Dietary Nucleotides Improve The Immune System Of Puppies at Weaning

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Summary

Animals have a continuous requirement for nucleotides, especially for systems that present a high risk of cell turnover, like the immune system. Nucleotides may become semi-essential nutrients under certain circumstances (e.g. weaning period) because endogenous synthesis may be insufficient to sustain normal function. In this case the immune function depends upon dietary nucleotide sources.

The aim of the present study was to test the impact of the nucleotide supplement, specifically designed to mimic nucleotides in bitch’s milk, on the immune function of weaned puppies.

A total of 21 beagle puppies weaned 8 weeks of age were allocated to 3 balanced groups and fed one of the following diets: standard commercial puppy diet (control diet); control diet supplemented with 900 ppm of nucleotides; or control diet supplemented with 1350 ppm of nucleotides. One week after weaning puppies were vaccinated with an heptavalent vaccine. During the study blood samples were taken in order to analyze plasmatic concentration of C reactive protein (CRP), non specific immunoglobulins and antibody titre parvovirus. Peripheral blood mononuclear cells (PBMC) stimulation test was also performed.

Supplement groups showed higher antibody titre against parvovirus 14 days after vaccination and higher unspecific immunoglobulin levels.

PBMC stimulation test a 35 days also showed higher results in the supplemented groups.

CRP 1 day after vaccination and haematocrit after 35 days showed better results for the supplemented groups.

In conclusion, dietary supplementation with nucleotides mimicking nucleotides of bitch’s milk, improved the immune response capacity of puppies at weaning.

Introduction

Nucleotides are the monomeric precursors units of RNA and DNA. They are compound by a nitrogenous base, a five-carbon sugar (ribose or deoxyribose) and 1 to 3 phosphate groups. The nitrogen-containing bases are derivatives of purines (adenine and guanine) and pyrimidines (cytosine, thymine and uracil). Nucleotides are not considered to be essential for humans or dogs and cats (NRC, 1986) because they can be synthesized de novo endogenously, via a process using amino acids and glucose. De novo pathway is metabolically costly (Yu V., 2002) and is limited in some tissues as intestinal mucosa, bone marrow haematopoiëtic cells and lymphocyte, with high rate of replication (Pérignon et al. 1987). These tissues depend more on the salvage pathway that produces nucleotides from either exogenous dietary nucleosides or endogenous purine and pyrimidine bases. Nucleotides might become essential nutrients for these tissues during periods of insufficient intake, high rate of physical growth or in the presence of disease. Therefore it’s possible that an adequate dietary intake of free nucleotides may optimize their physiological function (Maldonado et al., 2001; and Yu V., 2002).

Dietary supplementation with nucleotides has already been used in animals and humans with proved health benefits. Dauy et al (1994) demonstrated that nuclotides promote intestinal growth and maturation in young rats. Pickedring et al (1995) and Schaller et al (2004) in a clinical study with children, showed a significantly higher antibody response.
to different vaccines (Haemophilus influenzae, Diphtheria, polio virus and tetanus) in children fed with an infant formula supplemented with nucleotides versus not children with non-supplemented formula. In a similar clinical study, Carver et al. (1991) showed significantly higher natural killer cell activity and interleukin-2 activity at 2 months of age in peripheral blood mononuclear cells of children supplemented with nucleotides.

**Material and Methods**

A total of 21 beagle male and female puppies weaned at 8 weeks of age (day 0 of the study) were allocated to 3 balanced groups (sex and weight) and fed during 35 d with 3 different diets: 1) a standard commercial diet for puppies (control diet, group C); 2) a control diet supplemented with 900 ppm of free nucleotides in same proportion as bitch milk (Bioiberica, Spain) (group A); or 3) a control diet with 1350 ppm of free nucleotides in same proportion as bitch milk (group B). One week after weaning, puppies were vaccinated against parvovirus, distemper, adenocircus, para influenza and leptospira with a heptavalent vaccine (Vanguard-7, Pfizer, Spain). Blood samples were taken on vaccination day (day 7 of study) and after 24-h (day 8 of study), 14-d (day 21 of study) and 28-d (day 35 of study). The plasmatic concentrations of C reactive protein (CRP) were analyzed before vaccination (7-d) and 24 h after with the Tridelta Phase™ range canine CRP kit (Tridelta development limited, Ireland) and final absorbance of samples was measured by a microtiter plate reader (Powerwave XS, Biotek Instruments, USA) at 450 nm using 630 nm as reference.

Non specific immunoglobulins (IgG, IgM, and IgA) and antibody titre against parvovirus and were analyzed and blood lymphocyte phenotyping on vaccination day, 14 and 28 days later. Lymphocyte proliferation assay was performed from blood of 28 days after vaccination.

Commercial ELISA kits were used for parvovirus antibody (INGEZIM PARVO CANINO, Ingenasa, Spain) and non specific immunoglobulins analysis (IgG, IgM, and IgA; Bethyl Texas). For Lymphocyte phenotyping peripheral blood mononuclear cells (PBMC) were collected after centrifugation over histopaque 1077 (Signa-Aldrich chemie Bmbh, Germany) and two-colour analyses were performed, with direct or indirect staining depending on the antibody used. For indirect one, cells were incubated with monoclonal antibodies (anti canine CD4, CD8; Serotec, Raleigh, NC), followed by incubation with FITC-conjugated sheep F(ab)2anti-rat (Serotec, Raleigh, NC). For direct staining, cells were incubated with labeled monoclonal antibodies (canine anti-CD3 and anti-CD21; Serotec, Raleigh, NC). Normal rata sera incubated with PE-conjugated goat F(ab)2anti-rat and isotype control antibodies were used to control non-specific labeling. After washing, the cells were resuspended with PBS FACSFlow Solution. Immunofluorescence intensites were measured by flow cytometry (FACSCalibur, Becton Dickinson) and data were analyzed using the CELLQuest™ software.

For Lymphocyte proliferation assay after isolation of peripheral blood mononuclear cells (PBMC) from heparinized blood samples by centrifugation over Ficoll-Hypaque (Histopaque 1077, Sigma, St. Louis, MO), cells were cultured in absence or presence of mitogen, phytohaemagglutinin (PHA) and pulsed with 10 M of 5-bromodeoxyuridine (BrdU). The cell proliferation was determined using a non-radioactive ELISA technique (Cell proliferation ELISA, BrdU (colorimetric), Boehringer Mannheim, Germany). Results were expressed as optical density (OD) units.

Data of lymphocyte proliferation were subjected to ANOVA according to the general linear model (GLM) procedure of SAS (1996). Repeated measures analyses of variance with time and treatment as the with-in subject factor was used to analyze plasmatic concentrations of C reactive protein, non specific immunoglobulins, antibody titer, blood lymphocyte phenotyping and haematocrit values using the model (MIXED-type TOEP) of SAS (1996), and LSMEANS follow-up test was used for comparisons of means. A two-tailed P-value of <0.05 was considered significant.
Results

A total of 21 dogs finalized the 35-day long feeding study. The evaluation of the human immunity included unspecific serum immunoglobulin concentration, (Table 1) and ELIZA quantification of parvovirus antibodies (Tables 2). Cellular immunity was evaluated through differentiation of lymphocyte subpopulations by flow cytometry (Table 3) and by the lymphocyte proliferation assay.

Lymphocyte proliferation assay baseline values were equal for all three groups (0.17 ± 0.0022; 0.18 ± 0.019 and 0.18 ± 0.0014 for control, 900ppm and 1350ppm groups) while stimulation values showed a significant increase in the 1350ppm group (1.28 ± 0.09; 1.39 ± 0.095 and 1.62 ± 0.117” for control, 900ppm and 1350ppm groups; p<0.05) as shown on figure 1.

The haematocrit results are shown on Table 4 while the results of the evaluation of the C-reactive protein can be found on Table 5.

Discussion & Conclusion

Nucleotides are considered as non-essentials for babies (although recently, the EU Scientific Committee issued supplementation guidelines for infant formula) and also for dogs and cats (NRC, 1985, 1986), because they can be synthesized endogenously from basic components (aminoacids and glucose), although by a metabolically expensive pathway (YU V 2002).

Several studies have taken place, mostly in infants, showing the benefits of dietary supplementation with nucleotides, in particular in highly replicating tissues (gastrointestinal tract, immune system, etc.) In the case of the immune system, dietary supplementation have shown an increase both in humoral immunity (increase in Ig concentration for several vaccines (Pickering 1995, Schaller 2004), increase of unspecific IgM and IgA values [Navarro 1999]) and in cellular immunity (increase in lymphocyte proliferation, increase in NK cells and IL-2 [Carver 1991]).

The present study tried to show the benefits of nucleotide supplementation both in humoral and cellular immunity in dogs. It also tried to evaluate the effect of dietary nucleotides on the overall health status of the animals.

The results obtained in this study showed a better humoral response in the supplemented groups. As indicated by the higher unspecific immunoglobulin levels (IgG, IgA, IgM) compared to the control group. The antibody titre for parvovirus was also higher in both supplemented diets compared to the control group. Although all three groups had antibody titre higher than 1/80 (which is considered as protective according to the vaccine manufacturer), both supplemented groups had significative higher levels of antibodies, thus probably showing improved humoral immunity in the supplemented groups.

Although flow cytometry did not show any significative difference between the groups, probably due to the high variablility of this technique and to the limited number of animals, the lymphocyte proliferation test (peripheral blood mononuclear cell stimulation test) did show an increased response after stimulation in the 1500ppm-nucleotide supplement group, thus probably showing a better cellular immune status of these supplemented animals.

When trying to evaluate the overall health status of the animals, the haematocrit showed an increased value in both supplemented groups. Although all the three groups had haematocrit levels in the normal range, the higher values for both supplemented groups probably indicate a better general health status of the two supplemented groups.

The analysis of the CRP 1 day after vaccination (acute response) showed an increased value in both supplemented groups, which normalized after 27 days. Although this interpretation may be controversial, we assume that this elevated acute values are an indicator of increased reactivity against the vaccine of the supplemented animals, thus indicating a better overall health status.
In conclusion, this study evaluated the effects of nucleotide-supplemented weaning diets in puppies, in particular on the immune system and on the overall health status of the animals. We can conclude that nucleotides improved biological markers of the immune response in puppies and helped to an improved overall health status.

References


