

## Blood Lipids, Fecal Fat and Chymotrypsin Excretion in the Dog: Influence of Age, Body Weight and Sex

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**ABSTRACT.** Effects of physiological variables (age, body weight and sex) on lipemia (total lipids, phospholipids, triglycerides and NEFA), fecal fat and chymotrypsin excretion were examined in dogs. On comparing various ages and body weights, they found statistically significant differences, while only total lipids showed a statistically significant difference between the two sexes. Moreover, there was a significant correlation between body weight and fecal fat and chymotrypsin excretion. The results obtained contribute to the evaluation of small intestine functionality, especially in relation to some diseases, as malabsorption, maldigestion and steatorrhea in the dog.

**KEY WORDS:** blood lipid, canine, fecal chymotrypsin, fecal fat.

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Knowledge of the physiological processes involved in digestion and absorption of different nutrients is very important for clinical explanation of some digestive disorders in the dog [14]. In fact, the effect of maldigestion and malabsorption is greater on fats than on other nutrients, as these processes are characterised by a specific sequence of events [17]. Therefore, total lipids, phospholipids, triglycerides, NEFA and fecal excretion are very important parameters in evaluation of small intestine functionality.

Moreover, we know that fecal fats are composed by endogenous fat excretion (colon desquamation cells, colon secretion, bacterial synthesis), which is relatively constant [8]. The tests which can be made on feces enable us to understand the functionality of the digestive system and associated glands. Chemical tests on feces enable us to evaluate the motor and secretory activity of the digestive system by determining some enzymes (amylolytic, lipolytic, proteolytic). Feces are mainly made up of residual food, bacteria, digestive gland secretions and desquamated enteric cells, and their content is influenced by diet, digestive capacity (physiological quantities of various enzymes) and intestinal motility [4, 5]. We can verify regular pancreatic functionality by determining blood p-aminobenzoic acid (PABA) concentration [21].

In the study of protein digestion, trypsinic activity is commonly determined in serum and feces, as it has been observed that a decrease in trypsinogen secretion is frequently an indicator of gross pancreatic exocrine insufficiency. However, several studies carried out on dogs in physiological and clinical conditions have shown that a number of biological variables, such as diet and the techniques used considerably influence the measuring of trypsinic activity [2, 16, 18, 20, 26]. Intestinal flora can destroy pancreatic enzymes during their regular intestinal transit and fecal proteolytic bacteria can simulate trypsinic activity and alter the results of this test. Some authors have obtained significant results on humans, by seeking chymot-

rypsin in feces, as it seems to be more stable than trypsin during intestinal transit [1, 7]. On the basis of this knowledge, we decided to study how some physiological variables, such as age, body weight and sex influence lipemia, fecal fat and chymotrypsin excretion in the dog, in order to contribute to the evaluation of digestive processes in dogs fed on commercial pellets.

### MATERIALS AND METHODS

**Animals:** Ninety-two clinically healthy adult dogs of different breed, age and body weight were used in this study (15 Great Danes, 8 males and 7 females, average age  $37 \pm 6$  months and average body weight  $62 \pm 2$  kg; 10 Neapolitan Mastiffs, 6 males and 4 females, average age  $44 \pm 6$  months and average body weight  $66 \pm 3$  kg; 16 German Shepherds, 6 males and 10 females, average age  $41 \pm 6$  months and average body weight  $39 \pm 2$  kg; 14 Boxers, 10 males and 4 females, average age  $42 \pm 6$  months and average body weight  $34 \pm 2$  kg; 10 German Dachshunds, 7 males and 3 females, average age  $43 \pm 5$  months and average body weight  $6 \pm 1$  kg; 27 mixed-breed dogs, 15 males and 12 females, average age  $46 \pm 9$  months and average body weight  $31 \pm 8$  kg). Dogs were divided into three groups, according to body weight: the first group weighed between 5 and 7 kg, the second between 18 and 48 kg and the third between 50 and 70 kg. Dogs were also divided into two groups according to age: the first group between 28 and 40 months, the second between 41 and 61 months. Lastly the animals were divided into two groups, according to sex: the first group included 52 males, the second 40 females (Table 1).

All dogs received regular vaccinations and were treated for endoparasites. They remained healthy over the duration of the study, based on routine physical and laboratory examinations.

**Diet:** Several batches of commercial pet food, complete

**Table 1.** Mean values of total lipids, phospholipids, triglycerides and NEFA in serum, fecal fats and chymotrypsin, expressed in their conventional units together with the standard deviations, in dogs (in parenthesis the number of subjects) of different age, body weight and sex

	Total lipids (g/l)	Phospholipids (mM/l)	Triglycerides (mM/l)	NEFA (mEq/l)	Fecal fats (g/24 hrs)	Chymotrypsin (u/g feces)
Age (months)						
1 28–40 (47)	3.45 ± 0.57	90.14 ± 7.42	0.86 ± 0.14	0.22 ± 0.08	9.61 ± 3.77	32.57 ± 13.27
2 41–61 (45)	3.29 ± 0.57	92.30 ± 7.73	0.83 ± 0.15	0.22 ± 0.08	8.34 ± 4.29	29.82 ± 14.43
Body weight (kg)						
1 5–7 (10)	2.82 ± 0.14 <sup>a-a'</sup>	92.66 ± 2.37	0.68 ± 0.07 <sup>b-a'</sup>	0.16 ± 0.03 <sup>c-a'</sup>	1.23 ± 0.26 <sup>d-a'</sup>	6.34 ± 0.95 <sup>d-a'</sup>
2 18–48 (57)	3.20 ± 0.51 <sup>a''</sup>	90.47 ± 8.62	0.81 ± 0.12 <sup>a''</sup>	0.20 ± 0.06 <sup>a''</sup>	8.00 ± 1.50 <sup>a''</sup>	28.18 ± 5.30 <sup>a''</sup>
3 50–70 (25)	4.00 ± 0.21	92.67 ± 6.22	1.01 ± 0.06	0.30 ± 0.06	14.39 ± 1.24	49.97 ± 2.22
Sex						
1 Males (52)	3.75 ± 0.46 <sup>d</sup>	91.44 ± 7.05	0.81 ± 0.18	0.22 ± 0.08	8.18 ± 4.58	28.60 ± 15.02
2 Females (40)	3.23 ± 0.57	91.20 ± 7.03	0.75 ± 0.20	0.20 ± 0.06	6.77 ± 4.41	25.17 ± 14.79

Significant differences:

group 1 Vs 2: a= p&lt;0.05; b=p&lt;0.01; c=p&lt;0.05; d=p&lt;0.001; group 1 Vs 3: a'= p&lt;0.001; group 2 Vs 3: a'' =p&lt;0.001.

and balanced for adult dogs were fed to the subjects during the pre-experimental (15 days) and experimental (1 week) periods. The amount of food administered to the dogs was determined considering the body weight classes: group 1 were fed between 70 and 115 g/day, dogs in group 2 between 180 and 330 g/day and dogs in group 3 between 335 and 420 g/day, as recommended from producing industries. Pet food had the following chemical composition: proteins (26%), fats (15%), minerals (7,5%) and cellulose (2,5%). The food was administered once a day and water was available *ad libitum*. Food was given to maintain a constant BW and body condition score of 3 on a scale of 5.

**Procedure:** Blood and feces samples were collected three times over a week and an average quantity from these three collections was considered for each dog, so to obtain 92 samples. After a 12-hr fasting, at 08:00, blood was drawn from each subject by radial venipuncture. Feces produced over 24 hr were collected, pooled in dark sampling bottles and maintained at 4°C.

**Laboratory analysis:** Spectrophotometry in U.V. was used to establish the concentration in each serum of the following parameters: total lipids, phospholipids, triglycerides and NEFA. Fecal fat concentration was also determined using Sobel's method [4], while spectrophotometry in U. V. was used to calculate chymotrypsin concentration in feces produced over 24 hr, using a colorimetric test (Boehringer Mannheim) [6, 12, 13, 15]. From 24-hr feces, 100 mg were used to determine the chymotrypsin activity, by means of the procedure described in explanatory notes of the kit.

**Statistical analysis:** Results were expressed as mean ± SD. Analysis of variance (one-way ANOVA) and Student's *t* test were used to determine significant differences among the studied parameters (total lipids, phospholipids, triglycerides, NEFA, fecal fat and chymotrypsin) on the basis of the considered variables (age, body weight and sex). A *p*-value < 0.05 was considered significant in all analyses. If ANOVA showed an acceptable level of significance (*P*<0.05), Bonferroni's multiple comparison test was applied for post hoc comparison. Linear regression analysis was

performed to compare the effect of body weight on fecal fat and chymotrypsin.

## RESULTS

During experimental periods, all animals remained healthy based on the absence of clinical signs and normal blood parameters. By applying the one-way ANOVA on the different body weight classes, we obtained the following results: for total lipids, *F*(2,89)=41.09, *P*<0.0001; for phospholipids, *F*(2, 89)=0.9063, *P*=0.4; for triglycerides, *F*(2,89)=48.46, *P*<0.0001; for NEFA, *F*(2,89)=32.77, *P*<0.0001; for fecal fats, *F*(2,89)=377.5, *P*<0.0001; for chymotrypsin, *F*(2,89)=405.4, *P*<0.0001. Table 1 shows the mean values of total lipids, phospholipids, triglycerides, NEFA, fecal fats and chymotrypsin together with the standard deviations, taking into consideration age, body weight and sex and with the post-hoc comparison (Bonferroni's multiple comparison) among the body weight classes.

On comparing males and females, total lipids only showed a statistically significant difference (*P*<0.001, Student's *t* test). Moreover, a linear correlation between fecal fats and body weight was observed with a correlation coefficient *r*=0.99, *F*=1% and a regression line *y*=0.036+0.23•*x* (Fig. 1). There was no significant correlation between the other parameters and age and body weight. A linear correlation between fecal chymotrypsin and body weight was observed with a correlation coefficient *r*=0.99, *F*=1% and a regression line *y*=2.75+0.74•*x* (Fig. 2).

## DISCUSSION

Our results indicate that lipemia in the dog is influenced by the variables we considered. Body weight and sex influence total lipids, while only body weight influences triglycerides and NEFA. Phospholipids do not show statistically significant differences for the variables we considered. Fecal fat excretion is influenced by body weight, not by sex. Fecal fat excretion and serum parameters studied are not

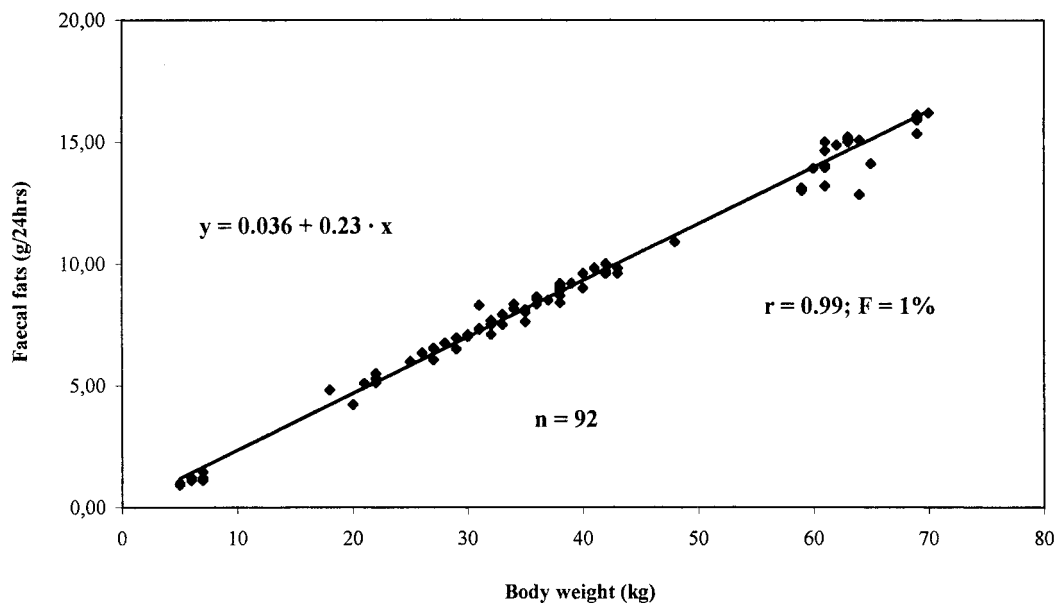


Fig.1 Correlation coefficient (r) with the relative statistical significance (F) and regression line between fecal fats and body weight.

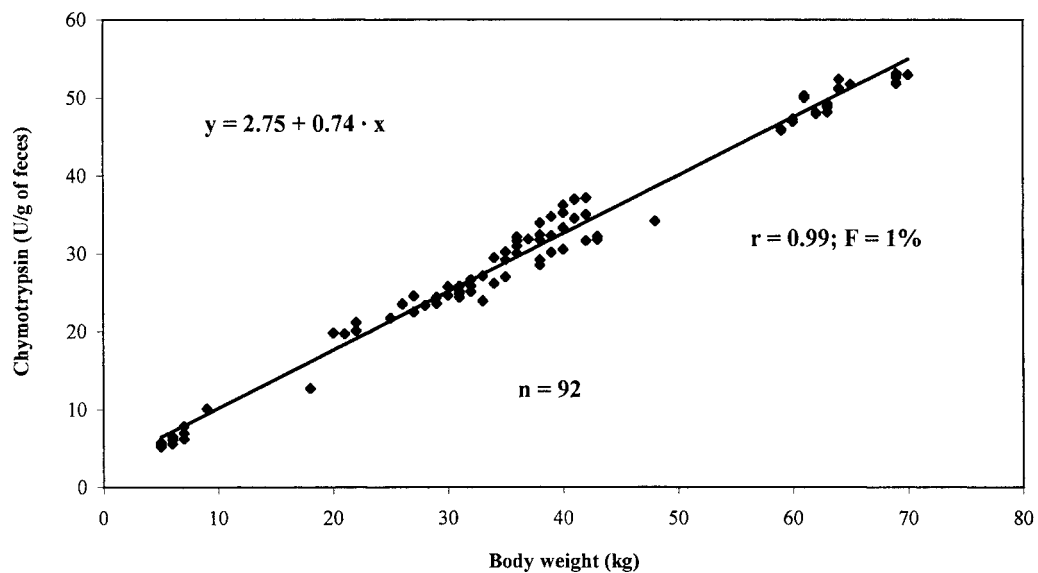


Fig.2. Correlation coefficient (r) with the relative statistical significance (F) and regression line between chymotrypsin and body weight.

correlated, as indicated in the literature by some authors [19]; they observed that lipemia returned to before feeding levels from 4–6 hr after feeding.

We observed a significant correlation between body weight and daily fecal fat excretion, as found in other studies [10, 14, 16, 23].

The higher apparent fecal fats and chymotrypsin excretion of larger dogs might be due to gastrointestinal length and absorptive surface area as well as variations in intestinal transit time or in nutrient absorption [25]. Data relating

intestinal length and body size in dogs are not available, but the relative mass of the gastrointestinal tract has been shown to be smaller in large dogs [25]. In a 5-kg dog, it contributes to about 7% of body mass, while in a 60-kg dog it represents only 2.8%; this would indicate that intestinal surface area (in  $\text{cm}^2/\text{kg}$ ) is larger in small dogs which does not fit with their lower digestive capacity. This could be explained with the more rapid intestinal transit time of small dogs which could affect nutrient utilization by reducing contact time between enzymes and chyme. On the other hand, recent studies have

reported similar rates of gastric emptying and oro-caecal transit time between small and large size breeds [24]. This would suggest that body size-related changes in digestibility coefficients would not be due to differences in upper gastrointestinal transit time. Further researches are needed to better explain the relationship between size and digestive capacities in dog. Lastly body weight influence chymotrypsin, while sex appears not to affect enzymatic activity.

Fecal chymotrypsin excretion in the healthy dog varies greatly (from a minimum of 5.21 to a maximum of 53.12 U/g of faeces), but is always greater than 3 U/g of feces, considered the threshold value of regular pancreatic functionality, valid both for man and dog [1]. Fecal chymotrypsin increases with higher body weight, as shown by the significant linear correlation shown in Fig. 2.

In conclusion, we can assert that some qualitative variables, such as age, body weight and sex, significantly affect the homeostatic processes which regulate fecal fat and chymotrypsin excretion. This knowledge makes a considerable contribution to evaluation of intestinal and pancreatic functionality, especially in the case of pathologies such as malabsorption, maldigestion and steatorrhea, which are common in the dog [22].

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