ADVANCE VETERINARY

RESEARCH REPORTS

A Research Update for the Veterinarian from Affinity Petcare

Puppy nutrition

Isabelle Jeusette, DVM, PhD
Victor Romano, DVM

The period of growth is a very sensitive period in the dog as in other species. During this period, puppies are at risk of orthopedic as well as infectious diseases and they have to acquire all the physiological functions of the adult.

The canine specie is very particular regarding the wide variation of body weight observed in adult dogs of different breeds.

INTRODUCCIÓN

The canine specie is very particular regarding the wide variation of body weight observed in adult dogs of different breeds. Consequently, the time to achieve the adult body weight is very variable between breeds. While a mini dog will finish his/her growth in up to 9 months, a giant dog will require more than 15 months. The growth curve is not linear, it can be divided in 2 phases: a rapid (exponential) growth phase followed by a slower phase. The exponential phase is particularly long and delicate in large breed dogs (Fig growth curve to add).

Compared to mini dogs, weaning in large breed dogs happens much earlier in their bone development. This explains that the post weaning risks of bone anomalies are different in small and large dogs. In the past, nutritional deficiencies were common in growing animals. Today, these deficiencies are exceptional and we observe more diseases related to nutritional excess.

Young animals are also at risk of viral, bacterial or parasitic infections but a good nutrition during gestation, lactation and growth can help to reduce the consequences.

Offering food to puppies may be started at 2,5 weeks at the earliest and should begin by week 4 at the latest. At that time, milk cannot support anymore an optimal nutrition for all the puppies. The aim of this paper is to review the nutritional requirements of the puppy- from the pre-weaning period until the end of growth- to help to limit the risk of growth diseases and to obtain adult dogs in the best shape. Nutrients that can help to improve the immunity and the development of the puppy will be also presented.

Nutritional requirements

Energy

During the first two weeks of life, puppies are at high risk of dehydration, hypothermia and hypoglycemia. The hepatic glycogen storage is low and they depend on the milk intake to survive. Providing a high-energy very palatable diet to the bitch at the end of the gestation (three last weeks) and during lactation is important to assure a good lactation and consequently a good development of the new born: in large breed bitches the capacity for food intake can limit energy intake; in small breed bitches, the diet's palatability can be a limiting factor.

Growing animals require more energy compared to adults. They have to cover the maintenance requirement and they need energy to assure the muscular and bone development. The growth rate of an individual depends on several factors : genetic potential,

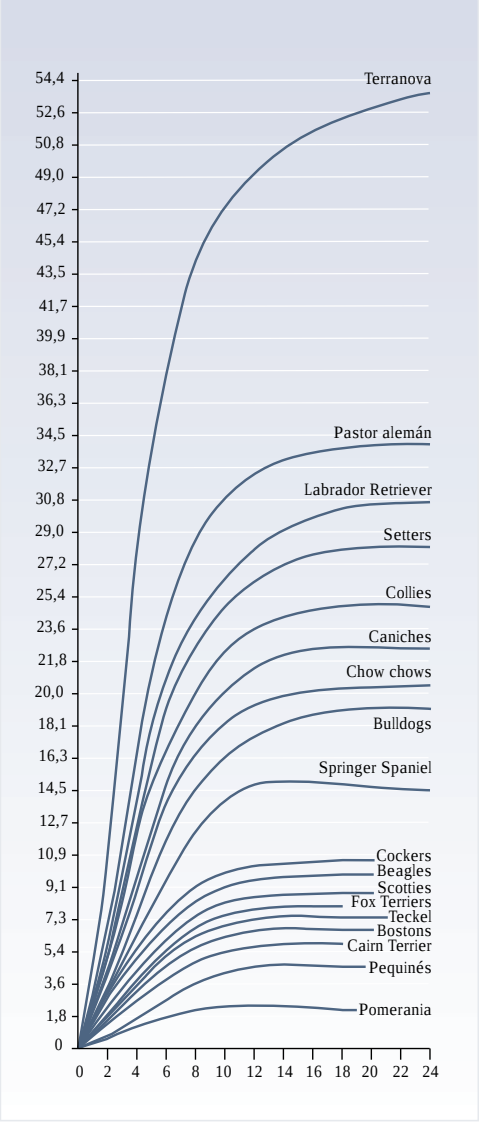


Figure 1: Growth curve to add

breed, sex, environment, nutrition, … Energy intake has a direct influence on it: the most the puppy eats, the more quickly he grows. But a rapid growth rate does not mean an optimal growth rate. In large breed dogs, excessive energy intake results in excess of body weight on an immature osteo-articular system that increases the mechanical stress on cartilages and growth plaques. Hip dysplasia, osteochondrosis, radius curvus, hypertrophic osteodystrophy are recognized to be linked to an excess of body weight during growth. But the mechanical stress is maybe not the only mechanism implicated.Obesity and excessive energy intake result also in modifications of hormonal secretions (IGF-1, thyroid hormones,…) whose consequences remains to be evaluated in dogs. In small size dogs, the consequences of a high-energy intake on the osteo-articular system are less important but that can lead to obesity in young adult.Early obesity and excessive energy intake in dogs predispose to several pathologies (insulin resistance, diabetes, dyslipideamia, hypertension, osteoarthritis, mammary cancer, kidney disease, …) and reduces life expectancy.

Practically, at weaning, growing puppies require 2 times as much energy/kg adult body weight as adult dogs of the same breed. Energy requirement decreases with time : a decrease at 1.6 and then 1.2 times maintenance when 50% and 80% of adult body weight is reached, respectively, is arbitrary recommended. But energy requirement are always individual. Ideally, as in adult dogs, the body condition score of the puppy has to be followed to be sure he/she is never too lean or too fat. The ribs have to be palpable but not visible *(Figure 2 BCS puppy).*

Dietary lipids

Fat is an important component of the animal diet. It is source of energy, essential fatty acids, liposoluble vitamins and it improves the palatability. Fats are important for the skin and hair condition, nervous development, inflammation regulation and immune function. In humans, it has been shown that obesity in children could be linked to an excess of dietary protein and a decreased in dietary fat, compared to milk composition, in the first months of growth. In newborn dogs, fat digestion is well developed.
Dietary fats can be saturated or unsaturated. Among the unsaturated fats, the essential polyunsaturated fatty acids (PUFA) are of particular interest, mainly the one from the omega-6 and the omega-3 families. Linoleic acid (LA) and arachidonic acids (ARA), for example, belong to the omega 6 family while alpha-linolenic acid (ALA) and docosahexaenoic acid (DHA) belong to the omega-3 family. An optimal omega-6/omega-3 ratio (5 to 10) during gestation, lactation and growth is important : an excess in omega-6 increases the production of pro

INMUNITY SUPPORT - NUCLEOTIDES

Immunity support

About 30% of the puppies die between birth and weaning. This impressive percentage justifies the necessity of improving the immunity of the puppy. After birth, puppies are exposed to a high diversity of antigens. The development of the puppy immunity starts in utero but is not sufficient to assure a complete protection after birth. Only small amounts of antibodies are able to pass the placental barrier. The main source of immunoglobulins is therefore colostrum. The diet of the bitch during gestation and lactation has to be adapted to assure a high quality lactation. The intake of colostrum normally protects the young animals for 1-2 months. After this period, the active immune system has to respond to environmental antigens. However, the concentration of maternal antibodies in the colostrum decreases quickly after birth and the maternal antibody are able to pass through the intestinal wall for about 24 hours. Consequently, all the puppies from a same litter are not equivalent from an immunological point of view. Puppies delivered first have a better chance to ingest large amount of concentrated colostrum, while latter puppies ingest less immunoglobulins. Colostrum and milk can also vary in quality: immunoglobulins unadapted to the puppy environment or in low concentration, poor nutritional quality,…

Primovaccination of the puppy takes place between 4-8 weeks of age, depending on the risk of disease in the environment. This period is particularly critical due to the “immune gap”: the colostral immunity is too low to offer a good protection but still interferes with the vaccine.

Another problematic period is the time of adoption. At that moment, the environment of the puppy changes a lot: new owner, new environment, eventually new diet,…Ideally, the diet cannot be changed without a transition period. Otherwhile, in addition to “normal stress”, a intestinal flora derivation, and consequently diarrhea is frequently observed, and the risk of bacterial or viral infection is higher.

Recent researches have shown that it is possible to improve the immune response of the puppy through nutrition.

Nucleotides

Nucleotides are structural components of RNA and DNA and therefore during cellular division, where the genetic material is being duplicated, there is an increasing need of nucleotides for this duplication. Nucleotides include 2 families: purines (adenosine, guanine) and pyrimidines (cytosine, thymine, uracil).

Highly replicating cells and in particular those of the immune system, have a high requirement of nucleotides, cause the genetic information is being duplicated in each cellular division.

These nucleotides may be synthesized “de novo”, from carbohydrates and amino acids, which is very expensive in terms of metabolic cost; or the nucleotides can be “reused”, either from

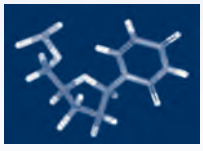


Figure 3: Representación 3D de un nucleótido.

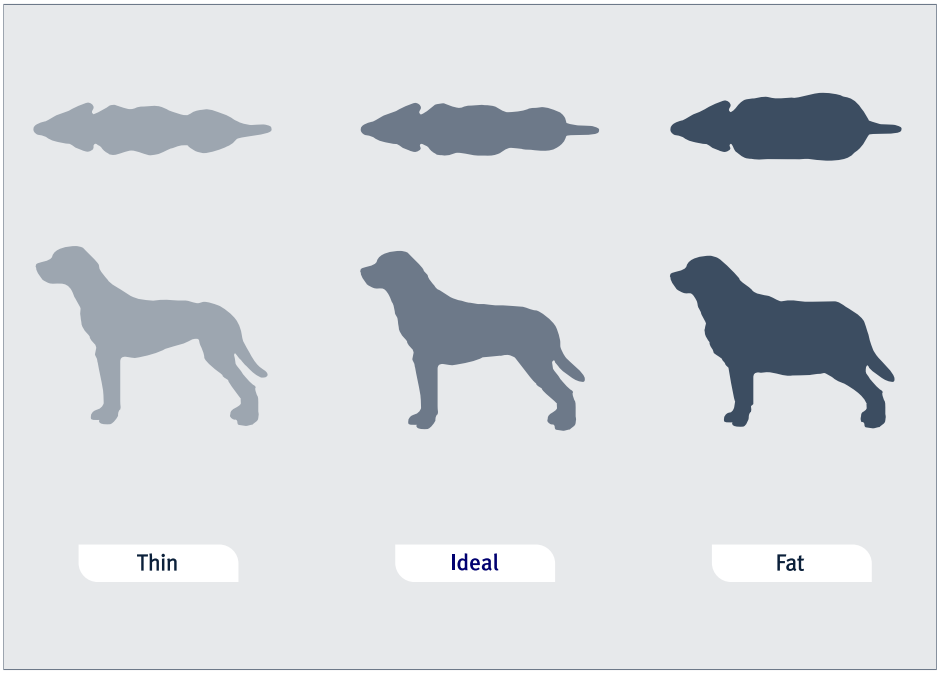


Figure 2: BCS puppy

inflammatory cytokine and in human babies, it has been shown that it favors **adipogenicity.**

Polyunsaturated fatty acids and neural development

Compared to humans, dogs are born much earlier in their development life. Significant brain and nervous development occurs during the neonatal period and large amount of long chain PUFA are required to support this neural development.

A recent study in dogs has shown that supplementation in DHA of the bitch and puppy's diets during gestation and until 16 weeks of age improves the puppy trainability.

Another study has also shown that bitch and puppy's diets enriched with long chain omega-3 PUFA during gestation, lactation and growth improved visual

the catabolism of nucleic acids or using dietary sources.

During the first days/weeks of age, puppies receive dietary nucleotides from their mother's milk. Milk has a specific proportion of nucleotides which is intrinsic to each specie (i.e. bitch's milk has a specific nucleotide's profile which is different from human's milk).

After weaning, when puppies do not any more eat mother's milk, nucleotide intake is reduced, cause the solid food contains a much smaller amount compared to mother's milk.

This reduced intake is important: the dog is still growing, which means that the requirements for nucleotides is still quite high, and the immune system is still maturating. During this important growth stage, the nucleotide availability relies therefore much more on the puppy's "de novo" synthesis capability. And this capability is very limited in immature animals.

The decrease in nucleotide's intake after weaning and the positive effect of supplementing diets with nucleotides has been already studied for several years.

Numerous researches in humans and in different animal species have shown the benefit of supplementing foods with nucleotides, in particular in highly replicating cells (enterocytes, lymphocytes, bone marrow, ...).

Supplementing diets with nucleotides in pigs increases microvilli size, increases brush border enzymes and decreases inflammation and cellular mortality in case of intestinal injury.

On the immune system, the effects of nucleotide supplementation in infants indicate an increased cellular immunity, as shown by an increase in lymphocyte proliferation.

Also in children, the humoral immunity was improved by supplementing with nucleotides: different studies showed increase in unspecific immunoglobulins (IgM, IgA) and improved response to vaccination, in particular against Haemophilus, diphtheria, tetanus and polio virus.

For this reason, recently (2003) the EU scientific committee issued recommendations to supplement infant formulae (baby milk-replacers) with nucleotides, to help growing-babies in the process of cellular replication and helping to increase their immunity.

Following the same principle, Romano et al (2006) studied the positive effects on weaning puppies of dietary supplementation with nucleotides, with the same profile as bitch's milk (both in concentration and proportion of the different nucleotides), in particular evaluating the immune response.

Supplementation of puppy food with nucleotides, with same concentration and proportion as bitch's milk, showed an enhanced immune response, both in humoral and cellular immunity.

Humoral immunity was studied by analyzing serum unspecific immunoglobulin levels (in particular IgG, IgA and IgM) and parvovirus-vaccine related antibodies (IgG).

Results showed a significant increase in unspecific circulating immunoglobulins (IgG, IgA and IgM) in the supplemented animals compared to the unsupplemented ones. The parvovirus

performances in developing dogs.

Protein and amino acids

Dietary proteins provide essential (that cannot by synthesized) and non-essential amino acids. Both are required for the synthesis of proteins in the body (muscles, hormones, neurotransmitters, neuromodulators, heme…), neoglucogenesis, energy, nucleotides,… Some amino acids have also their own function.

The optimal protein requirement during gestation, lactation and early growth have not been extensively studied. The quality of the mother diet (protein level) could have an effect on the glycogen storage of the new born puppy. Protein digestion is different in the young puppy compared to adult. The capacity of the newborn to produce gastric acid is underdeveloped. The weak acidity seems sufficient for the coagulation of milk protein and the digestion by endopeptidase but the risk for passage of pathogenic bacteria is higher. Moreover, the renal capacity is limited until 3-8 weeks of age. A highly digestive protein source is recommended.

In growing dogs, there is no scientific data to support a deleterious effect of protein, by contrast to excessive energy intake. In the contrary, a deficit in dietary protein can affect negatively the growth.

Carbohydrates

Pregnant bitches require dietary carbohydrates for optimal reproductive performances and survivability of puppies. During the first weeks of life, puppies are at high risk of hypothermia and hypoglycemia. Hepatic glycogen is the main energy source in the newborn because they have limited fat stores. Hepatic glycogen covers a great part of the energy required for temperature regulation and maintains blood sugar level. However, hepatic glycogen storage is quite low. Carbohydrates (from colostrum) are required to provide glycogenic precursor. New born dogs have high capacity to digest milk (lactose and fat). The lactase enzymatic activity is high in young puppies. Then, lactase activity decreases with age while amylase activity increases to reach adult levels at about 12 weeks of age. In young suckling, saccharose and starch cannot be fully digested and consequently could induce digestive disturbance at high level (fermentative diarrhea). They have to be introduced moderately.

Calcium, Phosphorus, vitamine D

The ratio calcium/phosphorous is particularly important in growing dogs. Phosphorus absorption depends on calcium absorption. The calcium/phosphorus metabolism is under the control of 3 hormones:

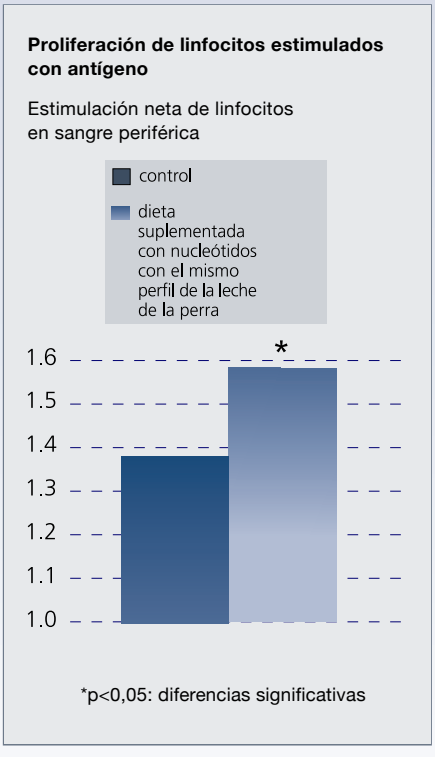


Figura 4: Inmunidad celular. Datos presentados en el encuentro de la American Academy of Veterinary Nutrition (AAVN); Kentucky, 2006. (Romano et al, 2006)

ELISA title was also significantly higher in the animals fed with nucleotides compared to the unsupplemented ones.

Cellular immunity was evaluated through a specific in vitro-test (lymphocyte proliferation test) which quantifies the proliferation capacity of lymphocytes before and after stimulation.

Animals receiving the nucleotide supplement showed a higher proliferation capacity than those receiving no extra nucleotides.

These results indicate that dietary nucleotides, in same proportion as homologous milk, increase the immune potential of the animals, both at humoral (as shown on immunoglobulin concentrations) and at cellular level (as shown on lymphocyte proliferation).

Antioxidants

Supplementation of the puppy diet (7 weeks old) with antioxidants increases the response to the distemper disease vaccine and improves the effective and long-term immunologic memory.

Probiotics and prebiotics

Probiotics (Lactobacillus or Enterococcus faecium for example) are microorganisms that normally belong to the healthy bowel flora and that have a beneficial effect on the health. They can work synergistically with prebiotics (fructo-oligosaccharides or inulin, for example) that are non-digestible dietary ingredients that stimulate the growth of the first ones. Prebiotics and probiotics are important for the gastro-intestinal health. More recently, it has also been shown that they have immune stimulant properties.

The supplementation of puppy diet (8 weeks old) with probiotics induces a better specific immune response : higher fecal Ig-A, canine distemper virus vaccine specific IgG and IgA and mature B cells concentration.

Short chain fructooligosaccharides supplementation in bitch and puppy's diets from 35th day gestation results in a higher IgM concentration in colostrums and milk. It tends also to increase serum IgM concentration in the bitches and to induce a better IgM specific response after kennel cough vaccination.

- Parathyroid hormone (PTH) : increases calcemia by increasing renal and bone reabsorption of calcium and by activation of vitamin D in the kidney; decreases phosphatemia

- Vitamin D (calcitriol) : increases calcemia and phosphatemia by increasing renal calcium reabsorption, helping PTH activity, increasing gut reabsorption of calcium and phosphorus

- Calcitonine : decreases calcemia by reduction of osteoclastic activity

Moreover, growth hormones and IGF stimulates also intestinal calcium absorption.

In young dogs, in addition to the normal very efficient active calcium absorption that happens all life long, a passive absorption is also observed until at least 6 months of age. This passive absorption depends on the dietary calcium concentration and explains that dogs are better protected against calcium deficit than calcium excess.

Passive absorption in puppy cannot be lower than 40% of ingested calcium quantity. Excessive calcium intake in growing dogs can have detrimental effects on the endocrine status (hypercalcemia, hypophosphatemia, decreased parathyroid activity, increased thyroid C cells activity, …), and on the skeletal metabolism (increased osteoblast, decreased osteoclast, increased bone mass and cartilage cones,…) that finally lead to skeletal diseases in large breed dogs (rickets, osteochondrosis, radius-curvus, enostosis, Wobbler syndrome).

Calcium deficiency induces secondary hyperparthyroidism and excess calcitriol. Clinically, severe calcium deficiency can result in pathological fractures, slower growth rate or osteomalacia. Today these affections are rare. It was mainly observed in the past with all meat diet characterised by a low calcium/ high phosphorus ratio.

Clinical signs of vitamin D deficiency are similar to calcium deficit.

Vitamin D excess induces ectopic calcifications. Vitamin D intoxication in puppies can lead to death in 2 to 5 weeks.

Vitamin A, E, copper and iron

After birth, puppies have only moderate hepatic iron, copper and vitamin A and E reserve. Exogenous supply is necessary.

Folic acid

In puppies, there is a risk of cleft palate mainly between day 25-28 of gestation. Folic acid before and during pregnancy decreases by 50% this risk.

Protein and amino acids

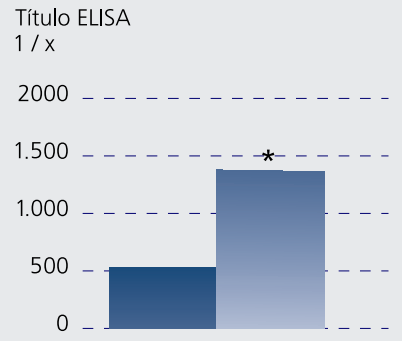
Protein/energy malnutrition is a frequent cause of secondary immuno-deficiency. It decreases both cellular and humoral immune response (cytokine and Ig production, antibody's affinity, complement activity, macrophages efficacy, …).

Some amino acids are particularly important. Arginine is an essential amino acids in dogs and its supplementation induces an increase in thymus weight and size. Glutamine is an N-transporter. It is a precursor of nucleotides synthesis and a regulator of protein metabolism. It is an important source of energy for the cells with a high rate of multiplication like enterocytes, macrophages, lymphocytes.

Glutamine supplementation results in an increase in polymorphonuclears, macrophages, lymphocytes, cytokine production, phagocytosis activity.

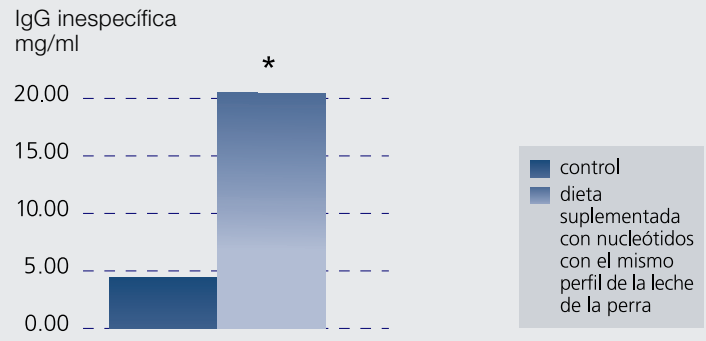
• ***Específica***

Anticuerpos Parvovirus (ParvoIgG)
Incremento 14 días después de la vacunación



• ***Inespecífica***

Producción inespecífica de IgG (mg/ml): diferencias entre el día 7 y el 21.
Incremento 14 días después de la vacunación



***p<0,05: diferencias significativas**

Figura 5

In practice

Ideally, offering solid food should start from 2.5-3 weeks of age. The first solid food should be highly digestible and have an intermediate composition between milk and growing diet.

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This solid diet should be rehydrated and warm on demand. Feeding amount should be adapted to weight development. To avoid digestive problem (vomiting, diarrhoea), food intake has not to be too high but moderate. At weaning (6-8 weeks of age), intake of solid food should be high enough to provide sufficient energy and nutrients without causing digestive problem. Weaning and adoption is a delicate period. Rapid change in diet should be avoid and food intake limited the first 3 days to avoid digestive problem.

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Parque de Oficinas St.Cugat Nord
Pl.Xavier Cugat, 2 - Edificio D, 3@ Planta
08190 St.Cugat Nord
BARCELONA

For more information: Tel. +34 93 492 70 00 - Fax. +34 93 492 70 01 - info@advanceveterinary.com