

Oxfendazole induces protein catabolism and gluconeogenesis in experimental neurocysticercosis

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ABSTRACT

Neurocysticercosis (NCC) is an endemic public health disease of the central nervous system highly related to epilepsy and seizures. *Taenia crassiceps* is an experimental model used for NCC and biochemical studies of the host-parasite relationship. For the past 50 years the NCC therapeutic treatment is performed with albendazole (ABZ) and praziquantel which opens a gap for new therapies due to parasitic resistance and other adverse effects of the drugs. Oxfendazole (OXF) is an albendazole derivative with efficacy against tissue cestodes of veterinary importance. The aim of this study was to determine the metabolic impact of OXF on *T. crassiceps* cysticerci intracranially inoculated in Balb/C mice. The animals were intracranially inoculated with *T. crassiceps* cysticerci and 30 days later received single dose oral treatment of OXF, ABZ and NaCl 0.9% (control group). The metabolic impact was quantified through the detection of metabolites from glycolysis, anaerobic fermentation of lactate and propionate, tricarboxylic acid cycle, protein catabolism, fatty acids oxidation. The differences observed in the concentrations of metabolites from the OXF treated group showed that the drug induced gluconeogenesis, increase in protein catabolism, fatty acids oxidation and propionate fermentation in comparison to the ABZ and control treated groups. In conclusion, OXF induced greater metabolic impact in *T. crassiceps* cysticerci than the standard NCC treatment, ABZ, showing that it may represent an alternative drug for its treatment.

1. Introduction

Neurocysticercosis (NCC) is a helminthic infection of the central nervous system (CNS) with high mortality and morbidity in endemic developing countries. This infection is related to poor hygienic conditions of life where there are deficient sanitary conditions. NCC is the main infectious cause of seizure and epilepsy which are mainly observed when the parasite located in the CNS parenchyma is in a degenerative phase (Carpio et al., 2021).

The severity of NCC is related to the number of cysticerci, their location and viability within the CNS. Viable cysticerci are able to induce an immunomodulation of the host's response and normally do not induce symptoms while the degenerative ones, which have already died, induce an inflammatory reaction in the CNS and are responsible for most of the symptoms and even death of the host (Carpio et al., 2018). The current NCC treatment is based on the detection of the cysticerci, its development stage and on the number of cysts within the CNS. When there are up to two viable parenchymal cysticerci it is recommended

albendazole treatment for 10–14 days. On the other hand, when there are more than two viable parenchymal cysticerci a combined treatment with praziquantel and albendazole for 10–14 days may be recommended. Also, it is necessary to follow up the patients in order to detect side effects such as leucopenia and hepatotoxicity. This therapeutical scheme should be monitored in order to ensure the parasitic clearance and to diminish the effects of the possible inflammatory boost caused by the parasite's death (White Jr et al. A.C. 2018).

Oxfendazole (OXF) is a benzimidazole anthelmintic drug that has been proved to present efficacy against intestinal and tissue infections and is currently approved for veterinary use (Gonzalez et al., 2019). A study has demonstrated that OXF can be used in mass chemotherapy program on pigs in order to prevent the transmission of the taeniasis-cysticercosis complex in highly endemic areas (Garcia et al., 2006). Also, OXF has been shown to present efficacy against *Taenia solium* cysticerci in pigs after a single oral dose treatment and to have a longer plasma half-life than albendazole which explains the higher cysticidal effect (An et al., 2019).

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Biochemical studies of the parasite and of the host-parasite interaction have been useful in order to help determine the drugs mode of action and may be used as a screening tool to aid determine the drug's efficacy against parasitic infections. Benzimidazole derivatives, such as albendazole and oxfendazole, are capable of impacting the polymerization of α and β subunits of tubulin which leads to cytoskeleton instability, tegument alterations, impairment of the mitotic spindle during cell division, struggle of intracellular transport of substances and several biochemical effects derived from the impact on nutrients uptake (Vinaud and Ambrosio 2020). Several metabolic pathways related to energy production such as glycolysis, tricarboxylic acid (TCA) cycle, protein catabolism, fatty acids oxidation and lactate and propionate fermentation have been used in the determination of the biochemical impact of drugs on parasites (Vinaud et al., 2008, 2009, 2020; Isac et al., 2020; Vinaud and Ambrosio 2020; Lima et al., 2021a).

Taenia crassiceps is a cestode with phylogenetic and immunogenic similarity to *T. solium* which allows its use as experimental model in cysticercosis studies. It has been widely used in biochemical studies of the host-parasite interaction, the effect of drugs on the parasite and its survival mechanisms (Vinaud and Ambrosio 2020). Therefore, the aim of this study was to determine the metabolic impact of oxfendazole on cysticerci intracranially inoculated in mice.

2. Materials and methods

2.1. Ethical considerations

This study was in accordance with the ethical principles of animal experimentation determined by the National Counsel of Control in Animal Experimentation (CONCEA) and was approved by the Ethics Committee in Animal Use from the Federal University of Goiás, Brazil, protocol number 064/19.

2.2. Maintenance of *taenia crassiceps*

The *T. crassiceps* cysticerci, ORF strain (Ontario Research Facility) has been maintained through peritoneal passages in 8–12 weeks old BALB/c female mice in the animal facilities of the Tropical Pathology and Public Health Institute of the Federal University of Goiás since 2002 according to Vaz et al. (1997).

2.3. Mice intracranial inoculation of *taenia crassiceps* cysticerci and treatments

Taenia crassiceps cysticerci were removed from the peritoneal cavity of mice used in the maintenance of the parasite and classified into morphological stages (Vinaud et al., 2008). Five initial stage cysticerci, i. e. without buddings and with translucent membrane, were inoculated in the intracranial cavity of 8–12 weeks old female Balb/c mice. The intracranial inoculation was performed after the trichotomy and anesthesia of the animals with a solution of ketamine and xylazine (0.1 ml/10 g). The hole in the skull was performed using a dental drill. After the inoculation the lesion was sutured. The mice received analgesic treatment with tramadol for a period of seven days after the inoculation and were daily observed (Matos-Silva et al., 2012). After 30 days of the infection the animals were treated with a single dose via gavage of: 0.9% NaCl (control group); 15 mg/Kg of albendazole (commercially available oral solution, Teuto®) or oxfendazole (diluted in injection water, Sigma-Aldrich®); 30 mg/Kg of albendazole or oxfendazole. Each treatment group was formed by 25 mice resulting in a total of 125 mice in this study.

24 h after the treatment the mice were euthanized through cervical dislocation and the cysticerci removed, weighed and frozen in liquid nitrogen for posterior biochemical analysis. The intracranial cavity of the mouse does not allow the inoculation of more than five cysticerci because it will induce an increase in the intracranial pressure and the

compression of the nervous system could be fatal to the mouse. Therefore, it is possible to retrieve a small number of cysticerci from each animal after 30 days of infection, which represent an insufficient amount of biological material for the biochemical analysis. To solve this, we formed a pool of cysticerci removed from five mice and weighted them. The variation in cysticerci number and size retrieved from the treated groups is minimized when the quantification of metabolites is adjusted per gram of cysticerci (Matos-Silva et al., 2012).

2.4. Biochemical analysis

The cysticerci were homogenized in Tris-HCl 0.1 M buffer pH 7.6, supplemented with a protease inhibitor (SigmaFast protease inhibitor cocktail tablets, EDTA-free, Sigma®). The cysticerci extract was centrifuged at 10,000 rpm per 10 min at 4°C. The organic acids were extracted through an ionic exchange solid phase column (Bond Elut® Agilent®) (Vinaud et al., 2008; Fraga et al., 2012).

The resulting samples were analyzed through high performance liquid chromatography (HPLC) through an exclusion BIORAD-Aminex HPX-87H column. The eluent was sulfuric acid 5 mM at 0.6 mL/min with spectrophotometric reading at 210 nm. The results were analyzed through the Star Chromatography Workstation software (Agilent®), previously calibrated for the following metabolites identification: pyruvate, lactate, citrate, oxaloacetate, fumarate and malate (Vinaud et al., 2008). The glucose, urea, creatinine and lactate dehydrogenase quantifications were performed through an Architect C8000 Plus device, using commercial kit protocols with enzymatic method.

2.5. Statistical analysis

All experiments were performed five times independently. Due to the differences in parasite load between treatments, all the quantifications were converted in values per gram of cysticerci. The statistical analysis was performed using the Graph Pad Prism 8.2 software. The descriptive analysis was performed as to determine the normal distribution and homogenous variation as well as mean and standard deviation. One Way repeated measures analysis of variance was performed followed by the Bonferroni post-test when comparing the treatments. The differences were considered significant when $p \leq 0.05$.

3. Results

The oxfendazole treatment (30 mg/kg) induced a significant increase in glucose and LDH concentrations in comparison to the other treatments and to the control group, while there was a significant decrease in pyruvate concentrations and no effect in the lactate one ($p < 0.05$) (Fig. 1).

Both concentrations of oxfendazole induced an intensification of the tricarboxylic acid cycle due to the significant increase in the concentrations of oxaloacetate, citrate, malate and alfa-ketoglutarate in comparison to the other groups ($p < 0.05$) (Fig. 2).

Alternative energy productions pathways were also intensified due to the increase in the concentrations of propionate (fermentation), beta-hydroxybutyrate (fatty acids oxidation), fumarate, urea and creatinine (protein catabolism) in the groups treated with oxfendazole in comparison to the other groups ($p < 0.05$) (Fig. 3).

4. Discussion

This study allowed the detection of the metabolic impact of two different concentrations of oxfendazole on *T. crassiceps* cysticerci intracranially inoculated in Balb/c mice in comparison to the therapeutic standard NCC treatment, albendazole. The effort to increase the antiparasitic drugs arsenal is important in order to evade parasitic resistance against widely used drugs (Codd et al., 2015). The

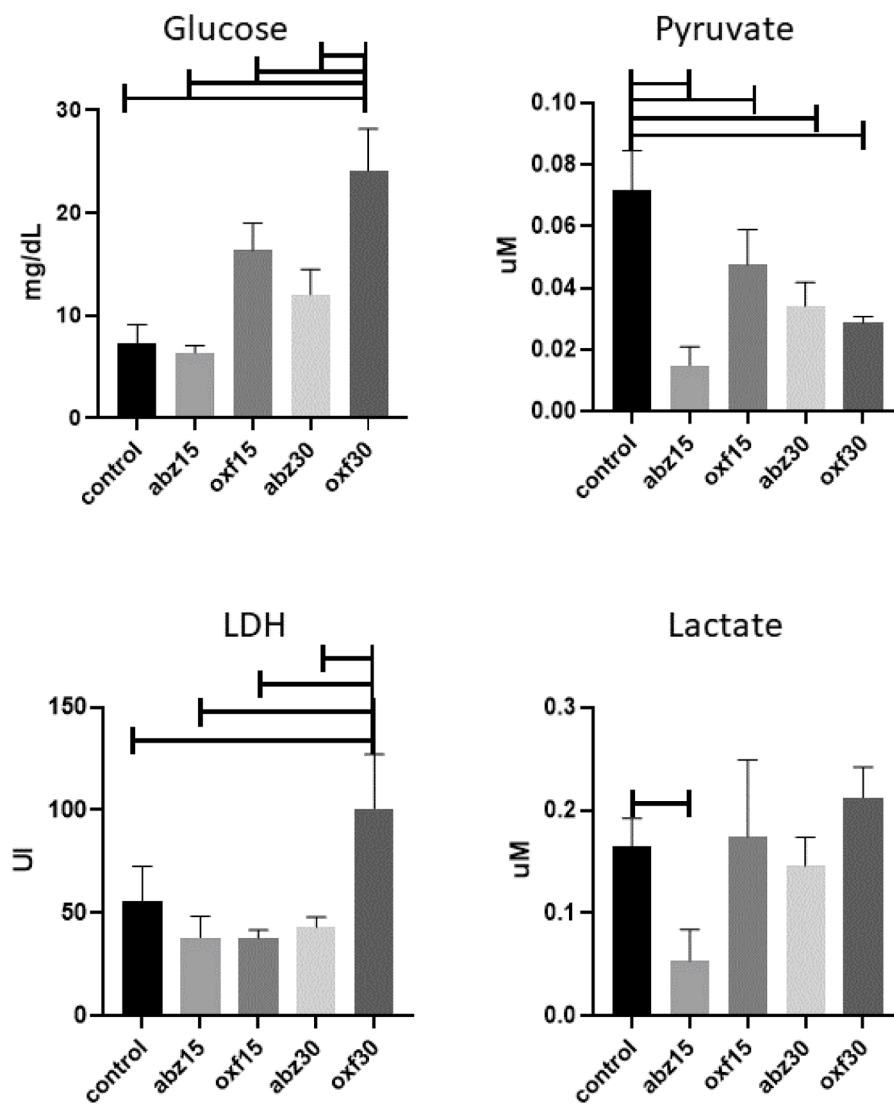


Fig. 1. Mean concentrations of glucose, pyruvate, LDH and lactate quantified in *Taenia crassiceps* cysticerci intracranially inoculated in BALB/c mice after treatment with albendazole or oxfendazole. Control group received treatment with NaCl 0.9%. abz15: treatment with albendazole 15 mg/kg; abz30: treatment with albendazole 30 mg/kg; oxf15: treatment with oxfendazole 15 mg/kg; oxf30: treatment with oxfendazole 30 mg/kg. Bars indicate statistical difference between groups ($p < 0.05$).

concentration of 30 mg/kg of OXF has proven to completely eliminate cysticerci in pigs showing better results when compared to the effects of a similar concentration of praziquantel, which is other standardized drug used in NCC treatment (Gonzales et al., 1996). This is in accordance to our results in which the concentration of 30 mg/kg of OXF showed greater metabolic impact on the parasite. Both ABZ and OXF are metabolized in the liver and the resulting metabolites (albendazole sulphoxide and fenbendazole sulphone) also present anthelmintic efficacy (Gonzalez et al., 2019).

Benzimidazole derivatives are able to impair the nutrients uptake, especially glucose, by the cellular membranes leading the parasite to enhance several metabolic survival mechanisms (Vinaud and Ambrosio 2020). The increase in glucose concentrations after the OXF treatment in our study may have occurred due to two different mechanisms: 1. Glycogenolysis; 2. Gluconeogenesis. Glycogenolysis may have occurred due to the consumption of the abundant glycogen reserves present in the parasite's tegument, allowing the maintenance of glucose levels in the cytosol and ensuring the ATP production (Swiderski et al., 2018).

On the other hand, the gluconeogenesis may be observed as a complex metabolic pathway linked to other metabolites quantified in our study. In order to compensate the lack or decrease on glucose uptake the parasitic cell may increase the activity of enzymes such as lactate dehydrogenase in a reverse action which convert lactate into pyruvate; pyruvate carboxylase which converts pyruvate into oxaloacetate, malate

dehydrogenase which convert malate into oxaloacetate, among others. The latter goes through a series of enzymatic reactions in order to end up as glucose (Yu et al., 2021). It was possible to observe in our results the increase in LDH, malate and oxaloacetate concentrations and the decrease of pyruvate concentration which is in accordance to the gluconeogenesis pathway. The induction of gluconeogenesis in *T. crassiceps* cysticerci by drugs has been described previously by our study group (Vinaud and Ambrosio 2020; Lima et al., 2021a).

Protein catabolism has been shown to be used as alternative energetic pathways by cestodes previously (Vinaud et al., 2009). Protein catabolism is an important source of fumarate, alpha-ketoglutarate and ammonia, which is then converted into urea in order to be excreted. Fumarate and alpha-ketoglutarate are intermediate metabolites of the TCA cycle and function as fuel in order to maintain it working (Da Poian et al. 2010). In our study we have induced a metabolic stress in the parasite's cells through the exposure to OXF and ABZ in different concentrations resulting, according to the benzimidazole derivatives mode of action, in tegument damage, nutrients uptake impairment and intracellular transportation deficiency (Vinaud and Ambrosio 2020). This forced the parasite to enhance other metabolic pathways as a survival mechanism and which was observed especially after the treatment with 30 mg/kg of OXF.

Other biochemical pathways enhanced by the OXF treatment were the fatty acids oxidation and the propionate fermentation. It was

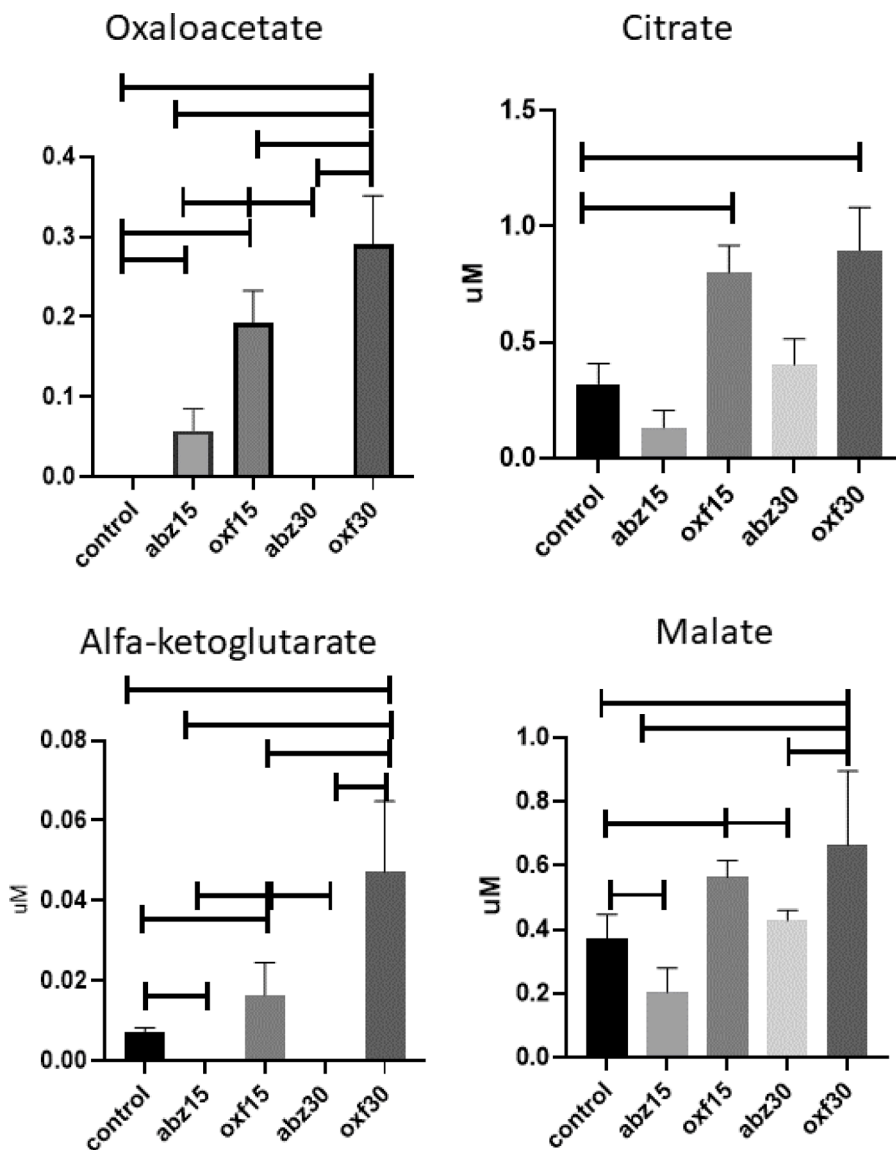


Fig. 2. Mean concentrations of tricarboxylic acid cycle metabolites - citrate, oxaloacetate, alfa-ketoglutarate and malate - quantified in *Taenia crassiceps* cysticerci intracranially inoculated in BALB/c mice after treatment with albendazole or oxfendazole. Control group received treatment with NaCl 0.9%. abz15: treatment with albendazole 15 mg/kg; abz30: treatment with albendazole 30 mg/kg; oxf15: treatment with oxfendazole 15 mg/kg; oxf30: treatment with oxfendazole 30 mg/kg. Bars indicate statistical difference between groups ($p < 0.05$).

possible to detect a significant increase in beta-hydroxybutyrate and propionate concentrations in the groups treated with OXF in comparison to the control group. Beta-hydroxybutyrate is an energy carrier observed in situations of low glucose levels as it may be metabolized into acetyl-CoA and its higher levels are related to a process called ketogenesis. Also, beta-hydroxybutyrate acts as regulator of the intracellular glucose and lipid metabolism (Newman and Verdin 2017). In our study the significant increase in beta-hydroxybutyrate may be a response to the impairment in glucose uptake and may act as a rapid cellular response in order to maintain the function of the TCA cycle.

The unbalance in the metabolic pathways generated by the presence of drugs in the parasitic cells leads to altered levels of acetyl-CoA that may be decarboxylated and phosphorylated into succinate which undergoes the action of enzymes such as propionyl-CoA carboxylase, methylmalonyl-CoA mutase and acyl-CoA transferase contributes to the production of propionate (Pietrzak and Szaz 1981). The alteration in this pathway due to the presence of benzimidazole derivatives has been described previously by our group (Picanço et al., 2019).

It is important to highlight that the metabolic alterations observed were more intense in the groups treated with OXF than the ones treated with ABZ. The better efficacy of OXF in comparison to ABZ has also been determined in a study comparing the cysticidal effect of these drugs on

cystic echinococcosis in sheep and goats (Njoroge et al., 2005). According to a review by Mkupasi et al. (2013) OXF (30 mg/kg) presents 100% of cysticidal effect in muscular and brain cysts in pigs while ABZ (30 – 50 mg/kg) presents a killing rate ranging from 89 to 99% in muscular cysts and a variable effect in brain cysts. These different responses may be related to OXF presenting a greater absorption rate in the gastrointestinal in comparison to other benzimidazole derivatives. Also, the differential target in subunits of microtubules, OXF impairs β subunits while ABZ impairs α subunit, may result in varied range of metabolic impact and cytoskeletal alterations in the parasite (Lima et al., 2021b).

In conclusion, it was possible to observe in this study an enhancement of alternative energetic pathways in *T. crassiceps* cysticerci intracranially inoculated in mice after in vivo treatment with oxfendazole in comparison to albendazole and control treatment groups. These results show that the metabolic impact of oxfendazole was greater than the observed after the albendazole treatment showing that this drug is a potential alternative therapeutic treatment for NCC. This study also confirms the biochemical studies may act as screening for drugs efficacy against parasites. It is important to highlight that in spite of this study evaluation of conserved biochemical pathways the metabolic responses observed in the parasites intracranially inoculated in mouse may differ

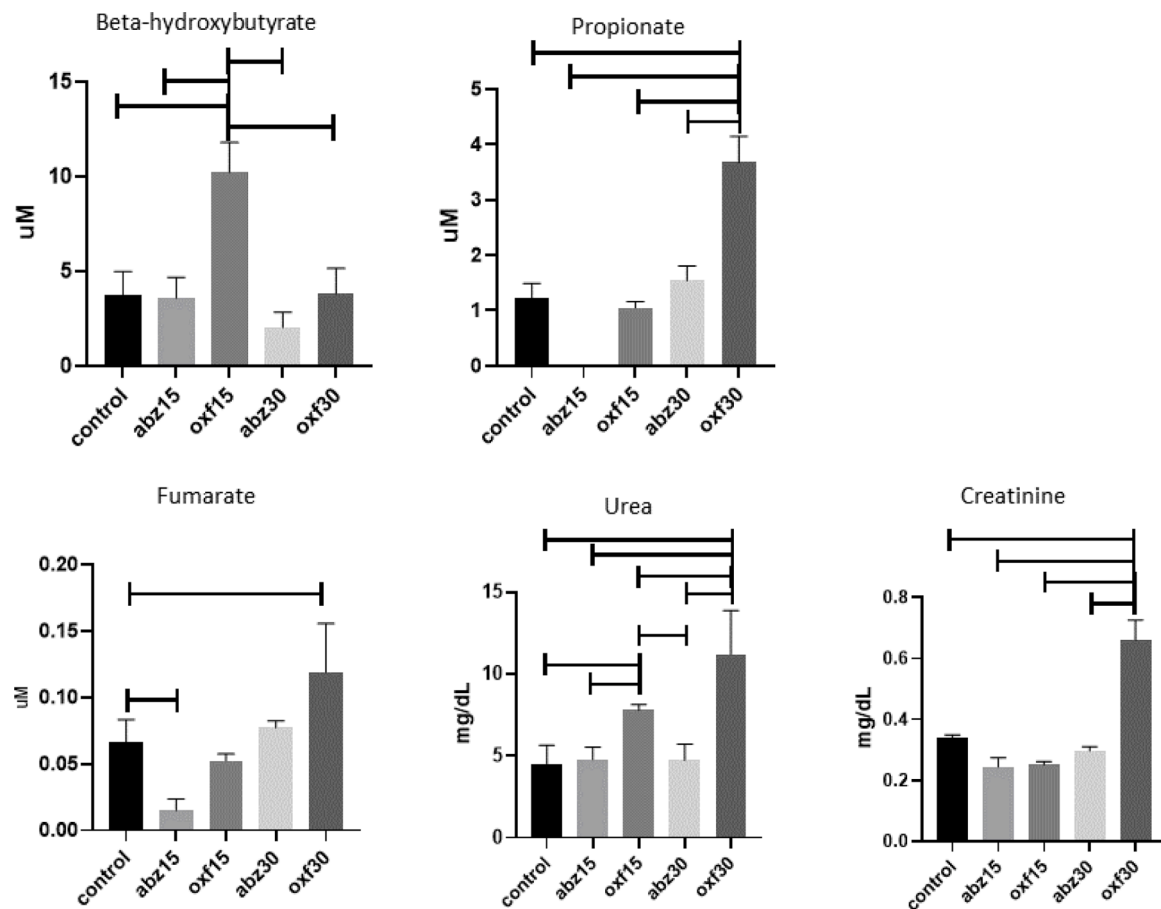


Fig. 3. Mean concentrations of beta-hydroxybutyrate, fumarate, propionate, urea and creatinine quantified in *Taenia crassiceps* cysticerci intracranially inoculated in BALB/c mice after treatment with albendazole or oxfendazole. Control group received treatment with NaCl 0.9%. abz15: treatment with albendazole 15 mg/kg; abz30: treatment with albendazole 30 mg/kg; oxf15: treatment with oxfendazole 15 mg/kg; oxf30: treatment with oxfendazole 30 mg/kg. Bars indicate statistical difference between groups ($p < 0.05$).

from the ones in human infections due to inherent differences between species. Therefore, more studies are needed in order to confirm these effects in human NCC.

CRediT authorship contribution statement

Laila Timina Bueno Correia: Data curation, Formal analysis, Investigation, Methodology, Writing – original draft. **Nayana Ferreira de Lima:** Data curation, Formal analysis, Investigation, Methodology, Writing – original draft, Supervision. **Taynara Cristina Gomes:** Data curation, Methodology. **Carlos Henrique De Sousa Guerra:** Data curation, Methodology. **Tatiane Luiza Costa:** Data curation, Methodology. **Marina C Vinaud:** Conceptualization, Funding acquisition, Writing – review & editing, Supervision, Validation, Visualization, Project administration.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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