Royal Canin research news

Folic acid and cleft palate in brachycephalic dogs

Aurélien Guilloteau, MEng
Royal Canin Research Center, Aimargues, France

Aurélien Guilloteau, graduated from the ESITPA (engineer in agriculture) in 2002. He finished his studies at the Royal Canin Research Center, investigating the development of a hypoallergenic food. Aurélien Guilloteau joined the Royal Canin Research Center to work with the Cat and Dog breeder network.

Eric Servet, MEng
Royal Canin Research Center, Aimargues, France

Eric Servet graduated from a French engineering school, specializing in food ingredients and food technology, in 1999. He was an engineer for dairy industries working on pilot development and product formulation from 1999 to 2001. After a year spent in the USA working for Royal Canin USA on product stability and palatability, Eric Servet joined the Royal Canin Research and Development Department in France as a research engineer in 2002.

Vincent Biourge, DVM, PhD, Diplomate ACVN and ECVCN
Royal Canin, Research Center, Aimargues, France

Dr. Biourge graduated from the Faculty of Veterinary Medicine of the University of Liège (Belgium) in 1985. He remained as an assistant in the Department of Nutrition for 2 years before moving to the Veterinary Hospital at University of Pennsylvania (Philadelphia, USA) and later to the Veterinary Medical Teaching Hospital of the University of California (Davis, USA). In 1993, he was awarded a PhD in Nutrition from the University of California and he became a Diplomate of the American College of Veterinary Nutrition. In 1994, he joined the Research Center of Royal Canin in Aimargues (France) as Head of Scientific Communication and Nutritionist. Since January 1999, Dr. Biourge has been in charge of managing the nutritional research program of Royal Canin.

Claude Ecochard, MEng
Royal Canin, Research Center, Aimargues, France

Claude Ecochard initially trained as a biochemist (Master's degree at the University of Lyon, France) and food processing engineer (Engineering and Postgraduate degrees in 1986 at the ENSBANA of Dijon: French Food engineer school specialized in Nutrition). He has gained over 18 years of experience working mainly in research and development projects for food processing companies. Since 2000, Claude Ecochard has played a key role at Royal Canin R & D, managing the development of new products and nutritional innovations.

Introduction

The generic term folates is used to designate various biological complexes (>100) that have a similar chemical structure and the same biological activity (folic acid is one of these compounds). Folic acid (vitamin B9) is a synthetic form not found in nature, but which is used in food supplementation and fortification.

When combined with vitamin B12, folates play an important role in the synthesis of nucleic acids (purine and pyrimidine bases of DNA), in cellular division and in the metabolism of some amino acids such as homocysteine. Their action is often associated with that of vitamin B12. Folates are essential to the synthesis of many neurotransmitters such as dopamine, noradrenaline and adrenaline and, in this respect, play an important role in the central nervous system (1).

In canine fetuses, cleft lips and/or palates result from failure of the nasal and maxillary buds to fuse during embryogenesis. This process normally takes place around the 33rd day of gestation (3) **(Figure 1)**. In dogs, cleft palates were experimentally induced by the administration of a folic acid antagonist (diazo-oxo-norleucine) from the 25th to 28th day of gestation (2).

Such pathologies can be due to multifactorial causes:

Environmental causes:
In women, exposure to teratogens can affect embryonic development. A relatively high intake of alcohol, nicotine, vitamin A, corticosteroids, alkylating agents, phenytoin and trimethadione-troxidone has been linked to cleft palates.

The mother's state of health can also contribute to the development of cleft palates/lips e.g. diabetes mellitus, myotonic dystrophy (3).

Genetic causes:
Studies have explained the appearance of cleft palates and/or lips by mutations of the MTHFR gene (methylenetetrahydrofolate reductase). MTHFR plays a central role in the folate cycle, where it enables the reduction of folate to its active form (4,5).

Cleft lips/palates have been observed in many canine breeds including Boxer, French Bulldog, English Bulldog, Cavalier King Charles, West Highland White Terrier, Collie, German Shepherd and Chihuahua. However, brachycephalic dogs such as the Boxer seem to be the most commonly affected (3). Similarly with cats, Persians and Siamese can be affected with this abnormality. 6.5% of kittens display deformities or congenital defects causing death, the most frequent being cleft palate (6).

Benefit of folic acid supplementation:
In human, the prevention of such conditions requires folic acid supplementation. Research has demonstrated a reduction of 48% in the risk of cleft palates in the children of women who have been taking multivitamin supplementation before conceiving or during the 1st month of pregnancy, although there is no reduction in this risk for women who began supplementation in the 2nd or 3rd month (7).

Dogs also appear to respond to folate supplementation. A retrospective observation study on Boston Terriers reports a reduction in the incidence of cleft palates in puppies from 17.6% (1974-1981) to 4.2% (1981-1993) after introduction of daily supplementation of bitches with 5 mg of folic acid from mating until 3 weeks after whelping. This intake does not lead to complete eradication of the risk (8). No adverse effects of feeding folic acid have been demonstrated (NRC, 1985). In the last edition (in press, 2006), the NRC does however advise not to exceed 1000 times the conventional recommendation of 0.18 mg/kg of dry matter.

The aim of the study was to validate the effect of a folic acid supplementation (5 mg of folic acid/day/dog) during 18 months in French Bulldog bitches on the prevalence of cleft palates/lips in their litters.

Materials and methods

Animals
45 French Bulldog bitches belonging to 5 breeders were monitored, i.e. a total of 66 litters born. 24 bitches whelped once, 15 bitches whelped twice and 4 bitches whelped 3 times.
The average weight of bitches was 11.5 ± 1.9 kg.

Diets

Two different diets were compared:

1. Control food: ROYAL CANIN premium maintenance diet: kibble containing folic acid at usual doses (0.9 mg/kg) of feed **(Table 1)**.

2. Supplemented food: ROYAL CANIN premium maintenance diet + Folic acid: kibble supplemented with folic acid **(Table 1)**.

The food supplemented with folic acid was prepared from the control food with the addition of a special coating containing 34.48 mg of folic acid/kg of food. This corresponds to 5 mg of folic acid per day, based on

Figure 1.
Clef palates in Pug puppies.
Credit : UMES (Unité de médecine de l'élevage et du sport, Alfort Veterinary School).

a consumption of approximately 145 g of food at 4108 kcal/kg for a bitch weighing 8 kg (standard breed weight).

Breeders gave only the food provided by us and the bitches received no additional intake than the diets being tested. The diets were coded and breeders did not know the code of the supplemented food (single blind test). Both diets were tested in all breeders.

Protocol

Two groups of bitches per breeder were split randomly between 2 diets.

The bitches were fed one of the 2 diets when they came into heat (i.e. around 2 weeks before the first mating/fertilization) and for the first 6 weeks of gestation. All the veterinary treatments to ensure the animal's wellbeing were recorded. Bitches with concurrent disease in which the treatment might have secondary effects on the puppies were excluded from the study.
At birth, the puppies were subjected to a thorough examination by the breeders or by the veterinarians carrying out the cesarean section in order to detect the possible presence of abnormality.

Statistical analysis

A descriptive analysis was performed on the data collected (number of puppies born, number of cleft palates) based on returned questionnaires.
In order to identify the significant differences between the 2 diets, an analysis of variance (ANOVA) was performed, using a commercial software (Statgraphics Plus). F-value with p-value lower than 0.05 is considered significant. The test for X^2 was used to assess the effect of the treatment on the prevalence of cleft palates in the litters.

Results

35 litters born under the control food and 31 litters born under the test food were monitored. Reproduction performances are summarized in **Table 2**. The prevalence of cleft palates was 8.57% for the control food and 4.41% for the supplemented food.

The difference in the number of puppies per litter between the 2 treatments is not significant **(Table 2)** (p-value = 0.3123, p>0.05).

The number of cleft palates per litter and per diet, across all litters, was significantly reduced with the folic acid supplemented food (p = 0.02, p<0.05) **(Figure 2)**.

Discussion and conclusion

The aim of this field study was to validate the benefit of folic acid supplementation (5mg/bitch/day) from 15 days before mating until the end of the gestation period of French Bulldog bitches on the prevalence of cleft palates in their litters.

In French Bulldogs, the results obtained after 1.5 years of research indicated that folid acid supplementation was associated with a 48.54% reduction in cleft palates. These results confirms Elwood's observations, which reported a 76% reduction in the risk of cleft palates in the Boston Terrier with a supplementation of 5 mg folic acid per day per bitch.

As in women, folate supplementation in bitches does not mean preventing all cases of cleft palate. This is probably explained by the multifactorial nature of this condition.

It is essential that folate supplementation is administered

Table 1.
Analysis of the diets

	Control food	Supplemented food
Moisture (%)	9	9
Protein (%)	25	25
Crude fat (%)	12	12
Fiber (%)	2.5	2.5
Starch (%)	25.2	25.2
Minerals (%)	6.3	6.3
Folic acid (mg/kg)	0.9	34.5
Vitamin B12 (mg/kg)	0.2	0.2

Ingredient list: *corn, corn meal, dehydrated poultry meat, animal fats, poultry liver, beet pulp, brewers yeast, vegetable oil, minerals, trace-elements, hydrolyzed yeast (a source of mannan-oligosaccharides), egg powder, vitamins.*

Table 2.
Reproduction performances

	Control food	Supplemented food
Number of bitches	35	31
Number of puppies	175	136
With cleft palate	15	6[a]
Dead at birth	4	6
Dead after birth	3	3
Other neonatal malformations	0	3
Abortion	0	2
Barren bitch	3	1
Number of puppies/bitch	5 ± 2.53	4.38 ± 2.10
Number of puppies/pregnant bitch	5.47 ± 2.09	4.85 ± 1.86

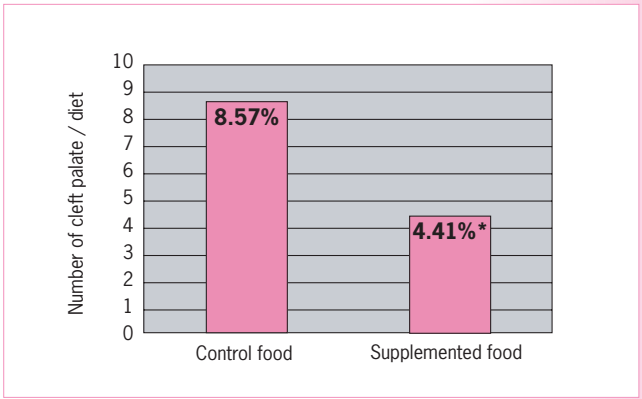
[a] *significantly different from the control group*

as soon as a bitch comes into estrus, since the medullary tube closes during the first months of gestation (before the 33rd day) in bitches.

We have not observed an effect of supplementation on litter size.

To conclude, this study confirms that folate supplementation in the first months of a bitch's pregnancy would significantly reduce the risks of cleft palate. ◆

Figure 2.
Number of cleft palate / diet



Number of cleft palate / group of puppies fed with each diet
** Significantly different by the X^2 test.*

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