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Anton C. Beynen

## **Gluten proteins for dogs**

*A considerable number of commercial dog foods makes a gluten-free claim. Gluten is the umbrella word for water-insoluble proteins found in the grains of wheat, barley, rye, oats and related plants (Note 1). Grain-free invariably is gluten-free, but gluten-free is not always grain-free. Dog food may only contain non-gluten grain(s), such as corn and/or rice. It is likely that many dog owners look for gluten-free food, or are attracted by this descriptor, because gluten hypersensitivity in humans, including celiac disease, is a well-known reason to avoid gluten (Note 2).*

*Gluten describes multiple, specified groups of proteins in wheat, barley, rye and oats. But it also is a generic term for grain proteins as in corn gluten or rice gluten. Some dog-food ingredient lists declare corn gluten (Note 3). None of the proteins in corn and rice are recognized by gluten immunoassays. Obviously, some wheat-gluten proteins are detected, while certain gluten proteins from barley, oats or rye likely will be (1, 2). Thus, corn and rice are demonstrably gluten-free. Canine hypersensitivity to corn, rice, barley, rye or oats has not been reported, but wheat allergy can occur in dogs (3), albeit at very low prevalence.*

*Supply and demand of gluten-free dog food overwhelm true need when it comes to gluten hypersensitivity. Wheat allergy has been shown in dogs, but the prevalence in the whole population may only be about 1 in 10,000. Among members of some Irish-Setter families, dietary wheat gluten causes poor body condition without or with chronic diarrhea. Some Border Terriers with attacks of involuntary movements improved upon dietary-gluten avoidance. It is prudent to feed a gluten-free food to high-risk dogs of the two breeds.*

*Dog food labeled as gluten-free might contain gluten due to ingredient cross contamination prior to or after arrival at the food-production site. Test kits for detection of gluten, in the form of a dipstick-style immunoassay, can be used for qualitative detection of gluten. Videos on the internet show the application and outcome for certain commercial, gluten-free dog foods (Note 4). Recent quantitative gluten analyses showed that 8 dog foods labelled as gluten- or grain-free were negative for gluten.*

*Wheat gluten in dog foods is well digestible, but it has a poor nutritive value due to its low content of lysine, an essential amino acid. However, good mixed foods containing wheat gluten meet the amino-acid requirements of dogs. More broadly, all four gluten-containing grains - wheat, barley, rye and oats - are suitable ingredients for dog food, but they are not necessary. Clearly, gluten-free food is indicated for dogs with apparent gluten hypersensitivity. Such dogs are small in number, but equally important.*

### **Gluten in grains**

Gluten proteins underlie the unique baking quality of wheat. They are composed of two major groups of numerous, related proteins high in glutamine and proline (4): the glutenins (glutelins) that are insoluble in aqueous alcohol, and the alcohol-soluble gliadins (prolamines). Gliadins are monomeric proteins with different primary structures. Glutenins have large, aggregated structures. Proteins similar to wheat gliadins are hordeins in barley, avenins in oats and secalins in rye, all being referred to as gluten.

With the use of RP-HPLC (analytical reversed-phase high-performance liquid chromatography), the two gluten-protein fractions, gliadins and glutenins, were measured in wheat, barley, rye and oats flour, each comprising a proportional mixture of four cultivars (5). The percentages of crude protein-gliadins-glutenins-gluten were 11.3-5.9-3.0-8.9 for wheat, 7.7-3.1-1.1-4.2 for barley, 7.1-2.5-0.6-3.1 for rye and 8.1-1.3-1.0-2.3 for oats. Gluten furnished 78% of crude protein in wheat, gluten's relative contribution being smaller for the other grain flours.

### **Gluten in dog food**

There are various commercially available immunochromatographic gluten assays (2, 6-8). The test kits are used to screen the presence of gluten in foods and beverages. Particular tests are based on antibodies that react either with both gliadins and glutenins (6) or with gliadins in wheat, rye and barley (7). The tests, which detect gluten levels as low as 10-20 mg/kg product (6, 7), showed that uncontaminated rice (6) and corn (8) are gluten-free. The test kits may be used for the detection of gluten in dog food (Note 5).

With the use of HPLC-HRMS (high-performance liquid chromatography coupled with mass spectrometry with high power of resolution), the amount of wheat flour equivalents was analysed in 10 commercial dog foods (9). Two dry foods with "cereals" as first ingredient - "cereals" was used as unspecified, collective term - contained 5.0 and 5.3 % wheat flour. For four dry and 4 wet foods, labelled as grain-free or gluten-free, the signal was below the quantification limit. However, that limit was 0.4%, which is relatively high (cf. Note 2).

### **Wheat-protein quality**

Wheat protein contains about 80% gluten. Dogs aged 9-12 weeks were fed semipurified diets containing 10% crude protein from either casein or wheat gluten as the sole protein source (10). The protein efficiency ratios (g weight change/g protein consumed) were 2.3 for casein and -0.4 for wheat gluten. When compared with the minimum, amino-acid requirements of 4- to-14-week-old puppies (11), the diet with wheat gluten was severely deficient in lysine (Note 6). In dogs with induced anemia plus hypoproteinemia, feeding wheat gluten versus casein lowered food intake or when eaten was unfavorable for blood protein production (12, 13).

With use of the difference method, three studies with adult dogs have determined the digestibility of crude protein in extruded (14), whole-ground (15) or autoclaved (16) wheat. The apparent, total-tract, crude-protein digestibilities were 83, 81 and 78% of intake. Two dog studies (17, 18) compared extruded, isonitrogenous diets containing either wheat gluten or poultry meal. Within studies, the inclusion rates of both protein sources were 46 or 33% and dietary crude protein levels were 22 or 23%. Apparent protein digestibilities for the diets with wheat gluten in place of poultry meal were 87.9 versus 82.4 (17) and 88.1 versus 84.0 % of intake (18).

### **Agenized wheat flour**

In 1937, Melnick and Cowgill (19) confirmed their earlier, serendipitous observations that dogs fed high-gliadin diets showed convulsive reactions. They suggested that the dogs' response was due to a protein sensitization. The gliadin used was isolated by means of 75% alcoholic extraction of wheat-gluten flour. In 1947, Newell et al. (20) published that generalized convulsions in dogs fed wheat gluten were associated with abnormalities in their electroencephalograms. Data obtained later indicate that the toxic gluten proteins must have been derived from agenized wheat flour.

The two papers (19, 20) do not mention the outbreaks of so-called canine hysteria, running fits or fright disease that occurred in the US, England and the Netherlands between 1920 and 1940.

Apparently healthy dogs experienced sudden, repeated anxiety attacks. In 1931, Klarenbeek (21) reported that the baked food given to affected dogs elicited the symptoms in 9 healthy laboratory dogs within a week. One dog died, but the others recovered within a few days after diet change. The offending, baked food was based on wheat flour (21).

In 1946, Mellanby (22) showed that agenzized wheat flour, or wheat flour treated with gaseous nitrogen trichloride, produced symptoms in dogs similar to fright disease, whereas the non-treated flour was not toxic. In those days, most wheat flour used to be agenzized as it produced a well-risen loaf of bread with smooth, crisp crust. Later studies (23) revealed that the toxic principle was not nitrogen trichloride itself, but methionine sulfoximine, a derivative of methionine.

### **Wheat allergy**

Today, grain-free dog food is mainstream, which by definition extends to gluten-free. The anti-grains conception is generally backed by six false arguments, one of them being that grains cause food allergy (24). Quite some dog foods make a “wheat-free” claim, which is inspired by common fear of wheat allergy. Food is often blamed for canine skin problems, clarifying the high demand for hypoallergenic foods in general, but also the interest in wheat-free foods. This demand is maintained by perceived effectiveness of hypoallergenic foods as a result of spontaneous recovery, a shift in season or associated interventions.

Veterinarian-guided feeding tests are necessary to diagnose food sensitivity. A hypoallergenic elimination diet should suppress the symptoms, followed by relapse when the dog is fed the original food. The prevalence of true canine food allergy has been rated at 0.05% (25). For 198 dogs with true food allergy, wheat was the culprit in 30 animals, or in 15% of the cases (3). Extrapolating that percentage to the whole dog population leads to a wheat-allergy prevalence of 0.0075% or one dog in 13,333.

### **Wheat-sensitive Irish Setters**

A 1984 paper (26) describes 10 Irish Setter dogs that were presented with poor weight gain or weight loss, with or without chronic diarrhea. Examination revealed that the animals had partial villus atrophy, increased intra-epithelial lymphocytes and selective alterations in brush border enzymes. These findings were interpreted as comparable to celiac disease in man.

In four litter mates bred from two of the 10 affected animals, wheat sensitivity was shown (27-29). When fed on meat and cereals, the offspring exhibited partial villus atrophy, which recovered on a commercial wheat-free diet and relapsed on subsequent challenge with wheat flour (20 g/day followed by 100 g/day for 6 weeks each). By one year of age, progeny (n= 6) reared exclusively on a grain-free diet had normal values for villus height, intraepithelial-lymphocyte density and brush-border alkaline phosphatase activity (30, 31). In that respect, they did not differ from clinically healthy, age-matched Irish Setters (n= 5) fed a wheat-containing diet.

Further studies indicated that Irish Setters with wheat sensitivity have greater permeability to <sup>51</sup>Cr-EDTA and probe sugars, and have lower anti-gliadin antibody concentrations than controls, irrespective of being fed a diet with or without wheat (30-34). Genetic studies concluded that gluten-sensitivity in Irish Setters does not resemble human celiac disease (35) and that inheritance is autosomal recessive (36). The above-mentioned studies concern Irish Setters found in England, but an affected Canadian family has been described also (37).

### **Gluten-sensitive Borden Terriers**

CECS (canine epileptoid cramping syndrome) is a paroxysmal movement disorder that occurs in Border Terriers. Affected dogs have episodes of abnormal, uncontrolled movement, often associated with signs of gastrointestinal disease, including intermittent vomiting, diarrhea, borborygmi and abnormal abdominal cramping (38). Many owners and breeders hold the view that a gluten-free diet reduces the frequency and severity of CECS episodes (38, 39). There is suggestive, experimental evidence that CECS in Border Terriers is gluten-sensitive.

Five privately owned Border Terriers with clinically confirmed CECS, and without a history of gluten-free feeding, were fed a commercial, exclusively gluten-free diet (39). After 9 months of strict dietary adherence, there was clinical improvement in all dogs, while serum anti-gliadin antibodies had dropped. In the open, non-controlled study, the five dogs underwent multiple dietary changes when going from their habitual, gluten-containing diet to the gluten-free diet. It was still concluded that CECS in Border Terriers is a gluten-sensitive movement disorder triggered and perpetuated by gluten (39).

CECS in Border Terriers is now termed paroxysmal gluten-sensitive dyskinesia as it is separate to epilepsy (40), but the effect of gluten as the only dietary variable has not yet been reported. Owners of Border Terriers changed their dog's diet, after a suspicion of CECS, to a commercial hypoallergenic diet/grain-free diet: 14 out of 19 owners thought to see a reduction in the frequency of episodes (41).

A Border Terrier with neurological signs, atopy and gastrointestinal disease was put on a gluten-free diet (40). Over the next 14 days, the owners reported no further abnormal episodes and also resolution of the pruritis and gastrointestinal signs. The serum level of anti-gliadin antibodies had decreased. The combination of neurological signs, atopy and gastrointestinal disease may constitute a particular syndrome in Border Terriers (42, 43).

### **Soft-Coated Wheaten Terriers**

Among Soft-Coated Wheaten Terriers, a familial syndrome of protein-losing enteropathy and/or nephropathy (PLE-PLN) has been reported (44). PLE is associated with inflammatory bowel disease and/or lymphangiectasia and PNE is the result of glomerulonephritis. Some breeders and owners held/hold the belief that the syndrome is related to gluten hypersensitivity (44), but a wheat-gluten challenge did not elicit adverse reactions (45). Thus, PLE-PLN in Soft-Coated Wheaten Terriers is not caused by a specific sensitivity to wheat gluten.

Soft-Coated Wheaten Terriers with familial PLE-PLN (n= 6), belonging to a research colony, were fed a diet free of wheat gluten for 6 weeks and then received 10 g supplemental gluten/day for 7 weeks (45). None of the dogs had more than an infrequent episode of vomiting, diarrhea or inappetence during gluten administration (Note 7).

In a provocative trial, six dogs from the research colony were fed cooked white rice and 7 different test meals, consisting of chicken, corn, cottage cheese, wheat, lamb, soy or milk (46). The test meals were consumed completely. All of the dogs exhibited once or more vomiting, diarrhea or pruritis, the most common reactions being to chicken and corn. It was concluded that food allergies are present in Soft-Coated Wheaten Terriers affected with the syndrome of PLE-PLN (46). That conclusion is based on a single challenge with each food item, but the outcome of a provocation-elimination-provocation test is weightier.

### **Note 1**

Gluten proteins similar to those found in wheat, barley, rye and oats are also present in other grains. These gluten-containing grains are triticale, a hybrid of wheat and rye, and ancient wheat varieties such as spelt and kamut (47). Corn (maize) and rice are gluten-free. Gluten-free alternative sources are the so-called minor cereals, sorghum, teff, millet and wild rice. Other gluten-free sources include the pseudocereals, quinoa, cañihua, chia and amaranth (48).

#### **Note 2**

The only known effective treatment of human celiac disease is a life-long gluten-free diet (cf. 8). Such a diet should contain less than 20 mg gluten per day to prevent a relapse of intestinal damage. To guarantee the safety of gluten-free products for people with celiac disease, various authoritative bodies recommend a threshold of 20 mg gluten/kg gluten-free products (cf. 8).

#### **Note 3**

Corn gluten (meal) contains about 60% protein. After removing oil, fiber and starch from corn kernels, the remaining corn gluten is ground into a meal. The production of rice gluten is similar. One may come across corn gluten or corn-gluten meal in the ingredient lists of some dry dog foods (a-d). A single dry dog food once declared rice gluten as ingredient (e, cf. 49).

Given the often used gluten-free claim on dog foods, which implies that gluten is bad for dogs, corn and rice gluten probably are considered label-unfriendly and thus kept out of ingredient lists or shoved under a collective ingredient term.

a. Sanimed Weight Reduction, Sanimed Weight Control.

<https://sanimedveterinarynutrition.nl/en/composition-sanimed-curative-dog.html>

b. Hill's Prescription Diet w/d Multi-Benefit. <https://www.chewy.com/hills-prescription-diet-wd-multi/dp/54717>

c. Diamond Hi-Energy. <https://www.diamondpet.com/dog/diamond/hi-energy/>

d. Retriever Hi Protein. <https://www.dogfoodadvisor.com/dog-food-reviews/retriever-dog-food/>

e. Wysong Anergén.

[http://www.dogfoodanalysis.com/dog\\_food\\_reviews/showproduct.php/product/333](http://www.dogfoodanalysis.com/dog_food_reviews/showproduct.php/product/333)

#### **Note 4**

The EZ Gluten® assay detects gluten levels in foods and beverages as low as 10 mg/kg product (6). Two videos show how to apply the test kit for checking dog foods labelled as gluten-free (f, g).

f. <https://www.youtube.com/watch?v=IrErS7nM5-o>

g. [http://www.glutenfreefox.com/articles/honest\\_kitchen\\_dog\\_food.html](http://www.glutenfreefox.com/articles/honest_kitchen_dog_food.html)

#### **Note 5**

Due to the omnipresent grain-free dog foods on the market, and the widely used no-wheat claim, wheat-containing dog foods have been relegated to the background, but they are provided. A few examples of complete dry foods with wheat are presented here. Foods with gluten carriers other than wheat can also be found on the petfood market.

Diamond Maintenance has wheat flour as the second ingredient, after chicken by-product meal (h). The extruded food, Pronature Original Adult, states wheat shorts as third ingredient, after chicken

meal and ground corn (i). The pressed food, ProCare Protection Puppy, declares wheat as third ingredient, after dehydrated poultry and corn (j). The baked food, Magnusson's Adult, declares whole wheat flour and barley meal as second and third ingredient, after fresh beef and pork meat (k).

Three foods having barley as part of their names also declare oat meal as ingredient (l-n). The dry dog food Burns Weight Control states a content of 55% oats (o), which may represent 1.27% gluten in the product. The extruded dog food Bosch Adult Geflügel & Dinkel (Poultry & Spelt) declares barley as first ingredient and four more gluten-containing ingredients as third, fourth, sixth and seventh ingredient, namely wheat meal, wheat, spelt and wheat bran (p). It seems that ingredient splitting took place with regard to wheat (cf. 50). Rye flour makes its appearance in some commercial dog biscuit treats (q-s) and in recipes for home-made dog treats (t-v).

h. <https://www.diamondpet.com/dog/diamond/maintenance/>

i. [http://www.pronature.ru/Pronature\\_new/en/dog-products/classic-adult2.html](http://www.pronature.ru/Pronature_new/en/dog-products/classic-adult2.html)

j. <https://www.prinspetfoods.com/dog/prins-procare-protection/prins-procare-protection-puppy>

k. <https://www.hundmat.com/en/products/magnussons-adult/>

l. Wellness Complete Health Lamb & Barley. <https://www.wellnesspetfood.com/natural-dog-food/product-catalog/complete-health-lamb-barley>

m. Nature's Recipe Rice & Barley. <https://www.amazon.com/NatureS-Recipe-Digest-Chicken-30-Pound/dp/B00AMG7YCY>

n. Purina Pro Plan Sport, Turkey & Barley. <https://www.purina.com/pro-plan/dogs/dry-dog-food/sport-active-27-17-high-protein-turkey-barley>

o. <https://fetch.co.uk/burns-weight-control-adult-senior-chicken-oats-dry-dog-food-100085011>

p. <https://www.heimfutterservice.de/de/bosch-standard/565-bosch-adult-geflugel-dinkel-3-kg.html>

q. Only Natural Pet, Fresh Kisses. <https://www.pawdiet.com/reviews/only-natural-pet-all-natural-health-treats-fresh-kisses-breath-tartar-biscuits-dog-treat/>

r. K9 Granola Factory Simply Biscuits. <https://www.amazon.com/K9-Granola-Factory-Simply-Biscuits/dp/B008D9WWIS>

s. Basil & Baxter's Peanut Butter Honey Large Dog Biscuits. <https://www.gotpetsupplies.com/products/basil-baxters-peanut-butter-honey-large-heart-dog-biscuits-10-lbs>

t. Vigil S. How to make dog treats at home that don't include whole-wheat flour. <https://dogcare.dailypuppy.com/make-dog-treats-home-dont-include-wholewheat-flour-3145.html>

u. Dog Treat Kitchen. Wheat free dog treats. Ham & cheese on rye dog cookie. <http://www.dogtreatkitchen.com/wheat-free-dog-treats-with-ham.html>

v. Miss Molly says. Dog lovin' homemade dog treats recipe. <https://missmollysays.com/dog-lovin-homemade-dog-treats-recipe/>

## **Note 6**

The study that measured protein efficiency ratios involved three semipurified diets containing 10% crude protein from either casein, soy-protein isolate or wheat gluten as the sole protein source (10). The table below compares the minimal requirements of the 10 essential amino acids for growth of puppies aged 4-14 weeks and the supply of these amino acids by the diets with casein, soy protein isolate or wheat gluten. The amounts of amino acids in the table are given as g/MJ metabolizable energy.

The experimental diets had a calculated energy density of 1.67 MJ/100 g (cf. 51). The National Research Council has set minimal amino-acid requirements (MR) and recommended allowances (RA) that are expressed as g/1000 kcal (11), or g/4.184 MJ. Published amino acid compositions of casein (52), soy-protein isolate (53) and whole wheat gluten (54) were used to calculate the amino-acid contents of the diets.

| Amino acid     | MR   | RA   | Casein | Soy  | Gluten |
|----------------|------|------|--------|------|--------|
|                | g/MJ |      |        |      |        |
| Arginine       | 0.38 | 0.47 | 0.27   | 0.46 | 0.14   |
| Histidine      | 0.19 | 0.23 | 0.23   | 0.16 | 0.13   |
| Isoleucine     | 0.31 | 0.39 | 0.41   | 0.29 | 0.24   |
| Leucine        | 0.62 | 0.77 | 0.71   | 0.46 | 0.41   |
| Lysine         | 0.42 | 0.53 | 0.58   | 0.36 | 0.07   |
| Methionine     | 0.17 | 0.21 | 0.21   | 0.08 | 0.11   |
| Met + cystine  | 0.33 | 0.42 | 0.24   | 0.15 | 0.23   |
| Phenylalanine  | 0.31 | 0.39 | 0.38   | 0.31 | 0.29   |
| Phe + tyrosine | 0.62 | 0.78 | 0.79   | 0.53 | 0.52   |
| Threonine      | 0.39 | 0.49 | 0.32   | 0.22 | 0.15   |
| Tryptophan     | 0.11 | 0.14 | 0.10   | 0.08 | 0.25   |
| Valine         | 0.32 | 0.41 | 0.52   | 0.28 | 0.06   |

MR = minimal amino-acid requirement; RA = recommended allowance. Casein, Soy and Gluten refer to diets with 10% crude protein (1.67 MJ/100 g) and either casein, soy-protein isolate or wheat gluten as the sole protein source.

The protein efficiency ratios (g weight change/g protein consumed) were 2.3 for casein, 1.2 for soy protein and -0.4 for wheat gluten (10). When compared with the minimal amino-acid requirements, the first limiting amino acids in the casein, soy and gluten diets were arginine, methionine + cysteine and lysine, the relative amounts being 71, 45 and 17%. As would be expected, a higher degree of protein deficiency was associated with a lower protein efficiency ratio.

#### **Note 7**

The abstract of the paper states that serum globulin concentration decreased significantly after prolonged administration of gluten (45). Gluten intake was associated with a decrease in serum globulin from 2.95 to 2.50 g/l, but the before and after measurements were not accompanied by a parallel control group.

#### **Note 8**

It has been reported that sera of dogs with lymphoplasmacytic enteritis (55) or intestinal T-cell lymphoma (56) had increased concentrations of antibodies against gliadin. Possibly, the dogs were hypersensitive to gluten-containing ingredients in their diet, which might have contributed to their condition.

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