Pet Inmunonutrition Guide

NUCLEOFORCE®
Pets

Bioiberica

Postnatal stage

The neonatal period in canines occurs during the first 3 weeks of life. This stage is considered critical since there is a high risk of mortality, mainly due to septicemia, in the first 3 days of life (5, 6). **See Figure 1.**

Between 10-30% of puppies die during birth or within 21 days of life

A puppy's survival during this period depends on the ability of the newborn to adapt to life outside the uterus. Puppies face 2 main challenges (7):

- Intestinal system development
- Immune system development

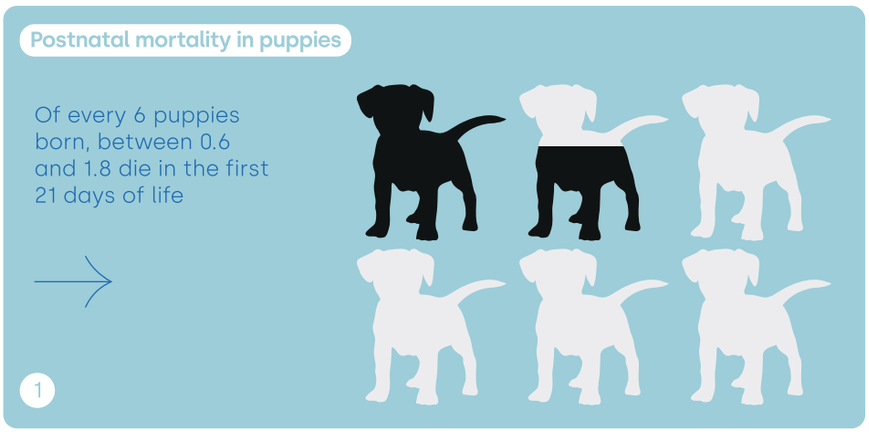
From a nutritional point of view, the flow of nutrients from the placenta discontinues at delivery and the supply of nutrients depends on the ability of the newborn to latch on, suckle, and assimilate colostrum and subsequently breast milk.

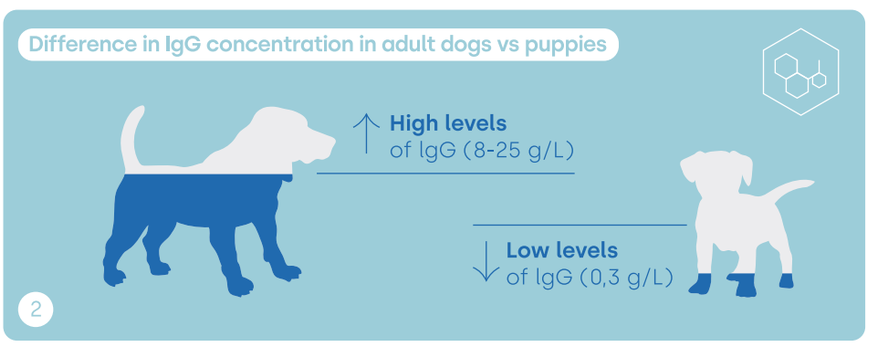
As regards developing immunity, the situation of newborn puppies is critical. This is due to the type of endotheliochorial placentation in canines that limits immune transfer from the mother to the foetus. Therefore, newborns are considered almost agammaglobulinemic since they present very low concentrations of immunoglobulins.

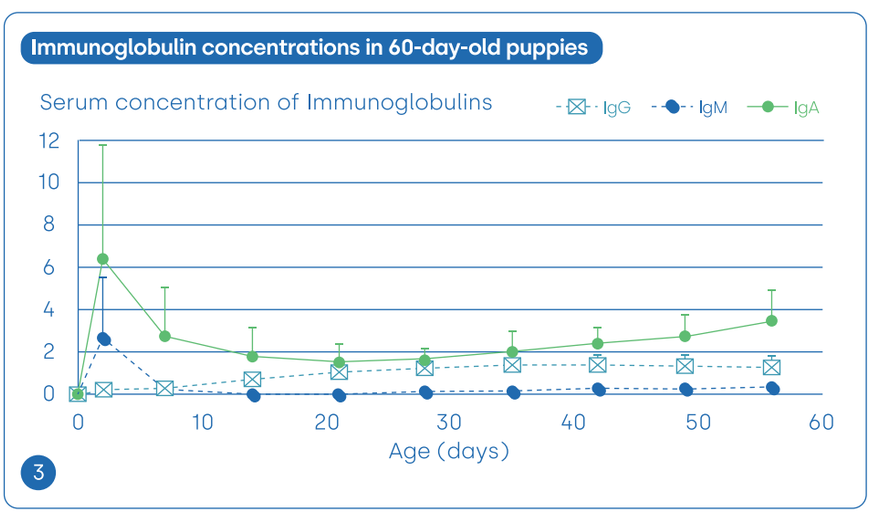
At birth, serum levels of IgG (the main Ig transferred through colostrum) are 0.3 g/L, while an adult dog has concentrations between 8-25 g/L (8.9). See Figures 2 and 3.

Considering the challenges mentioned above, **the importance of colostrum ingestion by puppies is evident, both nutritionally and immunologically, as it is both a highly digestible source of nutrition and the main pathway for maternal antibodies to reach the puppy.** Most antibodies (97%) are transferred through colostrum (the remaining 3% are transferred through the placenta) (10).

Absorption of immunoglobulins from colostrum occurs during the first hours of the puppy's life, this absorption decreases until the intestine becomes less permeable to large molecules such as antibodies (immunoglobulins), this occurs between 16-24 hours after birth (7).





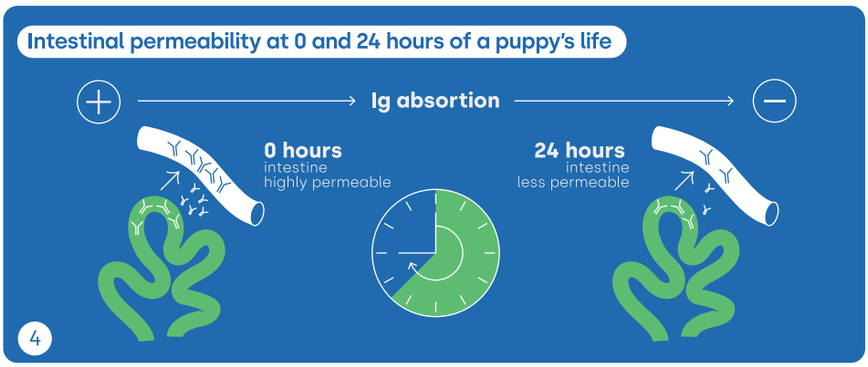


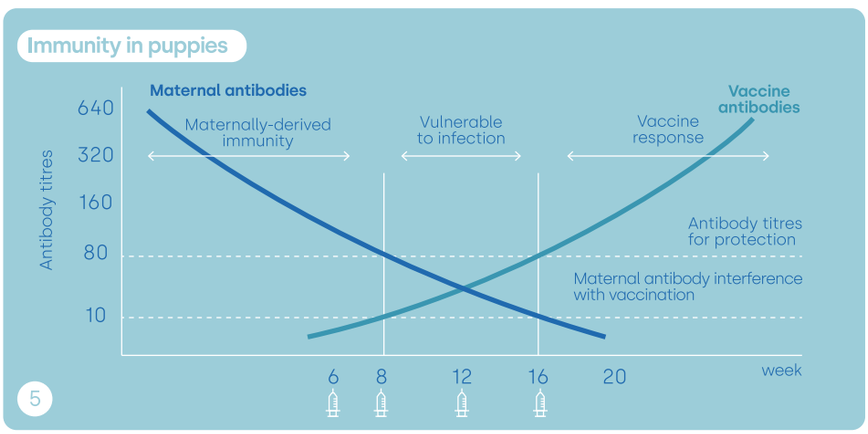
See Figure 5. These immunoglobulins transferred by the mother will progressively degrade, so that there will come a point at which the serum concentration of antibodies in the puppy will begin to decline, and the puppy's protection will be reduced. Many of these antibodies may have an inhibitory effect on the effectiveness of the vaccines administered to puppies (10). This is why the main vaccines are administered over several stages. See Figure 5.

This situation creates a period known as the **immune gap, or window of vulnerability, which runs from 3 to 4 weeks of age until the puppy is 12 to 14 weeks old.** During this period dogs are more susceptible to infection.

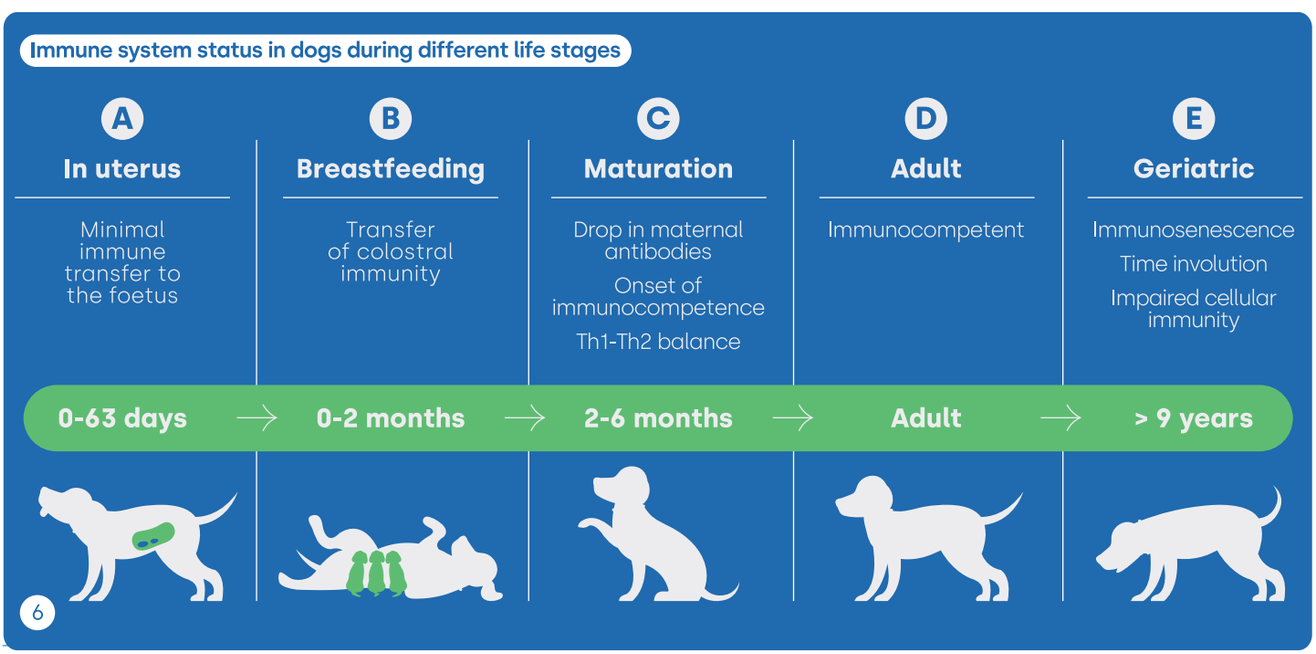
Adult stage

As can be seen in **figure 6**, once the dogs reach adulthood they will be considered immunocompetent, so they will be able to face various microbiological challenges properly.





The immune system, like any other system, is constantly adapting and remodeling, so it needs adequate nutrition to function.



Geriatric stage

When pets reach the geriatric stage of life, the structure and functionality of their immunocompetent state, established through microbial challenges or vaccination, will begin to decline. This may compromise the animal's immune response to antigenic challenges such as infections or vaccines. During this period, as well as during the first stage of life, immunity is key, therefore **strategies that contribute to improving immunity in both young and senior animals should be implemented.** One of these strategies is known as immunonutrition.

Immunonutrition is an emerging field focused on preparing the immune system through supplementation with specific nutrients. This usually focuses on improving intestinal health and immune response (11).

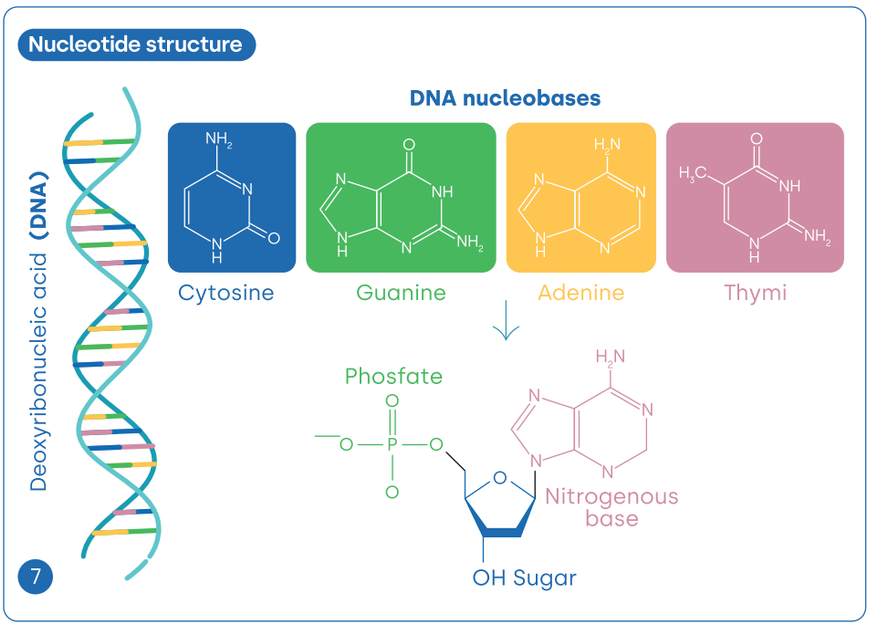
The concept of immunonutrition in pets is little studied, but can potentially be of great importance to improve the immune system in puppies and geriatric animals, in addition to favouring the animal's response to other challenges such as infectious diseases or states of immunosuppression, among others. There are several nutrients that can be used as immunonutrients and this guide focuses on **nucleotides.**

Nucleotides, a functional microingredient.

Nucleotides are low molecular weight bioactive compounds that play an essential role in most biochemical pathways and biological functions (metabolic energy, cell signaling, enzyme cofactors, etc.) (1, 2). In addition, they are the structural units that constitute the nucleic acid chains containing genetic information (3). This is especially important for the coding of proteins, biomolecules and cellular compounds. **See Figure 7.**

Nucleotides and nucleic acids are constantly forming and degrading. They can be synthesized endogenously by the organism. However, their **synthesis is a process that involves a high energetic cost to the animal.** In addition to this, **the intestinal and immune systems** have a limited capacity for nucleotide synthesis (3).

During situations of physiological stress, there is a high rate of cell replication, therefore the demand for nucleotides to form genetic material will be very high and endogenous synthesis will not be able to cover this demand (2).



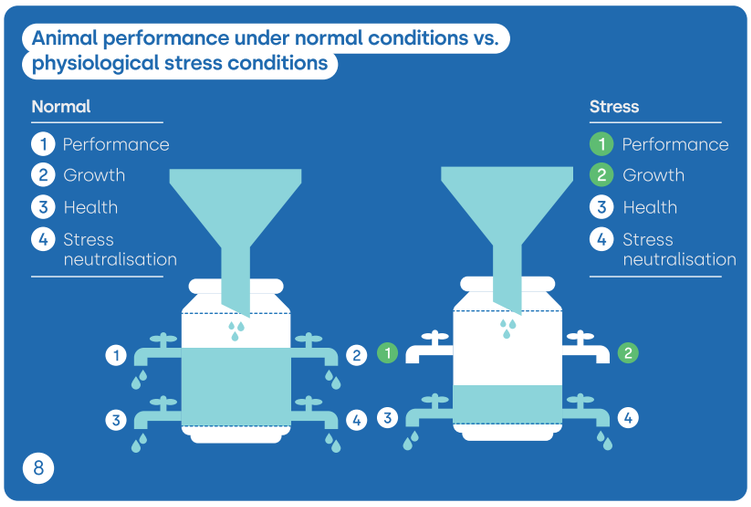
This is why nucleotides are considered conditionally essential biomolecules.

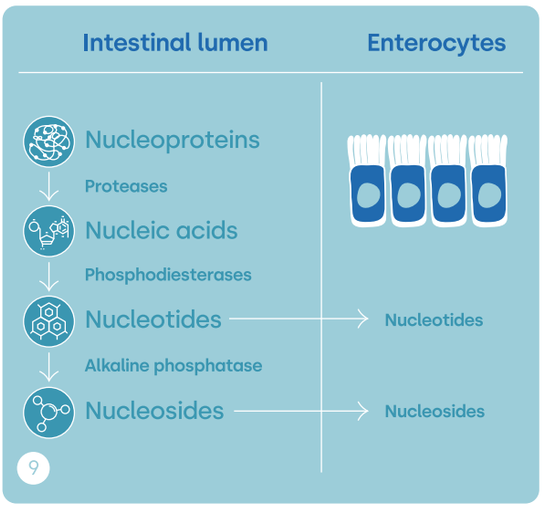
In pets, supplementing with nucleotides is important at many stages of life because **nucleotides help the animal to cope with the high demand caused by challenges,** such as growth in puppies, maternal gestation, infections or immunosuppression (30, 31). **See Figure 8.**

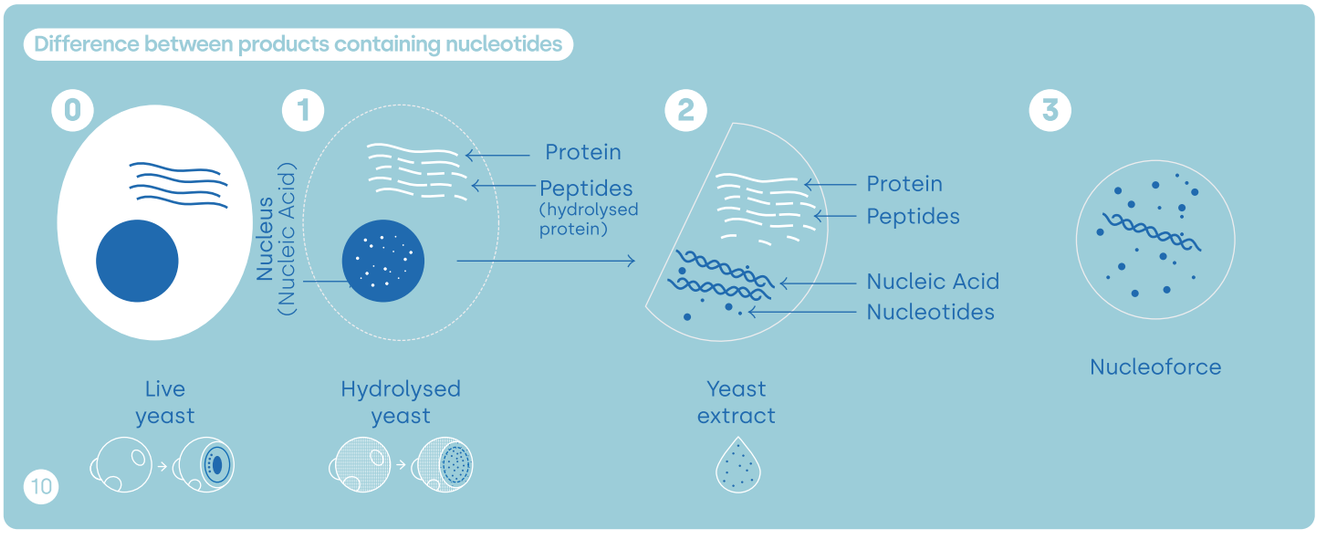
Differences between nucleotide-containing products.

There is a wide range of products on the market containing nucleotides, however, **it is important to differentiate between products containing a high percentage of free nucleotides (highly bioavailable) and those containing nucleic acids expressed as total nucleotides (poor bioavailability).** This is important because products with a high concentration of free nucleotides will have a very low inclusion dose, as opposed to products with a low content of free nucleotides, which will require a

higher inclusion rate. **See Figure 9.** To understand these differences, the manufacturing process of products containing nucleotides, mostly derived from yeast, is explained below. **See Figure 10.**







Nucleotide-containing products are usually derived from yeasts such as *Saccharomyces cerevisiae*. Yeast is hydrolysed, causing cellular components such as proteins or nucleic acids to partially fragment. In the case of Nucleoforce, this hydrolysis is of the enzymatic type, as this type of hydrolysis avoids yeast cellular components deteriorating. At this point the resulting product is a hydrolysed yeast, which has low nucleic acid content (nucleotides with poor bioavailability). Next, the insoluble fraction (cell walls) is separated from the soluble fraction or cytoplasm (yeast extract). The soluble fraction contains partially hydrolysed proteins and partially hydrolysed nucleic acids. The resulting product is known as yeast extract, which has a somewhat higher concentration of nucleic acids than hydrolysed yeast and a low concentration of free nucleotides.

For the manufacture of Nucleoforce, the soluble fraction, or yeast cytoplasm, is subjected to ultrafiltration, thus concentrating the nucleic acids, which will then be subjected to a second enzymatic hydrolysis, obtaining a product with a high concentration of free nucleotides, easily assimilated by pets.

Nucleotide transfer from colostrum and breast milk.

Human breast milk is known to contain nucleotides, which is why in 1991 the European Commission's Scientific Committee on Food approved nucleotide supplementation in infant milk (12).

In the canine species, as well as in other species, there is a transfer of nucleotides to puppies through colostrum and mother's milk. The nucleotides with the highest concentration in canine milk are CMP and UMP (pyrimidines). Furthermore, in canine species, unlike other species studied (human, bovine, ovine and porcine), the concentration of nucleotides increases during the course of lactation. **See Figure 11**. This may be associated with the rapid growth rate in puppies, as birth weight doubles 9 days after birth, suggesting the importance of nucleotides for optimal development (13).

In general, ingredients with high protein concentration, as well as those containing cellular elements, are potentially sources of nucleotides in their nucleoprotein form (low bioavailability) (29). Generally speaking, ingredients containing cellular elements are potential sources of nucleotides in their nucleoprotein form.

There is a problem: the bioavailability of nucleic acids decreases considerably by up to 30% when bound to proteins. In addition, these poorly bioavailable nucleic acids must be broken down into nucleotides and nucleosides to be absorbed at the intestinal level (4). This hinders their absorption, especially in young animals, which lack these enzymes. See Figure 9.

Changes in nucleotide concentration in breast milk during lactation

	Breastfeeding day					
Nutrient	**1**	**4**	**8**	**12**	**16**	**20**
Total milk solids [%]	24.2^{a}	19.6^{b}	20.5^{b}	19.4^{b}	21^{ab}	22.4^{ab}
Crude protein [%]	13.1^{a}	7.4^{b}	7.5^{b}	6.6^{b}	6.6^{b}	7.7^{b}
Nucleotide 5-monophosphate[µmol/100 ml]						
AMP	0,3	0,7	0,2	0,4	0,6	2.6
CMP	0.4^{a}	13.2^{ab}	32.5^{ab}	43.9^{ab}	49^{ab}	50.9^{b}
UMP	2.6^{a}	8.2^{a}	14.4^{ab}	28.2^{bc}	31.9^{c}	39.2^{c}
Total nucleotides	3.3^{a}	22.2^{a}	47.1^{ac}	71.9^{bc}	81.5^{bc}	95.7^{bc}

Different superscript letters indicate statistically significant differences (p<0.05).

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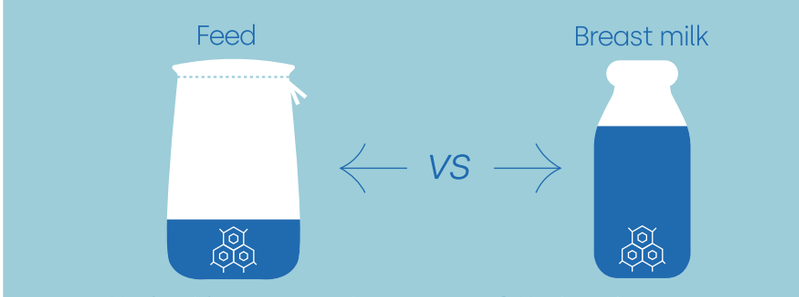
It is important that puppies continue to receive nucleotides after weaning. However, they switch from mother's milk (a source of high-content species-specific nucleotides) to eating feed made from ingredients that usually have a low concentration of nucleotides (30). See Figure 12.

Nucleotide concentration in feed vs. breast milk



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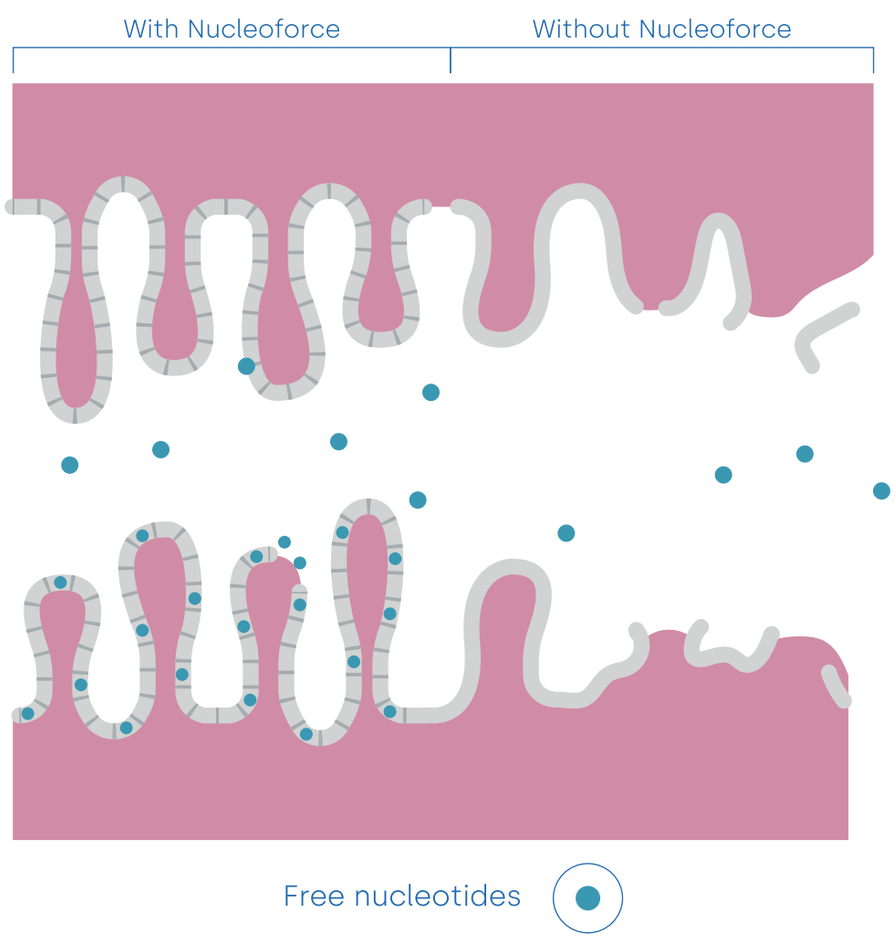
Importance of nucleotides to intestinal health.

At the intestinal level, exogenous nucleotides are important due to the rapid division of mucosal cells. In addition, these cells have very limited endogenous nucleotide synthesis (30, 31).

Considering the above, nucleotides, as precursors of nucleic acids, play an important role in cell replication, but also activate expression pathways that contribute to the correct maintenance of intestinal integrity. An example of this is that, after supplementing with nucleotides, messenger RNA levels are increased and this boosts the production of tight junction proteins, which are important for proper intestinal integrity (30, 31).

The high replication of intestinal cells is reflected in the greater length of intestinal villi and consequently a greater absorption area. This, together with the improved integrity of the intestinal barrier, will favour the absorption of nutrients by the pet, improve resistance to enteric pathogens, which will lead to a reduction in the incidence of diarrhoea (29, 30, 31). See Figure 13.

Representation of the effect of Nucleoforce Pets on intestinal integrity



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Role of nucleotides in vaccine response in puppies.

Bioiberica has studied the benefits on immunity in puppies after supplementing their diet with **Nucleoforce** at a ratio similar to that found in canine breast milk. The objective of the test was to analyse serum immunoglobulin levels to assess humoral immunity and canine parvovirus antibody concentration to measure the immune response 14 days post-vaccination (14).

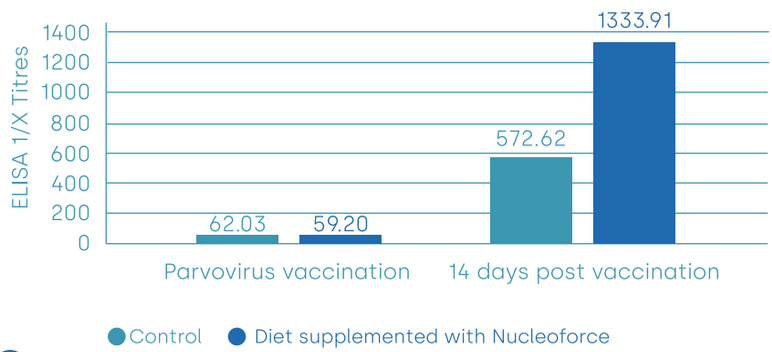
Humoral response in puppies supplemented with **Nucleoforce** in a ratio similar to that of canine breast milk was better compared to the control group (no nucleotides). This is due to the higher concentration of non-specific immunoglobulins (IgA, IgG and IgM) in the group that received **Nucleoforce** (14).

The group that received Nucleoforce had a better response to vaccines as the titres for antibodies offering protection against canine parvovirus were higher compared to the control group. See Figure 14.

According to in-vitro and in-vivo mouse studies, the explanation for the increase in antibody production is the fact that supplementation with **Nucleoforce** allows these to be incorporated into the nucleotide pool of the immune system and can act at the level of the T-helper cells during antigenic presentation and consequently improve T-cell response to antigenic stimuli by increasing the production of immunoglobulins (15). **See Figure 15.**

Effect of Nucleoforce Pets on vaccine response

Specific response Increased serum antibodies against Parvovirus at 14 days post-vaccination



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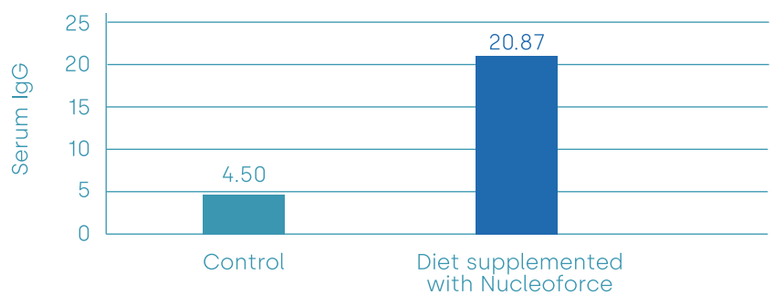
Effect of Nucleoforce Pets on vaccine response

Nonspecific response Serum IgG difference at 14 days post vaccination



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Role of Nucleoforce in the response to canine leishmaniasis.

Leishmaniasis is an emerging, uncontrolled zoonotic disease caused by a parasite, *Leishmania infantum*, which has dogs as its main reservoir host. This disease can be transmitted to humans becoming a zoonotic disease of significance to public health. There are effective pharmacological treatments against canine leishmaniasis such as pentavalent antimonials, allopurinol and miltefosine. However, these do not contribute to the total elimination of the parasite. Furthermore, their use is associated with side effects and antimicrobial resistance in both dogs and humans (16).

Dogs infected with *Leishmania* may develop clinical disease, but may also remain in a subclinical phase (clinically infected but healthy). Asymptomatic dogs may begin to develop signs, requiring monitoring, especially in endemic areas (16). Bioiberica has done several studies assessing the effectiveness of nucleotide supplementation in dogs infected with *Leishmania infantum* (17, 18).

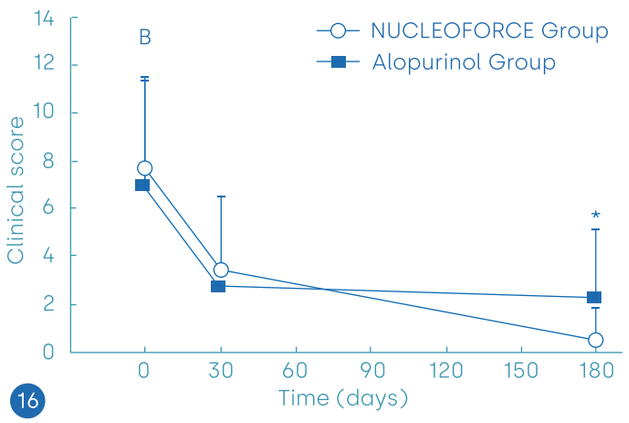
In the first study, dogs that tested positive for Leishmania using ELISA, PCR and cytology were divided into 2 groups:

- N-methylglucamine antimoniate (MGA) combined with allopurinol (10 mg/kg PO BID). (Conventional treatment against canine leishmaniosis).

- MGA + Impromune (Nucleoforce + Immunactive)

MGA was administered at 50 mg/kg SC BID for the first 28 days in both groups and followed up for 180 days. **One of the limitations of conventional treatment is the adverse effects caused by allopurinol,** such as the high urinary concentration of xanthine and hypoxanthine, which can lead to nephrolithiasis and urolithiasis (20).

Clinical score changes in dogs with *leishmaniasis* treated with Nucleoforce + Immunactive (AHCC) or with Allopurinol for 180 days



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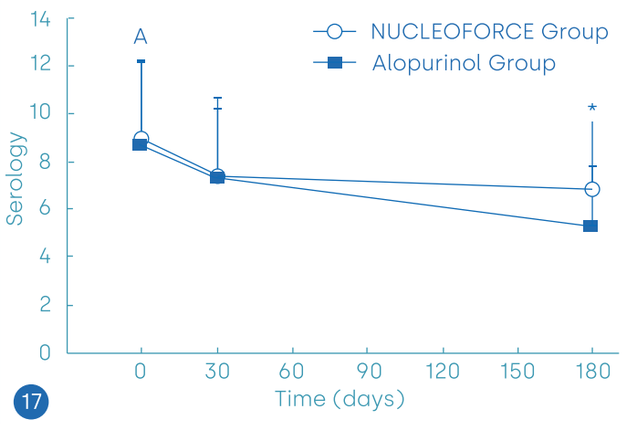
Changes in antibody titres in dogs with *Leishmaniasis* treated with Nucleoforce + Immunactive (AHCC) or with Allopurinol for 180 days



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It was observed that the group receiving the Nucleoforce + Immunactive supplement for between 60 and 180 days showed differences in the clinical score compared to the group that received allopurinol. In addition, at the end of the study it was observed that there was a significantly higher proportion of dogs with a clinical score of less than 5 in the group that received Nucleoforce compared to the group that received allopurinol (96% vs 72% P=0.031) (20). See Figure 16. The Nucleoforce group had a significantly higher concentration of antibodies compared to the allopurinol group. See Figure 17.

In the **second study,** clinically healthy, infected dogs that had not received medical treatment in the 2 months prior to the start of the study and had not received vaccination against canine leishmaniasis were studied. The dogs were divided into 2 groups (17).

- Placebo treatment
- Treatment with Nucleoforce + AHCC

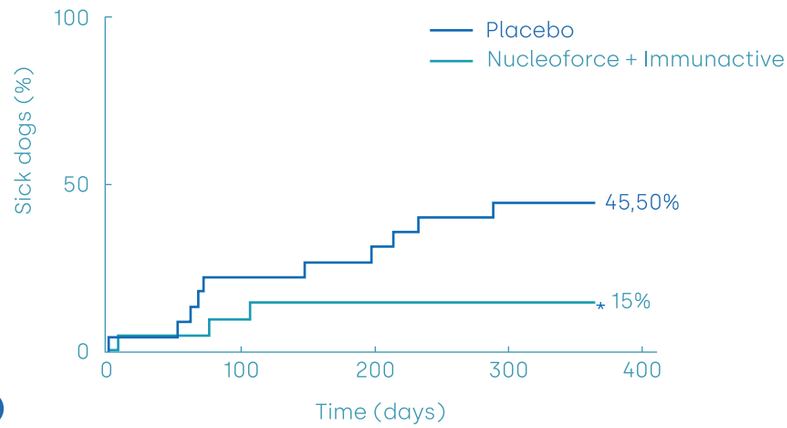
The treatment lasted 365 days. The changes related to disease progression can be seen in the following image. Over the course of the study **only 3 dogs out of 20 developed clinical signs in the Nucleoforce group,** whereas in the placebo group 10 of 22 dogs developed clinical signs (15% vs. 45.50% P= 0.047) (17). **See Figure 18.**

The reason why nucleotides contribute to improving the response against *Leishmania* is described in a study by Segarra et al. (17), in which macrophages and lymphocytes from mice stimulated with *L. infantum* antigen were cultured with and without the presence of **Nucleoforce**. This study showed that Nucleoforce contributed to increase certain cytokines in *L. infantum* infected cells such as IL-1, IL-2, in addition to releasing IFN-γ, TNF-α and macrophage colony stimulating factors (M-CSF). This favours cellular response (Th1) and macrophage activation that will increase reactive oxygen species (ROS) in peripheral blood mononuclear cells (PBMC) and result in the destruction of *Leishmania*. **See Figure 19.**

It should be noted that Nucleoforce doesn't have a direct leishmanicidal action, but rather acts by stimulating macrophages (16,19).

Bioiberica's research team has developed a useful scheme for the multimoda management of canine leishamniosis in an endemic area (18). **See Figure 20.**

Progression of canine *leishmaniasis* in dogs according to treatment group over 365 days



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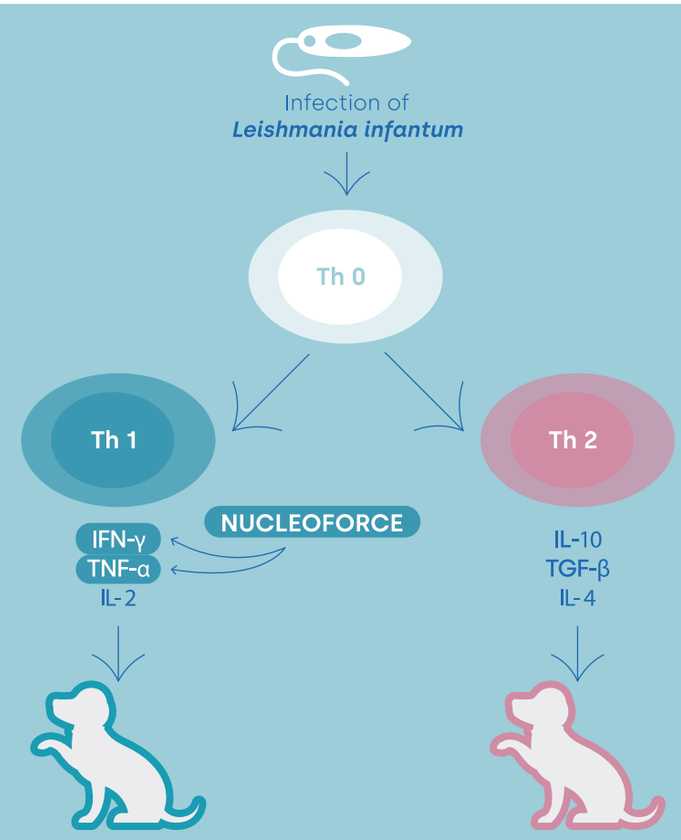
Nucleoforce-mediated stimulation of cellular response (Th1) with or without AHCC.



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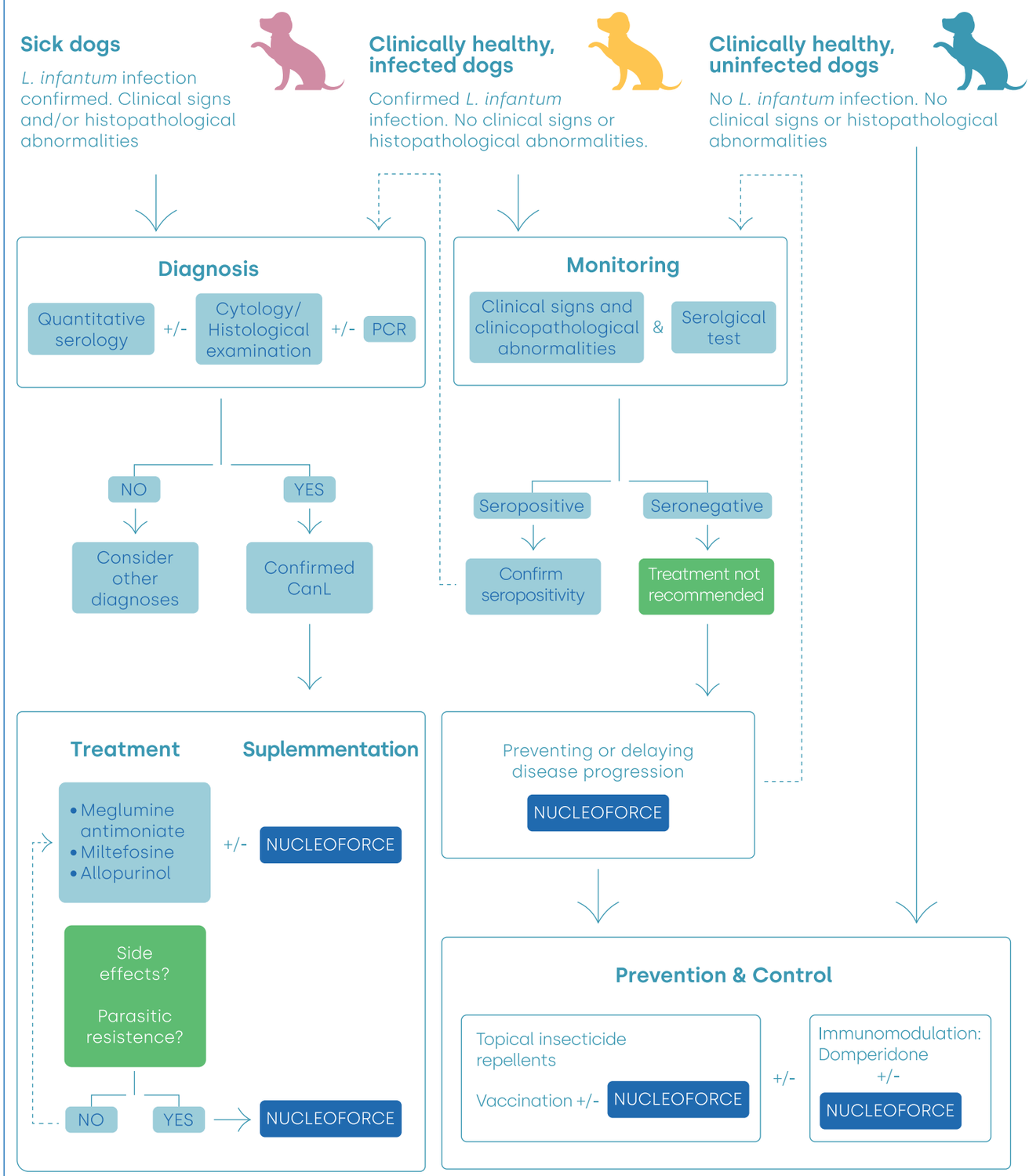
Suggested algorithm for multimodal management of canine *leishmaniasis* in an endemic area.



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In addition, from April 2022 onwards, the use of dietary nucleotides has been included in the LeishVet guidelines for the treatment of canine leishmaniasis. **See Figure 21.**

Consult us at: **https://www.leishvet.org/**

LEISHVET recommendations for the management of *Leishmania*.

Drugs/Supplements	Dose	Side effects
Meglumine antimoniate	100 mg/kg SC, SID or divided in 2 doses for 4-6 weeks (reduced initial dose for 2-3 days may be useful for detecting adverse effects);	• Potential nephrotoxicity • Pain and inflammation in the injection area
Miltefosine	2 mg/kg PO SID for 28 days	• Dysorexia • Vomits • Diarrhea
Allopurinol	10 mg/kg PO, BID for 6-12 months (depends on phase and tolerance)	• Urolithiasis • Kidney mineralization • Nephrolithiasis
Domperidone	0.5 mg/kg PO SID for 1 month	Galactorrhea
Dietary nucleotides with AHCC	Follow manufacturer's instructions PO 6-12 months	**Without secondary effects**

21

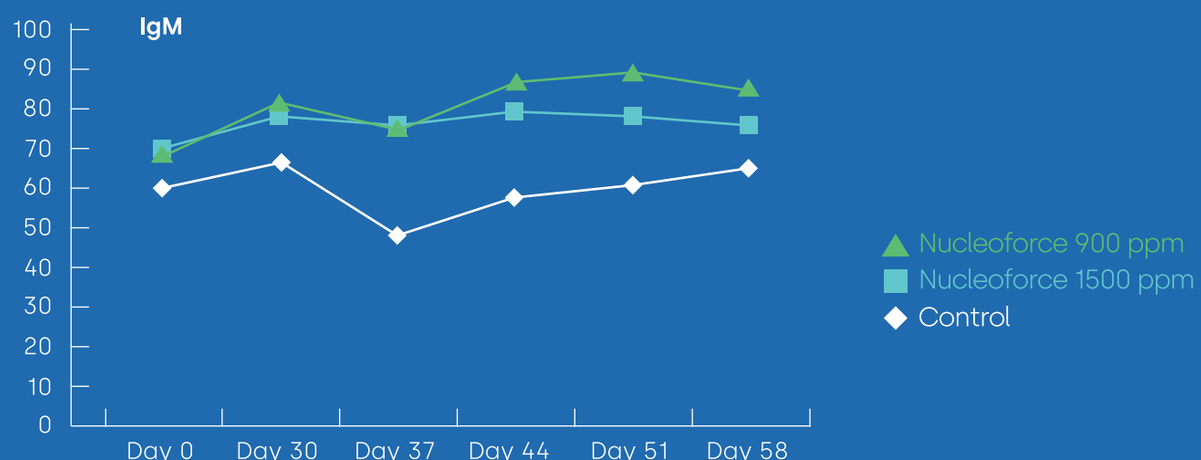
Role of Nucleoforce in immunosuppressed dogs

One of the side effects of chemotherapy is depression of the immune system. As has been seen, **Nucleoforce** contributes to the maintenance of the immune system, since nucleotides play an important role in extracellular molecular signaling and consequently in the modulation of immune and inflammatory responses (21).

Bioiberica carried out a study to test the effect of **Nucleoforce** on immune function in dogs receiving chemotherapeutic treatment (lomustine and cyclophosphamide). The dogs were divided into 3 groups. 2 groups received a diet supplemented with nucleotides for 30 days prior to chemotherapy. The other group received a placebo-supplemented diet 30 days prior to chemotherapy (22).

It was observed that **groups that received Nucleoforce in the diet 30 days before undergoing chemotherapy had a higher concentration of IgM, indicating a higher level of humoral protection,** which is important due to the immunosuppressive effects caused by chemotherapy. In terms of the lymphocyte proliferation index, the groups that received Nucleoforce compared to the control group. **See Figure 22.**

Evaluation of humoral immunity (Immunoglobulin M) after chemotherapy treatment.



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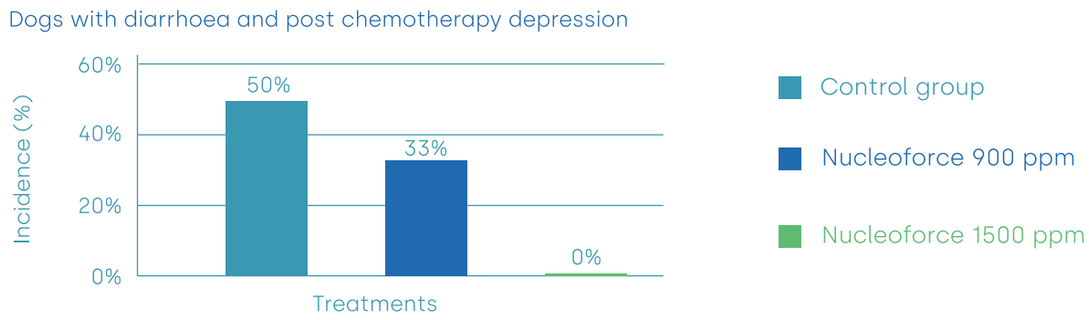
This improved immune status in the dogs receiving Nucleoforce was reflected in the lower incidence of clinical signs in the dogs after chemotherapy. See Figure 23.



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Role of Nucleoforce for senior animals

The increased life expectancy of pets has led to the recognition of an increased susceptibility to disease due to the deterioration of the immune system known as immunosenescence.

There are many causes that may be related such as thymic involution and decreased production of immature cells in the bone marrow and thymus (9).

Age-related changes impair cell-mediated immune response. In addition, there is a decline in the humoral response due to decreased Th cell functionality. What does seem to persist is the ability to generate humoral immune responses, this is because there is a persistence of protective vaccine antibody titres and the response to booster vaccination with an increase in antibody titre (9, 23).

Commonly, geriatric dogs exhibit impaired immune responses to novel antigenic challenges, such as infections and vaccines, probably related to a reduced peripheral pool of immature T-cells and low diversity of the T-cell receptor repertoire (24). This could be observed when the rabies vaccine was administered for the first time to a group of geriatric dogs that showed a significant decrease in antibody titres suggesting vaccination failure (25).

On the other hand, geriatric dogs are more susceptible to oxidative damage, due to a lower response to oxidative stress. Therefore, strategies should be sought to reduce the animal's proinflammatory state, as well as supplementing with antioxidative agents. (26)

In this case, nucleotide supplementation can contribute to the circulating pool of available nucleotides. This nucleotide pool is very small in resting cells, however, **additional nucleotide supply is required to support DNA replication, as well as the synthesis of RNA and continuously replicating proteins. In the case of the immune system, this will stimulate the production of leukocytes, which are important for protecting the body against infectious diseases** (27). This is especially relevant in geriatric animals, whose immune response is in a senescent state.

There are also studies suggesting that nucleotides may play an important role in the prevention of oxidant-induced DNA damage because they can prevent lipid oxidation through increased synthesis of RNA, which is responsible for the synthesis of enzymes required to cope with oxidative stress (28).

Conclusions

Supplementation with Nucleoforce Pets in pets' diets during certain life stages such as weaning or old age, as well as in situations that may be challenging for our pets, can contribute to the maintenance of the immune system and intestinal health. This will lead to a better quality of life for pets and their owners.

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