CLINICAL IMPROVEMENT AND NORMALIZATION OF ACUTE PHASE RESPONSE AFTER TREATMENT WITH GLUCANTIME® AND DIETARY NUCLEOTIDES AND AHCC IN DOGS WITH CLINICAL LEISHMANIOSIS

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BACKGROUND AND OBJECTIVE

Canine leishmaniosis (CanL) due to *Leishmania infantum* is a major global zoonosis potentially fatal to humans and dogs.[1] **Acute phase proteins (APPs)** can be used as early markers in patients with CanL and also for monitoring response to treatment, since serum levels of APPs are usually higher in infected dogs and tend to decrease as they respond to therapy.[2,3] More specifically, paraoxonase-1 (PON1), ferritin and C-reactive protein (CRP) are APPs that reflect changes in inflammatory and oxidative stress response in these dogs.[4]

The first-line recommended treatment for CanL is currently a combination of subcutaneous N-methylglucamine antimoniate (MGA) for 4-6 weeks with oral allopurinol for at least 6-12 months.[1,5] However, **allopurinol can induce an increase in urinary xanthine levels**, which might eventually lead to urolithiasis and renal mineralisation,6–8 and it has also been reported to stimulate the growth of Leishmania promastigotes in vitro.[9] Moreover, allopurinol resistance has recently been reported in *L. infantum* parasites isolated from dogs undergoing allopurinol treatment, and associated with clinical relapse, which raises a public health concern.[10] There is, therefore, a need for alternative therapies that can be effectively and safely administered long-term in CanL patients.

Among possible options, dietary nucleotides and Active Hexose Correlated Compound (**AHCC**) could be considered. Orally administered **nucleotides** modulate the immune response, positively influencing lipid metabolism, immunity, and tissue growth, development and repair.[11,12] AHCC is used in humans for its ability to stimulate the immune system, and it enhances Th1 cell response.[13]

Since the type of immune response against *L. infantum* plays a key role in the disease progression and outcome of CanL-infected patients, being a stronger Th1 (cellular) response associated with a better prognosis,[1,5] the objective of the study was to **evaluate the effects of an immunomodulatory oral supplement containing dietary nucleotides and AHCC (IMPROMUNE®, Bioiberica SA, Barcelona, Spain) together with MGA in dogs with clinical leishmaniosis, with especial interest in variations in APPs levels**.

MATERIALS AND METHODS

Sixty-nine dogs with naturally-occurring clinical CanL were included in this multicentre, open-label, allopurinol-controlled clinical trial and randomized into two groups: **allopurinol (Control) group** (10 mg/kg allopurinol PO BID for six months) or **supplement group** (17 mg/kg AHCC and 32 mg/kg dietary nucleotides PO SID for six months). All dogs received 50 mg/kg MGA (Glucantime®, Merial Laboratorios SA, Barcelona, Spain) SC every 12 hours during the first 28 days.

At 0, 30 and 180 days of treatment, dogs were clinically evaluated, and a variety of analytes were measured from blood (CBC, biochemical profile, and APPs including PON1, ferritin, CRP and albumin), urine and bone marrow samples.

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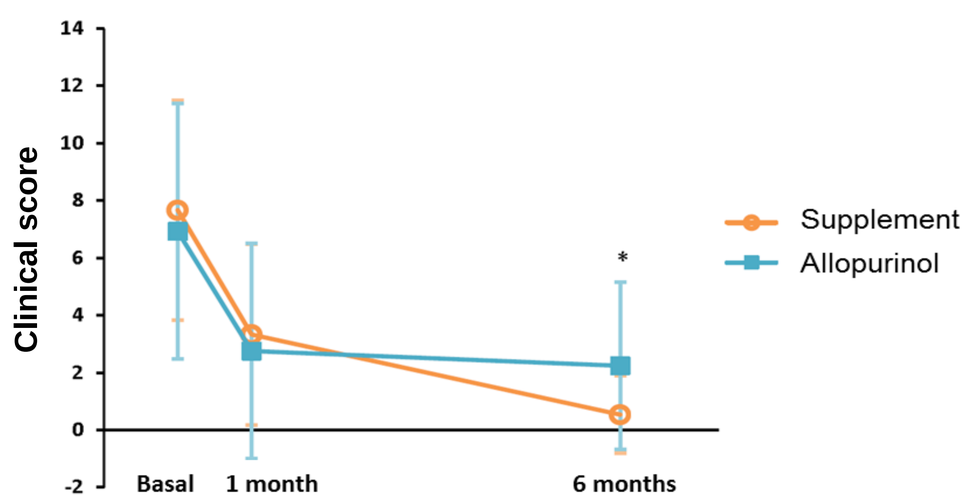
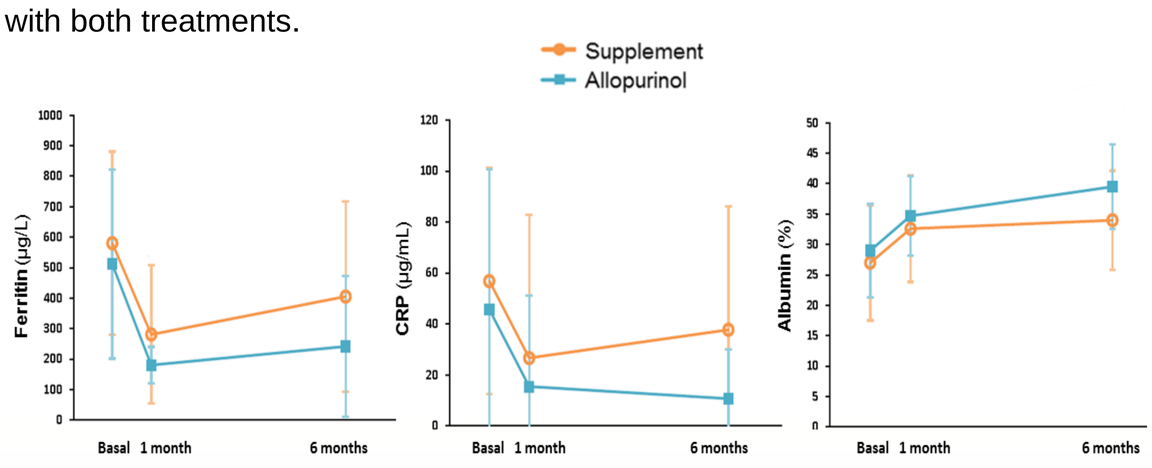
RESULTS

- A significantly **lower clinical score was observed with the supplement** at the end of the study (p=0.005)



- **A total of 12 patients (41%) from the allopurinol group developed xanthinuria while none did with the supplement (0%) (p = 0.000).**
- No significant differences were found between study groups in the distribution of study patients according to their IRIS staging of renal disease.
- Both treatments resulted in an increased CD4+/CD8+ ratio.
- Both treatments provoked a significant **increase in serum PON1 activity** after 30 and 180 days, compared to baseline (p<0.01), but there were no differences in PON1 evolution between treatments (p>0.05). **Serum ferritin and CRP levels decreased** along the treatment in both groups. The ferritin reduction was significant (p<0.05) for both treatments at 30 and 180 days, whereas the decrease in CRP was significant with both treatments after 30 days but only with allopurinol at the end of the treatment. Compared to baseline, both treatments significantly decreased total serum protein concentrations (p=0.000) after 30 and 180 days. **Albumin concentrations also increased** after 30 and 180 days with both treatments.



- Overall, both study compounds were well tolerated and no side effects related to these treatments were reported in any patient.

DISCUSSION AND CONCLUSIONS

The present study shows consistent evidence that the tested treatment of **nucleotides with AHCC, along with four weeks of MGA, leads to a clear improvement in clinical and clinicopathological parameters in CanL patients and a normalization of the acute phase response**. An association between clinical recovery and improvements in APPs levels has been reported previously,[3] as it also happened in both of the herein study groups. Normalization of ferritin and PON1 serum levels, as seen in this study, have already been described in prior studies in CanL treated patients.[4] Based on the findings of this study,

the efficacy of the tested supplement is at least equivalent to allopurinol and superior in some respects, showing a better safety profile and hence becoming a possible therapeutic alternative for treating CanL patients.

Further studies are warranted to assess its efficacy in a longer study period.

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