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RESEARCH ARTICLE

Effects of feeding diets with different protein levels on preference and some blood parameters in dogs

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Farklı protein düzeyleri ile beslemenin köpeklerde tercih ve bazı kan değerleri üzerine etkisi

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Öz

Amaç: Çalışma köpeklerde farklı protein düzeyleri ile beslemenin etkilerini gözlemek amacıyla yapıldı.

Gereç ve Yöntem: Canlı ağırlıkları 15-30 kg arasında olan kısırlaştırılmış 30 adet genç yetişkin erkek köpek kullanıldı. Kuru maddesinde %18, 21, 25 ve 28 ham protein içeren 4 ekstrude mama üretildi ve bir ay boyunca 28 köpeğe yedirildi. Çalışmanın başında ve sonunda kan örnekleri alındı.

Bulgular: Kan üre nitrojeninin (BUN) protein düzeyinden önemli düzeyde etkilendiği; kreatinin, total protein, albumin ve fosfor düzeylerinin ise değişmediği tespit edildi. Köpeklerin yüksek proteinli mamayı daha çok tercih ettikleri belirlendi.

Öneri: Bir ay süreyle yedirilen yüksek proteinli mamaların kan değerleri üzerine olumsuz etkilerinin olmadığı gözlendi. Yüksek proteinin böbrek fonksiyonları üzerine etkilerinin belirlenebilmesi için farklı yöntemler uygulanmalıdır.

Anahtar kelimeler: Köpek maması, protein, kan üre azotu, tercih

Abstract

Aim: This study aimed to establish the effect of different dietary protein levels on certain blood parameters and food preference of dogs.

Materials and Methods: A sample of 30 neutered, adult male dogs, with live weights of approximately 15-30 kg. Twenty-eight of dogs were fed four manufactured diets that respectively contained 18%, 21%, 25%, and 28% crude protein in dry matter for a period of one month. Blood samples were taken at the beginning and at the end of the study.

Results: The blood urea nitrogen levels were found to be significantly affected by the protein level in the diet, however, creatinine, total protein, albumin, and phosphorus levels were not found to be directly affected by dietary protein. It was determined that dogs prefer food with higher protein content in the preference test conducted with thirty dogs.

Conclusion: There were no adverse effects on the blood parameters of the different protein levels. The effects of high protein foods on kidney function should be monitored using different methods.

Keywords: Dog food, crude protein, blood urea nitrogen, preference

Introduction

The European Pet Food Industry Federation (FEDIAF 2013) reports nutritional guidelines for adult dogs and states that food must contain at least 18% protein in dry matter (DM). The percentage of protein in commercial dog foods, however, ranges from 15 to 60% in DM (Debraekeleer et al 2000). Once amino acid needs are met, the excess amino acids are not stored as protein in the body and are instead deaminated in the liver. The by-products of protein catabolism pass through and are excreted by the kidneys. The remaining keto acid analogues are used as an energy source or stored as fat or glycogen. The contribution of excess dietary protein to the progression of subclinical kidney disease is not well understood. Studies in humans have shown that protein restriction slows the progression of kidney disease (Case et al 2011). The restriction of protein in the early stages of kidney disease effectively improves the general condition (Leibetseder and Neufeld 1991). In a four-year long study (Finco et al 1994), no difference was found in the renal function of dogs that consumed 18% and 34% protein in DM. However, histological examinations revealed increased mesangial matrix scores and fibrosis in individuals that consumed the higher protein diets. Otherwise, it was reported that in glomerulonephritis and chronic interstitial nephritis had mesangial proliferation (Finco et al 1994).

The National Research Council (NRC 2006), states that the maintenance protein requirement of an adult dog is at least 2.62 g/ $BW^{0.75}$/day. The minimum crude protein (CP) requirement depends on digestibility and quality. For example, 12% CP in DM is sufficient for a food with a digestibility of 75%. In that case, the bioavailability of the protein should be higher in foods that contain less than 12% CP. In foods with an energy density of 4 kcal/g, the suggested daily maintenance protein consumption for adult dogs is approximately 10% in DM (Case et al 2011). Over a 10-week period, Williams et al (2001) fed 2- and 8-year old dogs with food that contained 16, 24, and 32% CP, and reported that diets that contain no more than 16% CP are required to provide nitrogen balance.

Food palatability for dogs may be positively correlated with protein level, no scientific research associated with it was found. This study aimed to determine the some blood parameters and the food choice of the dogs fed the diets with different protein levels.

Material and Methods

This study was conducted at Selcuk University Veterinary Faculty Research and Application Farm's Dog Research Unit with the permission of the local ethics committee (No: 2014/53). A total of 30 mixed-breed male dogs (firstly 28 dogs for feeding trial during 30 days; and then two dogs were added for preference test) were used in the study. Dogs were selected from healthy young adults, 1-3 years old, had been neutered. Animals were weighed, and they were dosed with internal and external parasite medicines prior to being moved to individual pens. The pens comprised 190 × 190 cm closed and 510 × 230 cm open areas, and a concrete floor. The animals were housed in individual pens to ensure consistency in the housing conditions. Food was given as at 1.8 times that of the daily recommended maintenance diet as per NRC (2006). Dogs were fed every day, once a day, and at the same time (10.00 a.m.) of day. Fresh water was provided ad libitum.

The dogs were divided into four similar groups, each consisting of seven to eight dogs, using the sensitive separating method (İnal 2005), and according to locations in the unit and live weights.

Foods

Four different experimental foods were manufactured at Bil-Yem Facilities using poultry meal and corn gluten as the protein source. The experimental foods contained 18%, 21%, 25%, and 28% CP in DM but were equal in energy levels (Table 1).

Analysis of nutrients

The analyses of dry matter (DM), ash, crude protein (CP), ether extract (EE), crude fiber (CF) and starch of the experimental food samples were performed according to the methods reported by the Association of Analytical Communities (AOAC 2003). The energy content of the samples was calculated with NRC (2006) formulas using the results of the analyses as following.
ME, kcal/kg = ((5.7 X CP X 10) + (9.4 X EE X 10) + (4.1 X (NFE X 10 + CF X 10))) X (91.2 - (1.43 X CF)) /100 - (1.04 X CP X 10)
ME: Metabolisable energy, NFC: Nitrogen-free extract

Blood analysis

Blood samples were taken from each animal before the commencement of the study (day 1) and at the end of the study (day 30). The blood serum was removed and tested at the Central Laboratory of Veterinary Faculty. Creatinine, blood urea nitrogen (BUN), total protein, albumin, and phosphorus levels were determined using the Biochemistry Analyzer (BT3000 PLUS).

Preference test

The method tested for two foods at the same time was applied. In this study, two foods that contained 21% and 28% CP in DM were used for the two-pan preference test. These two foods were chosen so that animal preferences can be clearly identified. Thirty animals were tested for statistical power. The amount of food that will meet the daily energy needs of adult and normal-activity dogs was estimated to be approximately 250-450 g. However, to determine their preferences clearly, each dog was offered 500 g of each food. Each of the 30 dogs were fed 500 g of both food types once every day, and at the same time

Table 1. Composition and calculated nutrients of experimental diets

Ingredient	18% CP	21% CP	25% CP	28% CP
Poultry meal, 59% CP	13.00	15.00	18.50	20.00
Barley	10.00	10.00	10.00	10.00
Corn	31.19	27.42	21.26	16.00
Corn gluten meal,56% CP	7.00	10.00	14.00	18.00
Corn starch	14.00	13.00	12.00	12.00
Rice	15.00	15.00	15.00	15.00
Whey	2.00	2.00	2.00	2.00
Sunflower oil	3.00	3.00	3.00	3.00
Beef tallow	3.00	3.00	3.00	3.00
Vitamin-mineral*	1.81	1.58	1.24	1.00
Calculated nutrients, in 100 g DM				
Crude protein, g	18.87	21.65	25.76	28.68
Energy, kcal	442	443	446	448
Crude fiber, g	2.27	2.26	2.23	2.20
Ether extract, g	10.59	10.76	11.05	11.10

*: Aminovit (per liter:Vitamin A 20.000.000IU, Vitamin D3 200.000 IU, Vitamin E 10.000 mg, Vitamin B1 2.500 mg, Vitamin B2 2.500 mg, Vitamin B6 500 mg, Vitamin B12 5 mg, Vitamin K3 500 mg, Vitamin H 15 mg, Pantotenic Acid 2.500 mg, Choline Chloride 70.000 mg, L-Arginine 600 mg, L-Cystine 100 mg, L-Leucine 600 mg, L-Valine 600 mg, L-Isoleucine 200 mg, L-Histidine 200 mg, L-Phenylalanine 500 mg, L-Proline 800 mg, L-Serine 100 mg, L-Tyrosine 200 mg, L-Treonine 500 mg, DL-Methionine 500 mg, L-Triptophane 20 mg, L-Lysine 3.000 mg, L-Glutamic Acid 4.000 mg, L-Alanine 1.000 mg); Minesol (per liter: Phosphorus 75.000 mg, Calcium 20.000 mg, Sodium 1.600 mg, Manganese 600 mg, Potassium 1.050 mg, Ferrous 1.600 mg, Magnesium 3.200 mg, Zinc 650 mg, Copper 250 mg, Cobalt 250 mg, Selenium 10 mg, Methionine 10.000 mg, Lysine 5.000 mg); potassium chloride; zinc proteinate; calcium iodate; sodium bicarbonate

of day, for 4 days. The dogs were taken to the outside areas of their pens while the food pans were placed, and then taken to the inside areas of their pens for the tests. Water was available for ad libitum consumption. Dogs could feed for a period of 1 h, after which the remaining quantities were weighed and the consumed quantities were calculated. The placement of the pans was alternated each day to eliminate any bowl-placement bias by the dogs. The highest overall consumption determined which food type was preferred. To determine the food's palatability, the consumption rate was calculated by the formula: Relative intake (%) = (Food 1 intake× 100) / (Food 1 intake + Food 2 intake). Dogs with a ratio greater than 0.51 were classified as preferring Food 1, and dogs with a ratio less than 0.49 were classified as preferring Food 2 (Griffin 1995, Dust et al 2005).

Statistical analysis

Independent t-tests (Student's t-tests) (v.22 SPSS) were used to test the differences between groups in the comparisons of blood analysis values. Dependent t-tests (Paired t-tests) (v.22 SPSS) were used to test the differences between the pre-trial and post-trial data.

Results

The results of the nutrient analyses of the experimental foods are presented in Table 2. Although the experimental foods were formulated to contain 18%, 21%, 25%, and 28% CP in DM, the CP analyses revealed that these values did deviate slightly in the final products (± 0.5%).

The results of the serum analyses of the BUN, creatinine, albumin, total protein, and phosphorus levels are listed and compared between the dogs fed with experimental CP diets (Table 3). No significant differences were found in the blood parameters, except in the BUN value which increased with increasing dietary protein ($P = 0.002$). The BUN levels also decreased significantly at the end of the experiment ($P < 0.05$) in groups that consumed 18% and 21% CP in DM. Creatinine levels ranged from 1.24-1.99 mg/dl, and were significantly increased in all groups at the end of the trial ($P < 0.01$). Recorded creatinine levels were above normal limits (0.50-1.70 mg/dl). Albumin levels were found to be significantly higher in the group that was fed 25% CP in DM ($P < 0.05$) at 30th day, however, no significant difference was found between the groups ($P = 0.299$). Blood phosphorus levels were found to be significantly lower in samples taken at the end of the trial in all groups, except for the group that was fed 25% CP in DM ($P < 0.05$), however, values still fell within the normal range (2.2-5.5 mg/dl). At 30th day, the total protein serum levels were only observed to have increased in dogs that were fed the 25% CP in DM diet ($P < 0.05$), and no significant difference was found between the groups ($P = 0.805$).

The determined preference ratios of the two foods from the two-pan preference test (21% and 28% CP) are presented in Table 4. Dogs preferred the 28% protein diet significantly.

Table 2. Nutrient analysis results of the foods, in 100 g DM

Diet	DM	Ash	EE	CF	CP	Starch	ME,kcal*
18% CP	92.55	5.20	7.58	3.48	19.08	55.84	378
21% CP	92.63	5.52	7.10	2.81	20.55	51.86	379
25% CP	92.38	6.29	7.17	2.72	25.42	44.19	379
28% CP	92.48	6.95	7.86	2.56	27.54	41.83	382

*: It was calculated with NRC 2006 formulas.

Table 3. The effects of different protein levels on some blood parameters

Blood parameters		Diet				
		18% CP	21% CP	25% CP	28% CP	P
BUN, mg/dl	1st day	21.29±1.41 A	21.86±1.97 A	22.14±2.30	22.14±1.87	0.987
	30th day	15.00±1.05 Bb	17.00±1.07 Bb	22.43±1.91a	23.00±1.95a	0.002
	P	<0.001	0.012	0.736	0.466	
Creatinine, mg/dl	1st day	1.29±0.13 B	1.31±0.06 B	1.26±0.11 B	1.24±0.06 B	0.953
	30th day	1.99±0.20 A	1.86±0.05 A	1.89±0.16 A	1.71±0.06 A	0.570
	P	<0.001	<0.001	0.001	<0.001	
Albumin, g/dl	1st day	3.81±0.09	3.69±0.12	3.41±0.19 B	3.80±0.10	0.133
	30th day	3.91±0.08	3.74±0.09	3.73±0.10 A	3.89±0.06	0.299
	P	0.267	0.637	0.031	0.482	
Phosphorus, mg/dl	1st day	5.46±0.31 A	5.59±0.65 A	5.21±0.22	5.80±0.49 A	0.826
	30th day	3.93±0.18 B	4.21±0.37 B	4.73±0.39	4.71±0.66 B	0.501
	P	0.009	0.004	0.344	0.012	
Total protein, g/dl	1st day	6.87±0.22	6.77±0.23	6.69±0.29 B	7.07±0.21	0.703
	30th day	7.31±0.22	7.10±0.20	7.36±0.25 A	7.29±0.08	0.805
	P	0.051	0.108	0.003	0.354	

a,b: Means within a row with no common letters differ significantly (P < 0.05), (n = 7)
A,B: Means within a column with no common letters differ significantly (P < 0.05), (n = 28)

Table 4. Effect of protein level on animal's preference

	21% CP	28% CP
Served food, g/day*	500	500
Refused, g/day*	301.5	149.7
Food intake, g/day*	198.5	350.3
Preference rate, %	32.6	67.4

*. Average of four days, n = 30

Discussion

The composition of dog food is variable; however, the demand and flow of this food is continuous. It is possible that the protein values of poultry meal or corn gluten meal in dog food may be higher or lower than predicted or calculated, as was the case in our study. The analyzed ether extracts were lower than the formulated. Because acid-hydrolysis was not applied before ether extract analysis. The reason for the differences in energy values; energy were calculated from the values given in the ingredient lists in Table 1, and according to the NRC formulas in Table 2. NRC methods for computing chemical compositions of food can be underestimate the ME content of low-fiber foods (Castrillo et al 2009). Analysis of the manufactured experimental food in our study showed that as the crude protein increased, the starch and crude fiber levels decreased. The ash values also increased due to the increase in poultry meal in food types with higher CP in DM. Energy content was found to be approximately the same in all experimental food types. The most commonly used barley, rice, corn (İnal et al 2017), vegetable and animal fats in dog diets were used as an energy source in experimental diets.

The reduction in BUN blood serum levels in first two experimental groups is thought to have been due to the fact that the dogs were fed diets containing up to 23% CP in DM prior to the commencement of the study, and is hence a result of the transition from this previous diet to the 18% and 21% CP experimental diets. The recorded BUN values ranged from 15.00-23.00 mg/dl, and fell within normal limits (8.00-28.00 mg/dl) (Kaneko et al 2008, Latimer 2011). Dos Reis et al. (2016) found significantly increased BUN levels (13.99 and 16.14 mg/dl, respectively) in dogs fed with foods that contained 25% and 35% CP in DM. In contrast, Romsos et al. (1976) found no difference in BUN levels of dogs fed on diets that contained 20-48% CP.

When compared to reference values (2.30-3.10 g/dl) (Kaneko et al 2008, Latimer 2011), the albumin values of all experimental groups were found to be relatively high at both at the beginning and at the end of our experiment. According to the Laboratory of Veterinary Diagnostic Medicine, University of Illinois (Swanson et al 2004), however, the recorded albumin values of our study fell within the normal range (2.10-4.30 mg/dl). The above-normal creatinine values reported in our study can be associated with the presence of excess dietary protein and the inactivity of the dogs.

Blood phosphorus levels have been seen to increase in kidney diseases (Case et al 2011). It was, therefore, a positive indicator, in terms of the health of the dogs used in this study, that recorded phosphorus levels were not high, and not as high as creatinine and albumin levels. Total protein levels (6.69-7.36 g/dl) remained within normal limits (5.40-7.50 g/dl) (Kaneko et al 2008, Latimer 2011). The findings of Swanson et al. (2004), i.e., total protein (6.99 g/dl), albumin (3.37 g/dl), and phosphorus (3.73 mg/dl), were similar to those of our study in dogs that were fed with foods that contained 22% CP.

Although it has been recommended that animals with chronic renal problems should be fed a diet that is reduced to 8% CP (Swanson et al 2004), depending on the severity of the condition, it has also been reported in various studies (Finco et al 1994, Polzin et al 1984, Robertson et al 1986, Finco et al 1992), that dietary protein levels do not affect renal lesions or renal function in various studies.

The two-pan preference test (that compared preference between foods that contained 21 and 28% CP) showed that the dogs significantly preferred the food type with the higher CP level. This higher-protein food contained more poultry meal and corn gluten. It is thought that the higher composition of poultry meal in the food is the influential factor on the preference towards the higher protein food type. Although dogs can become accustomed to high carbohydrate diets, they are natural carnivores.

Conclusion

If other essential nutrients are balanced in adult dog food, the CP level can be reduced to 18% in DM. It can also be raised to 28% at the same time. There were no adverse effects on the blood parameters of the different protein levels. All values are within normal limits.

Further studies are needed to evaluate the health implications of feeding dogs a diet that is high in protein. The effects of high protein foods on kidney function should be monitored using different methods.

In this study, dogs obviously preferred high protein food, 28% CP versus 21% CP.

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