ImPROmune®

SUMMARY OF PUBLISHED CLINICAL STUDIES

EFFICACY OF IMPROMUNE® IN DOGS TREATED WITH CHEMOTHERAPY

Evangelio Sanchez E., Borda E., Tecles Vicente F., Martínez-Puig D., Ceron Madrigal J.J., Chetrit C. A dietary nucleotide formula improves the immune status of dogs receiving a chemotherapy treatment. 10th Congress of The European Society of Veterinary clinical Pathology, Barcelona, 2008.

Purpose of the study

To evaluate the efficacy of IMPROMUNE® on the immune system of immunodepressed dogs secondary to chemotherapy treatment with lomustine/cyclophosphamide.

Materials and methods

Twelve Beagle dogs were used. A commercial diet, supplemented with placebo (Control group) or with IMPROMUNE®, was administered over a period of 60 days.

Each group consisted of 6 animals.

On day 30 of the study, all animals were given a single dose of chemotherapy: lomustine (70 mg/m2) and cyclophosphamide (180 mg/m2).

Samples of peripheral blood were collected on days 30, 37, 44, 51 and 58, to assess:

- Acute phase proteins
- Humoral immunity
  - Antibody levels
- Cell immunity:
  - T and B lymphocyte subpopulations
  - Lymphocyte proliferation
- Liver, blood and biochemistry parameters

Results

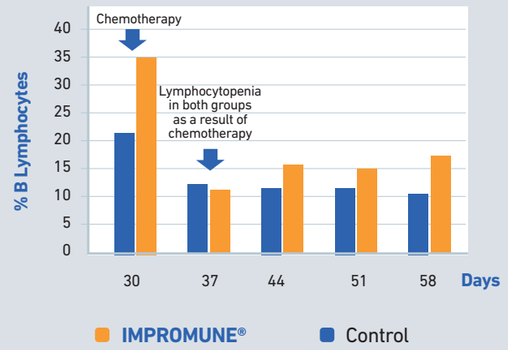


fig 1. *Distribution of blood B lymphocytes in the Control and Impromune® groups (Group B). The group taking the Impromune® supplement had a significantly higher percentage of B lymphocytes than the control group before administering chemotherapy. In the following days, lymphocyte levels decreased but the Impromune® group recovered to levels approaching the initial normality sooner than the other group.*

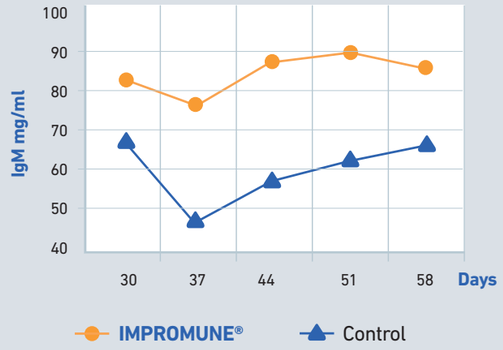


fig 2. *Blood IgM concentration in the Control and Impromune® groups. In the Control group, chemotherapy caused a drop in IgM, while in the Impromune® group the drop was less pronounced, and recovery was complete by the following week.*

The group taking the IMPROMUNE® supplement presented **higher percentages of B lymphocytes** than the Control group, and a **greater capacity for IgM antibody synthesis.**

Diarrhoea and depression were detected in 50% of the dogs in the Control group but **none in the IMPROMUNE®** group.

Conclusion

IMPROMUNE® improves the efficacy of humoral and cell immune responses by increasing B lymphocytes and IgM and therefore the immune status of the animal.

Administering IMPROMUNE® to dogs undergoing lomustine/cyclophosphamide chemotherapy treatment **decreases the frequency of side effects** such as vomiting and diarrhoea.

EFFICACY OF NUCLEOFORCE® IN PUPPIES

Romano, V., Martínez-Puig, D., Torre, C., Iraculis, N., Vilaseca, LI, Chetrit, C. Dietary nucleotides improve the immune status of puppies at weaning. Journal of Animal Physiology and Animal Nutrition Journal compilation, 2007.

Purpose of the study

To evaluate the efficacy of Nucleoforce® on immune parameters in recently weaned puppies.

Materials and methods

Twenty-one Beagle puppies weaned at 8 weeks of age, with no previous vaccinations, were included. There were 7 subjects in each of the 3 groups: Diet supplemented with 1,000 ppm nucleotides (Nucleoforce®), diet supplemented with 1,500 ppm nucleotides (Nucleoforce®), and a Control.

At 9 weeks of age they were vaccinated with a pentavalent vaccine (distemper virus, parvovirus, adenovirus, parainfluenza, leptospira).

Blood was collected on days 7, 14 and 28 of the study, to observe the efficacy of the product through plasma Ig concentration, post-vaccination seroconversion, post-vaccination levels of acute phase proteins and in vitro activation of T lymphocytes with concanavalin A.

Results

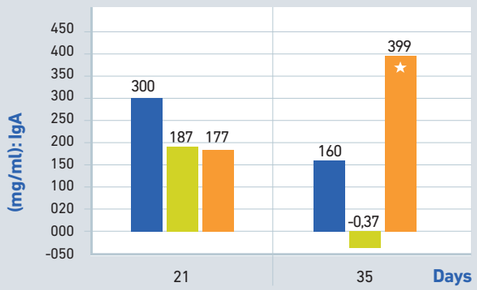


fig 1. *Non-specific IgA serum concentration. At 21 days, no differences were observed in the IgA levels of the three groups. However, at 35 days, the 1,500 ppm Nucleoforce® group showed a statistically significant higher concentration (*) than the Control group and the 1,000 ppm Nucleoforce® group.*

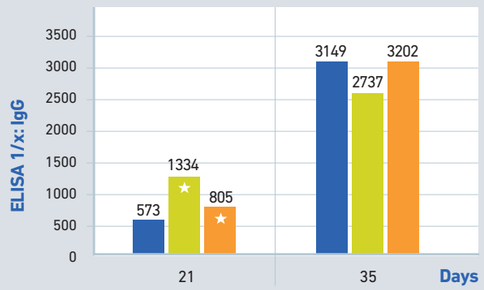
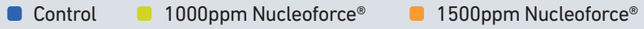


fig 1. *Parvovirus-specific IgG serum concentration. There was no difference in response to the vaccine at 35 days between the three treatment groups. It was found that the two groups supplemented with Nucleoforce® showed a higher antibody count at 21 days.*



Conclusion

- **Nucleoforce® improves humoral and cell immune responses** after vaccination in puppies.

EFFICACY OF NUCLEOTIDES IN DOGS TREATED WITH CHEMOTHERAPY

Burkhard, M.J., Sanchez, N., Torre, C., Couto, C.G. 2011. Dietary nucleotides in dogs undergoing anticancer chemotherapy. American Society for Veterinary Clinical Pathology (ASVPC) 46th and American College of Veterinary Pathologist (ACVP) 62nd. Abstract 29.

Purpose of the study

To evaluate the effects of nucleotides on haematology and immune function in dogs with cancer.

Materials and methods

Twenty-seven dogs undergoing chemotherapy treatment (11 with lymphoma and 16 with osteosarcoma) using the Wisconsin-Madison-19 and suramin/doxorubicin protocols, respectively, were selected. The subjects received nucleotides or placebo randomly. All dogs underwent physical, haematological and immunological examinations 8 weeks from the start of the study, for monitoring purposes.

Results

	Placebo group		Nucleotides Group	
Haematocrit	↓	52.2% --> 49.54%	↑	49% --> 49.9%
Platelets	↓	266.5 --> 239.5 µ/l	↑	278.7 --> 334.8 µ/l
White blood cells and neutrophils	↓*	4.8 µ/l	↓	9.6 --> 6.5 µ/l
Serum concentration levels of IgA and IgM	↓*	(P = 0,008)	↑*	(P = 0,025)
Percentage of CD3 lymphocytes	=	No changes in percentage	↑	50.2% --> 63.9%
Percentage of CD4 lymphocytes	=	No changes in percentage	↑	29.2% --> 37.6%

*Statistically significant

Conclusion

- Oral administration of nucleotides **improves leukopenia and neutropenia** caused by chemotherapy.
- **Nucleotides improve cell** (CD3 and CD4) **and humoral (IgA and IgM) immunity** in treated animals.

IMPROMUNE® FOR THE MANAGEMENT OF PATIENTS WITH CANINE LEISHMANIASIS: A TREATMENT FOCUSED ON IMPROVING IMMUNE RESPONSE AGAINST PARASITES

Segarra S, Miró G, Montoya A, Pardo-Marín L, Boqué N, Ferrer L, Cerón J. Randomized, allopurinol-controlled trial of the effects of dietary nucleotides and active hexose correlated compound in the treatment of canine leishmaniosis. Veterinary Parasitology 239 (2017) 50–56

Purpose of the study

Canine leishmaniasis caused by *Leishmania infantum* is a potentially fatal global zoonosis in humans and dogs (Solano-Gallego et al., 2011).

Among the recommended treatment protocols, there is good scientific evidence for a combination of subcutaneous meglumine antimoniate (MgA) and oral allopurinol (Noli and Saridomichelakis, 2014; Solano-Gallego et al., 2009). However, administering allopurinol may induce an increase in urinary xanthine levels, which could eventually lead to urolithiasis and renal mineralisation (Torres et al., 2016). In addition, parasite resistance to allopurinol has recently been reported in samples from dogs treated with allopurinol, who relapsed, which would implicate a public health problem (Yasur-Landau et al., 2016).

Therefore, new therapeutic options are required that can be administered effectively and safely in the long term for these patients.

Impromune® (Bioibérica SA, Barcelona) is an oral supplement based on nucleotides and AHCC *(active hexose correlated compound)* that modulates the immune response. Nucleotides positively influence lipid metabolism and immunity, and tissue growth, development and repair (Fontana et al., 2010; Gil, 2002). AHCC is used in humans for its ability to stimulate the immune system and enhance cell immune response (Ulbricht et al., 2013).

The aim of this multicentre, prospective, randomised, controlled study was to compare the effects of Impromune® with those of allopurinol in dogs with leishmaniasis.

Materials and methods

For this purpose, 69 dogs with clinical leishmaniasis were divided into two groups: an allopurinol group (positive control) (10 mg/kg allopurinol orally every 12 hours for 6 months) or an Impromune® group (17 mg/kg AHCC and 32 mg/kg nucleotides orally, once daily for 6 months). All dogs also received 50 mg/kg of MgA (Glucantime) subcutaneously every 12 hours for the first 28 days. At 0, 30 and 180 days of treatment, dogs were clinically assessed using a clinical scoring system (the greater the severity, the higher the score) and a range of analytes were measured using blood, urine and bone marrow samples.

Fontana, L., Martínez-Augustin, O., Gil, Á., 2010. Role of Dietary Nucleotides in Immunity. Funct. Food Rev. 2, 91–100.
Gil, A., 2002. Modulation of the immune response mediated by dietary nucleotides. Eur. J. Clin. Nutr. 56 Suppl 3, S1-4.
Noli, C., Saridomichelakis, M.N., 2014. An update on the diagnosis and treatment of canine leishmaniosis caused by Leishmania infantum (syn. L. chagasi). Vet. J. 202, 425–35.
Solano-Gallego, L., Koutinas, A., Miró, G., Cardoso, L., Pennisi, M.G., Ferrer, L., Bourdeau, P., Oliva, G., Baneth, G., 2009. Directions for the diagnosis, clinical staging, treatment and prevention of canine leishmaniosis. Vet. Parasitol. 165, 1–18.
Solano-Gallego, L., Miró, G., Koutinas, A., Cardoso, L., Pennisi, M.G., Ferrer, L., Bourdeau, P., Baneth, G., 2011. LeishVet guidelines for the practical management of canine leishmaniosis. Parasit. Vectors 4, 86–102.
Torres, M., Pastor, J., Roura, X., Tabar, M.D., Espada, Y., Font, A., Balasch, J., Planellas, M., 2016. Adverse urinary effects of allopurinol in dogs with leishmaniosis. J. Small Anim. Pract. 57, 299–304.
Ulbricht, C., Brigham, A., Bryan, J.K., Catapang, M., Chowdary, D., Costa, D., Culwell, S., D'Auria, D., Giese, N., Iovin, R., Isaac, R., Juturu, V., Liu, A., Mintzer, M., Rusie, E., Shaffer, M., Windsor, R.C., 2013. An evidence-based systematic review of active hexose correlated compound (AHCC) by the Natural Estándar Research Collaboration. J. Diet. Suppl. 10, 264–308.
Yasur-Landau, D., Jaffe, C.L., David, L., Baneth, G., 2016. Allopurinol Resistance in Leishmania infantum from Dogs with Disease Relapse. PLoS Negl. Trop. Dis. 10, e0004341.

Results

After 6 months, a significantly lower clinical score was observed with Impromune® ($p = 0.005$). In addition, 12 subjects (41%) in the allopurinol group developed xanthinuria, while none developed xanthinuria on Impromune® (0%) ($p = 0.000$).

On the other hand, no significant differences were found between groups with regard to the distribution of patients according to the IRIS (International Renal Interest Society) classification of chronic kidney disease. Both treatments resulted in a significant reduction of the parasite load measured by RT-QPCR, an increase in the CD4+/CD8+ ratio and a tendency towards normalisation of the electrophoretic patterns and acute phase protein levels. Apart from the urine changes already mentioned, no treatment-related adverse effects were observed in either group.

Conclusion

Although several treatment options are now available, the management of subjects with canine leishmaniasis remains complicated. Some of the most commonly used therapies, such as allopurinol, can lead to undesirable effects.

In this study in dogs with clinical leishmaniasis initially receiving MgA, administering Impromune® was clinically more effective than allopurinol after 6 months of treatment. Furthermore, unlike allopurinol, Impromune® did not stimulate the development of xanthine in urine.

These results suggest, therefore, that **Impromune® could be considered as a new alternative in the treatment of dogs with clinical leishmaniasis**. Based on what has been observed, its use would seem to be particularly indicated in those patients who have already shown undesirable effects associated with allopurinol, although its use in combination with other current therapies cannot be ruled out.

Each patient is different and, as in human medicine, in veterinary medicine there is an increasing tendency towards personalised, or rather individualised medicine; a treatment tailored to each patient. Therefore, having an additional tool can be particularly useful in designing the optimal treatment protocol for each individual case and can also increase the chances of success.

Results

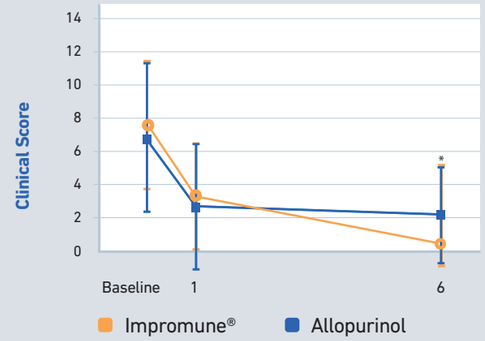
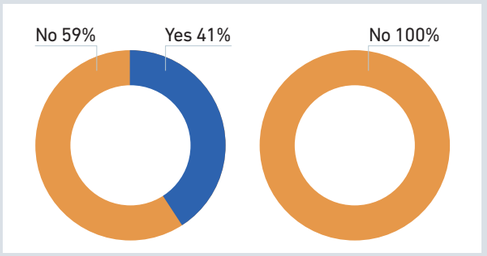


fig 1. *Clinical score (mean and standard deviation) at baseline and at 1 and 6 months, in each of the treatment groups.*



Development of xanthinuria in the **Allopurinol** group

Development of xanthinuria in the **Impromune®** group

fig 2. *Percentage of cases that developed xanthine in urine during the 6 months of the study, in each of the treatment groups.*

IMPROMUNE® REDUCES LEISHMANIA PROGRESSION IN CLINICALLY HEALTHY INFECTED DOGS

Segarra S, Miró G, Montoya A, Pardo-Marín L, Teichenné J, Ferrer L, Cerón J. Prevention of disease progression in Leishmania infantum-infected dogs with dietary nucleotides and active hexose correlated compound Parasites & Vectors 2018 11:103

Purpose of the study

Canine leishmaniasis (CanL) caused by *Leishmania infantum* is a major global zoonosis that is potentially fatal to humans and dogs[1]. **Clinically healthy infected dogs** have no clinical signs or clinicopathological abnormalities despite confirmed infection.
Although these patients are not sick and some of them do not develop a clinical disease, they pose a therapeutic challenge. They may pose a risk of parasite transmission, especially if they progress to clinical leishmaniasis, and should be managed appropriately[2]. However, the widespread use of leishmaniasis drugs is not recommended in these dogs with subclinical infection to avoid the development of resistance[1].

Orally administered **nucleotides** modulate immune response, positively influencing lipid metabolism, immunity and tissue growth, development and repair[3]. Active hexose correlated compound **(AHCC)**, an alpha-glucan-rich compound extracted from the mycelia of shiitake mushrooms *(Lentinula edodes)*, is used in humans for its ability to stimulate the immune system[4].

In a recent randomised, controlled trial of allopurinol in dogs with clinical leishmaniasis who received an initial 28-day course of methylglucamine antimoniate (MgA), it was shown that oral treatment with nucleotides plus AHCC has similar efficacy to allopurinol, achieving better clinical results after six months and without producing xanthinuria[5]**. The aim of the present study was to evaluate the long-term effects of nucleotides plus AHCC in patients with clinical CanL and to determine whether this intervention could have a preventive effect that would protect them from becoming ill and starting to show clinical signs.**

Materials and methods

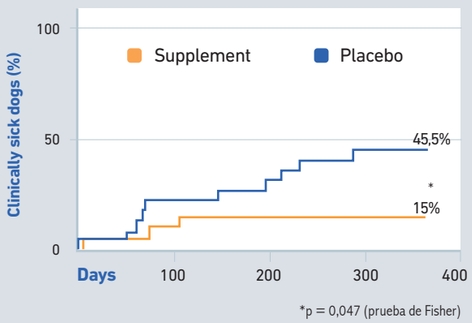
Forty-six privately-owned dogs with naturally occurring *L. infantum* infection were included in this randomised, multicentre, double-blind, placebo-controlled trial.

The dogs included were randomly assigned into **two groups** and received a **single daily oral administration of placebo or a dietary supplement (IMPROMUNE®**, Bioiberica S.A.U., Barcelona, Spain; 32 mg/kg nucleotides plus 17 mg/kg AHCC) for 365 days. **Disease progression (change from being clinically healthy to being sick) was monitored in each group during the study and the result was used to assess the preventive effect of the supplement.** At 0, 60, 180 and 365 days of treatment, dogs were assessed using a validated objective clinical score5 and various analytes were measured in blood, urine (UPC, density and abnormalities) and bone marrow (swab, qualitative PCR and RT-PCR) samples. Dogs with disease progression were excluded from the study and for the final data analyses only their data up to the last visit when they showed no progression to becoming sick, were used.

Results

Initially, the two groups were the same in terms of demographic and baseline characteristics of the subjects. The breeds included in the supplement/placebo groups were: boxer (2/3), German shepherd (2/2), American Staffordshire terrier (2/0), Rottweiler (1/1), Siberian husky (1/0), Doberman (2/2), Breton spaniel (1/0), English bulldog (1/0), Labrador (1/0), mixed breed (6/7), dogue de Bordeaux (1/0), cocker spaniel (1/1), Mallorcan ratter (0/1), bullmastiff (0/1), pit bull (0/2), Andalusian bodeguero/ratter (0/1), French bulldog (0/1), English setter (0/1), St Bernard (0/1), beagle (0/1). At baseline there were no significant differences ($p > 0.05$) between the groups in any of the parameters studied. One dog was excluded from the supplement group and three from the placebo group for reasons unrelated to disease progression.

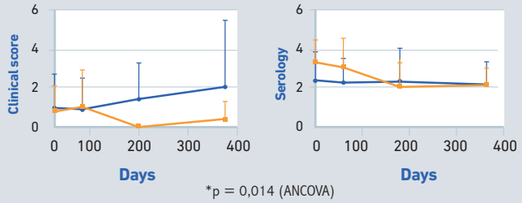
• *During the study,* **3 dogs showed PROGRESSION OF DISEASE in the supplement group compared to 10 in the placebo group (15% vs. 45.5%; p = 0.047).**



*p = 0,047 (prueba de Fisher)

• *Due to the clinical worsening of the dogs in the placebo group,* **a lower CLINICAL SCORE** *was observed after 180 days* **with the supplement** *(p = 0.014).*

• **ELISA serology decreased significantly ($p < 0.01$)** *compared to baseline only in the* **supplement group after 60, 180 and 365 days of treatment.**



*p = 0,014 (ANCOVA)

Supplement Placebo

• *No significant differences were observed between groups or over time in CD4 and CD8 blood levels, CD4/CD8 ratio, serum proteinograms, parasite load, complete blood count and biochemistry, IRIS staging of chronic kidney disease, temperature or body weight.*

• **Both the supplement and placebo were well tolerated,** *and no side effects related to these compounds were reported in any patient.*

Conclusion

Oral administration of **IMPROMUNE®** for 365 days to clinically healthy, *L. infantum* infected dogs is safe and achieves a significant reduction in the rate of disease progression and in ELISA serological counts of Leishmania infection antibodies. These findings point to a possible preventive effect of **IMPROMUNE®** in clinically healthy CanL dogs, preventing them from becoming sick patients.

1. Solano-Gallego L, Miró G, Koutinas A, et al. LeishVet guidelines for the practical management of canine leishmaniosis. Parasit Vectors. 2011;4:86-102.
2. Miró G, Petersen C, Cardoso L, et al. Novel areas for prevention and control of Canine Leishmaniosis. Trends Parasitol. 2017;33:718–30.
3. Gil A. Modulation of the immune response mediated by dietary nucleotides. Eur J Clin Nutr. 2002;56 Suppl 3:S1-4.
4. Ulbricht C, Brigham A, Bryan JK, et al. An evidence-based systematic review of active hexose correlated compound (AHCC) by the Natural Standard Research Collaboration. J Diet Suppl. 2013;10(3):264-308.
5. Segarra S, Miró G, Montoya A, et al. Randomized, allopurinol-controlled trial of the effects of dietary nucleotides and active hexose correlated compound in the treatment of canine leishmaniosis. Vet Parasitol. 2017;239:50-56.

ACTS ON BOTH INNATE AND ADAPTIVE RESPONSES

Innate response

+FAST
- SPECIFIC

TISSUE DAMAGE

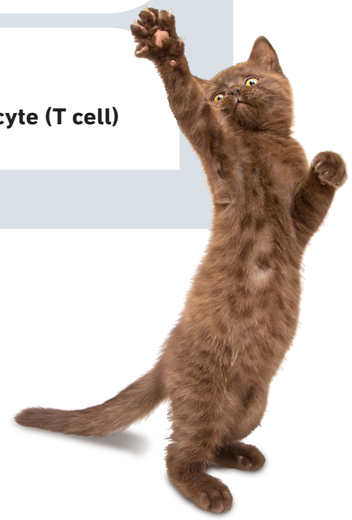
- **MACROPHAGES** • Fibroblasts • Endothelial cells
- **Leukocytes**
- **NK CELLS**

CYTOKINES

ACUTE PHASE PROTEINS

AHCC
- **NK cell activity**
- **Macrophage proliferation**

NUCLEOFORCE®
- **Phagocytic activity**
- **Macrophage T Lymphocyte (T cell) Interaction**



PRESENTER CELL ANTIGEN

Adaptive Response

- FAST
+ SPECIFIC

LTh0 Undifferentiated

IFN-γ
TFN-γ
IL-12

Th1 CELL IMMUNITY

Developed for intracellular agents and tumour cells

Activation of:
- NK cells
- Cytotoxic T cells

B-lymphocytes (low antibody production, mainly IgG2)

Th2 HUMORAL IMMUNITY

Developed for extracellular parasites and bacteria

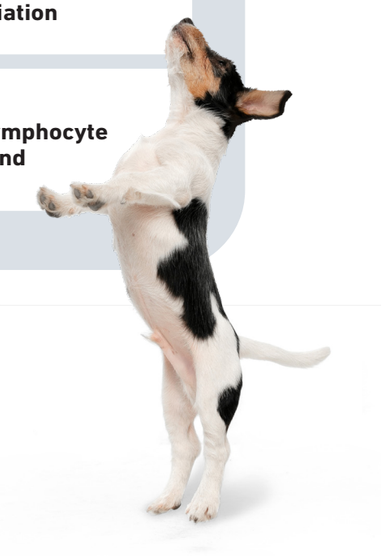
Activation of:
- B-lymphocytes (high production of IgE, IgA, IgG1 antibodies)

Deactivation of:
- NK cells
- Cytotoxic T cell

IL-4
IL-5
IL-13

AHCC
- **Promotes T (T cell) to Th1 Lymphocyte differentiation**

NUCLEOFORCE®
- **Promotes mature T-lymphocyte (T cell) proliferation and activation**



YOUR REFERENCE PRODUCT FOR THE IMMUNE SYSTEM[1]

NUCLEOFORCE® · AHCC

Presentation:
In containers of 20 or 40 tablets,
pouches of 200 tablets, and 30 ml of palatable paste

Each clinical package contains
20 blister packs of 10 tablets and 12 dispenser sachets.

Your reference product for the immune system[1]

IMPROMUNE® contributes to the correct development of the IMMUNE system as it acts SYNERGICALLY[2]:

- Both innate and adaptive responses
- Both cell and humoral responses

IMPROMUNE® provides all the essential ingredients that contribute to the correct development of the immune system in various situations [3,4,5]:

- Infections
- Medical immunosuppression
- Physiological stress
- Pets likely to suffer from any of the above situations

UNIQUE combination:

- Specific nucleotide profile.
- Patented formula, synergic effect: Nucleotides + AHCC
- Excellent safety profile

PALATABLES presentations

Ingredients:

Each palatable tablet contains:

- Nucleoforce® Yeast extract from *S. cerevisiae* with Nucleotides).....585 mg
- AHCC (*Lentinus edodes*, mushroom).....315 mg
- Magnesium stearate

Each ml of paste contains:

- Nucleoforce® (Yeast extract from *S.cerevisiae* with Nucleotides).....14,62%
- AHCC (*Lentinus edodes*, mushroom).....7,88%

Storage conditions:

Store in a cool, dry place.

Description:

IMPROMUNE® is a food supplement recommended for cats and dogs to strengthen their immune systems and to boost any innate or adaptive response[2].

Nucleoforce® is a specific Nucleotide formulation developed after studying the specific needs of different species of mammals. Nucleotides are semi-essential immunonutrients which, when taken orally, contribute to the correct development of the immune system. Nucleoforce® regulates phagocytic activity of macrophages as well as the interaction between these T lymphocytes. It also regulates the maturation, proliferation and activity of T lymphocytes. AHCC is an extract from the mycelium of Lentinus edodes, which regulates NK cell activity, as well as macrophage proliferation and differentiation of T to Th1 lymphocytes. Different studies have shown that in combination, Nucleoforce® and AHCC have a synergic effect that acts on innate and adaptive responses. This SYNERGIC EFFECT has been patented.

Recommended use[3,4,5]:

Recommended for use in immunocompromised dogs and cats such as:

	Use of IMPROMUNE®
Infectious processes	
• Bacteria (e.g. pyogenic infection), viruses (e.g. calicivirus, papillomatosis), Parasites (ex: demodicosis, giardia), fungal infections (ex: ringworm, Malassezia)	Minimum duration 15-20 days
• Leishmania	Minimum duration 6 months
Acquired immunosuppression	
• Administration of immunosuppressants (e.g. glucocorticoids)	Before and after treatment, 15-20 days
• Chemotherapy	Before, during, and after chemotherapy, 15-20 days
• Elderly animals, immunosenescence	Minimum duration 30 days
• Stress	
Postoperative/post-multiple trauma conditions (e.g. Caesarean, pyometra)	Minimum duration 30 days
Reproduction (e.g. lactation, large litters)	Minimum duration 30 days
Puppies (e.g. around vaccine period)	Minimum duration 30 days
Malnutrition	Minimum duration 30 days

Posology and method of administration:

IMPROMUNE® is easy to administer because it is very palatable. The design of the tablets means they can be split to make it easier for smaller animals to ingest.

Depending on the situation, and the animal's progress, the administration period can be extended at the veterinarian's discretion.

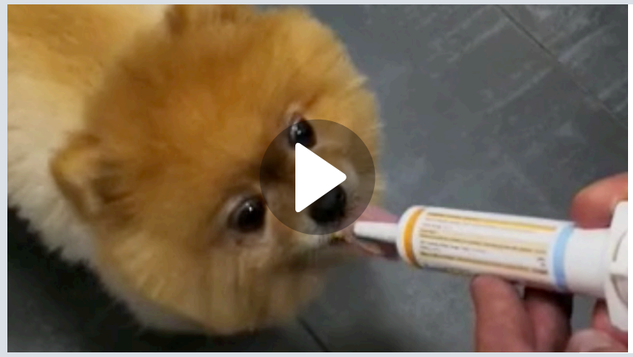
Weight of animal	Tablets per day	Daily dose
< 10 kg	½	2 ml
11 - 25 kg	1	4 ml
> 26 kg	2	8 ml

IMPROMUNE® does not cause adverse effects

1. PetSellout Report March 2020.
2. Evangelio Sanchez E., Borda E., Tecles Vicente F., Martínez-Puig D., Ceron Madrigal J.I., Chetrit C. 10th Congress of The European Society of Veterinary clinical Pathology, Barcelona, 2008.
3. Romano V, Martínez-Puig D, Torre C, Iraculis N, Vilaseca LI, Chetrit C. Journal of Animal Physiology and Animal Nutrition Journal compilation, 2007
4. Segarra S, Miró G, Montoya A, et al. Randomized, allopurinol-controlled trial of the effects of dietary nucleotides and active hexose correlated compound in the treatment of canine leishmaniosis. Vet Parasitol. 2017;239:50-56.

VIDEOS

Reaction of small dog on Impromune® palatable paste



www.vimeo.com/498297602

Introduction to Impromune® palatable paste

To view the video you need to enter the password IMMUNITY

www.vimeo.com/499232782

ImPROmune®

YOUR REFERENCE PRODUCT FOR THE IMMUNE SYSTEM[1]

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