

# Questionnaire-Based Pet Owner Evaluation of Gastrointestinal Tolerance of a New High Protein-Low Carbohydrate Diet Range in Adult Dogs

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## ABSTRACT

The present survey, based on a multiple-choice questionnaire, was undertaken to assess the gastrointestinal tolerance of four commercial diets from the Veterinary HP-M™ diet range. The four diets were tested in 284 adult dogs over a 28-day period and compared to their usual diets. Online evaluations were performed at the beginning of the study, at the end of the 4-day diet transition, and at 7, 14 and 28 days. Digestive sensitivity with the dog's usual food was reported in 28% of the enrolled dogs. At least 94% of the pet owners declared they were satisfied with the transition to the tested diets. Average stool consistency scores with the tested diets remained steady at the optimal value of 2.2. Globally acceptable stool odour and small or normal stool volume were reported in over 70% of the dogs.

Unchanged or decreased faeces odour and volume were described in more than 70% of the dogs and unchanged or increased stool consistency was reported in more than 77% of the dogs in comparison to their previous diet. Faecal consistency and stool volume were not significantly different between the different time points. The number of dogs showing flatulence was significantly lower on days 7, 14 and 28 than on day 0.

In conclusion, a safe diet transition and a high digestive tolerance was reported after feeding various-sized adult dogs of different breeds with the tested Veterinary HPMTM diets.

## INTRODUCTION

Faecal characteristics are important parameters for dog owners to assess diet quality and digestibility. Various factors such as the source, amount, and quality of proteins, starches, fibres, and minerals can affect the digestibility of dry-expanded diets (Goudez, 2011). Large breed adult dogs, and particu-

**Table 1.** Recorded parameters for the assessment of dog gastrointestinal tolerance

	Time of Recording				
	Day 0	Day 4	Day 7	Day 14	Day 28
Digestive sensitivity	x				
Previous dietary transition within the last 12 months	x				
Owner's perception about dietary transition between the usual diet and the test diet		x			
Faecal consistency	x	x	x	x	x
Evolution of faecal consistency compared to the usual diet			x	x	x
Stool odour	x		x	x	x
Evolution of stool odour compared to the usual diet			x	x	x
Stool volume			x	x	x
Evolution of stool volume compared to the usual diet			x	x	x
Flatulence	x		x	x	x

larly sensitive ones, can exhibit higher faecal moisture, increased frequency of soft stools, as well as an increased number of defaecations compared to small dogs (Weber et al., 2006; Nery, 2012). Thus, some authors have underlined the need to develop new nutritional strategies to improve faecal quality in large and sensitive dogs (Nery, 2012).

On the other hand, small breeds have a relatively lower digestive capacity. Thus, more digestible foods could be of interest for small breed dogs (Weber, 2003).

The principle of High Protein and Low Carbohydrate (HP-LC) diets has been the subject of research in dogs for several decades. According to the literature, HP-LC diets can be of interest for weight stabilisation, maintenance of muscle mass, and regulation of blood sugar (Diez, et al. 2002; Blanchard, et al. 2004; Chaix, et al. 2014; Hill, et al. 2001; Wakshlag et al. 2003; Kronfeld et al. 1977; Hill, et al. 2009; Hewson-Hughes, et al. 2011; Roudebush & Schoenherr, 2010; Prélaud & Harvey, 2006). The new Veterinary HPM diet range (Virbac SA, France) are HP-LC diets that offer dogs a nutritious alternative to high carbohydrate diets.

Recently, a study (Chaix, et al. 2016)

demonstrated the digestive tolerance of 3 diets from the Veterinary HPM diet range in various sized puppies and growing dogs of different breeds. Preliminary experimental studies have already shown that Veterinary HPM diets can be used safely in adult dogs. The aim of the present study was to provide evidence of the gastrointestinal tolerance of 4 diets from the Veterinary HPM diet range in adult dogs in field conditions.

## MATERIALS AND METHODSS

### Survey Design

Dog owners were recruited by an independent company specialised in customer satisfaction research. Each owner was asked to test a new diet issued from the Veterinary HPM range for 28 days. During the test period, owners had to complete a multiple-choice questionnaire online. The questions focused on the gastrointestinal tolerance of their dogs with their usual diet (D0), at the end of the 4-day diet transition (D4), and with the test diet alone after 7 (D7), 14 (D14), and 28 (D28) days (Table 1). Stool consistency, odour and volume with the test diet were assessed by the dog owners using a 1-to-5 scoring standard scale (Fig. 1), with a classification into five categories for odour

**Table 2. Tested diets ingredients and guaranteed analysis**

Ingredients	ADST		ADNST		ADLM		ADNLM	
Guaranteed analysis*	Moisture*	9	9	9	9	9	9	9
	Proteins*	35	35	35	35	35	35	35
	Animal to vegetable protein ratio	89/11	89/11	89/11	89/11	89/11	89/11	89/11
	Fat*	11	14	14	17	14	14	14
	Minerals*	8	7.5	7.5	8	7.5	7.5	7.5
	Crude cellulose*	5	9.5	9.5	5	9.5	9.5	9.5
	Nitrogen free extract (NFE) *	26	25	25	26	26	25	25
	Starch*	22	20	20	22	20	20	20
	Calcium*	1.3	1.3	1.3	1.3	1.3	1.3	1.3
	Phosphorus*	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Ca/P	1.3	1.3	1.3	1.3	1.3	1.3	1.3	
Measured metabolisable energy (kcal/100g)	382	329	329	382	329	329	329	
Energy from protein (%)	34	37	37	34	34	37	37	
Energy from fat (%)	40	36	36	40	40	36	36	
Energy from NFE (%)	25	27	27	25	25	27	27	

\*% on a crude matter basis

**Table 3** Origin of usual diet

	ADST	ADNST	ADLM	ADNLM
Total number of dogs	46	36	126	76
Veterinary practice*	3 (7%)	2 (6%)	1 (1%)	5 (7%)
Other specialized distribution channel*†	16 (35%)	13 (36%)	62 (49%)	31 (41%)
Mass retailer*	27 (59%)	21 (58%)	63 (50%)	40 (53%)

\* Number of dogs (percentage)

† Other specialized distribution channels included pet shops, garden centres, internet and others

(very slightly odorous (very acceptable), slightly odorous (acceptable), mildly odorous (slightly acceptable), odorous (slightly bearable), and very odorous (unbearable)), and a classification into three categories for volume (small, normal or large). Appreciation of stool odour was defined as “globally acceptable” when it was slightly acceptable, acceptable, or very acceptable. Stool consistency, odour, and volume were compared between the test diet and the dog’s usual diet as decreased, unchanged, or increased. When flatulence was observed under the test diet, the owners were asked if the frequency was increased or decreased compared with the usual diet.

### Test Diets

Four HP-LC diets specially formulated for adult dogs issued from the Veterinary HPM™ diet range (Virbac SA, France) were tested:

- Adult Dog Small & Toy (ADST)
- Adult Dog Neutered Small & Toy (ADNST)
- Adult Dog Large & Medium (ADLM)
- Adult Dog Neutered Large & Medium (ADNLM)

Ingredients and guaranteed analysis of the diets are presented in Table 2.

The test diets were packed in neutral bags labelled with the appropriate feeding table. The daily ration was offered to the animals in one or several meals in accordance with the pet owners’ habits. The proportion of the test diet in the usual diet was progressively increased during a diet transition over the first 4 days of the study as

follows: 25% vs 75% on the first day, 50% vs 50% on the second and third days; 75% vs 25% on the fourth day. From the fifth day of the study onwards, the test diet was the exclusive food.

### Analytical Method

Statistical analyses were performed using SAS 9.3. Likelihood ratio chi-square tests were used to compare gastrointestinal tolerance parameters between the different time points (D0=usual diet, D7/D14/D28=test diet) for each diet (ADST, ADNST, ADLM, and ADNLM). A linear mixed model with time as fixed effect and subject as random effect was used to compare the mean consistency faecal scores between the different time points for each diet. A threshold value of  $\alpha = 0.05$  was used to define significance.

## RESULTS

### Animals

Adult dogs between 1 and 12 years old and used to eating dry commercial kibble diets were enrolled in the study.

The origin of the dogs’ usual diets is detailed in Table 3. Most diets were bought in supermarkets or specialised shops, and much less frequently in veterinary practices.

The questionnaire was administered to 284 household dog owners. Forty-six, 36, 126, and 76 of them were provided with ADST, ADNST, ADLM, and ADNLM, respectively. Ninety-two percent of the dogs were pure breeds of various sizes. All the characteristics of the tested animals are presented in Table 4.

**Table 4. Testing animal characteristics per test diet**

		ADST	ADNST	ADLM	ADNLM
Total number of dogs		46	36	126	76
	Beauceron			2 (2%)	2 (3%)
	Belgian Shepherds†			6 (5%)	5 (7%)
	Border Collie			8 (6%)	2 (3%)
	Boxer				2 (3%)
	Bulldogs‡		1 (3%)	15 (12%)	1 (1%)
	Cane Corso			2 (2%)	1 (1%)
	Carlin	3 (7%)	1 (3%)		
	Cavalier King Charles	2 (4%)	2 (6%)		
	Cocker Spaniels§			13 (10%)	3 (4%)
	Coton de Tulear	4 (9%)	2 (6%)		
	Dalmatian			2 (2%)	2 (3%)
	Doberman			3 (2%)	1 (1%)
	Dutch Shepherd			1 (1%)	2 (3%)
	German Shepherd			4 (3%)	14 (18%)
	Golden Retriever			12 (10%)	8 (11%)
	Lhasa Apso	2 (4%)			
	Labrador Retriever			15 (12%)	10 (13%)
	Pinsher	2 (4%)			
	Pointers¶			3 (2%)	2 (2%)
	Ratter		2 (6%)		
	Setters**			7 (6%)	5 (7%)
	Shih Tzu	12 (26%)	6 (17%)		
	Spaniels††	1 (2%)		9 (7%)	1 (1%)
	Terriers‡‡	11 (24%)	12 (33%)	7 (6%)	
	Wirehaired Pointing Griffon			2 (2%)	1 (1%)
	Other breeds§§	3 (7%)	2 (6%)	10 (8%)	10 (13%)
	Crossbred	6 (13%)	8 (22%)	5 (4%)	4 (5%)
Sex N* (%)	Female	20 (43%)	11 (31%)	71 (56%)	46 (61%)
	Male	26 (57%)	25 (69%)	55 (44%)	30 (39%)
Age (years)	Mean (+/- SD¶¶)	4.50 (+/- 2.27)	4.36 (+/- 1.69)	4.52 (+/- 2.80)	4.86 (+/- 2.70)
	Minimum	2.00	2.00	1.00	1.00
	Maximum	8.00	7.00	12.00	12.00
Weight (kg)	Mean (+/- SD¶¶)	6.78 (+/- 1.81)	6.88 (+/- 1.97)	24.66 (+/- 9.13)	28.53 (+/- 8.13)
	Minimum	3.80	3.90	10.00	11.00
	Maximum	10.00	11.00	52.00	58.00

\* Number of dogs

† Belgian Shepherds included Groenendael, Malinois, and unidentified Belgian Shepherds

‡ Bulldogs included English and French Bulldogs

§ Cocker Spaniels included American, English, Tibetan and Springer Cocker Spaniels

¶ Pointers included German Shorthaired, Weimar, and unidentified Pointers

\*\* Setters included English and Irish Setters

†† Spaniels included Brittany, French and unidentified Spaniels

‡‡ Terriers included Bull, Cairn, Fox, Irish, Jack Russel, Parson Russel, Scottish, West Highland White, and Yorkshire Terriers

§§ Other breeds, each represented by only one dog for each test diet, included Bichon, Dachshund, and Poodle for ADST, Dachshund and Maltese for ADNST, Australian Shepherd Dog, Basenji, Basset Fauve de Bretagne, Chow Chow, Landseer, Portuguese Water Dog, Rottweiler, Samoyed, Siberian Husky, and White Suisse Shepherd Dog for ADLM, and Arigeois, Australian Shepherd Dog, Berger de Beauce, Australian Cattle Dog, Chow-Chow, Dogue de Bordeaux, Siberian Husky, Rhodesian Ridgeback, Rottweiler, and Shar Pei for ADNLM

¶¶ Standard deviation

**Fig. 1.** 1-to-5 scoring standard scale for assessment of faeces quality



Score 1 : Hard and dry stools



Score 2 : Well-formed and firm stools



Score 3 : Well-formed and loose stools



Score 4 : Unformed stools



Score 5 : Liquid stool

## Usual Diet

### *Digestive Sensitivity*

Thirty-seven percent, 28%, 29%, and, 21% of the adult dogs selected for testing ADST, ADNST, ADLM, and ADNLM, respectively, were described by their owners as being digestively sensitive. Manifestation of gastrointestinal sensitivity included diarrhoea in 65%, 60%, 75%, and 63% of the dogs, respectively, vomiting in 35%, 50%, 19%, and 31% and/or flatulence in 24%, 20%, 42%, and 50% of the dogs, respectively.

### *Faecal Consistency*

The faecal consistency scores with the dogs' usual diets are presented in Fig. 2. The great majority of the dogs (96%, 97%, 98%, and 93%, respectively) had well-formed stools (faecal scores 1 to 3). The mean faecal consistency scores with the usual diet were very similar in the four groups of dogs (2.1, 2.1, 2.2, and 2.2 respectively) (Fig. 3).

### *Stool Odour*

Appreciation of stool odour with the dogs' usual diets is presented in Fig. 5. In most

dogs (93% for ADST, 85% for ADNST, 86% for ADLM, and 75% for ADNLM), the owners described stool odour as globally acceptable.

### *Flatulence*

Flatulence with the usual diet was reported by 54%, 47%, 68%, and 66% of the dog owners selected for testing ADST, ADNST, ADLM, and ADNLM, respectively.

### **Previous Experience of Diet Transition**

Forty-seven percent, 40%, 56%, and 52% of the pet owners participating in the study for testing ADST, ADNST, ADLM, and ADNLM, respectively, had already experienced previous diet transitions when changing their dog's diet.

On this occasion, gastrointestinal troubles were observed in 13%, 20%, 25%, and 19% of the dogs, respectively: soft faeces (in 0%, 50%, 46%, and 100% of the dogs, respectively) and/or diarrhoea (50%, 0%, 31%, and 0% of the dogs, respectively), and/or flatulence (in 50%, 0%, 77%, and 0% of the dogs, respectively).

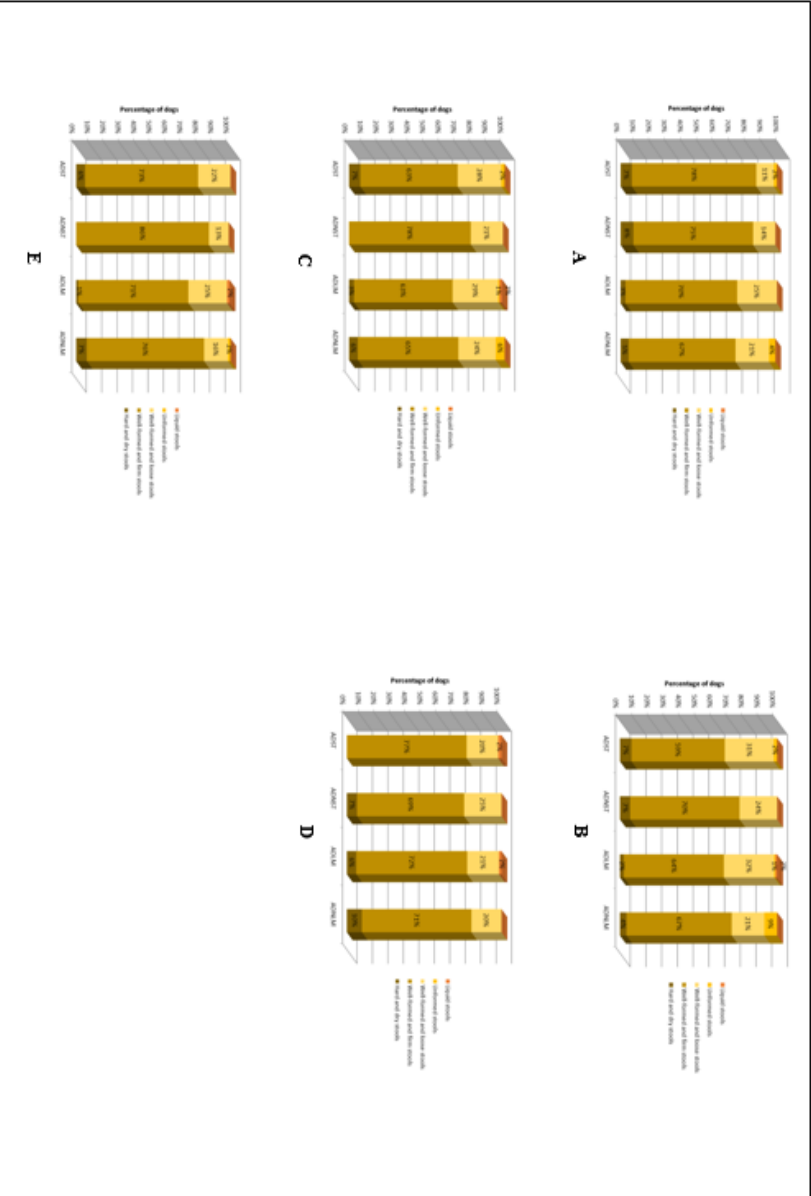
### **Test Diets**

The number of available owner appreciations during the course of the study from D4 to D28, varied from 44 to 32 for ADST, 33 to 29 for ADNST, 121 to 98 for ADLM, and 75 to 60 for ADNLM.

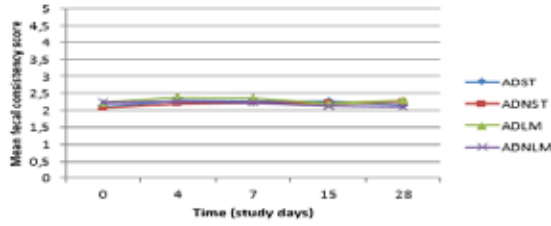
### *Faecal Consistency*

With all the test diets, 92 to 100% of the dogs had well-formed stools from D4 to D28 (Fig. 2). The faecal consistency assessment according to the 1-to-5 scoring standard scale (Fig. 1) did not significantly differ between the different time points, except between D0 and D4 for ADLM with a decrease in the number of dogs showing

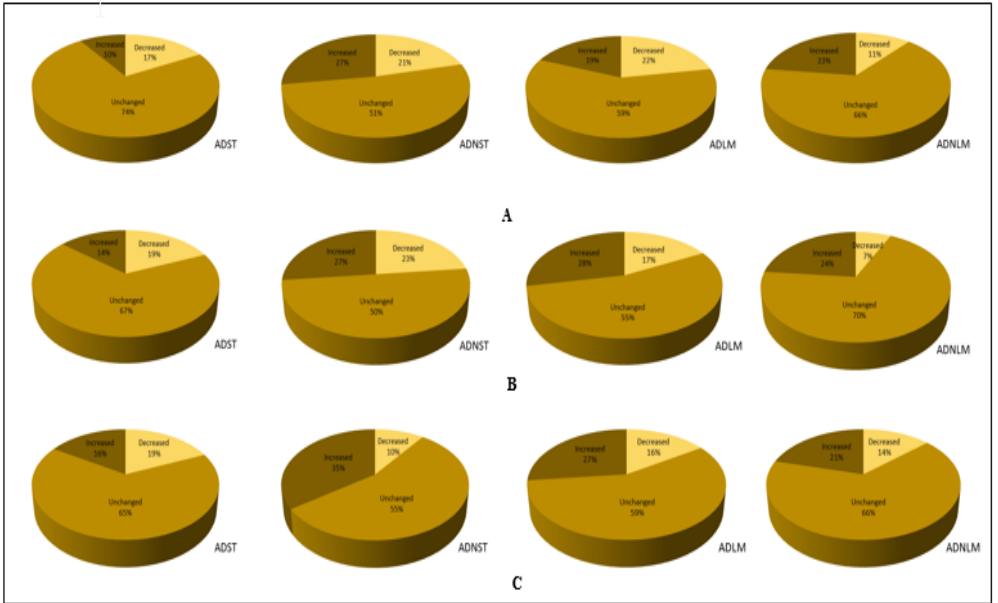
**Fig. 2**  
**Distribution of fecal consistency scores with the usual diet (A), on D4 at the end of the dietary transition (B), and on D7 (C), D14 (D), and D28 (E) with the test diet alone**



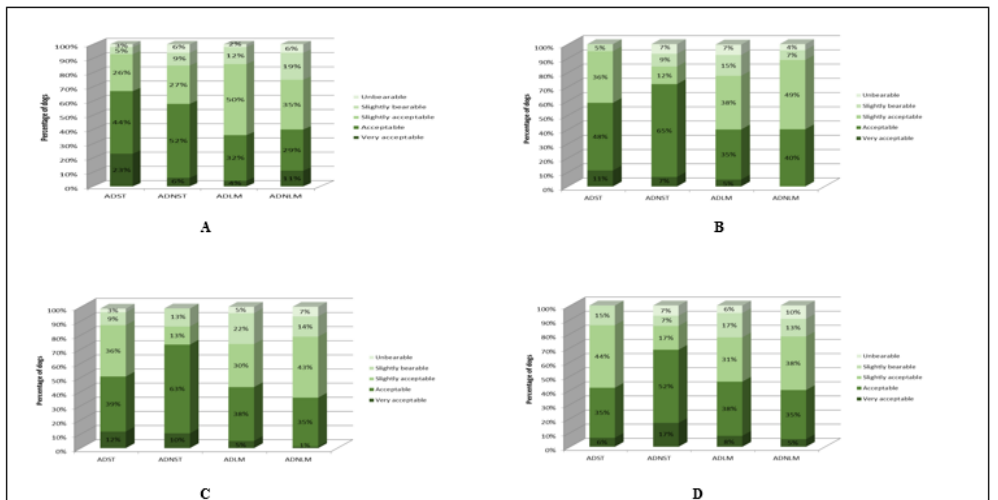
**Fig. 3**  
**Mean fecal consistency scores with the usual diet, on D4 at the end of the dietary transition, and on D7, D14 and D28 with the test diet alone**



**Fig. 4**  
**Evolution of fecal consistency on D7 (A), D14 (B), and D28 (C) with the test diet compared to the usual diet**



**Fig. 5**  
**Appreciation of stool odour with the usual diet (A) and on D7 (B), D14 (C) and D28 (D) with the test diet alone**





well-formed and firm stools and an increase in those exhibiting well-formed and loose stools ( $p = 0.04$ ). However, the mean faecal consistency scores remained between 2.1 and 2.4 in all groups throughout the study with no significant differences between the different time points (Fig. 3). Compared to the usual diet, unchanged or increased stool consistency was reported in 77 to 94% of the dogs (Fig. 4).

### **Stool Odour**

Seventy-three to 95% of the owners reported globally acceptable stool odour when the test diets were administered alone between D7 and D28 (Fig. 5). When considering all test diets, unchanged or decreased faeces odour was described in 74 to 97 % of the dogs, in comparison to their previous diet (Fig. 6).

### **Stool Volume**

Stool volume was reported as small or normal in 72% to 94% of the dogs throughout the study (Fig. 7). Whatever the test diet, stool volume was not significantly different between the different time points (D7, D14, D28). Compared to the usual diet, the volume of faeces did not change or decreased in 72% to 86% of the dogs (Fig. 8).

### **Flatulence**

Sixty-six to 89% of the dogs had no flatulence from D7 to D28. Moreover, a decreased frequency of flatulence was observed in 2 to 8% of the dogs (Fig.9). In most cases, the few dogs experiencing flatulence with the test diets had already experienced it with their usual diet and the proportion of dogs with flatulence was significantly lower at D7, D15, and D28 in all groups compared to D0 ( $0.0001 < p < 0.0124$ ).

### **Satisfaction**

Ninety-four percent, 94%, 95%, and 100% of the pet owners whose dogs were fed with ADST, ADNST, ADLM, and ADNLM, respectively, were satisfied with the dietary transition.

The satisfaction rates and scores at the end of the study were 91% (7.4/10), 91% (7.7/10), 83% (7.3/10), and 83% (7.5/10)

with ADST, ADNST, ADLM, and ADNLM, respectively.

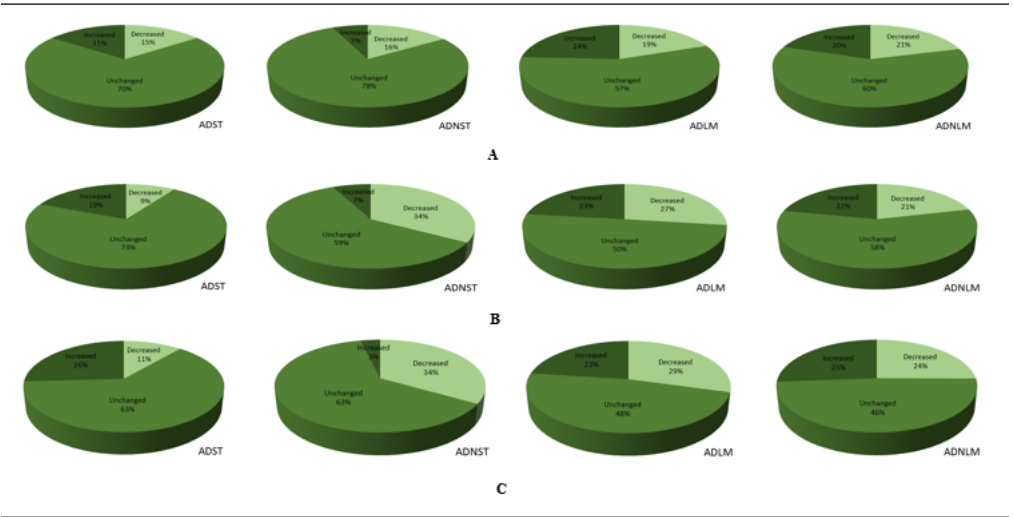
## **DISCUSSION**

Diet selection in animals has long been evaluated in relation to energy intake. Research over the past 2 decades has demonstrated the critical role of macronutrient balance (Hewson-Hughes, 2013). Recent studies show that, given the choice, dogs and cats show a clear preference for high animal protein foods (Dillizer et al., 2011). Based on these observations, biologically appropriate pet foods should be rich in protein or have meat as the top ingredient, and be low in carbohydrates.

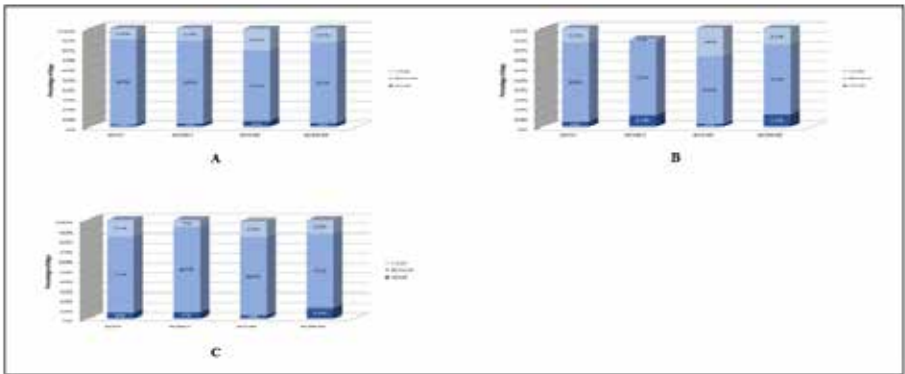
The FEDIAF Guide (FEDIAF, 2014) considers that dogs do not require carbohydrates in their diets, and according to the National Research Council's Committee on Animal Nutrition (2006), "there appears to be no requirement for carbohydrates provided sufficient protein is given". The development of the HP-LC Veterinary HPM range diets is based on this nutritional approach. A good digestive tolerance of these diets has already been demonstrated in young animals of various breeds (Chaix, et al. 2016). Even if higher apparent digestibility has been shown in adults compared to puppies (Weber et al., 2003), the safety of these diets remains to be proven in adult dogs in a number of various breeds and weights. This is all the more important as a significant effect of body size on faecal quality has been reported in adult dogs (Weber et al., 2006; Nery, 2012).

Compared with small dogs, a lower faecal consistency and higher faecal moisture in large dogs have been pointed out in several studies, more specifically in certain sensitive breeds such as German Shepherds, Labrador Retrievers, Great Danes and Giant Schnauzers, (Weber, et al., 2003 ; Weber, et al., 2006 ; Nery, et al., 2010 ; Goudez, et al. 2011 ; Nery ,et al., 2012). Many hypotheses on the mechanisms affecting faecal quality in large breeds have been proposed. Because the relative mass of the gastrointestinal tract is lower in large dogs (3–4% of their

**Fig. 6**  
Evolution of stool odour on D7 (A), D14 (B), and D28 (C) with the test diet compared to the usual diet



**Fig. 7**  
Evaluation of stool volume on D7 (A), D14 (B), and D28 (C) with the test diet



**Fig. 8**  
Evaluation of stool volume on D7 (A), D14 (B), and D28 (C) with the test diet compared to the usual diet

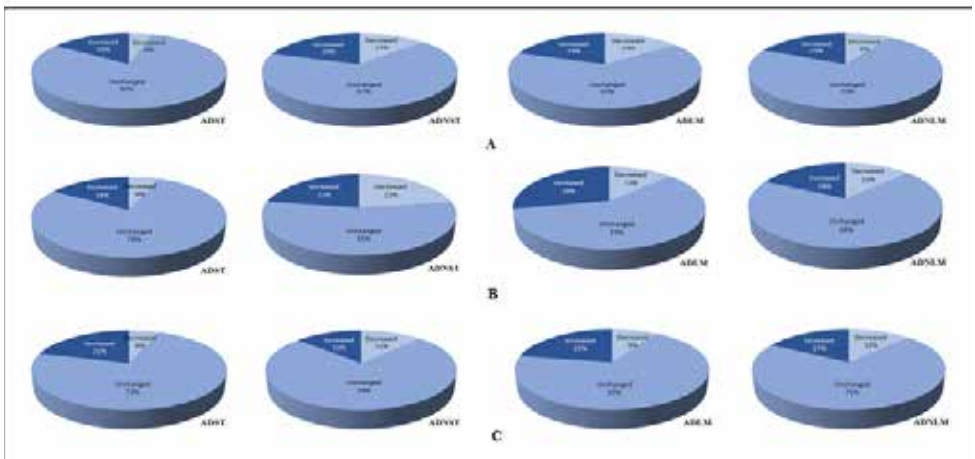
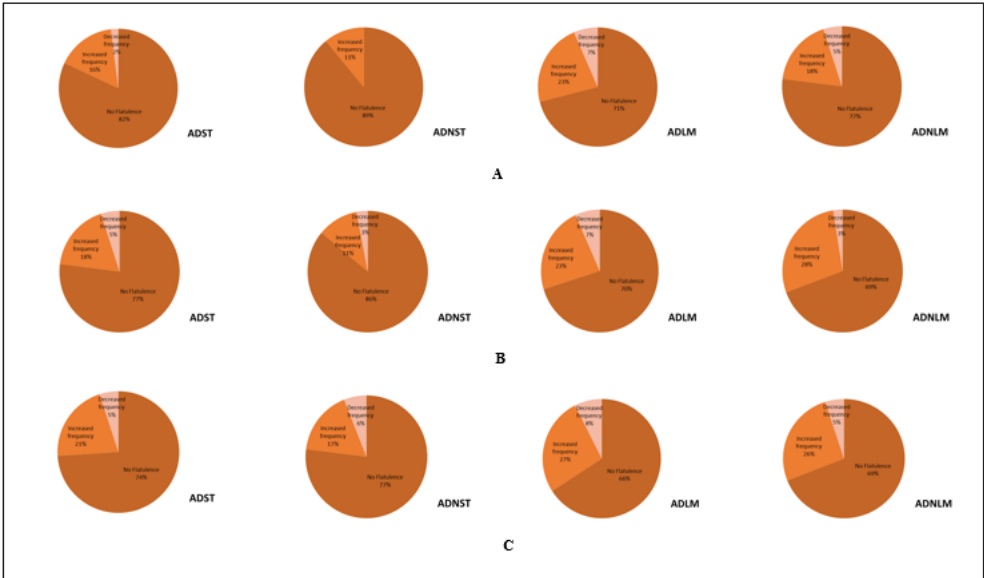


Fig. 9  
Frequency of flatulence on D7 (A), D14 (B), and D28 (C) with the test diet compared to the usual diet



bodyweight) compared to smaller ones (6–7% of their bodyweight), a lower digestive efficiency in larger dogs seemed to be the more obvious explanation (Weber et al., 2003). However, except for crude fat, Weber et al. (2003) demonstrated that apparent digestibility increased with the dog's body size. Since apparent digestibility is a crude reflection of the processes occurring within the gastrointestinal tract, which include the contribution of colonic fermentation, some authors (Weber, et al., 2003 ; Weber, et al., 2006 ; Nery, et al., 2010 ; Goudez, et al. 2011 ; Nery, et al., 2012) hypothesized that higher digestibility coefficients in larger dogs as well as poor faecal quality could result from more extensive carbohydrate and protein colonic fermentation. On the basis of the results of a previous study reporting a lower apparent absorption and higher faecal loss of sodium and potassium in Great Danes than in Beagles, the same authors put forward another supposition. Absorption of electrolytes, mainly sodium ions, across the intestinal or colonic mucosa being a major determinant of water uptake, a low absorption of those ions in large breed dogs could also explain a net higher secretion of water

into the gut lumen through osmotic pressure (Weber, et al., 2003 ; Weber, et al., 2004 ; Weber, et al., 2006 ; Nery, et al., 2010 ; Goudez, et al. 2011 ; Nery, et al., 2012).

Lastly, Weber, et al., 2006, suggested that an increased intestinal permeability and a prolonged colonic transit time could also be, at least in part, a reason of poor digestive tolerance in large breeds.

From the previous observations, it appears important to limit colonic fermentative activity in large dogs. This could be achieved, firstly by using highly digestible diet, which will reduce the quantity of residues arriving in the colon, and secondly by reducing the quantity of fermentable fibre in diet (Weber, et al., 2006; Nery, et al., 2010). A diet formulated with highly digestible proteins led to reduced concentrations of protein-based fermentation products in faeces together with improved faecal quality in dogs, especially in large sensitive ones (Nery, et al., 2012). Thus, when formulating a diet, the digestibility of the protein sources is an important factor to be considered to improve faecal quality in dogs of different body sizes and digestive tolerance (Nery, et

al., 2010). Conversely, increased dietary protein concentrations are suspected to lead to a higher faecal score by affecting the quantity of substrate available for colonic fermentation (Nery, et al., 2010).

In fact, the results of the present study show no change or even improvement in faecal consistency between the usual diet and the HP-LC tested diets in over 77% of the dogs. Faeces with the HP-LC Veterinary HPM Virbac range diets had a normal appearance with mean stool consistency scores remaining between 2.1 and 2.4, very close to the ideal score (i.e. between 2 and 3). This observation also applied to large breed dogs. Indeed, amongst the 25 Labradors and the 18 German Shepherds enrolled in the study, only two dogs showed a faecal consistency score of 4 (unformed stools) at only one time point, while the other dogs had well-formed stools throughout the study period. Moreover, the safety of the dietary transition from many varieties of canine foods was confirmed by owner satisfaction (more than 94% declared they were satisfied) and the stability of faecal scores before the start of the study and at the end of the transition period.

There were also very few changes in stool odour and volume during the course of the study. Faecal quality (consistency, volume, and odour) was not affected by the increased amount of proteins in the tested diets, reflecting good digestive tolerance of this new range of diets in adult dogs. This was confirmed by the significant decrease in the frequency of flatulence with the test diets (flatulence in about 11 to 34% of the dogs) compared to the usual diets (flatulence in about 47 to 68% of the dogs) from D7 to D28.

The assessment of faecal quality based on surveys and evaluations by the owners is certainly more subjective than evaluations made in experimental studies. Nevertheless, subjectivity bias was significantly limited in this study by the use of a clear score chart illustrated by photographs for the assessment of faecal consistency, by an evaluation pro-

cess that compared the same parameters in both the test diets and the dogs' usual diets, and by the choice of closed ended questions limiting the number of possible answers.

In conclusion, the average stool consistency scores remained stable at the optimal value of 2.2 during the 24-day period when the test diets were administered alone. Faeces odour and volume were unchanged or improved in most dogs and the proportion of dogs with flatulence significantly decreased compared to D0. The 4 Veterinary HPM Virbac products were demonstrated to have a digestive tolerance at least similar or even higher than that of the dogs' usual diets in small, medium and even large breed dogs.

In summary, it can be considered that the new formulations of the Veterinary HPM Virbac range diets have a good gastrointestinal tolerance in adult dogs. These results confirm similar previous observations reported in puppies and growing dogs (Chaix, et al. 2016).

## CONCLUSION

HP-LC diets have been demonstrated to be of interest for helping to prevent some health problems (Chaix, et al., 2014; Leriche, et al., 2014; Hill, et al., 2001; Wakshlag, et al., 2003; Kronfeld, et al., 1977; Reynolds, et al., 1999; Paquin, 1979; Pibot, 1988; Funaba, et al., 2002; Hill, et al., 2009; Hewson-Hughes, et al., 2013). As previously reported in puppies and growing dogs, the formulation of the Veterinary HPM Virbac range diets, as it is close to the natural diet of carnivores, may explain their safe use during the critical time of diet transition as well as their high digestive tolerance in various sized adult dogs of different breeds observed in the present study.

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