

Full Length Research Paper

## Activities of enzyme adenosine deaminase in serum of lambs experimentally infected with *Haemonchus contortus* and treated with selenium and copper

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The aim of this study was to evaluate adenosine deaminase (ADA) activity in serum of lambs experimentally infected with *Haemonchus contortus* and treated with selenium and copper. We used 33 lambs, divided into five groups: Group A was composed of five healthy animals (not infected). The groups B, C, D and E comprised seven animals each infected with *H. contortus*. Group B was not treated (positive control), group C was supplemented with sodium selenite, group D with copper and group E with both sodium selenite and copper. Blood collection was performed at days 0, 20, 40, 60 and 80 post-infection (PI) for analysis of ADA activity. ADA activity reduced in the serum of animals infected with *H. contortus* when compared to not-infected at days 20, 40 and 60 PI. At day 80 PI, ADA activity only decreased in group B. Furthermore, during infection by *H. contortus* in lambs ADA activity was observed to reduced in serum. It can be concluded that supplementation with selenium and copper influenced ADA activity, directly or indirectly.

**Key words:** *Haemonchus contortus*, adenosine, copper, sodium selenite, lambs.

### INTRODUCTION

Gastrointestinal parasitism by *Haemonchus contortus* is responsible for large losses in the productivity of small ruminants (Bambou et al., 2009). This parasite penetrates the surface of abomasal mucosa to feed on host blood (Jasmer et al., 2007). The systemic effects observed due to infection by this nematode includes decreased levels of erythrocyte parameters and total plasmatic proteins, particularly albumin (Blackburn et al., 1992). The use of immune response as an auxiliary tool to combat infection by *H. contortus* may delay the

resistance, besides reducing residues of frequent anti-helminthic treatments (Camargo et al., 2010).

Intracellular parasites most often invoke a Th1-type response and helminthes parasites a Th2-type response (Miller and Horohov, 2006). According to researchers, when selenium, vitamin E and copper are administered, immunity can be activated and serum oxidative damage parameters may be reduced in sheep infected with *H. contortus* (Camargo et al., 2010; Leal et al., 2010; Soli et al., 2010). Copper is an essential trace element for the

maintenance of several biological processes, such as energy metabolism, iron homeostasis and antioxidant protection (McDowell, 1992). Sodium selenite is a common dietary form of selenium, recognized as an essential nutrient for animal and human nutrition (Combs and Gray, 1998; Combs, 1999). In the form of amino acid selenocysteine, it is a component of many antioxidant enzymes, and has been associated with functions of the immune system such as induction of apoptosis and production of reactive oxygen species (ROS) (Arner and Holmgren, 2000; Marsden and Strasser, 2003). Researchers discovered that selenium may influence the activity of many enzymes and the concentration of nucleotides and nucleosides (Moore and MacKenzie, 2008; Belle et al., 2011). This may be related to purinergic system changes (including alterations in enzymes activity) and its role in the immune response, which has been previously described (Franco et al., 1997; Xaus et al., 1999; Codero et al., 2001).

Adenosine is a molecule able in regulating cell metabolism, triggering a variety of physiological effects and participating in apoptosis, necrosis and cell proliferation. It also acts as an endogenous regulator of innate immunity, protecting the host from excessive tissue injury associated with strong inflammation (Burnstock, 2006; Desrosiers et al., 2007). Adenosine deaminase (ADA: EC 3.5.4.4) is considered a key enzyme in the metabolism of purines, catalyzing the irreversible deamination of adenosine into inosine (Franco et al., 1997). ADA is ubiquitous, but it can be found with high activity in sera, thymus, lymphoid tissues and peripheral lymphocytes. It has been demonstrated that this enzyme plays an important role in lymphocyte function and it is essential for the normal growth, differentiation and proliferation of T lymphocytes (Franco et al., 1997; Codero et al., 2001). In previously published study, it was observed a decrease in ADA activity in serum of lambs infected with *H. contortus* (Da Silva et al., 2013). Therefore, the objective of this study was to evaluate ADA activity in serum of lambs experimentally infected with *H. contortus* and treated of selenium and copper.

## MATERIALS AND METHODS

### Compounds

Sodium selenite was acquired from Merck Brazil in powder and diluted in physiologic solution (0.9%) resulting in a final concentration of 1.67%. Copper was acquired from Laboratórios Agroinsumos (Buenos Aires, Argentina). The substrate adenosine was obtained from Sigma-Aldrich.

### Animals

The study used 33 crossbred Corriedale x Texel male lambs that were five months old. The animals were kept in holding pens (one per group) at the Veterinary Hospital of Universidade Federal de Santa Maria (UFSM) for 40 days to diet [concentrated oat hay (*Avena sativa*), 15% of crude protein] and experimental environment adaptation. During this period, animals received anthelmintic

treatment (two doses with an interval of 15 days) with a combination of closantel and albendazole (Closalben®, Ceva Santé Animale, Paulínia, São Paulo, Brazil). The copper and selenium content in feed was determined by the Chemical Analyses Laboratory of UFSM. The procedure was approved by the Ethics and Animal Research Committee from UFSM, number 82/2009.

### Experimental groups

The animals were divided into five groups: Group A consisted of five healthy animals (not infected) used as a negative control; Group B ( $n=7$ ), animals were infected with *H. contortus* and not-treated (positive control); Group C ( $n=7$ ), animals were infected with *H. contortus* and supplemented with sodium selenite (0.2 mg/kg of body weight - BW) delivered intramuscularly (IM) (Merck Brasil, Jacarepaguá, RJ, Brazil); Group D ( $n=7$ ), animals were infected with *H. contortus* and supplemented with copper (3.5 mg/kg of BW) delivered subcutaneously (SC); Group E ( $n=7$ ), animals were infected with *H. contortus* and supplemented with sodium selenite (0.2 mg/kg of BW) IM and copper (3.5 mg/kg of BW).

Sodium selenite supplementation was carried out 20 days before the beginning of the experiment and repeated at day 0 of experiment (T0). That is because, according to the literature, the selenium dose reaches peak blood within 60 days after administration (Ramirez-Bribiesca et al., 2005). Copper was administered at T0 and 30 days later. Already copper, reaches its sanguine peak well before, so this difference between treatments (Eisler, 2007).

### *Haemonchus contortus* infection

All animals from groups B - E were infected orally with 500 larvae (L3) per animal, every two days, for a period of 20 days (Rowe et al., 2008), starting at T0. The larvae were obtained from the Parasitic Diseases Laboratory of Federal University of Paraná. They were obtained by coproculture technique (Roberts and O'Sullivan, 1950).

### Sample collection

Blood was collected by the Vacutainer® system in tubes without anticoagulant. The samples were obtained at days 0 (T0, before parasite infection), 20 (T1), 40 (T2), 60 (T3) and 80 (T4). After blood was collected the samples were centrifuged to obtain serum and then stored at -20°C for further analysis of ADA activity.

### Adenosine deaminase activity

ADA activity was measured spectrophotometrically in serum of all animals by the method of Giusti and Gakis (1971). The reaction was started by the addition of the substrate (adenosine) to a final concentration of 21 mmol/l and incubations were carried out for 1 h at 37°C. The reaction was stopped by adding 106 mmol/l/0.16 mmol/l phenol-nitroprusside/ml solution. The reaction mixtures were immediately mixed to 125 mmol/l alkalinehypochlorite (sodium hypochlorite) and vortexed. Ammonium sulphate 75  $\mu$ mol/l was used as ammonium standard. The ammonia concentration is directly proportional to the absorption of indophenol at 650 nm. The specific activity is reported as U/L.

### Parasitology of feces

Fecal samples for quantification of eggs per gram (EPG) were collected at T0, T1, T2, T3, T4 and processed according to the

**Table 1.** Adenosine deaminase (ADA) activity in serum of sheep *Haemonchus contortus* infected.

Days post-infection	Means and standard deviations of ADA activity in serum (U/L)				
	Group A	Group B	Group C	Group D	Group E
T0 = Day 0	18.58 ± 3.90 <sup>a</sup>	17.06 ± 3.69 <sup>a</sup>	16.20 ± 4.40 <sup>a</sup>	16.85 ± 4.10 <sup>a</sup>	16.61 ± 4.00 <sup>a</sup>
T1 = Day 20	20.51 ± 5.00 <sup>a</sup>	13.30 ± 4.09 <sup>b</sup>	11.89 ± 2.20 <sup>b</sup>	13.40 ± 2.70 <sup>b</sup>	10.66 ± 3.50 <sup>b</sup>
T2 = Day 40	21.35 ± 4.00 <sup>a</sup>	13.93 ± 3.29 <sup>b</sup>	14.20 ± 1.88 <sup>b</sup>	18.20 ± 2.60 <sup>ab</sup>	14.40 ± 2.90 <sup>b</sup>
T3 = Day 60	19.60 ± 4.74 <sup>a</sup>	13.20 ± 3.50 <sup>b</sup>	14.60 ± 4.88 <sup>ab</sup>	12.30 ± 3.00 <sup>b</sup>	17.10 ± 4.12 <sup>ab</sup>
T4 = Day 80	18.76 ± 5.04 <sup>a</sup>	12.30 ± 2.40 <sup>b</sup>	20.20 ± 3.68 <sup>a</sup>	20.71 ± 5.40 <sup>a</sup>	18.34 ± 4.00 <sup>a</sup>

Group A: Animals not-infected; group B: infected by *H. contortus*; Group C: infected by *H. contortus* and supplemented with selenium; group D: infected by *H. contortus* and supplemented with copper; group E: infected by *H. contortus* and supplemented with copper and selenium. Data are expressed as mean ± standard deviation. Means in the same line followed by different letters are statistically different among group them by Tukey's test at 5% probability ( $P < 0.05$ ).

technique described by Gordon and Whitlock (1939). Five days after the end of experiment three, animals from each group were euthanized (10 mg intravenous (IV) acepromazine; 2g IV sodium thiopental; then 100 ml IV potassium chloride), and their parasite loads were determined (Ueno and Gonçalves, 1998).

#### Statistical analysis

Results of EPG do not present normal distribution and therefore were transformed to logarithms. Subsequently, the ADA activity and EPG results were subjected to one-way analysis of variance (ANOVA) and by Tukey Post-hoc test. Values with probability (P) less than 5% were considered statistically different.

## RESULTS

### Adenosine deaminase activity in serum (ADA)

No differences in ADA activity were observed among groups at day 0 (Table 1). At day 20 PI, ADA activity significantly ( $P < 0.05$ ) decreased in serum from all groups infected with *H. contortus* when compared to group A, not-infected animals (Table 1). At day 40 PI ADA activity decreased in groups B, C and E (Table 1), and at day 60 PI it decreased in groups B and D (Table 1). At the end of the experimental period (80 days PI) only group B maintained ADA activity significantly decreased ( $P < 0.05$ ) when compared to negative the control and the other groups supplemented with selenium and/or copper (Table 1).

### EPG

The EPG data are described in Table 2. After infection it was observed positive EPG for all sheep infected with *H. contortus*. At days 20, 40 and 60 PI no significant differences in EPG were observed in the groups. At day 80 PI a reduction in EPG in group E (supplemented with selenium and copper) was observed when compared to other groups infected with *H. contortus* (Table 1). The parasite load of animals is shown in Table 2. The number of parasites significantly reduced in the group E.

## DISCUSSION

In this experiment it was observed that infection by *H. contortus* alters ADA activity. The evaluation of ADA activity is a great tool in the diagnosis of tuberculosis in humans (Mello et al., 2000) and has been studied in protozoan infections as *Trypanosoma* sp. and *Leishmania* sp. (Ozcan et al., 1998 ; Da Silva et al., 2011). In this study, reduction of ADA activity was observed in serum of sheep infected with *H. contortus* and supplemented with copper and selenium. Therefore, this study aimed to verify the influence of infection by *H. contortus* on the ADA, as well as the influence of supplementation with minerals on the activity of the enzyme.

The reduction in ADA activity may cause an increase in the extracellular concentrations of adenosine, which would be converted into inosine. Adenosine acts as a sensor and provides information to the immune system regarding tissue damage or acute inflammatory changes occurring in the vicinity of the immune system (Kumar and Sharma, 2009). Reduction in the activity of ADA and elevated adenosine levels would lead to agonism of adenosine receptors which exist in many cell types, with possible anti-inflammatory effects, including the inhibition of Th1 and/or Th2 immune response. In infection caused by *H. contortus* there is a predominance of Th2 and cellular response with production of interferon and cytokines (Miller and Horohov, 2006). However, inhibition of this response by the action of extracellular adenosine in purinergic receptors could be a compensatory effect, attenuating inflammation and tissue damage.

A reduced ADA activity was observed in infected but not supplemented group (group B) during all experimental period, but the same did not occur in the infected groups and supplemented with copper and/or selenium (group C, D and E) which had ADA activity similar to negative control group (group A) at day 80 PI. Therefore, ADA activity return to basal level may be related directly or indirectly to supplemented copper and selenium. According to the study of Belle et al. (2011), in experiments involving activation of the immune system, the organic selenium compound would modulate ADA activity,

**Table 2.** Fecal egg count in sheep infected with *Haemonchus contortus*.

Parameter	Time	Means and standard deviations of fecal egg count				
		Group A	Group B	Group C	Group D	Group E
EPG	T0	0.00 ± 0.00 <sup>a</sup>	0.00 ± 0.00 <sup>a</sup>	0.00 ± 0.00 <sup>a</sup>	0.00 ± 0.00 <sup>a</sup>	0.00 ± 0.00 <sup>a</sup>
	T1	0.00 ± 0.00 <sup>a</sup>	714.28 ± 320 <sup>b</sup>	471.43 ± 210 <sup>b</sup>	385.71 ± 150 <sup>b</sup>	614.28 ± 240 <sup>b</sup>
	T2	0.00 ± 0.00 <sup>a</sup>	8328.57±2300 <sup>b</sup>	6700 ± 2900 <sup>ab</sup>	4557.14±1600 <sup>c</sup>	5214.3±2500 <sup>ab</sup>
	T3	0.00 ± 0.00 <sup>a</sup>	5300.00±2800 <sup>b</sup>	4728.57±2100 <sup>b</sup>	5628.57±1700 <sup>b</sup>	3957.15±2400 <sup>b</sup>
	T4	0.00 ± 0.00 <sup>a</sup>	7028.57±1900 <sup>b</sup>	4671.43±2500 <sup>bc</sup>	6257.14±1200 <sup>b</sup>	3542.86 ± 610 <sup>c</sup>
Parasite Load†	T4	0.00 ± 0.00 <sup>a</sup>	567.33 ± 154 <sup>b</sup>	526.66 ± 136 <sup>b</sup>	430 ± 102 <sup>b</sup>	346 ± 46 <sup>c</sup>

Group A: animals not-infected; group B: infected by *H. contortus*; Group C: infected by *H. contortus* and supplemented with selenium; group D: infected by *H. contortus* and supplemented with copper; group E: infected by *H. contortus* and supplemented with copper and selenium. Data are expressed as mean ± standard deviation. Means in the same line followed by different letters are statistically different among group them by Tukey's test at 5% probability ( $P < 0.05$ ). T0 refers to day 0, T1 day 20, T2 day 40, T3 day 60 and T4 day 80.

and thus contribute to the maintenance of cellular integrity during inflammatory processes. According to researchers, supplementation with selenium and copper in lambs infected with *H. contortus* may contribute to modulate the immune response (Leal et al., 2010; Galindo-Barboza et al., 2011) and confers protection against oxidative stress (Camargo et al., 2010). The interaction of copper with adenosine monophosphate (AMP), adenosine diphosphate (ADP), adenosine triphosphate (ATP) and adenosine has been reported (Onori and Blidaru, 1985; Onori, 1987), which may consequently alter the activity of ectonucleotidases and ADA as observed in this study. Thus, indirectly, supplementation with these compounds may have contributed to keep ADA activity in serum similar to normal basal levels, compared to the negative control (group A).

In this study we observed that the supplementation with selenium and copper were able to enhance the reduction of EPG, as well as the number of parasites in the abomasum of the animals of these groups. This is because probably the copper orally has a direct action on the parasite and thus reducing the parasitic load (Soli et al., 2010). Already, selenium has been shown to stimulate and improve immunity of animals infected with *H. contortus* (Camargo et al., 2010), which the authors may reflect the reduced number of parasites. Note that mineral supplementation was able to increase the activity of ADA with the chronicity of the disease and caused a reduction in parasite load (T4), as can be seen in Table 2. But studies are needed to verify the association between ADA activity and parasite load, as well as ADA and selenium supplementation and copper.

Based on the results of this study we can conclude that lambs infected with *H. contortus* showed reduced ADA. This change may be related to the inflammatory response and/or clinical signs of haemonchosis. Administration of selenium and copper may have influenced ADA activity, directly or indirectly. We assume that the supplementation of selenium associated with copper may have reduced pathogenicity (decreased worm burden and EPG) of

*H. contortus* infection in sheep by increasing the immune response and normalizing ADA activity.

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