible]	[illegible]
3	1100	4900	2800	0	8800	7	3200	26600	1500	0	[illegible]
4	2700	10600	5600	100	19000	8	1300	10300	4000	0	15600
						9	2500	24000	1100	700	[illegible]
average	2325.00	16450,00	8000,00	75,00	26850,00		2760,00	21180,00	5700,00	140,00	30760,00
	865.51	13406,89	7424,26	95,74	21545,38		[illegible]	[illegible]	[illegible]	[illegible]	[illegible]

L_4 = 4th larval stage L_5 = 5th larval stage

Table 2 - Mean body weight, body water and body composition in calves before and after infection with 1000 L_3 and 2000 L_3 *H.placei*/kg of live body weight. Data found out by means of tritiated water.

Treatment	Live body weight (Kg)	T ½ (hours)	Water turnover (l day⁻¹)	Total body water (l)	Body fat (% pv)	Body free of fat (% pv)
Infectado (1000 L_3 n=4)	97.12±6.35	95.37±01.91	15.52±1.11[a]	88.87±5.77[a]	32.90±2.02	67.10±2.02
Não Infectado (n=4)	93.16±6.60	116.96±12.71	10.43±1.15[b]	71.16±4.64[b]	43.62±3.65	56.40±3.65
Infectado (2000 L_3, n=5)	109.68±4.73[a]	120.28±18.02	13.27±1.89	88.78±4.55	40.42±3.42	59.58±3.42
Não Infectado (n=5)	100.5±5.41[b]	110.56±9.92	11.73±0.50	76.84±4.64	44.14±0.70	55.90±0.70

n= number of calves Different superscripts letters mean p<0.05

Hematocrit: As shown in Figure 3, after Day 28 post infection, there were some differences in the mean values of hematocrit which became greater in animals of Experiment I. These differences were significant ($p<0.05$) on days 31 and 33. They were less significant ($p=0.06$) on days 29 and 35 post infection.

In Experiment I the initial value was 27.5% ± 1.73, the lowest mean value occurring on Day 25 (16.25% ± 3.77). After it, the values showed some recovery up to the end of the trial reaching the level of 21.25% ± 2.63.

In Experiment II within the first week of infection the hematocrit dropped about 3.2%. This difference increased on the second week when the mean value was 21.4% ± 0.89 on Day 14. On the third week the initial hematocrit value of 28.8% ± 1.79 dropped to the average of 18.2% ± 6.10. On the fourth week the mean values dropped to the low level of 13.4% ± 2.97. At the end of the trial they were also very low (13.8% ± 3.9)

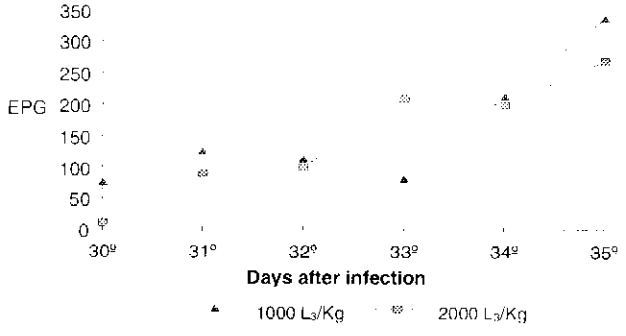

Fig. 1- Mean number of eggs per gram of feces (EPG) in calves infected with 1000 L3 and 2000 L3 *H. placei*/kg of body weight throughout the experiment.

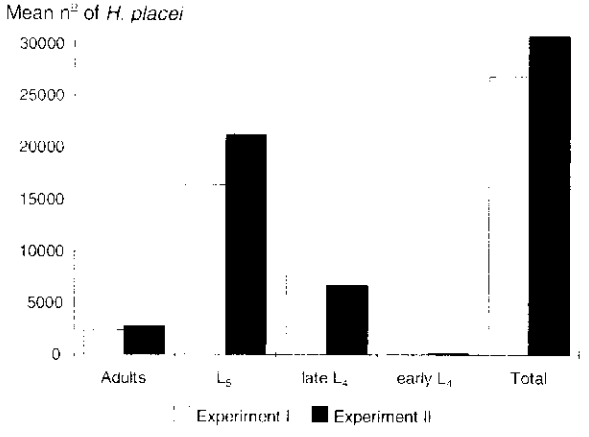

Fig. 2 - Mean number of *H. placei* at different development stages and mean worm burden in calves of experiment I and II. 35 days after infection.

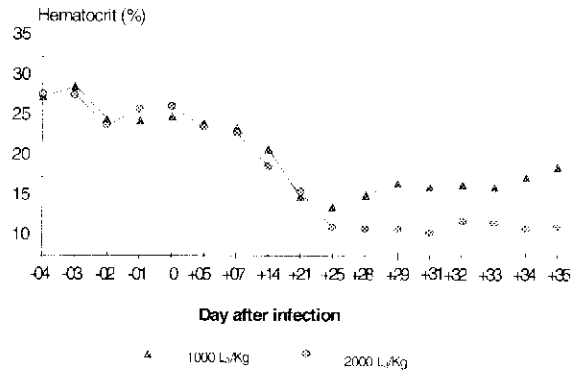

Fig. 3 - Mean values of hematocrit (%) in calves infected with 1000 L_3 and 2000 L_3 *H. placei*/ kg of live body weight before and throughout the experiments.

Body weight: Mean body weight gains were 3.9 kg and 9.1 kg in the first phase (non infected) and the second phase (infected) of Experiment I and Experiment II, respectively (Table 2). The differences between phases were significant ($p<0.05$) only in Experiment II.

Body water and body composition: After infection, the total body water (TBW) significantly ($p<0.05$) increased in the animals infected with 1000 L3, the same occurring with the water turnover (Table 2). Such differences represented an average increase of 24.8% of TBW and 48,8% in the water turnover. The time of the biologic half-life of tritiated water (T1/2) and the proportion of body fat, both undergone a reduction of mean values after infection. Considering the decrease of body fat, there was an increase of the mean value of body weight without fat, but such difference was not significant.

None of the variables such as T 1/2, daily water turnover, TBW, body fat and body weight free from fat, showed statistically significant differences between the two phases with calves infected with 2000 L3 (Table 2). Aside, it should be noted that these variables showed a similar behavior to that found in animals from Experiment I, except T1/2 values.

Water balance: Water produced by the internal metabolism (metabolic water) increased from 4.64±2.78 to 6.07 ± 2.18 per day ($p>0.05$): water loss due to evaporation increased from 6.31± 1.67 to 10.27 ± 2.32 ($p=0.07$) per day after the experimental infection. Such differences are increments of 30.8% and 62.75% respectively, when compared to the first phase but were not statistically significant.

Water produced by the internal metabolism (metabolic water) decreased from 3.18 ± .78 to 2.78 ± 1.99 per day; Water loss due to evaporation increased from 5.97 ± 1.26 to 6.21± 2.25 per day after infection. Such differences were not statistically significant.

During infection, the water from feed of animals infected with 1000 L3 was significantly ($p<0.05$) larger than in the previous phase, before infection (Table 5).

Feed intake, excretion of feces and urine: Calves of Experiment I significantly ($p<0.05$) drank 62.9% more water after infection than in the first phase (Table 3). The total output of water

through feces and urine showed a significant ($p<0.05$) trend for increase, reaching 27.4% after infection. The intake and excretion of dry matter and consequently the coefficient of digestibility and feces excretion did not show statistically significant differences in the two experiments.

In Experiment II, the single variable showing statistically significant ($p<0.05$) difference was fecal excretion that showed 13% increase, i.e., from 3.6 kg ± 0.33 to 4.07 kg ±0.52 per day after infection (Table 3). There were not statistically differences among other variables such as water intake and output or dry matter, between the two phases. However, it is important to notice that there was an increase in water consumption and water excretion after infection in both trials. Statistically significant ($p<0.05$) differences of 22.69% and 23.25% were observed in Experiment I and Experiment II, respectively.

Table 3- Mean daily intake and excretion of water and dry matter plus fecal excretion in calves before and after infection with 1000 L_3 and 2000 L_3 *H.placei*/kg of live body weight.

Treatment	Water intake ($l\ d^{-1}$)	Water excretion ($l\ d^{-1}$)			Dry matter ($Kg\ d^{-1}$)		Fecal output ($Kg\ d^{-1}$)
		Urine	Feces	Total	Input	Output	
Infected (**1000 L_3**, n=4)	9.45±0.57[a]	2.13±0.31	3.12±0.28	5.25±0.59	2.57±0.11	0.75±0.04	3.87±0.32
Non infected (n=4)	5.80±0.60[b]	1.64±0.31	2.48±0.07	4.12±0.40	2.62±0.08	0.66±0.02	3.15±0.79
Infected (**2000 L_3**, n=5)	10.49±1.63	3.70±0.61	3.35±0.46	7.05±0.99	2.63±0.16	0.72±0.07	4.07±0.52[a]
Non infected (n=5)	8.55±1.30	2.90±0.40	2.83±0.28	5.72±0.50	2.75±0.06	0.75±0.05	3.60±0.33[b]

n- number of calves. Different superscripts letters mean $p<0.05$.

Nitrogen balance: Table 4 shows that nitrogen intake by calves infected with 1000 L3 significantly ($p<0.05$) decreased from 64.72 g to 58.58 g per day, thus occurring a trend to reduce nitrogen retention. Other variables such as nitrogen excretion through feces and urine, and nitrogen balance, did not undergo significant changes after infection with 1000 L_3 or either 2000 L_3.

Table 4- Daily means of nitrogen balance in calves before and after infection with 1000 L_3 and 2000 L_3 *H.placei*/kg of live body weight.

Treatment	Nitrogen intake ($g\ d^{-1}$)	Nitrogen excretinon ($g\ d^{-1}$)			Nitrogen balance ($g\ d^{-1}$)
		Fezes	Urina	Total	
Infected (**1000 L_3**, n=4)	58.58±2.10[a]	10.69±0.46	12.43±1.92[a]	23.12±2.34	35.46±2.27
Non infected (n=4)	64.72±1.90[b]	9.55±0.43	14.80±1.11	24.36±1.52	40.40±0.81
Infected (**2000 L_3**, n=5)	61.35±3.06	10.42±1.02	18.00±1.33[b]	28.42±2.25	32.94±2.23
Non infected (n=5)	61.50±3.17	9.42±0.23	17.50±1.77	26.91±1.58	34.60±3.20

n- number of calves. Different superscripts letters mean $p<0.05$.

Comparison between calves infected with 1000 L_3 and 2000 L_3 *H.placei*: Comparison between variables of Experiment I and Experiment II did not show significant differences related to body water, body composition, water intake and excretion, dry matter, fecal output and nitrogen balance.

Table 4 shows that only nitrogen excretion through urine was significantly ($p<0.05$) smaller in calves infected with 1000 L_3 than those infected with 2000 L_3. This leads to a discreet increase of nitrogen retention, although not statistically significant ($p>0.05$).

Data from Table 3 shows a trend for a smaller excretion of water through the urine in calves infected with 1000 L_3 when compared to those infected with 2000 L_3, although not statistically significant ($p=0.07$). The same trend was observed regarding the excreted water (Table 5). However, when the mean figures of metabolic water are compared, they look significantly ($p<0.05$) larger in calves infected with 1000 L_3 (Table 5).

Table 5- Water balance in calves before and after infection with 1000 L_3 and 2000 L_3 *H.placei*/kg of live body weight.

Treatment	Water from food (l/d)	Water excreted (l/d)	Metabolic water (l/d)	Evaporated water (l/d)
Infected (**1000 L_3**, n=4)	9.45±0.57[a]	5.25±0.59	6.07±2.18[a]	10.27±2.32[a]
Non Infected (n=4)	5.80±0.60[b]	4.12±0.40	4.64±1.34	6.31±1.67
Infected (**2000 L_3**, n=5)	10.49±1.63	7.05±0.99	2.78±1.99[b]	6.21±2.25[b]
Non infected (n=5)	8.55±1.30	5.72±0.50	3.18±2.78	5.97±1.26

n- number of calves. Different superscripts letters mean $p<0.05$.

DISCUSSION

In both trials, clinical signs showed by animals after the experimental infection agree with the classic pathological aspects of haemonchosis in domestic ruminants. This refers to the gradual development of a submaxillary oedema, increasing pallor of the visible mucous membranes, decreasing packed cell volumes and development of a persistent anemic condition (ALLOMBY & DARGIE, 1973).

The decreasing of PCV values in the second week post infection, that have also been mentioned by several authors (HARNESS *et alii*, 1971; BARONI & SANTIAGO, 1983; ABBOT *et alii*, 1988; GENNARI *et alii*, 1991) was more intense and persistent in animals of Experiment II. According to HARNESS *et alii* (1971) this is caused by the population development related to large doses of infective larvae in which the competition by food and the concurrent host resistance extend the patent period and blood spoliation by the parasites. The prepatent period is also prolonged as observed in two calves infected with 2000 L_3/kg of body weight passing out eggs of *H. placei* late in feces. The reduced proportion of *H. placei* adult females in the parasitic population would explain the low fecal egg counts.

BARGER & LE JAMBRE (1985) observed that the establishment of infective larvae and their further survival are not affected by the size of the infective dose. This fact agrees with the hematocrit findings and more evident clinical signs in animals of Experiment II (2000 L_3/kg of body weight). This leads to the assumption of high mortality among the parasitic population occurring in exponential scale along the time, being proportional to the infective dose. The recovery of 26.85% and 13.59 % nematodes occurred in animals infected with 1000 L_3 and 2000 L_3/kg of body weight, respectively.

These populations composed mostly by nematodes of the 5th larval stage produced some changes such as the significant ($p<0.05$) mean increase of about 24.88% and non significant ($p>0.05$) increase of 15.53% of TBW in animals of Experiment I and Experiment II, respectively.

The significant increase of TBW and the water turnover in calves infected with 1000 L_3 showing clinical signs of haemonchosis confirmed the observations of VIEIRA-BRESSAN *et alii*,(1992) in calves showing subclinical infections by *H. placei* with very mild symptoms of such alterations.

These changes also confirm the suspicion raised by the observations related to the increase of intravascular plasma volume in the papers by ABBOTT *et alii* (1984; 1985b; 1986a,b; 1988) on sheep and GENNARI *et alii*, (1991) in cattle suffering from haemonchosis that suggested the possibility of TBW increase.

Changes of such nature may occur as part of the mechanism to the maintenance of body water homeostasis without influencing the water proportion in the adjacent tissues. Therefore, it is possible to suppose that the increase of intravascular plasma volume extending to the tissues probably occurs because of the duration and severity of the parasitic infection. The infection prolongs and enhances its effect by enough time making possible that the intravascular plasma volume spreads through all the body tissues (SILVEIRA, 1988).

According to HOUPT (1984), by the time it occurs, some strong reduction of blood volume or even if there is some increment of osmotic pressure, granulomatous cells from the juxtaglomerular kidney apparatus release rennin protease in large quantities to make the conversion of the plasmatic protein angiotensinogen into angiotensin. The last one will act in the cortical region of the adrenal glands increasing the synthesis and release of aldosterone into the blood. Aldosterone will act in the distal area of the collecting tubuli of kidney's nephrons producing an increasing reabsorption of sodium and chlorine ions, thus increasing plasma osmolarity. In concert, vacuolized nerve cells of the hypothalamus, act as osmoreceptors recording changes of the blood osmotic pressure and releasing the antidiuretic hormone ADH through the hypophysis. ADH will help a better water reabsorption by kidney's tubuli and collecting ducts.

In this way, the increase of sodium retention producing expansion of extracelullar space and the regulation of plasma osmolarity by water reabsorption, both concur for a larger body water retention, keeping the concentration of Na constant into the blood and increasing the plasma volume (SILVEIRA, 1988).

It should be kept in mind, that an average increment of 24.88% in the TBW of animals used in Experiment I does not mean a continuous accumulation of water into the body. It means that a change in the body composition occurred, animals undergoing a reduction of solids such as fat, proteins and minerals, replacing these materials by the liquid constituent (water).

If we assume that: Water consumed (liters per day - l/ d) + Metabolic water (l/ d) = Excreted water (l/ d) + Evaporated water (l/ d) = Water turnover (l/ d), and replace the mean values in both phases of the two experiments it is deducted that even after the infection by *H. placei* the animals excreted the same amount of water that was ingested, i.e., they reached a perfect water balance. The idea that infected animals stored water in a constant way, becoming turgid or edematous should be discarded.

An important fact allowing to make such deduction was the associated study of body water with water balance demonstrating that they are complementary. The combination of values whose variables were depending upon the radioisotope study to others whose precision was dependent on the careful handling of the experimental calves (assays independent of the studies with tritium) made possible a better understanding and the observation of the hydric behavior of experimental animals.

In order to maintain the water balance facing the prolonged blood spoliation by *H. placei* in Experiment I, the animals required 62.9% more water. Consequently, this caused some changes in other hydric parameters to get adjusted to such requirements. Moreover, an increase of more than 30% in the production of metabolic water contributed to a large storage of water into the body. This resulted in the need to move it quicky, accelerating the turnover in about 48% in comparison to the preinfection values. Losses due to excretion increased and there was a reduction of the mean biological T 1/2 of tritiated water, from 116 to 95 hours.

Although some of the parameters did not show statistical significant differences under individual variations, their general behavior cannot be ignored, because there is a risk to miss the understanding of other significant parameters when making an appraisal of the whole mechanism.

In Experiment II, in which only live body weights showed significant variation, the body water behavior may be analyzed under the same focus. At the second phase of the study with tritiated water, the little mean increase of water consumption, accompanied by the reduction on the production of metabolic water, caused some small amount of water storage generating a discreet increment in the quantity of TBW (15.53%). This produced some turnover acceleration accompanied by a small increase of water loss by excretion.

The increase of biological T1/2 of tritiated water, from 110 to about 120 hours, besides the little reduction in the production of metabolic water, suggests an apparent reduction of the hydric metabolism. It also suggests that the animals hold (did not accumulate) for some time the water into their bodies.

Regarding to body water, water turnover, T 1/2 and body fat after the clinical infection by *H. placei*, an important fact to be noticed is that the behavior of these variables in both

experiments is the same as that observed by VIEIRA-BRESSAN *et alii* (1992). There was an exception with the T1/2 of tritiated water that increased in Experiment II. There was an increase of the differences in amplitude of those parameters becoming statistically significant in some of them during the infection with 1000 L_3 (Experiment I).

HOLMES & BREMNER (1971) observed opposite results regarding the hydric metabolism in sheep infected with *Ostertagia circumcicta*. In their trials the increase of biological T1/2 of tritiated water accompanied the reduction of water intake; the water input being significantly smaller in animals with parasitic infections. Animals with less parasitism showed an opposite behavior in regard to the T1/2 thus suggesting the same behavior regarding the turnover of body water. PARKINS *et alii* (1990) obtained similar results in cattle infected by *Ostertagia* sp. and *Cooperia* sp. There was an increase of body water just after infection. Although this increase has been reported and confirmed by several authors (HALLIDAY *et alii*, 1965; BAKER *et alii*, 1965; ENTROCASSO *et alii* 1986), the reduction of parasitic burden suggested an apparent return to normality.

It can be said that diarrhea concurs to maintain the body hydric homeostasis, being a strong opponent of the regulation mechanisms of body water.

BREMNER (1982) observed that animals showing parasitic diarrhea caused by gastrointestinal helminths were capable to overcome the hydric deficit caused by the diarrhea itself. Although there is not a deeper study about changes in the proportion of TBW, the reduction of plasma volume and hemoconcentration supply valuable evidences to suppose about changes occurring in the body composition.

In other studies referring to body water and gastrointestinal parasitism with the appearance of diarrhea (BAKER *et alii*, 1965; HALLIDAY *et alii*, 1965; TAYLOR *et alii*, 1989; PARKINS *et alii*, 1990) the turnover values decreased while those of T1/2 of tritiated water increased during the patent period. Even without information on other parameters to explain the mechanism of hydric regulation it is possible to suppose that those animals hold the water for some more time in their bodies to activate the endocrine and neurohormonal water replacement mechanisms. This is a good help and compensation for water loss caused by diarrhea and inappetence.

BAKER *et alii*, (1965) also observed that the diarrhea ceased after anthelmintic treatment and there was an increase of turnover, reduction of both the biological T1/2 of tritiated water and the proportion of total body water.

Although animals of Experiment I did not show diarrhea, feces became less consistent after infection. The loss of water through feces and urine increased although there was some increment in water consumption. Simultaneously, the turnover values of body water increased and the T1/2 of tritiated water diminished. In Experiment II, the single calf with diarrhea, showed the same pattern of animals belonging to Experiment I regarding the above mentioned variables. This shows that a reduction of water turnover can also be associated with the increment of the proportion of body water.

The values found out in this study for the proportion of total body water before infection, are similar to those mentioned by other authors (SPRINGELL, 1968; KAMAL & SEIF, 1969; SEARLE, 1970) that worked with young domestic ruminants without parasitism. The same applies to the increments in the proportion of total body water, when compared to the results of HALLIDAY *et alii* (1965), BAKER *et alii* (1965), TAYLOR *et alii* (1989) and PARKINS *et alii* (1990) in animals harboring gastrointestinal helminths.

Regarding to nitrogen balance, the results agree to those found by ABBOT *et alii* (1984, 1988) in sheep with haemonchosis, in which nitrogen losses by excretion did not overcome ingested levels. In the second paper (1988) by this author, nitrogen retention was greater in infected animals.

Negative nitrogen balances were observed by ABBOT *et alii* (1985b), ROWE *et alii* (1988), but in both studies animals were fed with a low protein diet while in the present study they received normal protein levels.

Studies on the effect of other gastrointestinal helminthosis, with single experimental infections such as sheep (PARKINS *et alii*, 1973) or cattle ostertagiosis (TAYLOR *et alii*, 1989; XIAO *et alii*, 1992a) and bovine cooperiosis (ARMOUR *et alii*, 1987) or multiple infections by *Ostertagia* sp. and *Cooperia* sp in cattle (VERSTEGEN, 1987; ENTROCASSO *et alii* 1986., PARKINS *et alii*, 1990) showed a reduction in nitrogen retention related to the low levels of nitrogen intake caused by anorexia that did not occur in the present study. Another common finding in some studies is the increment of plasmatic urea concentration (HOLMES & BREMNER, 1973; PARKINS *et alii*, 1973; ABBOT *et alii*, 1988; XIAO *et alii*, 1992 c) that according to most authors is the the major factor contributing to nitrogen losses by excretion and therefore, for a negative balance of this element. However, ABBOT *et alii* (1988) observed a negative nitrogen balance in sheep with acute haemonchosis in which the increase of urea concentration did not correspond to the concurrent loss increase by excretion.

ROWE *et alii* (1988) observed that the conversion of food or endogenous nitrogen in ammonia increased more than 3.5 times in animals suffering from haemonchosis. Most of the ammonia was absorbed in the small intestine and metabolized increasing urea synthesis. This author mentions that VERNON & PEAKER (1973) suggested that "lost proteins", i. e., those not digested as a consequence of parasitism, when reaching

the large intestine were taken by enteric bacteria and converted to ammonia. This compound after absorption reached the liver where it was converted to urea that could be excreted or used in a new aminoacid synthesis.

In the present paper there is no data to explain in a definite way the nitrogen metabolism in both experiments, but based on the above mentioned results it is possible to understand the absence of a nitrogen negative balance in calves with clinical haemonchosis. According to XIAO *et alii* (1992a) the study of protein and energetic balance in ruminants is quite difficult because their rumen bacteria are an endogenous source of feed. XIAO *et alii* (1992a,b,c) observed that in cattle with clinical ostertagiosis there was a smaller intake of dry matter accompanied by reduction of nitrogen retention. However, in these animals there was an increase of urea nitrogen in plasma and more nitrogen was excreted through the urine.

Because of the lack of alterations of intake, excretion and balance of nitrogen in calves of Experiment II, the apparent reduction in the nitrogen balance in animals of Experiment I should not have happened for digestibility problems. It might have been caused by the reduction of ingested nitrogen by the infected animals.

Moreover, according to HOUPT (1984) a factor helping absorption of nitrogen into the rumen is the antidiuretic hormone (ADH), probably by improving mucosal permeability. In this way, perhaps the eventual increase of ADH secretion triggered by the changes in the proportion of total body water contributed to improve nitrogen absorption and then produced a positive nitrogen balance.

According to HOLMES (1987) the main deleterious effect of gastrointestinal parasitism is the deviation of the major activity of protein synthesis for muscular development (in growth) to the production of essential proteins required for cell proliferation in the gastrointestinal tract.

These factors among some others not less important such as the replacement of plasma proteins, fat, minerals, water and building of antibodies require energetic expenses that are difficult to estimate because of the wide number of enbroiled variables.

Changes in the proportion of body fat which was reduced in the patent phase of infection, more noticeable in Experiment I, are probably related to the increase of animal's energetic demands caused by blood spoliation and consequent issues. As fat, plus carbohydrates are the main components of energetic reserves (HOUPT, 1984) it is very possible that in both experiments fat was used to supply the energy requirements, even under a positive nitrogen balance. In spite of its availability, probably only the nitrogen was not sufficient to satisfy those needs.

The reason for the differences in the mobilization of fat in Experiment I (24.5%) and Experiment II (8.43%) in the second phase, when both experimental groups harbored nearly the same parasitic burden may be the same that justifies the differences which were noticed in the other parameters. Believing that the establishment of the parasitic burdens was not influenced by any of the studied factors and that mortality ocurring in the infective population was larger in Experiment II, about half of the initial population was present in the infected animals on the second phase.

In such way, the reduction on the source of metabolic stress (parasites) to a small proportion of its initial population, associated with some degree of resistance and adaptation, allowed that animals belonging to Experiment II were probably through a recovery period. At this time the magnitude of the ocurred alterations of the studied parameters in Experiment I had already happened before observations of the second phase of studies with tritiated water, possibly a short time after the initial agression with a double dosage of *H. placei* infective larvae.

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SUMÁRIO

O metabolismo da água, balanço hídrico e de nitrogênio (N) foram estudados em bezerros com dois níveis de infecção com *Haemonchus placei*. Nove bezerros machos da raça Holandesa Preta e Branca, com 5 meses de idade, receberam, via oral, 1000 (n=4) e 2000 (n=5) L_3 de *H. placei*/Kg de peso vivo. De acordo com metodologia anteriormente descrita, os estudos fisiopatológicos foram realizados em dois períodos distintos: antes e após a infecção, sendo os animais experimentais controles deles mesmos. Durante esses períodos, de 5 dias cada, com os animais em gaiolas metabólicas para avaliação do metabolismo da água através da injeção de água tritiada e dos balanços hídrico e de N foram feitas colheitas de sangue, alimento, água, fezes e urina, assim como aferições da ingestão de alimento e água e excreção de fezes e urina. Aos 35 dias após a infecção, os animais foram

sacrificados para determinação da carga parasitária. Não se evidenciaram diferenças entre as populações de nematóides adultos em relação à dose infectante. No entanto, a população de adultos jovens (L_4) foi significativamente maior que a de adultos em ambas dosagens. Houve decréscimo do hematócrito após 2 semanas da infecção, sendo significativamente menores e mais persistentes os valores encontrados na infecção com 2000 L_3. Ocorreu aumento significante da água corpórea total (ACT) e da taxa de turnover da água, e redução da meia vida biológica, porém houve um equilíbrio hídrico nos animais com 1000 L_3. Na infecção com 2000 L_3 ,apenas a redução do ganho de peso corporal foi significativa e as pequenas alterações observadas sugerem uma aparente redução do metabolismo hídrico, indicando uma retenção de água por mais tempo no organismo. As perdas de N pela excreção não superaram os níveis ingeridos devido a alimentação conter níveis adequados de proteinas. A redução na porcentagem da gordura corpórea durante a patência da infecção com 1000 L_3 foi interpretada como maior demanda energética dos animais face à expoliação causada pelos parasitos e indução da resposta imune.

PALAVRAS-CHAVE: *Haemonchus placei*, metabolismo da água, balanço de nitrogênio, balanço hídrico, níveis de infecção, bovinos.

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